Historical Population dynamics of red snapper (*Lutjanus campechanus)* in the northern Gulf of Mexico

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HISTORICAL POPULATION DYNAMICS OF RED SNAPPER (*LUTJANUS CAMPECHANUS*) IN THE NORTHERN GULF OF MEXICO

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Abstract.—A total of 313 young-of-the-year red snapper (*Lutjanus campechanus*) belonging to the 1999 year class were sampled from three geographic regions in the northern Gulf of Mexico and assayed for haplotype variation in mitochondrial (mt)DNA. Analysis of molecular variance revealed that only a small proportion (0.24%) of the genetic variance was distributed among regions; accordingly, the corresponding Φ_{ST} value did not differ significantly from zero. Exact tests of homogeneity of haplotype distributions also were non-significant. Tests for departure from a neutral Wright-Fisher model of genetic polymorphism, however, were significant, and a 'mismatch' distribution of nucleotide-site differences in mtDNA indicated that the departure from neutrality could be due to population expansion. Estimates of the time since expansion ranged from ≈270,000 to ≈420,000 years before present. The latter is consistent with the hypothesis that red snapper likely colonized the continental shelf in the northern Gulf following a glacial retreat. The observed departure from a neutral Wright-Fisher model also may suggest that insufficient time has lapsed for red snapper in the northern Gulf to attain equilibrium between mutation and genetic drift. However, the temporal signature provided by the 'mismatch' distribution is far older than the last glacial retreat which began ≈18,000 years ago. If the departure from neutrality reflects events occurring after the last glacial retreat, tests of present-day population or stock structure may well be compromised. The same may be true for other marine fish species in the northern Gulf.

Red snapper (*Lutjanus campechanus*) is an important, highly exploited marine fish distributed primarily along the continental shelf in the Gulf of Mexico from the Yucatan Peninsula in Mexico to the northeastern Florida coast (Hoese & Moore 1998). Although the species has provided an important fishery since the early 1900s, red snapper in U.S. waters have declined by an estimated 90% since the 1970s (Goodyear & Phares 1990). Factors impacting red snapper abundance include overexploitation by directed commercial and recreational fisheries, juvenile mortality associated with bycatch in the shrimp fishery, and habitat change (Gallaway et al. 1999; Ortiz et al. 2000). Management of red snapper resources in U.S. waters is currently based on a unit stock hypothesis (GMFMC 1989). Whether red snapper in fact comprise a single stock across the northern Gulf, however, remains an issue. Separate management of regional stocks, if they exist, would be a desirable goal to avoid regional over-exploitation and to conserve adaptive genetic variation (Carvalho & Hauser 1995; Hauser & Ward 1998).

Previous genetic work generally has been consistent with the existence of a single stock of red snapper in the northern Gulf (Camper et al. 1993; Gold et al. 2001) and with the hypothesis that significant gene flow occurs at one or more life-history stages (Goodyear 1995; Gold & Richardson 1998a). The hypothesis of significant gene flow is not consistent with a number of tagging studies that have shown adult red snapper to be sedentary and exhibit high site fidelity (Szedlmayer & Shipp 1994; Szedlmayer 1997). However, Patterson et al. (2001) recently documented extensive movement of adult red snapper in the northeastern Gulf and suggested that movement of adults might be sufficient to facilitate mixing across the northern Gulf. A second hypothesis is that observed genetic homogeneity reflects historic rather than contemporary gene flow, and that present-day red snapper could be isolated yet have been in sufficient genetic contact in the past to remain genetically indistinguishable (Camper et al. 1993; Gold & Richardson 1998a). In such situations, populations may not have reached equilibrium between mutation and genetic drift, and if so, would be expected to depart from expectations of the neutral Wright-Fisher model of genetic polymorphism (Fu 1997).

This study examined the alternate hypothesis by assessing patterns of mitochondrial (mt)DNA variation among red snapper sampled from three geographic regions in the northern Gulf and asking whether mtDNA haplotype distributions deviated from those expected under mutation-drift equilibrium. Populations that are expanding or declining typically are not in mutation-drift equilibrium (Fu 1997), and in such situations may leave a characteristic 'mismatch' distribution signature (Rogers & Harpending 1992). Consequently, this study also examined the 'mismatch' distribution of nucleotide site differences in mtDNA between pairs of individuals in order to assess whether red snapper in the northern Gulf had expanded or declined demographically. Red snapper were likely precluded from occupying most of the contemporary continental shelf in the northern Gulf during Pleistocene glacial advance (Gold & Richardson 1998a), and colonization of shelf waters following glacial retreat could have generated conditions conducive to population expansion.

Materials and Methods

Young-of-the-year red snapper were procured in the fall of 1999 during a demersal trawl survey of the northern Gulf carried out by the

National Marine Fisheries Service (NMFS). Individual fish were sampled from the catch of a 12 m shrimp-trawl net, frozen onboard and returned to College Station where tissues were removed and stored at −80°C. Specimens were obtained from different offshore localities corresponding to three geographic regions (Fig. 1) representing the northwestern Gulf (south Texas coast, 14 trawls, n = 127, range/trawl = 4-12, mode = 8), the northcentral Gulf (Louisiana coast, 14 trawls, n = 123, range/trawl = 1-20, mode = 10), and the northeastern Gulf (Mississippi-Alabama coast, 9 trawls, n = 63, range/trawl = 1-13, mode = 10). Genomic DNA was isolated from frozen tissues as described in Gold & Richardson (1991).

Assay of mtDNA employed single strand conformational polymorphism or SSCP (Orita et al. 1989). Regions within the NADH-4 (ND-4) and NADH-6 (ND-6) protein-coding genes were sequenced and the Lasergene software package *Primer Select* was used to design polymerase-chain-reaction (PCR) primers that amplified mtDNA fragments less than 250 base pairs (bp) in size. The fragments were 163 bp from ND-4 and 122 bp from ND-6. PCR primers (forward primer first, then reverse primer) were as follows: ND-4 (5' - CAAAACCTTAATCTTCTACAATGCT - 3'; 5' - CAGGGGGTCTGTTGCTAT - 3') and ND-6 (5' - CGAAGCGTCCCCCGACT - 3'; 5' - CGGTTGATGAACTAGGTGATTTTTC - 3'). PCR conditions followed those used for red snapper microsatellites (Gold et al. 2001), except that annealing was carried out at 58°C and both primers for each fragment amplified were radioactively labelled. Following PCR, 5μL of stop solution (95% formamide, 0.05% bromophenol blue and xylene cyanol, 10 mM NaOH) was added to 10μL of PCR product. This solution was heat denatured at 100°C for 10 min and then snap-chilled in ice water. Varying gel composition and electrophoresis conditions optimized resolution of electromorphs. Adequate resolution was provided by electrophoresing PCR products at 500 V for 16 h on 8% non-denaturing polyacrylamide gels (37.5:1 acrylamide:bis-acrylamide, 0.5X TBE), supplemented with 5.0% glycerol (4.0% for NADH6) and run in 0.5X TBE buffer. The ND-4 and ND-6 electromorphs were best resolved by electrophoresis at 12°C. Efficiency of SSCP procedures to identify sequence variants was assessed by sequencing multiple representatives of each electromorph and comparing patterns of sequence divergence among them. Representatives of each electromorph were run on subsequent SSCP gels as reference controls.

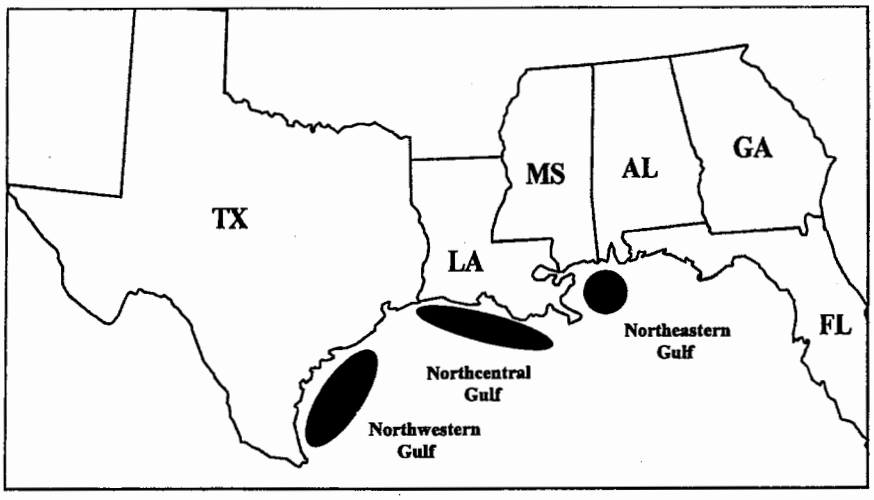

Figure 1. Collection localities of young-of-the-year red snapper (*Lutjanus campechanus*) from the northern Gulf of Mexico: northwestern Gulf (n = 127), northcentral Gulf (n = 123, and northeastern Gulf (n = 63).

MtDNA haplotype (nucleon) and nucleotide diversity were estimated after Nei (1987). The former represents the probability that any two individuals drawn at random will differ in mtDNA haplotype, whereas the latter represents the number of nucleotide differences per site between two randomly chosen sequences. Private haplotypes were tabulated and a V test (DeSalle et al. 1987) was used to test whether the proportion of private haplotypes differed significantly among regional samples. Homogeneity of mtDNA haplotype distributions among regions was assessed via analysis of molecular variance and exact tests (based on a Markov-chain procedure). For AMOVA, significance of the variance among samples and of Φ_{ST} was assessed by permutation (10,000 replicates). Both tests of homogeneity were carried out using ARLEQUIN (Schneider et al. 2000).

Deviation from mutation-drift equilibrium was assessed via Fu & Li's (1993) D^* and F^* and Fu's (1997) F_S measures of selective neutrality. Tests of significance of Fu and Li's D^* and F^* and Fu's F_S statistics were performed using DNASP (Rozas et al. 2003) and ARLEQUIN, respectively, and were based on 1,000 (D^* and F^*) and 10,000 (F_S) randomizations. Mismatch-distribution analysis (Rogers & Harpending

1992) was used to assess population expansion. As populations at mutation-drift equilibrium are expected to have ragged mismatch distributions (Rogers & Harpending 1992), the r measure of 'raggedness' (Harpending 1994) was calculated using ARLEQUIN; tests of $r = 0$ were carried out by parametric bootstrapping (10,000 replicates), also using ARLEQUIN.

RESULTS

Twelve electromorphs (A-L) of the 163 bp ND-4 fragment and fourteen electromorphs (A-N) of the 122 bp ND-6 fragment were identified via SSCP. Sequences of all electromorphs may be found in Table 1. All electromorphs of the ND-4 fragment differed by no more than a single nucleotide substitution from the most common electromorph (designated 'A'); for the ND-6 fragment, two electromorphs ('F' and 'G') differed by more than one nucleotide substitution from any other electromorph. Multiple representatives of each electromorph (both fragments) were sequenced but no variation within an electromorph type was detected.

A total of 32 composite mtDNA haplotypes were identified (Table 2). Haplotypes AA, BB, and AC were the most common, occurring at frequencies within regions of >0.300 (AA), 0.190 – 0.331 (BB), and 0.134 – 0.238 (AC). Twenty-one private haplotypes were observed; the number of private haplotypes per regional locality was 8 (Texas), 10 (Louisiana), and 3 (Mississippi/Alabama). None of the private alleles occurred at a frequency greater than 0.017, and the proportion of private haplotypes did not differ significantly among regions ($V_{[2]} = 0.657$, $P > 0.05$). Nucleon and nucleotide diversities among regions were 0.770 (Texas), 0.776 (Louisiana), and 0.798 (Mississippi/Alabama), and 0.006 (Texas), 0.007 (Louisiana), and 0.006 (Mississippi/Alabama), respectively.

Analysis of molecular variance revealed that only 0.24% of the molecular variation was distributed among samples rather than within samples; the Φ_{ST} value of 0.002 did not differ significantly ($P = 0.253$) from zero. An exact test of homogeneity in mtDNA haplotype distribution among regions also was non-significant ($P = 0.307$). Given the absence of heterogeneity in the distribution of mtDNA haplotypes among samples, all mtDNA haplotypes were pooled into a single sample for all subsequent analysis.

Table 1. Sequence electromorphs of NADH-4 and NADH-6 (mitochondrial DNA haplotypes) identified from red snapper (*Lutjanus campechanus*). Sequences are from the light strand of the molecule and are listed in the 5' to 3' direction. Note that NADH-6 is encoded on the heavy strand (i.e., codon positions would be reversed).

NADH4

```
Haplotype A   AAAAATCCTAATTCCAACCCTAATGCTCGTCCCAACAACTTGGCTGACCCCCGCCAAATGACTCTGACCTACAGCCCTTCTA
Haplotype B   ............................................................................C...
Haplotype C   ............................................................................
Haplotype D   ............................................................................
Haplotype E   ...........................................................................G.....
Haplotype F   ........................................................T...........................
Haplotype G   .....................................................................T..........
Haplotype H   ............................................................................
Haplotype I   ..........................................A.................................
Haplotype J   ..........................................................................A.....
Haplotype K   ..................G.....................................................................
Haplotype L   ............................................................................

Haplotype A   CACAGCCTAGTAATTGCACTTGCTAGCCTCACCTGATTGAAAAACCTCTCAGAAACAGGCTGGTCCTGCCTAAACCCCTAC
Haplotype B   ............................................................................
Haplotype C   ............................................................................G.....
Haplotype D   ........T...................................................................
Haplotype E   ............................................................................
Haplotype F   ............................................................................
Haplotype G   ............................................................................
Haplotype H   ....................................A.....................................
Haplotype I   ............................................................................
Haplotype J   ............................................................................
Haplotype K   ............................................................................
Haplotype L   ..................A.....................................................
```

Table 1. Cont.

NADH6

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Haplotype A   CAACCCCCGTGTCAACTCCAGCACTACAAACAAAGTAAGTAAAAGAACTCATGCACTAATCAC
Haplotype B   ............................................................
Haplotype C   ..G.............A..........................................
Haplotype D   ............................................................
Haplotype E   ..G.........................................................
Haplotype F   .............T....A.........................................
Haplotype G   ..................A.........................................
Haplotype H   ............................................................
Haplotype I   ............................................................
Haplotype J   ............................................................
Haplotype K   ..................A.........................................
Haplotype L   ..................A.....G.....................................
Haplotype M   ..G.........................................................
Haplotype N   ..G.....................................  ....................

Haplotype A   TAGTATCCCGCCCCCTAATGAGTACATCAACGCAACGCCCCCAATATCCCCCCGAAGCGT
Haplotype B   ..........................................................G.....
Haplotype C   ............................................................
Haplotype D   ..........................................................A.
Haplotype E   ............................................................
Haplotype F   ............................................................
Haplotype G   ..................A.....G...................................
Haplotype H   .........A..................................................
Haplotype I   ..........................................................G.....
Haplotype J   ..........................................G.................
Haplotype K   ..........................................................T.....
Haplotype L   ............................................................
Haplotype M   .............G..............................................
Haplotype N   ..A.........................................................
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Table 2. Frequencies of mitochondrial (mt)DNA haplotypes from age 0-1 red snapper (*Lutjanus campechanus*) sampled from three regions in the northern Gulf of Mexico. Sample region and number of individuals are northwestern Gulf (TX, n = 127), northcentral Gulf (LA, n = 123), and northeastern Gulf (MS-AL, n = 63). First letter (A-L) represents sequence eletromorphs at ND-4; second letter (A-N) represents sequence electromorphs at ND-6. Electromorph sequences may be found in Table 1.

MtDNA haplotype	TX	LA	MS-AL	MtDNA haplotype	TX	LA	MS-AL
AA	0.306	0.328	0.333	AJ		0.008	
BB	0.331	0.319	0.190	AK	0.008		
AC	0.165	0.134	0.238	AL		0.008	
AB	0.066	0.017	0.079	AM	0.008		
BA	0.008	0.017	0.048	BH	0.008		
AD	0.008	0.017	0.032	BN		0.008	
AE	0.016	0.017		CC			0.016
CA	0.008	0.017	0.016	FC	0.008		
AF	0.008	0.008		GC		0.008	
AG		0.008	0.016	GH		0.008	
AH		0.008	0.016	HA	0.008		
BC	0.016			IA	0.008		
BD	0.016			IC		0.008	
DA		0.017		JF		0.008	
EA		0.017		KA		0.008	
AI		0.008		LA			0.016

Fu & Li's (1993) D^* and F^* and Fu's (1997) F_s measures of selective neutrality were negative and significant for the pooled samples (D^* = -2.85, P = 0.019; F^* = -2.73, P = 0.007; F_s = -22.59, P = 0.000), consistent with demographic growth of a population (Fu 1997). Population growth (expansion) also was indicated by the unimodal mismatch distribution (Fig. 2) and by Harpending's (1994) raggedness index (r) which was non-significant (r = 0.107, P = 0.070). The time at which demographic expansion in red snapper might have occurred was estimated via the relationship $\tau = 2ut$ (Rogers & Harpending 1992). The value τ is the crest or peak of a unimodal mismatch distribution (measured in units of $1/2u$ generations), u is the mutation rate/generation of the region under study, and t is time in generations. The estimate of τ (2.412) was obtained from ARLEQUIN; u was estimated as the product of $m_T\mu$, where m_T is the number of nucleotides assayed (285) and μ is an estimate of the mutation rate per nucleotide. For estimate(s) of μ, the molecular-clock calibrations for mitochondrial protein-coding genes developed by Bermingham et al. (1997) were used and employed two rates (1.0%/10^6 yr and 1.5%/10^6 yr) for the (combined) ND-4 and ND-6 sequences from red snapper. For generation time, 15 and 20 years were used, framing the hypothesized generation time in red snapper of 17-19 years (J. Cowan, Louisiana State University, pers. comm.). Estimates

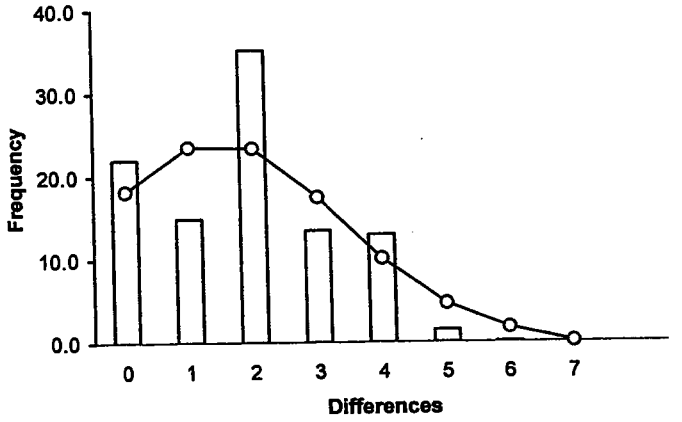

Figure 2. Mismatch distribution observed for mitochondrial DNA sequences (haplotypes) of young-of-the-year red snapper (*Lutjanus campechanus*) from the northern Gulf of Mexico. Bars represent observed frequency of differences between sequences; line represents the expected distribution assuming demographic expansion.

of u ranged from 1.5 x 10^{-7}/generation (μ = 1.0%/10^6 yr, 15 yr/generation) to 3.0 x 10^{-7}/generation (μ = 1.5%/10^6 yr, 20 yr/generation). Estimates of the time when demographic expansion in red snapper could have occurred ranged from ≈200,000 yr (u = 3.0 x 10^7/generation) to ≈540,000 yr (u = 1.5 x 10^7/generation). Despite uncertainties surrounding appropriateness of the molecular clock calibrations (Martin & Palumbi 1993; Rand 1994), and issues with use of pairwise-difference parameters such as τ (Felsenstein 1992), estimates of the time since demographic expansion in red snapper fit well within the Pleistocene epoch.

DISCUSSION

The observed homogeneity of mtDNA-SSCP haplotype frequencies among sample localities is consistent with the hypothesis that red snapper constitute a single stock in the northern Gulf. Similar findings were reported by Camper et al. (1993) based on restriction-site analysis of whole mtDNA and by Gold et al. (2001) based on analysis of microsatellites. Because genetic homogeneity typically implies sufficient gene flow to offset genetic divergence, continuous movement of red snapper at various life-history stages has been hypothesized (Goodyear 1995; Gold & Richardson 1998a; Patterson et al. 2001).

The significant departure of mtDNA variation from expectations of

the neutral Wright-Fisher model of genetic polymorphism indicates that red snapper in the northern Gulf have not attained equilibrium between mutation and genetic drift. Moreover, the negative values for the 'neutrality' indices, particularly Fu's (1997) F_S index, suggest that the departure from neutrality stems from population growth. However, in addition to population growth, the D^* and F^* indices of Fu and Li (1993) and the F_S index of Fu (1997) also can signify either background selection or genetic hitchhiking, respectively (Fu 1997). Neither seems plausible in this case, in part because data are from mtDNA which is inherited as a single gene and independently from all nuclear genes, and in part because the mismatch distribution and Harpending's (1994) raggedness index were consistent with the hypothesis of historical population expansion. In addition, because red snapper were precluded from occupying much of the contemporary continental shelf in the Gulf when sea levels during Pleistocene glaciations were at least 100 m lower than they are today (CLIMAP 1976; Rezak et al. 1985), colonization of shelf waters and opening of favourable habitat following glacial retreat would be expected to generate conditions conducive to population expansion. This scenario is consistent with the estimated time of $\approx$200,000 – 540,000 years ago, given that the Pleistocene Epoch began approximately 1.8 million years ago (http://vulcan.wr.usgs.gov/Glossary/geo_time_scale.html).

Camper et al. (1993) and Gold et al. (2001) suggested that the genetic homogeneity observed among present-day red snapper in the northern Gulf might reflect historical rather than current gene flow. Briefly, genetic homogeneity among putatively isolated, present-day populations could be sustained provided there has been both insufficient time since colonization of continental-shelf waters and sufficiently large effective population sizes such that allele frequency differences arising via mutation have not reached mutation-drift equilibrium. However, the time since expansion indicated from the mismatch distribution ($\approx$200,000 – 450,000 years ago) would seem too long for genetic divergence not to have arisen, assuming there has been no gene flow among localities and that effective population sizes are even one-tenth to one-hundredth of the current estimated census size of 7 – 20 million individuals. Unfortunately, estimating approximately how long it would take for genetic divergence to arise in this situation is problematic, given the absence of estimates of the effective (female) size of red snapper populations in the northern Gulf and the possibly unrealistic assumptions that red snapper form 'idealized' populations that exhibit an infinite-

island model of population structure. On the other hand, the last glacial retreat and the (re)opening of the continental shelf in the northern Gulf was only within the last 18,000 years (Rezak et al. 1985), a time period that is potentially too short for genetic divergence to occur if effective (female) sizes are only 1-2 orders of magnitude smaller than current census size and particularly if there is periodic gene flow among (semi-) isolated stocks.

There are a number of caveats to the above inferences. The first is that immigration of rare, genetically distinct mtDNA haplotypes also could generate negative D^*, F^*, and F_s values (Skibinski 2000). However, such immigration would be expected to lead to multimodal mismatch distributions (Marjoram & Donelley 1994), unlike the unimodal distributions observed here. A second caveat is that declining rather than expanding populations also can produce unimodal mismatch distributions. However, the 'wave' of a unimodal distribution of a declining population is expected to have an extremely steep leading edge, often with several secondary peaks that have large values (Rogers & Harpending 1992), a pattern not observed in the mismatch distribution generated from mtDNA sequences. Finally, the tests of neutrality may not necessarily measure the same temporal period as the mismatch distribution. The latter indicated a period of population expansion that occurred between $\approx$200,000 and 450,000 years ago, whereas the tests of neutrality could reflect an expansion dating to the last glacial retreat. At present, there is no way to distinguish among these alternatives.

Assuming red snapper in the northern Gulf deviate from mutation-drift equilibrium because of demographic expansion following the last glacial retreat, the question arises as to how prevalent are the same genetic patterns and demographic histories in other marine fishes in the northern Gulf. Grant & Bowen (1998) hypothesized that the combination of high haplotype diversity and low nucleotide diversity for mtDNA was indicative of a population bottleneck followed by rapid growth (their Category 2), and assigned two species that are common in the northern Gulf (red drum, *Sciaenops ocellatus*, and greater amberjack, *Seriola dumerili*) to this category. They erroneously assigned red snapper to Category 1 (low haplotype diversity and low nucleotide diversity) based on an error in reading Table 3 in Camper et al. (1993). Given the range of haplotype (0.770 – 0.798) and nucleotide (0.006 – 0.007) diversity found here, red snapper clearly belong in Category 2. A review of the literature reveals that many other fishes in the northern Gulf also appear

to belong to Grant and Bowen's Category 2: Gulf toadfish, *Opsanus beta* (cf. Avise et al. 1987); Spanish sardine, *Sardinella aurita* (cf. Tringali & Wilson 1993); common snook, *Centropomus undecimalis* (cf. Tringali & Bert 1996), and black drum, *Pogonias chromis*, spotted seatrout, *Cynoscion nebulosus*, and king mackerel, *Scomberomorus cavalla* (synopsized in Gold & Richardson 1998b). Analysis of selective neutrality and of mismatch distributions of mtDNA datasets may demonstrate that these species also have undergone demographic expansions that could be dated approximately to changes in habitat availability during or following Pleistocene glaciation. Consequently, it may be that the (spatial) genetic homogeneity observed for many fishes in the northern Gulf of Mexico owes more to historical than contemporary gene flow, and that stocks meriting independent management may have gone unnoticed. A final important point to note that these current results do not necessarily reflect contemporary trends or contradict the documented decline of present-day red snapper stocks (Goodyear & Phares 1990), as evidence of historic demographic expansion is not necessarily affected by even severe bottlenecks that occur subsequent to population expansion (Rogers 1995; Lavery et al. 1996).

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