Population Structure of Red Snapper from the Gulf of Mexico as Inferred from Analysis of Mitochondrial DNA

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SEDAR74-RD62

March 2021

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Abstract.—Variation in mitochondrial DNA (mtDNA) was examined among 707 red snapper *Lutjanus campechanus* representing 16 samples taken during 3 years from localities in the northern and western Gulf of Mexico. Ninety-two composite mtDNA haplotypes were revealed by 13 restriction enzymes (representing 93 inferred restriction sites). Significant heterogeneity ($P = 0.042$) in mtDNA haplotype frequencies was detected among the 16 samples; however, homogeneity tests of mtDNA haplotype frequencies between or among samples taken in different years at the same locality and among samples at different localities within the same year, were not significant. No phylogeographic structure of haplotypes was evident, nor were rare haplotypes clustered geographically. Spatial autocorrelations did not differ significantly from those expected when no correlation exists. These findings are consistent with the hypothesis that a single breeding population of red snapper inhabits the northern Gulf of Mexico. Intrapopulational mtDNA diversities, however, differed significantly among samples, suggesting that red snapper in the Gulf may not be drawn from a single population. Red snapper in the Gulf of Mexico may possibly include recently derived populations for which there has been insufficient time for accumulation of significant differences in mtDNA haplotype frequencies.

Red snapper *Lutjanus campechanus* is one of the most economically important fish species in the Gulf of Mexico (hereafter, Gulf; GMFMC 1989, 1991) that historically has supported both commercial and recreational fisheries (Goodyear and Phares 1990). Although the species is distributed in the western Atlantic essentially from the Yucatán peninsula to North Carolina, its center of abundance is the Gulf (Hoese and Moore 1977). Restrictions recently have been placed on red snapper harvest in U.S. waters because of apparent decreases in abundance (Nichols 1989; Goodyear 1992). The apparent decreases have been attributed to several factors, including the directed fishery and the bottom-trawl fishery for shrimp (in which juvenile red snapper are discarded as bycatch) (GMFMC 1989; Goodyear and Phares 1990; Goodyear 1992).

Assessment and management of red snapper within the Gulf of Mexico Exclusive Economic Zone (EEZ) and adjoining Territorial Sea are based on a unit (single) stock hypothesis (GMFMC 1989, 1991). Although few data addressing the issue of stock structure were available when the management plan was drafted, subsequent genetically based studies of red snapper from the northern Gulf have been consistent with the presumptive existence of a single stock (population) with considerable gene flow among sample localities. These studies have employed both nuclear-encoded allozyme loci (Johnson 1987) and mitochondrial DNA (Camper et al. 1993; Gold et al. 1994).

Mark–recapture and sonic tracking experiments suggest that postlarval stages (i.e., juveniles, subadults, and adults) of red snapper are relatively sedentary, nonmigratory, and usually associated with specific substrates or structures (Bradley and Bryan 1975; Beaumariage and Bullock 1976; Fable 1980; Szedlmayer and Shipp 1994; Szedlmayer, in press). Collectively, results of these studies are not consistent with the notion of considerable gene flow across the northern Gulf or with the observed spatial genetic homogeneity. However, inshore–offshore movements of postlarval red snapper are recorded, and individuals occasionally move considerable distances (Beaumariage 1969; Bradley and Bryan 1975; Beaumariage and Bullock 1976; Gutherz and Pellegrin 1988). In addition, red snapper eggs and larvae are pelagic (Leis 1987) and could be dispersed hydrodynamically (Goodyear 1992).

In this study, we expand our survey of mtDNA variation among red snapper by comparing samples taken from the same localities in multiple years. The objectives were to further test the hypothesis that all red snapper in the northern Gulf are a single stock (population) and to assess temporal stability of mtDNA haplotype frequencies. The use of mtDNA as a means of identifying subdivision within species is well documented (Avise 1987; Avise et al. 1987; Ovenden 1990).

Methods

Sixteen samples, totalling 707 red snapper, were procured during 1990, 1991, and 1992 from nine
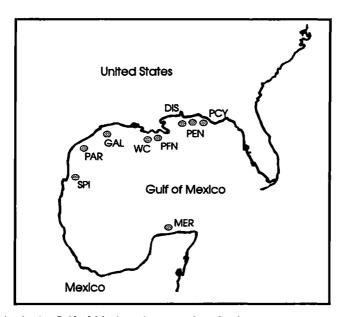
FIGURE 1.—Localities in the Gulf of Mexico where samples of red snapper were procured. Acronyms are as follows: MER (Mérida, Mexico); SPI (Port Isabel, Texas); PAR (Port Aransas, Texas); GAL (Galveston, Texas); WC (West Cameron, Louisiana); PFN (Port Fourchon, Louisiana); DIS (Dauphin Island, Alabama); PEN (Pensacola, Florida); and PCY (Panama City, Florida). Sample sizes taken in 1990 and 1991 are given in Camper et al. (1993) and Gold et al. (1994). Sample sizes taken in 1992 were as follows: SPI, 52; PAR, 52; GAL, 50; PFN, 50; DIS, 50; and PCY, 50.

locations by angling and from fishers (Figure 1). Heart and white muscle tissues were removed from each specimen sampled in U.S. waters and immediately frozen in liquid nitrogen. Tissues from specimens obtained in Mexico were stored in a −20°C freezer in a fish house in Mérida until transported on wet ice to Mexico City where they were frozen on dry ice for transport to College Station. All material ultimately was transferred to an ultracold freezer at Texas A&M University and stored at −80°C.

Methods of mtDNA assay were those of Gold and Richardson (1991). Basic procedures involved digestion of mtDNA with 13 restriction enzymes (Appendix 1), agarose electrophoresis, Southern transfer to nylon membranes, and hybridization with a red snapper mtDNA probe. The probe used was the entire red snapper mtDNA molecule cloned into bacteriophage lambda. All 93 restriction sites detected were mapped (Kristmundsdottir et al. 1996). Fragment (digestion) patterns produced by single digestions with each restriction enzyme are given in Appendix 1.

A restriction site presence–absence matrix for individual mtDNA haplotypes was generated by using the Restriction Enzyme Analysis Package (REAP) of McElroy et al. (1992). The methods of Nei and Tajima were used to estimate nucleotide sequence divergence among mtDNA haplotypes (Nei and Li 1979) and intrapopulational (within sample) nucleotide sequence diversities (Nei and Tajima 1981). The latter values represent average nucleotide sequence divergence (distance) between individuals within samples. Standard errors of within-sample, nucleotide sequence diversities were generated by numerical resampling (bootstrapping) with 100 iterations (replicates) for each sample (Weir 1996). The distribution of bootstrap replicates within each sample was tested for skewness and kurtosis (normality) with the g_1 and g_2 indices, respectively. Homogeneity among bootstrap-generated mean values was tested using both single-classification (Sokal and Rohlf 1966) and Kruskal–Wallis (Siegel 1956) analyses of variance.

Significance testing of mtDNA haplotype frequencies among samples and between or among samples taken in different years at the same locality was carried out by using a Monte Carlo randomization (bootstrap) procedure (Roff and Bentzen 1989). Significance levels for multiple tests performed simultaneously were adjusted by using the sequential Bonferroni approach (Rice 1989). A minimum-length, parsimony network of mtDNA

haplotypes was constructed by connecting composite haplotypes in increments of single-site gains or losses.

Spatial autocorrelation analysis of frequencies of common mtDNA haplotypes was carried out by using the Spatial Autocorrelation Analysis Program (SAAP) of Wartenberg (1989). Noise generated by low-frequency mtDNA haplotypes was minimized by employing only those haplotypes (10 total) that occurred in nine or more individuals; individuals sampled in different years at each locality were pooled. Four distance classes were used in each of two SAAP runs: the first run employed equal numbers of pairwise comparisons (five each) in each distance class; the second employed equal geographic distances between distance classes. Pairwise comparisons in each distance class in the latter run were 5, 9, 3, and 4. Expected values for Moran's I in the absence of autocorrelation were estimated according to the method of Sokal and Oden (1978).

Results

Mitochondrial DNA fragment patterns from single digestions with the 13 restriction enzymes produced 92 composite mtDNA haplotypes (genotypes) among the 707 specimens of red snapper surveyed (Appendix 2). Haplotype 1 was the most common, occurring in 340 of the 707 individuals (48.1%). Haplotypes 10, 31, and 3 were the next most common, occurring in 92 (13.0%), 39 (5.5%), and 29 (4.1%) of the individuals, respectively. Of the remainder, 30 haplotypes were found in 2–17 individuals (21.1%) and 58 haplotypes were found in only one individual each (8.2%). Estimates of percent nucleotide sequence divergence among the 92 haplotypes ranged from 0.15 to 1.33 (mean ± SE = 0.55 ± 0.02).

Intrapopulational mtDNA diversities (bootstrapped mean ± SE) varied from 0.132 ± 0.031 in the 1991 sample from Port Aransas, Texas, to 0.293 ± 0.056 in the 1990 sample from Pensacola, Florida (Table 1). In general, mtDNA diversities were slightly higher in samples from the northeastern Gulf. Several mean values (Table 1) differed from one another by more than two standard errors, suggesting significant differences among samples in intrapopulational mtDNA diversity. We examined this further by testing distributions of bootstrap replicates within samples for normality and then testing homogeneity of (bootstrapped) mean values. After correction for multiple tests, all g_1 (skewness) and g_2 (kurtosis) values were not significant ($P > 0.05$). Both single classification

TABLE 1.—Intrapopulational (mtDNA) nucleotide sequence diversities among samples from the Gulf of Mexico from bootstrap resampling (100 replicates); diversity values in parentheses represent means estimated according to the method of Nei and Tajima (1981). Sample acronyms are defined in Figure 1.

Sample	Year sampled	Number of haplotypes (individuals)	Nucleotide sequence diversity (mean % ± SE)
MER-1	1991	15 (44)	0.225 ± 0.031 (0.237)
SPI-1	1991	16 (52)	0.185 ± 0.029 (0.187)
SPI-2	1992	15 (43)	0.209 ± 0.032 (0.216)
PAR-1	1991[a]	7 (25)	0.132 ± 0.031 (0.140)
PAR-2	1991	14 (35)	0.190 ± 0.036 (0.188)
PAR-3	1992	20 (52)	0.248 ± 0.031 (0.255)
GAL-1	1991	12 (47)	0.191 ± 0.029 (0.198)
GAL-2	1992	12 (50)	0.175 ± 0.030 (0.173)
WC-1	1991	14 (54)	0.197 ± 0.030 (0.207)
PFN-1	1990[a]	17 (36)	0.281 ± 0.047 (0.282)
PFN-2	1992	10 (50)	0.174 ± 0.031 (0.178)
DIS-1	1991	19 (53)	0.221 ± 0.032 (0.232)
DIS-2	1992	16 (50)	0.231 ± 0.038 (0.236)
PEN-1	1990[a]	5 (25)	0.293 ± 0.056 (0.311)
PCY-1	1991	15 (50)	0.262 ± 0.035 (0.271)
PCY-2	1992	16 (41)	0.240 ± 0.039 (0.249)

[a] Data reported in Camper et al. (1993).

and Kruskal–Wallis analyses of variance revealed significant heterogeneity among mean values ($F = 143$; df = 15, 1584; $P < 0.001$; and $H = 914$; df = 15; $P < 0.001$; respectively).

Haplotype frequencies varied significantly ($P = 0.042$) among the 16 samples. However, tests of homogeneity of mtDNA haplotype frequencies between or among samples taken in different years at the same locality and among samples at different localities taken in the same year (1991 and 1992) were not significant (Table 2). The heterogeneity among the 16 samples appears to stem from temporal variation between the two samples from Port Fourchon (PFN), Louisiana (Table 2). We then employed V-tests (DeSalle et al. 1987) to test homogeneity of frequencies of individual haplotypes. No significant differences in frequencies of any haplotype found in four or more individuals were detected either across all 16 samples or across the nine localities from which samples from different years were pooled. Finally, we searched for regional subdivision of mtDNA haplotype frequencies in the Gulf by pooling samples into three defined subregions and then testing for haplotype frequency homogeneity. Subregions (pooled samples) were the northeastern Gulf (Panama City, Pensacola, and Dauphin Island), the north-central Gulf (West Cameron and Port Fourchon), and the northwestern Gulf (Galveston, Port Aransas, and South Padre Island). No significant difference (P

TABLE 2.—Tests of temporal and spatial homogeneity of mtDNA haplotype frequencies between and among samples from the Gulf of Mexico. Probability was based on bootstrap analysis according to the method of Roff and Bentzen (1989). Locality acronyms are defined in Figure 1.

Test group	Number of samples	Number of haplotypes tested	Homogeneity P
Temporal			
SPI	2	26	0.299
PAR	3	29	0.982
GAL	2	18	0.420
PFN	2	23	0.048[a]
DIS	2	29	0.914
PCY	2	25	0.236
Spatial			
1991 samples	7	52	0.187
1992 samples	6	49	0.324

[a] Not significant ($P > 0.05$) when corrected for multiple tests.

= 0.190) in mtDNA haplotype frequency among subregions was detected.

A minimum-length, parsimony network of mtDNA haplotypes (Figure 2) revealed no phylogeographic structure of haplotype lineages. The most common haplotype (haplotype 1) was the major hub, and other common haplotypes (i.e., haplotypes 3, 10, 17, and 31) formed smaller hubs. All of these common haplotypes (hubs) are found across the Gulf, and none of the hubs were restricted geographically. Virtually all of the low-frequency haplotypes occurred in more than two localities, and in no case were low-frequency haplotypes restricted to two or three geographically contiguous localities (Table 3). As examples, haplotypes 14, 19, and 38 (observed in five, four, and four individuals, respectively) were found off of South Padre Island, Texas, and off of Panama City, Florida (Table 3). This distribution of rare haplotypes is inconsistent with a model of restricted gene flow and population substructuring wherein low-frequency haplotypes are expected to be partitioned spatially into one or a few geographically contiguous localities (Slatkin 1989).

Spatial autocorrelation analysis generated 40 Moran's I-values in each run (10 haplotypes × 4 distance classes). When equal numbers of pairwise comparisons were used, two significant ($P < 0.05$) I-values were obtained; both were negative and occurred in the third and fourth distance classes. When equal geographic distances between distance classes were used, three significant values were found. All three were negative: two occurred in the third distance class and one occurred in the

fourth distance class. Mean I-values were negative in all four distance classes and did not differ significantly from expected values of I in the absence of autocorrelation.

Discussion

Spatial and temporal patterns of mtDNA haplotype variation among red snapper in the northern Gulf are consistent with the hypothesis of a single breeding population (unit stock). Frequencies of mtDNA haplotypes were essentially uniform between years and across the sampling area, and phylogeographic structure of haplotype assemblages or of low-frequency haplotypes was not apparent. Similar findings have been reported for a variety of other marine fish species, including several with demersal life history phases. Examples include New Zealand hake *Merluccius australis* (Smith et al. 1979), orange roughy *Hoplostethus atlanticus* (Smolenski et al. 1993; Elliot et al. 1994), Pacific damselfish *Stegastes fasciolatus* (Shaklee 1984), and a deepwater snapper *Pristipomoides filamentosus* (Shaklee and Samollow 1984) and the emperor snapper *Lutjanus sebae* (Johnson et al. 1993).

Genetic homogeneity and absence of spatial patterns in allele distributions in marine fishes typically have been interpreted to indicate existence of a single breeding population or gene pool (Graves et al. 1992a, 1992b; Baker et al. 1995). Most authors (e.g., Graves et al. 1992b; Camper et al. 1993) note either or both of the major caveats to this interpretation: (1) one cannot prove a null hypothesis; and (2) observed genetic homogeneity may reflect historical rather than present-day gene flow. The latter means that populations could be isolated at present but have had sufficient genetic contact in the recent past to remain indistinguishable in allele frequencies and distributions. In nearly all of the studies of marine fishes wherein genetic homogeneity has been documented, it typically is suggested that one or more aspects of the life history of the species promotes gene flow. For species with sedentary or demersal adult phases, hydrodynamic transport of eggs or larvae is often inferred as the mechanism promoting gene flow (Shaklee and Samollow 1984; Johnson et al. 1993; Elliot et al. 1994). This mechanism would be expected to promote gene flow in red snapper because eggs and larvae are pelagic (Leis 1987). Goodyear (1992) also has suggested that short-range, nonseasonal movement of adult red snapper could occur and lead to gradual dispersal from localized centers of abundance.

Intrapopulational mtDNA diversities differed

FIGURE 2.—Minimum-length parsimony network of mtDNA haplotypes of red snapper from the Gulf of Mexico. Branch lengths connecting haplotypes are proportional to the number of restriction site changes (either one or two) required to connect adjacent haplotypes, except for haplotypes 3, 10, 17, and 31, all of which differ from haplotype 1 by one restriction site. Common haplotypes inferred to be hubs are shaded.

significantly among samples of red snapper. This measure represents the average nucleotide sequence divergence (distance) between any two individuals drawn at random from a sample (Nei and Tajima 1981) and is hypothesized (Avise et al. 1988; Ball et al. 1990) to provide a relative measure of effective female population size ($N_e m_f$). Consequently, our finding may suggest spatial or temporal differences in the effective number of

female parents or ancestors associated with individual sample localities. Thus, although the lack of phylogeographic structure in the distribution of mtDNA haplotypes indicates that no major barriers to gene flow among red snappers exist in the northern Gulf, the significant heterogeneity in intrapopulational mtDNA diversities suggests that red snapper in the Gulf may not be drawn from a single population. The possibility that red snapper in the

TABLE 3.—Distribution of mtDNA haplotypes among localities from the Gulf of Mexico. Only haplotypes found in 4–17 individuals are shown. Locality acronyms are defined in Figure 1. Dashes are sight guides only.

Locality	Occurrences of mtDNA haplotype:													
	4	6	7	13	14	15	17	19	27	29	30	38	55	68
MER-1	1	–	2	1	–	–	–	–	1	–	1	–	–	–
SPI-1	–	1	1	1	1	–	–	–	1	–	–	1	–	–
SPI-2	–	2	–	2	–	1	–	1	2	–	1	–	–	–
PAR-1[a]	1	–	–	1	–	–	–	–	1	–	–	–	–	–
PAR-2	–	–	1	1	–	1	–	–	–	1	–	1	1	–
PAR-3	–	–	–	1	2	–	–	1	1	1	–	–	3	1
GAL-1	–	4	1	–	–	–	–	–	2	–	1	–	–	1
GAL-2	–	–	–	–	–	1	1	–	2	1	1	–	–	–
WC-1	3	–	–	1	–	–	2	–	1	1	–	–	–	2
PFN-1[a]	–	–	–	–	–	1	2	1	1	1	–	–	–	–
PFN-2	–	–	–	–	–	–	3	–	–	–	1	–	–	–
DIS-1	1	–	1	1	–	1	2	–	1	–	–	1	–	–
DIS-2	–	–	1	2	–	–	1	–	–	–	–	–	1	–
PEN-1[a]	1	1	1	1	1	–	–	–	–	–	–	–	–	–
PCY-1	3	–	2	1	1	–	4	–	–	–	–	–	1	–
PCY-2	–	1	1	–	–	1	2	1	–	1	–	1	–	1
Totals	10	9	11	13	5	6	17	4	13	6	5	4	6	5

[a] Data reported in Camper et al. (1993).

northern Gulf may not represent a single population also is suggested by a recent study (Bortone and Chapman 1995) of restriction site variation in a 558-base-pair fragment of the mitochondrially encoded 16S rRNA gene. In brief, Bortone and Chapman (1995) examined 197 red snapper sampled from seven localities across the northern Gulf and one locality near the Yucatán peninsula. Ten mtDNA haplotypes were identified, and frequencies of several haplotypes varied significantly among localities. Bortone and Chapman (1995) noted that their results differed from those reported by Camper et al. (1993) and speculated that the observed genetic differences could have stemmed from nonrandom sampling of genetically related individuals.

The bulk of the genetic data obtained to date is consistent with the interpretation that red snapper comprise a single population in the northern Gulf. In our view, the major, biological caveat to this interpretation is that the observed genetic patterns may reflect historical rather than present-day gene flow. For red snapper, recent historical events that could have affected present-day distribution primarily include Pleistocene glaciations when environmental changes significantly altered biotic communities (Graham and Mead 1987) and when waters on a reduced continental shelf (Rezak et al. 1985) were much cooler. Colonization of newly available habitat in the northern Gulf after the last glacial retreat could lead to a situation wherein recently derived populations of red snapper have had insufficient time to accumulate haplotype frequency differences. Because of the economic importance of red snapper in the Gulf, this possibility should be tested. We suggest that genetic molecules that evolve more rapidly than mtDNA (e.g., microsatellite loci) should be investigated.

Marine Fishes" and is contribution 50 of the Center for Biosystematics and Biodiversity.

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Appendix 1: Mitochondrial DNA Fragment Patterns

TABLE A1.1.—Fragment patterns and sizes from red snapper mtDNA after single digestion with each of 13 restriction enzymes. Fragment sizes are in base pairs. Parentheses indicate fragments not normally seen in autoradiographs but known to exist from mapping.

| | Fragment size for pattern: | | | | | | | | |
A	B	C	D	E	F	G	H	I	J
				Apa I					
15,800	8,800	13,500	14,200	12,900	9,400	10,700			
1,000	7,000	2,300	1,600	2,900	6,400	5,100			
	1,000	1,000	1,000	1,000	1,000	1,000			
				Bcl I					
12,300	10,900	12,300	15,400	8,300	12,300	12,300	13,700		
3,100	3,100	4,500	1,400	4,000	3,100	1,600	3,100		
1,400	1,400			3,100	1,100	1,500			
	1,400			1,400	(300)	1,400			
				Dra I					
9,200	9,200	9,200	6,700	9,200	8,100	6,400			
2,800	3,300	2,200	2,800	2,600	2,200	3,300			
2,200	2,200	2,000	2,500	2,200	2,000	2,800			
1,200	1,200	1,200	2,200	1,200	1,200	2,200			
900	900	900	1,200	900	1,100	1,200			
(500)		(800)	900	(500)	900	900			
		(500)	(500)	(200)	(800)				
				Hind III					
8,100	9,700	8,100	8,100						
4,100	4,100	3,000	4,600						
3,000	3,000	2,800	4,100						
1,600		1,600							
		1,300							
				Hpa I					
12,000	6,000	16,800	12,000	8,300	12,000				
4,800	6,000		4,500	4,800	4,000				
	4,800		(300)	3,700	(800)				
				Nco I					
14,800	10,600	14,800	16,800	11,700					
2,000	4,200	1,400		3,100					
	2,000	(600)		2,000					
				Nhe I					
9,100	9,100	9,100	4,700	5,200	6,800	4,900	9,100		
4,700	4,700	3,900	4,700	4,700	4,700	4,700	3,000		
1,300	1,200	1,300	4,300	3,900	2,300	4,200	1,700		
1,200	1,000	1,200	1,300	1,300	1,300	1,300	1,300		
(500)	(500)	(800)	1,200	1,200	1,200	1,200	1,200		
	(300)	(500)	(500)	(500)	(500)	(500)	(500)		
				Pvu II					
12,100	16,800	16,000							
3,900		800							
800									
				Sca I					
6,000[a]	6,000[a]	6,000	6,000[a]	6,000[a]	6,000	6,000			
2,200	2,200	3,800	2,400	2,400	3,600	5,700			
1,200	1,800	2,200[a]	1,800	2,400	2,400	2,200			
(600)	(600)	1,200	(600)	(600)	2,200	1,200			
(600)	(200)	(600)	(600)		1,200	(600)			
(200)		(600)			(600)	(600)			
		(200)			(200)	(200)			
				Sma I					
8,600	8,600								
3,700	7,200								
3,500	1,000								

TABLE A.1.1.—Continued.

				Fragment size for pattern:					
A	B	C	D	E	F	G	H	I	J
				Sst I					
10,800	10,800	13,500							
3,300	2,700	3,300							
2,700	2,100								
	1,200								
				Stu I					
5,600	4,300	5,600	8,300	4,100	6,300	5,600	5,600	5,600	5,600
4,100	4,100	2,700	4,100	3,400	4,100	4,800	4,100	4,400	4,100
2,700	2,700	2,400	1,700	2,700	2,700	2,700	2,000	4,100	2,300
1,700	1,700	1,700ᵃ	1,200	2,200	1,700	1,700	1,700	1,200	2,000
1,200	1,300	1,200	700	1,700	1,200	1,200	1,200	700	1,200
700	1,200	700	(600)	1,200	(600)	(600)	700ᵃ	(600)	700ᵃ
(600)	700	(600)	(200)	700	(200)	(200)	(600)	(200)	(200)
(200)	(600)	(200)		(600)			(200)		
	(200)			(200)					
				Xba I					
7,200	7,200	9,000	7,200	7,200					
4,800	3,600	4,800	4,800	4,800					
3,000	3,000	3,000	4,800	3,000					
1,800	1,800			1,300					
1,200	1,200			(500)					

ᵃ Fragment "doublets" determined from mapping.

Appendix 2: Occurrence and Distribution of Haplotypes

TABLE A2.1.—Distribution of mtDNA composite digestion patterns (haplotypes) among samples of red snapper.ᵃ Letters, from left to right, are digestion patterns (from Appendix Table A1.1) for the restriction enzymes *Apa* I, *Bcl* I, *Dra* I, *Hind* III, *Hpa* I, *Nco* I, *Nhe* I, *Pvu* II, *Sca* I, *Sst* I, *Stu* I, and *Xba* I. Locality acronyms are defined in Figure 1. Dashes are sight guides only.

Haplo-type	Composite mtDNA digestion pattern	Haplotype occurrences for sample:												
		MER-1	SPI-1	SPI-2	PAR-2	PAR-3	GAL-1	GAL-2	WC-1	PFN-2	DIS-1	DIS-2	PCY-1	PCY-2
1	AAAAAAAAAAAAAA	18	26	21	18	20	26	29	28	28	25	26	18	18
2	AEABAAAABAAAA	–	–	–	–	–	–	–	–	1	–	–	–	–
3	AABAAAAAAAAAA	3	–	2	1	4	1	1	3	2	–	2	5	1
4	AACABAAAAAAAA	1	–	–	–	–	–	–	3	–	1	–	3	–
6	BACAAAAAAAAAA	–	1	2	–	–	4	–	–	–	–	–	–	1
7	AADAAABABAAAA	2	1	–	1	–	1	–	–	–	1	1	2	1
10	AACAAAAAAAAAA	6	10	5	4	7	5	6	5	6	7	6	9	5
13	AAAAAAAAAABAA	1	1	2	1	1	–	–	1	–	1	2	1	–
14	AADAAAAAAAAAA	–	1	–	–	2	–	–	–	–	–	–	1	–
15	BAAAAAAAAAAAA	–	–	1	1	–	–	1	–	–	1	–	–	1
16	AAAAAAAABBAAA	–	–	–	–	1	–	–	–	–	–	–	–	1
17	AAAAAAABAAAAA	–	–	–	–	–	–	1	2	3	2	1	4	2
19	AAAABAAAAAAAA	–	–	1	–	1	–	–	–	–	–	–	–	1
20	ABAAAAAAAAAAA	–	1	–	–	–	–	–	–	–	–	–	–	–
27	AAABAAAABAAAA	1	1	2	–	1	2	2	1	–	1	–	–	–
29	AAEAAAAAAAAAA	–	–	–	1	1	–	1	1	–	–	–	–	1
30	AAAAAADAAAACA	1	–	1	–	–	1	1	–	1	–	–	–	–
31	AAAAAAAABAAAA	4	3	–	2	2	2	5	4	5	4	3	1	4
32	AFAAAAAAAAAAA	1	–	–	1	–	1	–	–	–	–	–	–	–
33	AEAAAAAAAAAAA	1	–	–	–	–	–	–	–	–	1	–	–	–
34	AAAAAAAABAAGA	1	–	–	–	–	–	–	–	–	–	–	–	–
35	AAAAAAAAAAAHA	2	–	–	–	–	–	–	–	–	–	–	–	–

TABLE A2.1.–Continued.

Haplo-type	Composite mtDNA digestion pattern	Haplotype occurrences for sample:												
		MER-1	SPI-1	SPI-2	PAR-2	PAR-3	GAL-1	GAL-2	WC-1	PFN-2	DIS-1	DIS-2	PCY-1	PCY-2
36	AACAAAAAAAAIA	1	–	–	–	–	–	–	–	–	–	–	–	–
37	AFAAAAAAAABAA	1	–	–	–	–	–	–	–	–	–	–	–	–
38	CACABAAAAAAAA	–	1	–	1	–	–	–	–	–	1	–	–	1
39	AAAAADAAAAAAA	–	1	–	–	–	–	–	–	–	–	–	–	–
40	AAAAAAAAAAADA	–	1	–	–	–	–	–	–	–	–	–	–	–
41	ACAADAAAAAAAA	–	1	–	–	–	–	–	–	–	–	–	–	–
42	AAAACAAAAAAAA	–	1	–	–	–	–	–	–	–	–	–	–	–
43	AAAAAEAABAAAA	–	1	–	–	–	–	–	–	–	–	–	–	–
44	AAAAAAAAAACAA	–	1	–	–	–	–	–	–	–	–	–	–	–
45	AAAAAEAAAAAAA	–	–	–	–	–	–	–	–	–	1	–	–	–
46	AAAAAAABBAAAA	–	–	–	–	–	–	–	–	–	1	–	–	–
47	AACAABAAAAAAA	–	–	–	–	–	–	–	–	–	1	–	–	–
48	AAAAAAAAAAAEA	–	–	–	–	–	–	–	–	–	1	–	–	1
49	AAAAAAAAEAAAA	–	–	–	–	–	–	–	–	–	1	–	–	–
50	ADAAAAAAAAAAA	–	–	–	–	–	–	–	–	–	1	–	–	–
51	AAABAAAABAAFA	–	–	–	–	–	–	–	–	–	1	–	–	–
52	AGAAAAAAAAAAA	–	–	–	–	–	–	–	–	–	1	–	–	–
53	AACCAAAAAAAAA	–	–	–	–	–	–	–	–	1	–	1	1	–
54	AACABAAAAAAAC	–	–	–	–	–	–	–	–	–	–	–	1	–
55	AAABAAAAAAAAA	–	–	–	1	3	–	–	–	–	–	1	1	–
56	AACAAAFAAAAAA	–	–	–	–	–	–	–	–	–	–	–	1	–
57	AFAAAAAADAAAA	–	–	–	–	–	–	–	–	–	–	–	1	–
58	CACAAAAAAAAAA	–	–	1	–	–	–	–	–	–	–	–	1	–
59	AACAEAAAAAAAA	–	–	–	–	–	2	–	–	–	–	–	–	–
60	DAAAAAAAAAAAA	–	–	–	–	–	1	–	–	–	–	–	–	–
61	BAAAAAAABAAAA	–	–	–	–	–	–	–	1	–	–	–	–	–
62	AAAAAAAACAAAA	–	–	–	–	–	–	–	1	–	–	1	–	–
63	AAAAAAABAAAAD	–	–	–	–	–	–	–	1	–	–	–	–	–
64	AACAFAAAAAAAA	–	–	–	–	–	–	–	1	–	–	–	–	–
65	AAAAAAACAAAAA	–	–	–	1	–	–	–	–	–	–	–	–	–
66	AAAAAGAAAAAAA	–	–	–	1	–	–	–	–	–	–	–	–	–
67	AACAAAAFAAAAA	–	–	–	1	–	–	–	–	–	–	–	–	–
68	AAAAAAAABAAAE	–	–	–	–	1	1	–	2	–	–	–	–	1
69	AAADAAAAAAAAA	–	–	–	–	–	–	–	–	2	–	–	–	–
70	AFAAAEAAEAAAA	–	–	–	–	–	–	–	–	–	–	1	–	–
71	EACAAAAAAAAAA	–	–	–	–	1	–	–	–	1	–	–	–	–
72	AAFAAAAAAAAAA	–	–	1	–	–	–	–	–	–	–	–	–	–
73	AHCAAAAAAAAAA	–	–	1	–	–	–	–	–	–	–	–	–	–
74	AAABAAAABAADA	–	–	1	–	–	–	–	–	–	–	–	–	–
75	AAAAAAAAAAAAE	–	–	1	–	–	–	–	–	–	–	–	–	–
76	AHAAAAAAAAAAA	–	–	1	–	–	–	–	–	–	–	–	–	–
77	AAAAAAAAFAAAA	–	–	–	–	–	–	1	–	–	–	–	–	–
78	AADAAABABAAAC	–	–	–	–	–	–	1	–	–	–	–	–	–
79	AAAAAAAAAAAJA	–	–	–	–	–	–	1	–	–	–	–	–	–
80	EAAAAAAAAAAAA	–	–	–	–	1	–	–	–	–	–	–	–	–
81	AHCAAAAACAAAA	–	–	–	–	1	–	–	–	–	–	–	–	–
82	AAGAAAAAAAAAA	–	–	–	–	1	–	–	–	–	–	–	–	–
83	AFAAAABAAAAE	–	–	–	–	1	–	–	–	–	–	–	–	–
84	AAAAAADAAAAAA	–	–	–	–	1	–	–	–	–	–	–	–	–
85	AACAAAAACAAAA	–	–	–	–	1	–	–	–	–	–	–	–	–
86	AABAAAHABAAAA	–	–	–	–	1	–	–	–	–	–	1	–	–
87	FAAAAAAAAAAAA	–	–	–	–	–	–	–	–	–	–	–	–	1
88	GADAAABABAAAA	–	–	–	1	–	–	–	–	–	–	–	–	1
89	FAABAAAAAAAAA	–	–	–	–	–	–	–	–	–	–	1	–	–
90	AABAAAABAAAAA	–	–	–	–	–	–	–	–	–	–	1	–	–
91	AAAAAAAAGAAAA	–	–	–	–	–	–	–	–	–	–	1	–	–
92	AFAAAAABAAAAD	–	–	–	–	–	–	–	–	–	–	1	–	–

[a] Distribution of mtDNA haplotypes in PAR-1, PFN-1, and PEN-1 are given in Camper et al. (1993), as are digestion patterns for mtDNA haplotypes 5, 8, 9, 11, 12, 18, 21–26, and 28; none of these haplotypes were observed in the samples listed in this table.