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Mitochondrial DNA Diversity And Divergence Among Sharpnose Sharks, Rhizoprionodon Terraenovae, From The Gulf Of Mexico And Mid-Atlantic Bight

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Abstract.—The Atlantic sharpnose shark, *Rhizoprionodon terraenovae,* is a small coastal shark that is harvested in both directed and nondirected fisheries throughout its range. Because pups of this species are found both along the southeastern U.S. Atlantic coast and the Gulf of Mexico, it is possible that multiple isolated breeding stocks exist. Restriction fragment length polymorphism analysis of mitochondrial DNA was used to test the hypothesis that Atlantic sharpnose sharks from the U.S. Atlantic coast and the western Gulf of Mexico have identical mitochondrial haplotype frequencies and therefore no apparent genetic stock structure. Seven mitochondrial haplotypes were detected among 52 individuals. The distribution of haplotypes between samples did not differ significantly from homogeneity (P= 0.694), indicating that the null hypothesis of a single breeding population could not be rejected.

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Mitochondrial DNA diversity and divergence among sharpnose sharks, *Rhizoprionodon terraenovae,* from the Gulf of Mexico and Mid-Atlantic Bight*

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The Atlantic sharpnose shark, *Rhizoprionodon terraenovae,* is a small (maximum length 110 cm total length) coastal shark that inhabits the east coast of North America from New Brunswick, Canada, to Yucatan, Mexico (Compagno, 1984). This species is abundant along the southern U.S. Atlantic coast and is second only to the sandbar shark, *Carcharhinus plumbeus,* in longline catches in Virginia (Musick et al., 1993). It supports a large recreational fishery off Texas (Parrack[1]) and is an important species in the Mexican shark longline fishery (Applegate et al., 1993). In addition to being caught in directed fisheries, the Atlantic sharpnose shark is frequently taken by shark longliners targeting large coastal species (Branstetter and McEachran, 1986; Russell, 1993) as well as by commercial shrimp trawlers (Branstetter, 1981; Parrack[1]); however, the implementation of turtle excluder devices (TED's) has produced the additional benefit of reducing bycatch of sharks (Branstetter[2]). Atlantic sharpnose sharks travel in sex-segregated schools, as noted by the disparate sex ratios of adults captured by longlines (Branstetter, 1981; Musick et al., 1993). The gestation period for this species is about ten to twelve months, and parturition takes place from April to June in the northern Gulf of Mexico (Branstetter, 1981; Parsons, 1983) and from May to June in South Carolina (Castro, 1993).

The most recent fishery management plan for sharks in the coastal Atlantic waters of the United States (NMFS[3]) divides sharks into three categories for management purposes: pelagic species, large coastal species, and small coastal species. Currently catches of small coastal species (predominantly the Atlantic sharpnose shark) are not regulated

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1 Parrack, M. L. 1990. A study of shark exploitation in U.S. Atlantic coastal waters during 1986–1989. NOAA, Natl. Mar. Fish. Serv., Southeast Fisheries Science Center, Miami, Florida.

2 Branstetter, S. 1995. Gulf and South Atlantic Fisheries Development Foundation, Suite 997, Lincoln Center, 5401 W. Kennedy, Tampa, Florida 33609. Personal commun.

3 NMFS. 1993. Fishery management plan for sharks of the Atlantic Ocean. U.S. Dep. Commer., NOAA, Southeast Regional Office, St. Petersburg, FL, p. 1–167.

because catch rates are assumed to be at or below maximum sustainable yield and because life history parameters predict a relatively high recruitment rate for this species in comparison to the large coastal species targeted by the U.S. Atlantic shark longline fishery (NMFS[3]). Recently Cortés (1995) challenged this assessment on the basis of a reevaluation of life history characteristics relevant to recruitment (age at maturity, fecundity, and longevity) and suggested that the Atlantic sharpnose shark may be more vulnerable to overfishing than was previously assumed.

Proper management of this species requires not only accurate estimates of standing stock and recruitment values but also an understanding of the reproductive population structure of the species. The observation that pups of this species are found both in the South Atlantic Bight as well as in the Gulf of Mexico, coupled with the small size and apparent lack of significant longshore migration, suggests that there may be isolated breeding populations of this species. Information on stock structure of marine fishes has traditionally relied on two approaches: tag and recapture studies and analyses of genetic variation. Although considerable information has been obtained on the movements of large sharks by means of tagging (Casey and Kohler, 1990), these tagging studies have generally neglected smaller species of sharks. Furthermore, to our knowledge the genetic structure of any population of small coastal shark has not been investigated. This study tests the hypothesis of genetic homogeneity in allele frequency in Atlantic sharpnose sharks between the Gulf of Mexico and Mid-Atlantic Bight by using restriction fragment haplotypes of mitochondrial DNA (mtDNA).

Materials and methods

Atlantic sharpnose sharks were collected with research longlines in the Mid-Atlantic Bight (n=23) as part of an ongoing shark research program of the Virginia Institute of Marine Science, from the recreational fishery of southern Texas (n=21) and from artisanal longline vessels from Veracruz, Mexico (n=8) (Fig. 1). Heart tissue samples from Atlantic sharpnose sharks caught in the Mid-Atlantic Bight were placed into cryovials and stored under liquid nitrogen in the field. In Texas and Mexico, whole hearts were collected on wet ice and stored frozen at (–20°C) until shipped to Virginia. All samples were stored at –70°C.

Mitochondrial DNA was isolated from tissue by following the rapid isolation protocol of Chapman and Powers (1983). Aliquots of mtDNA were digested with ten restriction enzymes (*Ava* I, *Ava* II, *Ban* I, *Bcl* I, *Bst*E II, *Dra* I, *Hin*d III, *Hpa* I, *Sca* I, and *Xho* I) by following the manufacturers' instructions. Restriction fragments were separated on 1.0% horizontal agarose gels run at 2V/cm overnight, then transferred after electrophoresis to a nylon membrane by means of Southern transfer according to the protocols of Sambrook et al. (1989). Filters were hybridized with highly purified mtDNA from tiger shark, *Galeocerdo cuvier*, nick-translated with biotin-14-dATP, and visualized with the BRL BlueGene Nonradioactive Nucleic Acid Detection System.

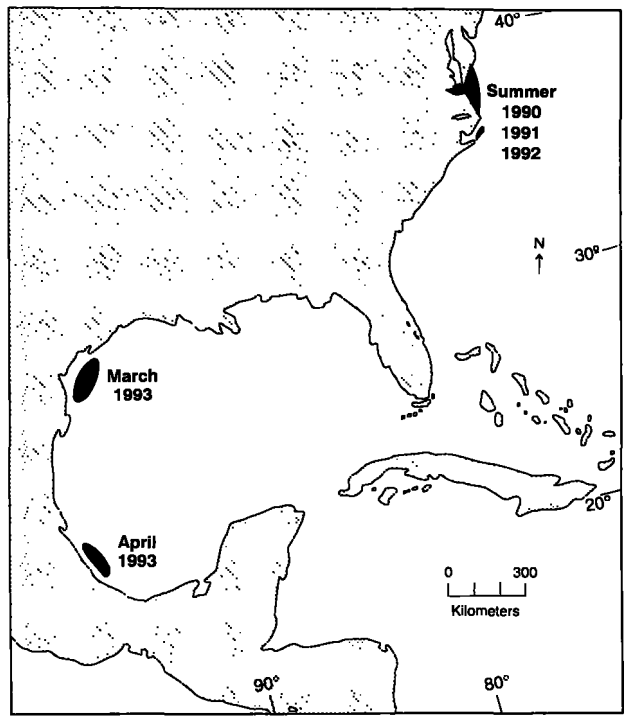

Figure 1
Locations and dates for collection of sharpnose shark, *Rhizoprionodon terraenovae*.

Fragment patterns were scored for each restriction enzyme and each individual was assigned a composite genotype based on the fragment patterns for all enzymes (Tables 1 and 2). The nucleon (haplotype) diversity was calculated for each sample and for the composite of all samples following Nei (1987). Nucleotide sequence diversity was calculated following the site approach of Nei and Miller (1990). Chi-square significance of the difference in genotypic frequencies between samples was computed by using the randomization protocol of Roff and Bentzen (1989). Nucleotide sequence diversities and divergences were calculated by using the REAP statistical analysis package (McElroy et al., 1991).

Table 1

Estimated size in kilobase pairs of mitochondrial DNA restriction fragments for various restriction enzymes in the Atlantic sharpnose shark, *Rhizoprionodon terraenovae*.

Ava I		*Ava* II	*Ban* I	*Bcl* I		*Bst*E II	*Dra* I		*Hin*d III	*Hpa* I				*Sca* I	*Xho* I
(A)	(B)	(A)	(A)	(A)	(B)	(A)	(A)	(B)	(A)	(A)	(B)	(C)	(D)	(A)	(A)
7.53	7.53	11.12	11.54	6.62	6.62	6.23	3.14	2.68	6.06	4.84	4.84	5.57	4.84	9.85	11.30
5.17	7.09	2.43	5.44	4.10	4.10	6.09	2.68	2.50	3.83	4.03	3.55	4.03	4.76	5.89	5.32
1.92	1.02	1.60	—	2.26	3.08	1.57	2.08	2.08	2.43	3.43	3.43	3.43	3.43	1.08	—
1.02	8.34	0.64	16.98	1.87	2.26	1.54	2.08	2.08	2.43	2.09	2.09	2.09	2.09	—	16.62
0.83	—	0.55		1.21	0.79	0.84	1.75	1.75	0.80	1.18	1.18	1.18	1.18	16.82	
—	16.48	—		0.79	—	0.65	1.56	1.56	0.68	0.73	0.73	—	—		
16.47		16.33		—	16.85	—	1.14	1.14	—	—	0.45	16.30	16.30		
				16.85		16.92	1.14	0.98	16.23	16.30	—				
							0.98	0.98			16.27				
							—	0.57							
							16.55	—							
								16.32							

Results

Ten restriction enzymes produced an average of 50 restriction fragments with a survey of 288 base pairs (bp) or 1.74% of the 16.6 kb mtDNA molecule (Table 1). Seven haplotypes were detected, resulting in a nucleon diversity of 0.640 and an overall nucleotide sequence diversity (NSD) of 0.13% (Table 2; Fig. 2). Four of the ten restriction enzymes revealed multiple restriction patterns; one enzyme, *Hpa* I, detected four different patterns. The single most common haplotype was found with similar frequencies in each sample and in each year within the Atlantic sample (range=0.50–0.67), and three less common haplotypes also occurred in all three geographic samples. Three rare haplotypes, each found in a different individual, were equally divided among the three sampling locations. Each haplotype differed from the common pattern by the gain or loss of a single restriction site (Fig. 2.) The chi-square probability of haplotype homogeneity among samples (Roff and Bentzen, 1989) was 0.694, indicating that the samples could have been drawn from a single population of mtDNA haplotypes. The nucleotide sequence divergences between the three samples, corrected for within sample diversity, were all less than 0.01%.

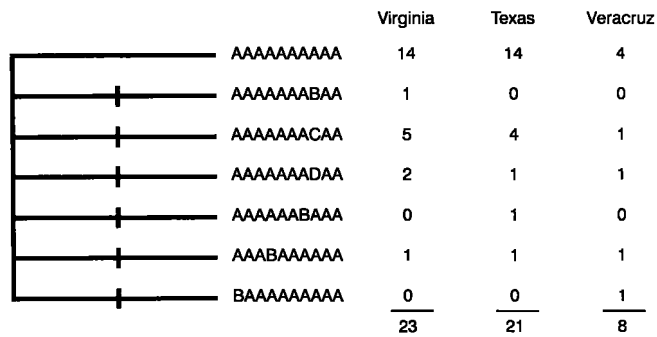

Figure 2

Composite mtDNA haplotypes, inferred phylogenetic relationships among haplotypes (vertical hatches represent restriction site differences), and distribution of haplotypes in samples of Atlantic sharpnose shark, *Rhizoprionodon terraenovae*. Composite haplotypes consist of restriction fragment patterns for the following enzymes (left to right): *Ava* I, *Ava* II, *Ban* I, *Bcl* I, *BstE* II, *Dra* I, *Hin*d III, *Hpa* I, *Sca* I, and *Xho* I.

Table 2

Nucleon diversity and percent nucleotide sequence diversity in the Atlantic sharpnose shark, *Rhizoprionodon terraenovae*.

Sample source	Nucleon diversity	Nucleotide sequence diversity (%)
Virginia	0.597	0.120
Texas	0.538	0.105
Veracruz	0.788	0.175
Total	0.640	0.133

Discussion

The results of this study indicate that historically there has been sufficient gene flow among sharpnose

sharks from the Gulf of Mexico to the Mid-Atlantic Bight to prevent significant divergence in mitochondrial DNA haplotypes. The frequencies of the most common alleles, as well as the occurrences of rare alleles, were nearly identical in each of the three samples. The nucleon and nucleotide sequence diversities were also similar among samples. The hypothesis that Atlantic sharpnose sharks collected from locations as distant as Veracruz and Virginia were members of a single homogeneous gene pool could not be rejected.

The distribution of mtDNA haplotypes has been used previously to infer patterns of gene flow in several other commercially important shark species in the Gulf of Mexico and southeastern (U.S.) Atlantic coast (reviewed in Avise, 1992). Gene flow in fishes is accommodated both by the active movement of juveniles and adults, as well as by the passive movement of eggs and larvae. Significant differences in mtDNA haplotype frequencies have been detected in species with limited adult migration and demersal eggs (marine toadfishes, *Opsanus* spp.; Avise et al., 1987) as well as in those with pelagic eggs and larvae (black sea bass, *Centropristis striata;* Bowen and Avise, 1990; and menhaden, *Brevoortia* spp., Bowen and Avise, 1990).

Sharpnose sharks are nektonic from the moment of parturition; therefore, gene flow is accommodated only by the active movements of juveniles and adults. Significant differences in haplotype frequencies were detected in redfish (*Scianops ocellatus*; Gold et al., 1993) of the Gulf of Mexico and southeast U.S. Atlantic coast but not in the hardhead catfish (*Arius felis;* Avise et al., 1987), two species with large active adults but with presumably little passive transport of eggs and larvae. In addition, Heist et al. (1995) detected no heterogeneity in mtDNA haplotype frequencies in the sandbar shark, *Carcharhinus plumbeus*, over the same geographic range.

In menhaden, the presence of two groups of genetically divergent mtDNA haplotypes in the Atlantic was interpreted as indicating complete isolation of these two groups in the past, followed by a mixture of stocks with divergent mtDNA haplotypes (Bowen and Avise, 1990). The close relationships among all haplotypes detected in the Atlantic sharpnose shark is consistent with the hypothesis of a single evolutionary lineage with no historical subdivision.

The lack of genetic divergence among Atlantic and Gulf of Mexico sharpnose sharks can not prove that separate stocks do not exist. An exchange rate of a small number (<20) of females per generation between isolated breeding populations is enough to prevent drift from establishing significant heterogeneity in allele frequencies (Allendorf and Phelps, 1981). Therefore fishery-relevant stocks can be maintained in the absence of statistically significant genetic divergence. Furthermore, if a single population has recently diverged into multiple stocks, there may not have been sufficient time for a significant level of genetic divergence to have become established. Perhaps by examining genetic characters that evolve more rapidly than whole mitochondrial DNA (such as direct sequencing of the mitochondrial control region or microsatellite analysis) stock structure may be eventually detected in this species. The only way, however, to determine the current level of gene flow in this species may be through a tag and recapture program. This information is necessary to determine whether regional exploitation of this species will be compensated by immigration from other regions and whether regional (state) regulations will be an effective means of conservation.

In order to perform robust tests of hypotheses concerning gene flow in organisms, the markers used must have sufficient intraspecific variation so that differences in the frequencies of alleles can be assessed between regions. The level of intraspecific variation in the Atlantic sharpnose shark (NSD= 0.13%) is considerably higher than the NSD of 0.036% reported by Heist et al. (1995) in the sandbar shark, *Carcharhinus plumbeus*, although lower than that detected by Heist et al. (1996) in the shortfin mako, *Isurus oxyrinchus* (NSD=0.38%). Although the number of individuals surveyed in this study is small, the similar amount of variation detected within each sample and the close agreement in frequencies between regions strongly suggest mtDNA haplotype homogeneity between sharpnose sharks of the Mid-Atlantic Bight and Gulf of Mexico. This study has demonstrated that this small coastal shark, with no passive larval transport, nevertheless exhibits mtDNA haplotype homogeneity across a broad geographic range.

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