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Genetic heterogeneity among blacktip shark, *Carcharhinus limbatus*, continental nurseries along the U.S. Atlantic and Gulf of Mexico

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Abstract Genetic population structure of the blacktip shark, *Carcharhinus limbatus*, a commercially and recreationally important species in the southeast U.S. shark fishery, was investigated using mitochondrial DNA control region sequences. Neonate blacktip sharks were sampled from three nurseries, Pine Island Sound, Terra Ceia Bay, and Yankeetown, along the Gulf of Mexico coast of Florida (Gulf) and one nursery, Bulls Bay, on the Atlantic Ocean coast of South Carolina (Atlantic). Sequencing of the complete mitochondrial control region of 169 neonates revealed 10 polymorphic sites and 13 haplotypes. Overall haplotype diversity and percent nucleotide diversity were 0.710 and 0.106%, respectively. Haplotype frequencies were compared among nurseries to determine if the high mobility and seasonal migrations of adult blacktip sharks have maintained genetic homogeneity among nurseries in the Atlantic and Gulf. Chi-square analysis and AMOVA did not detect significant structuring of haplotypes among the three Gulf nurseries, $P(\chi^2) = 0.294$, $\Phi_{ST} = -0.005$ to -0.002. All pairwise AMOVA between Gulf nurseries and the Atlantic nursery detected significant partitioning of haplotypes between the Gulf and Atlantic ($\Phi_{ST} = 0.087$–0.129, $P < 0.008$), as did comparison between grouped Florida Gulf nurseries and the Atlantic, $\Phi_{CT} = 0.090$, $P < 0.001$. Based upon the dispersal abilities and seasonal migrations of blacktip sharks, these results support the presence of philopatry for nursery areas among female blacktip sharks. Our data also support the treatment of Atlantic and Gulf blacktip shark nursery areas as separate management units.

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Introduction

The blacktip shark, *Carcharhinus limbatus*, is a cosmopolitan tropical and subtropical species common to coastal waters along the U.S. Atlantic from South Carolina to the Florida Keys, the Gulf of Mexico, and the Caribbean Sea (Castro 1996). It is the most frequently harvested species commercially [> 4,500,000 lb (ca. 2,041 t) dry weight commercial landings 1997–1999] and recreationally (> 180,000 individuals 1997–1999) in the U.S. Atlantic Ocean and Gulf of Mexico large coastal shark fishery (Cortes 2000; NMFS 2001). Life history traits such as moderately slow growth rates and low fecundity (females produce approximately 4–6 pups every other year) (Castro 1996), limit recruitment abilities and make blacktip shark populations susceptible to stock collapses from overfishing. In 1999, NMFS reduced quotas and bag limits for the blacktip shark complex (NMFS 1999) in response to growing concern over the sustainability of blacktip shark populations targeted by the large coastal shark fishery. The species was deemed overfished and stock assessments in 1998 estimated populations at only 44–50% of their maximum sustainable yields (NMFS 1998, 2001). Recent assessments indicate Atlantic blacktip shark populations are not currently overfished (NMFS 2003). Despite the commercial importance of the species and concern regarding population levels, the stock structure of blacktip shark populations in these areas is not known. Knowledge of stock structure is essential for the effective management of a species as deviations from panmixia can require treatment of subpopulations as separate management units.

Juvenile and neonate blacktip sharks utilize continental nursery areas along the coasts of the Florida Gulf

and South Carolina during summer months and make seasonal migrations from these areas to wintering grounds off southern Florida and the Florida Keys (Castro 1993, 1996; Hueter and Manire 1994; Hueter 1999; Heupel and Simpfendorfer 2002). Winter habitats have been poorly characterized and the extent to which blacktip sharks from the Atlantic and Gulf of Mexico mix during winter months is not known. Previous tagging studies documenting the high mobility of blacktip sharks along the U.S. Atlantic coast and within the Gulf of Mexico provided direct evidence of blacktip shark movements between these regions (Kohler et al. 1998). However, a much smaller percentage of tagged individuals moved between the Gulf of Mexico and Atlantic Ocean relative to species such as the sandbar shark, *Carcharhinus plumbeus*. Movements of up to 1,159 nautical miles (2,148 km) were observed for blacktip sharks, but the sex of the dispersing individuals was not reported and it is unknown if females and males display similar movement patterns. Despite the seasonal migratory behavior and long distance movements of this species, the regularity with which female blacktip sharks from the Gulf and Atlantic come into contact or move between areas remains unclear (Carlson and Brusher 1999), as does the degree of site-fidelity (philopatry) pregnant females exhibit for Gulf or Atlantic nurseries or natal nurseries (natal homing) (Hueter 1998; Hueter et al. 2003) during summer migrations. The high mobility of blacktip sharks may have produced genetic homogeneity among Gulf and Atlantic nurseries as fewer than ten migrants per generation can prevent genetic drift from producing significant genetic differences between locations (Allendorf and Phelps 1981).

Studies involving a diverse range of taxa have used genetic data to investigate population structure in marine organisms between the Gulf of Mexico and western Atlantic Ocean (review by Avise 1992; Lankford et al. 1997; Gold and Richardson 1998; Seyoum et al. 2000; Young et al. 2002). Genetic divergence between regions is commonly attributed to vicariant events and limited dispersal abilities (Saunders et al. 1986; Bowen and Avise 1990; Reeb and Avise 1990; Young et al. 2002). Significantly different allele frequencies among populations have been used as criteria for managing populations as separate stocks (Moritz 1994). Species with high dispersal abilities often do not exhibit genetic variation between the Gulf and Atlantic (Avise et al. 1986; Diaz et al. 1997; Buonaccorsi et al. 2001). Behavioral traits which limit gene flow, such as philopatry, can result in genetic divergence between populations of marine species which appear to have high dispersal potential (Baker et al. 1990; Meylan et al. 1990; review by Palumbi 1994).

Investigations into the genetic structure of shark populations between the Atlantic and Gulf of Mexico have been hampered by low levels of intraspecific variation in these taxa (Heist et al. 1995, 1996a; Heist and Gold 1999) potentially resulting from slow rates of evolution in sharks (Martin et al. 1992). Genetic homogeneity was observed between Atlantic and Gulf of Mexico populations of sandbar, Atlantic sharpnose, *Rhizoprionodon terraenovae*, and lemon, *Negaprion brevirostris,* sharks (Heist et al. 1995, 1996a; Heist and Gold 1999; Feldheim et al. 2001) and results were attributed to the dispersal capabilities of sharks throughout the study areas. However, the low intraspecific diversity of the genetic markers used in the sandbar and sharpnose studies may have led to underestimates of population structure in these species (Heist et al. 1995, 1996a; Heist and Gold 1999).

The low levels of intraspecific variation observed in previous studies demonstrate the need to use genetic markers with high mutation rates and increased resolution capabilities when investigating stock structure among shark populations. The maternally inherited mitochondrial control region displays high levels of intraspecific variability (Stepien 1995), resulting from relaxed selection on the noncoding hypervariable ends (Brown 1986; Lee et al. 1995). Sequencing of this molecule has been suggested as a useful tool for investigating genetic structure in sharks (Heist et al. 1996b) and a study using control region sequences of white sharks, *Carcharodon carcharias*, detected significant heterogeneity between South African and Australian/New Zealand populations (Pardini et al. 2001).

This study examines the degree of genetic homogeneity among blacktip shark nurseries located along the Gulf of Mexico coast of Florida and between U.S. Atlantic and Gulf of Mexico nurseries using the mitochondrial control region sequences of neonate blacktip sharks. We are testing the null hypothesis that the high mobility and seasonal migrations/wintering habits of adult blacktip sharks have led to genetic homogeneity among blacktip shark nurseries along the Atlantic coast of South Carolina and the Gulf of Mexico coast of Florida. Alternatively, reproductive philopatry for Gulf and Atlantic regions may have resulted in significant stock structure despite the high dispersal indicated by tag/recapture studies.

Materials and methods

Sample collection and storage

Neonate blacktip sharks were sampled within three nurseries along the Gulf of Mexico coast of Florida (Pine Island Sound, Terra Ceia Bay, and Yankeetown) from May to August and within one nursery off the Atlantic coast of South Carolina (Bulls Bay) from May to October in 2000 and in 2001 (Fig. 1). The number of neonates analyzed (n) from 2000 and 2001 ranged from 8 to 30 individuals (Table 1). Neonates were collected using gillnets or rod and reel. Approximately 1 cm^2 of tissue was removed from the dorsal fin of each individual and stored in 20% DMSO saturated with NaCl. Immediately following tissue collection the live sharks were tagged and released. Any dead animals were retained for other studies.

DNA extraction and amplification

Total genomic DNA was isolated from approximately 25 mg of fin tissue using the QIAGEN DNeasy Tissue kit. The polymerase chain reaction (PCR) was used to selectively amplify the mitochondrial

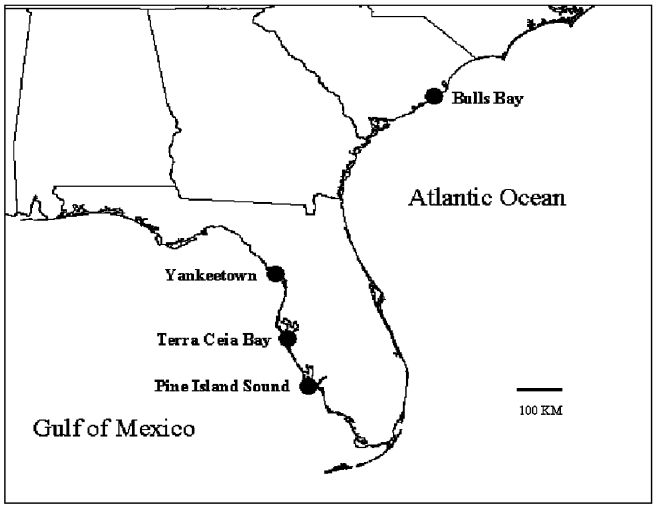

Fig. 1 Blacktip shark, *Carcharhinus limbatus*, nursery locations sampled in 2000 and 2001

Table 1 Number of individuals blacktip sharks, *Carcharhinus limbatus*, collected in 2000 and 2001 (*n*) analyzed from each nursery and results of within nursery temporal homogeneity tests. P_{rb} is the probability of haplotype frequency homogeneity between samples collected in 2000 and 2001 within each nursery based upon 1,000 pseudoreplications (Roff and Bentzen 1999). $P_{\Phi ST}$ is the probability of finding greater haplotype variance between samples collected in 2000 and 2001 within each nursery by chance based upon 1,000 permutations (Excoffier et al. 1992)

Nursery	2000 (*n*)	2001 (*n*)	P_{rb}	$P_{\Phi ST}$
Bulls Bay, S.C.	8	26	0.367	0.646
Pine Island Sound, Fla.	30	15	0.374	0.234
Terra Ceia Bay, Fla.	30	15	0.483	0.261
Yankeetown, Fla.	30	15	0.391	0.892

control region using primers within the proline tRNA (light strand primer Pro-L: 5′-AGG GRA AGG AGG GTC AAA CT-3′) and 12S rRNA (heavy strand primer 282:5′-AAG GCT AGG ACC AAA CCT; J.C. Patton, unpublished data) genes. PCR amplification was performed in 50-μl reactions containing approximately 50 ng template DNA, 1× *Taq* buffer (50 mM KCl, 10 mM Tris-HCl, 0.1% Triton X-100, pH 9.0), 200 μM each dNTP, 2 mM $MgCl_2$, 0.5 μM each primer, and 1.25 units *Taq* DNA polymerase. Amplification was performed with a Precision Scientific GTC-2 thermocycler and consisted of an initial denaturation of 2 min at 95°C, followed by 30 cycles of 1 min at 95°C, 1 min at 60°C, and 1 min at 72°C, and a final extension for 6 min at 72°C.

Gel extraction and sequencing

PCR products were separated on 1.4% agarose gels at 90 V for 90 min and extracted from the gel using the QIAGEN QIAquick Gel Extraction Kit. DNA sequencing of the entire mitochondrial control region was performed using the two initial PCR primers and two additional internal light strand primers: primer DloopF2 (5′-TCC ACA TTA ATC TAC TAT CAG C-3′) and primer DloopF3 (5′-GTC CTC TAG TTC CCT TTA ATG GC-3′). Prior to sequencing, primers were end-labeled with ^{32}P using polynucleotide kinase at 37°C for 30 min and 90°C for 2 min. Sequencing reactions were performed using the *fmol* DNA Sequencing System from Promega. Cycle-sequencing reactions were performed on a Hybaid Omn-E thermocycler using the following cycling conditions: initial denaturation of 4 min at 95°C, followed by 30 cycles of 30 s at 95°C, 30 s at 53–59°C, and 1 min at 70°C. Sequencing reactions were electrophoresed in 6% denaturing polyacrylamide gels at approximately 1,300 V for 2–8 h on a C.B.S. Scientific dedicated height nucleic acid sequencer. Gels were then dried and exposed to autoradiography film. PCR primers Dloop F5-L (5′-CCC TCT CGG AAA GAA ACC TCC-3′) and Phe-H (5′-AGG GGT TTT TCG AGA CAA CC-3′) were used to amplify a 206 base pair region containing a stretch of 11 adenine nucleotides which had an ambiguous 12th adenine in three individuals. One primer was end-labeled with ^{32}P and PCR amplification was carried out with an annealing temperature of 60°C. PCR reactions were electrophoresed in 6% denaturing polyacrylamide gels at approximately 1,300 V for 5 h to investigate potential length polymorphism. This region was also resequenced in these three individuals using the BigDye Terminator v.3.1 flourescence-based cycle sequencing kit (Applied Biosystems) and electrophoresed on an ABI PRISM 377 DNA Sequencer. Both of these methods supported the presence of DNA containing 11 and 12 adenine nucleotides. Whether the additional adenine nucleotide represented actual size heteroplasmy in these sharks or was merely an amplification artifact could not be determined and these individuals were treated as having 11 adenine nucleotides for all analyses (treatment as 12 adenine nucleotides did not affect results of any analyses).

Analysis of mitochondrial DNA control region data

Control region sequences were read manually and aligned using the ClustalW Alignment program of MacVector 6.5 (Oxford Molecular 1998). Summary statistics (number of haplotypes, haplotype frequencies, number of polymorphic sites, number of transitions and transversions, and nucleotide composition) were calculated with the Arlequin 2.0 program (Schneider et al. 2000). Haplotype diversity (*h*), the probability that two randomly selected haplotypes in the sample are different, and nucleotide sequence diversity (π), the probability that two randomly selected homologous nucleotides in the sample are different, were calculated following Nei (1987) as implemented in Arlequin 2.0. The chi-square (χ^2) Monte Carlo resampling procedure of Roff and Bentzen (1989) was used to test for temporal haplotype homogeneity between year 2000 and 2001 cohorts within nurseries, and spatial homogeneity among the three Florida nurseries. Temporal haplotype homogeneity within nurseries was also tested using analysis of molecular variance (AMOVA) (Excoffier et al. 1992) and probabilities of haplotype homogeneity were determined by comparing values to a null distribution of 1,000 samples generated by permuting haplotypes between years with Arlequin 2.0. After no significant temporal differences were observed within nurseries, cohorts from 2000 and 2001 were pooled within each of the four nurseries for all spatial analyses among nurseries. The proportion of haplotype variation allocated among nurseries versus within nurseries (Φ_{ST}) was calculated between each pair of Gulf and Atlantic nurseries using AMOVA with Arlequin 2.0. Probabilities of haplotype homogeneity for Φ_{ST} values were calculated by comparison to a null distribution of 1,000 samples generated by permuting haplotypes among populations. The initial $\alpha = 0.05$ significance level was adjusted for simultaneous pairwise comparisons using the sequential Bonferroni approach (Rice 1989). After χ^2 and all pairwise Φ_{ST} tests failed to reject genetic homogeneity among Florida Gulf coast nurseries, the three Gulf nurseries were grouped and hierarchical Φ statistics were calculated using AMOVA with Arlequin 2.0 to determine the proportion of haplotype heterogeneity partitioned among Gulf nurseries (Φ_{SC}) and between the Gulf and Atlantic (Φ_{CT}).

Results

The mitochondrial control region from a total of 169 individuals ranged from 1,066 to 1,067 bp. The

Table 2 Polymorphic nucleotide positions for 13 haplotypes. Haplotype numbers (*Haplo*) are listed in the left column and the positions of polymorphic base pairs are listed across the top row. The nucleotide at each position is given for haplotype 1. Only nucleotides different from haplotype 1 are given for all other haplotypes. Nucleotides identical to haplotype 1 are indicated with periods (.) and deletions are indicated with dashes (-). Complete haplotype sequences are deposited in GenBank (accession numbers: AY208861-AY208873)

Haplo	53	88	139	144	324	550	776	844	910	1052
1	T	T	T	C	G	A	T	T	A	A
2	.	.	.	.	.	.	.	C	.	.
3	.	.	.	.	.	.	.	C	.	G
4	C	.	.	.	.	.	.	C	.	.
5	.	.	.	.	.	.	.	C	-	.
6	.	.	.	T	.	.	.	C	-	.
7	.	.	A	.	.	.	.	.	.	.
8	.	C	.	.	.	.	.	C	-	.
9	.	.	.	.	.	G	.	.	.	.
10	.	.	.	.	.	G	.	C	.	G
11	.	.	.	.	A	.	.	.	.	.
12	.	.	.	.	.	.	C	C	.	G
13	.	.	.	.	.	.	.	.	.	G

Table 3 Total number of individuals analyzed (n), the number of haplotypes revealed, haplotype diversity (h), and percent nucleotide sequence diversity ($\%\pi$) for each nursery

Nursery	n	No. haplotypes	h	$\%\pi$
Bulls Bay, S.C.	34	2	0.371	0.035
Pine Island, Fla.	45	10	0.785	0.120
Terra Ceia, Fla.	45	8	0.720	0.106
Yankeetown, Fla.	45	9	0.796	0.134

nucleotide composition of the control region consisted of 13% guanine, 32% adenine, 35% thymine, and 20% cytosine (33% GC content). Sequence analysis revealed 10 polymorphisms (8 transitions, 1 transversion, and 1 insertion/deletion), resulting in 13 haplotypes (Table 2). Three individuals (one from Pine Island Sound and two from Yankeetown) possessed an ambiguous potential 12th adenine nucleotide at base pair 933 following a stretch of 11 consecutive adenine nucleotides found in all samples. Overall haplotype diversity and percent nucleotide sequence diversity for the four sample sites were 0.710 and 0.106%, respectively. Sample sizes (n), the number of haplotypes, haplotype diversities, and percent nucleotide sequence diversities for each nursery are listed in Table 3. The two most common haplotypes occurred in all four nurseries, while 11 haplotypes observed in Gulf nurseries were absent from the Atlantic (Table 4). All χ^2 and AMOVA probabilities of haplotype homogeneity within nurseries between year 2000 and 2001 cohorts were not significant ($P > 0.23$) (Table 1).

χ^2 spatial analysis failed to detect significant haplotype heterogeneity among the three Gulf nurseries ($P = 0.294$). The proportion of haplotype variation attributed to differences among the three Gulf nurseries by AMOVA was $\Phi_{SC} < 0.001$, $P = 0.521$. All pairwise AMOVA results between sites within the Gulf were not significant and Φ_{ST} values ranged from -0.005 to -0.002 (Table 5). All pairwise comparisons between Florida Gulf nurseries and the Atlantic nursery indicated a significant proportion of haplotype variation was dis-

Table 4 Geographic distribution of control region haplotypes among 2000 and 2001 blacktip shark samples from Atlantic (Bulls Bay) and Gulf of Mexico (Pine Island, Terra Ceia, and Yankeetown) nurseries. Haplotype numbers refer to haplotypes listed in Table 2

Haplotype	Bulls Bay		Pine Island		Terra Ceia		Yankeetown		Total
	2000	2001	2000	2001	2000	2001	2000	2001	
1	7	19	10	6	16	4	12	5	79
2	1	7	11	2	9	4	4	4	42
3	0	0	2	1	2	2	5	3	15
4	0	0	1	0	0	0	0	0	1
5	0	0	3	2	1	1	4	0	11
6	0	0	2	0	0	1	0	1	4
7	0	0	0	0	1	1	0	1	3
8	0	0	0	0	0	0	1	0	1
9	0	0	0	1	0	0	2	1	4
10	0	0	0	0	0	0	2	0	2
11	0	0	1	1	0	0	0	0	2
12	0	0	0	1	1	1	0	0	3
13	0	0	0	1	0	1	0	0	2
Total	8	26	30	15	30	15	30	15	169

Table 5 Pairwise spatial haplotype variation between nurseries and probabilities of haplotype homogeneity. *SC* Bulls Bay, South Carolina, *PI* Pine Island Sound, Florida, *TC* Terra Ceia Bay, Florida, *YT* Yankeetown, Florida. Numbers above dashed lines (-) are the proportion of haplotype variation allocated between nurseries (Φ_{ST}) for each pair of nurseries. Numbers below the dashed lines are the probabilities of finding greater haplotype variance between nurseries by chance based upon 1,000 permutations (Excoffier et al. 1992). Probabilities marked with * are significant after adjusting initial $\alpha = 0.05$ significance level using the sequential Bonferroni correction for multiple simultaneous comparisons (Rice 1989)

	SC	PI	TC	YT
SC	–	0.120	0.087	0.129
PI	< 0.001*	–	−0.005	−0.003
TC	0.007*	0.556	–	−0.002
YT	< 0.001*	0.473	0.420	–

tributed between nurseries and Φ_{ST} values ranged from 0.087 to 0.129 (Table 5). AMOVA between grouped Gulf nurseries and the Atlantic nursery indicated a significant proportion of haplotype heterogeneity between the Gulf and Atlantic, $\Phi_{CT} = 0.090$, $P < 0.001$.

Discussion

This study demonstrated genetic heterogeneity between blacktip shark nurseries in the Gulf of Mexico and along the Atlantic coast of South Carolina and revealed the highest degree of genetic differentiation between Gulf and Atlantic shark populations to date (Heist et al. 1995, 1996a; Heist and Gold 1999; Feldheim et al. 2001). Female blacktip sharks do not appear to be dispersing randomly between these regions during summer migrations to nurseries and there is a tendency for related females to return to the same region. Although the movement of low numbers of Atlantic females into the Gulf of Mexico may occur, as Atlantic haplotypes were common along the Florida Gulf, numbers have not been large enough to result in genetic homogeneity and Florida Gulf coast nurseries may receive strays from elsewhere in the Gulf of Mexico. As even low levels of genetic heterogeneity among marine populations may not indicate sufficient migration levels to rebuild local population depletions (Waples 1998), our results indicate the level of migration between the Atlantic and Gulf is too low for depleted blacktip shark stocks in the Atlantic to be replenished with the aid of migration from the Gulf (and vice versa) and the regional nurseries represent separate management units (Moritz 1994). Therefore, loss of regional nursery areas can result in depletions of local populations and concern over the conservation of nursery areas should be a management priority for this species.

Significant genetic heterogeneity was not detected among the three Florida Gulf coast nurseries potentially indicating a greater degree of straying among these proximate sites located along continuous nursery habitat. The low number of strays needed to homogenize genetic signals indicate the data from this study should not be interpreted as evidence that Florida Gulf females are returning randomly to nurseries along the Gulf coast of Florida.

Sampling methodology and genetic marker selection were crucial to the amount of genetic variation detected in this study. Neonates were sampled before leaving nurseries (Heupel and Hueter 2001) to ensure genetic data reflected differences among nursery usage and mitochondrial gene flow, not the movements of adults. By using a maternally inherited marker, the genetic makeup of adult female blacktip sharks utilizing Atlantic and Gulf nurseries could be inferred. Similarities in genetic structure within nurseries between years provided support that our results reflect geographic differences in the genetic structure of nurseries and are not heavily biased from temporal heterogeneity within nurseries. Also, sampling in consecutive years decreased full-sibling bias from sampling offspring of relatively few breeding females (e.g., the Allendorf-Phelps effect of Waples 1998). Female blacktips do not give birth in consecutive years due to gestation periods greater than 11 months (Castro 1996). The control region displayed sufficient variation to detect genetic heterogeneity. However, other mtDNA regions were not tested for variability and may also have been appropriate for investigating population structure in blacktip sharks. Although the majority of studies involving sharks have detected low levels of mtDNA variation, this is not true for all shark species (Heist et al. 1996b) and may partially reflect demographic fluctuations experienced by coastal species.

Palumbi (1994) outlined several mechanisms leading to population structure in marine species with high dispersal potential, such as blacktip sharks. The swimming abilities and documented movements of blacktip sharks (Kohler et al. 1998) suggest a lack of physical barriers to gene flow between the Gulf and Atlantic for this species, and the observed genetic heterogeneity likely resulted from aspects of blacktip shark behavior. Female blacktip sharks may remain in the Atlantic or Gulf while making seasonal migrations and utilize nurseries solely within these areas. If males and females display different migratory behaviors, our results may indicate sex-biased dispersal, but nuclear data is needed to test this hypothesis. Also, females may move between the Atlantic and Gulf or mix during winter months, but have strong tendencies to return to nurseries within one of these regions. This behavior, often developing from natal homing tendencies, has been well-documented among marine animals (Baker et al. 1990; Meylan et al. 1990). For genetic differentiation to occur, generations of related females would have to return to nurseries within either the Gulf or Atlantic. Both of these scenarios, females remaining within one region instead of dispersing throughout the species range and females consistently returning to nursery regions despite movements out of the regions, are forms of philopatry. Philopatry has been directly observed in juvenile

blacktip sharks from tag returns and a large percentage of blacktip pups which survived their first summer and were tagged within Terra Ceia Bay returned to their natal nursery after 1 year (30%) and 2 years (50%) at liberty (Hueter 1998; Hueter et al. 2003). Evidence also exists of philopatry in other species of sharks (Pardini et al. 2001; Feldheim et al. 2002). The demonstrated philopatry of juvenile blacktip sharks as shown through tag returns, repeated use of seasonal nurseries by adult female blacktip sharks, and genetic differences between Gulf and Atlantic nurseries, support a hypothesis of nursery area philopatry in adult female blacktip sharks within the area of this study. Although more of a concern when panmixia cannot be rejected by genetic data, consistency between biological data, such as tagging studies, with genetic results reduces the risk of inaccurate management decisions based solely on genetic data (Hauser and Ward 1998).

The observed genetic heterogeneity resulted from the relatively low level of genetic diversity in the Atlantic compared to Gulf nurseries. Out of 13 haplotypes, only the two most common haplotypes were found in the Atlantic (Table 4). Haplotype and nucleotide diversity were much lower in the Atlantic nursery than within any of the three Gulf nurseries and cannot be explained by sampling only 11 fewer individuals in the Atlantic versus each Gulf nursery. The same haplotypes were encountered in South Carolina during both years of sampling, indicating the relatively low diversity in the Atlantic was not a result of biased sampling. Low genetic diversity in the Atlantic may have resulted from a previous bottleneck event which substantially decreased the effective female population size in this area. Blacktip numbers along the Atlantic may be much smaller than within the Gulf of Mexico resulting in lower genetic diversity and increased risk of stock collapse. However, as we have sampled only one Atlantic nursery region, data from additional Atlantic nurseries should be collected before strong conclusions can be drawn regarding genetic diversity of Gulf of Mexico versus Atlantic blacktip shark populations.

The lower diversity we observed in the Atlantic is consistent with a hypothesis of post-glacial colonization/recolonization of suitable nursery habitats from southern Gulf of Mexico/Caribbean refugia after the last Pleistocene glacial maximum (less than 18,000 years ago) with diffusion effects (Palumbi 1994) resulting in fewer haplotypes present within Atlantic nurseries. Initial colonization of a nursery by a small number of founders can allow genetic drift to rapidly decrease variation. Also, decreases in genetic diversity can be expected to accompany increases in latitude in species with population structure as colonizing stocks from the south may stop along the coast during northern migrations (Buonaccorsi et al. 2001). Although adult blacktip sharks are encountered in waters as far north as New England, they become increasingly rare at latitudes higher than South Carolina (Castro 1996) and the sample site at Bulls Bay, South Carolina may approach the northern extent of Atlantic pupping grounds. Since samples were not examined from extreme southern Atlantic coast nurseries, it is unknown if there is a latitudinal decrease in genetic diversity among nurseries along the Atlantic or if the entire Atlantic coast exhibits low levels of variation relative to Gulf of Mexico nurseries. In several species, individuals from the southern Atlantic coast of Florida were genetically similar to individuals from the Gulf of Mexico, but distinct from individuals from the Atlantic north of central Florida (Avise 1992). The location of Bulls Bay, South Carolina near the northern limit of known blacktip pupping grounds may also limit diversity within nurseries by decreasing the number of pregnant females straying from proximate nurseries versus along the Florida Gulf coast where nurseries are abundant (Carlson and Brusher 1999; Hueter and Manire 1994; Hueter 1999). Caution needs to be exercised in the interpretation of genetic data from populations that are potentially recent (post-glacial) as populations may not have attained mutation/drift equilibrium and, more importantly for the present study, genetic equilibrium may not have been reached among populations with regard to drift and migration. However, the significantly lower diversity observed in South Carolina despite the yearly migrations and potential for straying of Gulf and Atlantic female blacktip sharks is evidence of minimal straying of Gulf female blacktips into South Carolina nurseries during pupping migrations. The magnitude of the difference in diversity observed between Florida Gulf and South Carolina nurseries in this study indicates contemporary gene flow is not homogenizing nurseries in these areas at a fast enough rate to be of importance to human fishery concerns and the Atlantic and Gulf nursery regions investigated in this study represent separate reproductive stocks.

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