ICES Journal of Marine Science Advance Access published June 21, 2016

ICES Journal of Marine Science

ICES Journal of Marine Science (2016), doi:10.1093/icesjms/fsw098

Population structure, gene flow, and historical demography of a small coastal shark (*Carcharhinus isodon*) in US waters of the Western Atlantic Ocean

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Portnoy, D. S., Hollenbeck, C. M., Bethea, D. M., Frazier, B. S., Gelsleichter, J., and Gold, J. R. Population structure, gene flow, and historical demography of a small coastal shark (*Carcharhinus isodon*) in US waters of the Western Atlantic Ocean. – ICES Journal of Marine Science, doi:10.1093/icesjms/fsw098.

Received 5 March 2016; revised 12 May 2016; accepted 13 May 2016.

Patterns of population structure, genetic demographics, and gene flow in the small coastal shark *Carcharhinus isodon* (finetooth shark) sampled from two discrete nurseries along the southeastern US coast (Atlantic) and three nurseries in the northern Gulf of Mexico (Gulf), were assessed using 16 nuclear-encoded microsatellites and 1077 base pairs of the mitochondrial DNA (mtDNA) control region. Significant heterogeneity in microsatellite allele distributions was detected among all localities except between the two in the Atlantic. Significant heterogeneity in mtDNA haplotypes was not detected, a result likely due to extremely low mtDNA diversity. The genetic discontinuities combined with seasonal movement patterns, a patchy distribution of appropriate nursery habitat, the apparent absence of sex-biased gene flow, and the occurrence of mating in the vicinity of nursery areas, suggest that both male and female finetooth sharks display regional philopatry to discrete nursery areas. Global and local tests of neutrality, using mtDNA haplotypes, and demographic model testing, using Approximate Bayesian Computation of microsatellite alleles, supported a range-wide expansion of finetooth sharks into US waters occurring less than ~9000 years ago. These findings add to the growing number of studies in a variety of coastally distributed marine fishes documenting significant barriers to gene flow around peninsular Florida and in the eastern Gulf. The findings also provide further evidence that the traditional model of behavioural ecology, based on large coastal sharks, may not be appropriate for understanding and conserving small coastal sharks.

Keywords: elasmobranch, fine-scale structure, post-glacial expansion.

Introduction

Observed patterns of population structure are the results of interactions among micro-evolutionary processes, in particular the balance between genetic drift and the homogenizing effects of gene flow (Slatkin, 1987). In natural systems, genetically independent populations are often bounded by physical barriers which impede the ability of individuals to disperse between suitable habitats (Irwin, 2002). In marine systems, however, hard barriers to dispersal often are not apparent and potential barriers such as areas of open, deep water for nearshore species or currents can be overcome by larval dispersal or movement of highly mobile juveniles and adults or both (Carr *et al.*, 2003). Consequently, a higher degree of connectivity in marine species over large spatial

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scales is often observed in comparison to terrestrial systems (Kinlan and Gains, 2003).

For elasmobranchs, which lack a larval phase, genetic connectivity depends on adult and sub-adult dispersal. A well-established paradigm in elasmobranch behavioral ecology, originating from the work of Springer (1967) on large coastal sharks, is that females are philopatric to nursery areas or regions, whereas males are not. As a result, gene flow is male mediated, often producing a pattern of heterogeneity in maternally inherited mitochondrial (mt)DNA haplotypes and relative homogeneity in bi-parentally inherited nuclear microsatellites when samples are from areas used for parturition (Portnoy and Heist, 2012). Genetic studies of both large coastal and large pelagic sharks support Springer's model (Pardini *et al.*, 2001; Keeney *et al.*, 2005; Portnoy *et al.*, 2010). The model, however, may have less applicability in small coastal sharks where adult males, females, and juveniles are more likely to co-occupy a near-shore environment (Knip *et al.*, 2010).

The finetooth shark, *Carcharhinus isodon*, is a small coastal shark distributed in US waters along the Atlantic coast of the southeastern US (Atlantic) and the northern Gulf of Mexico (Gulf) (Ebert *et al.*, 2013). During summer, all life stages, particularly neonates, are common in estuarine and shallow nearshore habitats, indicating that these areas are important nursery grounds (Ulrich *et al.*, 2007; Bethea *et al.*, 2015). Unlike large coastal sharks, adult finetooth shark males also are present in nursery areas (Bethea *et al.*, 2015) and mating takes place soon after parturition (Castro, 1993). Given an estimate of female longevity of 14–18 years (Drymon *et al.*, 2006) and the apparent variability between individual females of 1- vs. 2-year reproductive cycles (Driggers and Hoffmayer, 2009), lifetime fecundity for individual females is likely no greater than ~85 pups.

The coastal distribution of finetooth sharks makes them easily accessible to commercial and recreational fishing. They are a common component of the gillnet fishery in the southeastern United States and are taken as bycatch in the menhaden purse-seine fishery in the Gulf (Neer and Thompson, 2004). A stock assessment based on catch data from 1981 to 2000 indicated that finetooth sharks were experiencing overfishing; however, a more recent assessment (NOAA, 2007) indicated overfishing was no longer occurring. This recent assessment indicated that finetooth sharks in US waters appear to be less abundant than other small coastal sharks and that data on life history and catch rates are neither thorough nor detailed. In addition, lifetime fecundity and estimated growth coefficients, which are low in comparison with other small coastal sharks (Carlson *et al.*, 2003), indicate that finetooth sharks could be susceptible to overfishing.

Differences in life-history, limited returns of tagged animals, and patterns of seasonal migration are consistent with occurrences of different populations or stocks of finetooth sharks in the Atlantic and Gulf. Life-history differences include size at age for neonates, age at 50% maturity, and observed maximum ages of both males and females (Drymon *et al.*, 2006); finetooth sharks tagged in either the Atlantic or Gulf also have yet to be recaptured in the other region (Kohler *et al.*, 1998; NOAA, 2007). Finetooth sharks in the Atlantic follow a north–south seasonal-migration pattern and move north from the east coast of Florida to the Carolinas in the summer and return to the south in the winter (Castro, 1993). A similar pattern, moving to coastal nursery and mating grounds in the spring and summer and departing in the winter, also occurs among finetooth sharks in the eastern Gulf (Carlson and Brusher, 1999), and it appears there are discrete areas of essential fish habitat for finetooth shark neonates throughout the Gulf (NOAA, 2016).

The primary objectives of this study were to test whether discrete genetic units (populations) of finetooth sharks occur in US waters and to assess whether gene flow was sex-biased. We documented patterns of variation in maternally inherited mtDNA and bi-parentally inherited microsatellites, assessed population structure in US waters and whether finetooth sharks exhibit a sexual bias in gene flow, and estimated historical genetic demographics to better understand patterns of contemporary genetic diversity. Last, we compared genetic variation in a sample from a known wintering area off Cape Canaveral, Florida, with samples taken during the summer in both the Atlantic and Gulf to assess potential mixing between the two regions.

Material and methods

A total of 345 finetooth sharks were sampled between 2010 and 2013 (Supplementary Figure S1) from five estuarine and near-shore localities where the species is found in abundance during the summer: nearshore waters off South Carolina (SC) and north-eastern Florida (AFL) in the Atlantic, offshore of northern Florida (GFL), and western Alabama (AL) in the eastern Gulf, and offshore of Texas (TX) in the western Gulf. Samples were taken during the late spring and summer (May to September) when mature individuals are present in coastal waters for parturition and mating. The exception was in the sample from TX where five individuals were sampled during the winter (December and February). In addition, 23 individuals (sub-adult and adult) were sampled in the winter and early spring (December to early May) off Cape Canaveral, FL (CC). Individual sharks were a mix of juveniles, adult males, and adult females; sex, maturity, and fork length were recorded when possible for each individual. Tissues (fin clips) were stored in 95% non-denatured ethanol or 20% DMSO buffer (Seutin, *et al.* 1991). DNA was extracted following a modified Chelex extraction protocol (Estoup *et al.*, 1996). After a final 2 min centrifugation at 13 000 *g*, 1–2 µl of supernatant was used as a template for PCR reactions.

A total of 16 microsatellites were assayed. The forward primer from each primer pair was labelled with one fluorescent label of 6-Fam, Hex, or Ned (Dye Set D; Applied Biosystems). Descriptions of primers and protocols of PCR amplifications may be found in Giresi *et al.* (2012). Amplicons were electrophoresed on 6% polyacrylamide gels, using an ABI Prism 377 sequencer (Applied Biosystems), with the Genescan 400HD ROX Size Standard (Applied Biosystems) included in each lane. Scoring was conducted manually, using Genescan 3.1.2 (Applied Biosystems) and Genotyper 2.5 (Perkin Elmer).

The complete mitochondrial control region (Ctr; 1077 base pairs) was amplified using the primers Pro-L (5'-AGGGRAAGGAGGGTCAAACT-3', complementary to a portion of the proline tRNA and located on the L strand), and 282H (5'-AAGGCTAGGACCAAACCT-3', complementary to a portion of the 12S rRNA on the H strand (Keeney *et al.*, 2003). Thirty microliter PCR reactions contained 1X reaction buffer (pH 8.5), 2 mM $MgCl_2$, 0.25 mM of each dNTP, 15 pmol of each primer, 0.1 U μl^{-1} *Taq* polymerase, and 2 µl of template. Reaction conditions consisted of an initial denaturation at 95 °C for 4 min followed by 45 cycles of 95 °C for 1 min, 58 °C for 1 min and 72 °C for 1 min, followed by a final extension of 72 °C for 10 min. Amplified products were sent to University of Florida's

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Interdisciplinary Center for Biotechnology Research (http://www.biotech.ufl.edu/) or Beckman Coulter (http://www.beckmangenomics.com) for cleaning and bi-directional sequencing.

Summary statistics

Conformance to Hardy-Weinberg expectations (HWE) was assessed for each microsatellite in each summer sample, using Genepop 4.0 (Rousset, 2008); significance was assessed at the 0.05 level, using exact tests with 1000 batches and 10 000 iterations per batch. Sequential Bonferroni adjustment (Rice, 1989) was employed to correct for multiple testing. Microchecker 2.2.3 (van Oosterhout *et al.*, 2004) was used to screen each microsatellite in each sample taken during the summer for null alleles or genotyping error or both. Number of alleles, expected heterozygosity (unbiased gene diversity), and rarefied allelic richness was estimated for each microsatellite in each sample taken during the summer, using Fstat 2.9.3.2 (Goudet, 2001). Wilcoxon signed-rank tests, implemented in JMP Pro 11.2.0 (SAS Institute Inc.), were used to test for homogeneity of gene diversity and allelic richness between pairs of localities. For control region sequences, Arlequin 3.5.1.2 (Excoffier and Lischer, 2010) was used to estimate nucleon diversity (h) and nucleotide diversity (π) at samples taken during the summer.

Population structure

Homogeneity in allele and genotype distributions (microsatellites) among samples taken during the summer was tested using a single-level, analysis of molecular variance (AMOVA), implemented in Arlequin. Pairwise F_{ST} values between samples, based on microsatellites, also were estimated using Arlequin. Significance of pairwise F_{ST} values at the 0.05 level was assessed by permuting individuals between localities 10 000 times; correction for multiple testing employed the Benjamini and Hochberg (1995) false discovery rate (FDR) procedure. Lositan (Antao *et al.*, 2008) was used to screen for F_{ST} outliers (candidate loci under selection) by comparing observed mean F_{ST} values at each microsatellite, corrected for locus-specific gene diversity (expected heterozygosity), against a 95% confidence interval of F_{ST} values (corrected for diversity) generated by simulation. Runs were implemented with a preliminary simulation of 50 000 steps and used to estimate the neutral mean F_{ST}, followed by a final simulation of 50 000 steps and a FDR of 0.05. Discriminant Analysis of Principal Components (DAPC) was used to visualize genetic differences among samples and was carried out using the Adegenet package (Jombart and Ahmed, 2011) in R 3.0.2 (R Development Core Team, 2013), with prior group membership defined by sample locality. A hierarchical AMOVA, as implemented in Arlequin, also was carried out to test homogeneity of allele (microsatellite) distributions between samples from the Atlantic and those from the Gulf and between or among localities within each region.

Homogeneity of mitochondrial DNA (mtDNA) haplotype distributions among samples taken during the summer was tested using a single-level AMOVA, implemented in Arlequin. Pairwise Φ_{ST} values were estimated using Arlequin, with significance determined as previously described. A minimum-spanning network was created with Network 4.510 (Fluxus-engineering.com), using the full median-joining algorithm (Bandelt *et al.*, 1999), to visualize relationships of mtDNA haplotypes among localities. Maximum parsimony was used to remove unnecessary alternate connections (Polzin and Daneshmand, 2003). Mantel tests (Smouse *et al.*, 1986), as implemented in Arlequin (100 000 permutations), were used to evaluate whether estimates of pairwise F_{ST} (microsatellites) and Φ_{ST} (mtDNA) were correlated.

Because sampling localities in the summer coincided with areas of parturition and because of possible site fidelity of females to specific nursey grounds, relatedness of individuals within each sample was assessed to ensure that signals of genetic heterogeneity were not confounded by the presence of kin within localities. Relatedness (r), based on microsatellite data, was estimated between pairs of individuals within each sample and between pairs of individuals among samples, using the maximum likelihood approach implemented in ML-Relate (Kalinowiski *et al.*, 2006). Distributions of relatedness values within and among samples were then compared using a Kolmogorov-Smirnov test of homogeneity. The percentage of individuals with r values higher than 0.25 (half sib or more related) within and among samples was recorded and compared.

Genetic demography

Tajima's D (Tajima, 1989) and Fu's F_S (Fu, 1997) metrics were estimated for each sample, using Arlequin, to search for departures from neutrality; significance of D and F_S was determined using 50 000 simulated samples. When range-wide expansion occurs the signal often may be obscured by local demographic processes (Städler *et al.*, 2009); consequently, both Tajima's D and Fu's F_S also were estimated for all sample localities pooled; significance was determined as earlier.

Five divergence scenarios (Fig. 1 a–d) were compared, based on microsatellite data, using Approximate Bayesian Computation (ABC) in a coalescent framework, as applied in DIYABC 2.0 (Cornuet *et al.*, 2014), to determine whether the data were consistent with population expansion and whether such an expansion was sudden or occurred over time in a step-wise manner. The first scenario involved a rapid split where all identified, independent genetic units diverged from an ancestral gene pool at time t in the past. This was then compared with scenarios that involved tree-like bifurcations occurring from t_4 to t_1 in the past. These scenarios differed in the timing of each population split, but always followed a step-wise model in which a population had to split from a common ancestor with its nearest geographic neighboring population. Each scenario was run with population sizes free to vary after divergence. Runs consisted of 10^7 simulated data sets (2×10^6 per scenario). Posterior probabilities of each scenario were compared to determine which scenario was most consistent with observed data. Posterior predictive simulations were used to assess congruence between proposed models and observed data (Gelman *et al.*, 2013). Briefly, 10 000 data sets were simulated using the posterior distributions of parameters from the selected model and plotted along the planes of a PCA as was the observed data. These planes were defined by data sets that used the priors for the parameters. Posterior distributions of all demographic parameters were then estimated from the selected model, using the 20 000 simulated data sets closest to the observed data.

The contemporary effective number of breeders (N_b) was estimated for each sample, using microsatellite data only from juveniles and the linkage disequilibrium approach implemented in NeEstimator 2 (Do *et al.*, 2014). Minor alleles at a frequency of

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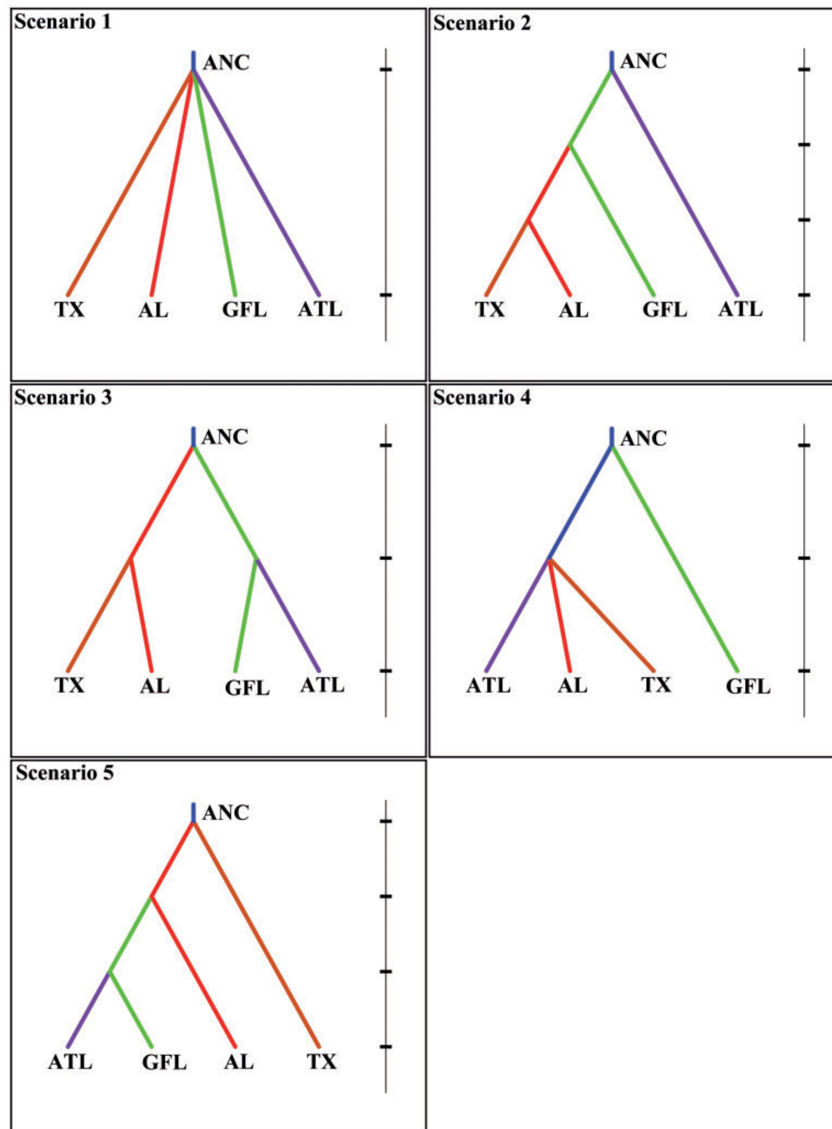

Figure 1. Demographic scenarios, tested in an approximate Bayesian framework, for samples from the Atlantic (ATL is SC and AFL pooled), northwestern Florida (GFL), Alabama (AL), and Texas (TX). For all scenarios N_{eLT} was free to change immediately after a split event; hash marks represent time steps in the past measured in generations; ANC is ancestral lineage.

0.02 or less were excluded and confidence intervals were obtained by jack-knifing. A global estimate of contemporary effective size (N_e) was obtained by pooling all samples and including individuals of all age classes and implementing the same linkage-based approach used to estimate N_b.

Dispersal

To assess whether a juvenile was the progeny of first-generation migrants or whether adults and sub-adults were first generation migrants, likelihood ratios were estimated as the likelihood of an individual's multi-locus (microsatellite) genotype originating in the population from which the individual was sampled over the highest likelihood of that genotype originating across all populations ($L' = L_{home}/L_{max}$) in GeneClass 2.0 (Piry *et al.*, 2004), using a Bayesian approach (Rannala and Mountain 1997). Significance was determined by simulating 10 000 individual multi-locus genotypes (Paetkau *et al.*, 2004) and generating a null distribution of L′ values in order to evaluate observed L′ values at $\alpha \leq 0.01$. The 23 adult individuals sampled in the winter off Cape Canaveral, Florida, were assigned back to a genetic group of origin, using the Bayesian clustering approach implemented in Structure 2.3.4 (Falush *et al.*, 2007). Initially, genetic groups were defined using multi-locus microsatellite genotypes of samples taken during the summer months. The no-admixture model was employed with 250 000 steps and a burn-in of 100 000 steps for $K = 1$–5; runs for each value of K were replicated three times and the correct K was selected by evaluating the mean likelihood scores for each value of K. Individuals sampled off Cape Canaveral were then assigned to groups, using the admixture model, and setting K to the selected number of groups (two) and employing 1 000 000 steps with a burn-in of 500 000 steps; assignment probabilities were determined as the mean across five replicates.

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Results

Summary statistics

Summary statistics for microsatellites appear in Supplementary Table S1. Genotypes at four microsatellite locus-sample pairs deviated from HWE prior to but not following Bonferroni correction. Analysis with MicroChecker did not indicate occurrence of null alleles, stutter, or large allele dropout at any of the 16 microsatellites. The mean number of alleles per locality varied from 12. 4 in GFL and SC to 9.0 in TX. Unbiased expected heterozygosity averaged across all loci ranged from 0.68 in TX to 0.66 in AL, while rarified allelic richness averaged across loci ranged from 9.2 in SC to 8.8 in TX. Pairwise tests of homogeneity in gene diversity indicated significantly greater diversity in TX than in AL ($P = 0.023$) and GFL ($P = 0.040$); all pairwise tests of homogeneity in allelic richness were non-significant. Summary statistics for mtDNA sequence data also appear in Table S1. Seven haplotypes were detected across all samples, with a single haplotype recovered in 92% of the individuals sampled. Estimates of h and π ranged from zero in SC (h and π) to 0.2764 (h) in AL and 0.00030 (π) in AFL. The distribution of haplotypes within each sample and Genbank accession numbers are given in Table Supplementary S2.

Population structure

Single-level AMOVA, based on microsatellites, revealed significant heterogeneity among samples ($\Phi_{ST} = 0.008$, $P < 0.001$, Table 1). Estimates of F_{ST} between pairs of samples were significant both before and after correction for all comparisons except SC vs. AFL (Table 2). No loci were identified as F_{ST} outliers. DAPC indicated either four or five genetic clusters based on microsatellite data (Fig. 2); the 95% ellipses for SC and AFL overlapped entirely, suggesting that four genetic clusters better explained the data. The test for homogeneity of relatedness, based on microsatellite data, was significant ($P = 0.030$); however, mean relatedness was greater among samples ($r = 0.039$) than within samples ($r = 0.038$) and the percentage of individuals with $r > 0.25$ was small both among (1.34%) and within (1.25%) samples. The single-level AMOVA, based on mtDNA haplotypes, was non-significant ($\Phi_{ST} = 0.019$, $P = 0.061$, Table 1). Pairwise Φ_{ST} values ranged from −0.0286 to 0.0606; the comparison of SC vs. AL differed significantly from zero before but not after correction for multiple tests (Table 2). The minimum spanning network (Figure 3) contained a central haplotype found in high frequency across all samples (92%). The remaining haplotypes were separated from the central haplotype by one substitution, except for one haplotype found in two individuals in AFL, which was separated from the central haplotype by two substitutions. The second most common haplotype was shared among all sample localities from the Gulf but was recovered from only five individuals (3.8%). No satellite haplotypes were shared between localities from the Atlantic and Gulf. A Mantel test between the two fixation indices, F_{ST} and Φ_{ST}, was non-significant ($P = 0.40$).

Genetic demographics

Tajima's D was negative for all samples and significant only in GFL ($D = -1.539$, $P = 0.022$); Fu's F_S was negative for all samples except AFL and was significant in GFL ($F_S = -2.716$, $P = 0.003$) and AL ($F_S = -2.550$, $P = 0.003$) (Table 3). Both measures were negative and significant when samples were pooled ($D = -1.472$, $P = 0.031$; $F_S = -7.532$, $P \leq 0.001$).

Sample localities AFL and SC were grouped into a single sample from the Atlantic (ATL), based on results of homogeneity testing, for ABC analysis. Scenario 1, where all populations diverged from an ancestral population at time t in the past (Figure 1), had the highest posterior probability based on both direct and logistic regression-based estimates; all other scenarios had <10% support (Supplementary Figure S2). Observed data also fell within simulated data for Scenario 1, suggesting a good fit for the model (Supplementary Figure S3).

The posterior distribution of long-term effective size (N_{eLT}) for all four (ATL, GFL, AL, and TX) samples was therefore estimated based on Scenario 1, as was the posterior distribution for the ancestral (ANC) N_{eLT} and time since divergence (t). Estimates of N_{eLT} and their high-density probability intervals (HDPI) were almost identical for all groupings (Table 4). In contrast, the point estimate of N_{eLT} for ANC was more than an order of magnitude smaller (916), and its HDPI did not overlap with sample-based HDPIs for GFL or AL (Table 4). The posterior probability distribution of t was well resolved; the modal value was 278 generations with 95% HDPIs ranging from 97 to 660 generations (Supplementary Figure S4). Estimates of N_b (Table 4) varied from 153 in AFL to 1208 in SC; N_b could not be estimated for GFL. All estimates had infinite upper bounds, except for AFL which had an upper bound of 1034. The global estimate of N_e was 12 798 and also had an infinite upper bound.

Dispersal

Only one individual in the entire data set was identified as a putative, first-generation migrant ($L' = 3.957$, $P = 0.0068$). The individual was an immature male captured in AL but with a genotype that had a greater likelihood of originating in GFL. Analysis using Structure revealed the most likely value of K was two ($P > 99\%$); the groups identified consisted of all samples from the Atlantic and all samples from the Gulf. All individuals except one sampled from CC during the winter were assigned to the Atlantic with high probability ($\geq 92\%$); the remaining individual was assigned to the Atlantic as well but with a probability of 83% (Figure 4).

Discussion

Patterns of genetic variation among finetooth sharks sampled at five different nursery areas along the US Atlantic coast (Atlantic) and northern Gulf of Mexico (Gulf) were assessed using both nuclear-encoded microsatellites and sequences of the maternally inherited mtDNA control region. Tests of genetic homogeneity with microsatellites indicated four genetically independent units: an Atlantic group composed of finetooth sharks from nearshore of South Carolina and North Florida, and three distinct groups within the Gulf–one from the northeastern Gulf (offshore of northern Florida), one from the northcentral Gulf (offshore of western Alabama), and one from the northwestern Gulf (offshore of Texas). Tests of homogeneity of mtDNA haplotypes were non-significant, a result likely due to extremely low mtDNA diversity. Departures from neutrality (mtDNA) and ABC analysis (microsatellites) supported an expansion of finetooth sharks in US waters occurring in the recent past, with an increase in long-term effective size of more than order of magnitude during the expansion.

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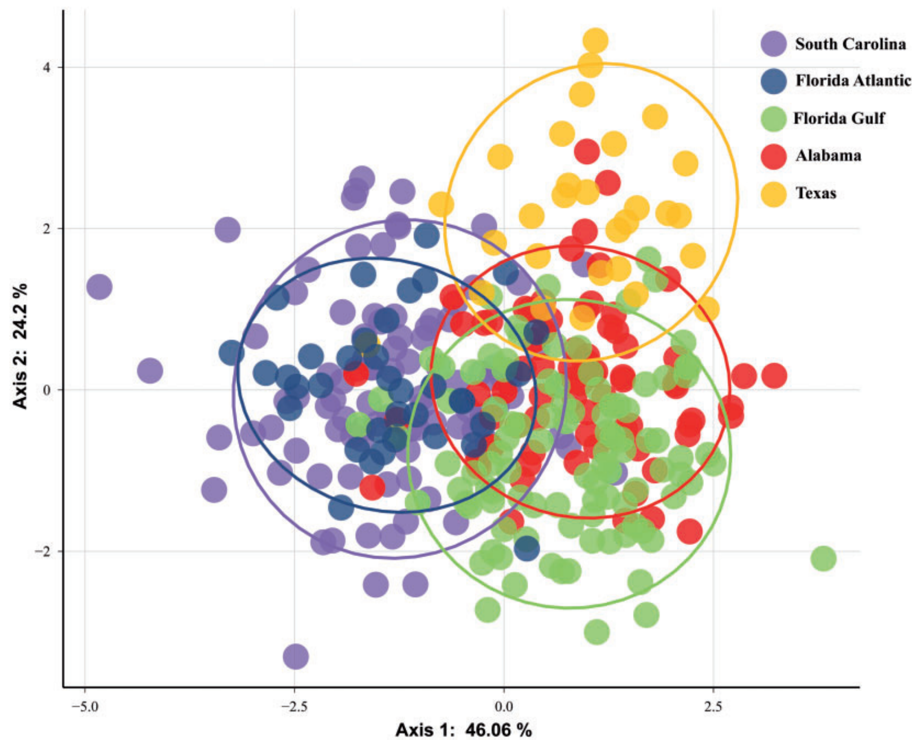

Figure 2. Results of discriminant analysis of principal components (DAPCs) of microsatellite data.

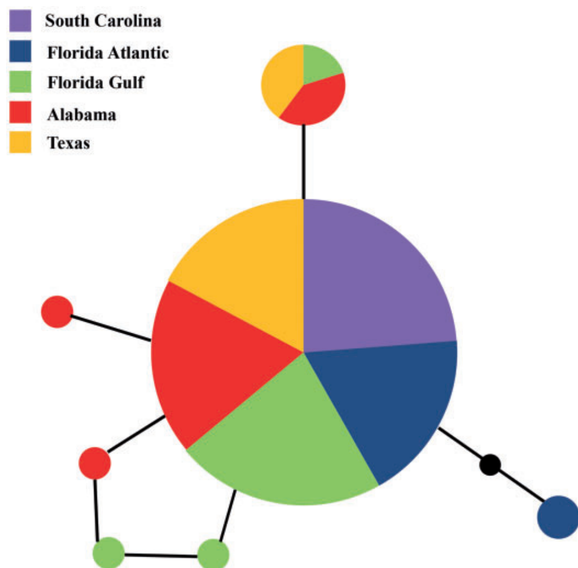

Figure 3. Minimum spanning network of seven mtDNA control region haplotypes from samples of finetooth shark. Sizes of circles are proportional to haplotype frequencies; branch lengths are proportional to number of mutations; black circle is an inferred haplotype.

Table 1. Results of single-level AMOVA, based on microsatellite genotypes and mtDNA sequences, among samples of finetooth shark (*C. isodon*)

Microsatellites	df	SS	VC	%V	F_{ST}	*P*
Among samples	4	43.32	0.042	0.78	0.008	<0.001
Within samples	685	3650.20	5.329	99.22		
Total	689	3693.51	5.371			
mtDNA	**df**	**SS**	**VC**	**%V**	Φ_{ST}	***P***
Among samples	4	0.61	0.002	1.86	0.019	0.061
Within samples	128	13.07	0.102	98.14		
Total	132	13.68	0.104			

df, degrees of freedom; SS, sum of squares; VC, variance component; and %V, percentage of variance.

Table 2. F_{ST} (microsatellite genotypes, above diagonal) and Φ_{ST} (control region sequences, below diagonal) values for pairwise comparisons between pairs of sample localities for finetooth sharks (*C. isodon*) sampled from five localities along the US Atlantic coast and northern Gulf of Mexico

	SC	AFL	GFL	AL	TX
SC	–	0.0013	**0.0090***	**0.0129***	**0.0068***
AFL	0.0553	–	**0.0112***	**0.0150***	**0.0066***
GFL	0.0158	0.0330	–	**0.0021***	**0.0050***
AL	**0.0225**	0.0324	−0.0081	–	**0.0059***
TX	0.0606	0.0433	−0.0002	−0.0286	–

SC, South Carolina; AFL, northeastern Florida; GFL, northwestern Florida; AL, Alabama; TX, Texas. Values significant at $\alpha = 0.05$ before correction are in bold; values significant after correction are denoted with an asterisk.

Population structure

The finding of significant genetic differences between finetooth sharks in the Atlantic and Gulf is consistent with known life-history differences between finetooth sharks in the two regions

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Table 3. Results of tests of neutrality (Tajima's *D* and Fu's F_S metrics) for finetooth sharks (*C. isodon*) sampled from five localities along the US Atlantic coast and northern Gulf of Mexico

	D	*P-value*	F_S	*P*
SC	NA	NA	NA	NA
AFL	−0.681	0.168	0.725	0.454
GFL	***−1.539***	***0.022***	***−2.716***	***0.003***
AL	−1.233	0.078	***−2.555***	***0.003***
TX	−0.662	0.228	−2.130	0.189
lobal	***−1.472***	***0.031***	***−7.532***	***<0.001***

SC, South Carolina; AFL, northeastern Florida; GFL, northwestern Florida; AL, Alabama; TX, Texas; Global, all samples pooled. *P* values for each metric are indicated; those significant at $\alpha < 0.05$ are bolded and in italics.

Table 4. Estimates of average, long-term effective population size (N_{eLT}), effective number of breeders (N_b), and generational effective size (N_e), based on microsatellites; lower bound (0.025) and upper bound (0.975) are 95% confidence intervals or HDPIs. ∞ is infinity

	0.025	N_{eLT}	**0.975**
ATL	12 600	22 000	37 600
GFL	16 600	28 800	40 600
AL	15 900	28 800	40 400
TX	11 600	22 600	37 800
ANC	218	916	13 700
	0.025	N_b	**0.975**
SC	430	1208	∞
AFL	58	153	1034
GFL	750	∞	∞
AL	193	617	∞
TX	134	647	∞
	0.025	N_e	**0.975**
Global	1690	12 798	∞

ATL (SC and AFL pooled); northwestern Florida (GFL); Alabama (AL); Texas (TX); and hypothetical ancestral population (ANC). Global is all samples pooled.

(Carlson *et al.*, 2003; Drymon *et al.*, 2006) and with tagging studies in which finetooth sharks tagged in one region have not been recovered in the other region (Kohler *et al.*, 1998; NOAA, 2007). In addition, all of the individuals sampled near Cape Canaveral in the winter were assigned to the Atlantic, further indicating little movement of finetooth sharks between the two regions. Limited movement, based on genetic data, between the two regions is now known in a number of coastal-marine fishes, including sharks, which differ in general life history (Gold and Richardson, 1998; Portnoy *et al.*, 2014). Suggestions regarding the origin and maintenance of the genetic discontinuity between the two regions include surface currents in the Florida Straits that could have an effect on larval dispersal or adult movement or both, the absence of suitable habitat along the southern Florida coast, and expansion into US waters by individuals from different glacial refugia (Gold and Richardson, 1998; Portnoy *et al.*, 2014). Genetic studies of large coastal sharks (Keeney *et al.*, 2005; Portnoy *et al.*, 2010), alternatively, have documented considerable gene flow around peninsular Florida, suggesting that increased capability for long-distance migration in these species and reduced predation risk because of their larger size render the barrier around peninsular Florida barrier less effective for these species.

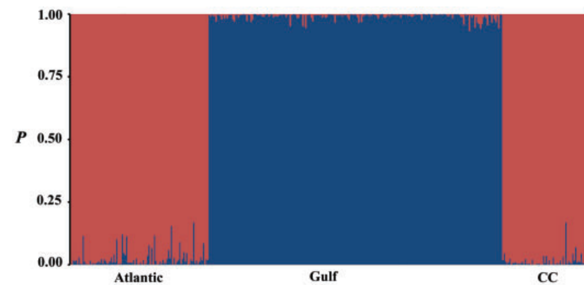

Figure 4. Probability (*P*) of ancestry of individuals in Atlantic or Gulf clusters, each line represents an individual collected either in the Gulf or Atlantic. CC samples are adults and sub adults collected off of Cape Canaveral, Florida, during the winter.

The two samples from the Atlantic were genetically homogeneous and appear to represent a single population. This is consistent with known seasonal migration patterns in the Atlantic where finetooth sharks move north from the east coast of Florida to the Carolinas in the summer and return south to Florida in the winter (Castro, 1993). In contrast, all three samples of finetooth sharks from the Gulf appear to represent genetically distinct populations. The two populations in the eastern Gulf (northern Florida and western Alabama) are roughly on either side of a marine-suture zone (Portnoy and Gold, 2012) where genetic and morphological differences on either side of the zone have been documented for several coastally distributed species, including other sharks (Karlsson *et al.*, 2009; Portnoy *et al.*, 2014), and where inshore fisheries-independent surveys indicate that finetooth sharks are relatively rare (Bethea *et al.*, 2015). Suggestions regarding the origin and maintenance of the genetic discontinuity across the zone include physical and ecological barriers stemming from the outflow of the Mississippi River at different points in the past 2–3 million years, the narrowing of the continental shelf around DeSoto Canyon, the transition from calcareous sediments in the eastern Gulf to terrigenous sediments in the western Gulf, and the periodic, often strong intrusion of the Loop Current into the eastern Gulf (Karlsson *et al.*, 2009; Portnoy and Gold, 2012; Portnoy *et al.*, 2014). Finally, a third population of finetooth sharks in the Gulf was detected off the coast of Texas in the western Gulf. The distance (>700 km) between the most eastern locality sampled off Texas and the locality off western Alabama exceeds the known distance of finetooth shark movement (NOAA, 2007). This, and the known distribution of essential finetooth shark habitat elsewhere in the western Gulf (NOAA, 2016), suggests there may be additional genetic units of finetooth sharks in the Gulf.

Gene flow and regional philopatry

We found no evidence of male-mediated gene flow, a dynamic detected in other sharks by increased divergence in mtDNA relative to nuclear-encoded sequences and concomitant discordance in patterns of population structure between the markers (Portnoy and Heist, 2012). The two marker types (mtDNA and microsatellites) in our study did differ in patterns of genetic divergence; microsatellite allele distributions across the region differed significantly, while mtDNA haplotype distribution did not. This finding could be interpreted to suggest female-mediated gene flow. However, the low level of mtDNA diversity observed in this study

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is extreme, even for sharks which are known to have a slow rate of mtDNA evolution (Martin *et al.*, 1992), suggesting instead that the mtDNA variation in finetooth shark is insufficient to detect genetic heterogeneity.

The genetic heterogeneity in microsatellites detected among finetooth sharks in the Gulf indicates limitations to gene flow in the region. Finetooth shark movement in the Gulf is seasonal (Carlson and Brusher, 1999) and related to migration of both males and females to nursery and mating grounds (Bethea *et al.*, 2015). This movement pattern, the patchy distribution of finetooth shark nursery habitat (NOAA, 2016), the apparent absence of sex-biased gene flow, and the occurrence of mating in the vicinity of nursery areas (Castro, 1993) suggest that both males and females may display regional philopatry to discrete areas. Chapman *et al.* (2015) reviewed much of the data regarding residency, site fidelity, and philopatry in sharks and noted that evidence for regional philopatry came from genetic studies documenting genetic heterogeneity between regions but where individuals were capable of moving between regions. Consistent with this, one individual was sampled off western Alabama but had a higher probability of originating in waters off northern Florida, and one individual tagged off St. Vincent Island on the north Florida coast was recaptured a few miles south of Biloxi Bay, Mississippi (NOAA, 2007). These limited observations indicate that finetooth sharks can move across the barrier separating the two populations in the eastern Gulf. However, as suggested by Knip *et al.* (2010), movement of small coastal sharks may not be related to return to specific sites but rather that population structure may be a function of seasonal migration, the dispersal range of individuals, and geophysical barriers to movement. Further studies addressing this issue in finetooth sharks and other small coastal sharks are clearly warranted.

Genetic demographics

A strong signal of recent expansion was detected using both mtDNA and microsatellites. Tests of neutrality (Tajima's D and Fu's F_S) were negative and significant when samples were combined but not significant in each sample, consistent with a recent, range-wide expansion (Städler *et al.*, 2009). Similarly, a model of rapid expansion from an ancestral gene pool (Scenario 1) was supported by ABC analysis rather than any of the gradual, stepwise models. The timing of expansion was estimated as occurring between 97 and 660 generations in the past. Given an estimated age of maturity between 3.9 and 6.3 years and estimates of longevity between 14 and 18 years (Carlson *et al.*, 2003; Drymon *et al.*, 2006), generation time in finetooth sharks could be up to 14 years, suggesting that expansion of finetooth sharks in US waters occurred less than 9250 years ago. Estimates of average, long-term effective population size (N_{eLT}) for individual samples were greater than 20 000 and more than an order of magnitude greater than the estimate of N_{eLT} for the ancestral population (916), further indicating recent population expansion. The overlap of estimated N_{eLT} for individual samples may indicate that local N_{eLT} cannot be estimated rigorously because of recent time of divergence and that these estimates may approximate the global N_{eLT} (Waples, 2010). The estimate of global N_e (contemporary effective size) was similar in magnitude (12 798).

These findings add to the growing number of studies documenting postglacial range expansions in marine systems (Maggs *et al.*, 2008), including benthic, demersal, and highly migratory species (Chevolot *et al.*, 2006; Hoarau *et al.*, 2007; Portnoy *et al.* 2014). Both the Atlantic and northern Gulf were impacted by climate change associated with glacial recession and by meltwater outflow events that persisted for thousands of years as the Laurentide Ice Sheet receded (Aharon, 2003). The presence of pulses of cold freshwater in the nearshore environment may have made nursery habitat currently used by finetooth sharks inaccessible and may explain why the estimate of the range expansion of finetooth sharks in US waters is more recent than the last glacial maximum ~20 000 years ago. Recent genetic studies of other species in the Atlantic and northern Gulf (Portnoy and Gold, 2012; Portnoy *et al.*, 2014), have reported similar patterns of genetic divergence consistent with recent, postglacial colonization, suggesting a common impact on semi-tropical coastal fishes.

Patterns of divergence in present-day species in genetic markers (microsatellites) presumed to be selectively neutral are related largely to the balance between gene flow and genetic drift over a large number of generations; consequently, patterns of divergence are impacted both by past and present-day dispersal and effective population size (Hauser and Carvalho, 2008). Locations and sizes of glacial refugia and patterns of recolonization may therefore be important drivers of current genetic variation within and between populations (Hewitt, 2004). In finetooth sharks, the ancestral effective size of 916 indicated by ABC analysis was an order of magnitude less than the estimates of average, long-term effective size in extant populations, suggesting that a small number of founding individuals could have colonized nursery habitat in the northern Gulf and Atlantic as it became available during glacial recession. Philopatric behavior and nursery habitat patchiness across the region in past and more recent times may then have reinforced patterns of genetic divergence.

The estimates of the contemporary effective number of breeding finetooth shark individuals (N_b) at each sample locality ranged from 153 to 1208. These estimates, however, should be viewed with caution, in part because upper confidence bounds in all but one sample were infinity, and in part because the samples contained individuals of mixed cohorts. In addition, while N_b can be related to N_e when life history parameters (e.g. lifetime reproductive success, age at maturity, adult life span) are well characterized (Waples *et al.*, 2013), it is difficult to relate the two parameters if aspects of life history are not well known, as is the case with finetooth sharks (NOAA, 2007).

Additional considerations

This study revealed discrete genetic units of finetooth shark in US waters, including three distinct units in the northern Gulf of Mexico. Understanding spatial population structure is important to management of fishery resources as genetically distinct populations within a fishery may possess unique, genetically-based physiological, behavioral, or other traits that promote distinctive differences in life-history characteristics such as growth rates, fecundity, or disease resistance (Stepien, 1995). Failure to understand how a fishery is structured genetically may then lead to over-exploitation and depletion of cryptic populations and loss of unique genetic resources (Hilborn *et al.*, 2003). Samples for this study were taken from areas in the Gulf identified as essential habitat for finetooth sharks. These areas are distributed discontinuously with at least three distinct areas (one large) along the Texas coast and one in the Florida Keys (NOAA, 2016), suggesting that additional genetic units of finetooth sharks may exist

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elsewhere in the Gulf. Finally, this and previous genetic studies of coastal sharks provide further evidence that small coastal sharks have different life histories, patterns of movement, and habitat usage relative to large coastal sharks (Knip *et al*., 2010) and that the traditional model of shark behavioural ecology, based on large coastal sharks (Springer, 1967), may not be appropriate for understanding and conserving small coastal sharks.

Supplementary data

Supplementary material is available at the *ICESJMS* online version of the article.

Acknowledgments

We thank M. Ajemian (Florida Atlantic University), G. Stunz (Harte Research Institute), M. Drymon (Dauphin Island Sea Lab), A. Brown and M. McCallister (University of North Florida), J. Bauman and S. Bauman (fishers), A. Shaw and E. Vinyard (South Carolina Department of Natural Resources), and the many interns and volunteers of the NOAA (National Oceanic and Atmospheric Administration) Fisheries Shark Population Assessment Group for assistance in procuring tissue samples. We also thank M. Giresi and A. Barker (Texas A&M University) for assistance in the laboratory. Finally, we would like to thank W. Stewart Grant and two anonymous reviewers for their helpful comments and suggestions. Work was supported by the National Marine Fisheries Service under Cooperative Research Grants (NA11NMF4540119 and NA12NMF4540080). Funding for the GULFSPAN Survey was provided in part by the Highly Migratory Species Division of the National Marine Fisheries Service, and partial funding for CMH was provided by the Harte Research Institute. This article is publication number 12 of the Marine Genomics Laboratory at Texas A&M University-Corpus Christi, number 106 in the series Genetic Studies in Marine Fishes, and contribution 748 of the South Carolina Marine Resources Center.

Data Accessibility

GenBank accession numbers for mtDNA sequences may be found in Supplementary Table S2 (online Supplementary Materials). Sampling locations, mtDNA haplotypes and microsatellites genotypes may found at DRYAD (doi:10.5061/dryad.99s52) under the file name 'Full data, finetooth sharks'.

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Table S1. Summary statistics for 16 nuclear-encoded microsatellites and a 1077 base-pair sequence of the mitochondrial control region for finetooth sharks (*Carcharhinus isodon*) sampled from five localities along the U.S. Atlantic coast and northern Gulf of Mexico: SC, South Carolina; AFL, northeastern Florida; GFL, northwestern Florida; AL, Alabama; TX, Texas. Microsatellites: *n*, sample size; #A, number of alleles; A_R, allelic richness; H_E, gene diversity (expected heterozygosity), and P_{HW}, probability of conforming to expected Hardy-Weinberg genotypic proportions. MtDNA: *n*, sample size; #H number of haplotypes; *s*, number of informative sites; *h*, nucleon diversity; and π, nucleotide diversity.

Microsat	SC	AFL	GFL	AL	TX
***Cac*03**					
N	99	36	101	79	29
#A	3	3	2	3	3
A_R	2.47	2.00	2.00	2.34	2.00
H_E	0.296	0.201	0.301	0.320	0.408
P_{HW}	1.000	0.353	0.514	0.776	0.639
***Cac*67**					
n	99	36	101	80	28
#A	33	30	35	31	22
A_R	24.02	26.31	24.06	23.65	21.71
H_E	0.960	0.962	0.953	0.958	0.945
P_{HW}	0.989	1.000	0.762	0.884	0.639
***Cis*102**					
n	99	36	101	76	29
#A	3	3	3	4	3
A_R	2.72	2.75	2.61	3.19	3.00
H_E	0.517	0.519	0.516	0.517	0.584
P_{HW}	0.885	0.504	0.050	0.822	0.586
***Cis*107**					
n	99	36	101	80	29
#A	12	8	12	11	10
A_R	9.94	7.92	9.56	9.38	9.86
H_E	0.857	0.810	0.850	0.773	0.826
P_{HW}	0.680	0.078	0.069	0.080	0.955

***Cis*108**					
n	99	36	101	80	29
#A	3	3	4	5	3
A_R	2.273	2.940	3.073	3.237	2.931
H_E	0.423	0.515	0.442	0.431	0.493
P_{HW}	1.000	0.867	0.455	0.718	0.399

***Cis*111**					
n	99	36	101	80	29
#A	5	3	4	5	3
A_R	3.362	2.940	2.715	3.926	3.862
H_E	0.442	0.456	0.509	0.545	0.485
P_{HW}	0.438	0.487	0.501	0.031	0.073

***Cis*121**					
n	99	36	101	80	29
#A	14	3	10	11	4
A_R	8.25	9.19	6.58	8.04	5.86
H_E	0.532	0.527	0.579	0.598	0.417
P_{HW}	0.403	0.205	0.386	0.822	0.282

***Cis*131**					
n	99	36	101	80	29
#A	7	6	6	4	3
A_R	5.125	5.44	4.00	3.34	3.00
H_E	0.696	0.703	0.579	0.598	0.600
P_{HW}	0.670	0.396	0.258	0.459	0.545

***Cis*139**					
n	99	36	101	80	29
#A	10	7	9	9	7
A_R	7.14	6.68	6.83	7.49	6.93
H_E	0.707	0.656	0.682	0.710	0.730
P_{HW}	0.048	0.965	0.499	0.135	0.256

***Cis*149**					
n	99	35	101	78	29

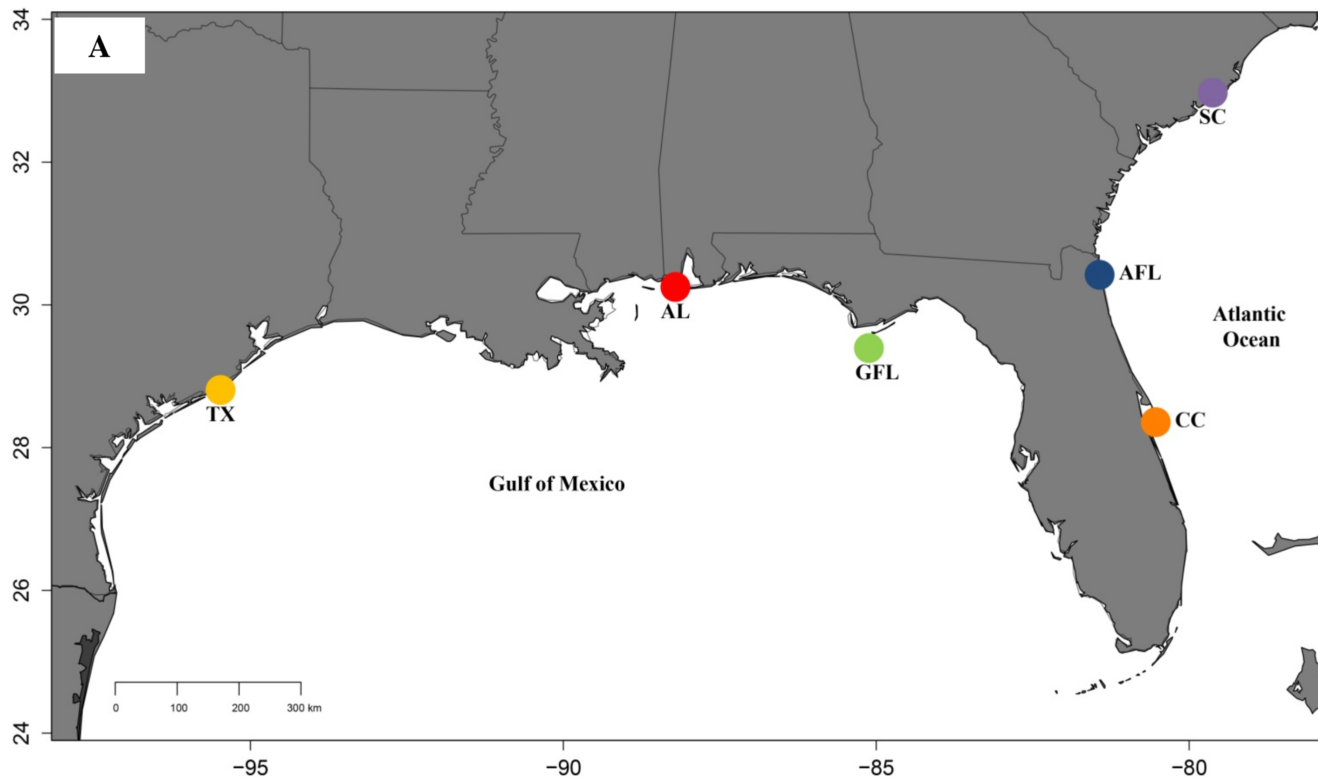
#A	8	9	8	8	7
A_R	6.987	8.304	6.68	6.78	6.93
H_E	0.746	0.702	0.694	0.728	0.784
P_{HW}	0.842	0.613	0069	0.441	0.650
***Cis*157**					
n	99	36	101	80	29
#A	7	5	8	6	4
A_R	5.22	5.00	5.81	5.31	3.93
H_E	0.602	0.675	0.565	0.492	0.575
P_{HW}	0.315	0.398	0.521	0.703	0.210
***Cis*161**					
n	99	35	100	80	27
#A	15	11	15	18	11
A_R	11.12	10.61	12.33	13.02	11.00
H_E	0.838	0.871	0.876	0.892	0.882
P_{HW}	0.825	0.158	0.514	0.282	0.784
***Cis*163**					
n	99	36	101	80	29
#A	41	32	47	41	29
A_R	28.07	27.88	28.12	26.75	27.95
H_E	0.964	0.958	0.956	0.944	0.969
P_{HW}	0.937	0.650	0.024	0.619	0.083
***Cis*168**					
n	99	36	101	80	28
#A	3	2	2	2	3
A_R	2.27	2.00	2.00	2.00	3.00
H_E	0.406	0.440	0.369	0.401	0.434
P_{HW}	0.720	1.000	1.000	1.000	1.000
***Cis*170**					
n	99	36	101	80	29
#A	17	13	20	16	16
A_R	13.77	12.75	14.85	13.76	15.58

H_E	0.887	0.906	0.898	0.885	0.911
P_{HW}	0.102	0.413	0.415	0.369	0.441
***Cis*175**					
n	99	36	101	80	29
#A	18	12	15	17	14
A_R	13.66	11.54	13.31	13.00	13.78
H_E	0.893	0.863	0.868	0.823	0.905
P_{HW}	0.074	0.622	0.408	0.955	0.256
MtDNA					
n	29	24	30	27	23
#H	1	2	4	4	2
s	0	2	3	3	1
h	0	0.159	0.193	0.276	.0166
π	0	0.00030	0.00024	0.00027	0.00015

Table S2. Spatial distribution of mtDNA haplotypes among finetooth sharks (*Carcharhinus isodon*) sampled from five localities along the U.S. Atlantic coast and northern Gulf of Mexico. SC, South Carolina; AFL, northeastern Florida; GFL, northwestern Florida; AL, Alabama; TX, Texas.

Haplotype	**SC**	**AFL**	**GFL**	**AL**	**TX**	**GenBank Accession #**
1	29	24	27	23	21	KU255136
2	-	-	1	-	-	KU255137
3	-	-	1	-	-	KU255138
4	-	-	1	2	2	KU255139
5	-	-	-	1	-	KU255140
6	-	-	-	1	-	KU255141
7	-	2	-	-	-	KU255142

Figure S1. Centroids of sampling localities for finetooth sharks along the U.S. Atlantic coast and northern Gulf of Mexico (A) and spread of sampling effort (B). SC, South Carolina; AFL, northeastern Florida; GFL, northwestern Florida; AL, Alabama; TX, Texas; CC, adults and sub adults sampled on wintering ground off Cape Canaveral, Florida.



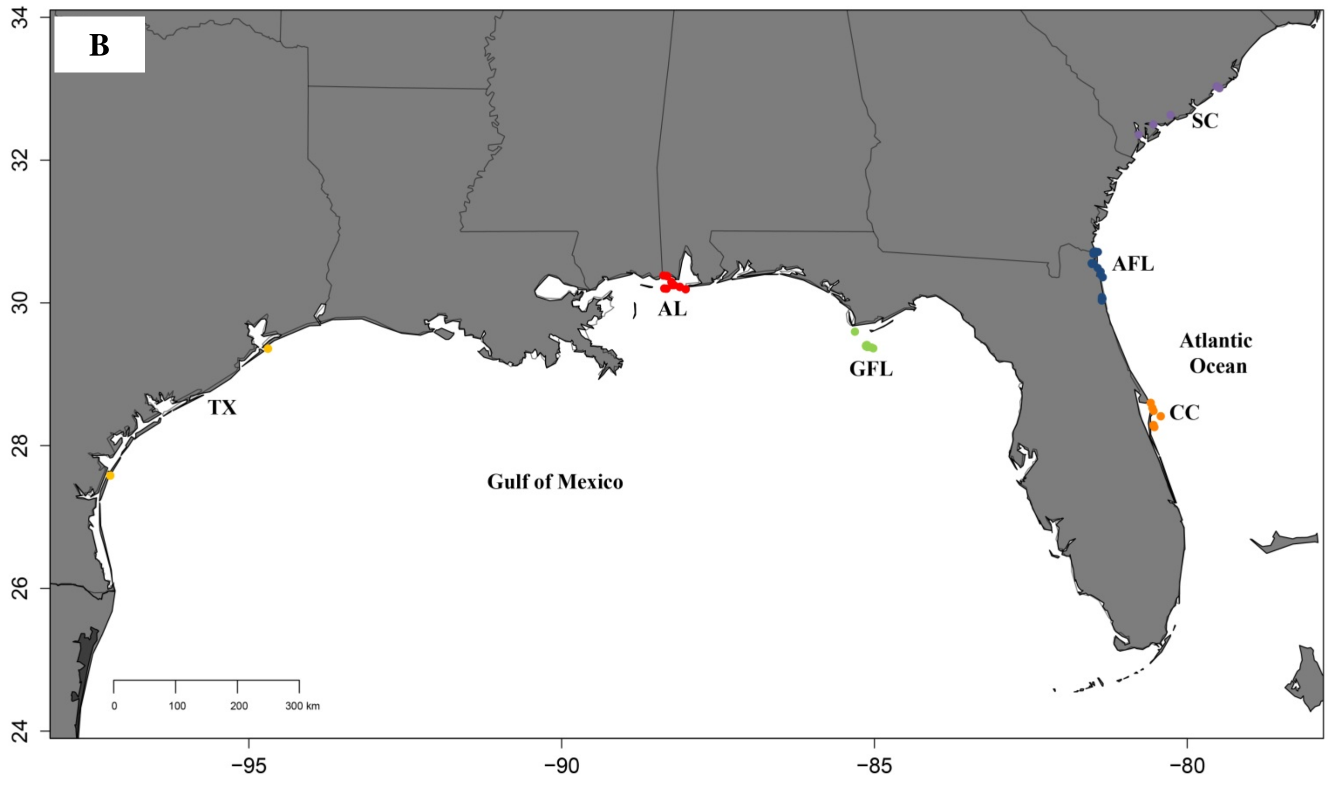
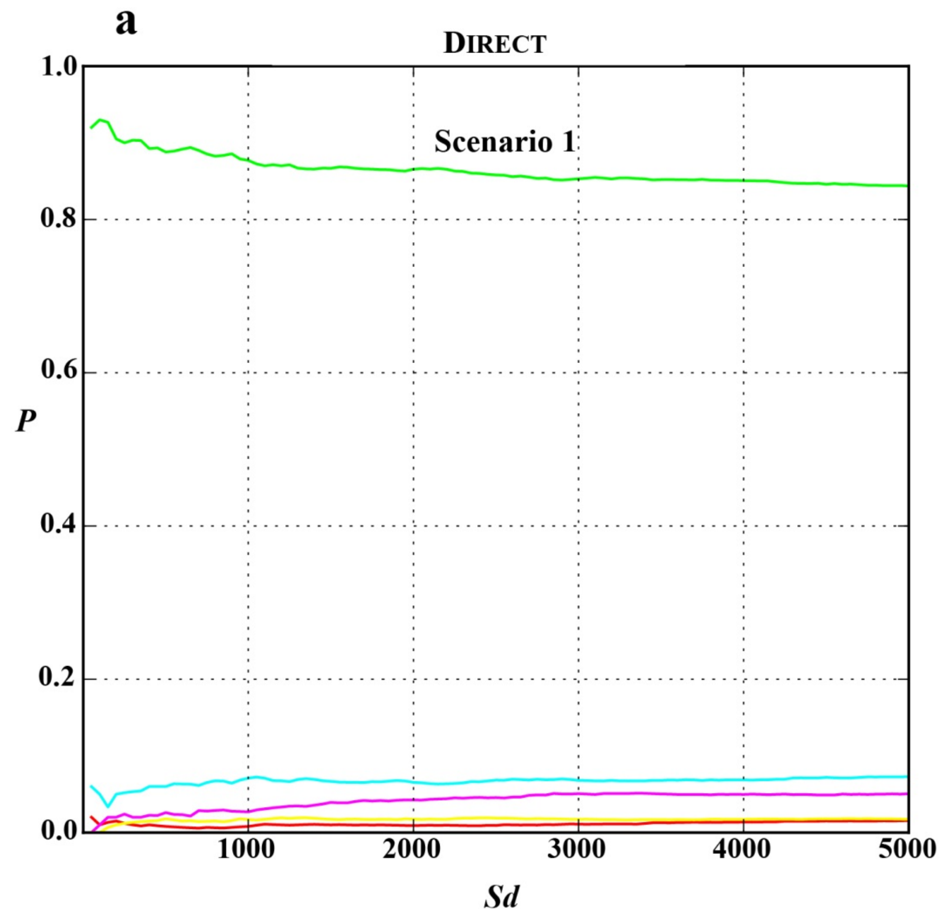

B
34
32
30
28
26
24
-95
-90
-85
-80
SC
AFL
AL
GFL
TX
Atlantic Ocean
CC
Gulf of Mexico
0
100
200
300 km

Figure S2. Results of model testing in an Approximate Bayesian Computation (ABC) framework: (a) direct estimate of posterior probability, (b) logistic regression estimate of posterior probability; ordinate is probability (*P*), abscissa is number of simulated data sets (Sd).



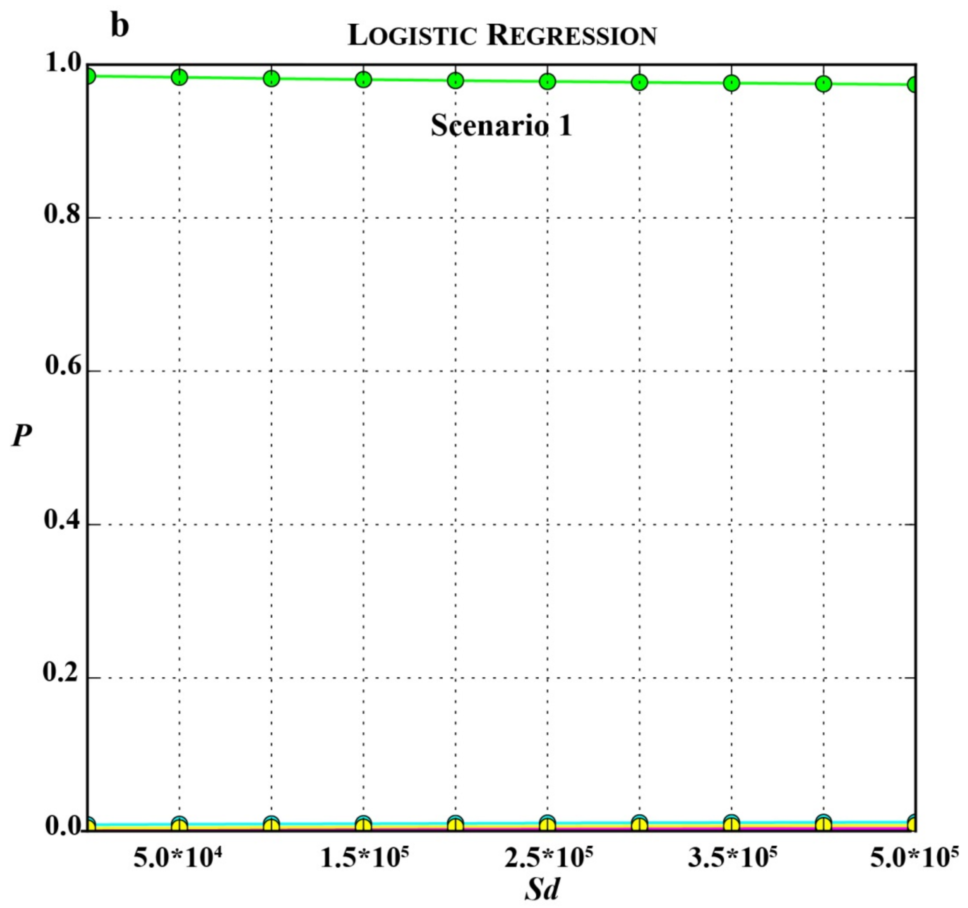

b
LOGISTIC REGRESSION
Scenario 1
1.0
0.8
0.6
0.4
0.2
0.0
P
5.0*10^4
1.5*10^5
2.5*10^5
3.5*10^5
5.0*10^5
Sd

Figure S3. Fit of observed data to model selected, using ABC analysis. Planes of principle components analysis were generated from 40 summary statistics from 10,000 simulations, using prior distributions of historical parameters. Open circles (Prior) are simulated data sets, generated using prior distributions of historical parameters; closed circles (Posterior) are simulated data sets, generated using the posterior distributions of historical parameters from the specified model. Closed yellow circle is observed data.

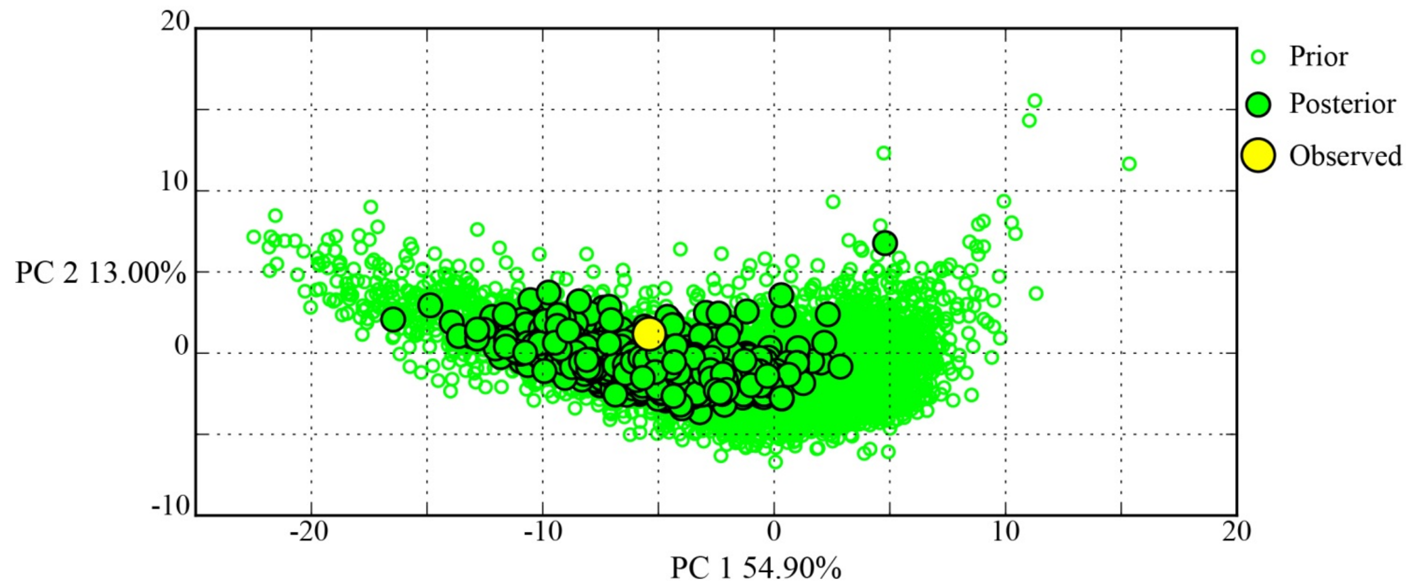


Figure S4. Posterior probability (P) distribution for estimate of time since divergence in generations (t) obtained from the selected scenario (Scenario 1).