FINAL REPORT

Competitive State Wildlife Grant Program

Project Title

Relative Abundance and Trophic Ecology of Two Sympatrically Distributed Sphyrnids, the Scalloped Hammerheads (*Sphyrna lewini*) and the Recently Discovered Carolina Hammerheads (*Sphyrna gilberti*) Within Known Nurseries Off the East Coast of the United States

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Abstract:

All proposed objectives in the proposal were met or exceeded; while there were some minor deviations from proposed objectives, none resulted in failure to complete the proposed objectives. As detailed in the executive summary and the final report below, we created a diagnostic panel of single nucleotide polymorphisms to readily identify scalloped (*Sphyrna lewini*) and Carolina (*S. gilberti*) hammerheads. We characterized the relative abundance, relatedness, stock structure, genetic diversity, effective number of breeders, diet, and trophic ecology for young-of-the-year (YOY) of both species. Carolina hammerheads were found to be most abundant in Bulls Bay, SC, although they were found throughout the southeast U.S., with the exception of the Tolomato River, FL, where only scalloped hammerheads were identified. Temporal differences in parturition may occur between the two species, and scalloped hammerheads are born, on average, 50 mm larger than Carolina hammerheads. Environmental variables (salinity, and dissolved oxygen) had no effect on abundance of YOY scalloped hammerheads but may influence abundance of Carolina hammerheads. There was significant overlap in the diet of YOY scalloped and Carolina hammerheads, indicating no partitioning of food resources in nursery areas. Results from analysis of stable isotopes that reflect maternal diet in YOY hammerheads indicate that adult female Carolina hammerheads likely reside further offshore, and feed at higher trophic levels than adult female scalloped hammerheads. An unexpected result was the finding of hybridization and backcrossing between the two species. Hybridization appears to be unidirectional, and when documented, female Carolina hammerheads had produced offspring sired by male scalloped hammerheads. Overall, the results of this study found that the nursery area in Bulls Bay, SC is critically important to Carolina hammerheads, although other nurseries contribute to the population. In the southeastern U.S., Carolina hammerheads have lower genetic diversity, and half the effective number of breeders as scalloped hammerheads. While this study focused on YOY hammerheads, our results give some insights into potential spatial and/or resource partitioning between adults of the two species. Our results, coupled with documented unidirectional hybridization, indicate that the Carolina hammerheads population is relatively small compared to scalloped hammerheads, and could be much more susceptible to overfishing, or habitat degradation of nursery areas. Future research will be needed to determine aspects of habitat utilization and life history information for juvenile and adult Carolina hammerheads. Based on the results of this research, Carolina hammerheads should be added to the Species of Greatest Conservation Need (SGCN) lists in North Carolina, South Carolina, Georgia and Florida

Executive Summary:

Next-generation sequencing was used to create a panel of 1,401 diagnostic single nucleotide polymorphisms (SNPs) that can be used to reliably distinguish between scalloped and Carolina hammerheads. A total of 578 scalloped hammerheads and 236 Carolina hammerheads were identified using the panel. Carolina hammerheads were found across the sampled range, with abundance highly concentrated in South Carolina. Of all identified Carolina hammerheads, 78.9% were sampled in South Carolina, and 71.3% were sampled within the Bulls Bay nursery specifically. First generation hybrids as well as backcrosses were also found across the sampled range. The identification of backcrosses indicates that hybrids are viable. Hybridization was both unidirectional and sex-biased; most instances of backcrossing involved scalloped hammerheads, and mitochondrial DNA haplotypes showed that the maternal species was a Carolina hammerhead in nearly all cases. Assessment of temporal patterns of Carolina hammerheads abundance relative to scalloped hammerheads abundance in Bulls Bay indicated that Carolina hammerheads abundance increased through the summer, beginning with average relative abundance of 0.453 in May and ending in 0.886 in August. Patterns of relative abundance across years were variable (0.312-0.870), but Carolina hammerheads were more abundant in Bulls Bay than scalloped hammerheads in all years but two.

Both scalloped and Carolina hammerheads young-of-the-year (YOY) arrived in the nursery area in early May, however abundance of Carolina hammerheads increased fourfold in mid-July, and Carolina hammerheads remained in the nursery area for at least a month after all scalloped hammerheads had left the Bulls Bay nursery. This indicates parturition may be prolonged in one or both species, and/or peak parturition may be offset in Carolina hammerheads. Differences in size of YOY were observed in the study, with scalloped hammerheads born ~50 mm larger than Carolina hammerheads, and these size differences were retained throughout the period of nursery usage. Unfortunately, we have insufficient data to determine whether differences in length are retained throughout Carolina hammerheads life-span. Determining if differences in length-at-age exist are important as many stock assessment inputs are based on length-based parameters (length-at-maturity, growth (von Bertalanffy) parameters, natural mortality estimates).

Genetic data was used to estimate relatedness of individuals within and between nurseries. A total of fourteen full-sibling and forty-thee half-sibling relationships were found among YOY scalloped hammerheads, and seven full-sibling and sixteen half-sibling relationships among YOY Carolina hammerheads. Patterns of relatedness across different sampling locations indicate scalloped hammerheads display some regional site fidelity but 45% of scalloped hammerheads half-siblings were detected across nursery sites, indicating straying between nursery occurs regularly. Carolina hammerheads exhibited a higher degree of fidelity to nursery sites, with only 30.5% of half-sibling relationships detected across different nursery sites. One parent-offspring relationships was identified between an adult male scalloped hammerhead sampled in 2019 and a YOY sampled in 2013, both off of South Carolina, suggesting males may exhibit some philopatric behavior.

Preliminary assessment of stock structure was performed for scalloped hammerheads on a dataset with randomly sampled siblings included as well as a dataset of completely unrelated individuals. Global F_{ST} values generated from single-level analyses of molecular variance (AMOVA) were significantly different from zero ($P < 0.001$) with both siblings included and removed, however no significant differences were found in pairwise comparisons of nurseries.

Discriminant analysis of principal components (DAPC) also did not support differentiation between nurseries.

Minimum estimates of the effective number of breeders (N_b) for scalloped hammerheads ranged from 492.7 (Cumberland Island, GA) to 1009.1 (Cape Canaveral, FL), and point estimates ranged from 1197.4 (Tolomato River, FL) to 32022.1 (Cumberland Island, GA). Minimum estimates of N_b for Carolina hammerheads ranged from 63.8 (Cape Canaveral, FL) to 1426.3 (Bulls Bay, SC), and point estimates ranged from 170.5 (Cape Canaveral, FL) to 1373.1 (Bulls Bay, SC). Point estimates of N_b for the overall northwest Atlantic region suggest scalloped hammerheads have more than double the effective number of breeders relative to Carolina hammerheads (4423.0 and 2114.0, respectively). Genetic diversity was estimated for each nursery in the form of observed heterozygosity (H_O), expected heterozygosity (H_S), and rarefied allelic richness (A_R). Scalloped hammerheads A_R in Cumberland Island was significantly greater than every other nursery ($P < 0.0001$), while all other nurseries were equal. H_o for scalloped hammerheads in Bulls Bay was significantly higher than all other nurseries, ($P = 0.0001$-0.0138) and Cumberland Island had less than Cape Canaveral and Tolomato River ($P < 0.0001$). No differences in H_S were observed between nurseries. Carolina hammerheads were less diverse than scalloped hammerheads by all measures of genetic diversity. Low diversity observed in Carolina hammerheads is consistent with low estimates of the effective number of breeders and suggests a reduced long-term effective size relative to scalloped hammerheads.

A total of 428 stomachs were collected from moribund or sacrificed Carolina (n=201), scalloped (n=181) and hybrid (n=46) hammerheads from estuaries and nearshore waters in North Carolina, South Carolina, Georgia and Florida to assess the diet of young-of-year (YOY) hammerheads along the southeast U.S. coast. The majority of the samples were collected in Bulls Bay, followed by nearshore GA waters, Cape Canaveral, nearshore SC and FL waters, Tolomato River and then NC. Muscle, liver, whole blood and plasma samples were taken from a subsample of the sharks from which stomachs were collected to perform stable isotope analysis ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$). Stable isotopes were used to investigate trophic ecology of YOY hammerheads (mainly via plasma samples due to having the shortest turnover rate of the sampled tissues) and to infer spatial and dietary patterns of mature females (mainly via muscle samples due to having the longest turnover rate). Dietary indices (%N, %W, %O, and %IRI) were calculated by species (Carolina, scalloped and hybrid; all locations combined) and then by region (all species combined per state).

YOY hammerheads can be considered generalist feeders along the southeast coast, and there was significant dietary overlap and niche overlap between hybrids, scalloped and Carolina hammerheads, with teleosts contributing the most to their diet, followed by crustaceans and then molluscs. For hybrids and the two species, star drum, penaeid shrimps and squid were the most important identified teleost, crustacean and mollusc, respectively. With both a large diet and niche overlap, there seems to be little habitat partitioning in estuarine and coastal waters between species, which could indicate increased competition for prey. According to isotopic signatures of plasma, Carolina and scalloped hammerheads may be foraging on different specific prey types or items across months, which may alleviate some competition through temporal resource partitioning. Hammerheads (species combined) from SC had more prey taxa in their diet than GA and FL, and the niche width of hammerheads decreases from SC to FL, which indicates that SC, and Bulls Bay in particular, may have more available resources (either dietary or habitat) for young hammerheads compared to the other states. The YOY hammerheads' relative condition remained consistent throughout their growth (as determined by mean condition by maturity stage

and by month caught) despite loss of their maternal provisions as they grew (as seen by an initial decrease in mean hepatosomotic index values across months and maturity stage), indicating they are foraging successfully.

Stable isotopic signatures from hammerheads caught in July, August and later reflect the diet of the YOY sharks, while the signatures from sharks caught in April-June have a maternal influence from provisioning, thereby reflecting their moms' signatures rather than their own diet. Carbon isotopic values provide information about basal resources, with relatively less enriched (more negative) values reflecting either 1) estuarine or offshore waters as compared to nearshore and coastal waters, or 2) pelagic foods web compared as compared benthic food webs. Nitrogen values infer relative trophic level. Trophic position was calculated using stable isotopes as well as from stomach contents, and the species have similar trophic levels as young sharks. However, looking at muscle tissue and isotopic signatures from sharks caught in April-June (which infer mature female trophic information), mature Carolina hammerheads appear to feed at a higher trophic level than scalloped hammerheads. The isotopic signatures from sharks caught in the later half of the summer reflect the stomach content results of a shared diet between YOY species in estuarine habitats, as indicated by a similar range of $\delta^{13}C$ values for Carolina and scalloped hammerheads. There is a significant difference between $\delta^{13}C$ values of the early-caught Carolina and scalloped hammerheads, with Carolina hammerheads having less enriched $\delta^{13}C$ compared to scalloped hammerheads. This indicates that the mature females of each species may be partitioning resources, and the Carolina hammerheads may be inhabiting more offshore waters or feeding more often from the pelagic food web compared to the scalloped hammerheads.

Objectives Synopsis:

The experimental objectives of the project were to: (1) establish baseline data pertaining to the relative abundance of two species of Sphyrnid sharks – scalloped and Carolina hammerheads – which are indistinguishable using external morphology using next-generation sequencing technology; (2) estimate the effective number of breeders and minimum number of female breeders in each nursery for each species; (3) investigate temporal and spatial utilization of nurseries by each species; and (4) determine diet, feeding ecology, trophic relationships and relative condition of each species. Conservation and management objectives included: (i) dissemination of project results to managers allowing the scalloped hammerheads species description to be updated and the Carolina hammerheads to be added to the Species of Greatest Conservation Need (SGCN) lists if warranted; (ii) dissemination of results via presentations (technical and general audiences) and technical and peer reviewed publications; and (iii) providing baseline information to managers for assessment and determination of population statuses. Specific objectives and a proposed timeline are listed below.

Objective 1:

Obtain tissues (fin clips) from ~100 young of the year (YOY) hammerheads per year from each of three known nursery areas: (i) Bulls Bay, SC, (ii) nearshore waters off Cape Canaveral, FL east coast, and (iii) Tolomato River, FL east coast. Survey efforts in the identified estuarine nurseries (Bulls Bay and Tolomato River) were expanded to increase spatial and temporal coverage, allowing individual nurseries to be further characterized, especially with respect to possible habitat partitioning. Additional surveys were conducted in estuarine waters of Georgia to identify potential new nursery areas. Tissues (fin clips) were collected from any GA hammerheads and included in genetic analyses. Over the duration of the project, we anticipated collecting a total of 600 to 800 fin clip samples. (year 1 to year 3).

Accomplishments Objective 1:

Tissue Collection

A total of 1,535 fin clips were collected from hammerheads (hereafter, hammerheads refers to scalloped and Carolina hammerheads; when scalloped or Carolina hammerheads is used, that indicates a genetically identified individual) in known nursery areas, and nearshore waters off the southeastern U.S. (Fig. 1). Fin clips were collected primarily by the project principal investigators, collaborators, and cooperators; however, samples were also collected from fishery independent surveys (NMFS Longline Survey, SEAMAP bottom trawl survey) and fishery dependent sources (NMFS longline observer program, GA SeaGrant trawl bycatch research). Of these, 938 samples were ultimately sequenced for species identification (Table 1). As proposed, the majority of these samples (877) were from young-of-year (YOY) hammerheads captured in or adjacent to nursery areas (Fig. 2, Table 2.). The majority of samples analyzed were from Bulls Bay, SC and the two nursery areas in Florida (Tolomato River, and Cape Canaveral). Despite the use of new gear and expanded sampling efforts, we were unable to obtain as many samples from estuarine waters off Georgia. The samples that were collected were from bycatch in commercial shrimp trawls as part of a study conducted by Georgia Southern University

personnel analyzing bycatch in this fishery. The large numbers of hammerheads documented as bycatch by this study indicate that the nearshore waters off Georgia are likely a nursery area for hammerheads. This would also explain why expanded sampling in estuarine waters by GADNR failed to encounter many hammerheads.

Expanded Sampling

Funding from the study was used to expand sampling efforts for three areas: the Tolomato River in Florida, estuarine waters off Georgia, and Bulls Bay, SC. From 2016 to 2019 a total of 272 longline sets (avg of 68 sets/year) were made in the Tolomato River by University of North Florida personnel. This was an increase of 30 sets/year over prior survey efforts. Longlines were 305 m long with 50 gangions (0.5 m 72 kg. test monofilament, 12/0 non-stainless circle hook) baited with Atlantic mackerel (*Scomber scombrous*). Gear was soaked for a maximum of 30 minutes to maximize survival of catch. These efforts resulted in the catch of 242 hammerheads, mostly YOY (n=195), with some juveniles encountered (n=34). The Tolomato River appears to be unique as it is the only documented nursery area found inshore (bound by land); further, hammerheads are the most frequently encountered species on longline sets in that location, whereas other species are more abundant in other sampled areas (Table 3).

Funding to Georgia Department of Natural Resources was used to expand their Cooperative Atlantic States Shark Pupping and Nursery Survey (COASTSPAN) by adding gillnet sampling to the ongoing hand-deployed longline survey. Gillnets used for the expanded sampling were 91.4m long, 3.7m tall, and constructed of #177 monofilament with a 10.2 cm stretched mesh. Fixed locations sampled for the COASTSPAN longline survey as well as experimental locations were used to sample for hammerheads (Fig. 3). Nets were set in depths of ~3.7m (net height) parallel to currents to avoid potential difficulties in managing the net due to the large tidal range (~2m) and current velocities experienced in the GA sounds. Gear was soaked for 20 minutes prior to retrieval to minimize mortality of captured individuals. From June to September (2016 and 2017) a total of 190 gill net sets were conducted. All elasmobranchs were removed from the net, identified to species and measured. During two years of expanded gillnet sampling a total of 305 sharks were captured by GADNR (5 species). While set in close proximity, the two gears (gillnets and longlines) captured a different species composition; longlines generally caught more Atlantic sharpnose (*Rhizoprionodon terraenovae*) and sandbar sharks (*Carcharhinus plumbeus*) and less bonnetheads (*S. tiburo*), blacktip sharks (*C. limbatus*) and finetooth sharks (*C. isodon*) than gillnets (Fig. 4). Catches of hammerheads were low in both gillnets and longlines (n=4, gillnets; n=5 longlines). Total catches by gear, and life stage are reported in Table 4. A masters thesis titled "Survey Gear Comparisons and Shark Nursery Habitat Use in Southeast Georgia Estuaries" fully details survey methods and results and is available at (https://digitalcommons.unf.edu/etd/731/).

While sampling by GADNR was unsuccessful at capturing hammerheads, a study investigating bycatch of elasmobranchs in commercial shrimp otter trawls conducted by Georgia Southern University found high bycatch of YOY hammerheads in the trawls. Georgia Southern University staff took fin clips and retained whole carcasses for use in this study. While we are unable to compare catch rates of hammerheads due to differing gear types (trawl/versus gillnet), based on the prevalence of hammerheads in the bycatch, the nearshore waters off of Georgia (particularly off of Cumberland Island), are likely a nursery area for hammerheads. Future research should investigate available data sources to confirm the presence of this nursery area.

The South Carolina Department of Natural Resources (SCDNR) initiated a new stratified random gillnet survey in Bulls Bay, SC to attempt to determine if habitat or resource partitioning was occurring between YOY scalloped and Carolina hammerheads. The estuarine and nearshore waters of Bulls Bay were stratified by depth and region and locations were assigned to all waters that could be sampled by gillnet (depths of 1-4 m). The Bay was divided into three regions to ensure randomly selected sites covered the bay. This resulted in a total of 214 sampling locations. Sites were randomly selected monthly, with the goal of sampling 6 sites/bay region (18 sets/month). Sampling was conducted from August 2016, to July 2018 and the bay was sampled from April-September when hammerheads were present. Gillnets were 100m long with a depth of 3.7m tall and constructed of #177 monofilament with a 10.2cm stretched mesh (same mesh size, and depth as GADNR gillnets). Sets were allowed to soak for 30 minutes prior to retrieval. All catch was measured and environmental water quality (salinity, water temperature, and secchi depth), depth, and latitude and longitude were recorded for each location. Whenever possible, random gillnet sets were conducted on the same days as ongoing SCDNR COASTSPAN sampling at an index site in Bulls Bay (Fig. 5). This site was sampled twice a month using a 231m gillnet (mesh size, and depth were the same as the random gillnet above). This site has been sampled twice a month (May-September) from 1998-present with an average of ten sets per month.

Over the course of the study, a total of 202 random gillnet sets were made in 124 locations (Fig. 5) covering most of the habitat that could be sampled by gillnet in Bulls Bay. This effort resulted in the capture of 717 sharks of 9 species (Table 5). Finetooth sharks were the most encountered species followed by Atlantic sharpnose sharks. Catches of hammerheads sharks were lower than expected with hammerheads only encountered at 19 of the 124 sites sampled (Fig. 6). Catches of hammerheads were lowest in southern Bulls Bay and highest in the northern part of the bay. Interestingly, catches were highest at sites closest to the location of the SCDNR COASTSPAN gillnet index station. The reasons for this are unknown, however it is likely due to the proximity to the largest creek in Bulls Bay (Five Fathom Creek). Over the same period as random gillnet sampling, the large gillnet sampling at the index station resulted in the capture of 158 hammerheads.

Significant Deviations:

There were no significant deviations. While additional sampling efforts in GA and SC were unsuccessful in catching large numbers of hammerheads, more than enough samples were obtained to allow successful completion of the other objectives.

Objective 2:

Generate reduced-representation libraries for outsourced Illumina (next-generation) sequencing in order to generate a highly replicable sample of many hundreds to thousands of polymorphic (variable) genetic markers located randomly across the genome of both species (year 1 to year 3).

Accomplishments Objective 2:

Bioinformatics and Species Identification

Genomic DNA was extracted using a Mag-Bind® Blood & Tissue DNA Kit (Omega Bio-Tek), and preparation of ddRAD libraries followed methods described in (Barker *et al.*, 2019). Following sequencing, individuals were demultiplexed using the script *process_radtags* (Catchen *et al.*, 2013), and the program *dDocent* (Puritz *et al.*, 2014) was used for *de novo* reference construction, read mapping and SNP calling. The reference was constructed from twenty-two individuals (17 scalloped hammerheads, 3 Carolina hammerheads, and 2 great hammerheads) sequenced on a paired-end run on an Illumina MiSeq sequencer with initial species identifications based on mitochondrial control region (mtCR) haplotypes. The twenty-two individuals used to construct the reference were subsequently screened for SNPs that could be used to distinguish scalloped, Carolina, and great hammerheads. Raw variants were filtered with VCFTools (Danecek *et al.*, 2011) for a minimum quality score of 20 and mean minimum depth of 10. Indels and sites with missing data were removed, and the dataset was thinned to retain only one SNP per contig. Two panels of diagnostic SNPs were identified, the first to distinguish great hammerheads from scalloped and Carolina hammerheads (panel 1) and the second to distinguish scalloped hammerheads from Carolina hammerheads (panel 2). Panel 1 was identified by calculating allele frequencies in GenoDive (Meirmans & Van Tienderen, 2004) and selecting SNPs that were completely fixed between great hammerheads and scalloped and Carolina hammerheads (grouped together). To identify panel 2, great hammerheads were removed from the dataset and allele frequencies were recalculated to identify SNPs that were completely fixed between scalloped and Carolina hammerheads. A total of 2,695 diagnostic SNPs were identified for panel 1 and 1,491 for panel 2.

Due to sequencing variation within and across runs, individuals varied in the number of diagnostics SNPs that were genotyped. Additionally, due to the small number of Carolina and great hammerheads used to identify diagnostic SNPs, individual variation, and potential species admixture, it is expected that at least some loci won't be completely fixed in all individuals. To determine the minimum number of diagnostic SNPs an individual must be genotyped at for accurate species identification, data from a subset of 127 previously identified individuals were resampled. For each panel of diagnostic SNPs, random subsets of loci of a range in numbers were selected (panel 1: 5-2000 loci; panel 2: 5-1200 loci). Individuals were identified again using the subsets of loci and compared to the original species determinations when all loci were used. This procedure was repeated for 1000 iterations, and the average number of correct identifications for each individual with each subset of loci was determined.

The remaining individuals were sequenced on eleven lanes of an Illumina HiSeq 4000 DNA sequencer. To determine species identity of each individual, *dDocent* was used to map reads and call SNPs. Raw variants were filtered to retain only diagnostic SNPs using VCFTools. Individuals were first identified as a great hammerheads, scalloped/Carolina hammerheads, or undetermined using a custom python script to compare genotypes to panel 1. Individuals identified as a great hammerheads or undetermined were removed from the dataset, and the remaining individuals were identified as a scalloped hammerheads, Carolina hammerheads or undetermined by comparing genotypes to panel 2 using a custom python script. A match of 95% to one species was required for positive species identification, and if an individual did not meet this threshold for any one species it was classified as undetermined. Individuals that were not genotyped at a minimum of 300 diagnostic SNPs were also classified as undetermined. Hybrids

were classified as F1 hybrids or backcrosses using NewHybrids (Anderson & Thompson, 2002) following methods described in Barker *et al*., (2019). Mitochondrial DNA haplotypes were assessed to determine the maternal species in a subset of hybrids as described in Barker *et al*., (2019). Due to issues in the fragment size selection step of ddRAD library preparation that led to the selection of fragments that were smaller than desired, one sequencing run could not be mapped to the *de novo* reference, and genotypes could not be called at the diagnostic loci. NewHybrids was used to determine species identity for pure species and hybrids for individuals from this sequencing run. Species identifications made with NewHybrids were validated by comparing to replicate individuals that were sequenced in other runs and identified with the diagnostic panel.

Significant Deviations Objective 2:

There were no significant deviations between the work proposed and the work completed. The presence of young-of-year great hammerheads (Barker *et al*., 2017) as well as documented hybridization between Carolina and scalloped hammerheads (Barker *et al*., 2019) was unexpected, and led to altering some approaches downstream; however, did not affect completion of the objective.

Objective 3:

Perform genetic analysis to accomplish the following objectives: (a) calculate relative abundance of each species in each sampled nursery area based on genetic identity, (b) develop a baseline estimate of genetic diversity for each species-nursery area combination for use in genetic monitoring, (c) detect parent-offspring relationships, (d) estimate the effective number of breeders and minimum number of female breeders in each sampled nursery, (e) provide preliminary data that may be useful for later stock structure analyses (both species) in the US. Since scalloped and Carolina Hammerheads have different numbers of vertebrae, vertebral counts will also be conducted to confirm that the diagnostic morphologic character and genetic characters align (year 1 to year 3).

Accomplishments Objective 3:

Objective 3 Methods

Following genetic species identification, scalloped and Carolina hammerheads were separated. All bioinformatic filtering and genetic analyses were conducted on each species separately. The *dDocent* pipeline was used to map individuals to species-specific references and call SNPs, and raw variants were filtered using VCFTools (Danecek *et al.*, 2011). Individuals with greater than 25% missing data were removed from the dataset. Sites with a sequence quality score less than 20 and genotypes with a quality score less than 30 were removed. Loci were filtered for a genotype call rate of 0.90, a minimum allele count of 3, a minimum depth of 5, a mean minimum depth of 15, and maximum depth of 200. Indels were removed and sites were filtered for mapping quality ratio, quality to depth ratio, allele balance, strand bias, and properly paired status. Loci with more than 10% (scalloped hammerheads) or 50% (Carolina hammerheads) missing data in any single sampling region were removed from the dataset, as well as loci with 15% (scalloped hammerheads) or 25% (Carolina hammerheads) missing data

within any sequencing library. Inbreeding coefficients (F_{IS}) were assessed to identify and remove problematic individuals. Low values of F_{IS} can be an indicator of sample contamination, while high F_{IS} suggests a high rate of false homozygotes. Carolina hammerheads with F_{IS} less than -0.25 and greater than 0.25 were removed. Scalloped hammerheads were removed if they had an F_{IS} value less than -0.5 or had both an F_{IS} value greater than 0.25 and more than 10% missing data. Library effects were evaluated by performing a PCA. If individuals grouped by sequencing run, the loci that contributed most to this pattern were removed. This was repeated until there was no longer any clear grouping by sequencing run. Finally, genotypes were phased into multi-allelic SNP-containing loci (hereafter loci) with the program rad_haplotyper to account for physical linkage as well as identify paralogous loci (Willis *et al.*, 2017). The final dataset of scalloped hammerheads contained 5,214 loci and 457 individuals. The final dataset of Carolina hammerheads contained 1,903 loci and 204 individuals. Data quality filtering was more stringent for conservation/population genetic analysis than what was required for species identification, thus fewer individuals were included in these analyses than analyses used to estimate relative abundance.

Relatedness

Relatedness coefficients and 95% confidence intervals were estimated using methods described in Wang 2002 (scalloped hammerheads) and the triadic likelihood method (Wang, 2007; Carolina hammerheads) as implemented in the R package *related* (Pew *et al.*, 2015). Pairs of individuals with relatedness coefficients > 0.4 were assumed to be parent and offspring or full siblings, and pairs of individuals with relatedness coefficients of 0.20-0.40 were assumed to be half siblings. Siblings were considered nonrandomly sampled if they were captured on the same day in the same location, and one individual from each nonrandomly sampled pair was removed for subsequent analyses.

Population Genetics Analyses

Carolina hammerheads were only commonly found in one location; thus population genetic analyses were performed for scalloped hammerheads only. Large juveniles (>1000 mm total length) and adults were removed from the dataset prior to analysis to mitigate the confounding effects of highly mobile individuals on the results of population genetic analyses.

A discriminant analysis of principal components (DAPC, Jombart *et al.*, 2010) as implemented in the R package Adegenet (Jombart, 2008) was used to identify genetic clusters. First, a *k*-means clustering approach was used to assign individuals into k = 2-4 groups. Next, two additional DAPC were conducted with group designations assigned a priori with individuals grouped by broad sampling region (South Carolina, Georgia, Northern FL, central FL) and by nurseries that had at least 20 individuals sampled (Bulls Bay, Cape Canaveral, Cumberland Island, Tolomato River). For each DAPC, a cross-validation analysis was conducted to ensure data was not over-fitted and determine the optimal number of principal components to retain for analysis.

To test for genetic differentiation among geographic samples, a single-level, locus-by-locus analysis of molecular variance (AMOVA) was conducted using Arlequin (Excoffier & Lischer, 2010). Significance of each test was evaluated using 1,000 permutations. Arlequin was also used to estimate pairwise F_{ST} between sampling regions and between nurseries, with

significance assessed as above and *p*-values adjusted for multiple comparisons using a false discovery rate procedure (Benjamini & Hochberg, 1995). F_{ST} based analyses were conducted on two datasets: one with nonrandomly sampled siblings removed, and one with all siblings removed.

Within Nursery Diversity

The effective number of breeders (N_b) was estimated for each nursery using a data set containing YOY and small juveniles and the linkage disequilibrium method (Hill, 1981; Waples, 2006; Waples & Do, 2010) as implemented in the program NeEstimator V2.1 (Do *et al.*, 2014). Nurseries with a minimum sample size of 20 were included, and one individual from each nonrandomly sampled sibling pair was removed prior to analysis. Alleles with a frequency of <0.02 were excluded from analysis, and 95% confidence intervals were generated using the jackknife method. An estimate of N_b for the overall NW Atlantic region was also calculated. The minimum number of female breeders (N_{mf}) for each nursery was estimated by adding the number of maternally related families to the number of unrelated YOY and juvenile individuals. The R package hierfstat (Goudet, 2005) was used to estimate observed heterozygosity (H_O), gene diversity (H_S) and allelic richness (A_R) for each scalloped hammerheads nursery. A Friedman test was used to determine if differences in diversity estimates exist among nurseries, and post-hoc Wilcoxon tests were used for pairwise nursery comparisons.

Objective 3 Results:

Species Identification and Abundance

Sequences were obtained from 938 individuals sampled in North Carolina (NC), South Carolina (SC), Georgia (GA), northern Florida (NFL), and central Florida (CFL, Table 1, Fig. 1). Sufficient sample sizes were obtained in four areas: Bulls Bay, SC (BB), Cumberland Island, GA (CI), Tolomato River, FL (TR), and Cape Canaveral, FL (CC, Table 2, Fig. 2); any nursery-specific analyses included these samples only. The nearshore waters off of Georgia have not been previously described as a nursery area, however the high number of YOY hammerheads encountered by trawlers in this area, indicates these nearshore waters likely serve as nursery habitat. Initial clustering using all loci identified three groups in the data that represented great hammerhead, Carolina hammerhead and scalloped hammerhead (Fig. 7) Therefore, two panels of diagnostic SNPs were identified, the first to distinguish great hammerheads from scalloped and Carolina hammerheads (panel 1) and the second to distinguish scalloped hammerheads from Carolina hammerheads (panel 2). Panel 1 was identified by calculating allele frequencies in GenoDive (Meirmans & Van Tienderen, 2004) and selecting SNPs that were completely fixed between great hammerheads and scalloped and Carolina hammerheads (grouped together). To identify panel 2, great hammerheads were removed from the dataset and allele frequencies were recalculated to identify SNPs that were completely fixed between scalloped and Carolina hammerheads. A total of 2,695 diagnostic SNPs were identified for panel 1 and 1,491 for panel 2. A total of 817 individuals were genetically identified with the panels of diagnostic SNPs (scalloped hammerheads = 578, Carolina hammerheads = 236, great hammerheads = 3; Table 6), 83 individuals were assigned into a hybrid category by NewHybrids (F1 hybrid = 37, scalloped hammerheads backcross = 38, Carolina hammerheads backcross = 8). NewHybrids also identified an additional 15 Carolina hammerheads and 13 scalloped hammerheads that could not

be identified (with 95% confidence) using the diagnostic panel. Further, details of hybrid analysis can be found in Barker et al. (2019). These individuals were included in species totals for relative abundance but were not included in genetic analyses. Both scalloped and Carolina hammerheads were found along the coast of the eastern U.S. from North Carolina to central Florida (Fig. 8), but Carolina hammerheads abundance was heavily concentrated in South Carolina. Of the Carolina hammerheads identified, 78.9% were sampled in South Carolina, with 71.3% sampled within the Bulls Bay nursery. Although Carolina hammerheads were found in other areas in the northern Florida region, as well as within nurseries both north and south of the area (Fig.8), none were identified in the Tolomato River nursery (Table 7).

Patterns of Carolina hammerheads abundance relative to scalloped hammerheads in Bulls Bay were examined across years and across months. On an annual basis, Carolina hammerheads were more abundant than scalloped hammerheads in all years but two, however the relative proportion of Carolina to scalloped hammerheads was variable, ranging from 31.2% in 2019 to 87% in 2012 (Table 8). Assessment of temporal patterns of Carolina hammerheads abundance relative to scalloped hammerheads in Bulls Bay from May to August indicated that Carolina hammerheads were relatively least abundant early in the season and increased through the summer, starting at an average of 0.453 in May and ending at 0.886 in August (Table 9).

Relatedness

A total of 14 full sibling relationships were identified in scalloped hammerheads, with the most found in the Cape Canaveral nursery (Table 10). Forty-three scalloped hammerheads half sibling relationships were detected, again with the most in Cape Canaveral. Of the half sibling pairs, three were YOY sampled in the same nursery in the same year, suggesting they belong to multiply sired litters. Multiple paternity has been previously documented in scalloped hammerheads, with observed rates of 46%-100% of the litters attributed to multiple sires (Rossouw *et al.*, 2016; Green *et al.*, 2017). The remaining pairs were sampled across different years, with 45% sampled in different nurseries, suggesting that females (or pups) stray between nearby nursery sites somewhat regularly. Seven full sibling and one half sibling pairs were nonrandomly sampled (same nursery on the same day), and one individual from each pair was removed prior to further analysis. Relatedness analysis of Carolina hammerheads revealed seven full sibling pairs and sixteen half sibling pairs, with the majority found in Bulls Bay (Table 11). Based on date and location of sampling, three half sibling pairs appear to belong to multiply sired litters. Multiple paternity has not yet been examined in Carolina hammerheads, but it is likely to occur given the high prevalence of genetic polyandry in elasmobranchs. For both scalloped and Carolina hammerheads, all multiply sired litters were found in Cape Canaveral. A higher degree of fidelity to nursery sites was observed in Carolina hammerheads, with 30.5% of relationships detected across different nursery sites. Four full and two half sibling pairs were determined to be nonrandomly sampled, and one individual from each pair removed prior to further analysis.

One of the scalloped hammerheads full sibling pairs was between two large mature males (total length 2620-2800 mm), both sampled near St. Helena Sound, SC in 2019. Further, a parent-offspring relationship was found between one of these males and a YOY sampled in Bulls Bay in 2013. Therefore, an avuncular relationship was supported between the same YOY and the other adult male sibling. The identification of adult male siblings and one of their offspring in a similar area suggests there may be some degree of male philopatry as well.

Population Genetics

After removing large juveniles (>1000 mm), mature individuals, and one individual from each nonrandomly sampled sibling pair, 403 scalloped hammerheads remained in the dataset for analysis. DAPC found no meaningful groupings among geographic sampling regions or among nurseries with a k-means clustering approach, nor with individual groupings assigned *a priori*. When randomly sampled siblings were retained for analysis, Tolomato River was significantly differentiated from both Bulls Bay (F_{ST} = 0.0004, P = 0.0147) and Cape Canaveral (F_{ST} = 0.0003, P = 0.0029, Table 12). Significant differentiation was also observed between Bulls Bay and Cape Canaveral (F_{ST} = 0.0005, P < 0.0001). When randomly sampled siblings were removed from the dataset, no comparisons were significantly different (Table 13). Global F_{ST} values generated from the single-level AMOVA were significantly different from zero (P < 0.001) with both siblings included and removed. The lack of structure indicated by DAPC coupled with the pairwise comparison results from the dataset of unrelated individuals suggests that there are no significant genetic differences among nurseries. The significant results observed when siblings are retained in the dataset are likely indicative of female philopatric behavior and not long-term gene flow, which is likely male-mediated (Daly-Engel *et al.*, 2012).

Within Nursery Diversity

For all scalloped and Carolina hammerheads nurseries, minimum estimates of N_b were finite and ranged from 492.7 (Cumberland Island) to 1009.1 (Cape Canaveral) for scalloped hammerheads and 63.8 (Cape Canaveral) to 1880.8 (Bulls Bay) for Carolina hammerheads (Table 14, Fig. 9). Point estimates for scalloped hammerheads ranged from 1197.4 (Tolomato River) to 32022.1 (Cumberland Island). Carolina hammerheads point estimates ranged from 170.5 (Cape Canaveral) to 3202.0 (Bulls Bay). Finite upper bounds were not obtained for scalloped hammerheads in Cumberland Island or Tolomato River, or for Carolina hammerheads in Cape Canaveral. Although the point estimate for scalloped hammerheads in Cumberland Island was quite high (32002.1), the minimum was below 500 (492.7). From a management perspective, minimum estimates should be monitored to ensure appropriate measures are taken for potentially imperiled populations. As expected, overall point estimates for the northwest Atlantic region indicate that the effective number of breeders is greater for scalloped hammerheads than for Carolina hammerheads (4423.0 and 2114.0, respectively). Interestingly, point estimates indicate that scalloped hammerheads have less than half the effective number of breeders than Carolina hammerheads in the Bulls Bay nursery (1373.1 and 3203.0, respectively). These estimates highlight the importance of Bulls Bay for continued persistence of Carolina hammerheads. The minimum number of female breeders for each nursery ranged from 23-107 (scalloped hammerheads) and 15-133 (Carolina hammerheads) but was largely a function of sample size (Table 15).

Genetic diversity in the form of rarefied allelic richness (A_R), observed heterozygosity (H_o), and expected heterozygosity (H_s) were estimated for both species in Bulls Bay and Cape Canaveral, and for scalloped hammerheads only in Cumberland Island and Tolomato River (Table 16). Wilcoxon tests indicated scalloped hammerheads in Bulls Bay had greater H_o than all other nurseries, (P = 0.0001-0.0138) and Cumberland Island had less than Cape Canaveral and Tolomato River (P < 0.0001). The Friedman's test for scalloped hammerheads H_s was significant

($P = 0.0214$), however pairwise comparisons between nurseries did not identify any differences. Scalloped hammerheads A_R in Cumberland Island was significantly greater than every other nursery ($P < 0.0001$), while all other nurseries were equal. Mean values for Carolina and scalloped hammerheads diversity estimates are reported in Table 16; statistical comparison between Bulls Bay and Cape Canaveral was not conducted due to the small sample size in Cape Canaveral. However, mean values indicated Carolina hammerheads were less diverse than scalloped hammerheads by all measures of diversity. Low diversity observed in Carolina hammerheads is consistent with the low effective number of breeders, and these results suggest a reduced long-term effective size.

Objective 4:

Randomly sacrifice a minimum of 30 hammerheads per species per estuary as well as utilize weak or moribund individuals that have been stressed by capture to determine information about diet and relative condition. Sacrificed individuals will be brought back to the lab where weights (whole, liver, and eviscerated) will be taken. The stomach will be removed and frozen and diet analysis performed.

Accomplishments Objective 4:

A total of 428 stomachs from Carolina (n=201), Scalloped (n=181) and hybrid (n=46) hammerheads caught between April through November and 2014 to 2019 were processed and analyzed. Samples were collected from 7 broad regions, including the 3 known nursery areas for young-of-year hammerheads (Bulls Bay, SC, Cape Canaveral, FL, and Tolomato, River FL) as well as nearshore areas off of South Carolina, Georgia, Florida and North Carolina. The goal of 30 hammerheads per species per nursery area was only met in Bulls Bay (Table 17). However, we were able to supplement regional samples by opportunistically collecting stomach and tissue samples from young-of-year hammerheads caught as bycatch in Georgia shrimp trawls (collected by Georgia Southern University).

In addition to the samples collected for this grant, samples for 5 other research projects were opportunistically collected from the moribund or sacrificed hammerheads. Vertebrae (n=196) were collected for an age and growth study of the Carolina and scalloped hammerheads along the southeast Atlantic coast (Cooperative Research Program, NMFS NA16NMF4540084). Paired eye and vertebrae (n=18) were collected for stable isotope and elemental analyses to better understand the spatial and temporal movements of each species off the southeast coast (State Wildlife Grant, USFWS SC-T-F19AF00723), fin clips (n=77) were taken for investigation of using near-infrared spectroscopy with tissue to determine if age could be estimated with fins, muscle samples (n=13) were collected for fatty acid analysis, and whole heads (n=76) were collected for gill and eye morphology as well as sensory analyses. In addition, 586 samples (including liver, muscle, brain, kidney and red blood cells) were collected for a contaminant and maternal offloading study (State Wildlife Grant, USFWS SC-T-F18AF00964).

Significant Deviations:

As documented in objective 1, efforts to sample scalloped hammerheads in estuarine waters off of GA were unsuccessful, however samples were opportunistically collected from

nearshore Georgia waters. These hammerheads were bycatch of commercial shrimp trawlers, and both stomachs as well as tissue samples were collected and analyzed. In the two nursery areas off Florida, efforts to achieve 30 of each species were unsuccessful as only scalloped hammerheads were found at the Tolomato River site. Carolina hammerheads were found off Cape Canaveral, however we were unable to collect enough to have 30 of each species. That said, we were able to obtain enough tissue and stomachs to provide a robust dataset for the analyses proposed in objective 6.

Objective 5:

Muscle, blood and liver samples will be taken, processed, and analyzed for stable isotope analysis to determine trophic status. (year 2 and year 3).

Accomplishments Objective 5:

A total of 1007 tissue samples (across muscle, liver, whole blood and plasma) were taken for carbon and nitrogen stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$, respectively) and 36 muscle samples were taken for sulfur isotope analysis ($\delta^{34}S$) (Table 18). Samples were taken from sharks caught in 6 broad regions (Bulls Bay, Cape Canaveral, Tolomato River, nearshore SC, nearshore GA and nearshore FL). There were 488 tissue samples collected from Carolina hammerheads, 442 samples from scalloped hammerheads and 77 samples collected from hybrids for $\delta^{13}C$ and $\delta^{15}N$ analysis.

Significant Deviations Objective 5:

An error was made during one of the sample processing runs that contained a large amount of tissue samples (including many from sharks collected in nearshore Georgia waters), which rendered the samples unusable for analyses. For this reason, as well as prioritizing sharks that were genetically identified, the number of samples analyzed for Georgia was low. In addition, the groups collecting samples in Florida and Georgia did not have the equipment necessary to get plasma samples, and therefore the number of blood samples from Georgia and Florida were not comparable to those taken in South Carolina. However, sufficient sample sizes were available for the analyses proposed in objective 6.

Objective 6:

Perform data analysis to accomplish the following objectives: (a) investigate temporal and spatial patterns in species abundance to study species-specific nursery utilization; (b) analyze abiotic factors to determine what factors affect species-specific distribution; (c) analyze diet data using direct quantification of stomach contents; (d) use relative condition and hepatosomatic indices to determine if either species is negatively impacted by competition for resources; and (e) conduct stable isotope analysis to determine if species-specific differences are present.

Accomplishments Objective 6:

Temporal and Spatial Patterns in Abundance

As reported in results from Objective 3a, there were nursery-specific differences in the abundance of Carolina hammerheads along the coast. The highest abundance of Carolina hammerheads was found in Bulls Bay, SC (59.5%) with decreasing abundance of Carolina hammerheads with latitude (Table 7). The exception was the Tolomato River, where no Carolina hammerheads were detected; this nursery is likely unique, as it is the only nursery area that occurs inshore in an entirely estuarine system. The other three known nurseries (Bulls Bay, nearshore Cumberland Island, and Cape Canaveral) are open ocean, although Bulls Bay is somewhat unique as it is a shallow embayment open to ocean influence.

While few hammerheads were captured in North Carolina waters, the animals sampled there were 33% Carolina hammerheads (Table 6). While this sample size is low, this likely indicates that center of abundance of YOY Carolina hammerheads is located in Bulls Bay, SC with decreasing abundance to the north or south. As reported in Objective 3a results, there were temporal differences in the abundance of Carolina and scalloped hammerheads (Table 9). To further investigate this, we examined catch per unit effort (CPUE, sharks captured/set) from the SCDNR COASTSPAN large gillnet survey on a biweekly basis. As relative abundance of hammerheads was variable over years, we pooled all catch data across years (2013-2018) to look at temporal trends standardized to effort. As not all hammerheads captured could be run for identification to species through genetics (due to funding and time constraints), the unidentified portion of catch is included as well. Parturition begins in early May, and YOY from both species enter the nursery area soon after; scalloped hammerheads are slightly more abundant from May through June, with both species increasing in abundance until abundance peaks in late July (Fig. 10). In early July, a surge of Carolina hammerheads enters the nursery area resulting in a fourfold increase in CPUE. After late July, scalloped hammerheads abundance rapidly decreases, and no scalloped hammerheads are found by the end of August (Fig. 10). While Carolina hammerheads abundance also decreases after the peak in July, they remain in the nursery area for at least a month longer than scalloped hammerheads in relatively high numbers. The increase in abundance of YOY for both species from May-July indicates that parturition likely occurs over several months with some females giving birth early, but most giving birth in mid- late June. Conversely, YOY could be born well outside of the nursery area, and their arrival may peak in mid-July.

While parturition likely occurs over the same timeframe, length data from the stratified random gillnet and SCDNR COASTSPAN gillnet survey indicate Carolina hammerheads are born at ~50 mm shorter length than scalloped hammerheads and these size differences are retained throughout nursery use (Fig. 11). The reason for these differences remains unknown, however it may be due to maternal litter size, differences in growth, or a reproductive strategy related to survivorship/foraging. Unfortunately, we have insufficient data to determine whether differences in length are retained throughout Carolina hammerheads life-span. Determining if differences in length-at-age exist are important as many stock assessment inputs are based on length-based parameters (length-at-maturity, growth (von Bertalanffy) parameters, and natural mortality estimates).

Abiotic Factors

Due to differences in survey gears, sample sizes, and effort as well as a lack of Carolina

hammerheads in all nursery areas, we were only able to model abiotic factors for the SCDNR COASTSPAN gillnet index station. While the stratified random survey was designed to investigate spatial partitioning and the effect of environmental variables on abundance, too few individuals of each hammerheads species were available for modeling. However, the stratified random gillnet survey was useful in modeling abundance of the more common species (finetooth sharks, Atlantic sharpnose, blacktip sharks and bonnetheads), and an ongoing undergraduate honors thesis at the College of Charleston is based on survey results.

The effects of water temperature, salinity, and dissolved oxygen on abundance of hammerheads was investigated using general linear models. As data were from a single fixed station, effects of depth, proximity to tidal creeks, and other variables could not be compared. Model results indicate that scalloped hammerheads presence is not affected by the environmental variables measured; however, salinity and dissolved oxygen had a significant effect on Carolina hammerheads abundance (Table 19). Carolina hammerheads abundance decreased with higher dissolved oxygen and increased with salinity (Fig. 12, Fig. 13). As these data were from a single sampling site, it is difficult to determine the significance of these results; however, they may, in part, explain the lack of Carolina hammerheads at the Tolomato River nursery area. The Tolomato River may undergo high fluctuations in salinity, and average salinities are consistently lower than those at Bulls Bay, SC. Future research should further examine the effects of these parameters on the abundance of these two species. As these environmental variables were not taken for the majority of captured hammerheads, we are unable to look at region wide trends.

Stomach Content Analysis

Each shark used for diet and trophic analysis was measured and weighed (wet weight in g), and the liver was excised and weighed. Stomachs were removed, contents were extracted from the stomach to halt further digestion, and then stomach and contents were frozen until analysis. Once thawed for analysis, the stomach was cut open and rinsed with water over a 500 μm sieve. Each prey item was identified to the lowest possible taxon and sorted into individual taxon groups. Prey items were enumerated, weighed (wet weight) and assessed for digestive state (4 states of varying degrees of digestion, 0 representing very little or no digestion and 4 representing mostly digested with hard structures used for identification such as otoliths and crustacean remains).

Multiple indices were calculated to assess diet through stomach contents. The frequency of occurrence (%O) calculates the proportion of stomachs containing one or more individual of each prey category and is expressed as a percentage of the total number of stomachs analyzed. Percent number (%N) is based on counts of a prey item and is expressed as the percentage of the total number of a prey item in all stomachs analyzed. Percent weight (%W) is the percentage of the weight of a prey item in relation to the total weight of all items for all stomachs. The %N overestimates small prey items eaten in large numbers and underestimates large food items which are eaten less frequently, while %W underestimates prey weights due to digestion and the presence of only hard parts that are used to identify prey (such as otoliths, squid beaks and shrimp rostra). Therefore, to get a less biased and more robust description of the hammerheads diet, the index of relative importance (IRI) was calculated using the combination of %N, %W and %O, and then expressed as a percentage (%IRI).

Dietary overlap and niche overlap were determined using Schoener's overlap index (α) and Morisita's index (C_D), respectively. The values range from 0 (no overlap) to 1 (complete

overlap) for both indices, and values above 0.6 are considered to suggest significant overlap. Significant dietary and niche overlap was found between young-of-year Carolina and scalloped hammerheads, as well as between both species with hybrid hammerheads in estuarine and nearshore waters of the southeast Atlantic coast (Table 20, Table 21). The large dietary and niche overlap suggests that there is little habitat partitioning in estuarine and coastal waters between species. Young-of-the-year scalloped hammerheads have a more diverse diet (as in individuals had more prey taxa per stomach) compared to Carolina hammerheads as well as seem to eat more often or voraciously, since they had a higher number of individual prey items per stomach (Table 22). The average total prey weight for all three species increases with maturity code (the state of umbilical scar healing), which indicates that the young sharks consume a higher amount of prey as they grow (Fig.14).

The two species and hybrids have similar niche widths across all regions according to Levin's index *B*, though scalloped hammerheads have a smaller niche width than Carolina or hybrid hammerheads (Table 23). When looking at region-specific values, SC hammerheads have the largest niche (*B*=6.30), followed by those in GA (*B*=4.55) and then FL (*B*=3.72). This could mean that there is a higher diversity and amount of prey available in SC waters or that the sharks in SC forage in more habitats than those in GA or FL. Though teleosts make up the highest proportion of their diet, both species can be considered generalist feeders due to a large number of prey taxa in their diet. There were no statistical differences between the diet of males and females for either Carolina or scalloped hammerheads (Schoener's overlap $\alpha = 0.82$ and 0.83, respectively). The trophic level (T_L) of the young hammerheads was calculated following Cortes (1999), and based on their stomach contents, scalloped hammerheads have a slightly higher trophic level than Carolina and hybrid hammerheads (T_L= 4.11, 4.07 and 4.05, respectively). This is likely due to a larger importance of crustaceans in the diet of the Carolina and hybrid hammerheads as compared to scalloped hammerheads (Table 20).

The sample sizes for each species in Georgia and Florida were too low to look at species-specific regional diet differences. However, because there was a high dietary overlap between species caught across all regions, Carolina, scalloped and hybrid hammerheads were then combined to investigate differences in diet composition by state. Sharks from South Carolina consumed 13 more prey taxa than those in Georgia and Florida and therefore seem to have a more diverse diet (Table 24). Carolina and scalloped hammerheads in SC have a higher dietary overlap than in GA or FL and a higher niche overlap than those in GA (Table 20). The niche width decreases with latitude (north to south) for all three species (Table 23).

Relative Condition and Hepatosomatic Indices

The hepatosomatic index (HSI), calculated as the ratio of liver weight to body weight, provides an indication on status of energy reserve in an animal. HSI changed by month as the sharks grew throughout the summer, with a continual decrease from April to July and then a slight increase from July to later in the year (Fig. 15). Carcharhinid sharks bear live young, and the pups are provisioned with maternal resources in means of an enlarged liver. The decrease in HSI reflects the loss of maternal provisioning as the young sharks grow and become better at capturing prey, while the increase is likely due to somatic growth once the maternal provisioning is depleted. The initial high HSI value in April and the steeper decline in Carolina HSI between May-July may indicate that the Carolina mothers likely provide greater energy reserves to their young than scalloped hammerheads.

The exact time frame of parturition is not known for the Carolina or scalloped hammerheads off the southeast U.S. coast, but the results of this study suggest that the earliest parturition the begins is late April and it may last through the mid-June. Therefore, sharks caught in June have a large potential age range. Because of this variability in age across days or months, the HSI was calculated by maturity stage to get a better picture of how the HSI changes with growth (Fig. 16). Looking at mean HSI, as the umbilical scars healed, the HSI decreased, revealing the loss of the maternal provisioning in the liver. The Carolina HSI was higher than that of scalloped across all maturity codes, reflecting the monthly plot in which Carolina has a higher initial HSI value and then maintains a higher HSI than scalloped hammerheads throughout the summer.

Condition factor is a morphometric index of an individual's condition or health and uses the ratio of body weight to length. The mean condition factor changed less by month for Carolina and scalloped hammerheads compared to the mean HSI (Fig. 17), and only slightly decreased with continued healing of the umbilical scar (Fig. 18). This indicates that although the young hammerheads are losing their maternal provisions in their liver, as shown by HSI, they are feeding sufficiently throughout their growth to maintain an overall healthy condition. Carolina hammerheads had a significantly higher condition factor in May than scalloped hammerheads (two sample t-test; $t(69) = 2.89$, $p < 0.01$), and a slightly higher condition factor than scalloped hammerheads in June and July as well as for the majority of the maturity states. This further supports the possibility that young Carolina hammerheads receive more provisioning from their mom, as they can maintain a healthier condition in the earliest part of life when learning how to forage proficiently.

Stable Isotope Analysis

Stable isotope analysis provides time-integrated information on the assimilated diet of an organism rather than just the snapshot of the diet, as is the case with stomach content analysis. As an organism consumes its prey, it incorporates the isotopic composition of that prey into its own tissues over time, and therefore the stable isotope ratio of the consumer tissues can be related in a predictive way to those of their diet. Nitrogen is enriched between trophic levels and therefore the isotopic ratio of nitrogen ($\delta^{15}N$) can be used to estimate relative trophic position of the consumer. Carbon is relatively conserved between trophic levels and the isotopic ratio of carbon ($\delta^{13}C$) can therefore track sources of primary carbon and provide information about foraging habitat, such as coastal versus offshore waters, or which food web(s) the consumer feeds from, such as the benthic or pelagic food web. Sulfur isotope ratios ($\delta^{34}S$) are even more conserved between trophic levels than $\delta^{13}C$, and can be used as an additional indicator of basal resource for trophic relationships within estuaries as it allows discrimination between benthic and pelagic producers at the base of the food webs (Connolly *et al*., 2004, Fry *et al*. 1982).

It can be difficult to study the trophic ecology of YOY sharks using stable isotopes due to long tissue turnover rates and maternal influence that can greatly affect their isotopic values. However, if mature individuals of a population are highly migratory and difficult to study, while their young inhabit a coastal nursery ground, then the isotopic values of these young sharks can be used to infer trophic information about mature females. Though neonate sharks begin to feed soon after birth, the composition of their tissues reflect that of the mother (or the provisioned reserves she provided), and therefore will have higher $\delta^{15}N$ values than if they were reflecting their own diet (Olin *et al*. 2011). In most cases, the $\delta^{15}N$ of neonate sharks will actually be higher

than the mother's isotopic signature ($\delta^{13}C$, $\delta^{15}N$) due to the provisioning. While tissues with a slower metabolic rate like muscle can be used to infer potential spatial and dietary information pertaining to adult females, tissues with a faster turnover rate like plasma can provide trophic information about YOY sharks. Tissues with turnover rates in between muscle and plasma, like whole blood and liver, can be used in conjunction with muscle and plasma to help estimate when the shark is reflecting its own true diet rather than its mothers'. Within the same individual, these four tissues have different isotopic signatures that reflect the varying turnover rates (Fig. 19 and Fig. 20). Looking across months for a particular species and location can help visualize the loss of maternal influence over time, which is represented by a decrease in $\delta^{15}N$ (Fig. 20). The shark tissue samples from June likely reflected the maternal isotopic signature, while those from August should be reflecting the shark's own diet rather than its mothers. When comparing within tissues, the August $\delta^{15}N$ values were consistently lower than the June values. To provide a reference for when the isotopic values of young-of-year sharks should reflect their own diet, tissue samples were collected for 10 male adult hammerheads (9 scalloped and 1 Carolina) caught in April and May off the SC coast. Their isotopic ratios were added the plots to compare the difference between mature and young-of-year samples.

The $\delta^{13}C$ and $\delta^{15}N$ range between the most- and least-enriched individual for each species provides information about the variability within and between populations. The average degree of trophic diversity within a species is represented by CD, or the mean distance to the centroid, while the standard ellipse area which is corrected for small sample size (SEAc) for each species was calculated as an estimate of isotopic niche width (Table 25). The carbon ranges for muscle tissue of Carolina and scalloped hammerheads were similar, indicating that the mature females of both species likely consume prey from similar food webs. The nitrogen ranges were both broad and similar between species as well, suggesting that Carolina and scalloped hammerheads both consume prey across multiple trophic levels. However, the muscle tissue CD and the SEAc were larger for the Carolina hammerhead, indicating that mature Carolina females may have a larger trophic diversity and niche width than scalloped hammerheads.

There was a significant difference between Carolina and scalloped hammerhead muscle isotopic values, in which Carolina hammerheads were less enriched in $\delta^{13}C$, but more enriched in $\delta^{15}N$ than the scalloped hammerheads (Fig. 21 and Fig. 22). When the individual isotopic signatures were removed and the ellipses (that represent the isotopic niche or the mean values with standard deviation) remain, there was a clear difference between species in the beginning of the summer, as seen by the lack of overlap between the ellipses of the two species in May and June (Fig. 22). Since muscle isotopic values, particularly those from sharks caught in the early part of the summer likely reflect the diet of mature females adults, this difference may be reflecting a difference in food research usage between mature females, with Carolina hammerheads feeding further offshore (less enriched $\delta^{13}C$), and/or in a more pelagic food web, as compared to scalloped hammerheads. The small overlap between the species' ellipses caught in July and August for muscle samples indicates that by this time, isotopic ratios seem to partially reflect the diet of the YOY sharks with more comparable $\delta^{13}C$ values, though there may still be some maternal influence due to the long turnover rate of muscle. The muscle isotopic signatures of hybrid individuals fell in the middle of the two true species, with many of the hybrids (mostly FI hybrids) expressing values more similar to Carolina hammerheads (Fig. 21). This mirrors the finding that the majority of hybrid hammerheads have a Carolina mother and a scalloped father, as the muscle samples reflect the maternal isotopic signature. The difference in carbon may be slightly attributed to latitudinal differences of isotopic values. Carbon ratios

change with latitude along the southeast U.S. coast, with less enriched (more negative) $\delta^{13}C$ values in South Carolina and more enriched $\delta^{13}C$ in Florida waters (Ceriani *et al.* 2014).

Sulfur isotopes ($\delta^{34}S$) are a stronger indicator of basal resource than carbon, with higher sulfur values associated with pelagic prey and lower values associated with benthic prey. When $\delta^{34}S$ was plotted against $\delta^{15}N$ of muscle for young-of-year Carolina and scalloped hammerheads that were grouped into late spring (i.e. younger sharks) and late summer (i.e. older sharks), a clear pattern emerged (Fig. 23). Carolina hammerheads had a higher $\delta^{15}N$ and $\delta^{34}S$ than most of the scalloped hammerheads, despite time period, suggesting that Carolina hammerheads feed more regularly on pelagic prey and scalloped hammerheads eat more benthic prey. When looking at %IRI for stomach contents, clupeids (*Brevoortia* spp.), anchovies and squid (pelagic species) all had a higher importance in the Carolina hammerheads diet, while sciaenid fishes (demersal species), in particular star drum, had a higher importance in the scalloped hammerheads diet (Table 20). The difference in $\delta^{15}N$ of muscle tissue between species suggests that mature Carolina hammerheads feed at a slightly higher trophic level than the mature scalloped hammerheads. The trophic position (TP) calculated for Carolina and scalloped hammerheads using the methods of Post (2002) were 4.04 and 3.88 for adults (mature females) and 3.77 and 3.78 for young-of year sharks, respectively. The TP for mature females was calculated using muscle isotopic values and the Atlantic brief squid, *Lolliguncula brevis*, as the most important prey, because squid was found to be important in the diet of older juveniles and adult scalloped hammerheads in other studies. The TP for young-of year hammerheads was calculated using plasma isotopic values and the star drum, *Stellifer lanceolatus*, as the most important prey because of stomach content analysis in this study. Calculated trophic positions indicated that there may be resource partitioning (spatial or dietary) between adult Carolina and adult scalloped hammerheads, but the YOY share similar resources.

The tissue turnover rates of liver and whole blood were in between the slow muscle turnover and the fast plasma turnover. When grouped by month, liver samples showed a similar pattern to muscle samples, with little overlap via ellipses in $\delta^{13}C$ values between Carolina and scalloped hammerheads caught in May and June but a high overlap with sharks caught in July and August (Figs. 24). As with muscle samples, this indicates that the younger sharks are still reflecting their maternal isotopic signature, while the older sharks are at least partially incorporating their own estuarine diet into their tissues. The large nitrogen ranges (Table 25) for the liver tissues may be reflecting the varying degrees of maternal provisioning, and the slightly larger nitrogen range for the Carolina hammerheads could indicate that the parturition period for the species is either across a longer time period or it begins slightly later than that of the scalloped hammerheads. Whole blood isotopic values from sharks caught in the early months, on the other hand, showed a higher overlap than liver samples between species across $\delta^{13}C$, though the Carolina hammerheads still exhibited higher $\delta^{15}N$ values (Fig. 25). There was also a higher overlap between species caught in the later months for both $\delta^{13}C$ and $\delta^{15}N$, consistent with the large dietary overlap found in stomach content analyses.

The $\delta^{13}C$ and $\delta^{15}N$ ranges of plasma for both species were similar to those in muscle, though the $\delta^{13}C$ mean for plasma is less enriched (Table 25; Fig. 19). This infers that the young-of-year sharks may be feeding from similar food webs to the mature sharks, though the less enriched values show that they are foraging in the estuaries versus in coastal waters. Carbon ratios are less enriched (more negative) offshore and within estuarine waters compared to coastal waters (Leakey *et al*. 2008). The plasma CD and SEAc values were larger for Carolina hammerheads, which is interesting because Carolina young-of-year had slightly less trophic

diversity in their diet according to stomach contents (Table 25). This highlights the importance of using multiple methods to study the trophic ecology of an organism, as the stomach contents provide just a snapshot of the diet while the stable isotopes reveal the assimilated diet.

The plasma samples showed a narrower range of $\delta^{13}C$ between species across all months due to the tissue's high turnover rate, though the sharks caught in May and June still seem to have a maternal influence since the $\delta^{15}N$ values were higher than those caught in July and August (Fig. 26 and Fig. 27). The isotopic signatures of adults for plasma had similar nitrogen values to the young-of-year sharks caught in July and August, indicating that the older young-of-year sharks have a similar trophic position to the adults. When plasma isotopic ratios are separated by species, an interesting pattern is seen by month for Carolina hammerheads (Fig. 28). May and June samples still partially reflected the maternal isotopic signature, but sharks caught between July-October likely fully reflected the diet of YOY. Most of the Carolina plasma samples were taken from sharks caught in Bulls Bay, SC, which suggests that perhaps there is a slight shift in diet over time for the young Carolina hammerheads in this nursery. The majority of scalloped plasma samples were also taken from sharks from Bulls Bay, however there was no clear pattern across months (Fig. 29). The difference in isotopic patterns could suggest that there is some resource competition between species (which is likely due to a high dietary overlap) in Bulls Bay, and Carolina hammerheads may be shift to different prey items over time. No Carolina or scalloped hammerheads stomachs were collected from Bulls Bay in October, however looking at count data there was a large increase in the number of squid found in Carolina stomachs from July to August. In addition, Carolina stomachs from July had a higher proportion of benthic prey (mostly benthic teleosts) than those from August. Benthic and pelagic prey are relatively comparable between July and August for scalloped hammerheads, indicating no large change in diet. Another possibility for the different plasma patterns between species is that the decrease in relative abundance of the scalloped hammerheads in July and August (Table 9) may reduce competition for certain prey items in Bulls Bay and the Carolina hammerheads can feed more often on the newly available prey.

Regional difference in stable isotopes could only be performed with scalloped hammerheads muscle samples. Tissue was collected from mature sharks off SC and young-of-year sharks in SC, GA and FL. Though there is a latitudinal gradient in $\delta^{13}C$ along the southeast U.S. coast with more enriched $\delta^{13}C$ values in FL, the change in $\delta^{13}C$ was not reflected in the isotopic values. This finding supports the idea that spatial partitioning from the shoreline (with mature Carolina hammerheads possibly located further offshore than the scalloped hammerheads) or trophic differences explain the difference in $\delta^{13}C$ between species. There was very high overlap between individuals from SC, GA and FL for both $\delta^{13}C$ and $\delta^{15}N$ (Fig. 30). It is not surprising that there is a large overlap in $\delta^{15}N$, as these are all young-of-year scalloped muscle samples that represent mature female diet. The high overlap in $\delta^{13}C$ could reflect constant movement up and down the coast as well as from inshore or offshore waters, thereby averaging the carbon signal over time and diminishing the latitudinal and nearshore-offshore differences. Most of the young-of-year samples were more enriched than the adults, which is interesting because the adults were all males. Since the isotopic signatures of the young sharks represent the diet of mature females, perhaps these data are showing a result of sexual segregation, with the scalloped hammerheads females inhabiting more nearshore waters than the males.

Significant Deviations Objective 6:

A small subset of muscle samples were analyzed for $\delta^{34}S$ to help elucidate differences across the basal resources for Carolina and scalloped hammerheads. The $\delta^{34}S$ results did clarify some of the $\delta^{13}C$ data and led to a better understanding and interpretation of the young-of-year trophic ecology.

Objective 7:

Complete yearly interim and final reports and disseminate results via presentations and peer reviewed publications.

Accomplishments Objective 7:

All annual reports as well as the final report have been submitted as required. The following list details presentations, posters and publications generated to date. Several manuscripts will be submitted for publication in peer-reviewed journals following the submission of this final report and multiple presentations will be presented at national fisheries meetings over the next year.

Talks

Barker, A.M., Frazier, B.S., Adams, D.H., Gelsleichter, J., and Portnoy, D.S. (2016) Identification and relative abundance of cryptic hammerheads sharks. Joint Meeting of Ichthyologists and Herpetologists, New Orleans, LA, July 2016

Shaw, A., Adams, D., Barker, A.M., Bedore, C., Gelsleichter, J., Portnoy, D.S. Reyier, E., Frazier, B.S. Diet analysis of sympatric Hammerheads species in the Southeast U.S. Marine Resources Division Conference of South Carolina Department of Natural Resources, Charleston, SC, March 29, 2017.

Shaw, A., Adams, D., Barker, A.M, Bedore, C., Gelsleichter, J., Portnoy, D.S., Reyier, Frazier, B. (2017) Trophic ecology and condition of sympatric hammerheads species in nursery habitats in the Southeast U.S. American Elasmobranch Symposium: Applications of Physiological Ecology in Elasmobranch Research, Joint Meeting of the American Society of Ichthyologists and Herpetologists, Austin, TX, July 2017

Portnoy, D.S., Barker, A.M., Adams, D.H. & Frazier, B.S. (2018) Hybridization between a cryptic species pair, *Sphyrna lewini* and *Sphyrna gilberti*, in the western North Atlantic. Sharks International, João Pessoa, Brazil, June 2018.

Barker, A.M., Frazier, B.S., Adams, D.H. & Portnoy, D.S. (2018) Hybridization between a cryptic species pair, *Sphyrna lewini* and *Sphyrna gilberti*, in the western North Atlantic. Joint Meeting of the American Society of Ichthyologists and Herpetologists, Rochester, NY, July 2018

Galloway, A., Barker, A.M.*, Bedore, C., Adams, D., Reyier, E., Gelsleichter, J., Portnoy, D.S., Frazier, B.S. (2019) Trophic ecology of the scalloped and Carolina hammerheads in coastal waters of the southeastern U.S. *Joint Meeting* of the American Society *of Ichthyologists and Herpetologists,*

Snowbird, UT, July 2019.

Barker, A.M., Adams, D., Bedore, C., Frazier, B., Gelsleichter, J., Kingon, K., Portnoy, D.S. (2019) Population structure and conservation genetics of scalloped hammerheads (*Sphyrna lewini*) in the U.S. Atlantic and Gulf of Mexico Joint Meeting of the American Society of Ichthyologists and Herpetologists, Snowbird, UT, July 2019.

Posters

Shaw, A. Adams, D., Barker, A., Bedore, C., Gelsleichter, J., Portnoy, D.S., Reyier, Frazier, B. (2016) Diet analysis of two cryptic Hammerheads species off the Southeastern United States. Joint Meeting of Ichthyologists and Herpetologists, New Orleans, LA, July 2016

Manuscripts

Barker, A. M., Adams, D. H., Driggers, W. B., Frazier, B. S., & Portnoy, D. S. (2019). Hybridization between Sympatric Hammerheads Sharks in the Western North Atlantic Ocean. *Biology Letters*, *15*, 20190004.

Barker, A. M., Frazier, B. S., Bethea, D.M., Gold, J.R., & Portnoy, D. S. (2017). Identification of Young-of-the-Year Great Hammerheads *Sphyrna mokarran* in Northern Florida and South Carolina. *Journal of Fish Biology 91 664-668.* doi: 10.1111/jfb.13356.

Significant Deviations Objective 7:

There were no significant deviations in reporting or dissemination of results. We will continue to work towards publication of results in peer-reviewed journals in subsequent months.

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Tables

Table 1. Total number of young-of-the-year (YOY), small juvenile (SM JUV), large juvenile (>1000 mm, LG JUV), and mature (MAT) scalloped and Carolina hammerheads sequenced in each region.

Location	YOY/SM JUV	LG JUV	MAT	Total
North Carolina	7	1	5	13
South Carolina	380	1	31	412
Georgia	93	1	10	104
Northern Florida	203	0	1	204
Central Florida	194	9	1	204
Unknown	0	1	0	1
Total	877	13	48	938

Table 2. Total number of young-of-the-year (YOY) and small juvenile (SM JUV) scalloped and Carolina hammerheads sequenced from nursery sites.

Nursery area	**YOY/SM JUV**
Bulls Bay, SC	350
Cumberland Island, GA	47
Tolomato River, FL	150
Cape Canaveral, FL	194

Table 3. Catch of sharks sampled by longline by the University of North Florida in the Tolomato River, FL. Species composition in numbers, percent of catch, numbers by sex, and by life stage are reported (Juv = juvenile).

Species	**Total caught**	**% Catch**	**Male**	**Female**	**Tagged**	**YOY**	**Juv**	**Mature**
Atlantic sharpnose shark	152	23.8	70	75	61	131	11	5
Bonnethead	12	1.9	4	8	9	3	8	1
Blacktip shark	63	9.8	24	36	56	38	23	
Bull shark	4	0.6	1		1		4	
Finetooth shark	73	11.4	34	36	63	26	44	1
Hammerhead	242	37.8	127	108	162	195	34	
Lemon shark	2	0.3		2	2		1	1
Nurse shark	4	0.6	2	1	4		1	1
Sandbar shark	60	9.4	33	27	58	12	43	
Smooth dogfish	1	0.2	1		1			
Atlantic stingray	4	0.6	2	1				2
Bluntnose stingray	9	1.4	1	8	3			5
Southern stingray	14	2.2	2	11	4		2	7
Total	640	100	301	313	424	405	171	23

Table 4. Catch by year and gear type for Georgia Dept. of Natural Resources shark survey. Water chemistry (salinity: parts per thousand; temperature: degrees Celsius; dissolved oxygen: mg/L) ranges and means for all species by life stage. Catch by year, gear, and life stage also shown for the aggregate catch.

Species		Longline		Gillnet		Salinity (ppt)		Temperature (°C)		D.O. (mg/L)	
	Total	'16	'17	'16	'17	Range	Mean	Range	Mean	Range	Mean
Bonnethead	251	56	44	49	102						
YOY	6	1	--	3	2	24.5-33.7	30.4	27.1-30.8	29.3	4.5-5.7	5.0
Juvenile	183	42	38	33	70	12.9-36.4	28.9	21.6-31.3	28.3	3.1-7.8	5.6
Adult	62	13	6	13	30	13.2-33.3	28.5	23-31.5	28.5	3.2-7.0	5.5
Finetooth	73	1	1	26	45						
YOY	66	--	1	22	43	22-33	30.6	22.8-31.3	29.5	4-6.7	5.5
Juvenile	7	1	--	5	1	13.2-34.4	28.7	29-30.8	30.1	4.7-7.0	5.5
Adult	3	--	--	2	1	25.5-32.7	28.8	24.2-27.1	25.8	4.5-6.7	5.9
Blacktip	61	5	11	9	36						
YOY	58	3	10	9	36	12.9-33.7	30.6	24.3-31.5	30.0	4.0-6.3	5.3
Juvenile	3	2	1	--	--	27.5-30	28.8	28-29.5	28.8	4.7-6.4	5.5
Atl. sharpnose	217	102	88	8	19						
YOY	137	72	58	2	5	12.9-36.2	30.2	26.1-31.2	28.4	3.3-7.3	5.5
Juvenile	29	7	17	3	2	25.5-34.8	31.9	27.8-30.8	29.7	4.3-8.4	5.6
Adult	52	24	13	3	12	22.2-32.2	27.6	23.2-30.7	26.6	3.3-7.0	6.0
Sandbar	25	17	5	3	--						
YOY	14	8	3	3	--	20.2-34.6	27.6	23.8-30.4	28.3	4.3-8.4	6.1
Juvenile	11	9	2	--	--	22.3-34.8	28.3	22.1-30.1	27.4	3.5-7.4	5.9
Blacknose	12	10	1	1	--						
Juvenile	1	1	--	--	--	--	36.2	--	28.9	--	5.5
Adult	11	9	1	1	--	28.9-32.7	29.8	27.1-28.2	27.7	4.5-6.9	6.0
Scalloped H.H.	9	--	5	2	2						
YOY	6	--	3	2	1	29.4-33.3	31.5	28.2-30.8	29.8	5.5-6.7	6.1
Juvenile	3	--	2	--	1	--	26.1	--	23.3	--	6.1
Lemon	2	2	--	--	--						
Juvenile	2	2	--	--	--	--	26.8	--	26.9	--	6.39
Spinner	1	1	--	--	--						
Adult	1	1	--	--	--	--	12.9	--	27.6	--	5.31
Aggregate Catch	654	194	155	101	204						
YOY	287	84	75	41	87						
Juvenile	239	64	60	41	74						
Adult	129	47	20	19	43						

Table 5. Catch of sharks and rays from the South Carolina Department of Natural Resources 100 m stratified random gillnet survey. Total number captured by species, sex and life stage (young-of-year (YOY), juvenile, and mature). The hammerhead category includes Carolina, scalloped and hybrid hammerheads.

Species	Total caught	Male	Female	Tagged	YOY	Juvenile	Mature
Atlantic sharpnose shark	166	112	49		87	5	74
Blacknose shark	12	2	10	9		5	7
Blacktip shark	97	36	55	70	42	51	3
Bonnethead	80	22	57	58		45	35
Finetooth shark	309	142	158	151	200	36	73
Hammerhead	42	23	17	3	41	1	
Sandbar shark	8	3	5	8	8		
Spinner shark	3	1	1		2	1	
Atlantic stingray	3		3				
Bluntnose stingray	1		1				
Bullnose ray	1	1					
Cownose ray	17	6	9				
Smooth butterfly ray	1	1					
Southern stingray	7	5	2				
Spotted eagle ray	1						
Grand total	748	354	367	299	380	144	192

Table 6. Species identifications in each region: North Carolina (NC), South Carolina (SC), Georgia (GA), northern Florida (NFL), and central Florida (CFL). Pure species identifications (Scalloped, Carolina, Great) are based on results of diagnostic SNP panels. Hybrid classifications (first generation hybrid (F1), Scalloped backcross, Carolina backcross) are based on results of NewHybrids analysis. BX indicates backcross. UND indicates the sample could not be identified using either method.

Location	Scalloped	Carolina	Carolina %	F1	Scalloped Bx	Carolina Bx	Great	UND
NC	8	4	33.3%	1	0	0	0	0
SC	153	198	56.4%	28	23	7	1	2
GA	72	17	19.1%	4	5	0	2	4
NFL	188	7	3.4%	0	1	0	0	3
CFL	169	25	12.9%	4	9	1	0	1
Total	590	251	29.4%	37	38	8	3	10

Table 7. Species identifications within nursery sites: Bulls Bay, SC (BB), Cumberland Island, GA (CI), Tolomato River, FL (TR), and Cape Canaveral, FL (CFL). F1 indicates first generation hybrid, BX indicates a backcross, and UND indicates the sample could not be identified with either the diagnostic panel or NewHybrids.

Location	Scalloped	Carolina	Carolina %	F1	Scalloped BX	Carolina BX	Great	UND
BB	122	179	59.5%	24	23	7	1	1
CI	32	9	22.0%	2	3	0	0	2
TR	148	0	0.0%	0	0	0	0	2
CFL	166	23	12.2%	4	9	1	0	0

Table 8. Relative abundance of scalloped and Carolina hammerheads in Bulls Bay, SC from 2012-2014 and 2016-2019 during the months May-August. N indicates the years' sample size for individuals abundance proportions.

	2012	2013	2014	2016	2017	2018	2019
Carolina	0.870	0.404	0.571	0.520	0.586	0.784	0.312
Scalloped	0.130	0.596	0.429	0.480	0.414	0.216	0.688
N	23	47	28	25	70	74	16

Table 9. Mean monthly relative abundance of scalloped and Carolina hammerheads in Bulls Bay, SC from May to August 2012-2014 and 2016-2019. N indicates the years' sample size for individuals' abundance proportions.

	May	June	July	August
Carolina	0.453	0.411	0.708	0.886
Scalloped	0.547	0.589	0.292	0.114
N	36	68	67	112

Table 10. Number of full (FS) and half (HS) scalloped hammerhead sibling pairs identified in nursery sites and adjacent nearshore waters.

Nursery	FS	HS
West Onslow Bay, NC	1	0
Bulls Bay, SC	1	8
St. Helena Sound, SC	1	0
Cape Canaveral, FL	8	13
Tolomato River, FL	3	8
Bulls Bay, SC/Kiawah Island, SC	0	1
Bulls Bay, SC/Cumberland Island, GA	0	1
Winyah Bay, SC/Cumberland Island, GA	0	1
Cumberland Island, GA, St. Augustine, FL	0	1
Cape Canveral, FL/Tolomato River, FL	0	4
Cape Canaveral, FL/ St. Augustine, FL	0	2
Cape Canaveral FL/Jacksonville, FL	0	2
Tolomato River, FL/St. Augustine, FL	0	1
Tolomato River, FL/Jacksonville, FL	0	1
Total	14	43

Table 11. Number of full (FS) and half (HS) Carolina hammerhead sibling pairs identified in nursery sites and adjacent nearshore waters.

Nursery	FS	HS
West Onslow Bay, NC	1	0
Bulls Bay, SC	3	8
Tybee Island, GA	1	0
Cape Canaveral, FL	2	4
Bulls Bay, SC/Kiawah Island, SC	0	1
Bulls Bay, SC/Cape Canaveral, FL	0	1
Cape Canaveral, FL/ St. Augustine, FL	0	2
Total	7	16

Table 12. F_{ST} (above the diagonal) and corrected *p*-values (below the diagonal) for pairwise comparisons between scalloped hammerhead nurseries (Bulls Bay = BB, Cape Canaveral = CC, Cumberland Island = CI, Tolomato River = TR) with randomly sampled siblings included. Significant values are denoted by *.

	BB	CC	CI	TR
BB		0.0005	-0.0001	0.0004
CC	0.0000*		0.0002	0.0003
CI	0.6059	0.3272		0.0000
TR	0.0147*	0.0029*	0.5918	

Table 13. F_{ST} (above the diagonal) and corrected *p*-values (below the diagonal) for pairwise comparisons between scalloped hammerhead nurseries (Bulls Bay = BB, Cape Canaveral = CC, Cumberland Island = CI, Tolomato River = TR) with siblings excluded.

	BB	**CC**	**CI**	**TR**
BB		0.0001	-0.0002	0.0000
CC	0.3701		0.0001	0.0001
CI	0.7598	0.6260		-0.0001
TR	0.6289	0.3926	0.8350	

Table 14. Estimated effective number of breeders (N_b) for scalloped (*Sl*) and Carolina (*Sg*) hammerheads nurseries and the northwest Atlantic region. Point estimates (mode) and minimum and maximum estimates based on 95% confidence intervals are shown. N indicates the number of individuals used to calculate estimates. Cumberland Island and Tolomato River estimates are not shown for Carolina hammerheads because no or few individuals were in those locations.

Location	**N**	**Min**	**Mode**	**Max**
NW Atlantic-*Sl*	403	3138.4	4423.0	7396.6
NW Atlantic-*Sg*	196	1426.3	2114.0	4001.2
Bulls Bay-*Sl*	84	718.0	1373.1	12129.0
Bulls Bay-*Sg*	116	1880.8	3203.0	10463.9
Cape Canaveral-*Sl*	113	1009.1	1737.5	5892.2
Cape Canaveral-*Sg*	19	63.8	170.5	Infinite
Cumberland Island-*Sl*	26	492.7	32022.1	Infinite
Tolomato River-*Sl*	118	573.8	1197.4	Infinite

Table 15. Estimated minimum number of female breeders (N_{mf}) for scalloped (*Sl*) and Carolina (*Sg*) hammerhead nurseries. N indicates the number of individuals used to calculate estimates.

Nursery Site	**N**	N_{mf}
Bulls Bay-*Sl*	84	75
Bulls Bay-*Sg*	143	133
Cape Canaveral-*Sl*	119	94
Cape Canaveral-*Sg*	21	15
Cumberland Island-*Sl*	26	23
Tolomato River-*Sl*	115	107

Table 16. Mean observed heterozygosity (H_O), Nei's unbiased gene diversity (H_S), and rarefied allelic richness (A_R) for scalloped (*Sl*) and Carolina (*Sg*) nurseries. Cumberland Island and Tolomato River estimates are not shown for Carolina hammerheads because no or few individuals were sampled in those locations.

Nursery Site	H_O	H_S	A_R
Bulls Bay-*Sl*	0.294	0.296	3.588
Bulls Bay-*Sg*	0.203	0.204	1.724
Cape Canaveral-*Sl*	0.292	0.296	3.593
Cape Canaveral-*Sg*	0.206	0.204	1.724
Cumberland Island-*Sl*	0.286	0.296	3.610
Tolomato River-*Sl*	0.291	0.296	3.590

Table 17. Numbers of stomachs processed from Carolina, scalloped and hybrid hammerheads by region. Asterisks (*) after the location denote previously known nursery areas for young-of-year hammerhead sharks.

Location	Carolina	Scalloped	Hybrid	Total
Bulls Bay, SC*	153	78	34	265
Cape Canaveral, FL*	11	24	1	36
Tolomato, FL*		16		16
Nearshore SC	15	9	2	26
Nearshore GA	14	38	8	60
Nearshore FL	8	16		24
Nearshore NC			1	1
Total	201	181	46	428

Table 18. Number of processed stable isotope samples by species (Carolina, scalloped and hybrid hammerheads), tissue and location. Bulls Bay, SC, Cape Canaveral, FL and Tolomato River, FL are known nursery areas for young-of-year hammerhead sharks. The majority of samples were processed for carbon and nitrogen isotope ratios ($\delta^{13}C$ and $\delta^{15}N$, respectively), with a small subset also processed for sulfur ($\delta^{34}S$) using muscle tissue.

	$\delta^{13}C$ and $\delta^{15}N$				$\delta^{34}S$
	Muscle	Liver	Whole blood	Plasma	Muscle
Carolina	**137**	**132**	**112**	**107**	**18**
Bulls Bay	104	104	100	96	9
Cape Canaveral	11	11			5
nearshore SC	11	10	10	9	2
nearshore GA	5	1			2
nearshore FL	6	6	2	2	
Scalloped	**139**	**121**	**92**	**90**	**18**
Bulls Bay	65	65	64	65	10
Cape Canaveral	22	23	3		7
Tolomato River	5	5	5	5	
nearshore SC	12	10	12	12	1
offshore SC	4	4	4	4	
nearshore GA	20	3	1	1	
nearshore FL	11	11	3	3	
Hybrid	**20**	**20**	**19**	**18**	
Bulls Bay	17	18	18	17	
Cape Canaveral	1	1			
nearshore SC	1	1	1	1	
nearshore GA	1				

Table 19. Summary statistics (β, p-value (p) and Pearson correlation coefficient(PCC)) for general linear models investigating the effect of environmental variables (water temperature (°C), salinity in parts per thousand (PPT), and dissolved oxygen (DO) mg/L) on abundance of Carolina and scalloped hammerheads.

	Carolina Hammerhead			Scalloped Hammerhead		
Parameter	β	p	PCC	β	p	PCC
Water Temp	-0.052	0.219	0.033	-0.004	-0.046	-0.196
Salinity	0.109	<0.001	0.223	0.950	0.275	0.274
DO	-0.474	<0.001	-0.227	0.001	-0.071	-0.067

Table 20. The Percent Index of relative importance (%IRI) values of prey items identified to the lowest possible taxon found in Carolina, scalloped and hybrid hammerheads combined from Florida, Georgia, South Carolina and North Carolina. Out of 428 stomachs analyzed, 16 were empty. %IRI values were calculated for 3 different groupings of prey items: by higher prey category (highlighted in dark gray), by identified family (highlighted by light gray), and by lowest possible taxon (unhighlighted). The presence of 0.00 within a category indicates that the prey item was found in stomachs of that species, however the prey was not common enough to be important as calculated by %IRI.

			---%Index of Relative Importance---		
Prey Category	**Family**	**Prey Item**	**Carolina**	**Scalloped**	**Hybrid**
Teleost			**84.34**	**90.83**	**84.21**
	Achiridae	Hogchoker		0.00	
	Arridae	Gafftopsail catfish		0.00	
	Atherinopsidae	Atlantic silverside	0.00	0.00	
	Clupeidae		0.59	0.28	0.47
		Brevoortia spp.	0.10	0.09	0.92
		Atlantic menhaden	0.17	0.07	
		Unidentified clupeid	0.00	0.01	
	Engraulidae		3.43	1.86	1.65
		Anchoa spp.	1.54	1.49	1.00
		Striped anchovy	0.27	0.07	0.40
		Bay anchovy	0.01	0.05	0.02
	Gerreidae	Unidentified mojarra	0.02		0.04
	Mugilidae	Unidentified mullet	0.01	0.01	
	Paralichthyidae		0.02	0.04	0.01
		Bay whiff	0.00	0.01	0.02
		Fringed flounder		0.03	
		Unidentified flatfish	0.01		
	Sciaenidae		59.90	83.93	76.35
		Star drum	17.87	32.21	34.85
		Spot	1.30	0.80	4.74
		Banded drum	0.85	0.04	0.12
		Menticirrhus spp.	0.73	2.13	
		Cynoscion spp.	0.08	0.90	0.34
		Atlantic croaker	0.03	0.64	3.20
		Unidentified Sciaenid	0.03	0.23	0.79
		Silver seatrout	0.01	0.06	
		Whiting		0.00	0.03
		Spotted seatrout		0.00	

		Silver perch	0.00	0.00	
	Sparidae		0.01		0.03
		Pinfish	0.01		0.06
	Trichiuridae	Atlantic cutlassfish	0.00		
	Other fishes	Unidentified teleost	59.82	48.33	33.48
		Unidentified snake eel	0.00		
Crustacean			**14.81**	**8.62**	**15.21**
	Penaeidae		32.87	12.14	19.78
		Penaeid shrimp	7.76	6.41	11.53
		White shrimp	3.41	1.53	2.95
		Brown shrimp	1.27	0.23	1.00
		Seabob	0.01	0.01	
	Paguridae	Flat-clawed hermit crab	0.00	0.00	0.09
	Squillidae	Unidentified mantis shrimp		0.00	0.03
	Upogebiidae	Flat-browed mud shrimp		0.01	
	Other crustaceans	Unidentified shrimp	0.95	1.66	1.52
		Unidentified hermit crab	0.01	0.01	
		Unidentified crustacean	0.36	0.27	0.15
		Unidentified mud shrimp	0.03	0.00	0.17
		Unidentified crab	0.01	0.25	0.02
Mollusc			**0.84**	**0.54**	**0.58**
	Loliginidae		3.16	1.72	1.64
		Loliginid Squid	3.34	2.71	2.49
		Atlantic brief squid			0.04
	Other molluscs	Unidentified conch	0.00		
		Unidentified clam	0.00		
		Unidentified mollusc		0.00	
Unknown taxon			0.01	0.01	

Table 21. Dietary overlap and niche overlap values calculated by Schoener's Overlap Index (α) and Morisita's index (C_D), respectively, between species across all states and then between species within each state. Indices were calculated using the total number of each prey type found within the specified set of stomachs. There was only 1 hybrid stomach analyzed from Florida, therefore the indices were not calculated between full species and hybrids.

Species Comparison			**State**	**Sex**	**α**	**C_D**
Carolina	vs.	ccalloped	All	All	0.80	0.96
Carolina	vs.	hybrid	All	All	0.83	0.98
Scalloped	vs.	hybrid	All	All	0.81	0.96
Carolina	vs.	ccalloped	SC	All	0.82	0.97
Carolina	vs.	hybrid	SC	All	0.78	0.96
Scalloped	vs.	hybrid	SC	All	0.74	0.91
Carolina	vs.	ccalloped	GA	All	0.71	0.87
Carolina	vs.	hybrid	GA	All	0.70	0.89
Scalloped	vs.	hybrid	GA	All	0.69	0.87
Carolina	vs.	scalloped	FL	All	0.78	0.97

Table 22. Summary of prey quantity and diversity in stomachs of young-of-year Carolina, scalloped and hybrid hammerheads across the southeast U.S. coast.

	Carolina	Scalloped	Hybrid
Number of stomachs	201	184	46
% of empty stomachs	4.5	3.3	4.3
% stomachs with more than 1 prey item	78	89	91
% stomachs with more than 2 prey item	59	77	83
% stomachs with more than 5 prey item	14	29	30
% stomachs with more than 1 prey taxa	71	84	91
% stomachs with more than 2 prey taxa	41	57	70
% stomachs with more than 3 prey taxa	20	32	20

Table 23. The niche width of each species by state as calculated with the Levin's Index using stomach content data.

	Carolina	Scalloped	Hybrid
All States	6.84	4.82	7.17
SC	7.02	5.06	8.23
GA	6.36	4.14	4.33
FL	2.92	3.61	x

Table 24. The % Index of relative importance (%IRI) values of prey items identified to the lowest possible taxon found in South Carolina, Georgia and Florida (all hammerhead species combined). Out of 427 stomachs analyzed, 16 were empty. %IRI values were calculated for 3 different groupings of prey items: by higher prey category (highlighted in dark gray), by identified family (highlighted in light gray), and by lowest possible taxon (unhighlighted). The presence of 0.00 within a category means that the prey item was found in stomachs collected from that state, however the prey was not common enough to be important as calculated by %IRI.

			----------%IRI----------		
Prey Category	**Family**	**Prey Item**	**FL**	**GA**	**SC**
Teleost			89.08	92.11	85.55
	Achiridae		0.08		
		Hogchoker	0.05		
	Arridae	Gafftopsail catfish	0.02		
	Atherinopsidae	Atlantic silverside			0.01
	Clupeidae		0.07	0.35	0.47
		Brevoortia spp.	0.05	0.26	0.10
		Atlantic menhaden			0.20
		Unidentified Clupeid		0.04	0.00
	Engraulidae		0.55	2.76	2.68
		Anchoa spp.	0.16	2.00	1.67
		Striped anchovy	0.01	0.13	0.20
		Bay anchovy	0.01	0.06	0.02
	Gerreidae	Unidentified mojarra			0.01
	Mugilidae		0.50		0.00
		Unidentified mullet	0.35		0.00
	Paralichthyidae		0.40	0.00	0.01
		Fringed flounder	0.12	0.01	
		Bay whiff	0.02		0.00
		Unidentified flatfish			0.00
	Sciaenidae		70.24	87.23	69.31
		Star drum	9.20	56.94	21.55
		Spot	0.65	0.11	2.07

		Banded drum			0.68
		Cynoscion spp.	1.43	0.01	0.39
		Menticirrhus spp.	0.42	4.45	0.51
		Atlantic croaker	0.31	0.71	0.21
		Unidentified Sciaenid	0.30	0.01	0.20
		Silver seatrout			0.05
		Silver perch			0.01
		Spotted seatrout			0.00
		Whiting			0.00
	Sparidae	Pinfish			0.01
	Trichiuridae	Atlantic cutlassfish			0.00
	Other fishes	Unidentified teleost	75.26	25.23	54.60
		Unidentified snake eel			0.00
Crustacean			10.37	7.22	13.74
	Penaeidae		25.62	7.91	25.04
		Penaeid shrimp	5.69	4.38	8.57
		White shrimp	2.16	0.80	3.02
		Brown shrimp	0.11	0.03	1.21
		Seabob		0.08	0.00
	Paguridae	Flat-clawed hermit crab			0.01
	Squillidae	Unidentified mantis shrimp			0.00
	Upogebiidae	Flat-browed mud shrimp			0.00
	Other Crustaceans	Unidentified shrimp	1.66	1.85	1.04
		Unidentified crustacean	0.25	0.23	0.30
		Unidentified hermit crab		0.04	0.00
		Unidentified mud shrimp			0.05
		Unidentified crab			0.00
Mollusc			0.55	0.67	0.71
	Loliginidae		2.52	1.75	2.46
		Loliginid squid	1.75	2.63	3.26
		Atlantic brief squid			0.00
	Other molluscs	Unidentified mollusc	0.01		
		Unidentified clam			0.00
		Unidentified conch		0.01	

Table 25. Summary of stable isotopic data by tissue and species. The standard deviation (±SD) are shown with the $\delta^{13}C$ and $\delta^{15}N$ means. CD, or the mean distance to the centroid, is the average degree of trophic diversity within a species and the SEAc, or standard ellipse area corrected for small sample size, represents an estimate of isotopic niche width.

Tissue	Species	n	$\delta^{13}C$ mean	$\delta^{15}N$ mean	C range	N range	CD	SEAc
Muscle	Carolina	136	-16.57±0.48	16.22±1.27	2.61	5.98	1.17	1.92
Muscle	Scalloped	131	-15.90±0.43	15.64±1.22	2.56	5.89	1.14	1.63
Muscle	Hybrid	20	-16.21±0.25	16.43±1.18	1.16	3.86	1.06	0.95
Liver	Carolina	131	-16.47±0.59	14.07±1.58	3.06	6.95	1.45	2.41
Liver	Scalloped	113	-16.02±0.60	13.89±1.13	3.07	5.52	1.11	1.94
Liver	Hybrid	20	-16.18±0.42	14.22±1.41	1.36	6.33	1.17	1.82
Whole blood	Carolina	111	-16.42±0.37	14.03±1.29	2.18	5.44	1.13	1.49
Whole blood	Scalloped	84	-16.20±0.42	13.87±1.13	2.18	4.59	1.06	1.28
Whole blood	Hybrid	19	-16.30±0.30	13.78±1.18	1.40	4.93	0.93	1.16
Plasma	Carolina	106	-17.41±0.61	13.48±1.73	2.93	6.41	1.59	3.32
Plasma	Scalloped	82	-17.23±0.56	13.52±1.52	2.76	5.86	1.43	2.66
Plasma	Hybrid	18	-17.17±0.31	13.20±1.61	1.11	5.83	1.23	1.53

Figures

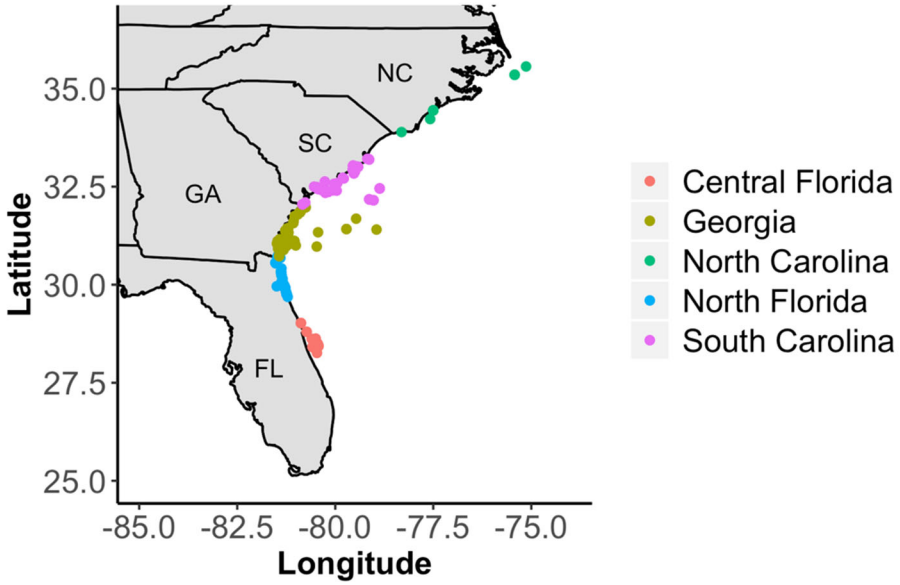

Figure 1. Map of sampling locations colored by region. Samples of scalloped and Carolina hammerheads were primarily collected by the P.I.s, collaborators and cooperators, but samples were also opportunistically collected from fishery independent and dependent sources.

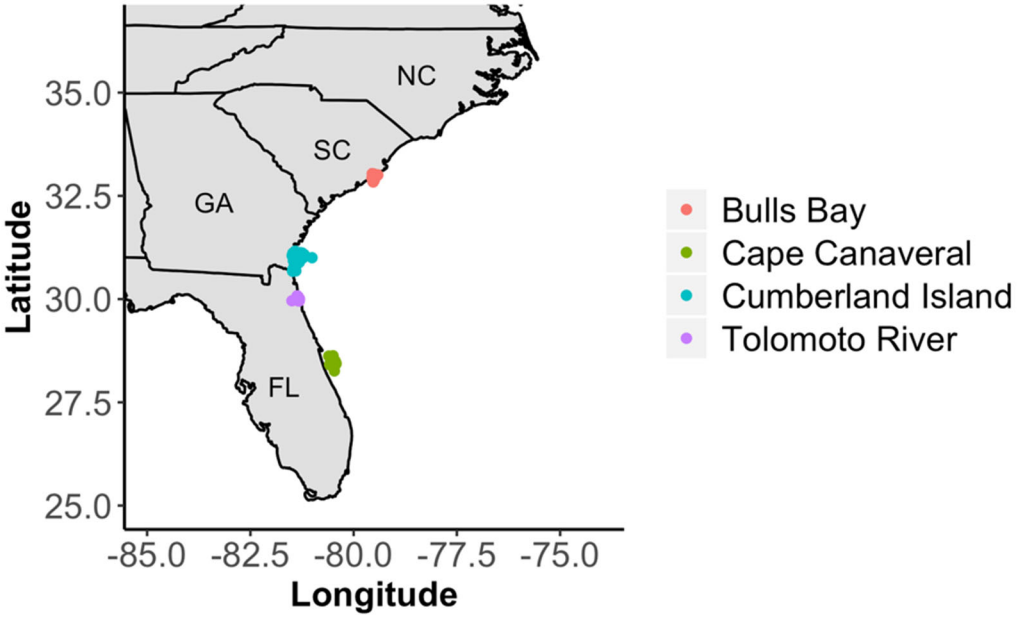

Figure 2. Map of samples taken in nursery sites. The nearshore waters off Georgia were not previously known to be a nursery area for scalloped and Carolina hammerheads.

Figure 3. Map of GADNR study site and sampling locations. Gillnet sets were paired with longline sets in established long-term study sites and were also set in experimental locations to attempt to determine potential hammerhead nursery areas.

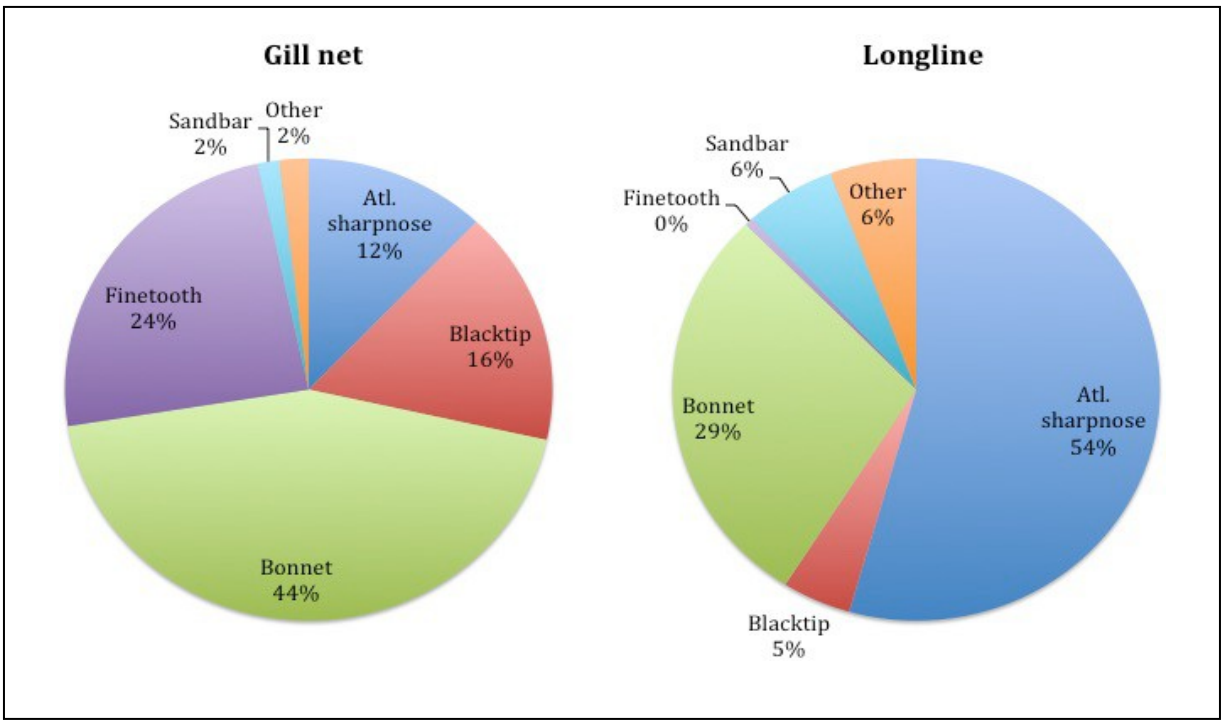

Figure 4. Catch of sharks from GADNR sampling by gear (n = 654) during the two-year study period. Gillnets were set in close proximity to longline sampling sites as well as in experimental locations.

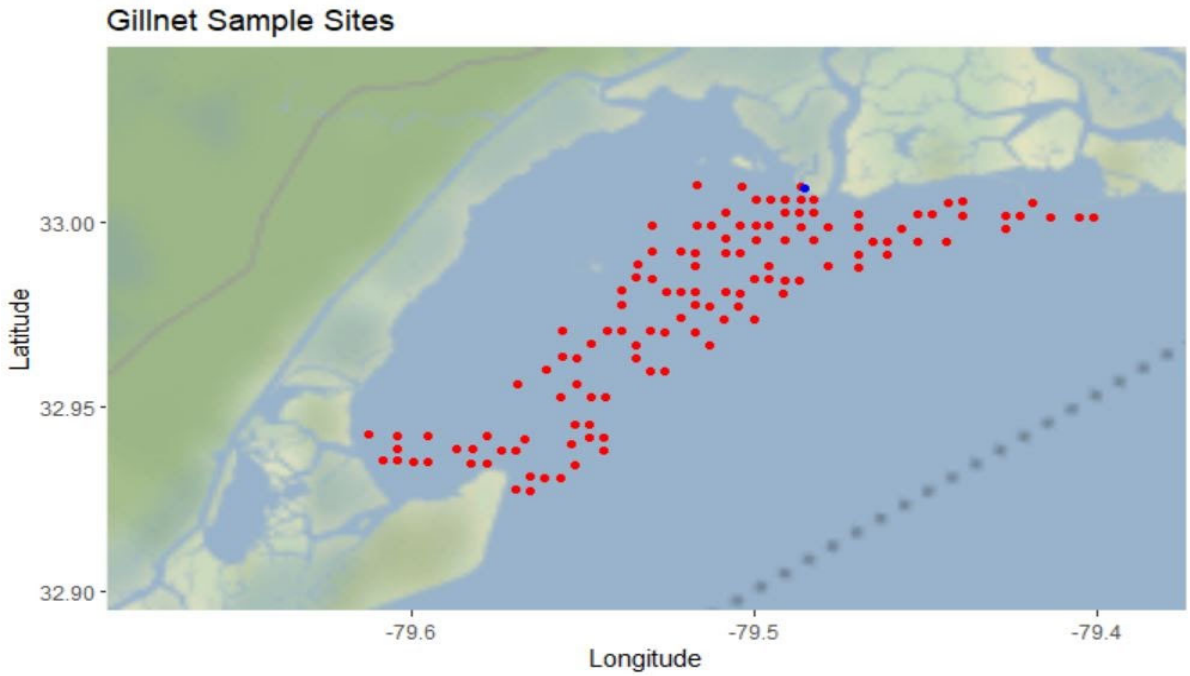

Figure 5. Locations of stratified random gillnet sets (red) and the South Carolina Department of Natural Resources index gillnet station (blue). Waters inshore of sampling sites were too shallow (<0.2 m at low tide), while waters offshore too deep for gillnet sampling (>4.0 m).

Figure 6. Catches of hammerhead sharks from stratified random gillnet sets in Bulls Bay, SC. Size of dots indicates number of hammerheads captured with small circles = 1, and the largest circles = 10. The majority of hammerheads captured were in close proximity to the South Carolina Dept of Natural Resources long-term large gillnet index station indicated by the asterisk (*) on the map.

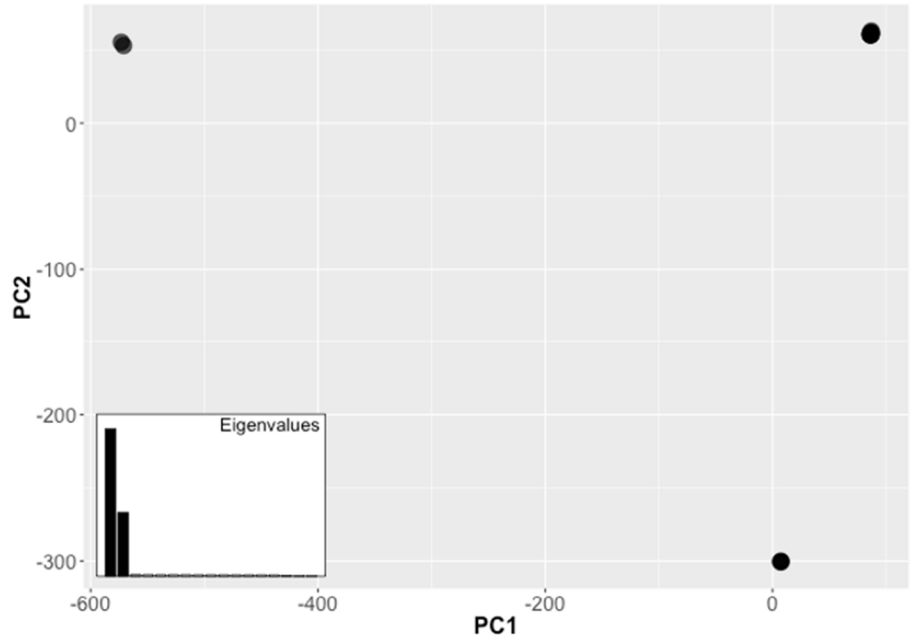

Figure 7. Results of PCA on SNP dataset generated from initial sequencing run demonstrating how well the species can be discriminated. Points in the upper left corner are great hammerheads, upper right are scalloped hammerheads, and bottom are Carolina hammerheads.

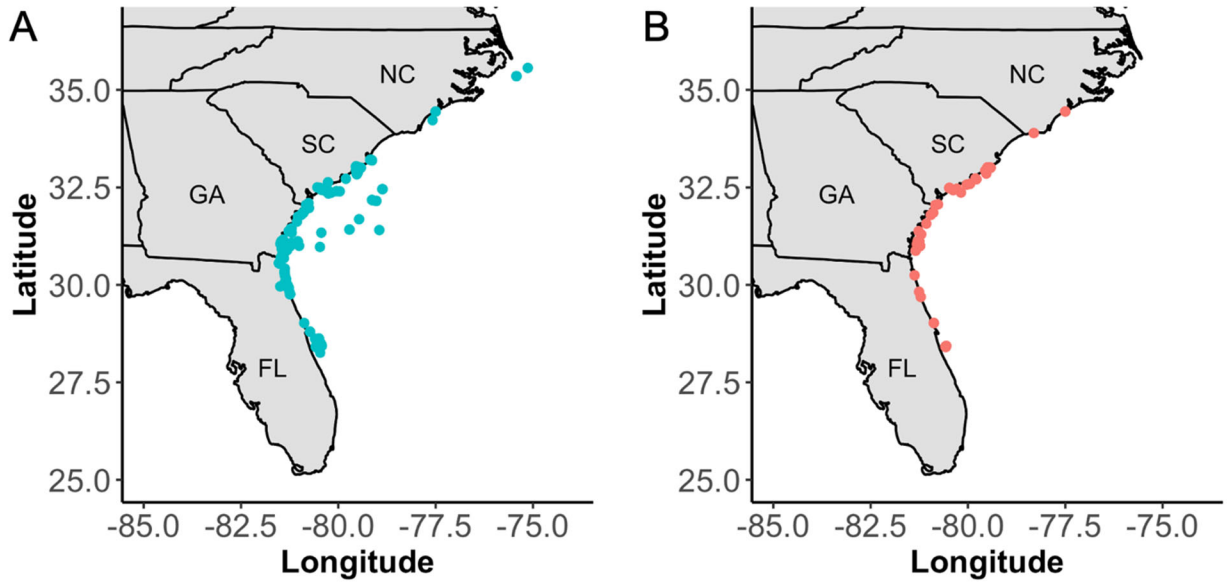

Figure 8. Distribution of scalloped (A) and Carolina hammerheads (B). While Carolina hammerheads were found along the same range as scalloped hammerheads, abundance of Carolina hammerheads was highest in South Carolina, an no Carolina hammerheads were captured in the Tolomato River in Florida.

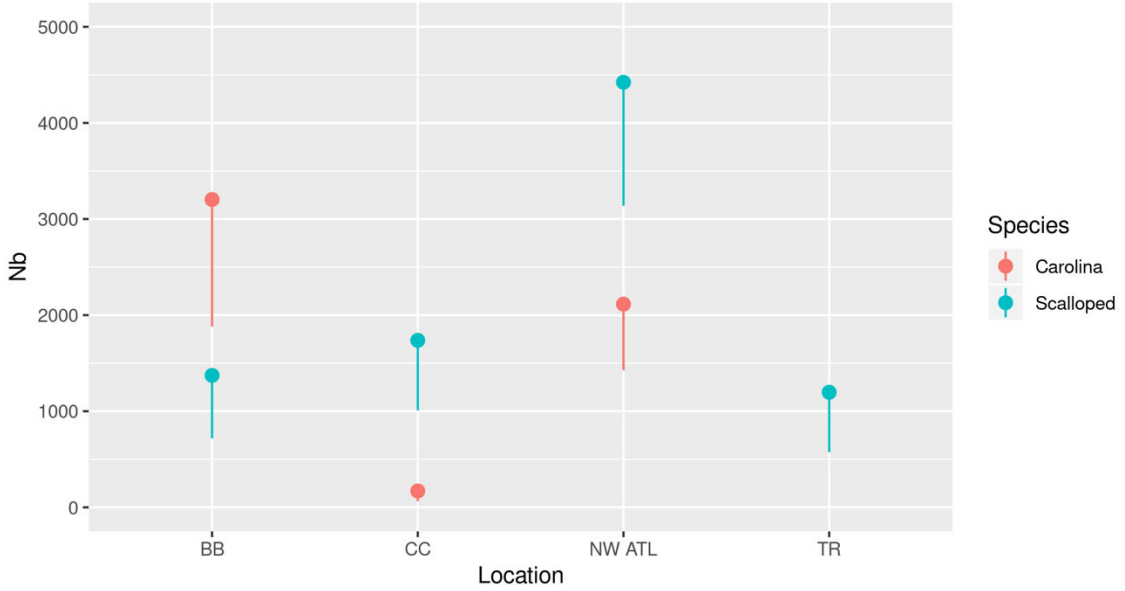

Figure 9. Effective number of breeders (N_b) for Bulls Bay, SC (BB), Cape Canaveral, FL (CC), Tolomato River, FL, and the northwest Atlantic overall (NW ATL). Point estimate and lower 95% confidence interval shown.

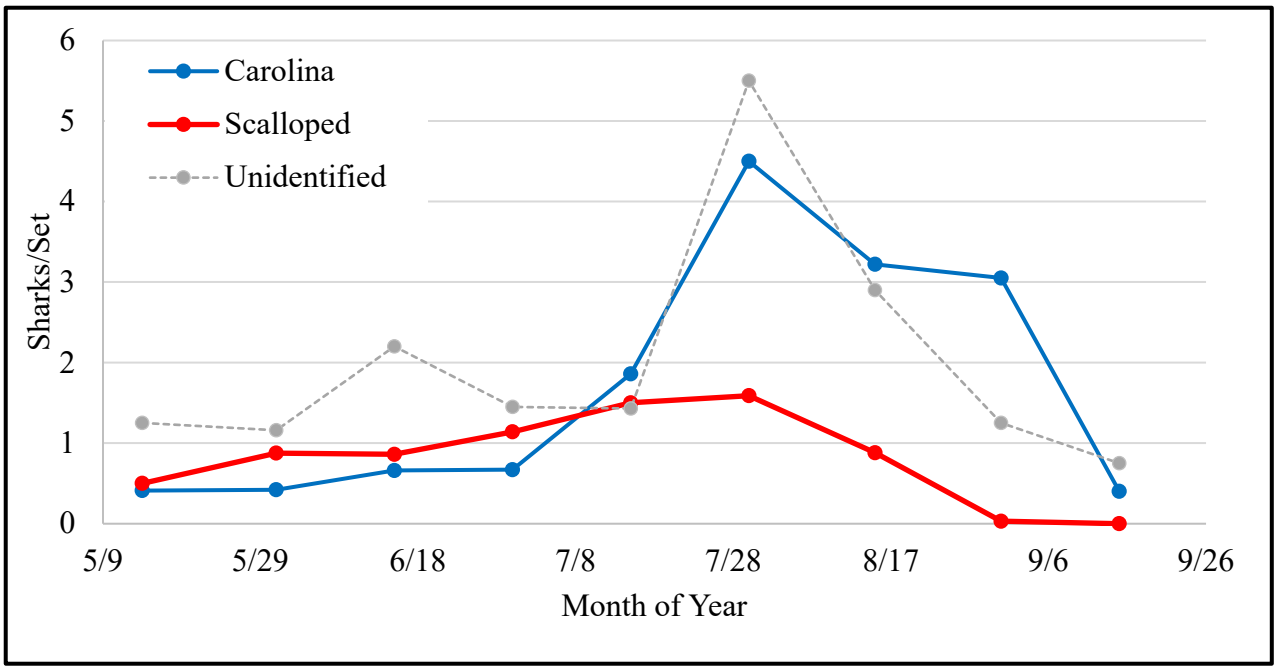

Figure 10. Catch of young of year Carolina and scalloped hammerheads per set for the South Carolina Department of Natural Resources large gillnet survey 2013-2018. Catches are binned into biweekly increments. Not all sharks captured could be genetically identified due to time and expenses, therefore CPUE of animals that were not analyzed genetically for species (unidentified) are also presented.

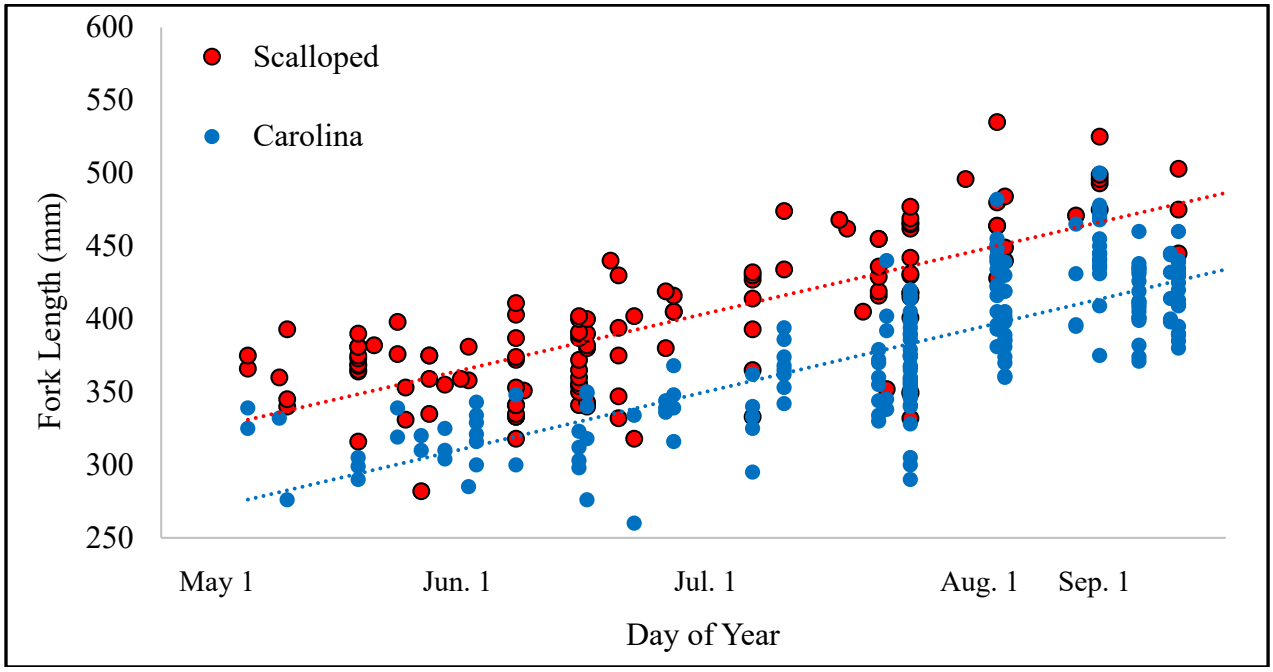

Figure 11. Fork length (mm) of young-of-year scalloped and Carolina hammerheads by day of year.

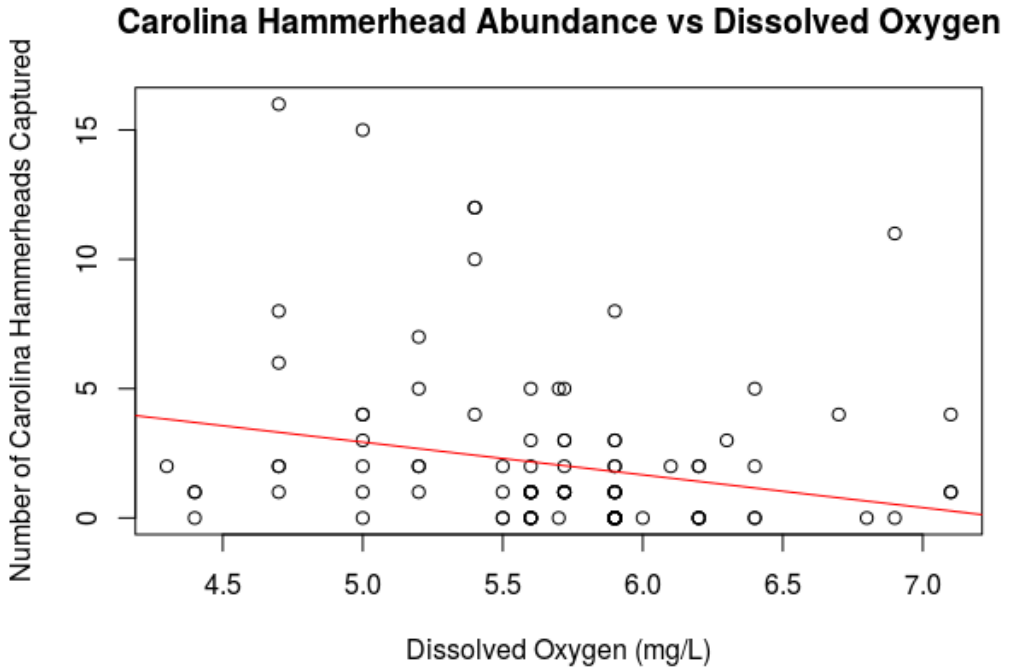

Figure 12. The relationship between dissolved oxygen and Carolina hammerhead abundance. Results indicate a slightly negative relationship between abundance and increasing dissolved oxygen (mg/L).

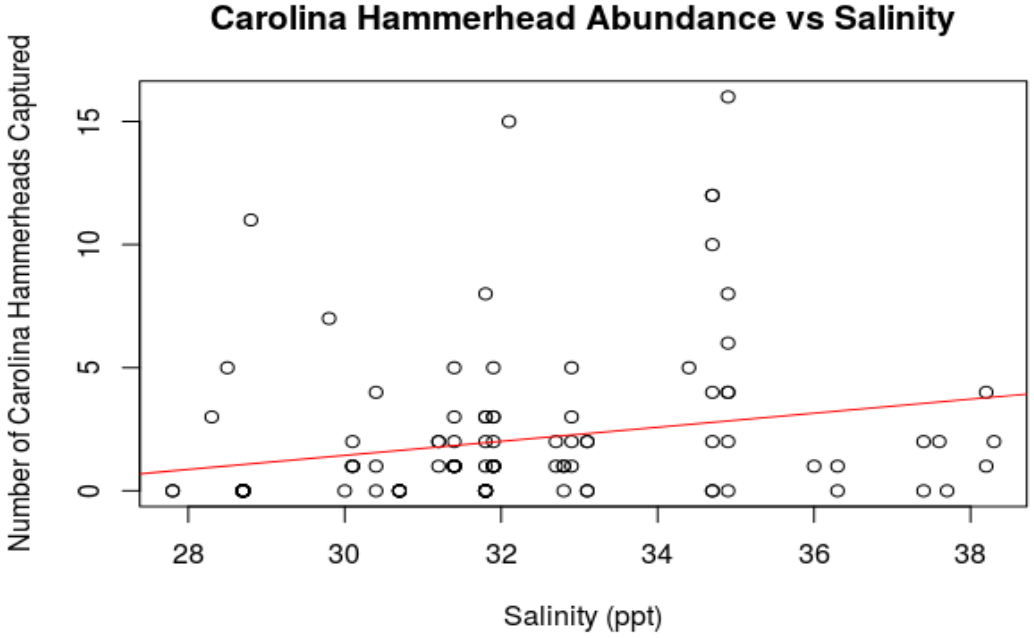

Figure 13. The relationship between salinity (parts per thousand) and Carolina hammerhead abundance. Results indicate a slightly positive relationship between abundance and increasing salinity.

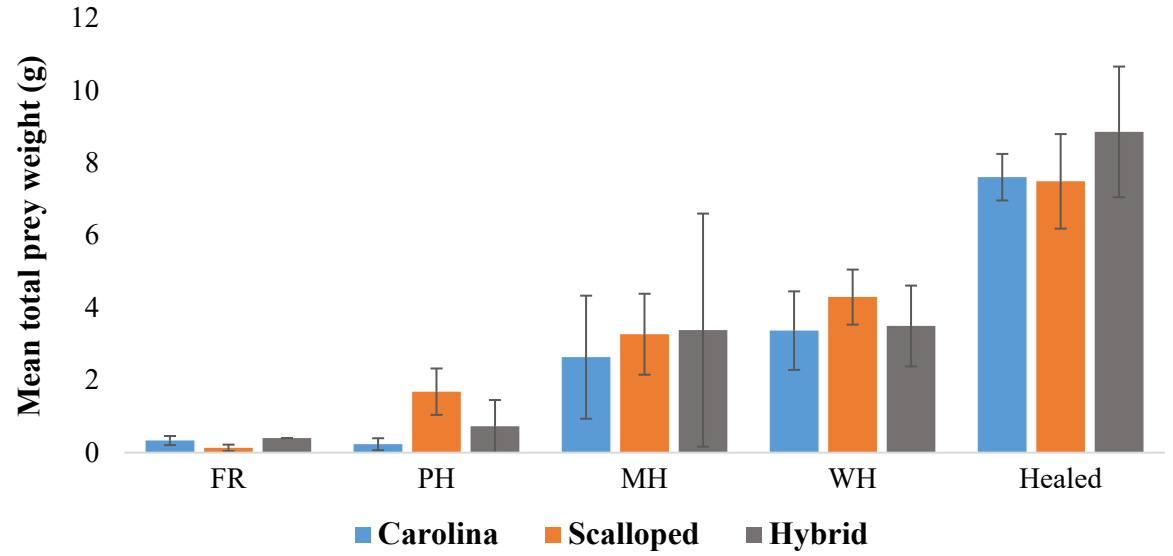

Figure 14. The mean total prey weight of stomach content with standard error for Carolina, scalloped and hybrid hammerheads for each maturity stage which is represented by the level of healing for their umbilical scar. FR = open umbilicus, PH = partially healed, MH = mostly healed, WH = well healed, Healed = no scar present.

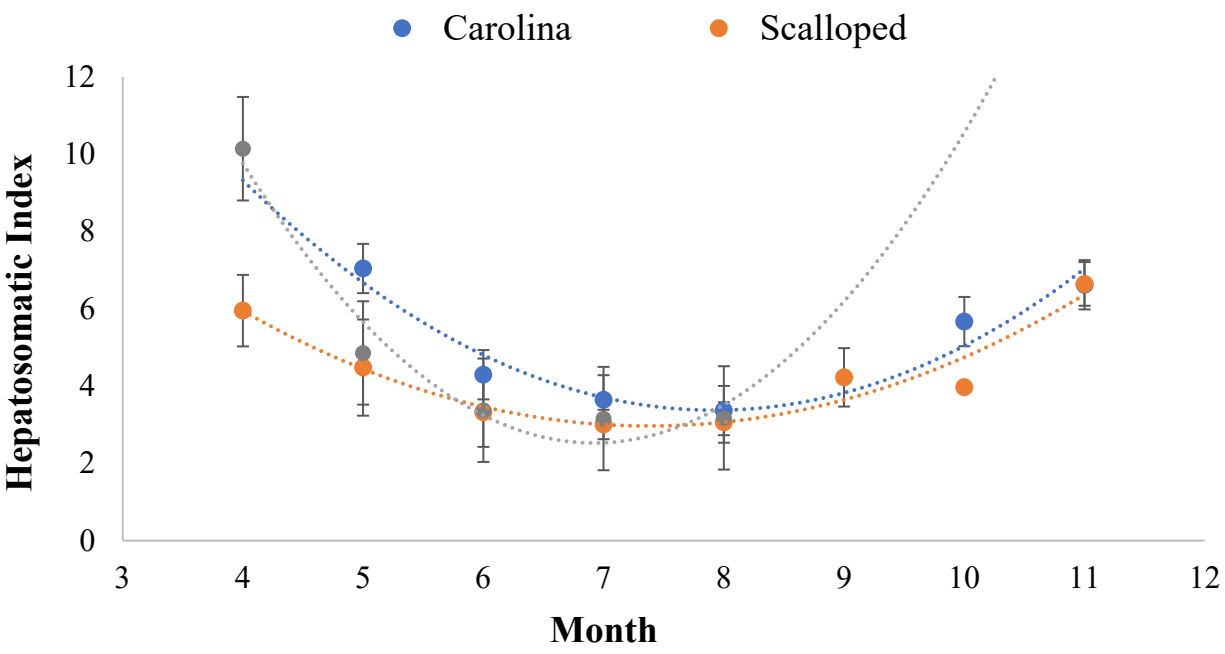

Figure 15. Mean and standard error of hepatosomatic index values (HSI) by month and species. The values of each species were fit with a polynomial regression. R squared values for Carolina, scalloped and hybrid hammerheads are 0.92, 0.92 and 0.96, respectively.

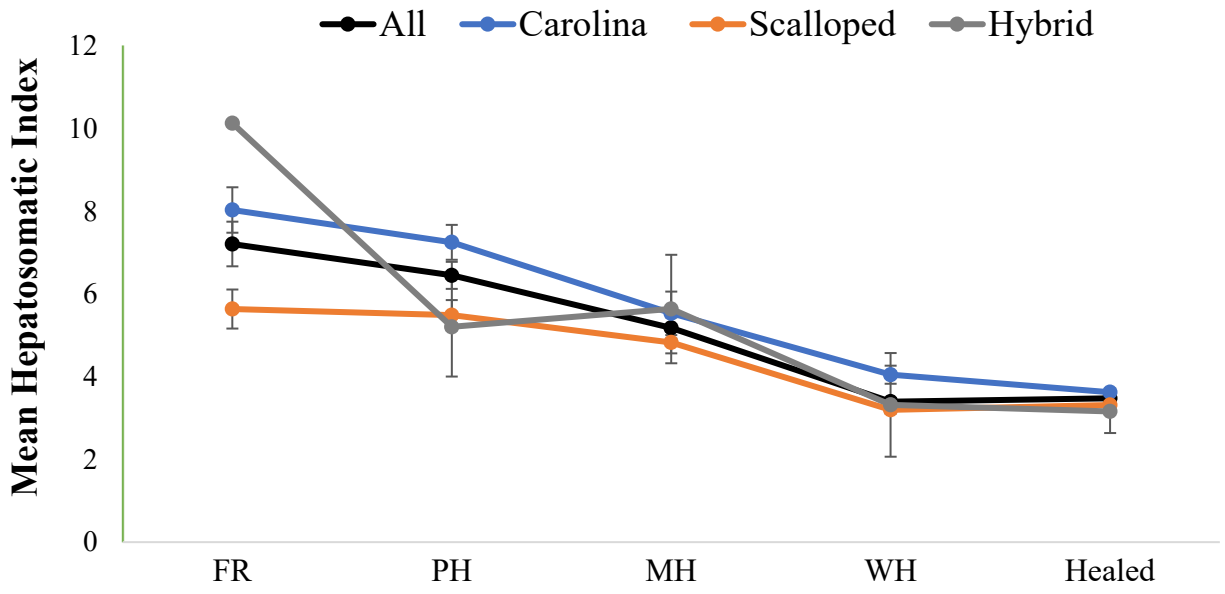

Figure 16. Mean and standard error of hepatosomatic index values (HSI) for Carolina, scalloped and hybrid hammerheads by maturity stage, (the level of healing of the umbilical scar, representing relative age). FR = open umbilicus, PH = partially healed, MH = mostly healed, WH = well healed, Healed = no scar present.

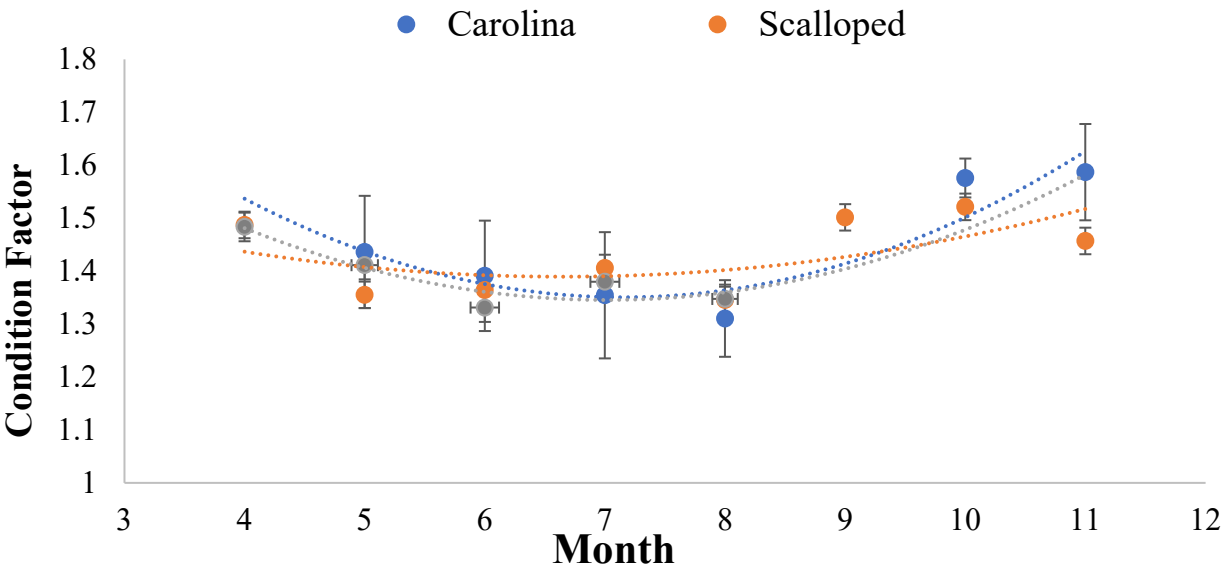

Figure 17. Mean and standard error of condition factor values by month and species. Values of each species were fit with a polynomial regression. R squared values for Carolina, scalloped and hybrid hammerheads are 0.85, 0.38 and 0.85, respectively.

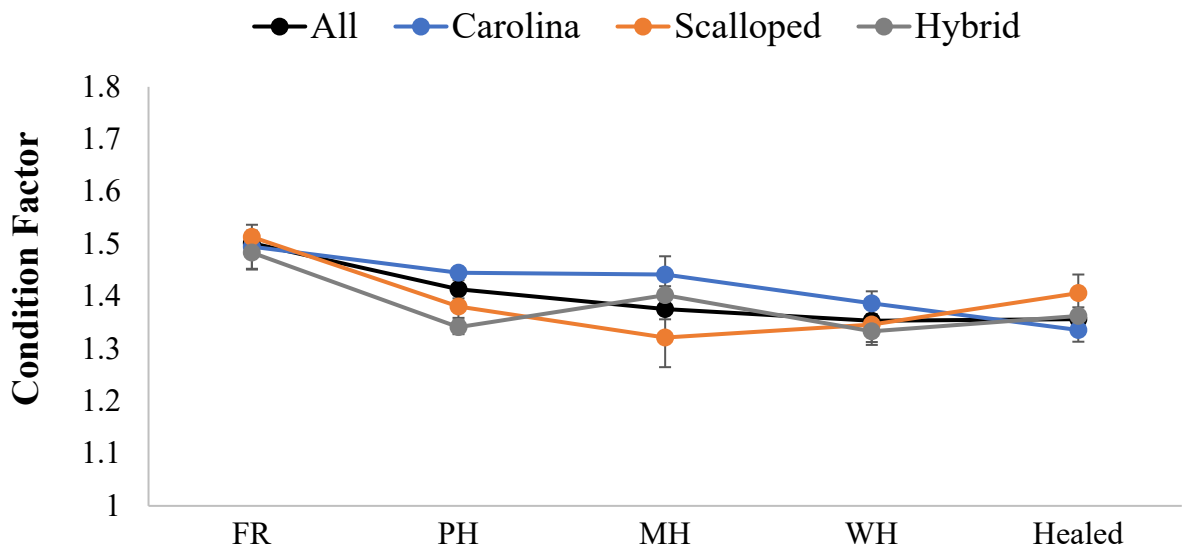

Figure 18. Mean and standard error of relative condition factor by for Carolina, scalloped and hybrid hammerheads by maturity stage, (the level of healing of the umbilical scar, representing relative age). FR = open umbilicus, PH = partially healed, MH = mostly healed, WH = well healed, Healed = no scar present.

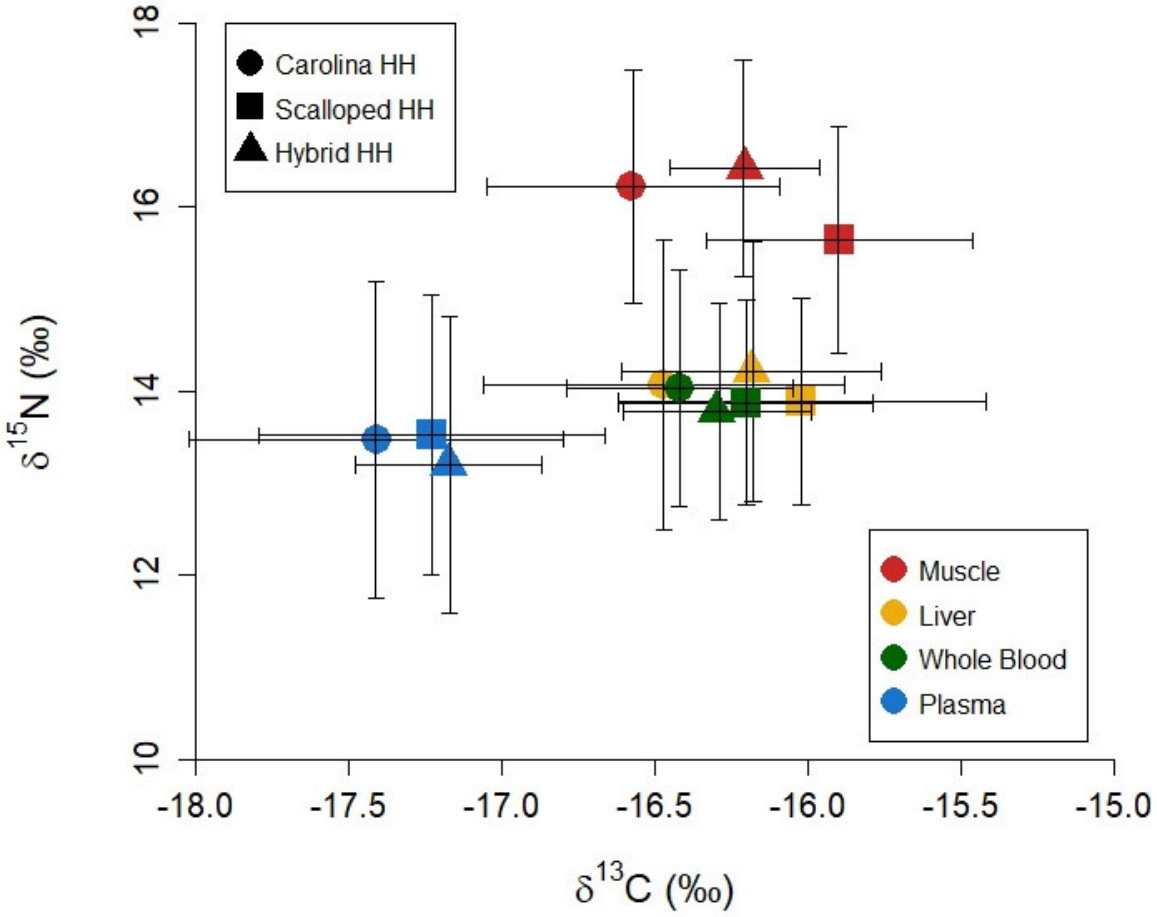

Figure 19. Mean (±SD) isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) of muscle, liver, whole blood and plasma tissues taken from Carolina, scalloped and hybrid hammerheads from the southeast U.S. coast (all states combined). Tissues are represented by different colors, and species are designated by circles, squares, and triangles for Carolina, scalloped and hybrid hammerheads, respectively.

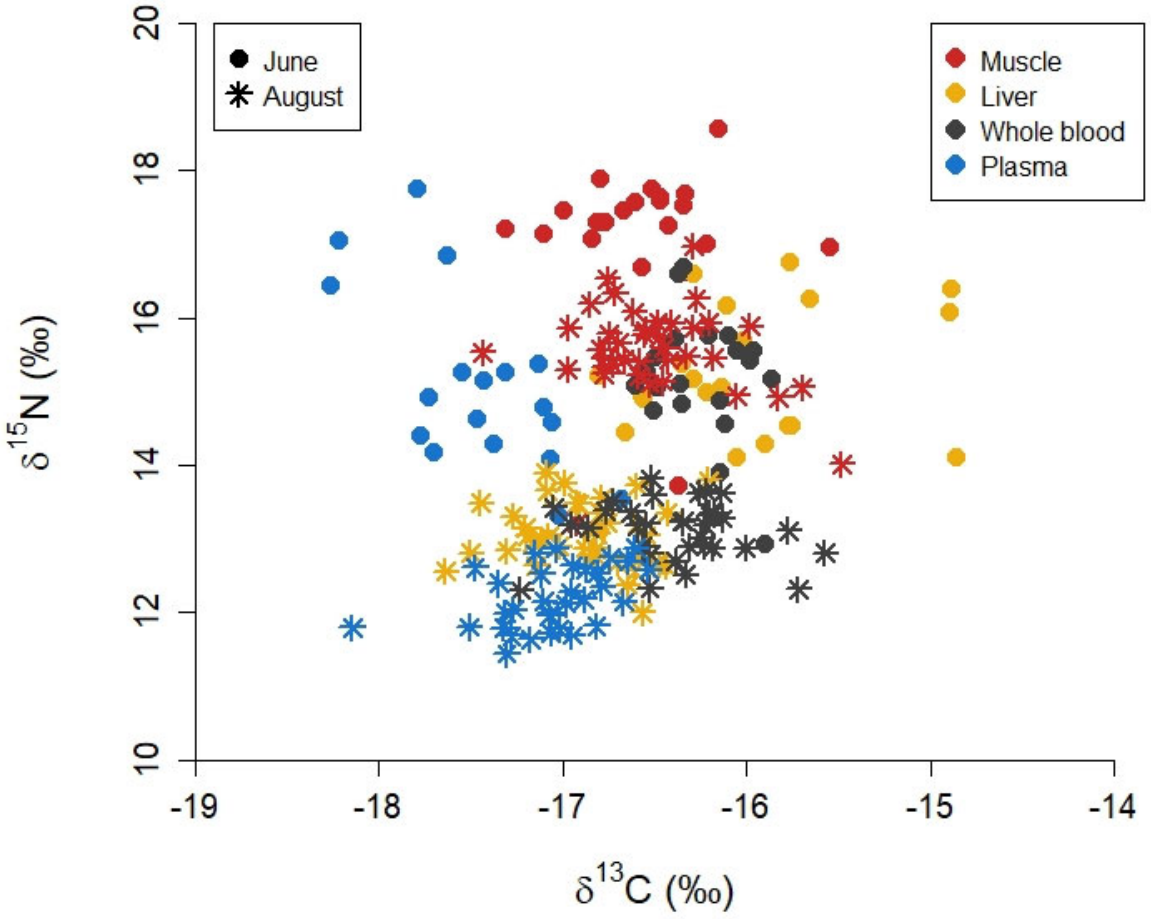

Figure 20. Isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) of muscle, liver, whole blood and plasma of Carolina hammerheads caught in June (solid circles) and August (stars). Tissues are represented by color. Muscle tissue has the slowest turnover rate, followed by whole blood, liver and then plasma. The samples from June represent sharks that have maternal influence on their isotopic signature, while those from August should be reflecting the shark's own diet rather than its mothers'.

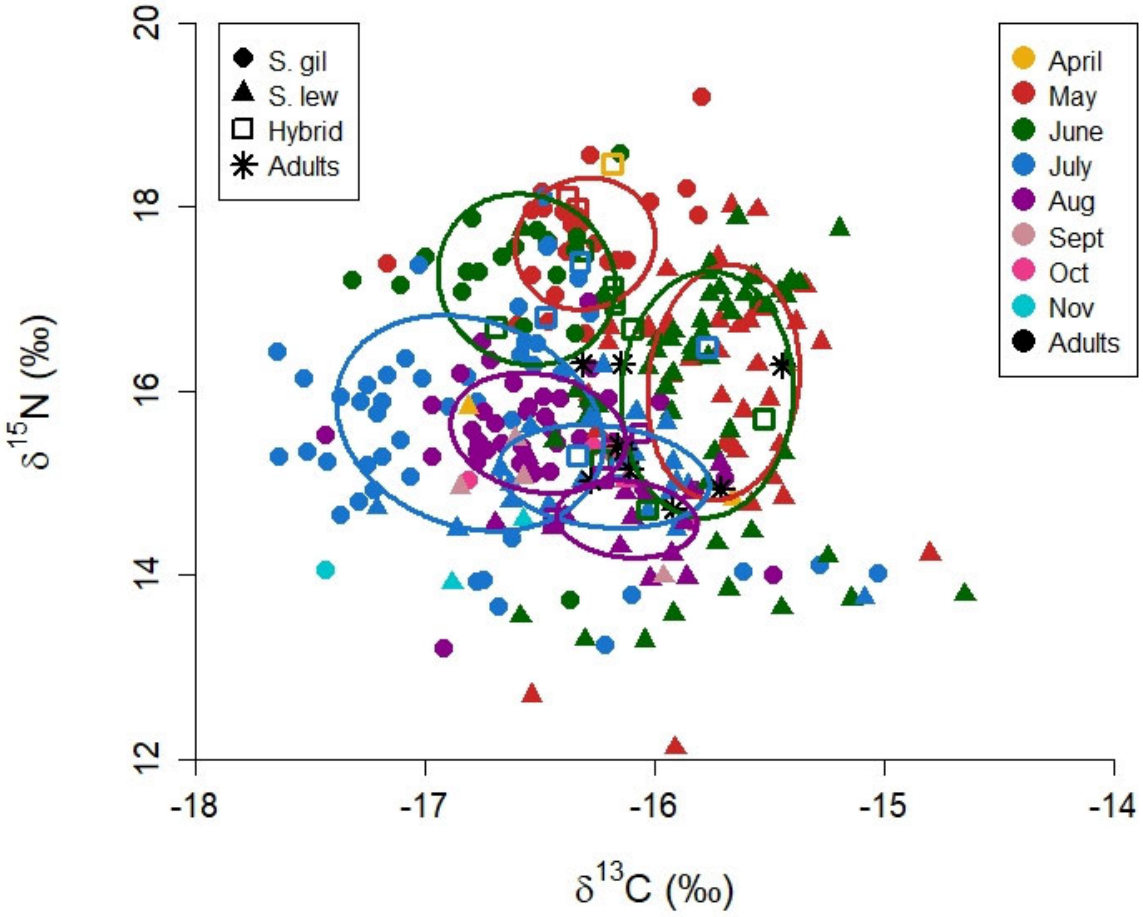

Figure 21. Muscle isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) grouped by month for Carolina, scalloped and hybrid hammerheads (represented by solid circles, solid triangles and open squares, respectively) from the southeast U.S. coast (all states combined). Adult samples (designated by black stars) were added (listed after the months in the legend) to compare to the young-of-year isotopic ratios. Ellipses represent the SEAc (corrected standard ellipse area), or the mean values with standard deviation, for the sharks caught in the months of May-August (color coded according to the month it represents). The ellipses around the circles and triangles represent the isotopic niche of the Carolina and scalloped hammerheads, respectively, during those months. Overlap between ellipses of the same color represent an overlap in isotopic niche between the species.

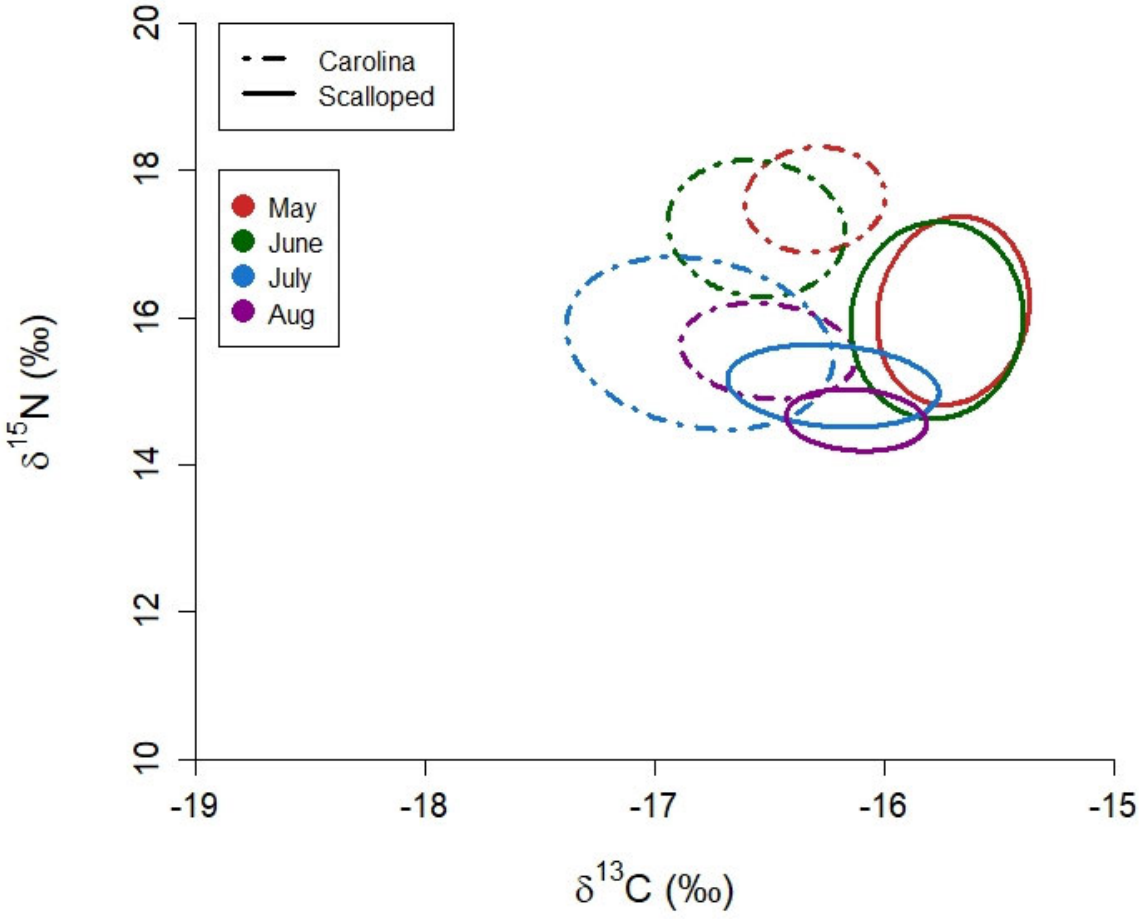

Figure 22. Standard ellipse areas corrected for small sample size (SEAc) for the sharks caught in the months of May-August for Carolina and scalloped hammerhead muscle samples from the southeast U.S. coast (all states combined). Ellipses represent the mean values of each group with standard deviation, or the isotopic niche, and overlap between ellipses represent an overlap in isotopic niche. Carolina and scalloped hammerheads are designated by dashed and solid lines, respectively.

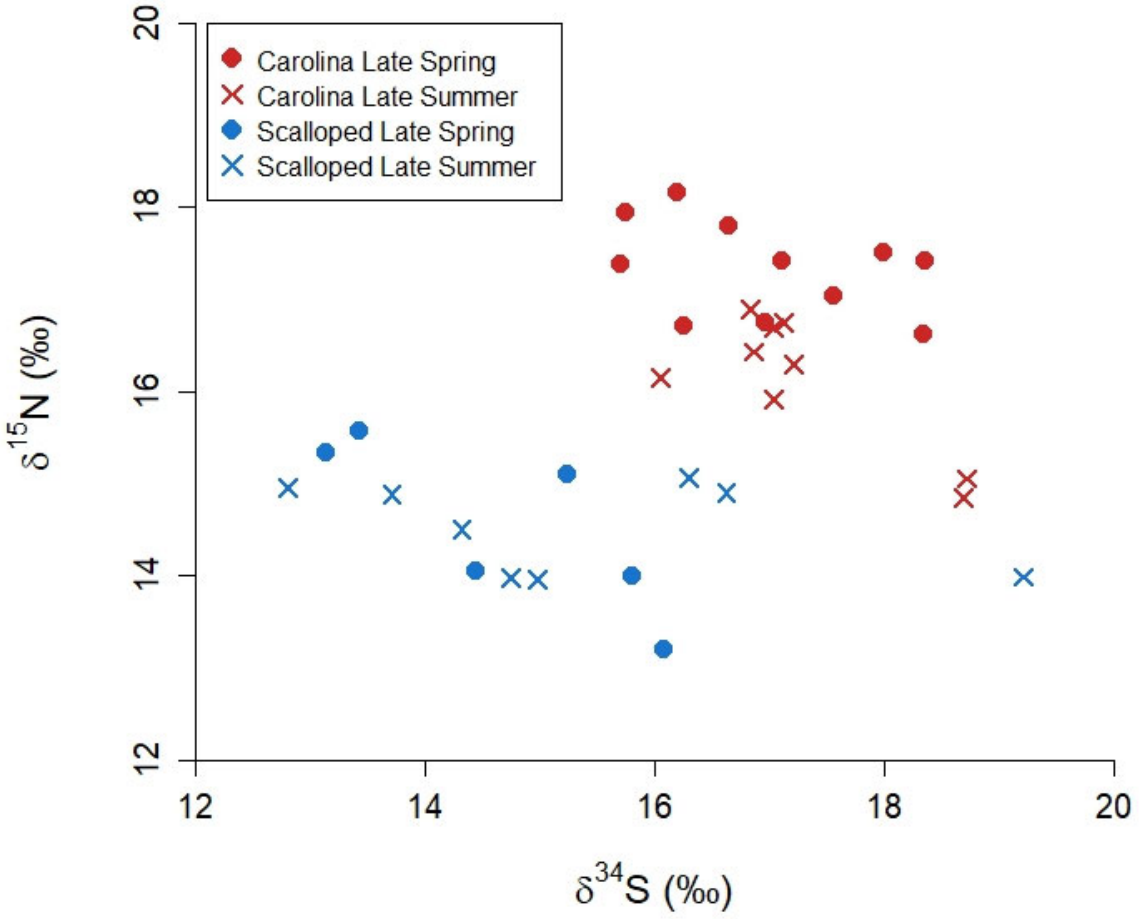

Figure 23. Sulfur ($\delta^{34}S$) and nitrogen ($\delta^{15}N$) ratios for Carolina and scalloped hammerheads (represented by red and blue, respectively) caught in late Spring (late April and May, represented by a solid circle) and late summer (August, represented by and 'x'). $\delta^{15}N$ infers trophic level while $\delta^{34}S$ distinguishes between basal resources (pelagic or benthic).

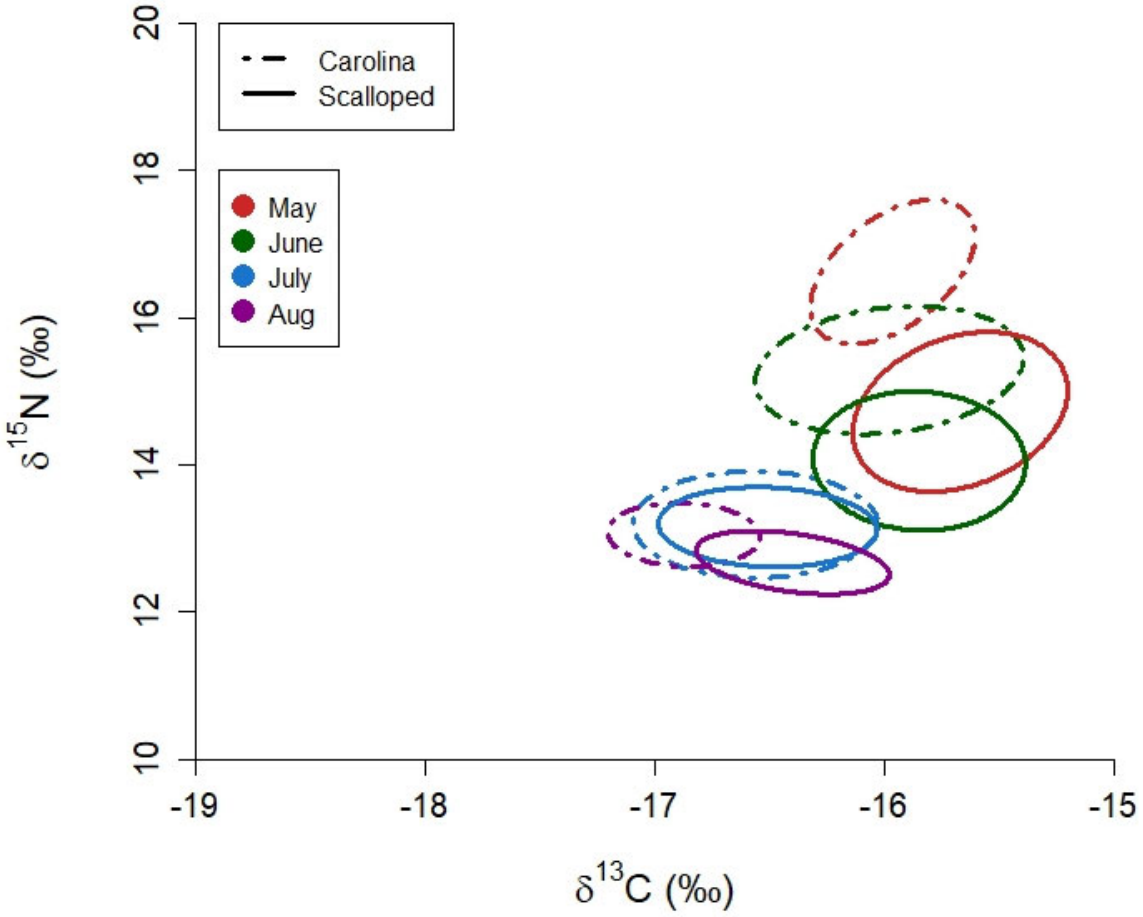

Figure 24. Standard ellipse areas corrected for small sample size (SEAc) for the sharks caught in the months of May-August for Carolina and scalloped hammerhead liver samples from the southeast U.S. coast (all states combined). Ellipses represent the mean values of each group with standard deviation, or the isotopic niche, and overlap between ellipses represent an overlap in isotopic niche. Carolina and scalloped hammerheads are designated by dashed and solid lines, respectively.

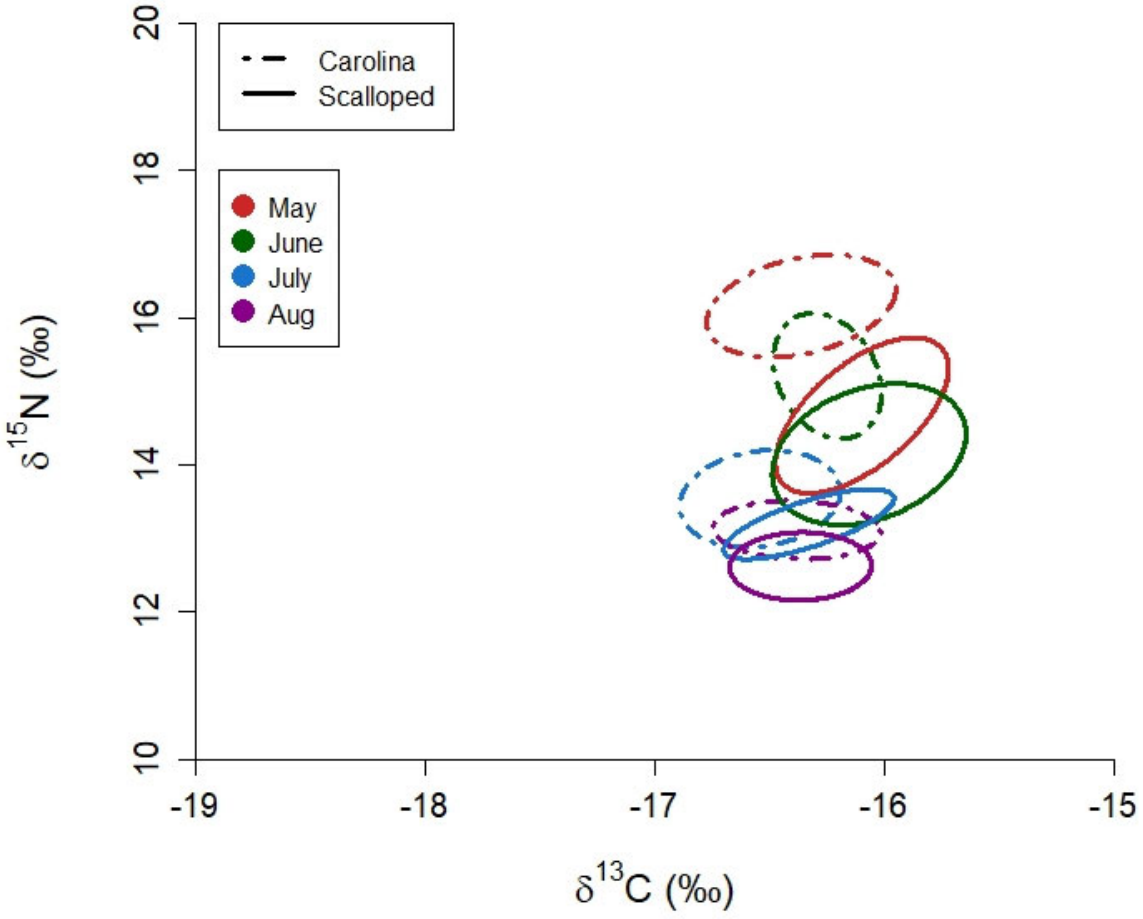

Figure 25. Standard ellipse areas corrected for small sample size (SEAc) for the sharks caught in the months of May-August for Carolina and scalloped hammerhead whole blood samples from the southeast U.S. coast (all states combined). Ellipses represent the mean values of each group with standard deviation, or the isotopic niche, and overlap between ellipses represent an overlap in isotopic niche. Carolina and scalloped hammerheads are designated by dashed and solid lines, respectively.

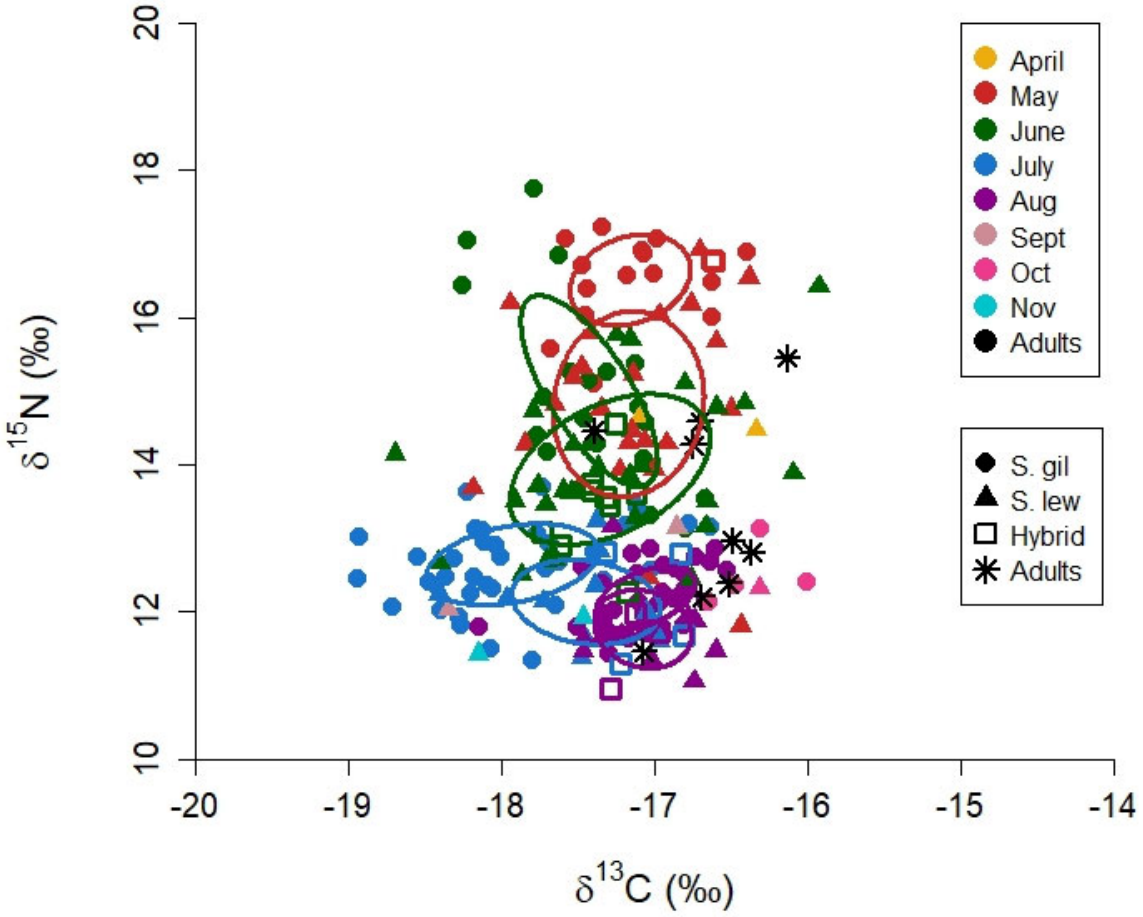

Figure 26. Plasma isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) by month of Carolina, scalloped and hybrid hammerheads (represented by solid circles, solid triangles and open squares, respectively) from the southeast U.S. coast (all states combined). Adult samples (designated by black stars) were added (listed after the months in the legend) to compare to the young-of-year isotopic ratios. Ellipses represent the SEAc (corrected standard ellipse area), or the mean values with standard deviation, for the sharks caught in the months of May-August (color coded according to the month it represents). The ellipses around the circles and triangles represent the isotopic niche of the Carolina and scalloped hammerheads, respectively, during those months. Overlap between ellipses represent an overlap in isotopic niche between the species.

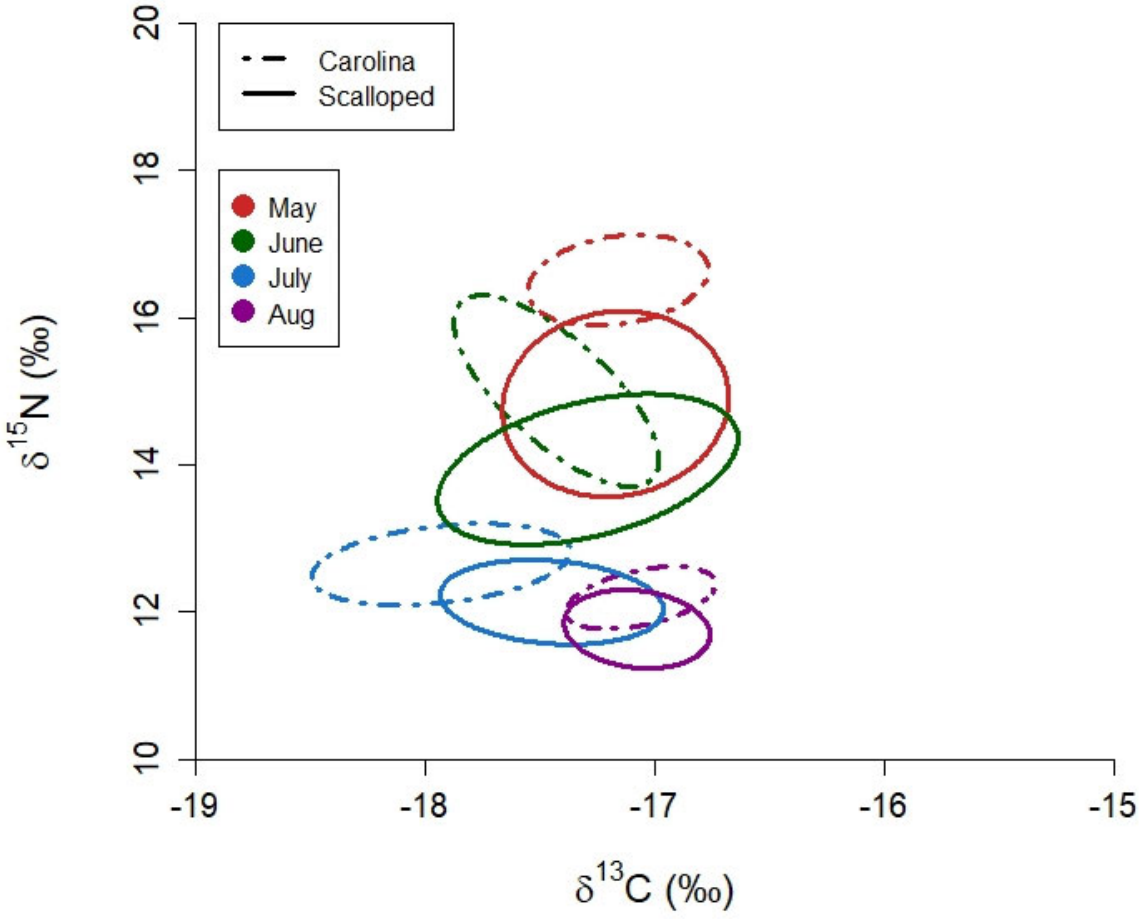

Figure 27. Ellipses calculated by SEAc (corrected standard ellipse area) for the sharks caught in the months of May-August (color coded according to the month it represents) for Carolina and scalloped hammerhead plasma samples from the southeast U.S. coast (all states combined). Ellipses represent the mean values of each group with standard deviation, or the isotopic niche, and overlap between ellipses represent an overlap in isotopic niche. Carolina and scalloped hammerheads are designated by dashed and solid lines, respectively.

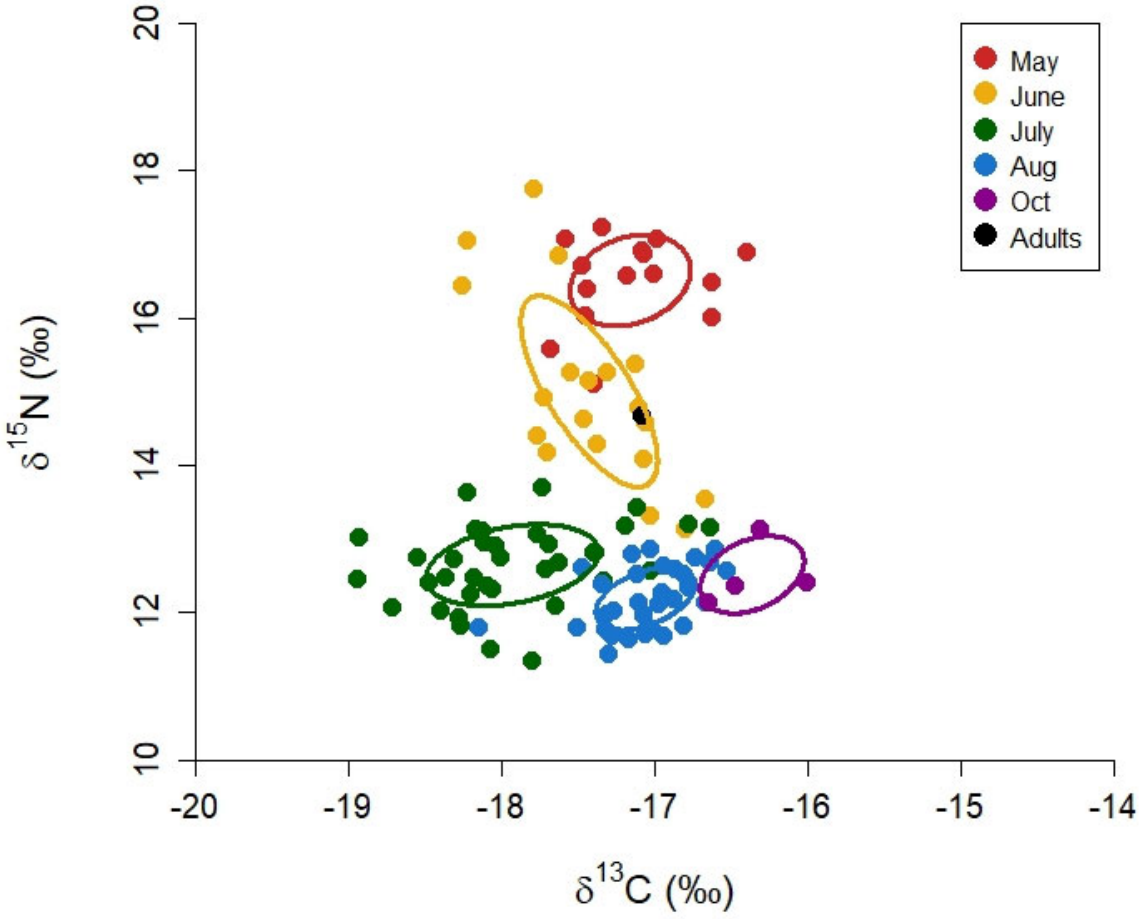

Figure 28. Plasma isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) grouped by month of Carolina hammerheads from the southeast U.S. coast (all states combined). The single adult sample (black circle) was added for comparison to the young-of-year isotopic ratios. The ellipses represent the isotopic niche as calculated by SEAc (corrected standard ellipse area) of the Carolina hammerheads by month they were caught. Overlap between ellipses represent an overlap in isotopic niche of Carolina hammerheads between months.

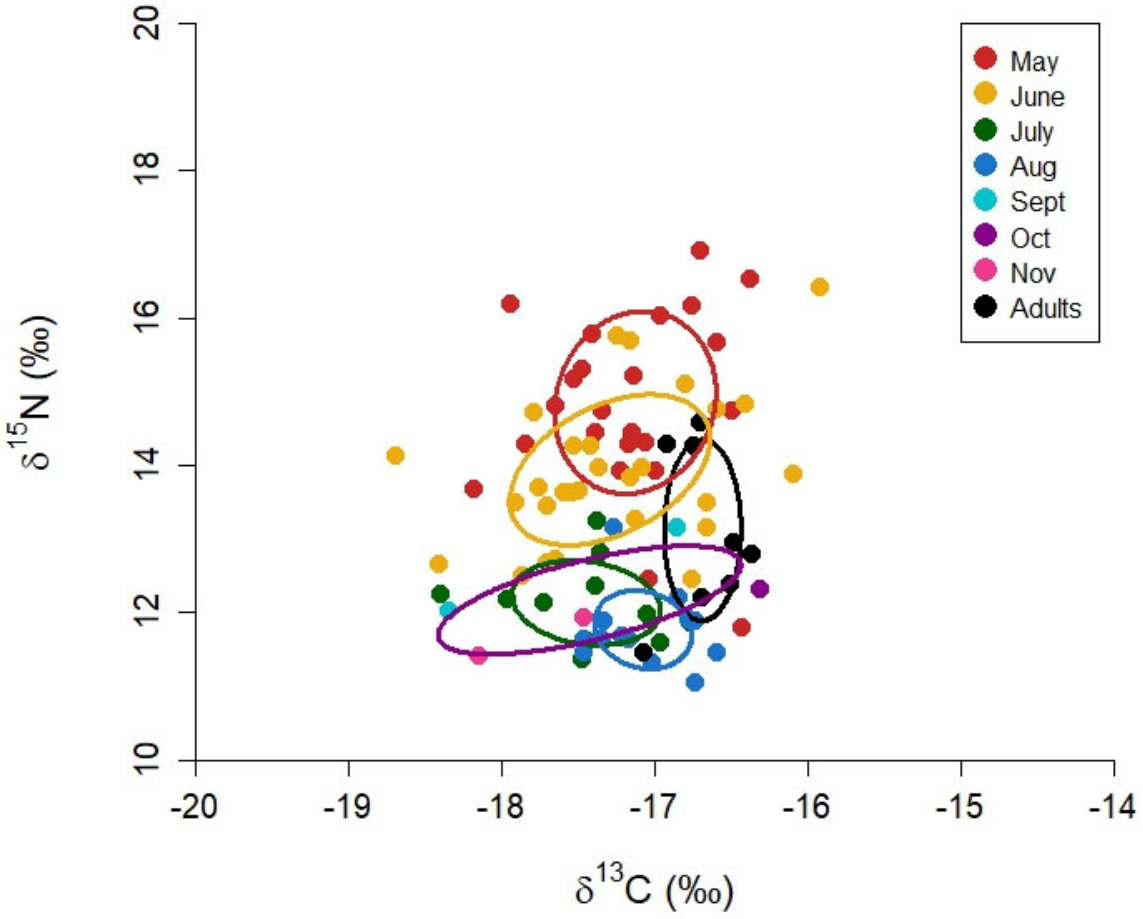

Figure 29. Plasma isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) grouped by month of scalloped hammerheads from the southeast U.S. coast (all states combined). The adult samples were added for comparison to the young-of-year isotopic ratios. The ellipses represent the isotopic niche as calculated by SEAc (corrected standard ellipse area) of the scalloped hammerheads by month they were caught with the exception of the black ellipse, which represents the isotopic niche of the adults (caught in April and May). Overlap between ellipses represent an overlap in isotopic niche of scalloped Hammerheads between months.

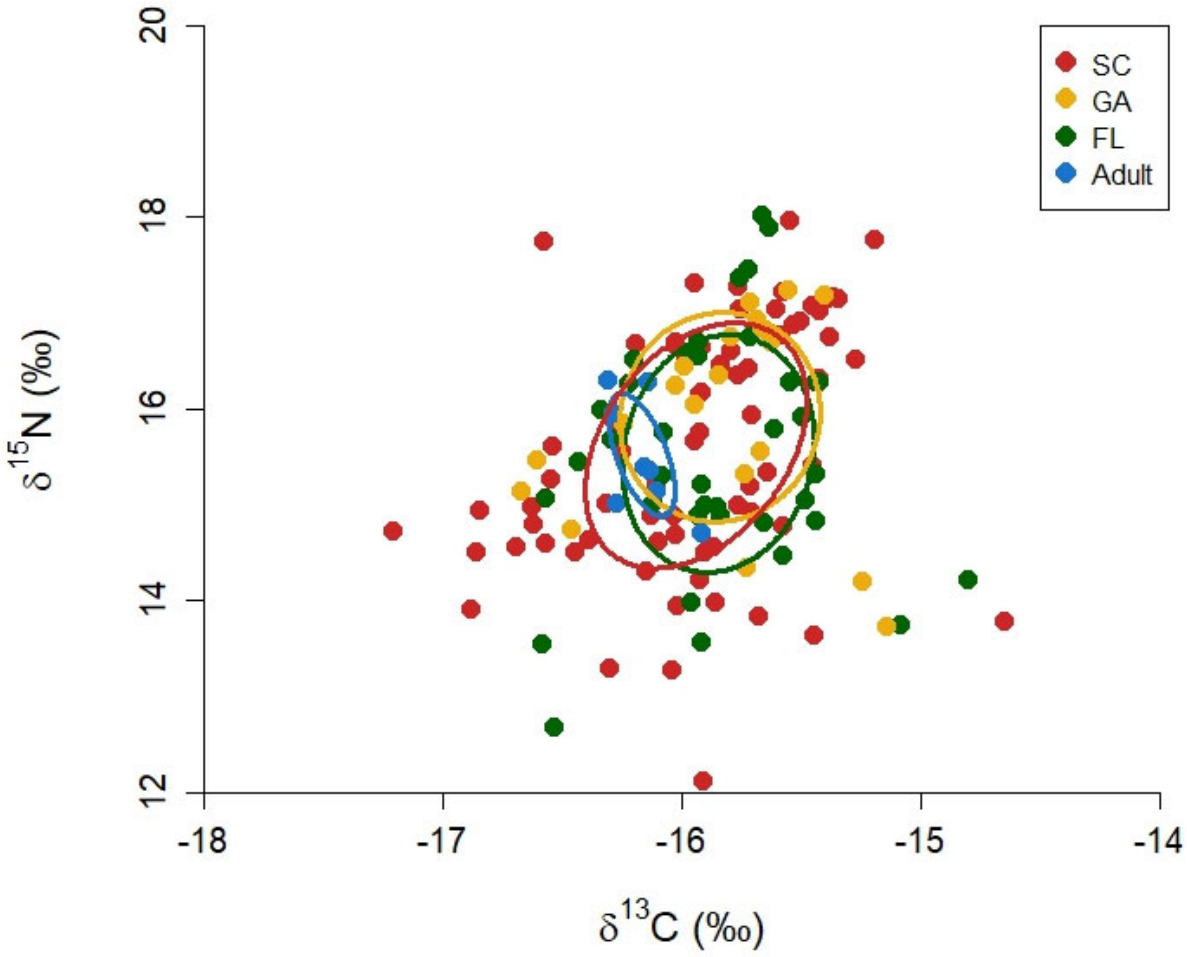

Figure 30. Muscle isotopic signatures for young-of year scalloped hammerheads collected from South Carolina, Georgia and Florida and mature male scalloped hammerheads from SC nearshore and offshore waters. The ellipses represent the isotopic niche as calculated by SEAc (corrected standard ellipse area) of the scalloped hammerheads by month they were caught with the exception of the blue ellipse, which represents the isotopic niche of the adults (caught in April and May). Overlap between ellipses represent an overlap in isotopic niche.

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Conservation biology

Hybridization between sympatric hammerhead sharks in the western North Atlantic Ocean

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Hybridization between closely related species has been documented across a wide range of taxa but has not been well studied in elasmobranchs. Hammerhead sharks have drawn global conservation concern because they experience some of the highest mortality rates among sharks when interacting with fisheries. Here we report on the detection of hybrids between the globally distributed scalloped hammerhead (*Sphyrna lewini*) and recently described Carolina hammerhead (*S. gilberti*) which are only known from the western Atlantic Ocean. Using a genomics approach, 10 first-generation hybrids and 15–17 backcrosses were detected from 554 individuals. The identification of backcrosses demonstrates hybrids are viable, and all backcrosses but one involved a scalloped hammerhead. All hybrids but one possessed Carolina hammerhead mtDNA, indicating sex-biased gene flow between species. Repeated hybridization and backcrossing with scalloped hammerheads could lead to the loss of endemic Carolina hammerheads.

1. Introduction

Hybridization between closely related species is ubiquitous in nature and occurs in at least 10% of animal and 25% of plant species [1]. Hybridization can be viewed as a constructive or destructive force, and potential consequences have been reviewed at length [2–5]. Positive outcomes of hybridization include movement of potential adaptive variation between species [6] and creation of novel genotypes that can lead to radiation of new species [7–10]. Negative effects of hybridization include reduction of fitness in endemic species via outbreeding depression [11], or reduction of biodiversity via genetic or demographic swamping [12,13]. A recent review on hybridization in marine fishes reported at least 111 hybrids involving 173 species, citing rarity of one parental species and ecological overlap as important factors leading to hybridization [14]. Little attention has been paid to hybridization in chondrichthyans in comparison with bony fishes, in large part because conserved morphology among phylogenetically related species makes hybrids difficult to identify based on morphology, and only a few studies have demonstrated contemporary hybridization using genetic techniques [15–19].

The scalloped hammerhead, *Sphyrna lewini*, is a circumglobally distributed shark found in tropical and warm temperate waters [20]. Scalloped hammerheads are dependent on coastal habitat as nursery grounds [21] and reproductively active individuals are known to aggregate [22], making them

vulnerable to fisheries when targeted or caught as bycatch [23]. Slow growth rates, low reproductive output [24], high fishing mortality [23,25,26] and high value of their fins [27] have resulted in declines in abundance throughout their range. As a result, scalloped hammerheads are listed as globally Endangered by the International Union for Conservation of Nature [23], and four out of six population segments are listed as Threatened or Endangered under the U.S. Endangered Species Act (ESA) [28].

Conservation efforts have been complicated by the recent discovery of a cryptic congener, the Carolina hammerhead, *Sphyrna gilberti*, which is sympatrically distributed with scalloped hammerheads in the western North Atlantic Ocean [29]. The species are differentiated morphologically by non-overlapping ranges of precaudal vertebrae counts (Carolina hammerhead: 83–91, scalloped hammerhead: 92–99) and estimated to have diverged 4.5 million years ago (95% CI *ca* 2–10 Ma) [30]. Carolina hammerheads are only known from specimens collected off the east coast of the USA from North Carolina to Florida, with the exception of three individuals captured off southern Brazil [30]. Little is known about the biology of Carolina hammerheads, but coastal waters from South Carolina to central Florida may be important nursery areas for this species [31], which is also critical nursery habitat for scalloped hammerheads [32,33].

As part of a study designed to investigate nursery habitat usage and relative abundance of scalloped and Carolina hammerheads in the US Atlantic and Gulf of Mexico (GoM), diagnostic single-nucleotide polymorphisms (SNPs) that were fixed between species were identified using double-digest restriction associated DNA sequencing (ddRAD). Individuals captured in nearshore habitats were genotyped at each SNP, but the identity of 33 young-of-the-year (YOY) individuals was equivocal. Inspection of genotypes of ambiguous individuals revealed some to be heterozygous at nearly all diagnostic loci and some with approximately 75% alleles from one species and 25% from the other, consistent with contemporary hybridization. In this study, patterns of hybridization between globally distributed scalloped hammerheads and endemic Carolina hammerheads in the western North Atlantic are assessed.

2. Methods

Fin clips were collected between 2010 and 2017 from 600 individuals identified as scalloped hammerheads *in situ* from the US Atlantic and GoM (figure 1), including 506 YOY, 83 juveniles and 11 adults. Genomic DNA was extracted with a Mag-Bind® Blood & Tissue DNA Kit (Omega Bio-Tek). Preparation of ddRAD libraries followed a modified Peterson *et al.* (2012) protocol [34] (electronic supplementary material, methods). The *dDocent* pipeline [35] was used to map reads to a de novo reference constructed from scalloped, Carolina and great (*Sphyrna mokarran*) hammerhead sequences, and call SNPs. Raw variants and individuals were filtered for quality using VCFtools [36] (electronic supplementary material, methods). Individuals were identified as scalloped hammerhead, Carolina hammerhead, great hammerhead or undetermined using a custom Python script and two panels of diagnostic SNPs, and a match of 95% to one species was required for identification. Four individuals identified as great hammerheads were removed from the dataset. After filtering, 554 individuals genotyped at 2512 SNPs remained in the dataset [37].

Hybrids were identified using the program NewHybrids, a Bayesian clustering method that estimates the posterior probability that an individual belongs to pure species or hybrid genotype classes [38]. Posterior probabilities were calculated for five genotype classes: pure scalloped hammerhead, pure Carolina hammerhead, F_1 hybrid, scalloped hammerhead backcross or Carolina hammerhead backcross. The F_2 genotype class (offspring of two hybrids) was not included owing to low frequency of putative F_1 hybrids suggested by the panel of diagnostic SNPs. Owing to computational limitations, the dataset of 2512 SNPs was reduced to a subset of 142 diagnostic SNPs for the NewHybrids analysis. Five independent runs were conducted with 1 000 000 sweeps following a 100 000 burn-in period, using Jeffreys-like priors for estimating allele frequencies and mixing proportions. Results of all runs were compared to ensure congruence. Individuals were considered to belong to a specific genotype class if the posterior probability for any single class was greater than 0.80.

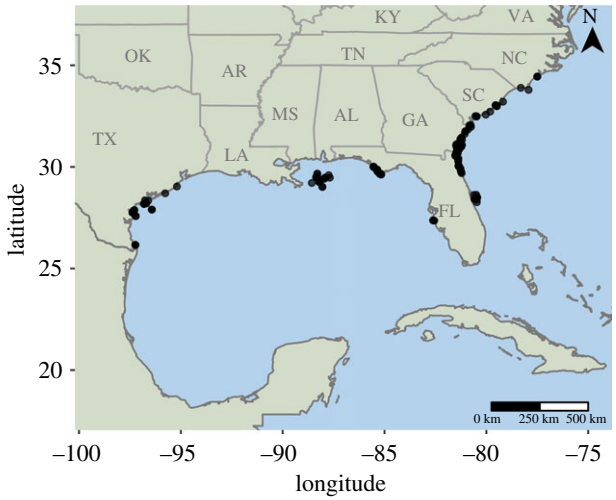

Figure 1. Map of sample locations. (Online version in colour.)

A discriminant analysis of principal components (DAPC) [39] was conducted using the R package Adegenet [40] as an additional method of hybrid identification. DAPC is a multivariate method that identifies genetic clusters by maximizing genetic differentiation between groups while minimizing variation within. Adegenet was used to simulate 100 individuals for each hybrid class to include in the DAPC using genotypes of pure individuals of each species. Following an initial principal component analysis to summarize variability among individuals, unsupervised clustering was performed for $K = 5$. One hundred and fifty principal components were retained, which resulted in both the lowest mean square error and highest mean success of group assignment in a cross-validation test.

The Bayesian clustering program Structure [41,42] was used to estimate individual admixture coefficients (q) and visualize admixture and distinctiveness between species. Five runs of 1 000 000 iterations following a 250 000 burn-in period were conducted for $K = 2$, using StrAuto [43] for automation and parallelization. Runs were summarized with Clumpak [44], and STRUCTURE PLOT [45] was used to visualize STRUCTURE and NewHybrids results. Pairwise F_{ST} between pure scalloped and Carolina hammerheads was calculated with the R package HIERFSTAT [46] using the Weir & Cockerham method [47].

To determine the maternal species of hybrids, a 683-base pair region of the mitochondrial control region (mtCR) was sequenced for seven F_1 hybrids, 12 scalloped hammerhead backcrosses and one Carolina hammerhead backcross, using the primers Pro-Shark (5′-GCCCTTGGCTCCCAAAGC-3′) and Phe-Shark (5′-TCATCTTAGCATCTTCAGTGCCA-3′). See electronic supplementary material, methods for PCR conditions.

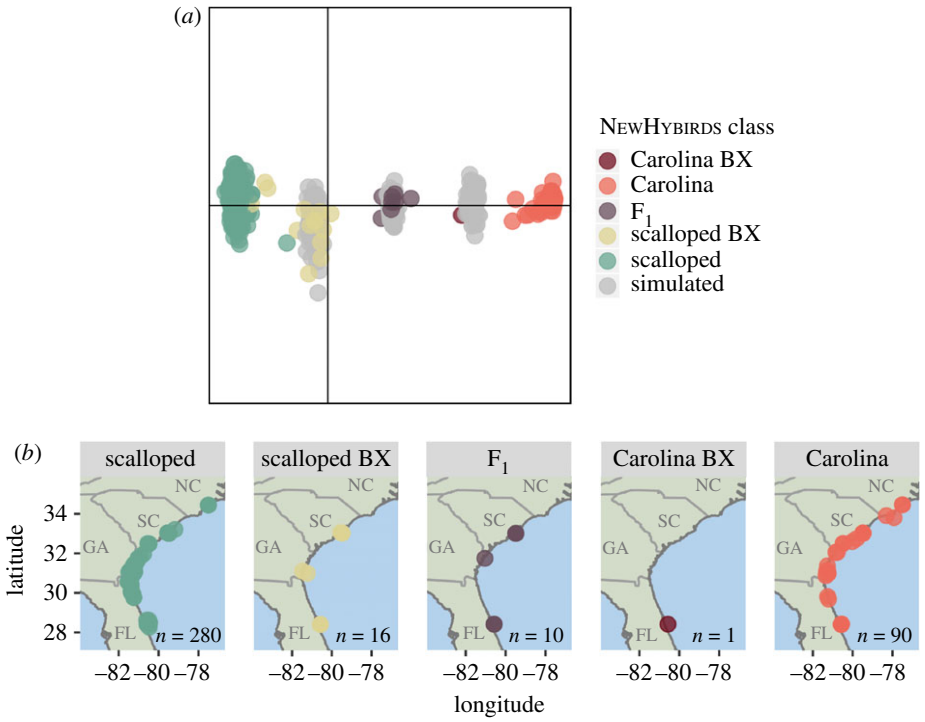

Figure 2. (*a*) DAPC results showing groupings of sampled and simulated individuals. Points are coloured according to the genotype class assigned in NewHybrids analysis. BX indicates backcross. (*b*) Map depicting sampling locations of F_1 hybrids, backcrosses (BX) and pure species individuals as determined by NewHybrids. The Gulf of Mexico is not shown because no hybrids or Carolina hammerheads were detected there.

3. Results

Of the 33 unidentified individuals, 27 were assigned to a hybrid class by NewHybrids (posterior probabilities greater than 0.98), and 25 by DAPC (figure 2 and table 1; group membership probabilities greater than 0.97), and all hybrids were YOY. Both methods detected the same 10 F_1 hybrids but differed slightly in the number of backcrossed individuals; differences are likely due to how each program handles missing data. NewHybrids ignores missing data, while DAPC requires no missing data, so mean allele frequencies were used to fill in missing genotypes. Owing to the comparatively large number of scalloped hammerheads in the dataset, individuals with missing data were skewed toward scalloped hammerhead genotypes; therefore NewHybrid results may be more accurate. For both analyses, scalloped hammerhead backcrosses (16 NewHybrids, 13 DAPC) were more common than Carolina hammerhead backcrosses (1 NewHybrids, 2 DAPC). The remaining unidentified individuals were classified as pure scalloped or Carolina hammerheads. Structure analysis indicated q was less than 1% for 503 individuals, 1–5% for 23 individuals, and 6–50% for the remaining 28 individuals that had been flagged as admixed by at least one of the two previous analyses (electronic supplementary material, figure S1). Pairwise F_{ST} between pure scalloped and Carolina hammerheads was 0.876. Analysis of mtCR haplotypes showed all individuals but one possessed a Carolina hammerhead haplotype (electronic supplementary material, data I; accession nos KY315827.1 and MK173053), indicating most observed instances of hybridization involved a female Carolina hammerhead.

Table 1. The number of individuals assigned to each genotype class by NewHybrids and DAPC.

genotype class	NewHybrids	DAPC
pure scalloped hammerhead	437	440
scalloped hammerhead backcross	16	13
F_1	10	10
Carolina hammerhead backcross	1	2
pure Carolina hammerhead	90	89

4. Discussion

Hybrids occurred where Carolina hammerheads are distributed in the US Atlantic (figure 2), with the greatest number in South Carolina. The overall proportion of sampled individuals assigned to a hybrid class was 4.5–4.9% (DAPC and NewHybrids, respectively). It should be noted that some individuals identified as hybrids were captured in the same location within a short timeframe (same day to two weeks apart). In other shark species, brood mates are known to associate for extended periods of time [48]; therefore, it is possible some hybrids belong to the same brood. Because the markers were diagnostic between species and Carolina hammerheads have very few mtDNA haplotypes present in the US Atlantic (four) [29,49], assessing sibling status was not possible. However, if full siblings are present in our data, the frequency of hybrid mating would be less than the frequency of hybrid individuals. Regardless, identification of YOY hybrids across multiple sampling years and nurseries suggests contemporary hybridization is not exceedingly rare. Low levels of admixture (1–5%) were detected in some individuals (approx. 5%), consistent with introgression between species. However, the species were strongly differentiated ($F_{ST} = 0.876$), and most individuals unambiguously assigned to one of the pure species groups. This suggests reproductive barriers exist, and the rate of admixture is not yet sufficient to homogenize gene pools.

Analysis of hybrid mtCR indicated Carolina hammerheads are nearly always the maternal species. Sex-bias in hybridization is common and there are many drivers of this phenomenon [50]. Rarity of conspecifics is thought to be a primary driver of hybridization [14,50] and when the relative abundance of hybridizing species differs, females of the rarer species often engage in interspecific mating because of increased contact frequencies with interspecific males relative to conspecifics [51]. Current knowledge regarding the range of Carolina hammerheads suggests they exist in a comparatively restricted region within the larger global distribution of scalloped hammerheads; thus it seems likely Carolina hammerheads are rare relative to scalloped hammerheads. However, more research defining the distribution, relative abundance and conservation status of Carolina hammerheads is needed to predict the effects of hybridization. Differences in parental investment in offspring can also drive unidirectional hybridization and theory predicts the high investment sex will resist interspecific mating when an adequate supply of conspecifics is available while the low investment sex will not [50]. Female scalloped hammerheads are live-bearing with relatively long gestation periods [24] and make long migrations to deliver young to appropriate nursery habitat [21], and may resist interspecific mating while male scalloped hammerheads may not.

Hybridization poses a challenge to conservation when species are threatened or endangered [52]. Difficulties arise in setting guidelines because circumstances (e.g. natural versus anthropogenic) and consequences of hybridization are context specific and no single policy can encompass every situation [52,53]. Hybridization can be a source of genetic variation for imperilled species [52] and introduce adaptive variants that facilitate species survival in changing environments [6]. Alternatively, introgressive hybridization threatens the genetic purity of parental species [54,55] and can result in loss of rare species [2]. Results of this study suggest hybridization is nearly unidirectional, with female Carolina hammerheads mating with male scalloped hammerheads, and F_1 hybrids nearly always backcrossing into scalloped hammerheads: a pattern that could lead to the loss of Carolina hammerheads over time. The identification of backcrossing and introgression in our data indicates F_1 hybrids are viable; however, if later generation hybrids have reduced fitness, hybridization could threaten Carolina hammerheads through wasted reproductive effort [2].

In the final determination, US ESA protection for scalloped hammerheads in the northwest Atlantic and GoM was not warranted [28]. However, this decision did not consider the presence of the sympatrically distributed and morphologically indistinguishable Carolina hammerhead, which has undoubtedly been included in previous assessments for scalloped hammerheads. Life-history data also likely contain information from both species, which could severely bias results that rely heavily on von Bertalanffy growth parameter estimates [56]. Future decisions regarding the conservation status of scalloped hammerheads will not only have to consider the presence and status of Carolina hammerheads, but should also consider the potential consequences of continued hybridization between these vulnerable species.

Ethics. Animals from Florida were collected under one of the following permits: SAL-12-0512SR, SAL-14-1409-SRP, SAL-15-1136A-SR, SAL-18-1292-SRP. Collections by South Carolina Department of Natural Resources were conducted under SCDNR Scientific Permit no. 2212. Samples from Texas Parks and Wildlife were sampled under Scientific Collection Authorizations. Animals collected by Texas A&M University-Corpus Christi were collected under Scientific Research Permit no. SPR-0614-111 and IACUC AUP no. 03-15.

Data accessibility. Panels of diagnostic SNPs, reference genome used for alignments and NewHybrids input data are available at https://github.com/ambarker/Sphyrna_Hybridization. mtDNA haplotypes are available on GenBank (accession nos KY315827.1 and MK173053). Raw ddRAD sequences and the filtered dataset are available upon request from the corresponding author (A.M.B.) and will be made publicly available at the conclusion of a separate ongoing study.

Authors' contributions. A.M.B., D.S.P. and B.S.F. conceived the study. A.M.B. and D.S.P. conducted laboratory work and data analysis. B.S.F., D.H.A. and W.B.D III contributed to data interpretation. All authors wrote the paper. All authors approved the final version of the manuscript and agree to be held responsible for the content herein.

Competing interests. We declare no competing interests.

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BRIEF COMMUNICATION

Identification of young-of-the-year great hammerhead shark *Sphyrna mokarran* in northern Florida and South Carolina

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Two sharks, visually identified in the field as young-of-the-year (YOY) scalloped hammerhead *Sphyrna lewini*, were identified as great hammerhead *Sphyrna mokarran* based on nuclear-encoded single nucleotide polymorphisms (SNP) and sequences of mtDNA. Individuals were captured and released in Bulls Bay, SC, and Saint Joseph Bay, FL, in 2013 and 2014, respectively. These findings indicate *S. mokarran* may be pupping in or around these areas and highlight new regions that may be a productive focus for future research on early life history of *S. mokarran*.

Key words: essential fish habitat; molecular identification; morphologically conserved species; shark nursery; *Sphyrna*.

Very little is known about the early life history of the great hammerhead, *Sphyrna mokarran* (Rüppell 1837). Locations of nursery grounds are not well defined and identification of these areas is of importance for management of the resource and conservation of the species (Miller *et al.*, 2014). Large coastal sharks that give birth to live neonates of small size (<70 cm) are expected to utilize discrete nurseries (Branstetter, 1990). The size at birth of *S. mokarran* (50–70 cm (Compagno, 1984) suggests that nursery use would be beneficial to neonates; pupping of *S. mokarran*, however, is thought to occur primarily in offshore waters (Hueter & Tyminski, 2007; Harry *et al.*, 2011). Young-of-the-year (YOY) *S. mokarran* have been observed using nearshore nurseries off the Gulf of Mexico coast of Florida as far north as Yankeetown (29·004467° N; 82·815062° W; Hueter & Tyminski, 2007). Young-of-the-year and juvenile *S. mokarran* < 200 cm total length (L_T) are not known to occur in coastal waters on the east coast of the U.S.A. (Castro, 2011).

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To investigate nursery habitat usage of two sympatrically distributed sphyrnids, the scalloped hammerhead *Sphyrna lewini* (Griffith & Smith 1834) and the recently discovered Carolina hammerhead *Sphyrna gilberti* Quattro, Driggers III, Grady, Ulrich & Roberts 2013, double-digest restriction associated DNA sequencing (ddRAD) was used to identify a panel of single nucleotide polymorphisms (SNP) that can be used to differentiate between the species. Because *S. lewini* and *S. gilberti* are conserved morphologically and differ only in the number of precaudal vertebrae (Quattro *et al.*, 2013), the panel allows for conclusive, non–lethal species identification.

Fin clips were collected from eighteen putative YOY *S. lewini* spread across four sites: Corpus Christi, TX (27·689378° N; 97·055843° W), Panama City, FL (29·7326667° N; 85·3691° W), Cape Canaveral, FL (28·389406° N; 80·586626° W), and Bulls Bay, SC (33·009500° N; 79·485346° W). Genomic DNA was extracted using a Mag–Bind Blood & Tissue DNA Kit (Omega Bio-Tek; www.omeganiotek.com). Double-digest restriction associated DNA sequencing (ddRAD) library preparation was conducted following a modified version of Peterson *et al.* (2012; Table S1, Supporting information). The library was sequenced as a paired–end run on one lane of a MiSeq DNA sequencer (Illumina; www.illumina.com). The dDocent pipeline (www.ddocent.com; Puritz *et al.*, 2014) was used for reference construction, mapping reads and SNP calling. A total of 39 011 SNPs were recovered from 4584 contigs (Table S2, Supporting information).

As an initial means of grouping individuals, PCA was run in Adegenet (Jombart, 2008) and three distinct genetic clusters were recovered (Fig. 1 and Table S3, Supporting information). The clusters were highly divergent across all loci ($F_{ST} = 0{\cdot}9–0{\cdot}98$). A total of 846 bp from the mitochondrial control region (mtCR) were sequenced from two to three individuals from each cluster to determine species identity (Table S4, Supporting information). Sequences were compared with haplotypes available on GenBank and three individuals were identified with 99% sequence identity as *S. lewini*, three with 99–100% sequence identity as *S. gilberti and* two with 100% sequence identity as *S. mokarran* (GenBank accession nos. KY315826–KY315830). The first individual identified as *S. mokarran* was captured in Bulls Bay, SC, on 9 July 2013 and the second in St. Joseph Bay, FL, on 5 August 2014 (Fig. 1). Total length was measured at 63·8 cm for the individual captured in SC and 67·0 cm for the individual captured in FL; both fell within the observed size range for neonate *S. mokarran* (Compagno, 1984). A neighbour-joining tree was created from mtCR data with MEGA7 (Kumar *et al.*, 2016) using a Jukes–Cantor substitution model with 500 bootstrap replicates (Table S5, Supporting information). Three groups were recovered with 100% support and were consistent with clusters identified using SNPs in the PCA (Fig. 2). Mean nucleotide divergence between the group identified as *S. mokarran* and other groups was *c.* 16% and mean divergence between *S. lewini* and *S. gilberti* was *c.* 5%. Within-group distances were negligible (0–0·1%; Table I).

Sphyrna mokarran is primarily a tropical species hypothesized to give birth offshore (Harry *et al.*, 2011; Hueter & Tyminski, 2007). The observation of two S. *mokarran* neonates in nearshore habitat of South Carolina and the northern Gulf of Mexico coast of Florida indicates that *S. mokarran* may use nursery habitat further north and further inshore than known previously. Little is known about the early life history of the species and, like other hammerhead sharks, *S. mokarran* is susceptible to over-exploitation (Denham *et al.*, 2007), making identification of essential fish habitat,

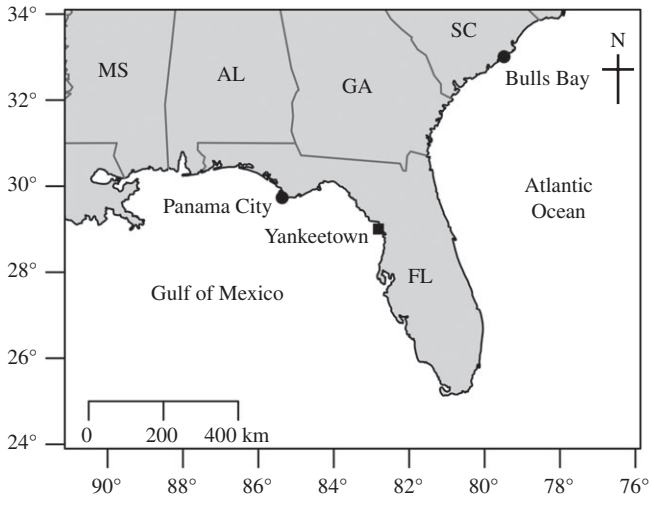

FIG. 1. Map indicating locations of *Spyrna mokarran* neonates identified in the present study (●), and location of previously known northernmost occurrence of *S. mokarran* neonates in the Gulf of Mexico (■).

such as nursery areas, a critical research topic. Given present data, it is not possible to characterize how important these two northern, inshore sites are to *S. mokarran.* Three scenarios may account for the presence of *S. mokarran* in these nurseries. First, it is possible that individuals were pupped elsewhere and subsequently moved into Bulls Bay and St. Joseph Bay after parturition. Given the size of the individuals, however, it is unlikely that they migrated a substantial distance. The capture date of the neonate in Bulls Bay occurred during the time of proposed parturition (Piercy *et al.*, 2010), meaning that the individual probably was born in close proximity to

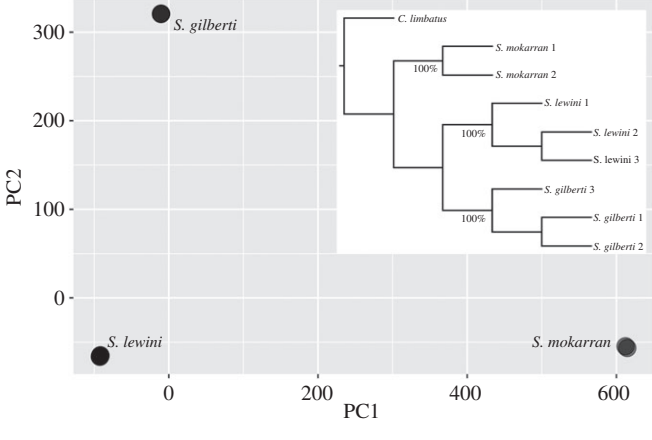

FIG. 2. Results of principal components (PC) analysis, using *Sphyrna* spp. single nucleotide polymorphism data, and a neighbour-joining tree constructed from mitochondrial control region data (mtCR). Both analyses identified three clusters that coincide with identification of three *Sphyrna* species using mtCR basic local-alignment search tool (BLAST) results. *Carcharhinus limbatus* was used as the out group.

TABLE I. Mean between and within-group nucleotide divergence among *Sphyrna* spp., based on mtCR sequences

	S. gilberti	*S. lewini*	*S. mokarran*
S. gilberti	0·001	0·049	0·165
S. lewini	0·049	0·000	0·159
S. mokarran	0·165	0·159	0·000

Bulls Bay. Second, these findings may indicate relatively new nursery habitat usage by *S. mokarran* due to a northward, coastal expansion in nursery usage. Third, several diagnostic features of *S. mokarran* (falcate pelvic fins and nearly straight anterior margin of the cephalofoil) are not as apparent in neonates, causing them to appear relatively similar to neonate *S. lewini* (Castro, 2011). It is possible that neonate *S. mokarran* have been caught in these areas previously but misidentified as *S. lewini*. Such misidentifications are common between morphologically conserved species, especially when one species is expected in a given region or habitat while the other is not (Branstetter, 1982; Tillett *et al.*, 2012). Other potential nursery sites for *S. mokarran* may not yet have been described, in part because of misidentification. Future work is needed to document how frequently neonate *S. mokarran* is encountered in these areas and to estimate the number of breeding females utilizing each site.

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Supporting Information

Supporting Information may be found in the online version of this paper:

Table S1. Method for double–digest restriction associated DNA sequencing (ddRAD) library preparation and data filtering.

Table S2. Single nucleotide polymorphisms recovered from *Sphyrna* spp.

Table S3. Genepop file used to identify genetic clusters.

Table S4. Mitochondrial control region (mtCR) sequences from two to three individuals from each cluster to determine species identity.

Table S5. Mitochondrial control region (mtCR) sequence alignments used to create neighbour-joining tree.

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