



## Distribution and relative abundance of scalloped (*Sphyrna lewini*) and Carolina (*S. gilberti*) hammerheads in the western North Atlantic Ocean

Amanda M. Barker<sup>a,\*</sup>, Bryan S. Frazier<sup>b</sup>, Douglas H. Adams<sup>c</sup>, Christine N. Bedore<sup>d</sup>, Carolyn N. Belcher<sup>e</sup>, William B. Driggers III<sup>f</sup>, Ashley S. Galloway<sup>b</sup>, James Gelsleichter<sup>g</sup>, R. Dean Grubbs<sup>h</sup>, Eric A. Reyier<sup>i</sup>, David S. Portnoy<sup>a</sup>

<sup>a</sup> Marine Genomics Laboratory, Department of Life Sciences, Texas A&M University-Corpus Christi, 6300 Ocean Dr., Corpus Christi, TX, 78412, USA

<sup>b</sup> South Carolina Department of Natural Resources, Marine Resources Research Institute, 217 Ft. Johnson Rd., Charleston, SC, 29412, USA

<sup>c</sup> Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Institute, Indian River Field Laboratory, 1220 Prospect Ave. #285, Melbourne, FL, 32901, USA

<sup>d</sup> Department of Biology, Georgia Southern University, 4324 Old Register Road, Statesboro, GA, 30458, USA

<sup>e</sup> Georgia Department of Natural Resources, One Conservation Way, Brunswick, GA, 31520, USA

<sup>f</sup> National Marine Fisheries Service, Southeast Fisheries Science Center, Mississippi Laboratories, 3209 Frederic St., Pascagoula, MS, 39567, USA

<sup>g</sup> University of North Florida, 1 UNF Drive, Jacksonville, FL, 32224, USA

<sup>h</sup> Florida State University Coastal and Marine Laboratory, 3618 Hwy 98, St. Teresa, FL, 32358, USA

<sup>i</sup> Herndon Solutions Group, LLC, NASA Environmental and Medical Contract, NEM-022 Kennedy Space Center, FL, 32899, USA

### ARTICLE INFO

Handled by: J Viñas

**Keywords:**

Sympatric  
Cryptic species  
Conservation  
Endangered species  
Shark management

### ABSTRACT

The scalloped hammerhead (*Sphyrna lewini*) and its cryptic congener, Carolina hammerhead (*S. gilberti*), are sympatrically distributed in the western North Atlantic Ocean. Because the species are indistinguishable based on external morphology, little research focused on Carolina hammerheads exists. In this study, the distribution of Carolina hammerheads in waters of the United States off the east coast (U.S. Atlantic) and Gulf of Mexico (Gulf) was examined and their abundance relative to scalloped hammerheads assessed by genetically identifying 1231 individuals using diagnostic single nucleotide polymorphisms. Both species were found in the U.S. Atlantic, where 27 % of individuals were Carolina hammerheads, but only scalloped hammerheads were identified in the Gulf. In Bulls Bay, SC, a well-known hammerhead nursery, assessment of relative abundance from May to September showed scalloped hammerheads were more abundant May-June and Carolina hammerheads more abundant July-September. Results of this study suggest Carolina hammerheads have a spatially limited distribution in the western North Atlantic and highlight the importance of Bulls Bay as a nursery for the species. In addition, the results suggest Carolina hammerheads may comprise a non-trivial proportion of what is considered the U.S. Atlantic scalloped hammerhead stock and should be considered in future decisions regarding management of the hammerhead complex.

### 1. Introduction

Advancements in molecular techniques have led to the discovery of hidden genetic diversity (cryptic species) within morphologically conserved taxa across metazoans and biogeographic regions (Pfenninger and Schwenk, 2007). Cryptic species are groups of evolutionary independent lineages that appear morphologically indistinguishable from

one another (Bickford et al., 2007; Sáez and Lozano, 2005). Genetic differentiation with a lack of morphological change is thought to occur when mating cues are nonvisual (e.g. chemical or auditory) or when there is strong selective pressure that promotes preservation of morphological characters (Bickford et al., 2007). Strong selection also could promote convergence in morphology, resulting in genetically distinct species that are similar in appearance (Fiser et al., 2018).

\* Corresponding author.

E-mail addresses: [abarker@islander.tamucc.edu](mailto:abarker@islander.tamucc.edu) (A.M. Barker), [frazierb@dnr.sc.gov](mailto:frazierb@dnr.sc.gov) (B.S. Frazier), [doug.adams@MyFWC.com](mailto:doug.adams@MyFWC.com) (D.H. Adams), [cbedore@georgiasouthern.edu](mailto:cbedore@georgiasouthern.edu) (C.N. Bedore), [carolyn.belcher@dnr.ga.gov](mailto:carolyn.belcher@dnr.ga.gov) (C.N. Belcher), [william.driggers@noaa.gov](mailto:william.driggers@noaa.gov) (W.B. Driggers), [GallowayA@dnr.sc.gov](mailto:GallowayA@dnr.sc.gov) (A.S. Galloway), [jim.gelsleichter@unf.edu](mailto:jim.gelsleichter@unf.edu) (J. Gelsleichter), [dgrubbs2@fsu.edu](mailto:dgrubbs2@fsu.edu) (R.D. Grubbs), [eric.a.reyier@nasa.gov](mailto:eric.a.reyier@nasa.gov) (E.A. Reyier), [david.portnoy@tamucc.edu](mailto:david.portnoy@tamucc.edu) (D.S. Portnoy).

Alternatively, cryptic species may have diverged too recently for morphological differentiation to take place (Fiser et al., 2018).

Cryptic species may have different habitat requirements, life history characteristics, and responses to disturbance and therefore pose a challenge to conservation and management. The inability to distinguish species based on external morphology makes it difficult to monitor populations (Lintott et al., 2016; Morningstar et al., 2014; Schönrogge et al., 2002) and appropriately manage stocks (Bickford et al., 2007; Rocha et al., 2007). Molecular methods have revealed several cryptic species complexes in commercially important marine fishes (e.g. grouper, Craig et al., 2009; rockfish, Hyde et al., 2008; amberjack, Martinez-Takeshita et al., 2015), and in some cases, these cryptic lineages co-occur (e.g. bonefish, Colborn et al., 2001; opah, Hyde et al., 2014). Additional challenges arise when cryptic species coexist in all or part of their range and, in these cases, research is needed to understand how potential differences in life history, reproductive ecology, behavior and habitat use may need to be accounted for in approaches to co-management.

Many groups of elasmobranchs (sharks, skates and rays) are morphologically conserved, and challenges associated with species-level identification have historically been an impediment to effective management. Recent estimates indicate 18.8 % of elasmobranchs assessed under International Union for Conservation of Nature (IUCN) Red List criteria are considered to be threatened with extinction (IUCN, 2019), and declines have been largely driven by targeted fisheries and mortality as bycatch (Dulvy et al., 2017). Catch records often lump sharks into broad categories based in part on morphological and or biological similarity, making it difficult to accurately assess what species were caught (Barker and Schluessel, 2005; Clarke et al., 2006). Complicating the issue is the growing number of cryptic lineages that have been revealed by molecular techniques, highlighting the importance of genetic methods in monitoring exploited elasmobranchs (Ovenden et al., 2015; Portnoy and Heist, 2012; White and Last, 2012). Molecular techniques have not only been used to identify cryptic elasmobranch species (e.g. wobbegong, Corrigan et al., 2008; skates, Griffiths et al., 2010; guitarfishes, Sandoval-Castillo et al., 2004; dogfishes, Ward et al., 2007), but also to understand distributions of morphologically conserved species and evaluate their relative abundance in regions in which they are sympatric (e.g. houndsharks, Giresi et al., 2015; blacktip sharks, Ovenden et al., 2010).

Sphyrnids, collectively known as hammerhead sharks, are a morphologically conserved group of international conservation concern. Great hammerheads (*Sphyrna mokarran*), scalloped hammerheads (*S. lewini*), and smooth hammerheads (*S. zygaena*) are often confused for one another despite having distinct morphological characteristics, and thus have been reported under a general category of "hammerheads" (Miller et al., 2013). Sphyrnids have experienced declines in abundance throughout their range due to slow growth rates and relatively low reproductive outputs compared to bony fish (Branstetter, 1987), high directed catch due to their desirability in the global fin trade market (Abercrombie et al., 2005), and high rates of at-vessel as well as post-release mortality associated with non-target commercial catch (Gallagher et al., 2014; Gulak et al., 2015; Morgan and Burgess, 2007). As a result, both scalloped and great hammerheads are considered Critically Endangered on a global scale by the IUCN and smooth hammerheads are considered Vulnerable (Rigby et al., 2019a, 2019b, 2019c).

Management and conservation of scalloped hammerheads in the Atlantic Ocean is further complicated by the presence of the sympatrically distributed cryptic congener, the Carolina hammerhead (*S. gilberti*). The existence of a cryptic hammerhead lineage in the Atlantic was first detected in the mid-2000s (Abercrombie et al., 2005; Duncan et al., 2006; Quattro et al., 2006), and the species was formally described in 2013 (Quattro et al., 2013). Scalloped and Carolina hammerheads are indistinguishable based on external morphology and can only be identified using precaudal vertebrae counts (83–91 Carolina

hammerhead, 92–99 scalloped hammerhead) or genetics (Quattro et al., 2013). Limited data suggest that Carolina hammerheads are found primarily off the southeastern United States (hereafter U.S.; Abercrombie et al., 2005; Duncan et al., 2006; Quattro et al., 2006), with the exception of three individuals reported near southern Brazil (Pinhal et al., 2012). Data collected from Carolina hammerheads have likely been included in previous stock assessments of scalloped hammerheads in the U.S. Atlantic (Hayes et al., 2009) and this could create a variety of problems. For example, Carolina hammerheads are thought to reach a smaller maximum size than scalloped hammerheads, (Quattro et al., 2013) and this could bias age and growth estimates that are important components of fisheries stock assessments (Cailliet et al., 2006; Pardo et al., 2013). Further, when a status review was conducted in 2013 to determine if protection under the U.S. Endangered Species Act (ESA) was warranted for scalloped hammerheads (Miller et al., 2013), the presence of a sympatrically distributed cryptic species was known, but a lack of data on distribution, abundance and life history for Carolina hammerheads prevented species-specific assessments and could not be factored into listing decisions. Under the ESA, listing decisions are applied to specific portions of a species range (distinct population segments), rather than listing the species as a whole. In the final determination, four out of six distinct population segments were listed as Threatened or Endangered, however protection was not warranted for scalloped hammerheads in the U.S. Atlantic and Gulf of Mexico (hereafter Gulf; NMFS, 2014), where the species are thought to overlap.

Both scalloped and Carolina hammerheads employ a reproductive strategy in which females utilize discrete coastal nursery habitats (Branstetter, 1987; Quattro et al., 2006). Shark nurseries are defined as areas where young sharks are encountered more commonly than other areas, remain resident for extended periods of time, and use the habitat repeatedly across years (Heupel et al., 2007). In the U.S. Atlantic, estuarine waters of South Carolina, most notably, Bulls Bay, SC (hereafter Bulls Bay), and nearshore waters of Cape Canaveral, Florida (hereafter Cape Canaveral) have been identified as primary nursery habitat for scalloped hammerheads (Adams and Paperno, 2007; Castro, 1993; Ulrich et al., 2007). Bulls Bay is a shallow estuarine system composed of *Sporobolus* sp. saltmarsh flats with anastomosing small creeks, large shallow mudflats, and barrier islands. The seafloor is primarily composed of fine sediments with occasional shell rakes, and gently slopes from exposed mudflats to 4 m deep (at low tide) 6 km offshore. The bay has minimal freshwater discharge, and waters are turbid with high (32–36 ppt) salinities. The nearshore nursery habitats off Cape Canaveral have no direct estuarine influence and extend approximately 5000 m offshore from the shoreline. The Southeast Shoal area is characterized by shallow, low-relief shoal habitat (1–5 m depths) with sand-shell substrate. Canaveral Bight, a deeper basin habitat (6–10 m depth) south of the shoals is characterized by more turbid water with fine sediments, and the shelf transition zone directly south of Canaveral Bight are also frequently used by scalloped hammerhead young-of-the-year (YOY; Adams and Paperno, 2007). Scalloped hammerhead YOY are also frequently observed in the Tolomato River, Florida (hereafter Tolomato River), a portion of the Atlantic Intracoastal Waterway that appears to serve as a unique, inshore primary nursery for this species based on comparisons of catch data from other northeast Florida estuaries (B.Wargat and J. Gelsleichter, unpublished data). Additional nursery habitat may exist in Georgia and North Carolina. In the Gulf, scalloped hammerhead YOY have been found in estuaries, bays, and beaches in Florida and Texas (Hueter and Tyminski, 2007). Coastal waters of South Carolina are also thought to provide important nursery habitat for Carolina hammerheads (Quattro et al., 2006), but their occurrence elsewhere has not been thoroughly evaluated.

In this study, a panel of diagnostic single nucleotide polymorphisms (SNPs) was generated using double-digest restriction associated DNA sequencing (ddRAD) to identify scalloped and Carolina hammerheads sampled in the U.S. Atlantic and Gulf, with a focus on known and potential nursery areas (hereafter nurseries). Data were used to describe

the distribution of Carolina hammerheads and determine their abundance relative to scalloped hammerheads. An understanding of the relative abundance of scalloped and Carolina hammerheads in U.S. waters will be needed in future assessments, and results of this study will help managers identify nursery areas for both species and are an important first step toward developing appropriate, species-specific management strategies.

## 2. Methods

A total of 1241 individuals were sampled in the U.S. Atlantic and Gulf (Fig. 1, Table 1) between 2010–2019. Tissues were stored in 20 % salt-saturated DMSO buffer (Seutin et al., 1991) or molecular grade EtOH initially and subsequently transferred to DMSO buffer for long-term storage. Sharks were captured using longlines, gillnet, otter trawl, or hook and line from a combination of targeted collection and fishery independent surveys. Fin clips were also obtained from mortalities in commercial shrimp trawls. Collections were made following animal care and use protocols of academic partners and standard operating procedures of state and federal agencies (see animal care statement). 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 dDOCENT pipeline was used for *de novo* reference construction, read mapping and SNP calling (Puritz et al., 2014). A *de novo* reference assembly was constructed from twenty individuals (15 scalloped hammerheads, 3 Carolina hammerheads, and 2 great hammerheads) sequenced as a paired-end run on an Illumina MiSeq sequencer, with initial species identifications based on mitochondrial control region (mtCR) haplotypes (Barker et al., 2017). Great hammerheads were included as the morphology of YOY great hammerheads is similar to that of the other two species and YOY great hammerheads are sometimes misidentified as scalloped hammerheads (Barker et al., 2017). The twenty individuals used to construct the reference assembly were subsequently screened for SNPs that could be used to distinguish scalloped, Carolina, and great hammerheads. Raw variants were filtered using VCFTools (Danecek et al., 2011) with a minimum quality score of 20 and mean minimum depth of 10. Indels and sites with any 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).

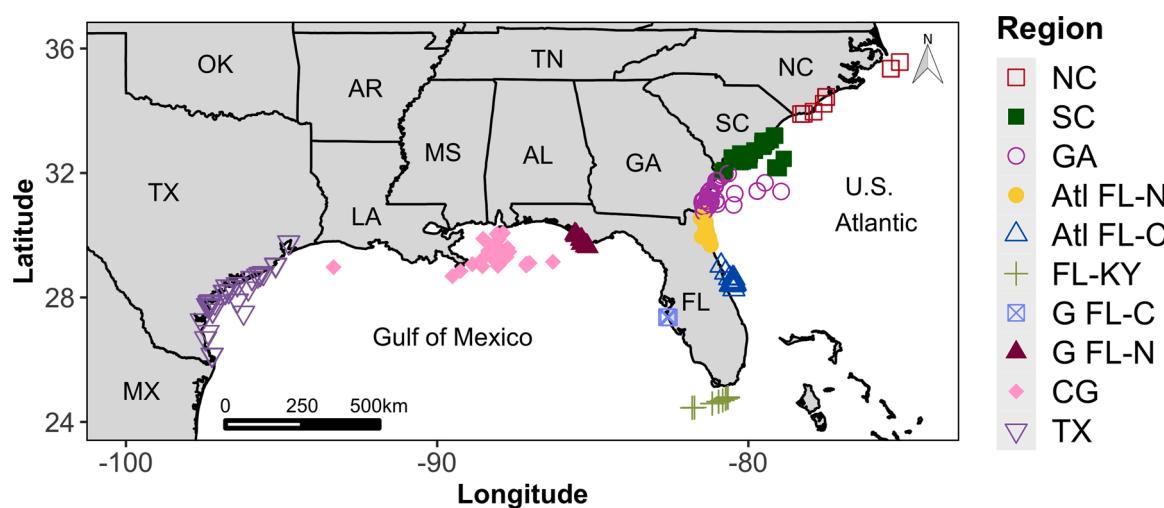
**Table 1**

Total number of young-of-the-year and small juveniles (Sjuv, 365–1000 mm stretch total length), large juveniles (Ljuv, 1021–1751 mm stretch total length), and mature (Mat, 1829–2750 stretch total length) individuals sampled in each region: North Carolina (NC), South Carolina (SC), Georgia (GA), Atlantic northern Florida (Atl FL–N), Atlantic central Florida (Atl FL–C), Florida Keys (FL–KY), Gulf central Florida (G FL–C), Gulf northern Florida (G FL–N), Central Gulf (CG), Texas (TX). One sample was from an unspecified location in the Atlantic (Atl-U).

Location	Sjuv	Ljuv	Mat	Total
NC	8	1	5	14
SC	389	1	30	410
GA	93	1	10	104
Atl FL-N	198	0	1	199
Atl FL-C	199	10	1	210
FL-KY	0	23	13	36
G FL-C	3	0	0	3
G FL-N	116	2	0	118
CG	0	33	20	53
TX	84	2	7	93
Atl-U	0	1	0	1
<b>Total</b>	<b>1080</b>	<b>74</b>	<b>87</b>	<b>1241</b>

Panel 1 was designed by calculating allele frequencies in GENODIVE (Meirman and Van Tienderen, 2004) and selecting SNPs that were completely fixed between great hammerheads and (scalloped + Carolina hammerheads). To design Panel 2, great hammerheads were removed from the dataset and allele frequencies recalculated to identify SNPs that were completely fixed between scalloped and Carolina hammerheads. A total of 2695 diagnostic SNPs were identified for Panel 1 and 1491 for Panel 2.

The remaining individuals were sequenced across 11 lanes on an Illumina HiSeq 4000 DNA. Each sequencing run contained a mix of individuals from different sampling locations to minimize library effects. dDOCENT was used to map reads and call SNPs and raw variants were filtered to retain only diagnostic SNPs using VCFTOOLS. Individuals were first identified as either great hammerhead or scalloped/Carolina hammerhead using composite genotypes of Panel 1 SNPs and a custom Python script (see Data Availability). Species identity was considered unknown if an individual had less than a 95 % match to a single category. Individuals identified as a great hammerhead or unknown were removed from the dataset, and the remaining individuals identified as either a scalloped hammerhead, Carolina hammerhead or undetermined by using composite genotypes of Panel 2 SNPs and a custom Python script. As above, a match of 95 % to one species was required for positive



**Fig. 1.** Map of sampling locations, colored by region: North Carolina (NC), South Carolina (SC), Georgia (GA), Atlantic northern Florida (Atl FL–N), Atlantic central Florida (Atl FL–C), Florida Keys (FL–KY), Gulf central Florida (G FL–C), Gulf northern Florida (G FL–N), Central Gulf (CG), Texas (TX).

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 removed from the dataset (see below). The program NEWHYBRIDS (Anderson and Thompson, 2002) was used to assess if undetermined individuals could be assigned into a hybrid (F1 hybrid, scalloped hammerhead backcross, Carolina hammerhead backcross) or non-hybrid category (scalloped hammerhead, Carolina hammerhead) following the methods of Barker et al. (2019).

Due to sequencing variation within and across runs, individuals varied in the number of diagnostic SNPs that were successfully genotyped. Additionally, due to the small number of Carolina and great hammerheads used to initially identify diagnostic SNPs and individual variation, as well as potential admixture between species (Barker et al., 2019), it was expected that at least some of the diagnostic SNPs would not be completely fixed in all individuals of a given species. To determine the minimum number of diagnostic SNPs required for accurate species identification, a resampling technique (custom script) was employed using the individuals from the first HiSeq library ( $n = 128$ ; great hammerhead = 1, scalloped hammerhead = 89, Carolina hammerhead = 27, mixed ancestry = 11). Random subsets of loci ranging in number from 5–2,000 loci in Panel 1 and 5–1,200 loci in Panel 2 were selected and individuals reidentified. This procedure was repeated for 1000 iterations, and the average number of correct identifications (i.e., matched original identification from the full panel of SNPs) for each individual with each subset of loci was determined.

Relative abundance (as a percentage) was determined by dividing the number of individuals of each species (scalloped hammerhead and Carolina hammerhead) by the total number of individuals across both species. Relative abundance was calculated for each region, as well as for known and potential nurseries, defined in this study as areas in which at least 20 YOY or small juveniles (stretch total length  $\leq 1000$  mm) were sampled. Regions were generally defined by state boundaries (North Carolina, South Carolina, and Georgia) with the exception of Florida, which was split into five regions (Atlantic northern Florida, Atlantic central Florida, the Florida Keys, Gulf central Florida, and Gulf northern Florida). Temporal trends in relative abundance of YOY over the sampling season for both species were assessed in Bulls Bay by pooling catch data across years (2013–2018) and standardizing to catch per unit effort (CPUE, sharks caught/gillnet set). Permutation tests implemented in the R package coin (Hothorn et al., 2008) were used to test for associations between species identity and environmental variables (salinity, dissolved oxygen, and water temperature) measured at the time of sampling using a YSI Pro 2030 (YSI Inc./Xylem Inc.). Generalized linear models were used to test for associations of environmental variables and month with the relative probability that a sampled individual was a scalloped or Carolina hammerhead. All models were compared against a null model and tested for goodness of fit and the optimal model was selected to minimize AIC values. Abundance trends and associations with environmental variables were evaluated only in Bulls Bay because YOY Carolina hammerheads were not identified in large enough numbers in other nurseries.

### 3. Results

Identification accuracy for Panel 1 was high even when very few SNPs were used, with an overall average of 99.1 % correct identification with five loci. The overall average correct identification for Panel 2 was similarly high for five loci (96.2 %), however, individual variation in correct identification was substantial (50.7–100.0 %; Fig. S1). Reliable and accurate identification (>99 % individuals identified correctly in >95 % of the iterations) with Panel 2 was not achieved until 300 loci were used, and 500 loci were required for 100 % of individuals to be identified correctly in >95 % iterations.

A total of 1120 individuals were identified with the panels of diagnostic SNPs (scalloped hammerhead = 878, Carolina

hammerhead = 236, great hammerhead = 6), and 83 individuals were assigned into a hybrid category by NEWHYBRIDS (F1 = 37, scalloped hammerhead backcross = 38, Carolina hammerhead backcross = 8; Table 2). NEWHYBRIDS also identified an additional 13 scalloped hammerheads and 15 Carolina hammerheads that could not be identified with the diagnostic panel, and these individuals were added to species totals for subsequent analysis. One individual could not be distinguished by Panel 1 and had a mix of scalloped/Carolina hammerhead alleles and great hammerhead alleles. The anomalous individual, which was sampled in the U.S. Atlantic near central Florida, was sequenced at the mitochondrial cytochrome oxidase subunit 1 (COI, see supplementary methods) gene and identified as a smooth hammerhead (*S. zygaena*; accession no MT863713). Nine individuals were genotyped at too few loci and were removed from the dataset. Scalloped hammerheads were identified in all areas sampled in both the U.S. Atlantic and Gulf. Carolina hammerheads were identified across the sampled area in the U.S. Atlantic, though not in every location, and were absent in the Gulf (Fig. 2). Carolina hammerhead abundance was heavily concentrated in South Carolina.

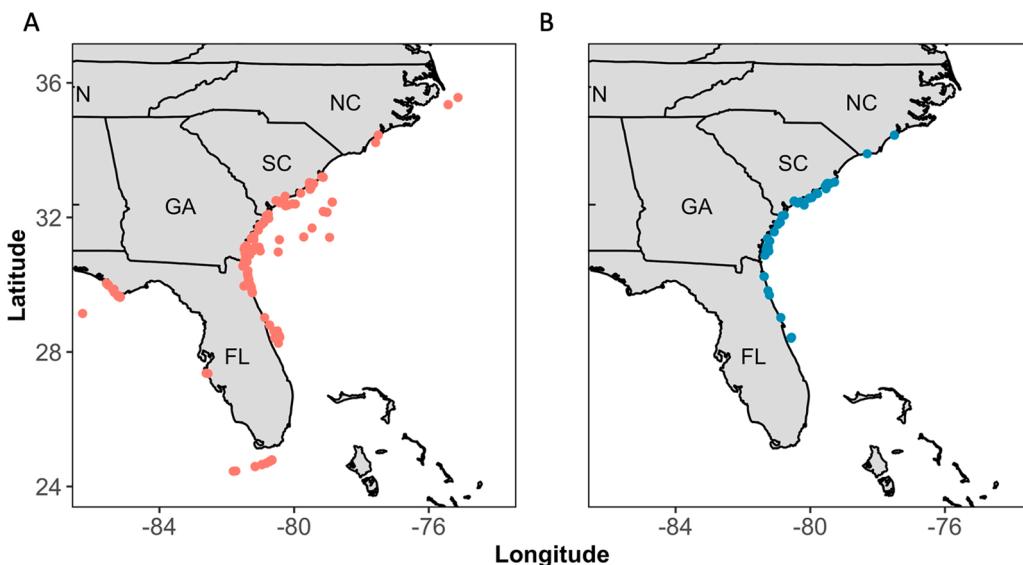
The relative abundance of Carolina to scalloped hammerheads was highest in South Carolina (56.4 %, total hammerheads = 351) and North Carolina (33.3 %, total hammerheads = 12; Table 3). Relative abundance of Carolina hammerheads roughly decreased along a latitudinal gradient, with the lowest relative abundance observed in the Florida sampling locations. However, Carolina hammerhead abundance was higher in central Florida (12.9 %, total hammerheads = 194) than northern Florida, where Carolina hammerheads were particularly rare (3.6 %, total hammerheads = 195). Relative abundance of Carolina hammerhead YOY was calculated for six nurseries in the U.S. Atlantic (Fig. 3) and was highest in Bulls Bay (61.0 %, total hammerheads = 287) and lowest in Tolomato River (0 %, total hammerheads = 148; Table 4).

In Bulls Bay, 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 87.0 % in 2012 (Table 5). Analysis of CPUE data from May to September showed that YOY of both species were present in Bulls Bay in May. Scalloped hammerheads were relatively more abundant from May through June, but both species increased in abundance during this time and abundances peaked at the end of July (Fig. 4). Scalloped hammerhead CPUE decreased rapidly after July, and the species was absent by the end of August. Carolina hammerhead CPUE increased sharply in July and declined through August and September. Permutation tests showed that salinity ( $Z = 4.636, P < 0.001$ ) and water

**Table 2**

Species identifications in each region: North Carolina (NC), South Carolina (SC), Georgia (GA), Atlantic northern Florida (Atl FL-N), Atlantic central Florida (Atl FL-C), Florida Keys (FL-KY), Gulf central Florida (G FL-C), Gulf northern Florida (G FL-N), Central Gulf (CG), Texas (TX). One sample was from an unspecified location in the Atlantic (Atl-U). Scal indicates scalloped hammerhead, Car indicates Carolina hammerhead, Great indicates great hammerhead, F1 indicates a first-generation hybrid, ScBX indicates scalloped hammerhead backcross, CarBX indicates Carolina hammerhead backcross and UND indicates the sample could not be identified using diagnostic panel or NewHybrids.

Location	Scal	Car	Great	F1	ScBX	CarBX	Und
NC	8	4	0	2	0	0	0
SC	153	198	1	27	23	7	0
GA	72	17	2	4	5	0	0
Atl FL-N	188	7	0	0	1	0	0
Atl FL-C	169	25	0	4	9	1	1
FL-KY	36	0	0	0	0	0	0
G FL-C	3	0	0	0	0	0	0
G FL-N	117	0	1	0	0	0	0
CG	53	0	0	0	0	0	0
TX	91	0	2	0	0	0	0
Atl-U	1	0	0	0	0	0	0
<b>Total</b>	<b>891</b>	<b>251</b>	<b>6</b>	<b>37</b>	<b>38</b>	<b>8</b>	<b>1</b>



**Fig. 2.** Sampling locations of scalloped (A) and Carolina hammerheads (B) in the U.S. Atlantic and eastern Gulf of Mexico.

**Table 3**

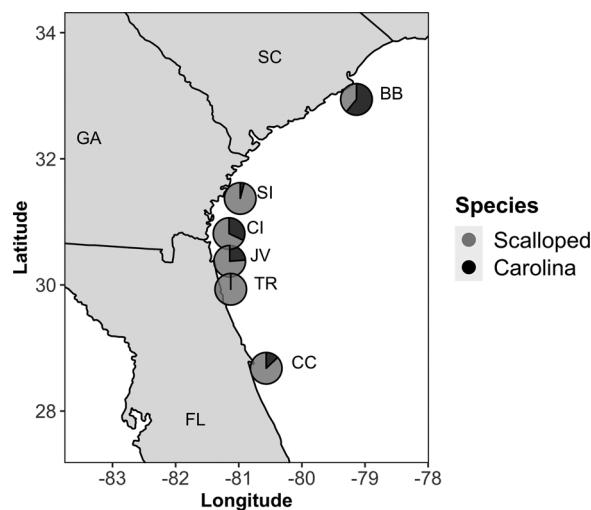
Relative abundance (as a percentage) of scalloped (Scal) and Carolina (Car) hammerheads in sampled regions of the U.S. Atlantic: North Carolina (NC), South Carolina (SC), Georgia (GA), Atlantic northern Florida (Atl FL-N), Atlantic central Florida (Atl FL-C). N indicates the total number of individuals used to calculate relative abundance. Locations are listed from highest to lowest latitude.

Location	N	Scal	Car
NC	12	66.7	33.3
SC	351	43.6	56.4
GA	89	80.9	19.1
Atl FL-N	195	96.4	3.6
Atl FL-C	194	87.1	12.9

**Table 4**

Relative abundance (as a percentage) of scalloped (Scal) and Carolina (Car) hammerhead young-of-the-year and small juveniles ( $\leq 1000$  mm stretch total length) in nurseries in the U.S. Atlantic: Bulls Bay, SC (BB), Sapelo Island, GA (SI), Cumberland Island, GA (CI), Jacksonville, FL (JV), Tolomato River, FL (TR), Cape Canaveral, FL (CC). N indicates the total number of individuals used to calculate relative abundance. Locations are listed from highest to lowest latitude.

Location	N	Scal	Car
BB	287	39.0	61.0
SI	24	95.8	4.2
CI	25	68.0	32.0
JV	21	76.2	2.8
TR	148	100.0	0.0
CC	177	87.0	13.0



**Fig. 3.** Relative abundance of Carolina and scalloped hammerheads in U.S. Atlantic nurseries: Bulls Bay, SC (BB, n = 287), Sapelo Island, GA (SI, n = 24), Cumberland Island, GA (CI, n = 25), Jacksonville, FL (JV, n = 21), Tolomato River, FL (TR, n = 148), and Cape Canaveral, FL (CC, n = 177).

temperature ( $Z = 5.059$ ,  $P < 0.001$ ) were significant predictors of species identity. The optimal generalized linear model also indicated salinity ( $Z = 3.771$ ,  $P < 0.001$ ) was significant, as well as month ( $Z = 2.360$ ,  $P = 0.018$ ) and these variables had a significant positive

**Table 5**

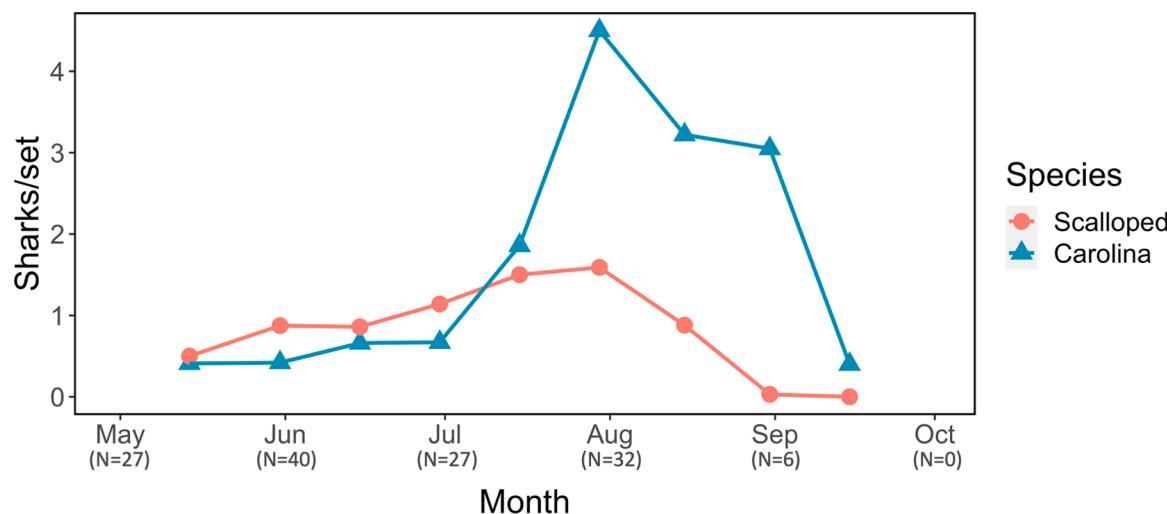
Relative abundance (as a percentage) of scalloped and Carolina hammerhead young-of-the-year in Bulls Bay, SC from 2012-2014 and 2016-2019 during the months May-August. N indicates the total number of individuals used to calculate relative abundance.

	2012	2013	2014	2016	2017	2018	2019
Scal	13.00	59.60	42.90	48.00	41.40	21.60	68.80
Car	87.00	40.40	57.10	52.00	58.60	78.40	31.20
N	23	47	28	25	70	74	16

association with Carolina hammerhead abundance, meaning that the relative probability that a sampled individual was a Carolina hammerhead increased with higher salinity and later months in the sampling period (Fig. S2).

#### 4. Discussion

Hammerhead sharks sampled along the U.S. Atlantic and Gulf of Mexico were genetically identified to describe the relative abundance and distribution of scalloped and Carolina hammerheads. In the U.S. Atlantic, 63.6 % of identified hammerhead sharks were scalloped hammerheads, 27.0 % were Carolina hammerheads, 8.9 % had mixed ancestry, and 0.4 % were smooth or great hammerheads. Scalloped and Carolina hammerheads were identified in the U.S. Atlantic from North Carolina to Florida. In the Gulf, 99 % of identified hammerhead sharks



**Fig. 4.** Catch per unit effort (defined as the number of sharks caught per gillnet set) of young-of-the-year Carolina and scalloped hammerheads in Bulls Bay, SC from May through September 2013–2018. N indicates the total number of gillnet sets performed in each month.

were scalloped hammerheads and no Carolina hammerheads were identified; the remaining 1% were great hammerheads. Carolina hammerheads were more abundant than scalloped hammerheads in South Carolina, but the relative abundance of Carolina hammerheads decreased with latitude.

Though the focus of the study was scalloped and Carolina hammerheads, genetic identifications revealed that four different species were sampled, with three great hammerheads sampled in the Gulf, and three great hammerheads and one smooth hammerhead sampled in the Atlantic. Most misidentified hammerheads were small juveniles (5 of 7 < 1000 mm STL), demonstrating that even experienced researchers and fishers may have difficulty differentiating among known sphyraenids, especially at small sizes. The shape of the anterior margin of the cephalofoil is one of the morphological characters used to distinguish among scalloped, great and smooth hammerheads, but cephalofoil shape changes with age and distinguishing features are not always apparent in small individuals (Castro, 2011; Gilbert, 1967). Data regarding the early life history of smooth and great hammerheads is limited, and minor diagnostic morphological differences present in YOY of different species may be easily missed when certain species are not reported to occur in a given area.

Relative abundance of Carolina hammerheads was highest in Bulls Bay (61%, n = 287), and more than 70% of all Carolina hammerheads identified in this study were sampled there. Reasons for such high abundance (relative and absolute) in a limited geographic area are not clear. One possible explanation is that Carolina hammerheads exhibit a high degree of natal philopatry, with a large proportion of breeding females in the western North Atlantic having been born in Bulls Bay and then returning to the same site to give birth. Philopatric behavior at a regional scale has been documented in a number of shark species (reviewed in Chapman et al., 2015), but evidence of natal philopatry is less common. A combination of tag-recapture and genetic data has shown that some lemon sharks (*Negaprion brevirostris*) in Bimini, Bahamas exhibit strong long-term site fidelity to their natal nursery, despite the availability of other appropriate nursery habitat nearby (Feldheim et al., 2014). Natal philopatry has also been suggested in blacktip reef sharks (*Carcharhinus melanopterus*) in Moorea, where some females made repeated migrations to the same nursery outside their usual home range (Mourier and Planes, 2013). Long term genetic profiling of Carolina hammerhead YOY and genetic reconstruction of parental genotypes would be needed to determine if the progeny of individual female sharks use Bulls Bay year after year.

In Bulls Bay the abundance of scalloped and Carolina hammerhead YOY varied across the sampling season. Both species were first

documented in the nursery in early May and both increased in abundance until peaking in July. Scalloped hammerheads were more abundant until mid-July, when a large spike in Carolina hammerhead abundance was observed (Fig. 4). Carolina hammerheads were more abundant for the remainder of the season and appeared to stay in the nursery at least a month longer than scalloped hammerheads. The increase in abundance for both species from May to July suggests parturition may occur over a prolonged period, but the surge of Carolina hammerhead abundance in July may indicate that time of peak parturition in Carolina hammerheads is offset from that of scalloped hammerheads. In addition, parturition in one or both species may occur at locations outside the nursery area, a reproductive strategy seen in Atlantic sharpnose sharks (*Rhizoprionodon terraenovae*; Ulrich et al., 2007) and bonnetheads (*S. tiburo*; Frazier et al., 2014). If Carolina hammerhead parturition occurs at a more remote location, the observed increase in July could reflect an offset in time of peak arrival. The observation of a temporal offset in abundance is consistent with temporal habitat partitioning, a mechanism that minimizes competition among species because shared limited resources are used at different times (Ross, 1986; Schoener, 1974). Temporal partitioning can occur on a diel scale, where competitors are active during different times of the day (Kronfeld-Schor and Dayan, 2003) or on a seasonal scale, such that peak abundance occurs during different times of the year (Paine, 1963; Sandercock, 1967). If Carolina hammerheads give birth later or delay movement into the nursery until scalloped hammerhead neonates have grown, it could minimize overlap of resource usage.

By contrast to the results seen in South Carolina, relatively few Carolina hammerheads were captured in northern Florida, with none found in the Tolomato River, even though the Tolomato River was well-sampled (n = 148), and Carolina hammerheads were found in areas north and south of the nursery, including adjacent coastal waters near Jacksonville. The Tolomato River differs from other sampled nurseries along the U.S. Atlantic in that it is behind a series of barrier islands and therefore not directly connected to the western North Atlantic. Instead, it only receives saltwater influx from the St. Johns River to the north and St. Augustine inlet to the south, both of which are approximately 20 miles in distance from the sampling site. Higher salinity was a significant predictor of Carolina hammerheads in Bulls Bay, and average monthly salinity was consistently lower in the Tolomato River than in Bulls Bay (Table 6). The other nurseries sampled in Florida occur in nearshore waters rather than estuaries, with stable salinities more closely matching oceanic conditions (Cape Canaveral ~36 ppt, Jacksonville ~32 ppt, Table 6; Iafrate et al., 2019). In Georgia nurseries, scalloped hammerheads were sampled in both estuaries as well as nearshore waters, while

**Table 6**

Average salinity in U.S. Atlantic nurseries during sampling seasons from August 2012-June 2019: Bulls Bay, SC (BB), Jacksonville, FL (JV), and Tolomato River, FL (TR).

Nursery	May	June	July	Aug	Sept
BB	32.06	31.45	31.33	32.52	30.70
JV	31.60	NA	32.04	32.83	32.39
TR	30.53	28.62	24.61	24.3	22.19

Carolina hammerheads were sampled almost solely in nearshore waters (Fig. S3). Salinity is one of the most important predictors of shark species abundance in estuaries and bays in the Gulf and U.S. Atlantic (Bethea et al., 2015; Ulrich et al., 2007), and a preference for higher salinity waters may explain the absence of Carolina hammerheads in the Tolomato River.

Carolina hammerheads also were absent in the Gulf, a result that is somewhat surprising given the high dispersive capability of hammerheads. One possibility is that Carolina hammerheads prefer temperate waters, which restricts their movement around the southern Florida Peninsula and into the Gulf. A preference for temperate water is consistent with the identification of Carolina hammerheads in southern Brazil, the only location that Carolina hammerheads have been identified outside the southeast U.S. (Pinhal et al., 2012), and the observation that the relative abundance of Carolina hammerheads decreased from north to south in the western North Atlantic. South of Cape Canaveral is a well-known biogeographic break that marks the transition from temperate to tropical fauna of south Florida and coincides with genetic divergence between Atlantic and Gulf populations of many coastal marine fishes, including sharks (Avise, 1992; Portnoy et al., 2014, 2015, 2016). Similarly, the range of the closely related smooth hammerhead was also thought to include mostly temperate water of the Atlantic (Castro, 2011), though the collection of one individual in the Gulf was recently reported (Deacy et al., 2020). Few samples of mature adult Carolina hammerhead were obtained, and it is possible that adult Carolina hammerheads move into the Gulf, but despite robust sampling ( $n = 303$ ) no YOY or juvenile Carolina hammerhead were caught, consistent with the species being absent. This is further supported by the fact that admixture between Carolina and scalloped hammerheads was detected in the Atlantic but not in the Gulf. An aversion to lower salinity waters could also partially explain the absence of Carolina hammerheads in the Gulf as there are several significant freshwater inflows in the northern temperate Gulf, including the Mississippi River, Mobile Bay and Atchafalaya River (Morey et al., 2003), but this does not explain the absence of Carolina hammerheads from more saline habitat along the West Florida Shelf and the coast of Texas. This potential lack of appropriate nursery habitat types or conditions coupled with philopatric behavior may have contributed to the present-day absence of Carolina hammerheads in the Gulf.

Research on Carolina hammerheads has thus far focused on immature individuals, making it difficult to relate relative abundances in nurseries to relative abundances within managed populations of mature hammerheads. Samples for this study were collected primarily from fishery-independent surveys in which large juveniles and adults are not commonly encountered ( $n = 87$ ; Atlantic = 47, Gulf = 40; Fig. S4). Three adult male Carolina hammerheads were captured offshore of South Carolina on longlines along with large juvenile and adult scalloped hammerheads. It is unclear if the species are spatially segregated as adults or if they use similar habitat at all life stages, but contemporary hybridization between species indicates some overlap of reproductive habitat (Barker et al., 2019). Recent methods using morphometrics and machine learning have been developed to aid in field identification of cryptic blacktip (*Carcharhinus limbatus*) and Australian blacktip sharks (*C. tilstoni*), and while large individuals were accurately identified 96 % of the time in field trials, identification was less successful for smaller sharks (<1200 mm total length; Johnson et al., 2017). Continued

research on Carolina hammerheads of all sizes may reveal subtle differences that may be useful in differentiating the species based on morphology and thus enable a further understanding of species-specific habitat utilization based on field identifications.

Differences in life history characteristics have been observed in cryptic shark species, and similar differences could exist between scalloped and Carolina hammerheads. For example, the blacktip shark and Australian blacktip shark are difficult to distinguish morphologically, but differ in length at maturity, maximum body size, habitat requirements, and time of parturition (Harry et al., 2012, 2019). These differences in life history and reproductive ecology have significant management implications because the species differ in their susceptibility to exploitation and respond differently to management measures (Harry et al., 2012, 2019). In addition to physiological differences suggested by the observed preference for higher salinity, Carolina hammerheads are reported to have a smaller size at birth than scalloped hammerheads (Quattro et al., 2013) and this may be indicative of life history differences between mature scalloped and Carolina hammerheads (e.g. differences in maternal length or brood size). Currently insufficient data exist to determine if size differences persist throughout their life span. Differences in body size can act as a mechanism that maintains species boundaries through assortative mating in sympatrically distributed species (Nagel and Schlüter, 1998), but hybridization between scalloped and Carolina hammerheads (Barker et al., 2019) indicates that if there are differences in adult size they do not act as a complete reproductive barrier.

The large panel of diagnostic SNPs used for species identification was reliable and accurate, but the molecular methods employed in this study would not be cost-effective for future studies focused only on species identification. MtDNA and nuclear ribosomal ITS2 are cheaper and more accessible methods that can be used to discriminate sphyrnids (Abercrombie et al., 2005; Quattro et al., 2006), however, neither of these methods can be used to identify hybrids and characterize backcrossing (Barker et al., 2019). Future work could use a subset of the diagnostic SNPs in a Genotyping-in-Thousands by sequencing (GT-seq) approach (Campbell et al., 2015) to genetically identify thousands of individuals in an economically efficient manner while discriminating hybrids. Recently developed CRISPR-based SHERLOCK methodologies may also be an accessible and cost-effective approach for rapid species identification in the future (Baerwald et al., 2020). Although the approach used in this study is more costly, data can be used simultaneously in a population genetics framework (Dimens et al., 2019; Portnoy et al., 2015), and to provide relevant information for conservation and management such as estimates of effective size (Waples et al., 2016) or abundance using a close-kin-mark-recapture framework (Bravington et al., 2016; Hillary et al., 2018).

Over 25 % of hammerheads sampled in the U.S. Atlantic were identified as Carolina hammerheads, and if mature individuals are found in a similar proportion, they would comprise a significant part of what is currently considered to be the U.S. Atlantic scalloped hammerhead stock. Scalloped hammerheads in the U.S. Atlantic are currently considered overfished with overfishing occurring and managed as part of the hammerhead shark complex, which also includes great and smooth hammerheads. Quotas for the hammerhead complex are set according to the total allowable catch of scalloped hammerhead (Hayes et al., 2009; NMFS, 2013). If past assessments include data from a second species that differs biologically, it would have significant implications for management of the hammerhead complex. There is also a critical need for Carolina hammerhead life history data. The results of this study suggest that Carolina hammerheads have a limited range in the western North Atlantic, and only three Carolina hammerheads have been identified in the South Atlantic (Pinhal et al., 2012). Although the effects are difficult to predict, ongoing hybridization and backcrossing with scalloped hammerheads could contribute to the loss of Carolina hammerheads over time (Barker et al., 2019). Further, given the observed limited range, unknown life history characteristics, and continued

directed and incidental fishing pressure, there is potential for Carolina hammerheads to be overfished in the U.S. Atlantic. Scalloped hammerheads are listed as Critically Endangered on the IUCN Red List of Threatened Species (Rigby et al., 2019c), and international trade is regulated by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, Appendix II). However, the status of Carolina hammerheads has not been assessed. Consideration of Carolina hammerheads separate from scalloped hammerheads in future national and international management is warranted but will likely require the development of methods to differentiate them in the field.

### Animal ethics and welfare

Animals collected by Texas A&M University-Corpus Christi personnel were collected under Scientific Research Permit No. SPR-0614-111 and IACUC AUP #03-15 and samples provided by Texas Parks and Wildlife were sampled under Scientific Collection Authorizations. Samples from the Florida State University Coastal and Marine Lab were collected under FSU IACUC Protocol 1718 and Florida Fish and Wildlife Conservation Commission Special Activities Licenses SAL-1345 and SAL-1092. Animals from the GULFSPAN Survey were collected under Florida Fish and Wildlife Conservation Commission Special Activities License number SAL-18-1292-SRP. Collection of specimens by South Carolina Department of Natural Resources were conducted under SCDNR Scientific Permit #2212. Samples collected by University of North Florida personnel were collected under FWC Special Activity Permit SAL-15-1136A-SR. Samples from Cape Canaveral were collected under NASA-KSC Ecological Program, SAL-12-0512SR, and SAL-14-1409-SRP. Samples from Georgia State University were collected under Georgia DNR Scientific Collecting Permit #1000520150 and Georgia Southern IACUC I17001. Samples from NMFS Southeast Fisheries Science Center Mississippi Laboratories were collected under permit NMFSPD 04-112-01.

### Funding

Work was funded by U.S. Fish and Wildlife Service Competitive State Wildlife Grant number SC-U2-F15AP00050 and NOAA/NMFS Cooperative Research Program Grant numbers NA14NMF4540063 and NA16NMF4540084. Work in Cape Canaveral, FL was conducted under NASA Environmental and Medical Contract #80KSC020D0023. Funding sources were not involved in the study design, collection, analysis, and interpretation of data, writing of the manuscript, or decision to submit the article for publication.

### Data availability

The panels of diagnostic SNPs, individual genotypes, scripts, and sample metadata are available at [https://github.com/marinegenomicslab/HH\\_abundance](https://github.com/marinegenomicslab/HH_abundance) [<https://doi.org/10.5281/zenodo.4959214>]. The COI haplotype is available on the GenBank Nucleotide Database at <https://blast.ncbi.nlm.nih.gov> and can be accessed with accession no MT863713.

### CRedit authorship contribution statement

**Amanda M. Barker:** Conceptualization, Formal analysis, Investigation, Writing - original draft, Visualization. **Bryan S. Frazier:** Conceptualization, Formal analysis, Resources, Writing - original draft, Supervision, Funding acquisition. **Douglas H. Adams:** Resources, Writing - original draft. **Christine N. Bedore:** Resources, Writing - original draft. **Carolyn N. Belcher:** Resources, Writing - original draft. **William B. Driggers:** Resources, Writing - original draft. **Ashley S. Galloway:** Resources, Writing - original draft. **James Gelsleichter:** Resources, Writing - original draft. **R. Dean Grubbs:** Resources, Writing - original draft, Funding acquisition. **Eric A. Reyier:** Resources, Writing

- original draft. **David S. Portnoy:** Conceptualization, Formal analysis, Investigation, Writing - original draft, Supervision, Funding acquisition.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Acknowledgements

We thank past and present members of the Marine Genomics Laboratory at Texas A&M University- Corpus Christi for assistance with troubleshooting laboratory work and many helpful conversations that have improved this manuscript. We thank J. Selwyn for help with statistical analysis. We thank M. Ajemian and G. Stunz (Texas A&M University- Corpus Christi), D. Bethea, B. Deacy, K. Hannan, C. Jones and L. Jones (National Marine Fisheries Service), M. Drymon (Mississippi State University), D. Wells (Texas A&M University- Galveston), J. Gardiner (Mote Marine Laboratory), F. Martinez-Andrade, C. Collins, N. Boyd, C. Troxler, C. Mace, B. Bartram, F. Grubbs, and M. Dunks (Texas Parks and Wildlife Department), and M. McCallister, C. Morgan, and B. Wargat (University of North Florida) for sample collection. This article is publication number 27 of the Marine Genomics Laboratory at Texas A&M University–Corpus Christi, number 120 in the series Genetic Studies in Fishes and contribution 847 of the South Carolina Marine Resources Center.

### Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.fishres.2021.106039>.

### References

- Abercrombie, D.L., Clarke, S.C., Shivji, M.S., 2005. Global-scale genetic identification of hammerhead sharks: application to assessment of the international fin trade and law enforcement. *Conserv. Genet.* 6, 775–788. <https://doi.org/10.1007/s10592-005-9036-2>.
- Adams, D.H., Paperno, R., 2007. Preliminary assessment of a nearshore nursery ground for the scalloped hammerhead off the Atlantic coast of Florida. *Am. Fish. Soc. Symp.* 50, 165–174.
- Anderson, E.C., Thompson, E.A., 2002. A model-based method for identifying species hybrids using multilocus genetic data. *Genetics* 160, 1217–1299.
- Avise, J.C., 1992. Molecular population structure and the biogeographic history of a regional fauna: A case history with lessons for conservation biology. *Oikos* 63, 62–76. <https://doi.org/10.2307/3545516>.
- Baerwald, M.R., Goodbla, A.M., Nagarajan, R.P., Goottenberg, J.S., Abudayyeh, O.O., Zhang, F., Schreier, A.D., 2020. Rapid and accurate species identification for ecological studies and monitoring using CRISPR-based SHERLOCK. *Mol. Ecol. Resour.* <https://doi.org/10.1111/1755-0998.13186>.
- Barker, M.J., Schluessel, V., 2005. Managing global shark fisheries: suggestions for prioritizing management strategies. *Aquat. Conserv. Mar. Freshw. Ecosyst.* 15, 325–347. <https://doi.org/10.1002/aqc.660>.
- Barker, A.M., Frazier, B.S., Bethea, D.M., Gold, J.R., Portnoy, D.S., 2017. Identification of young-of-the-year great hammerhead shark *Sphyrna mokarran* in northern Florida and South Carolina. *J. Fish Biol.* 91, 664–668. <https://doi.org/10.1111/jfb.13356>.
- Barker, A.M., Adams, D.H., Driggers, W.B., Frazier, B.S., Portnoy, D.S., 2019. Hybridization between sympatric hammerhead sharks in the western North Atlantic Ocean. *Biol. Lett.* 15 <https://doi.org/10.1098/rsbl.2019.0004>.
- Bethea, D.M., Ajemian, M.J., Carlson, J.K., Hoffmayer, E.R., Imhoff, J.L., Grubbs, R.D., Peterson, C.T., Burgess, G.H., 2015. Distribution and community structure of coastal sharks in the northeastern Gulf of Mexico. *Environ. Biol. Fishes* 98, 1233–1254. <https://doi.org/10.1007/s10641-014-0355-3>.
- Bickford, D., Lohman, D.J., Sodhi, N.S., Ng, P.K.L., Meier, R., Winker, K., Ingram, K.K., Das, I., 2007. Cryptic species as a window on diversity and conservation. *Trends Ecol. Evol.* 22, 148–155. <https://doi.org/10.1016/j.tree.2006.11.004>.
- Branstetter, S., 1987. Age, growth and reproductive biology of the silky shark, *Carcharhinus falciformis*, and the scalloped hammerhead, *Sphyrna lewini*, from the northwestern Gulf of Mexico. *Environ. Biol. Fishes* 19, 161–173. <https://doi.org/10.1007/BF00005346>.
- Bravington, M.V., Skaug, H.J., Anderson, E.C., 2016. Close-kin mark-recapture. *Stat. Sci.* 31, 259–274. <https://doi.org/10.1214/16-STS552>.
- Cailliet, G.M., Smith, W.D., Mollet, H.F., Goldman, K.J., 2006. Age and growth studies of chondrichthyan fishes: the need for consistency in terminology, verification,

- validation, and growth function fitting. Environ. Biol. Fishes 77, 211–228. <https://doi.org/10.1007/s10641-006-9105-5>.
- Campbell, N.R., Harmon, S.A., Narum, S.R., 2015. Genotyping-in-Thousands by sequencing (GT-seq): a cost effective SNP genotyping method based on custom amplicon sequencing. Mol. Ecol. Resour. 15, 855–867. <https://doi.org/10.1111/1755-0998.12357>.
- Castro, J.I., 1993. The shark nursery of Bulls Bay, South Carolina, with a review of the shark nurseries of the southeastern coast of the United States. Environ. Biol. Fishes 38, 37–48. <https://doi.org/10.1007/BF00842902>.
- Castro, J.I., 2011. The Sharks of North America. Oxford University Press, New York, NY.
- Catchen, J., Hohenlohe, P.A., Bassham, S., Amores, A., Cresko, W.A., 2013. Stacks: an analysis tool set for population genomics. Mol. Ecol. 22, 3124–3140. <https://doi.org/10.1111/mec.12354>.
- Chapman, D.D., Feldheim, K.A., Papastamatiou, Y.P., Hueter, R.E., 2015. There and back again: a review of residency and return migrations in sharks, with implications for population structure and management. Annu. Rev. Mar. Sci. 7, 547–570. <https://doi.org/10.1146/annurev-marine-010814-015730>.
- Clarke, S.C., McAllister, M.K., Milner-Gulland, E.J., Kirkwood, G.P., Michielsens, C.G.J., Agnew, D.J., Piñitch, E.K., Nakano, H., Shivji, M.S., 2006. Global estimates of shark catches using trade records from commercial markets. Ecol. Lett. 9, 1115–1126. <https://doi.org/10.1111/j.1461-0248.2006.00968.x>.
- Colborn, J., Crabtree, R.E., Shaklee, J.B., Pfeiler, E., Bowen, B.W., 2001. The evolutionary enigma of bonefishes (*Albula* spp.): cryptic species and ancient separations in a globally distributed shorefish. Evolution 55, 807–820.
- Corrigan, S., Huveneers, C., Schwartz, T.S., Harcourt, R.G., Beheregaray, L.B., 2008. Genetic and reproductive evidence for two species of ornate wobbegong shark *Orectolobus* spp. On the Australian east coast. J. Fish Biol. 73, 1662–1675. <https://doi.org/10.1111/j.1095-8649.2008.02039.x>.
- Craig, M.T., Graham, R.T., Torres, R.A., Hyde, J.R., Freitas, M.O., Ferreira, B.P., Hostim-Silva, M., Gerhardinger, L.C., Bertoncini, A.A., Robertson, D.R., 2009. How many species of goliath grouper are there? Cryptic genetic divergence in a threatened marine fish and the resurrection of a geopolitical species. Endanger. Species Res. 7, 167–174. <https://doi.org/10.3354/esr00117>.
- Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A., Handsaker, R.E., Lunter, G., Marth, G.T., Sherry, S.T., McVean, G., Durbin, R., 1000 Genomes Project Analysis Group, 2011. The variant call format and VCFtools. Bioinformatics 27, 2156–2158. <https://doi.org/10.1093/bioinformatics/btr330>.
- Deacy, B.M., Moncrief-Cox, H.E., Carlson, J.K., 2020. First verified record of the smooth hammerhead (*Sphyrna zygaena*) in coastal waters of the northern Gulf of Mexico with a review of their occurrence in the western North Atlantic Ocean. Southeast. Nat. 19 <https://doi.org/10.1656/058.019.0105>.
- Dimens, P.V., Willis, S., Dean Grubbs, R., Portnoy, D.S., 2019. A genomic assessment of movement and gene flow around the South Florida vicariance zone in the migratory coastal blacknose shark, *Carcharhinus acronotus*. Mar. Biol. 166, 86. <https://doi.org/10.1007/s00227-019-3533-1>.
- Dulvy, N.K., Simpfendorfer, C.A., Davidson, L.N.K., Fordham, S.V., Bräutigam, A., Sant, G., Welch, D.J., 2017. Challenges and priorities in shark and ray conservation. Curr. Biol. 27, R565–R572. <https://doi.org/10.1016/j.cub.2017.04.038>.
- Duncan, K.M., Martin, A.P., Bowen, B.W., De Couet, H.G., 2006. Global phylogeography of the scalloped hammerhead shark (*Sphyrna lewini*). Mol. Ecol. 15, 2239–2251. <https://doi.org/10.1111/j.1365-294X.2006.02933.x>.
- Feldheim, K.A., Gruber, S.H., DiBattista, J.D., Babcock, E.A., Kessel, S.T., Hendry, A.P., Piñitch, E.K., Ashley, M.V., Chapman, D.D., 2014. Two decades of genetic profiling yields first evidence of natal philopatry and long-term fidelity to parturition sites in sharks. Mol. Ecol. 23, 110–117. <https://doi.org/10.1111/mec.12583>.
- Fisher, C., Robinson, C.H., Malard, F., 2018. Cryptic species as a window into the paradigm shift of the species concept. Mol. Ecol. 27, 613–635.
- Frazier, B.S., Driggers, W.B., Adams, D.H., Jones, C.M., Loeber, J.K., 2014. Validated age, growth and maturity of the bonnethead *Sphyrna tiburo* in the western North Atlantic Ocean. J. Fish Biol. 85, 688–712. <https://doi.org/10.1111/jfb.12450>.
- Gallagher, A.J., Serafy, J.E., Cooke, S.J., Hammerschlag, N., 2014. Physiological stress response, reflex impairment, and survival of five sympatric shark species following experimental capture and release. Mar. Ecol. Prog. Ser. 496, 207–218. <https://doi.org/10.3354/meps10490>.
- Gilbert, C.R., 1967. A revision of the hammerhead sharks (Family Sphyrnidae). Proc. U. S. Natl. Mus. 119, 1–88.
- Giresi, M.M., Grubbs, R.D., Portnoy, D.S., Driggers III, W.B., Jones, L., Gold, J.R., 2015. Identification and distribution of morphologically conserved smoothhound sharks in the northern Gulf of Mexico. Trans. Am. Fish. Soc. 144, 1301–1310. <https://doi.org/10.1080/0028487.2015.1069212>.
- Griffiths, A.M., Sims, D.W., Cotterell, S.P., Nagar, A.E., Ellis, J.R., Lynghammar, A., McHugh, M., Neat, F.C., Pade, N.G., Queiroz, N., Serra-Pereira, B., Rapp, T., Wearmouth, V.J., Genner, M.J., 2010. Molecular markers reveal spatially segregated cryptic species in a critically endangered fish, the common skate (*Dipturus batis*). Proc. Biol. Sci. 277, 1497–1503.
- Gulak, S., de Ron Santiago, A., Carlson, J., 2015. Hooking mortality of scalloped hammerhead *Sphyrna lewini* and great hammerhead *Sphyrna mokarran* sharks caught on bottom longlines. Afr. J. Mar. Sci. 37, 267–273. <https://doi.org/10.2989/1814232X.2015.1026842>.
- Harry, A.V., Morgan, J.A.T., Ovenden, J.R., Tobin, A.J., Welch, D.J., Simpfendorfer, C.A., 2012. Comparison of the reproductive ecology of two sympatric blacktip sharks (*Carcharhinus limbatus* and *Carcharhinus tilstoni*) off north-eastern Australia with species identification inferred from vertebral counts. J. Fish Biol. 81, 1225–1233. <https://doi.org/10.1111/j.1095-8649.2012.03400.x>.
- Harry, A.V., Butcher, P.A., Macbeth, W.G., Morgan, J.A.T., Taylor, S.M., Geraghty, P.T., 2019. Life history of the common blacktip shark, *Carcharhinus limbatus*, from central eastern Australia and comparative demography of a cryptic shark complex. Mar. Freshw. Res. 70, 834–848. <https://doi.org/10.1071/MF18141>.
- Hayes, C.G., Jiao, Y., Cortés, E., 2009. Stock assessment of scalloped hammerheads in the western North Atlantic Ocean and Gulf of Mexico. North Am. J. Fish. Manag. 29, 1406–1417. <https://doi.org/10.1577/M08-026.1>.
- Heupel, M., Carlson, J., Simpfendorfer, C., 2007. Shark nursery areas: concepts, definition, characterization and assumptions. Mar. Ecol. Prog. Ser. 337, 287–297. <https://doi.org/10.3354/meps337287>.
- Hillary, R.M., Bravington, M.V., Patterson, T.A., Grewe, P., Bradford, R., Feutry, P., Gunasekera, R., Peddemors, V., Werry, J., Francis, M.P., Duffy, C.A.J., Bruce, B.D., 2018. Genetic relatedness reveals total population size of white sharks in eastern Australia and New Zealand. Sci. Rep. 8, 2661 <https://doi.org/10.1038/s41598-018-20593-w>.
- Hothorn, T., Hornik, K., van de Wiel, M.A., Zeileis, A., 2008. Implementing a class of permutation tests: the coin package. J. Stat. Softw. 28 <https://doi.org/10.18637/jss.v028.i08>.
- Hueter, R.E., Tyminski, J.P., 2007. Species-specific distribution and habitat characteristics of shark nurseries in Gulf of Mexico waters off peninsular Florida and Texas. Am. Fish. Soc. Symp. 50, 193–223.
- Hyde, J.R., Kimbrell, C.A., Budrick, J.E., Lynn, E.A., Vetter, R.D., 2008. Cryptic speciation in the vermillion rockfish (*Sebastodes miniatus*) and the role of bathymetry in the speciation process. Mol. Ecol. 17, 1122–1136 <https://doi.org/10.dz3hz7>.
- Hyde, J.R., Underkofler, K.E., Sundberg, M.A., 2014. DNA barcoding provides support for a cryptic species complex within the globally distributed and fishery important opah (*Lampris guttatus*). Mol. Ecol. Resour. 14, 1239–1247. <https://doi.org/10.1111/1755-0998.12268>.
- Iafrate, J.D., Watwood, S.L., Reyier, E.A., Ahr, B.J., Scheidt, D.M., Holloway-Adkins, K.G., Provancha, J.A., Stolen, E.D., 2019. Behavior, Seasonality, and Habitat Preferences of Mobile Fishes and Sea Turtles within a Large Sand Shoal Complex: Insights From Traditional Sampling and Emerging Technologies (No. OCS Study BOEM 2019-043). US Department of the Interior, Bureau of Ocean Energy Management, Sterling (VA).
- IUCN, 2019. IUCN Red List Assessment [WWW Document]. URL [https://www.iucnssg.org/g/uploads/5/4/1/2/54120303/r1\\_assessment\\_results\\_190124.pdf](https://www.iucnssg.org/g/uploads/5/4/1/2/54120303/r1_assessment_results_190124.pdf) (Accessed 8.1.20).
- Johnson, G.J., Buckworth, R.C., Lee, H., Morgan, J.A.T., Ovenden, J.R., McMahon, C.R., 2017. A novel field method to distinguish between cryptic carcharhinid sharks, Australian blacktip shark *Carcharhinus tilstoni* and common blacktip shark *C. limbatus*, despite the presence of hybrids. J. Fish Biol. 90, 39–60. <https://doi.org/10.1111/jfb.13102>.
- Kronfeld-Schor, N., Dayan, T., 2003. Partitioning of time as an ecological resource. Annu. Rev. Ecol. Evol. Syst. 34, 153–181. <https://doi.org/10.1146/annurev.ecolsys.34.011802.132435>.
- Lintott, P.R., Barlow, K., Bunnefeld, N., Briggs, P., Gajas Roig, C., Park, K.J., 2016. Differential responses of cryptic bat species to the urban landscape. Ecol. Evol. 6, 2044–2052. <https://doi.org/10.1002/ece3.1996>.
- Martinez-Takeshita, N., Purcell, C.M., Chabot, C.L., Craig, M.T., Paterson, C.N., Hyde, J.R., Allen, L.G., 2015. A tale of three tails: cryptic speciation in a globally distributed marine fish of the genus *Seriola*. Copeia 103, 357–368. <https://doi.org/10.1643/CI-124-224>.
- Meirmans, P.G., Van Tienderen, P.H., 2004. Genotype and Genodive: two programs for the analysis of genetic diversity of asexual organisms. Mol. Ecol. Notes 4, 792–794. <https://doi.org/10.1111/j.1471-8286.2004.00770.x>.
- Miller, M.H., Carlson, D.J., Cooper, P., Kobayashi, D.D., Wilson, J., 2013. Status Review Report: Scalloped Hammerhead Shark (*Sphyrna lewini*), Report to National Marine Fisheries Service, Office of Protected Resources, Silver Spring, MD.
- Morey, S.L., Martin, P.J., O'Brien, J.J., Wallcraft, A.A., Zavala-Hidalgo, J., 2003. Export pathways for river discharged fresh water in the northern Gulf of Mexico. J. Geophys. Res. 108, 3303 <https://doi.org/10.1029/2002JC001674>.
- Morgan, A., Burgess, G.H., 2007. At-vessel fishing mortality for six species of sharks caught in the Northwest Atlantic and Gulf of Mexico. Gulf Caribb. Res. 19 <https://doi.org/10.18785/gcr.1902.15>.
- Morningstar, C.R., Inoue, K., Sei, M., Lang, B.K., Berg, D.J., 2014. Quantifying morphological and genetic variation of sympatric populations to guide conservation of endangered, micro-endemic springsnails. Aquat. Conserv. Mar. Freshw. Ecosyst. 24, 536–545. <https://doi.org/10.1002/aqc.2422>.
- Mourier, J., Planes, S., 2013. Direct genetic evidence for reproductive philopatry and associated fine-scale migrations in female blacktip reef sharks (*Carcharhinus melanopterus*) in French Polynesia. Mol. Ecol. 22, 201–214. <https://doi.org/10.1111/mec.12103>.
- Nagel, L., Schlüter, D., 1998. Body size, natural selection, and speciation in sticklebacks. Evolution 52, 209–218. <https://doi.org/10.1111/j.1558-5646.1998.tb05154.x>.
- NMFS, 2013. Highly migratory species; Atlantic shark management measures; Amendment 5a; final rule. Fed. Regist. 78, 40318–40350.
- NMFS, 2014. Endangered and threatened wildlife and plants; threatened and endangered status for distinct population segments of scalloped hammerhead sharks; final rule. Fed. Regist. 79, 38214–38242.
- Ovenden, J.R., Morgan, J.A.T., Kashiwagi, T., Broderick, D., Salini, J., 2010. Towards better management of Australia's shark fishery: genetic analyses reveal unexpected ratios of cryptic blacktip species *Carcharhinus tilstoni* and *C. limbatus*. Mar. Freshw. Res. 61, 253–262. <https://doi.org/10.1071/MF09151>.
- Ovenden, J.R., Berry, O., Welch, D.J., Buckworth, R.C., Dichmont, C.M., 2015. Ocean's eleven: a critical evaluation of the role of population, evolutionary and molecular genetics in the management of wild fisheries. Fish Fish. 16, 125–159. <https://doi.org/10.1111/faf.12052>.
- Paine, R.T., 1963. Trophic relationships of 8 sympatric predatory gastropods. Ecology 44, 63–73. <https://doi.org/10.2307/1933181>.

- Pardo, S.A., Cooper, A.B., Dulvy, N.K., 2013. Avoiding fishy growth curves. *Methods Ecol. Evol.* 4, 353–360. <https://doi.org/10.1111/2041-210x.12020>.
- Pfenninger, M., Schwenk, K., 2007. Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. *BMC Evol. Biol.* 7, 121. <https://doi.org/10.1186/1471-2148-7-121>.
- Pinhal, D., Shivji, M.S., Vallinoto, M., Chapman, D.D., Gadig, O.B.F., Martins, C., 2012. Cryptic hammerhead shark lineage occurrence in the western South Atlantic revealed by DNA analysis. *Mar. Biol.* 159, 829–836. <https://doi.org/10.1007/s00227-011-1858-5>.
- Portnoy, D.S., Heist, E.J., 2012. Molecular markers: progress and prospects for understanding reproductive ecology in elasmobranchs. *J. Fish Biol.* 80, 1120–1140. <https://doi.org/10.1111/j.1095-8649.2011.03206.x>.
- Portnoy, D.S., Hollenbeck, C.M., Belcher, C.N., Driggers, W.B., Frazier, B.S., Gelsleichter, J., Grubbs, R.D., Gold, J.R., 2014. Contemporary population structure and post-glacial genetic demography in a migratory marine species, the blacknose shark, *Carcharhinus acronotus*. *Mol. Ecol.* 23, 5480–5495. <https://doi.org/10.1111/mec.12954>.
- Portnoy, D.S., Puritz, J.B., Hollenbeck, C.M., Gelsleichter, J., Chapman, D., Gold, J.R., 2015. Selection and sex-biased dispersal in a coastal shark: the influence of philopatry on adaptive variation. *Mol. Ecol.* 24, 5877–5885. <https://doi.org/10.1111/mec.13441>.
- Portnoy, D.S., Hollenbeck, C.M., Bethea, D.M., Frazier, B.S., Gelsleichter, J., Gold, J.R., 2016. Population structure, gene flow, and historical demography of a small coastal shark (*Carcharhinus isodon*) in US waters of the western Atlantic Ocean. *ICES J. Mar. Sci.* 73, 2322–2332. <https://doi.org/10.1093/icesjms/fsw098>.
- Puritz, J.B., Hollenbeck, C.M., Gold, J.R., 2014. *dDocent*: a RADseq, variant-calling pipeline designed for population genomics of non-model organisms. *PeerJ* 2, e431. <https://doi.org/10.7717/peerj.431>.
- Quattro, J.M., Stoner, D.S., Driggers, W.B., Anderson, C.A., Priede, K.A., Hoppmann, E.C., Campbell, N.H., Duncan, K.M., Grady, J.M., 2006. Genetic evidence of cryptic speciation within hammerhead sharks (Genus *Sphyrna*). *Mar. Biol.* 148, 1143–1155. <https://doi.org/10.1007/s00227-005-0151-x>.
- Quattro, J.M., Driggers, W.B.I., Grady, J.M., Ulrich, G.F., Roberts, M.A., 2013. *Sphyrna gilberti* sp. nov., a new hammerhead shark (Carcarhiniiformes, Sphyrnidae) from the western Atlantic Ocean. *Zootaxa* 3702, 159. <https://doi.org/10.11646/zootaxa.3702.2.5>.
- Rigby, C.L., Barreto, R., Carlson, J., Fernando, D., Fordham, S., Francis, M.P., Herman, K., Jabado, R.W., Liu, K.M., Marshall, A., Pacourea, N., Romanov, E., Sherley, R.B., Winker, H., 2019a. *Sphyrna mokarran*. The IUCN Red List of Threatened Species 2019: e.T39386A299. <https://doi.org/10.2305/IUCN.UK.2019-3.RLTS.T39386A299.en>. Accessed 24 May 2020.
- Rigby, C.L., Barreto, R., Carlson, J., Fernando, D., Fordham, S., Herman, K., Jabado, R.W., Liu, K.M., Marshall, A., Pacourea, N., Romanov, E., Sherley, R.B., Winker, H., 2019b. *Sphyrna zygaena*. The IUCN Red List of Threatened Species 2019: e.T39388A292. <https://doi.org/10.2305/IUCN.UK.2019-3.RLTS.T39388A2921825.en>. Accessed 24 May 2020.
- Rigby, C.L., Dulvy, N.K., Barreto, R., Carlson, J., Fernando, D., Fordham, S., Francis, M.P., Herman, K., Jabado, R.W., Liu, K.M., Marshall, A., Pacourea, N., Romanov, E., Sherley, R.B., Winker, H., 2019c. *Sphyrna lewini*. The IUCN Red List of Threatened Species 2019: e.T39385A2918526. Accessed 16 January 2020.
- Rocha, L.A., Craig, M.T., Bowen, B.W., 2007. Phylogeography and the conservation of coral reef fishes. *Coral Reefs* 26, 501–512. <https://doi.org/10.1007/s00338-007-0261-7>.
- Ross, S.T., 1986. Resource partitioning in fish assemblages: a review of field studies. *Copeia* 1986, 352–388. <https://doi.org/10.2307/1444996>.
- Sáez, A.G., Lozano, E., 2005. Body doubles. *Nature* 433, 111. <https://doi.org/10.1038/43311a>.
- Sandercock, G.A., 1967. A study of selected mechanisms for the coexistence of *Diaptomusspp.* in Clarke Lake, Ontario. *Limnol. Oceanogr.* 12, 97–112. <https://doi.org/10.4319/lo.1967.12.1.0097>.
- Sandoval-Castillo, J., Rocha-Olivares, A., Villavicencio-Garayzar, C., Balart, E., 2004. Cryptic isolation of Gulf of California shovelnose guitarfish evidenced by mitochondrial DNA. *Mar. Biol.* 145, 983–988. <https://doi.org/10.1007/s00227-004-1378-7>.
- Schoener, T.W., 1974. Resource partitioning in ecological communities. *Science* 185, 27–39. <https://doi.org/10.1126/science.185.4145.27>.
- Schönrogge, K., Barr, B., Wardlaw, J.C., Napper, E., Gardner, M.G., Breen, J., Elmes, G.W., Thomas, J.A., 2002. When rare species become endangered: cryptic speciation in myrmecophilous hoverflies. *Biol. J. Linn. Soc. Lond.* 75, 291–300. <https://doi.org/10.1046/j.1095-8312.2002.00019.x>.
- Seutin, G., White, B.N., Boag, P.T., 1991. Preservation of avian blood and tissue samples for DNA analyses. *Can. J. Zool.* 69, 82–90. <https://doi.org/10.1139/z91-013>.
- Ulrich, G.F., Jones, C.M., Driggers III, W.B., Drymon, J.M., Oakley, D., Riley, C., 2007. Habitat utilization, relative abundance, and seasonality of sharks in the estuarine and nearshore waters of South Carolina. *Am. Fish. Soc. Symp.* 50, 125–139.
- Waples, R.K., Larson, W.A., Waples, R.S., 2016. Estimating contemporary effective population size in non-model species using linkage disequilibrium across thousands of loci. *Heredity* 117, 233–240. <https://doi.org/10.1038/hdy.2016.60>.
- Ward, R.D., Holmes, B.H., Zemlak, T.S., Smith, P.J., 2007. DNA barcoding discriminates spurdogs of the genus *squalus*. In: Last, P.R., White, W.T., Pogonoski, J.J. (Eds.), *Descriptions of New Dogfishes of the Genus *Squalus* (Squaloidea: Squalidae)*. CSIRO Marine and Atmospheric Research, pp. 117–130.
- White, W.T., Last, P.R., 2012. A review of the taxonomy of chondrichthyan fishes: a modern perspective. *J. Fish Biol.* 80, 901–917. <https://doi.org/10.1111/j.1095-8649.2011.03192.x>.