

Research



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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 (*Sphyraena 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, *Sphyraena 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

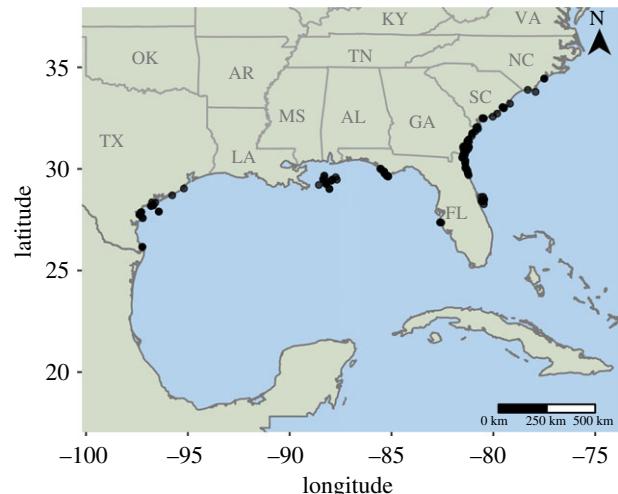


Figure 1. Map of sample locations. (Online version in colour.)

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, F₁ hybrid, scalloped hammerhead backcross or Carolina hammerhead backcross. The F₂ genotype class (offspring of two hybrids) was not included owing to low frequency of putative F₁ 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.

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 F₁ hybrids, 12 scalloped hammerhead backcrosses and one Carolina hammerhead backcross, using the primers Pro-Shark (5'-GCCCTTGGCTCCAAAGC-3') and Phe-Shark (5'-TCATCTTAGCATTTCAAGTGCCA-3'). See electronic supplementary material, methods for PCR conditions.

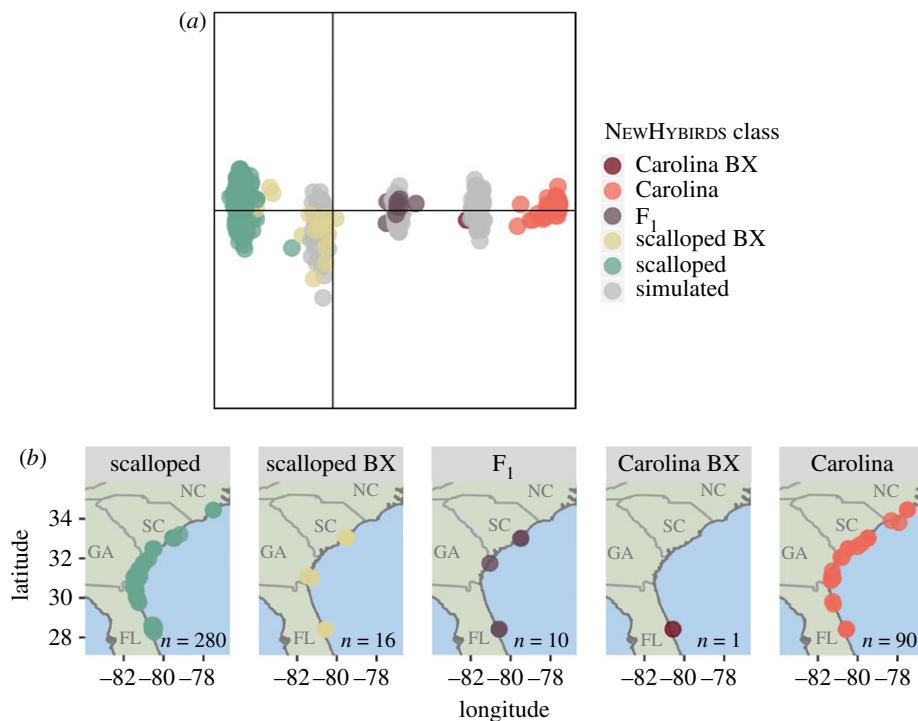


Figure 2. (a) DAPC results showing groupings of sampled and simulated individuals. Points are coloured according to the genotype class assigned in NEWHYBRIDS analysis. BX indicates backcross. (b) Map depicting sampling locations of F_1 hybrids, backcrosses (BX) and pure species individuals as determined 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.

4. Discussion

Hybrids occurred where Carolina hammerheads are distributed in the US Atlantic (figure 2), with the greatest number in

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

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 F₁ 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 F₁ 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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