Using Acoustic Telemetry and Population Genetics to Investigate Cobia Stock Structure in the Southeast U.S.

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ABSTRACT

GALLAGHER, RILEY. Using Acoustic Telemetry and Population Genetics to Investigate Cobia Stock Structure in the Southeast U.S. (Under the Direction of Dr. Jeffrey Buckel).

We used telemetry tagging, genetic analyses and collaborative receiver networks on the U.S. east coast to address questions about cobia (*Rachycentron canadum*) stock structure. From May 2018 to September 2019, we surgically implanted acoustic transmitters in 98 cobia caught in North Carolina and Virginia. Receiver networks between Florida and New Jersey detected 88% of these fish over the course of the study. The majority of tagged cobia were detected in Chesapeake Bay during summer months while a smaller number remained in ocean habitats during this period. In winter 2018-2019, 26% of tagged cobia were detected south of the current stock boundary but within the recognized stock mixing zone, while 7% of Cobia overwintered south of the purported mixing zone. We observed 20% of cobia tagged in 2018 near the continental shelf break off North Carolina during winter, likely in Gulf Stream associated waters. North Carolina- and Virginia-tagged cobia that used Chesapeake and ocean habitats in 2018 showed high site fidelity to those same habitats in 2019; however, we did not find any evidence of genetic differences between these two groups or between groups based on capture locations, but sample sizes were small. For rare occurrence species, we highlight the importance of using a multidisciplinary approach (e.g. tagging, genetics, etc.) to aid in sub-structure determinations. Our results confirmed previous conclusions regarding the stock boundary, but provided new information on the extent that northern-tagged cobia use Chesapeake Bay and the mixing zone and reveal a mechanism for stock sub-structure to develop. We anticipate our results being used for cobia monitoring, assessment, and management such as informing the spatio-temporal distribution of monitoring surveys and management.

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by
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APPROVED BY:

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BIOGRAPHY

Riley grew up in the Pacific Northwest and completed his undergraduate degree in Wildlife Biology (aquatic emphasis) from the University of Montana in 2013. He spent summers in Alaska guiding fly-fishing during his matriculation as an undergraduate student, and was often seen on campus with a fly rod in hand. After graduation, Riley alternated seasons guiding in Alaska, Washington, and Chilean Patagonia until his curiosity led him towards fisheries technician work throughout the inter-mountain west. Riley assisted with field research in cold-water lakes, rivers, and streams for Montana State University and multiple state agencies. In 2017, he accepted a graduate research assistantship at North Carolina State University under the advising of Dr. Jeffrey Buckel. Upon completion, he will earn a Master's of Science degree in Fisheries, Wildlife, and Conservation Biology. Riley accepted an offer to pursue a PhD program at the University of Auckland, New Zealand and will be investigating the movement and behavior of Australasian snapper *Pagrus auratus* using acoustic telemetry. Riley aims to unite anglers and scientists towards a common goal of improving the sustainability of fisheries resources.

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1. Introduction

Stock assessments are conducted on data within defined stock boundaries. Most assessment models assume uniformity in demographic rates across this discrete stock area (Cadrin et al. 2005). Proper delineation of stock boundaries requires a thorough understanding of a fishery as well as the biological distribution and spatial heterogeneity of a species (Ricker 1958; MacCall 1990; Cao et al. 2017). Improper specification of stock boundaries can lead to biased assessments and misguided management recommendations (Begg & Waldman 1999; Clark 1999; Williams 2002). In extreme cases, biased assessments can result in failed recovery of exploited stocks (Secor 2015).

Migratory marine species often exhibit complex spatial behaviors that create difficulties for assessment and management (Cadrin & Secor 2009). These behaviors include (or result in) metapopulations (*Oncorhynchus spp*. Cooper & Mangel 1999), cryptic sub-stocks (*Sebastes mystinus*, Burford et al. 2011), and intrapopulation migratory groups (*Morone saxatilis*, Secor 1999). Given these complexities, there has been a call to use multiple stock delineation approaches (Dizon et al. 1992; Hohn 1997; Begg & Waldman 1999) to aid in determination of stock boundaries in marine fish.

Marine species commonly exhibit site fidelity, undergoing large recurring migrations to specific functional habitats for feeding, pupping or breeding (Secor 2015). Specific types of site fidelity (e.g. natal, breeding) provide a mechanism for genetic divergence, as repeated spatial segregation can result in genetically isolated subpopulations (Waples 1991). Persisting isolation through time may ultimately lead to philopatry – the repeated seasonal recurrence of subpopulations to specific habitats across multiple generations (Secor 2015). Recent studies combining interdisciplinary approaches (e.g. genetics, tagging, otolith microchemistry) have

provided critical insight to spatially complex movements of pelagic fishes (Hueter et al. 2005; Nelson et al. 2010).

Cobia (*Rachycentron canadum*) are a moderately sized pelagic fish, seasonally abundant throughout marine waters of the U.S. Atlantic and Gulf of Mexico (GOM). Cobia spawn in polyhaline inshore waters (Joseph et al. 1964; Smith 1995; Lefebvre & Denson 2012) and offshore waters (Hassler & Rainville 1975), from April to September (Brown-Peterson et al. 2001). Cobia in the Atlantic reach peak spawning conditions in May in South Carolina (SC), June in North Carolina (NC), and July in Virginia (VA; Joseph et al. 1964; Smith 1995; Brown-Peterson et al. 2001). In fall, adults are known to move south to southern overwintering waters in Florida (FL; Shaffer & Nakamura 1989) or to deeper continental-shelf waters (Jensen & Graves unpublished).

Cobia are highly sought after by recreational anglers for their aggressive fight and excellent table fare. In the U.S. mid- and south- Atlantic, cobia landings are dominated by the recreational fishing sector (92% rec.; 8% comm.), yielding an annual coast-wide rec. quota of ~73,000 fish. NC and VA combined represent ~77.5% of the recreational harvest of Atlantic cobia (SEDAR 58 2020). Recreational landings in mid-Atlantic states have steadily increased in recent years (> 2002), occurring in pulses from early May to late June in NC and from mid-May to late September in Chesapeake Bay (Shaffer & Nakamura 1989, Smith 1995).

Much has been learned about the stock structure and movements of cobia since Smith's (1995) recommendation of a comprehensive tagging program for cobia. The stock boundary between the Atlantic and GOM stocks in Amendment 18 to the Fishery Management Plan for Coastal Migratory Pelagic Resources was the Dade/Monroe, FL county line (near the Florida Keys); largely for management ease (SAFMC 2011). In 2013, tagging and genetics data were

summarized and the stock boundary for Atlantic cobia was moved to the Georgia/Florida (GA/FL) state border (SEDAR 28 2013); the GOM stock was delineated from Brevard County, FL (near Cape Canaveral) south and west. Additionally, a "mixing zone", where Atlantic and GOM fish were found to overlap was identified between Brevard County and the GA/FL border (Figure 1), yet no information on timing was available. In 2018, a cobia stock identification workshop reexamined updated life history, tagging, and genetics information (SEDAR 58 2020). The updated information did not provide any new information to change the stock and mixing zone boundaries for Atlantic and GOM stocks; removals (landings and dead discards) used in the Atlantic cobia stock assessment to generate future quota levels do not include landings south of the GA/FL state border. In addition to the stock boundary information, the tagging and genetics data suggested sub-structure within the Atlantic stock; Perkinson et al. (2019) provided evidence that SC and VA cobia caught in estuarine waters during spawning season differ genetically and those groups differ from cobia caught in the ocean.

The majority of tagging data used to delineate and confirm the stock boundary came from conventional and acoustic tagging data from GOM and southeastern states (FL to SC) (Perkinson & Denson 2012; SEDAR 28 2013; SEDAR 58 2020). Research recommendations from this work highlighted the need for electronic tagging in the northern portion of Atlantic cobia range (i.e. NC & VA). Additionally, there was interest in further stock structure research from recreational anglers given the potential for reduced quota with the northward movement of the southern stock boundary.

Here, we use acoustic telemetry to investigate the stock boundary and sub-structure of cobia tagged in the northern part of the Atlantic stock range. Additionally, we examine for sub-structure within the Atlantic stock using population genetics on cobia that differed in their

capture location as well as habitat use during the summer spawning season. Our results for northern-tagged cobia confirm earlier conclusions regarding the stock boundary and mixing zone and provide evidence that northern-tagged cobia represent at least two unique groups whose members exhibit similar, repeated migratory behaviors.

2. Methods

2.1 Tagging

We surgically implanted acoustic transmitters in cobia to track cobia migratory behavior. Tagging occurred along coastal estuaries and nearshore regions of NC and VA, USA during spring and summer 2018 and 2019. We caught cobia using natural bait (live or dead on 9/0 circle or j hook) or artificial jigs often in cooperation with local charter boat captains. We used a landing net to subdue cobia and keep them submerged while surgery materials were prepared. For each cobia captured, we recorded fork length (FL, mm), capture location (GPS coordinates), fight time (from hooking to landing), surgery time (from removal from water to return to water), and release condition (Table 1); the release condition was based on that used by Heupel and Simpfendorfer (2002).

We qualitatively assessed all cobia in the landing net for active respiration and did not tag cobia showing signs of distress or bleeding. We divided cobia into two groups based on length: telemetry and conventional tag candidates (FL > 760 mm) and conventional tag only candidates (FL ≤ 760 mm). To bolster telemetry tag sample size in 2019, we reduced our minimum tagging FL from 760 to 690 mm. We secured cobia ventral side up into a padded, v-shaped surgery cradle and used a modified hose extension fitted to either a bilge or wash-down pump to irrigate seawater across the gills. Prior to surgery, a local anesthetic (1.5 mL sodium bicarbonate + 1.5 mL lidocaine) was introduced at incision locations. We performed surgeries *in situ* and

implanted uniquely coded acoustic tags (Vemco V16-4H, battery ~ 1613 d, 69 kHz, 30-90 s random delay), adhering to surgical implantation techniques for electronic tags in fish (Wagner et al. 2011) under NC State University (NCSU) IACUC protocol # 16-205-O. Per recommendation from NCSU College of Veterinary Medicine staff, we deviated from Wagner et al. by closing incisions with skin staples (Conmed Reflex One stapler, 35 mm wide) opposed to sutures. In 2019, ovarian biopsies were performed by inserting a plastic cannula into the urogenital opening and sex was deemed unknown if no ovarian tissue was observed. We transferred biopsy samples into 50 mL conical tubes containing 10% neutral buffered formalin and sent samples to be sectioned, dyed, and mounted at the Histology Laboratory located in the College of Veterinary Medicine at NCSU.

As part of a separate project to estimate mortality rates, all cobia were conventionally tagged with two $100 high reward red nylon wire core tags: one internal anchor (FM-95W) and one dart (FIM-65, Floy Inc.). We inserted internal anchor tags through an incision in the posterior abdomen opposite the telemetry incision and inserted the dart tag into the dorsal musculature. Each tag displayed a unique identification number, toll-free phone number and the statement "CUT TAG $100 REWARD."

We released all cobia within 100 m of the original capture site. Active cobia were released head first immediately following surgery, whereas less active cobia were held submerged with a lip-grip tool while the boat was under power and cobia were released upon observed attempts to swim independently. All NCSU-tagged cobia followed the surgery protocol.

We relied on collaborators at VIMS to implant NCSU telemetry tags in 2018 (n = 20) and 2019 (n = 10), consistent with the procedures outlined above. In addition to NCSU telemetry

tags, VIMS researchers implanted data storage tags (model G5 DST, 8 x 31 mm, Cefas Technology Limited) to investigate seasonal temperature and depth preferences for cobia in 2018; for the VIMS-tagged cobia in 2018, NCSU anchor tags were replaced with a VIMS lime green nylon loop tag. Fight and surgery times were not recorded for VIMS-tagged cobia, but we observed similar fight and surgery times while on joint tagging trips.

2.2. Acoustic receiver arrays and detections

We obtained cobia tag detection data from a variety of Vemco VR2-type receivers within our receiver array off NC, as well as from other acoustic receiver owners spanning the US Atlantic coastline (New Jersey (NJ) to FL) from May 2018 to December 2019 (Figure 1). The majority of receiver array owners between NC and FL submitted cobia detection data to the 'FACT' network; FACT consolidated, formatted, and sent detection data to tag owners over seasonal intervals. Array owners from NJ to VA commonly identified cobia tags using the Atlantic Cooperative Telemetry (ACT) tag and researcher databases and contacted us directly.

In 2018 and 2019, the NC array included receivers between Cape Hatteras and Cape Fear, NC. These were maintained by our group (NCSU), National Oceanic and Atmospheric Administration, University of NC - Wilmington, NC Division of Marine Fisheries (NC DMF) and the NC Aquarium. The location of these sites varied in depth from three to 66 m; from inshore sounds to offshore hard bottom sites near the continental shelf break. We attached Vemco VR2W receivers to three types of fixed attachments; 1200 mm sand-augers, channel marker posts, and Aids to Navigation (ATON) buoys. VR2AR acoustic release receivers were deployed at artificial reefs or deeper offshore areas of the shelf in Raleigh and Onslow bays in NC; each VR2AR receiver logged temperatures hourly. We analyzed these bottom temperature data by converting to daily means and averaging across depth bins; nearshore < 30 m,

intermediate 30 to 50 m, shelf break > 50 m; we also analyzed sea surface temperature (SST) data for these same depth bins. In 2018, the number of NCSU deployed receivers (n = 72) was larger as part of a separate project focused on inshore sounds throughout NC. As coverage needs changed, we considerably reduced inshore receivers and expanded the array offshore to its final size (n = 37 receivers) by July 2019. The Vemco receivers deployed in NC by other groups during our study period used similar mooring approaches.

We downloaded detections from NCSU-deployed VR2Ws quarterly and VR2ARs biannually. Each retrieved receiver was cleaned of biofouling, downloaded, battery replaced if needed, clock reinitialized, then taped and painted to minimize biofouling. We temporarily removed all receivers attached to ATONs or channel marker posts in preparation for major hurricanes (Florence 2018, Dorian 2019), until conditions permitted reattachment (< 3 weeks). In addition to stationary receivers described above, we also obtained cobia detections from a mobile platform. In winter 2018-2019 and December 2019, the Bureau of Ocean Energy Management (BOEM) provided acoustic detections collected off the coast of FL by a Vemco Mobile Transceiver (VMT) attached to an autonomous Wave Glider (SV3, Liquid Robotics).

2.3. Movement and undetected cobia analyses

We consolidated all cobia detections into a master database in R 3.6.2 (R Core Team, 2020) and ran them through a false detection analyzer, which omitted three suspect detections in heavily trafficked areas surrounded by multiple receivers. We standardized time settings into Coordinated Universal Time (UTC) and for each cobia; we calculated the average daily position for and set the initial release location to represent the first detection. All analyses, plots, and tables were constructed using ArcMAP 10.7.0 (ESRI, 2020) or RStudio 1.2.1 (RStudio Team, 2018).

We used a generalized linear model (GLM) with a Gaussian distribution to investigate the potential mechanism(s) for undetected (n = 12) and briefly detected (n = 3) cobia. We modelled the number of undetected and detected cobia as a function of multiple covariates including: condition factor, surgery time, fight time, and if the hook was left in the mouth. Determination of detected individuals was based on a binary outcome (detected = 1, undetected = 0). We compared models using forward and backward selection and used Akaike's Information Criterion (*AIC*) to select the best fitting model with fewest degrees of freedom (*df*; Burnham & Anderson 2002). We calculated percent deviance explained by subtracting the residual deviance from the null deviance then dividing by the null deviance and multiplying the result by one-hundred (Stoner et al. 2001). To test for differences in size (FL) of inshore vs. offshore fish, we used Welch's two sample t-tests (Welch 1938). All analyses were performed in R 3.6.2 (R Core Team, 2020).

Given previous evidence of genetic sub-structure in cobia reported by Darden et al. (2014) and updated by Perkinson et al. (2019), we were interested in testing for mechanisms leading to sub-structure in the northern portion of the Atlantic cobia stock. Cursory investigation of detected cobia showed two general migratory behaviors during the summer spawning period that informed our data analysis. Irrespective of tagging location (NC or VA), the majority of 2018 telemetry-tagged cobia were in the Chesapeake for much of the 2018 summer; those that were not in the Chesapeake were in the ocean (see Results below). Given this, we established two groups, 'Chesapeake' and 'ocean,' based on the "inshore" and "offshore" groups presented in Darden et al. (2014) and Perkinson et al. (2019).

These groups were defined by the following criteria. For cobia tagged in NC during 2018, a "Chesapeake' assignment required individuals to be within the Chesapeake Bay for at

least two weeks between 1 June and 31 August (Figure 2A). The two-week time period was based on a natural break in the data, but a one-month criterion would have given a similar result (Figure 2A). For cobia tagged within Chesapeake during 2018, a "Chesapeake" assignment required individuals to occur within the Bay for a shorter time period, one week, given that they were already in the Bay when tagged (Figure 2B); thus, cobia tagged after ~ 23 August did not have the potential to be included in the "Chesapeake" group. We assigned cobia to the ocean group if detections showed evidence of continued ocean residency for at least two weeks between 1 June and 31 August. Two ocean individuals did go into the Chesapeake but not for more than three days. The Chesapeake spatial domain was defined as a raster polygon outlining the Chesapeake Bay watershed with a line between Cape Charles and Cape Henry as the boundary separating Chesapeake and ocean detections; the point.in.poly() function from the spatialEco package (Evans & Ram 2019) in R was used to exclude detections outside of the polygon. Of the known live cobia, we were unable to assign a spatial group to eight individuals tagged in 2018 and 23 tagged in 2019 as they failed to meet the "Chesapeake" and "ocean" criteria. At the time of this analysis, individuals that did not satisfy these criteria generally lacked sufficient detection data during summer months.

We hypothesized that individual cobia assigned to Chesapeake and ocean groups in summer 2018 would return to those spatial domains in summer 2019; we used the criteria described above to assign cobia to the two groups in summer 2019. We tested for site fidelity by comparing the observed spatial domain locations in summer 2019 for individual cobia to the expected based on the hypothesized 100% site fidelity using chi-square tests (Zar 1974).

2.4. Genetic tissue collection and analyses

We collected tissue samples for genetic analyses from telemetry tagged cobia to

investigate sub-structure within the Atlantic stock. Tissue samples (~ 5x5 mm) were collected from the anal fin and stored in vials containing sarkosyl-urea preservative solution (8 M urea, 1% sarkosyl, 20 mM sodium phosphate, 1 mM EDTA) and sent to the SC Department of Natural Resources (SC DNR) for analyses. All DNA isolation, microsatellite amplification, and genotyping methods followed previous work on cobia from Darden et al. (2014). We isolated DNA from all samples using a magnetic bead isolation procedure. We amplified twenty polymorphic microsatellite loci via polymerase chain reaction (PCR) in three multiplexed groups on an iCycler thermal cycler (Bio-Rad Laboratories, Hercules, CA). We conducted PCR in 11 µL reactions with 1x HotMaster buffer with 2.5 mM Mg 2+ , 0.2 mM dNTPs, 0.025 units HotMaster Taq polymerase (5 Prime, Inc., Gathersburg, MD), 0.5 mM MgCl 2 , 0.20 mg/mL BSA, 0.3 µM forward and reverse primers, and 1 µL of 1:10 diluted DNA template. Individual primer concentrations differed among loci (Table 2). We labeled forward primers for all loci with WellRED fluorescent dyes (Beckman Coulter, Inc.). Thermal cycling for PCR used a modified 60°C touchdown protocol (from Renshaw et al. 2006) consisting of an initial denaturation step at 94°C for 2 min, followed by 34 cycles of denaturing at 94°C for 30 s, annealing at 60°C, 57°C, and 54°C (7, 7, and 20 cycles, respectively) for 1 min, and extension at 64°C for 2 min, followed by a final extension step at 64°C for 60 min. We separated both size standards (Genome Lab DNA Size Standard Kit 400, Beckman Coulter, Inc.) and reaction products on a CEQ 8000 automated sequencer (Beckman Coulter, Inc.) with fragment analysis performed using CEQ 8000 Fragment Analysis Software (Beckman Coulter, Inc.). Two independent readers manually scored all visual representations of the DNA samples (chromatograms) produced by the sequencer. We resolved discrepancies between readers in conference, or we re-ran samples to obtain an unambiguous genotype for all individuals.

To test for genetic differentiation, we examined for differences in alleles at 20 microsatellite loci among groups that we assigned based on capture location during the spawning season (NC = May to July; VA = June to August). Specifically, we assigned two offshore groups; Oregon Inlet (NCOIO) and Cape Lookout (NCCLO) and two inshore groups; Cape Hatteras (NCCHI) and Chesapeake Bay (VACBI), similar to Perkinson et al. (2019; Figure 3). Given distinct, repeated trends in location during the summer spawning period, we also wanted to investigate groups based on observed site fidelity. Thus, we assigned individuals into 'Ocean' and 'Chesapeake' groups for our second analysis. Standard population genetic statistical analyses were applied to the resulting sample datasets (Weir & Cockerham 1984, Goudet 1995). We tested loci for deviations from Hardy-Weinberg equilibrium and for linkage disequilibrium using an exact test (Guo & Thompson 1992) as implemented in Genepop 4.7.2 (Rousset 2017). Population genetic structure was evaluated using pairwise F_{ST}-style statistics calculated in GenAlEx 6.503 (Peakall & Smouse 2006) and Arlequin 3.5.1.2 (Excoffier & Lischer 2010), exact *G-tests* in Genepop and with the clustering algorithms implemented in STRUCTURE 2.3.4. We reported both F_{ST} and R_{ST} metrics from Arlequin. The clustering model assignment employed in the program STRUCTURE using a hierarchical approach with the assistance of the web-based software STRUCTURE HARVESTER 0.6.94 (Earl & von Holdt 2012) was used to identify the most appropriate number of distinct populations (K) of each run. All analyses were conducted from $K = 1$ to $K = \#$ of collection locations. Simulations were run with and without the locprior (collection location) parameter for all analyses, with three replicates for each K, the length of the burn-in period set at 100,000, and the number of Markov chain Monte-Carlo repetitions after burn-in set at 200,000, as described in Guo and Thompson (1992).

3. Results

3.1 Tagging

From May 2018 to September 2019, we telemetry tagged 98 cobia ranging in size from 690 to 1346 mm FL (mean FL = 923 ± 127 mm; Table 3) and conventionally tagged an additional 47 ranging from 440 to 810 mm FL (mean FL = 641 ± 82 mm) over 32 trips. Ninety-five percent of cobia were tagged within known summer spawning periods (e.g. May to September) and the remaining 5% were captured in September (Figure 4). We confirmed sex of 31 telemetry-tagged individuals, yielding 16 males to 15 females. Surgery times ranged from 2:00 to 15:27 min with a mean of 5:52 min. As the study progressed, surgery times decreased, and release conditions improved.

We telemetry-tagged 51 cobia in the NC ocean from May to early June 2018 and 2019 near Beaufort, Hatteras, and Oregon inlets (Figure 4). We tagged 17 cobia in NC inshore areas (Bogue (n = 2) and Pamlico (n = 15) sounds) from 30 May to 1 September 2019. The sizes (FL) of inshore and offshore tagged cobia did not differ (t = -1.14, df = 88, p = 0.26).

In 2018 and 2019, our collaborators at VIMS telemetry-tagged (n = 30) cobia within the VA portion of Chesapeake Bay between 6 June and 26 September. We did not tag any cobia in ocean waters of VA. Catches were high in June and August, and lower in July (Figure 4). The sizes (FL) of cobia tagged in NC and VA were not significantly different (t = -0.47, df = 75, p = 0.64; Figure 5).

Mortalities from injuries suffered during capture or surgery were the most likely reason for undetected and briefly detected cobia. The most parsimonious model (lowest AIC score) examining the probability of being detected included release condition and deep hooking. Two

of three fish with a condition factor of 4 and two of nine fish with the hook left in were never detected; fish in conditions 1, 2, and 3 had a higher probability of being detected (Figure 6A). Out of thirteen total reported recaptures of conventionally-tagged cobia, 11 were reported from Chesapeake Bay in summer/early fall and one each was reported from NC and SC in June. For 12 of these fish that were also telemetry-tagged, conventional tag returns confirmed the inferred location from telemetry tag detections.

3.2 Spatio-temporal distribution and movement

The geographic extent of cobia detections (n = 88,514) ranged from Seaside, NJ to Jupiter, FL, encompassing the known mixing zone and a portion of the GOM stock territory in eastern FL. Eighty-eight percent of tagged cobia were detected over the study period. The observed movement patterns for fish tagged in 2018 and 2019 were similar; therefore, the data for the two years were combined. Between May and early June, over half (58%) of cobia tagged in NC migrated to Chesapeake Bay (Figure 7A) and a smaller percentage remained in the ocean, ranging from NC to NJ (Figure 7B). Out of 30 cobia tagged in the Chesapeake, 26 remained exclusively within the estuary throughout the purported spawning period. The mean time spent in the Chesapeake was around two months (mean = 58 d) and egress from the estuary occurred gradually from August through October (mean egress = 27 August; Table 4). Movement data from cobia tagged in NC estuaries (Back & Pamlico sounds) was too sparse to infer general trends during summer, yet in fall these cobia made nearshore southwesterly migrations along the coast, overlapping in space and time with cobia tagged in other locations.

In fall, individual cobia showed remarkably similar timing in southwesterly migrations between Chesapeake and locations to the south, generally arriving on NC receivers from early to

mid-October (mean = 12 October). Once in NC, two movement patterns emerged; cobia either remained in ocean waters in NC, or continued south along the coastline, arriving in SC from late October to early November, GA mid-November and FL as early as late November. Concerted movement along the coastline corresponded with decreasing ocean temperatures. As bottom temperatures dropped below ~20°C, cobia were no longer detected on nearshore receivers but detections did occur on shelf break receivers during this time; bottom and surface temperatures were warmer at these deeper receiver sites (Figure 8).

From December 2018 through March 2019, we detected 20 unique individuals representing 19.8% of all detections for the entire study. Detection data revealed two distinct locations during winter months (Figure 9). We detected cobia on offshore NC receiver sites near the continental shelf break (n = 9, ~60 m depth) and south of the GA/FL stock boundary (n = 11). Bottom temperature data from offshore receivers ranged from 15.1°C to 23.5°C (mean = 19.4°C) when cobia were detected. Winter spatial segregation occurred independently of unique summer behaviors; both Chesapeake and ocean cobia redistributed during winter either offshore or near the stock boundary. For example, of the 11 cobia that wintered in FL, two were in ocean and seven were in Chesapeake during summer 2018; of the nine cobia that wintered near the shelf break in NC, two were in ocean and two were in Chesapeake in summer 2018. Mixing zone ingress, egress, and residency duration were highly variable. More than one quarter (26%) of 2018-tagged fish were detected in the mixing zone from November to May and three individuals were detected south of the mixing zone in GOM managed waters. These three individuals were among the earliest to arrive in FL (11 November to 8 December). Of the eleven cobia detected south of the GA/FL stock boundary, we detected all but one cobia after the

overwintering period. To date, no harvests have been reported during winter months and no detections occurred in the Gulf of Mexico.

In spring of 2019, there was a clear directed northward movement of cobia that wintered in the mixing zone and a westward movement of cobia that wintered over on continental shelf off NC. Cobia overwintering in the mixing zone were detected in GA late April, SC early to mid-May, NC in May, and VA in June, residing primarily in Chesapeake for the majority of the summer (Figure 10). Detection data evinced markedly directed seasonal migrations, with fish moving ~ 50 km day^{-1} until arrival to distinct summer habitats. NC overwintering cobia migrated west from the continental shelf break in concert with north migrating mixing zone cobia.

3.3 Inter-annual summer site fidelity

During the purported VA spawning period from June to September 2018, telemetry data revealed two disparate behaviors within the Atlantic stock that we grouped as Chesapeake (n = 31) and ocean (n = 8). We detected 24 of these 39 fish in 2019 and those detections suggest strong fidelity to Chesapeake and ocean habitats. Specifically, 94% of cobia assigned to the Chesapeake in 2018 returned to the Chesapeake in 2019 and 100% of cobia assigned to the Ocean group in 2018 remained in the ocean in 2019 (Figure 11). No cobia were detected in other coastal estuaries (e.g. Pamlico Sound, Delaware Bay). One cobia assigned to the Chesapeake group in 2018 was assigned to the ocean in 2019. Observations for Chesapeake (χ^2 = 0.0555, df = 1, p = 0.814) and Ocean fish (χ^2 = 0, df = 1, p = 1) did not deviate significantly from the expected proportions based on the hypothesized 100% summer site fidelity to these habitat groups.

3.4 Genetic and histological analyses

We analyzed 97 tissue samples from telemetry tagged individuals in both genetic analyses based on capture locations and telemetry-informed groups. All loci were in Hardy-Weinberg equilibrium at all capture locations except for one locus (Rca1-G02). We retained this locus for further analysis, as it was isolated to only one location (VACBI). No pairwise comparisons of F_{ST}, R_{ST}, and exact *G-test* resulted in significant genetic differences following Bonferroni correction ($p < 0.008$; Tables 5 & 6). Differences between offshore samples collected near Oregon Inlet, NC and Cape Lookout, NC showed the most differentiation ($p = 0.054$), followed by differences between Chesapeake Bay and Oregon Inlet ($p = 0.063$). STRUCTURE results indicated only one panmictic population among cobia sampled ($K = 1$; Figure 12A).

We found similar results with the telemetry informed groups (i.e. Chesapeake (n = 16) vs. Ocean (n = 6)). All loci were in Hardy-Weinberg equilibrium. No pairwise comparisons of F_{ST}, R_{ST}, and exact *G-test* resulted in significant genetic differences (Tables 5 & 6). STRUCTURE results suggested only one genetic population ($K = 1$; Figure 12B). Overall, genetic differentiation was not significantly different, whether between capture location groups or from site-fidelity groups identified using telemetry.

Out of six telemetry-tagged females that were successfully biopsied in NC in May, we observed various stages of vitellogenic development in five fish. Three of the five were in capable spawning condition upon capture. Histology samples from these three fish revealed oocytes in final stages of maturation characterized by lipid coalescence, migration of the nucleus to the periphery of the oocyte and relatively large diameter (550-725 μm). One individual

tagged on 16 May showed breakdown of nuclear membranes, yolk coalescence, and hydration in oocytes ranging in diameter from 725 to 775 µm. We observed vitellogenic oocytes intermixed with oocytes in primary (75-175µm) and secondary (275-350 µm) development stages, representative of batch spawning. No histology samples provided discrete evidence of spawning prior to capture (e.g. no post-ovulatory follicles observed). One early developing female biopsied on 29 May was caught and released a month later in the Chesapeake. The remaining three fish that showed evidence of current or imminent spawning were not detected in the Chesapeake or on any nearshore receivers.

4. Discussion

4.1 Telemetry-tagging in north supports location of current stock boundary

Our telemetry tagging of cobia in the northern portion of the Atlantic stock spatial area confirmed previous conclusions on the location of the stock boundary. The 2013 Atlantic cobia stock assessment (SEDAR 28 2013) established the GA/FL border as the unit stock boundary between Atlantic and GOM cobia for management ease. The best available genetics and tagging data lacked resolution to confirm the biological boundary, yet suggested that it occurred in the mixing zone between Cape Canaveral, FL and the GA/FL border where both Atlantic and GOM cobia were found to overlap. In 2018, a stock identification (ID) workshop for Atlantic and GOM cobia used updated life history, genetics, and tagging data to reexamine the boundary and degree of mixing between the two stocks (Perkinson et al. 2019; SEDAR 58 2020). The stock ID workshop (SEDAR 58 2020) concluded that the stock boundary would remain at the GA/FL border, although further telemetry-tagging and increased receiver coverage in this region were recommended. This past work was influenced by conventionally- and telemetry-tagged fish that

were mostly released in areas in the southern portion of the Atlantic stock. Our northern-tagged cobia had similar movements to those described previously; during summer, northern-tagged cobia were within the Atlantic stock spatial area but during winter occurred both in the stock area and south of the stock boundary.

Our results for northern-tagged cobia differed in the percentage of fish using the mixing zone; during winter, we documented a substantial portion of northern-tagged cobia in the mixing zone south of the current Atlantic/GOM stock boundary. The use of the mixing zone was higher for NC/VA tagged cobia than from prior findings from SC/GA telemetry-tagged cobia (Perkinson et al. 2019). Of the fish tagged in NC/VA in 2018, 26% were found in the mixing zone during winter while only 4% of SC/GA tagged cobia (Perkinson et al. 2019) were found in the mixing zone. These disparities could result from differential use of the mixing zone by northern- vs. southern-tagged Atlantic cobia, inter-annual variability in use of the mixing zone as a result of environmental (e.g. temperature) and biological factors (e.g. prey concentrations), or differences in detection probability. Given that these two studies were conducted during different periods, the differences in percent detections are more likely a result of differences in detection probability resulting from changes in receiver number in the mixing zone. During the timeframe of our study, receiver array owners bolstered receiver coverage in northern FL (e.g. Jacksonville gate, St. Augustine gate, BOEM Wave Glider), which increased acoustic detections of cobia and helped refine the stock boundary.

Cobia that used the mixing zone during winter returned to the Atlantic stock boundary during spring, summer, and fall months. Telemetry detections confirmed that 10 out of 11 (91%) winter 2018/2019 mixing-zone cobia returned to northern regions within the Atlantic stock boundary by summer 2019. The one cobia that we did not detect returning northward was also

undetected on its fall 2018 southward migration to the mixing zone, suggesting that it may migrate through deeper water devoid of receivers. Future detection data may confirm this cobia returning to areas within the Atlantic cobia stock boundary, as acquisition of telemetry data suffers from delayed download and processing time. To date, we have not received telemetry detections of cobia in the Gulf of Mexico, although it is possible that GOM detections from tagged-cobia have yet to be downloaded or distributed.

Although there have been multiple approaches used to identify the stock boundary for Atlantic cobia there are others that might hold promise in defining the boundary and the percent contribution of Atlantic and GOM cobia. For example, otolith shape (DeVries et al. 2002; Shepard et al. 2010) and otolith chemistry (Patterson et al. 2004) proved to be effective stock markers to refine the monthly stock contribution to the mixing zone of Atlantic and GOM stocks of king mackerel (*Scomberomorus cavalla*; SEDAR 38 2014). Currently, king mackerel landings in the winter mixing zone are split equally among Atlantic and GOM stocks from November to March. A multidisciplinary approach using tagging and otolith-based methods may similarly improve our understanding of the winter spatial variability and stock contribution to the cobia stock mixing zone.

4.2 Novel findings on cobia biology

We observed considerable overlap in the timing of individual cobia migrations during fall and spring. Migration timing was approximately synchronous among Chesapeake and ocean groups, providing evidence for a mixed stock dynamic in which groups intermix outside of reproductive periods (Shepard et al. 2010; Secor 2015). The location and frequency of detections of NC-offshore, NC-inshore, and VA-inshore tagged cobia occurred in concert. Weather fronts did not appear to motivate departure from Chesapeake, as egress occurred

gradually, not abruptly. Further investigation of photoperiod, temperature, or timing of prey migrations may provide insight into fall egress from estuaries and northern ocean habitats. The number of detected cobia decreased as the southward migration progressed, suggesting a fraction of individuals transitioned from nearshore to offshore areas devoid of receiver coverage. Indeed, receivers in NC documented 20% of 2018-tagged cobia moving from nearshore waters along the Outer Banks, NC to deeper sites adjacent to Cape Lookout, NC. Trends in detection data suggest major geographic features (e.g. capes) facilitate the areas where cobia move to deeper waters. We hypothesize that cobia use capes along the Atlantic coast (e.g. Cape Hatteras, NC, Cape Romain, SC, and Cape Canaveral, FL) to transition offshore. Future investigations into offshore habitat usage should consider deployment of receivers on known natural or artificial reefs near capes along the continental shelf break. In addition to moving offshore, the decrease in detected cobia during southward migration could also result from reduced detection probabilities of receivers in southern regions of the study area.

We provide direct evidence that individual cobia overwinter off NC and describe their winter thermal habitats. Previous cobia overwintering locations were inferred from researcher surveys and angler reports (Smith 1995) or tag recaptures (Perkinson 2019). As discussed in the stock ID workshop, cobia tagged in SC and GA were not detected in winter, suggesting overwintering in areas lacking receiver coverage (i.e. deeper water). Jensen and Graves (unpublished) used pop-off satellite tags to provide fishery-independent documentation of cobia overwintering locations in shelf break areas off NC and SC. Insight gained from their study provided impetus for our receiver deployments along the continental shelf break adjacent to Cape Lookout, NC.

Our results advance our understanding about the individual movement offshore, habitat use during wither months, and movement returning inshore. During the coldest winter months, cobia were exclusively found near the continental shelf break (Figure 8). Although most detections came from a receiver located at an artificial reef site, cobia were also detected on a receiver that was located on a nondescript, soft bottom habitat. An additional 16% of cobia detected in NC in fall migrated out of detection range until the spring, suggesting these cobia overwintered in deeper, warmer Gulf Stream waters or shelf habitats devoid of receivers. Using a depth-integrated habitat model, Crear et al. (2020b) found that 30 to 50% of suitable cobia habitat during winter occurs in shelf waters off North and South Carolina. The presence of cobia near the shelf break during winter is likely a function of depth and temperature preferences, as cobia are most often described as being found in depths between 20 to 60 m (Crear et al 2020b) and temperatures ≥ 20°C (Richards 1967; Shaffer & Nakamura 1989). During winter in NC, those temperatures in the ocean would be associated with Gulf Stream-influenced waters most often found near the shelf break. However, cobia detections occurred even as bottom temperatures approached 15°C, which had been previously documented by Smith (1995). It is also possible that cobia were in warmer water (e.g. surface), but close enough to receivers to be detected; indeed, our analysis of SST showed much warmer surface waters at the shelf break. Our data suggest that cobia likely respond to fine-scale shifts in local ocean temperature (e.g. cold fronts) by adjusting depth or latitudinal position along the shelf break.

4.3 Evidence of site fidelity provides potential mechanism for genetic sub-structure

Cobia showed high site fidelity to Chesapeake Bay and ocean habitats across years. Robust receiver coverage and known locations during summer provide strong support for Chesapeake Bay as an important summer habitat; this is likely for spawning (Joseph et al. 1964)

but the continued use of the Bay (September to mid-October) after the purported spawning period suggests it may be important for other reasons such as foraging. Cobia homing to the Chesapeake and seldom exiting during the breeding period may pass on similar traits to their progeny. Fidelity to spawning regions supports Perkinson et al. (2019) report of sub-structure in the Atlantic cobia stock between inshore and offshore captured cobia and provides a mechanism for genetic divergence. The results from our genetic analyses did not indicate differences between Chesapeake and ocean groups; observed site fidelity may therefore be phenotypically or environmentally driven within a panmictic (randomly mating) Atlantic stock. Alternatively, our sample size may not have been sufficiently robust to detect true genetic differences.

In addition to using telemetry to define genetic groups (i.e. ocean vs. Chesapeake), we also used capture location and time to define groups, similar to Perkinson et al. (2019). The lack of genetic differences in that comparison may be explained by differences in the time of year when samples were taken. Two thirds of Perkinson et al. (2019) samples were collected in NC ocean waters from June to September, whereas only five of our cobia samples came from the ocean after the large pulse in May (Figure 13). Of those five, only one cobia was later detected in Chesapeake, suggesting that cobia captured in NC after May/early June have a decreased likelihood of moving into the Chesapeake. Darden et al. (2014) speculated that Chesapeake bound individuals were generally present during collection of NC offshore individuals; thus, obtaining samples during the spawning season to look for genetic sub-structure (ocean vs. inshore) would be confounded with continued migration. Our detection data confirm this phenomenon, as most cobia tagged in NC ocean habitats in May were detected in Chesapeake Bay in June; we recommend that telemetry data be used to refine putative subpopulation groups assigned *a priori* in analyses of genetic data. This result highlights the importance of using a

multidisciplinary approach (i.e. genetics, telemetry, etc.) to address questions about population sub-structure.

4.4 Caveats and future research

The importance of NC estuaries to the greater Atlantic cobia stock is largely unknown. At the time of this study, cobia tagged in NC estuaries had yet to complete a full migration cycle. Currently, telemetry detections suggest Pamlico-tagged cobia may be part of or exhibit behavior similar to the Chesapeake inshore group. We suggest two working hypotheses: 1) cobia remain in Pamlico Sound for the duration of the summer spawning period, or 2) Chesapeake or ocean cobia use Pamlico Sound to forage during seasonal migrations. The earliest detections of Pamlico-tagged cobia occurred on the Hatteras Inlet buoy during fall, suggesting estuarine residency until water temperatures cooled. Furthermore, preliminary information from 2020 detections and conventional tag returns suggest site fidelity to Pamlico sound for cobia tagged in that system in 2019. Compilation of the full suite of 2020 detections will be needed to confirm site fidelity to Pamlico Sound.

Detections of cobia tagged in NC estuaries shed light on inshore spawning groups. The best available genetic data provide evidence that cobia may spawn in inshore NC and VA during their northward migration (Perkinson et al. 2019; Figure 3). No cobia tagged in NC or VA were ever detected inshore in SC, suggesting that mixing between the distinct population segment in SC (see Darden et al. 2014) and northern-tagged cobia is rare. SC established the Southern Cobia Management Zone in 2016 that restricts inshore harvest in May. Recent monitoring shows that very few adult males and almost no large gravid females have returned to Port Royal and St. Helena sounds in SC (pers. comm. M. Perkinson; SC DNR). With fishing effort

increasing, it is critically important to further investigate purported estuarine aggregations in NC and consider their susceptibility to the fishery. If identified, differences in vital and demographic rates between inshore and offshore cobia may warrant a two-tiered approach to assessment and management (Darden et al. 2014). Further investigation into the mechanisms driving sub-structure should combine genetics, histology, otolith chemistry, and tagging.

Recreational cobia anglers from Atlantic states formally raised concerns about the contribution of Atlantic stock landings off Northeastern FL (NEFL) towards GOM quota (Public comments, SEDAR 58 2020). Low percentages of tag recaptures (0%) from our study and (4%) Perkinson et al. (2019) suggest a low susceptibility to the NEFL fishery, although our telemetry data revealed a substantial proportion (> 25%) of tagged cobia south of the GA/FL stock boundary between November and March. Increased receiver coverage in NEFL and offshore relocations from the BOEM Wave Glider continue to improve our understanding of the spatio-temporal distribution of Atlantic cobia throughout the mixing zone. Wave Glider detections suggest that cobia associate with the Gulf Stream influenced waters near the shelf break in NEFL, which offers anglers under GOM regulations a unique opportunity to catch Atlantic cobia at smaller sizes (FL $\geq$ 33 in.) and higher bag limits (6 boat^{-1} day^{-1}) than allowed under Atlantic regulations. Although there were no reported northern-tagged cobia harvested in winter, the large percentage of Atlantic cobia in the mixing zone has implications for stock assessment and management. Currently, removals from the mixing zone are not included in the Atlantic cobia stock assessment. We recommend that future stock assessments of Atlantic cobia examine sensitivity to the inclusion of removals (landings and dead discards) from the mixing zone during winter and that the fishery in the mixing zone be monitored for changes in effort and removals. Telemetry data may be used to inform landings and discards needed for these analyses.

Depending on the assessment model sensitivity, outcomes may suggest annual catch limits be split between Atlantic and GOM anglers, similar to that of king mackerel (*Scomberomorus cavalla*; SEDAR 38 2014). Ultimately, managers should carefully monitor and enforce cobia removals in NEFL, given their potential to influence the Atlantic stock of cobia.

The stock assessment report noted difficulties in developing abundance indices for Atlantic cobia for multiple reasons (e.g. limited geographic extent, reporting bias), including the identification of targeted recreational cobia trips for effort determination (SEDAR 58 2020). As such, data workshop panelists (SEDAR 58) rejected all but one proposed fishery-dependent and -independent index of abundance for use in the Atlantic cobia assessment model. The sole fishery-dependent Southeast Region Headboat Survey (SRHS) accepted for the assessment provided reliable landings and discard data across the majority of the stock range from 1976 to 2015. Concerns were raised about the data being derived from fishery-dependent self-reported sources and that the index cannot be used in years when there is a fishery closure (e.g. 2016 cobia closure in federal waters). Subsequently, panelists recommended that the development of a new fishery-independent index of abundance be given top priority. Our telemetry tagging suggests that the bulk of northern-tagged fish use Chesapeake Bay during summer and early fall; although geographically limited, a directed fishery-independent monitoring program (e.g. gill net) in Chesapeake Bay may be more logistically feasible than a coast-wide study.

5. Conclusion

The benchmark stock assessment for Atlantic cobia, completed in January 2020, reported the status of cobia as not overfished and that overfishing was not occurring (SEDAR 58 2020). The projections show the Atlantic population can be fished at 75% of $F_{40\%}$. The management transition from the SAFMC to the Atlantic States Marine Fisheries Commission (ASMFC) in

2019 provided considerable flexibility for individual states to enforce specific management objectives. As such, states should carefully monitor seasonal catch and effort during spawning periods. For example, Chesapeake Bay will likely continue to be an important spawning and foraging habitat for Atlantic cobia in summer/early fall and its importance may increase in response to ocean warming (Crear et al. 2020a). If needed, regulations during this longer period of aggregation in easily accessed inshore waters may have a disproportionately higher effect on reducing fishing mortality. Similarly, the percent contribution of the Atlantic cobia landings in FL during winter may elicit modification to size or bag limits in the mixing zone consistent with ASMFC guidelines. Our study confirms the current placement of the unit stock boundary between FL/GA border as reasonable and appropriate, yet clearly shows the biological boundary occurs farther south near the Brevard county line. Current efforts to increase receiver coverage and continue participation in telemetry networks will be critical in further refining the stock boundary and providing estimates of vital rates (i.e. discard and total mortality; pers. comm. J. Krause). Our evidence of spawning site fidelity reveals a mechanism for genetic divergence and presence of sub-stocks (e.g. inshore, ocean) of Atlantic cobia. We recommend continued use of multiple approaches including telemetry-tagging, telemetry-informed genetic classification and analyses, histology, and tag recapture studies to provide additional insights into this recreationally and economically important fishery.

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Tables

Table 1. Condition values subjectively assigned to telemetry-tagged cobia at release post surgery.

Condition	Description
1	No revival time required, rapid swimming upon release, usually vigorous splash
2	No revival time required, slow but strong swimming upon release
3	Short revival time (< 30 s) required, slow or atypical swimming upon release
4	Long revival time (> 30 s), limited or no swimming observed upon release but respiration functional

Table 2. Multiplex panel, locus, allelic size range, number of alleles, and primer concentrations (μM) for 20 cobia-specific microsatellite loci.

Multiplex Panel	Locus	Allelic Size Range	Number of alleles	Primer Concentrations
1	Rca1-H10	116-138	10	0.106
	Rca1-H04	153-167	5	0.029
	Rca1B-A10	175-190	4	0.029
	Rca1-D08	172-174	2	0.039
	Rca1-A04	180-208	8	0.029
	Rca1B-F06	253-325	15	0.039
	Rca1B-E02	301-315	7	0.029
2	Rca1-F11	119	1	0.008
	Rca1-A11	166-198	14	0.047
	Rca1B-H09	169-225	15	0.066
	Rca1B-E08A	215-229	6	0.038
	Rca1-E05	239-273	9	0.038
	Rca1B-E06	307-321	7	0.047
	Rca1B-C06	341-413	17	0.057
3	Rca1B-D10	144-244	25	0.060
	Rca1-D07	149-159	3	0.020
	Rca1-E11	167-181	6	0.060
	Rca1-C04	221-255	12	0.060
	Rca1-G02	241-245	3	0.040
	Rca1-G05	269-285	6	0.060

Table 3. Summary of telemetry study results for cobia including the state, location, year, sample size, first and last dates of release, median fork length (FL) in mm with range, median detections (det.) per each tagged cobia with range, total det., and median number of detection days per each tagged cobia with range.

State	Location	Year	*n*	Release date	Median FL (range)	Median det. (range)	Total det.	Median days (range)
NC	Nearshore	2018	34	5/11 – 5/31	889 (762 – 1,350)	912 (4 – 6,238)	37,898	29 (1 – 120)
		2019	17	5/15 – 6/9	915 (705 – 1,270)	256 (18 – 1,041)	5,267	8 (2 – 42)
NC	Inshore	2019	17	5/30 – 9/1	860 (690 – 1,080)	70.5 (2 – 1,341)	3,809	3 (1 – 24)
VA	Inshore	2018	20	6/28 – 9/26	932 (860 – 1,120)	680 (69 – 2,007)	38,837	45 (7 – 84)
		2019	10	6/23 – 8/29	885 (750 – 1,280)	482 (27 – 6,548)	2,703	10 (2 – 31)
Total		2018-2019	98	5/11 – 9/26	900 (690 – 1,346)	561 (2 – 7,194)	88,514	17 (1 – 120)

Table 4. Median arrival and departure dates and residence times of telemetry-tagged cobia in Chesapeake Bay by state and year tagged.

State & Year tagged	**Arrival Year to CB**	***n***	**Median Arrival (range)**	**Median Departure (range)**	**Median Residence (range)**
NC 2018	2018	15	6/8	9/28	120 d
			(6/1 – 6/19)	(7/26 – 10/16)	(23 – 130 d)
NC + VA 2018	2019	28	5/30	8/21	66 d
			(5/18 – 8/3)	(6/17 – 10/10)	(18 – 140 d)
VA 2018	2018	20	na	9/25	41 d
				(7/1 – 10/9)	(3 – 88 d)
VA 2019	2019	10	na	9/17	26 d
				(7/26 – 9/30)	(9 – 86 d)

Table 5. Pairwise comparisons of F_{ST} values (below the diagonal) and associated p-values (above the diagonal) following Bonferroni correction ($p < 0.008$). **Left:** Capture Locations in the ocean: near Cape Lookout, NC (NCCLO) and Oregon Inlet, NC (NCOIO) and inshore: near Cape Hatteras, NC (NCCHI) and in Chesapeake Bay, VA (VACBI). **Right:** Site fidelity groups from Chesapeake Bay (TGI) and ocean (TGO).

	NCCHI	**NCCLO**	**NCOIO**	**VACBI**
NCCHI	—	0.198	0.126	0.279
NCCLO	0.012	—	0.054	0.243
NCOIO	0.019	0.022	—	0.063
VACBI	0.007	0.006	0.019	—

	TGI	**TGO**
TGI	—	0.910
TGO	0	—

Table 6. Exact *G-tests* of allelic frequency distributions *p*-values following Bonferroni correction ($p < 0.008$). **Left:** Capture Locations in the ocean: near Cape Lookout, NC (NCCLO) and Oregon Inlet, NC (NCOIO) and inshore: near Cape Hatteras, NC (NCCHI) and in Chesapeake Bay, VA (VACBI). **Right:** Site fidelity groups from Chesapeake Bay (TGI) and ocean (TGO).

	NCCHI	**NCCLO**	**NCOIO**	**VACBI**
NCCHI	—			
NCCLO	0.368	—		
NCOIO	0.618	0.313	—	
VACBI	0.287	0.039	0.112	—

	TGI	**TGO**
TGI	—	
TGO	0.557	—

Figures

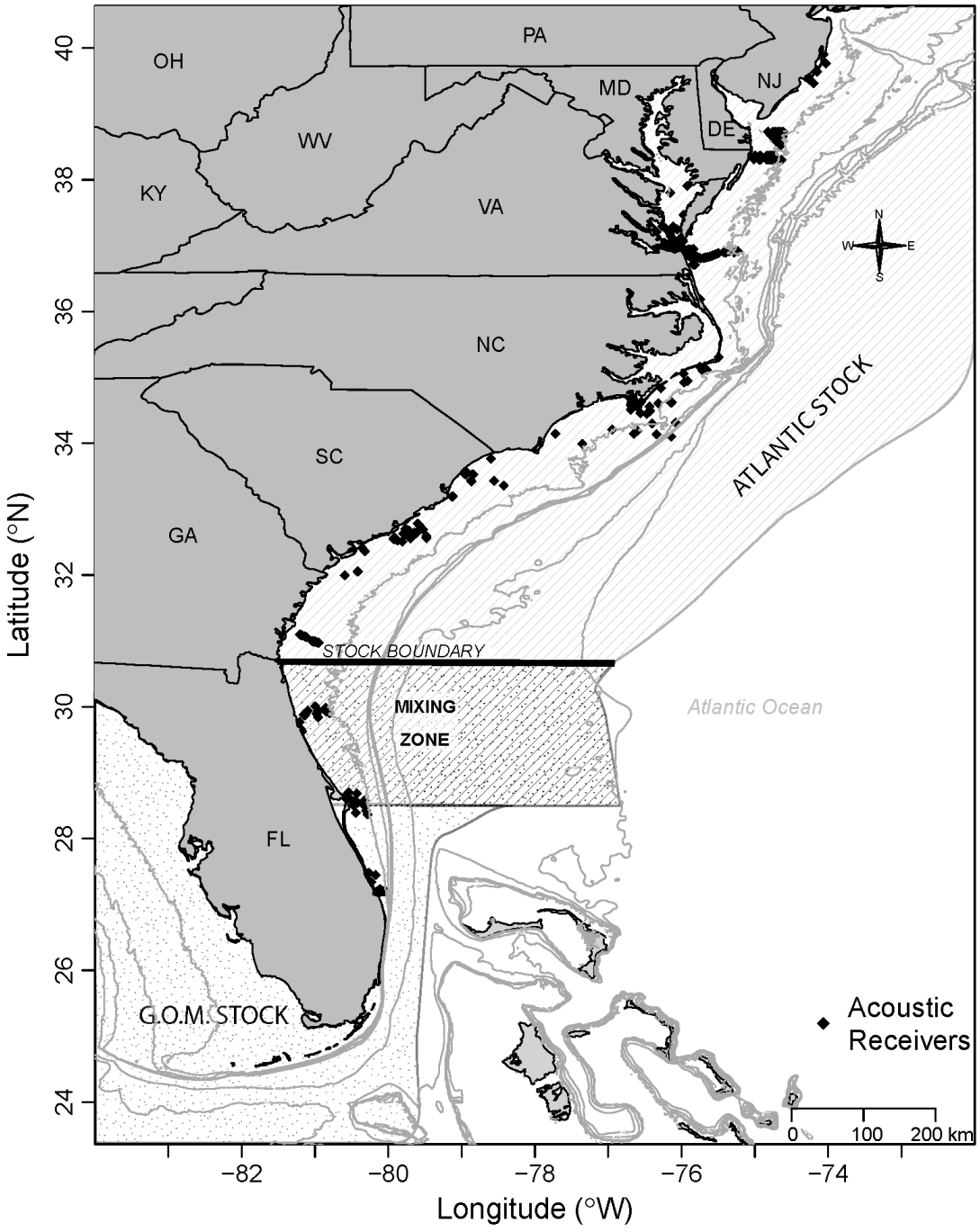


Figure 1. Cobia distribution throughout the US. Two stocks have been identified: Atlantic (diagonal bars) and Gulf of Mexico (grey dots). The mixing zone (bars + dots) between the two stocks occurs from the FL/GA stock boundary to Cape Canaveral, FL.

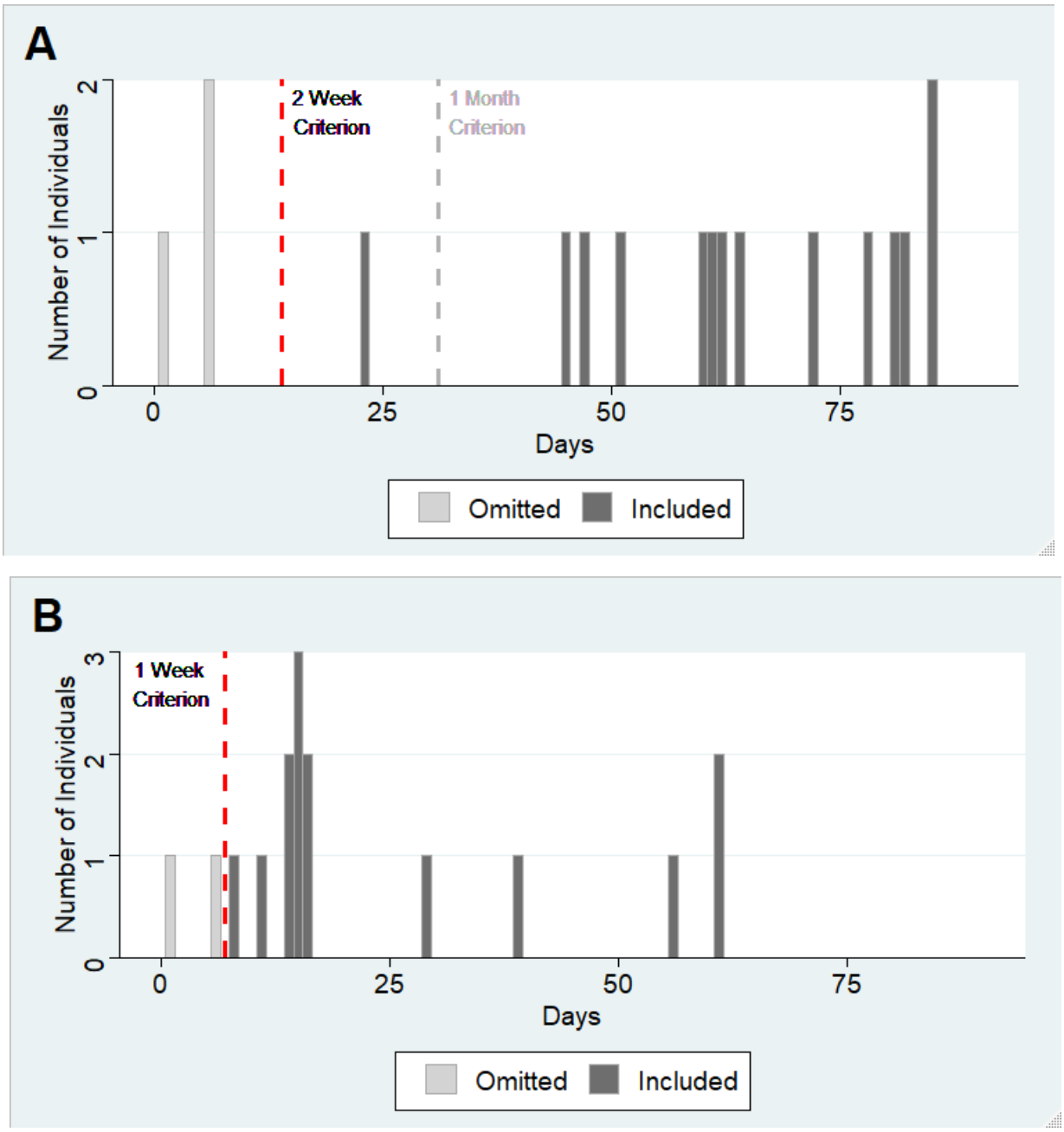


Figure 2. Date ranges of Chesapeake Bay residence times for **A.** NC-tagged and **B.** Chesapeake Bay-tagged cobia. Cobia with residence durations less than two weeks for NC-tagged and less than one week for Chesapeake Bay-tagged were omitted from site fidelity groups.

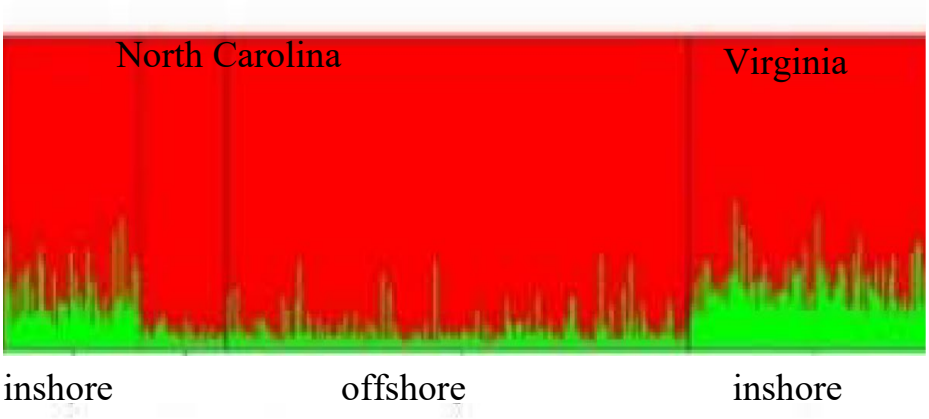


Figure 3. Population ancestry plot from Perkinson et al. 2019 based on STRUCTURE results of $K = 2$. Each vertical bar represents a single individual in the plot with colors indicating percent ancestry to each genetic group.

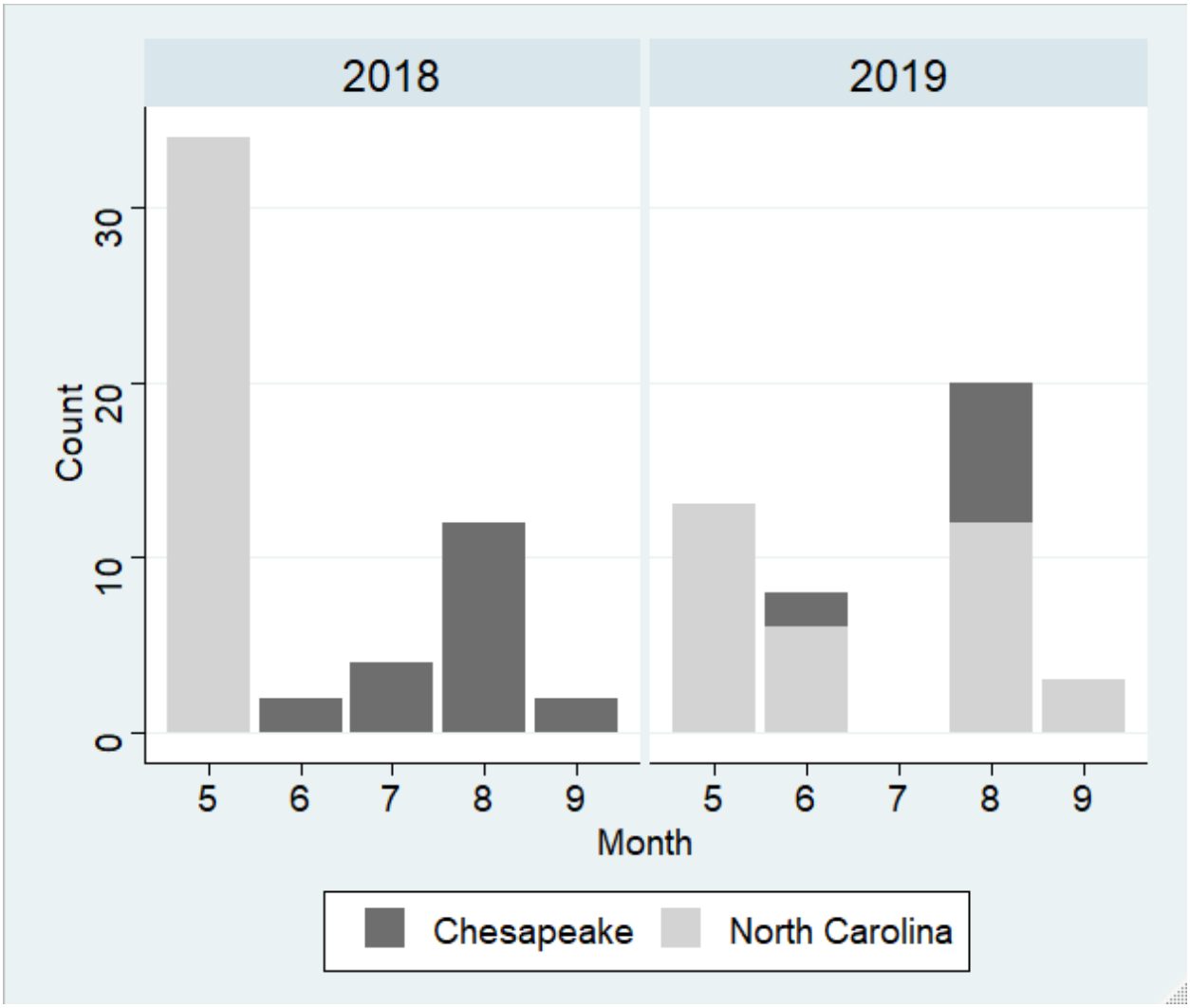


Figure 4. Number of cobia that were telemetry tagged by month and year in Chesapeake Bay, VA and North Carolina. Number of telemetry-tagged cobia by tagging region, month, and year.

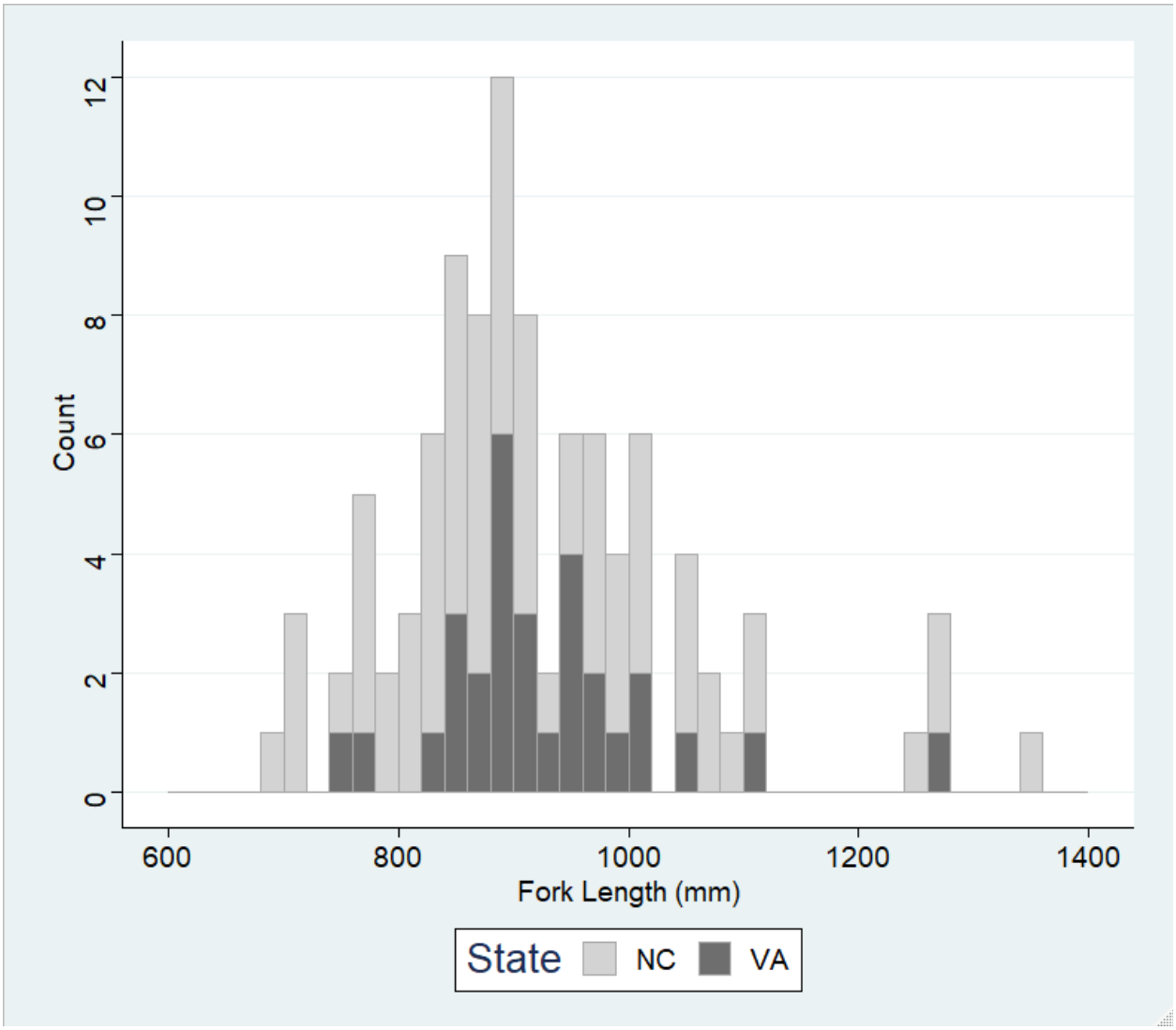


Figure 5. Fork length distributions of telemetry-tagged cobia by state (NC = light grey, VA = dark grey).

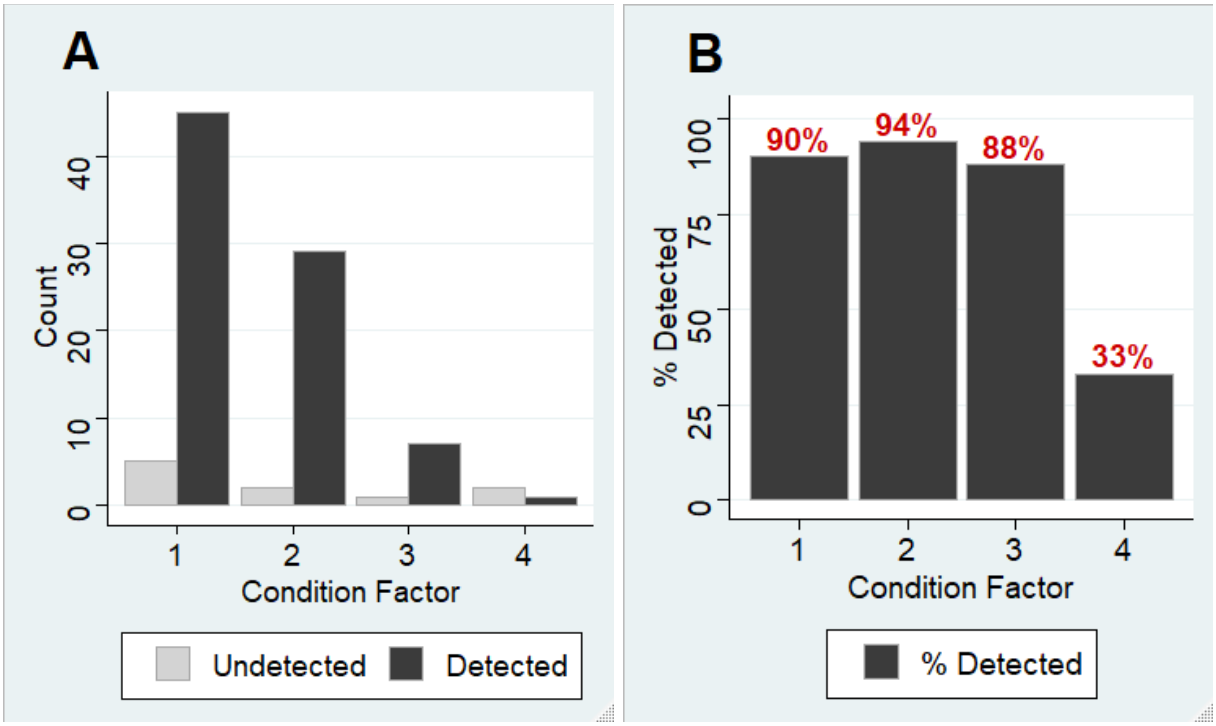


Figure 6. **A.** Number of cobia detected on acoustic receivers based on the assigned condition value (1-4) assigned upon release post-surgery. Conditions were defined as 1. Rapid swimming upon release 2. Slow but strong swimming 3. Slow, atypical swimming 4. Limited or no swimming **B.** Percent detected out of total cobia released based on condition factor.

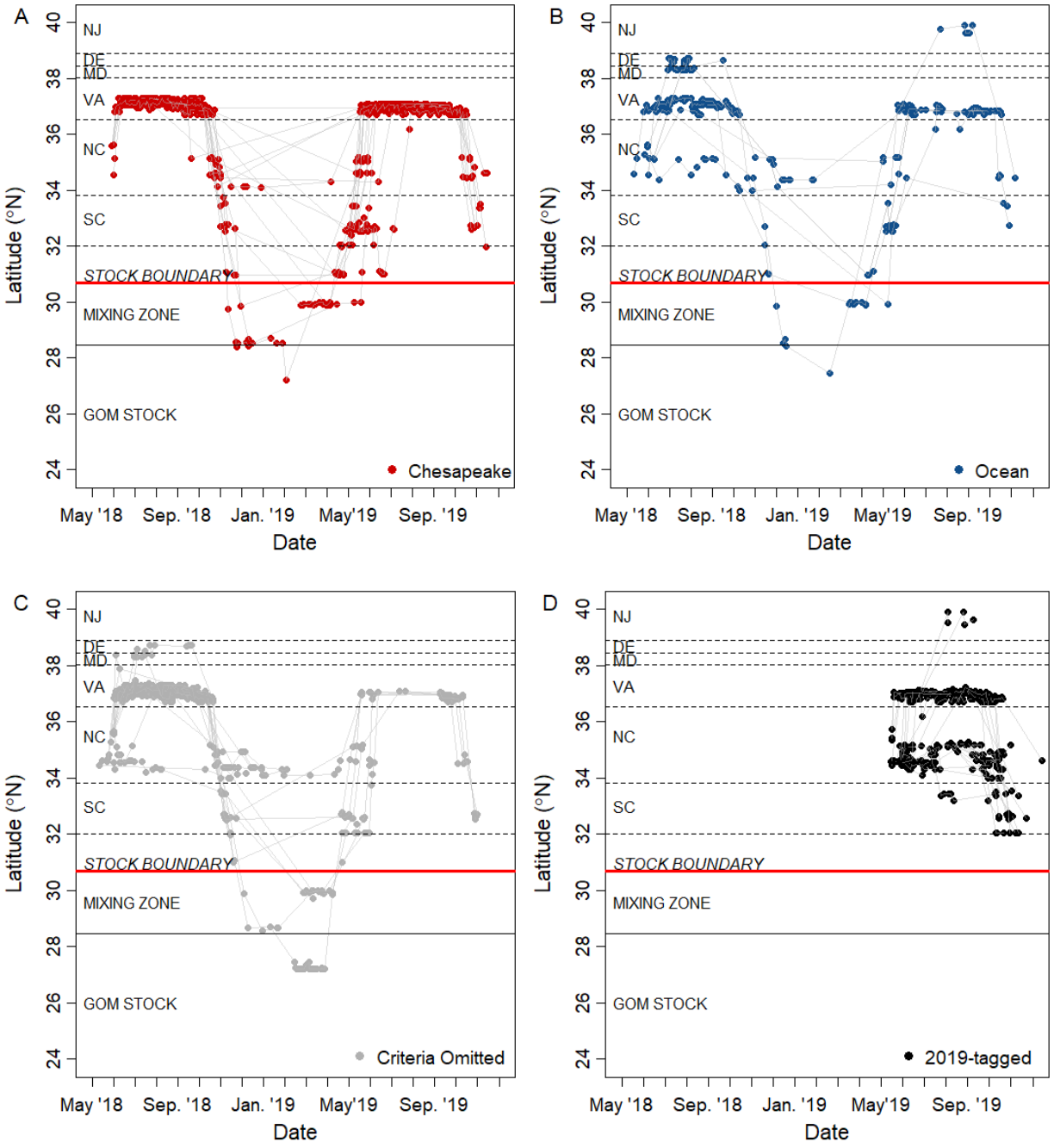


Figure 7. Receiver detections of individual 2018 telemetry-tagged cobia by latitude and date for **A.** Chesapeake-assigned **B.** ocean-assigned **C.** unassigned cobia omitted by criteria **D.** 2019-tagged cobia. Dashed lines represent state boundaries and solid line represents the southern boundary of the mixing zone. The red line represents the current stock boundary at the Georgia-Florida state boundary.

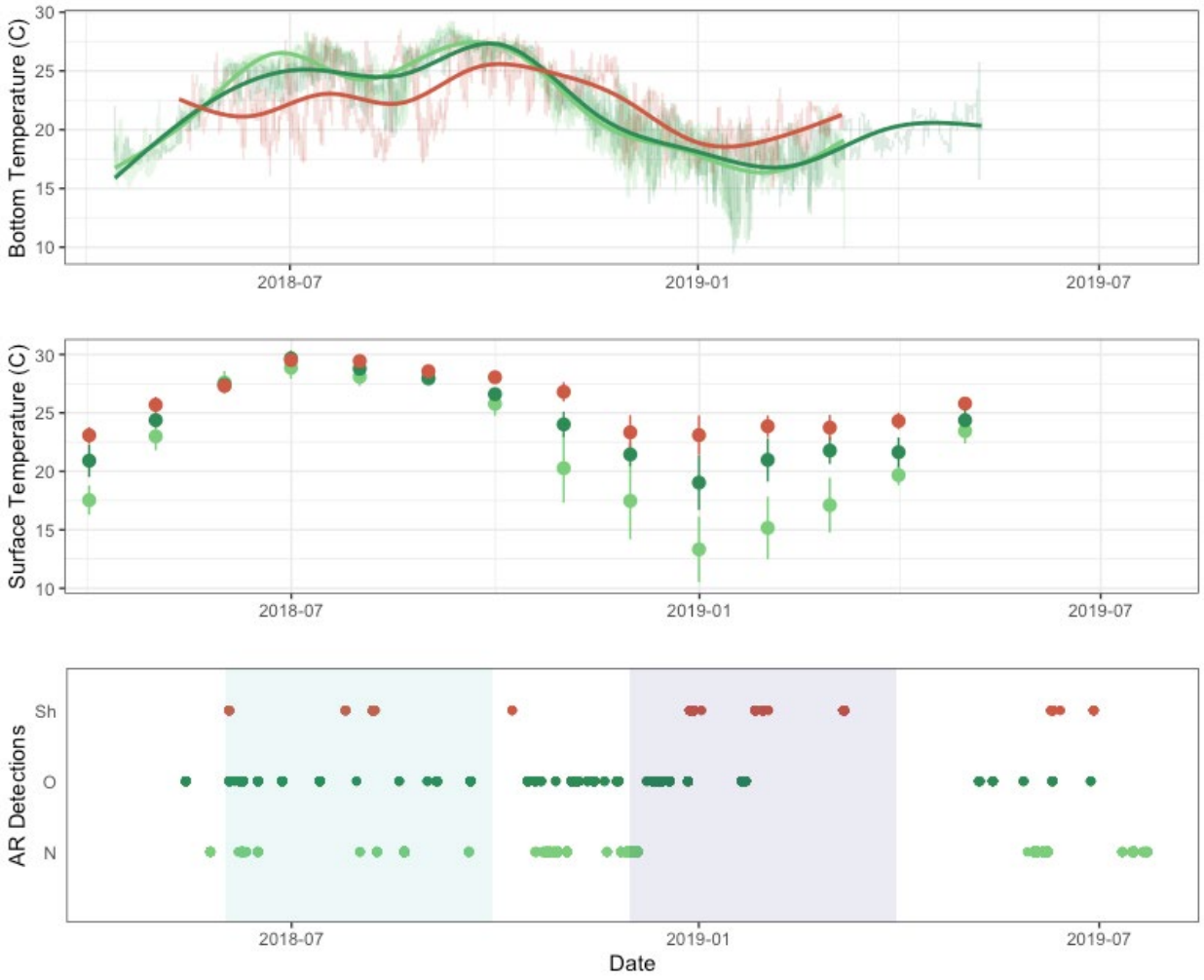


Figure 8. Bottom and surface temperatures and detections at NCSU acoustic release receivers by depth group. The top panel shows hourly temperatures recorded by the bottom-deployed acoustic release receivers with GAM fit lines. These profiles are divided by depth (light green: nearshore; dark green: offshore; orange: shelf). The middle panel shows average monthly satellite-recorded surface temperatures divided by depth (light green: nearshore; dark green: offshore; orange: shelf) with points and one standard deviation ranges. In the bottom panel, all detections (9381 detections, n = 34) recorded at each NCSU VR2AR receiver are plotted. Depth regions are denoted by dot color (light green: nearshore (N); dark green: offshore (O); orange: shelf (Sh)). The light green section indicates the 2018 spawning period (June through August) and the purple section denotes the winter (December through February).

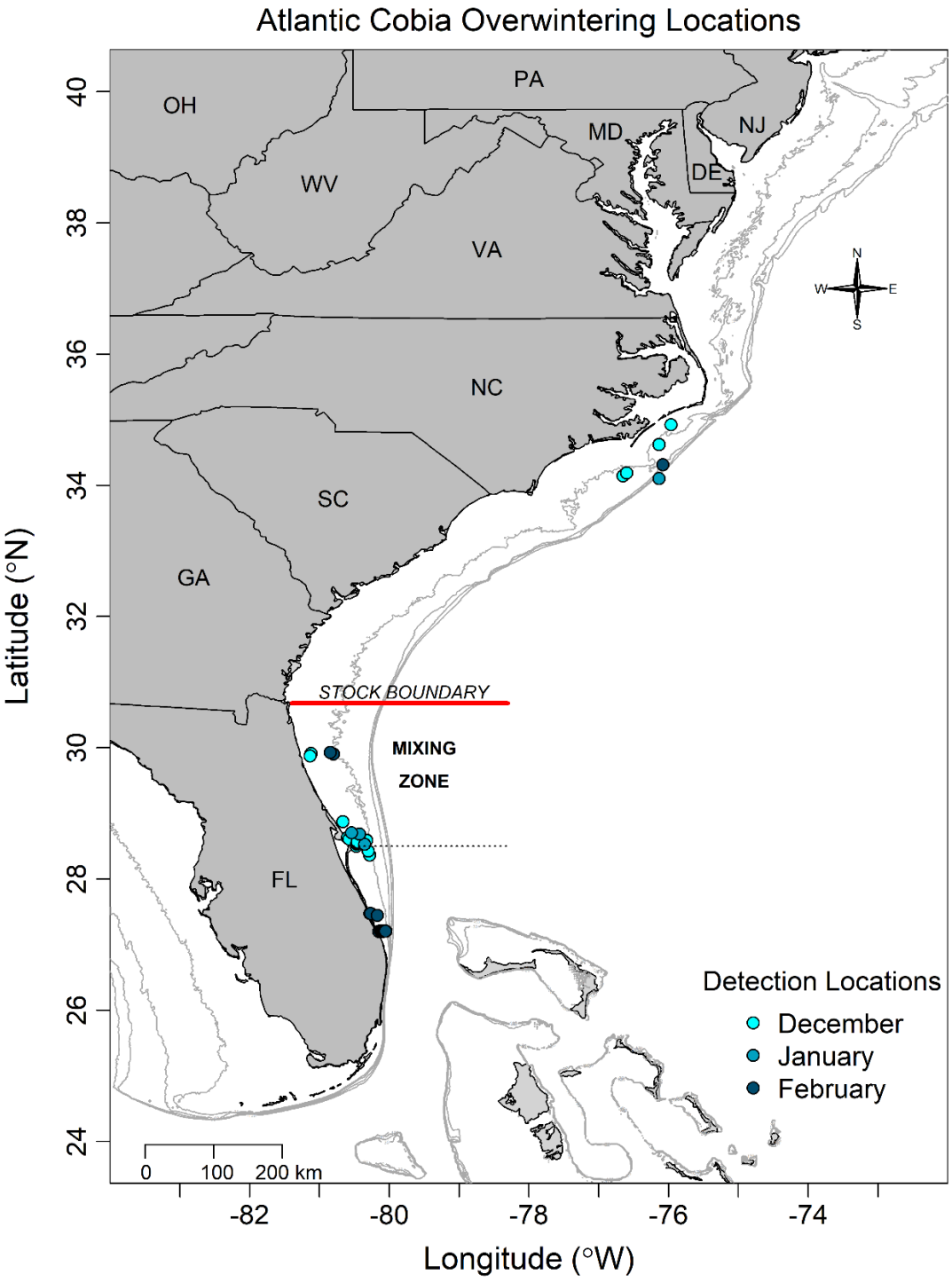


Figure 9. Location (circles) of receiver detections for individual tagged cobia during December, January, and February. The mixing zone is located between the Georgia-Florida stock boundary (red line) and Cape Canaveral, Florida (dotted line).

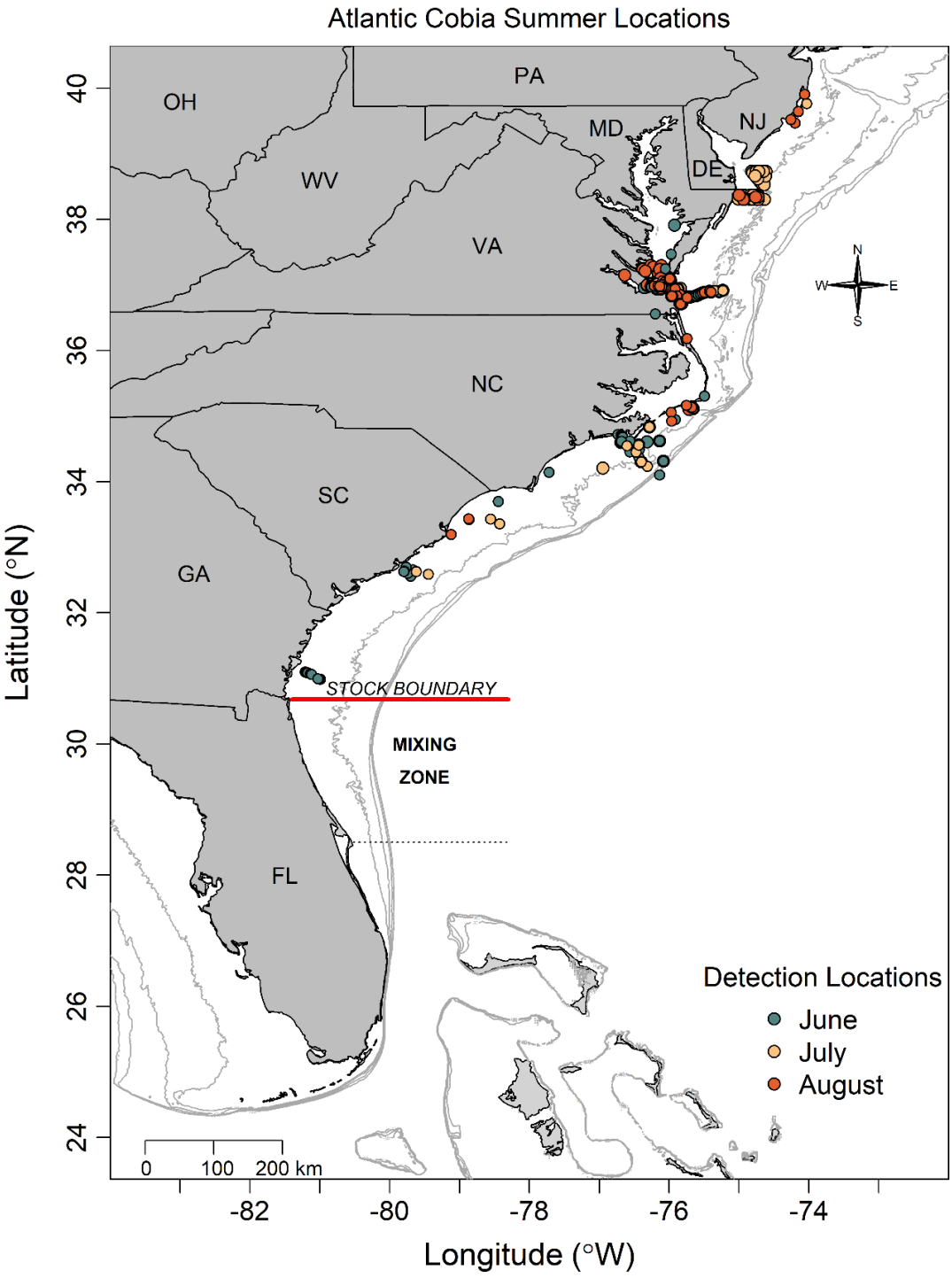


Figure 10. Location (circles) of receiver detections for individual tagged cobia during June, July, and August. The mixing zone is located between the Georgia-Florida stock boundary (red line) and Cape Canaveral, Florida (dotted line).

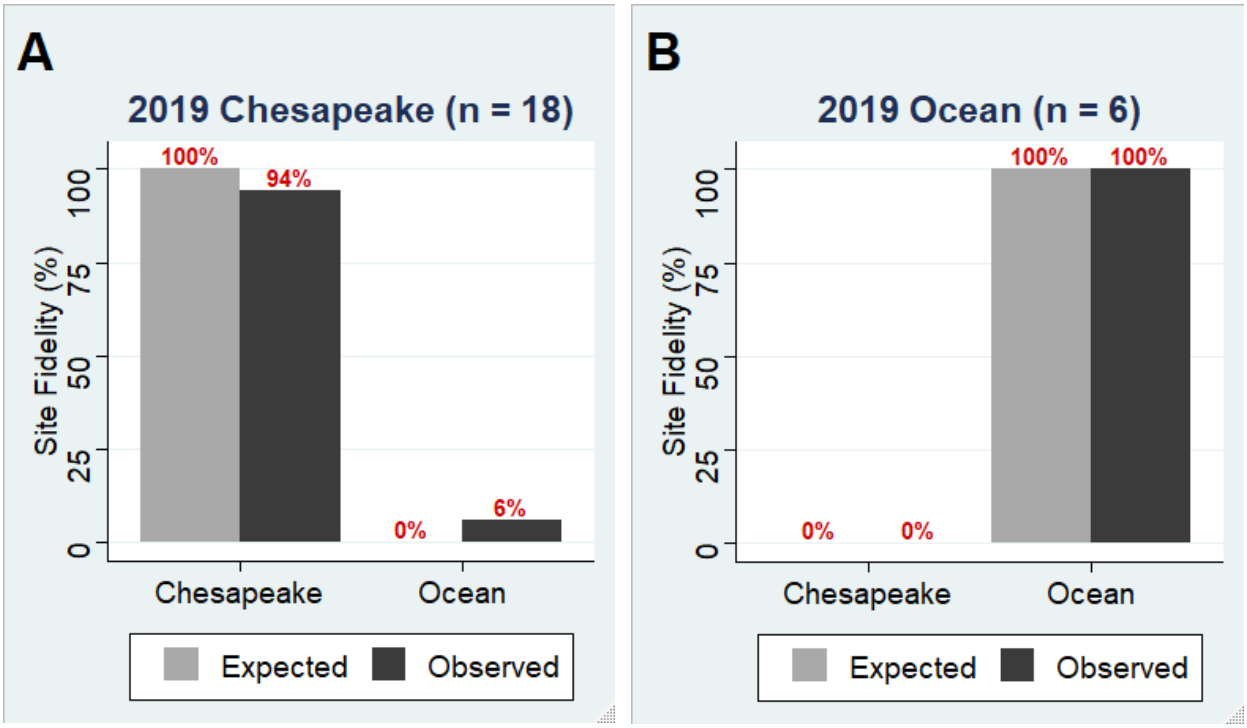
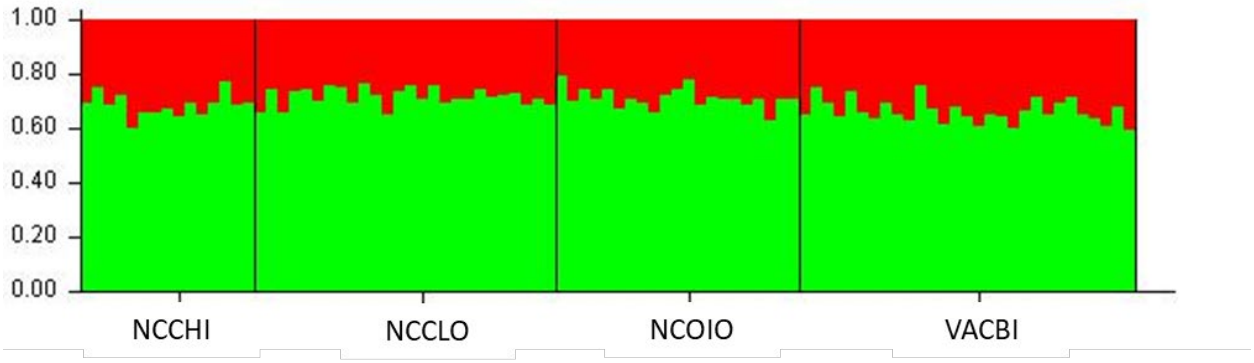


Figure 11. Degree of site fidelity in summer 2019 exhibited by cobia that were assigned to **A.** Chesapeake and **B.** ocean habitats in 2018.

A.



B.

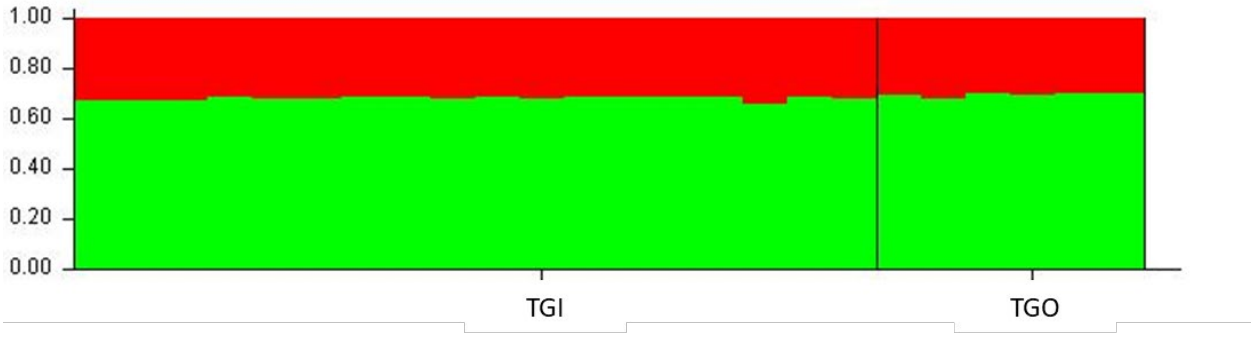


Figure 12. Population ancestry plot based on STRUCTURE results of $K = 2$ for visualization purposes of the true $K = 1$ ancestry. Each vertical bar represents a single individual in the plot with colors indicating percent ancestry to each genetic group based on **A.** Collection locations (VACBI = Chesapeake Bay, VA (inshore), NCOIO = Oregon Inlet, NC (offshore), NCCLO = Cape Lookout, NC (offshore), Cape Hatteras, NC (inshore)) and **B**. Telemetry groups (TGI = Inshore, TGO = Offshore) that showed site fidelity to specific regions.

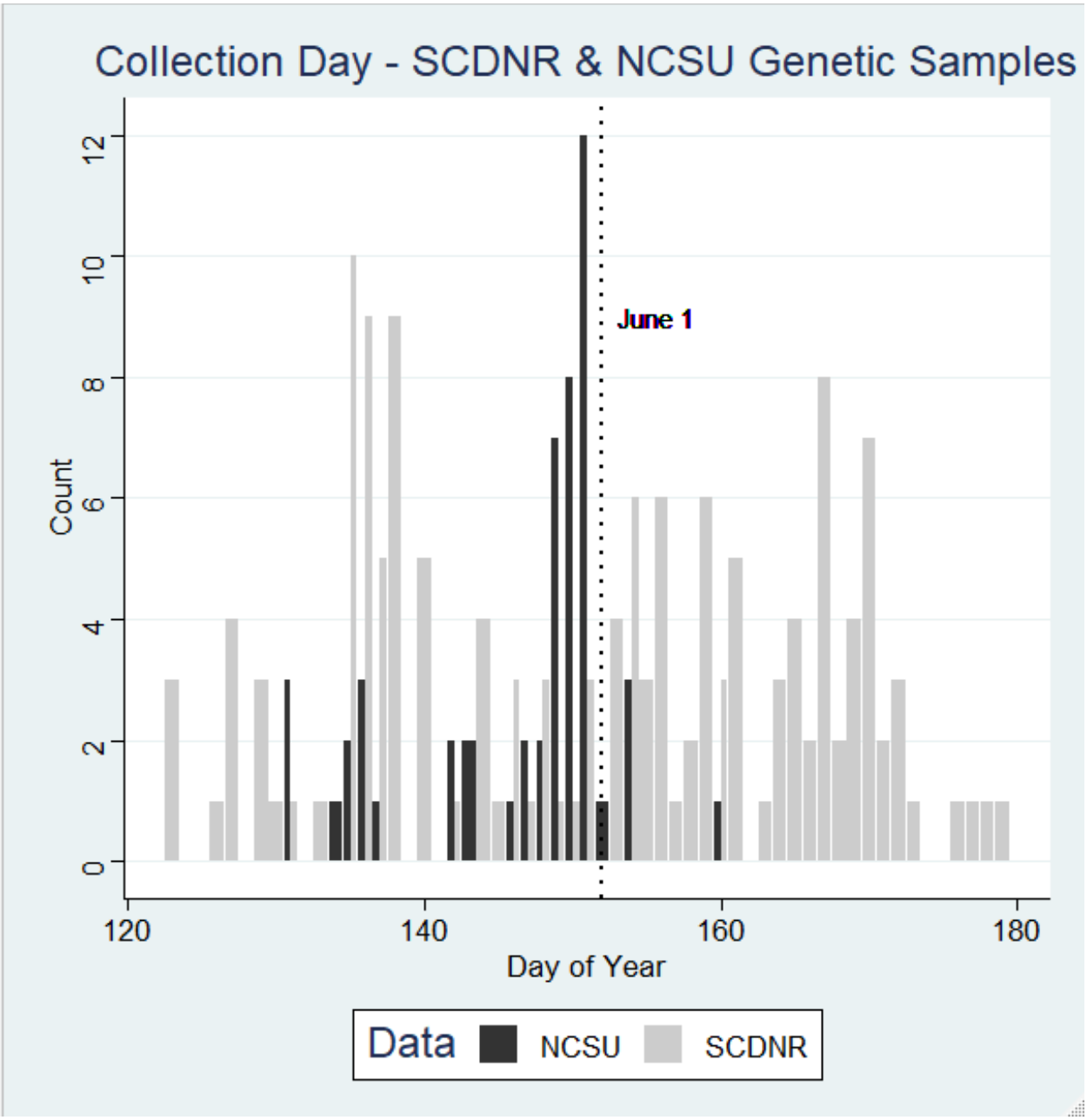


Figure 13. Histogram comparing cobia tissue collection dates between NCSU caught in the ocean in NC and NC ocean samples used in SEDAR 28.