Delineation of Coastal Shark Habitat within North Carolina Waters Using Acoustic Telemetry, Fishery-Independent Surveys, and Local Ecological Knowledge

by

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Abstract

Knowledge of shark habitat preferences and use patterns is important to effectively manage shark fisheries and account for interactions between sharks and species targeted by other fishery management or conservation efforts. To gain baseline knowledge of the availability and spatial extent of shark habitat in North Carolina waters, habitat was assessed at three spatial scales: coastal nearshore, whole estuarine, and estuarine microhabitat. The local ecological knowledge of North Carolina fishermen was also incorporated and compared with data collected using fishery-independent means. Catch data and acoustic telemetry were used to assess shark habitat at the coastal nearshore scale. Sharks captured near Cape Hatteras could be grouped into warm water and cold water assemblages. Juvenile Dusky (*Carcharhinus obscurus*) and Sandbar (*Carcharhinus plumbeus*) Sharks were tracked using acoustic telemetry, and 12 of 15 tagged Sandbar Sharks were detected over an area from New York City to Savannah, Georgia. The spatial extent of potential juvenile Sandbar Shark habitat was influenced by a combination of sea

surface temperature and chlorophyll-a concentrations, with habitat restricted to an area in the northern portion of Raleigh Bay near the Hatteras Bight during winter. At the estuarine scale, boosted regression tree modeling of shark catch and environmental data from North Carolina Division of Marine Fisheries (NCDMF) gillnet and longline surveys were used to spatially delineate potential habitat for six species within Pamlico Sound. Inlet distance and temperature were among the most influential environmental factors associated with species presence, while salinity was the most influential factor on abundance. Potential habitat for most species was located on the east side of the sound near the inlets with the exception of the Bull Shark (*Carcharhinus leucas*), which showed hot spots of potential habitat near sources of freshwater input. At the estuarine microhabitat scale, catch and environmental data from a fishery-independent survey conducted in Back and Core Sounds and a small-scale acoustic array deployed around Middle Marsh in Back Sound were used to identify associations between species and with specific habitat types. Spatial overlap between species was generally low and species assemblages separated based on water temperature. Smooth Dogfish (*Mustelus canis*), and Spiny Dogfish (*Squalus acanthias*) occurred primarily during mid-afternoon and Blacknose Sharks (*Carcharhinus acronotus*) occurring most often at night. A large Bull Shark tagged in another study accounting for the majority of tag detections and occurred within the array primarily during nighttime hours, most often on receivers deployed at two oyster reef sites and one sand flat site. Local ecological knowledge (LEK) of shark distributions and habitat preferences was gathered using structured interviews with North Carolina fishermen and their observations were compared

with data from fishery-independent surveys and primary literature to assess their accuracy. All hypotheses generated from LEK were classified as either supported or plausible. Overall, water temperature was identified as an important influence on shark species presence and assemblage composition, with factors such as salinity and potential interspecific interactions more important at finer habitat scales. The locations and spatial extents of shark habitats in North Carolina are influenced by dynamic environmental factors and may be affected by large-scale perturbations such as climate change.

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A Dissertation

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Introduction

The Ecological Importance of Sharks

As a group, sharks (Order Selachii) are typically upper-level predators in marine food webs, filling roles as either high secondary or tertiary consumers (Cortés 1999). The predatory role of sharks may make them important regulators of the populations of their prey and competitors, through both direct predation and indirect effects on prey behavior (Heithaus et al. 2010, Heithaus and Vaudo 2012). Sharks are suspected of facilitating cascading effects through marine food webs, though the strength of these effects can vary with community and environmental factors (Bascompte et al. 2005). In ecosystems with a diversity of predators, cascading effects of the loss of sharks can be mediated by other high-level predators such as tunas and billfish (Kitchell et al. 2002), but in systems in which one or two species of sharks are the dominant top predator, predator-prey interactions involving these species can affect even primary producers (Burkholder et al. 2013).

Declines in shark populations resulting from overfishing and other anthropogenic factors are suspected of causing significant food web changes due to the removal of top-down control on marine mesopredators (Baum and Worm 2009). In one high-profile example, declines in large shark populations off of North Carolina were implicated in the crash of the scallop fishery (Myers et al. 2007). However, not all shark species are affected by fishing pressure in the same way. Populations of some species, such as Bull (*Carcharhinus leucas*) and Tiger

(*Galeocerdo cuvier*) Sharks, have stabilized or are increasing, while other species such as Dusky Sharks (*Carcharhinus obscurus*) and Hammerheads (*Sphyrna* sp.) continue to decline (Hayes et al. 2009, Baum and Blanchard 2010, NMFS 2012a, Carlson et al. 2012). Though life history traits vary widely between shark species and can determine susceptibility to fishing pressure, juvenile survival rate tends to have the greatest influence on population growth or decline regardless of species (Cortés et al. 2012).

Defining Shark Habitat

Sharks may show fidelity to certain habitat types or specific locations for a variety of reasons (Chapman et al. 2015). The broad types of habitat use considered in this study include nursery, foraging, and migratory habitat. For the purposes of classifying habitat, nursery habitat is considered to benefit reproductive success, foraging habitat benefits foraging efficiency and growth, and migratory habitat represents the range of paths followed by sharks while traveling between more resident locations. However, these habitat definitions likely overlap. For example, the migrations of pelagic species may follow areas of high productivity that may provide foraging opportunities (Block et al. 2011).

Nursery habitat has been defined in a marine fisheries context an area that contributes more to the adult population than other available habitat through a combination of juvenile density, enhanced growth, increased juvenile survival, and movement from nurseries into adult habitats (Beck et al. 2001). These areas can be further classified as primary nursery habitat, where birth occurs, and secondary

nursery habitat, where juveniles occur consistently, though these two nursery types often overlap (Bass 1978). Because of the strong influence of early life survivorship on population dynamics (Cortés et al. 2012), nursery areas are critical habitat for sharks.

Foraging habitat is broadly defined as an area of relatively high food availability. As highly mobile predators, sharks are rarely resident in any particular location but may return to foraging habitats repeatedly over the course of their seasonal migrations (Meyer et al. 2010, Werry et al. 2014) or preferentially spend time in certain habitat types that are conducive to capturing prey (Heithaus et al. 2002, Hammerschlag et al. 2010). Trophic level and predation risk scale with body size for many shark species, so foraging habitat preferences may differ between life stages (Heupel et al. 2014).

Migratory habitat can be defined as the area animals travel through during directed movements between locations, which can be nursery and foraging habitats, generally in response to changing environmental conditions (Dingle and Drake 2007). Sharks have been documented traveling long distances between foraging habitats (Meyer et al. 2010) and returning over long distances to specific primary nurseries (Hueter et al. 2005). Long-distance movements may also follow specific environmental ranges or food sources (Block et al. 2011, Kajiura and Tellman 2016). Changes in migration routes or destination habitats may have considerable impacts on shark life cycle and population dynamics (Hueter et al. 2005, Chapman et al. 2015).

Criteria originally developed to classify shark nursery habitat can be adapted to identify other types of habitat. The presence of certain species and demographic groups of that species can be a coarse measure of habitat use. For example, shark nurseries have been identified by the presence of juveniles and pregnant females (Castro 1993). Fine-scale habitat selection and carrying capacity can be driven by a combination of abiotic environmental factors, food availability, and predation risk (Heithaus 2007). The three criteria used to identify nursery habitat by Heupel et al. (2007) can be used to define shark habitat beyond simple observations: 1) sharks are more abundant than in other areas, 2) sharks remain or occur repeatedly for extended periods of time, and 3) use of the area by sharks is consistent across years. Habitats often host multiple shark species, though the species assemblage may change with season (Ulrich et al. 2007) and individuals may move between habitats on a seasonal basis (Conrath and Musick 2008).

Sharks are often found in dynamic nearshore and estuarine habitats (Knip et al. 2010). Microhabitat use within these heterogeneous environments can vary along ecoclines, defined as gradual transitions along environmental gradients, and ecotones, which are separated by sudden transitions between abiotic conditions or biotic communities (van der Maarel 1990). Differences in abiotic habitat preferences can facilitate resource partitioning and reduce competition (Dunson and Travis 1991), though species distributions may overlap at boundary areas where their environmental tolerances overlap (Attrill and Rundle 2002). Distribution among co-occurring shark species can vary by abiotic ecoclines such as dissolved oxygen, temperature, and salinity, and ecotones such as depth strata and

the proximity of static features such as inlets (Wiley and Simpfendorfer 2007, Belcher and Jennings 2010, Froeschke et al. 2010).

Though abiotic environmental preferences are generally consistent, biotic interactions may structure shark habitat use patterns in ways not predicted by abiotic factors. Forging efficiency and prey availability may differ between microhabitat types (Lipcius and Hines 1986), particularly those that provide shelter from predation such as seagrass beds and oyster reefs (Grabowski et al. 2005). However, ecotone edges between protective and relatively exposed habitats may increase foraging opportunities (Hammerschlag et al. 2010). In turn, juveniles and small-bodied species must balance foraging opportunities with protection from their own predators (Heithaus 2007). Predation risk can exert a strong influence on habitat use and foraging patterns (Dill et al. 2003), but risk tolerance may vary by prey life history (Frid et al. 2012). Sharks may not co-occur with their most important prey to avoid competition with other species (Gutteridge et al. 2011) and may occupy habitats with sub-optimal prey availability in order to avoid predators (Bush and Holland 2002). Other species, however, seem to prioritize foraging over risk-avoidance and regularly move through habitats where potential predators may occur (Carlson et al. 2008).

Sharks readily move between different nursery habitats, which may serve to buffer the effects of degradation of any one habitat on species populations (Yates et al. 2012). However, many habitats are in nearshore or inshore waters where juvenile sharks may be more exposed to fishing pressure and other anthropogenic stressors than in non-nursery habitats (Knip et al. 2010). Even species that range

widely can show fidelity to specific nursery or foraging habitats or migration routes, which gives these particular areas a disproportionate influence over the behavior and population dynamics of those species (Hueter et al. 2005, Chapman et al. 2015). For these reasons, in United States waters the National Marine Fisheries Service (NMFS) has prioritized identification of nursery habitats as essential fish habitat (EFH) for coastal sharks (NMFS 2009).

Sharks in North Carolina

Little is currently known about shark habitat in North Carolina waters, but local physical and geographical features make areas of the state's coast well-suited as potentially productive shark habitat. Due to the influence of the Labrador Current and Gulf Stream, as well as the narrow continental shelf, Cape Hatteras sits at the dividing line between two major marine ecoregions: the Virginian temperate region to the north and the Carolinian subtropical region to the south (Hayden et al. 1984), also known among U.S. fishery managers as the Northeast and Southeast U.S. Continental Shelf Large Marine Ecosystems (Fautin et al. 2010). Fish assemblages differ significantly north and south of Cape Hatteras (Love and Chase 2007), and North Carolina waters form the border between different stocks of species that occur in both northern and southern waters (Baker et al. 2007).

An assessment by NMFS found evidence for primary nursery habitat within North Carolina waters for 12 shark species, of which Atlantic Sharpnose Sharks (*Rhizoprionodon terranovae*), Smooth Dogfish (*Mustelus canis*), and Sandbar Sharks (*Carcharhinus plumbeus*) were the most common (Jensen et al. 2002). Cape

Hatteras appears to be an important overwintering habitat for juvenile Sandbar and Dusky Sharks, which are common bycatch in local sink gillnet fisheries (Jensen and Hopkins 2001). Sandbar Sharks in particular have been tracked to this area from primary nurseries in the Chesapeake (Conrath and Musick 2008) and Delaware (McCandless et al. 2007) Bays. The University of North Carolina (UNC) longline survey, running since 1972 in the vicinity of Cape Lookout, has documented 22 species of sharks, and individuals within juvenile size ranges have been captured for 14 of them (Schwartz et al. 2013). Thorpe et al. (2004) determined that nearshore waters around Cape Fear regularly provided primary and secondary nursery habitat for Atlantic Sharpnose, Spinner (*Carcharhinus brevipinna*), Blacknose (*Carcharhinus acronotus*), and Dusky Sharks, and served as occasional nursery habitat for Blacktip (*Carcharhinus limbatus*) and Scalloped Hammerhead (*Sphyrna lewini*) sharks.

Shark presence and abundance in the inshore waters of North Carolina are not currently well-understood, but coastal sharks are captured during gillnet and longline surveys within Pamlico Sound (Lee Paramore, NCDMF, personal communication) and small coastal species are among the bycatch in the inshore shrimp fishery (Brown 2010). The freshwater-tolerant Bull Shark (*Carcharhinus leucas*) has been encountered throughout Pamlico Sound and within adjacent rivers (Schwartz 2012). Several shark species were documented in the Cape Fear River in the 1970s, including juvenile Blacknose, Dusky, and Atlantic Sharpnose Sharks (Schwartz 2000).

Shark habitats in North Carolina waters are potentially impacted by fisheries (Jensen and Hopkins 2001, Thorpe and Frierson 2009, Brown 2010) and may face

increasing pressure from coastal development, particularly impending fossil fuel exploration and extraction. Additionally, North Carolina's position at the edge of temperate and subtropical waters makes the state's waters ideal for observing species range shifts in response to changing climate (e.g. Pinsky et al. 2013) and some evidence already exists for recent shark species assemblage changes in the area (Schwartz et al. 2010). With the exception of the UNC longline survey focused in the vicinity of Cape Lookout (Schwartz et al. 2013), North Carolina does not currently engage in regular, targeted sampling for coastal sharks. Despite this lack of monitoring attention relative to other commercially and recreationally-sought fish species, North Carolina ranks in the top four states in terms of percentage of commercial shark fishing permit holders and several coastal species are specifically targeted by charter fishermen in state waters (NMFS 2012b). An updated assessment of shark nursery habitat in North Carolina waters is needed to best inform management of these species in the face of changing environmental and economic patterns.

U.S. Atlantic Coastal Shark Fisheries

U.S. commercial coastal shark fishing effort in the Atlantic is distributed along the mid-Atlantic and southeastern regions, though most targeted fisheries occur from North Carolina south (NMFS 2012b). Gear types used in coastal shark fisheries include bottom longlines, drift gillnets, strike nets, and sink gillnets, with bottom longlines and sink gillnets the most commonly-used gear types (NMFS 2012b, Mathers et al. 2013). Bottom longline gear characteristics can vary widely

by region, but typically consist of a mainline ranging from 0.5 to 13.7 km with 4 to 1,000 gangions usually consisting of heavy monofilament leader and either a circle or J-hook, with soak times ranging from 10.9 to 20.3 hours (Hale et al. 2011, NMFS 2012b). In the sink gillnet fishery, gear measures between 182 to 2,645 m in length and 2.4 to 15.2 m in depth, and total fishing time including soak and haul-back time ranges from 1.9-10.2 hours (NMFS 2012b, Mathers et al. 2013). Blacktip Sharks consistently have been the most-landed species in large coastal shark fisheries, while Atlantic Sharpnose Sharks historically have made up the majority of landings in small coastal shark fisheries (NMFS 2012b). These two species currently are the most commonly captured and retained species in the Atlantic bottom longline fishery, while Smooth Dogfish are the most commonly retained shark species in Atlantic sink gillnet fisheries, some of which target the species (NMFS 2014).

Recreational fisheries may represent a considerable and increasing source of fishing pressure on Atlantic coastal shark populations. Sharks have long been recognized as a target of recreational fishing effort in the Gulf of Mexico, and is of high economic importance to the region (Fisher and Ditton 1993). Recreational shark fisheries on the Atlantic coast are less developed, but interest has increased since the 1960s. Sharks may be caught by rod and reel from shore, fishing piers, and vessels, and charter fishing for coastal sharks is becoming increasingly popular (NMFS 2012b). Though the recreational fishery for sharks is generally catch-and-release, some species are commonly kept for consumption. In the Atlantic, Blacktip and Spinner Sharks make up the majority of large coastal sharks harvested recreationally (NMFS 2014). Among small coastal shark species, Atlantic Sharpnose

and Bonnethead (*Sphyrna tiburo*) Sharks make up the majority of species captured and retained in Atlantic recreational fisheries (Cortés 2007, NMFS 2014).

Management of Coastal Sharks in U.S. Atlantic Waters

At a Federal level, management of shark fisheries in U.S. Atlantic waters is administered by the NMFS Highly Migratory Species (HMS) management division. The first interstate fishery management plan (FMP) for sharks was developed in 1993 and grouped all managed species into three fishery complexes based on size and habitat in which the species were most often encountered: large coastal sharks, small coastal sharks, and pelagic sharks (NMFS 1993). Annual and semi-annual quotas were set by fishery complex, and for recreational fisheries a daily limit of four sharks per vessel was established. In 1999, NMFS introduced several new regulations regarding sharks as part of the FMP for Atlantic tuna, swordfish, and sharks (NMFS 1999). These included a reduced daily limit of one shark per vessel for recreational fisheries (with an allowance for one extra Atlantic Sharpnose Shark), a commercial size limit of 137 cm for ridgebacked coastal species (including Sandbar, Silky (*Carcharhinus falciformis*) and Tiger Sharks), limited-access permits for commercial shark fisheries, and a prohibition on landing sharks without fins attached in Atlantic waters. In addition, the 1999 FMP began separating species out of the fishery complexes: Porbeagle (*Lamna nasus*) and Blue Sharks (*Prionace glauca*) were given separate quotas from the rest of the pelagic shark complex, and landings were prohibited for 19 species of conservation concern, including Sand Tiger (*Carcharias taurus*), White (*Carcharodon carcharias*), and Dusky Sharks

(NMFS 1999). An amendment to this FMP added a time-area closure to sink gillnets off of Virginia, North Carolina, and South Carolina from January-July to protect pupping grounds used by Sandbar and Dusky Sharks (NMFS 2003).

Currently, shark fisheries are managed by the Consolidated Atlantic Highly Migratory Species FMP (NMFS 2006) and its amendments. The initial FMP maintained most of the previous regulations (NMFS 1999, 2003), added mandatory identification workshops for commercial fishermen and dealers involved with shark fisheries, and established a framework for assessing Finetooth Sharks (*Carcharhinus isodon*) separately from the small coastal shark complex. Amendments focused on sharks have largely concentrated on breaking up the shark fishery complexes and establishing species-specific management measures. Amendment 2 prohibited recreational and commercial landing of sandbar sharks with the exception of vessels involved in a limited-access research fishery with total fishery observer coverage (NMFS 2007). Amendment 3 formally removed Blacknose Sharks from the small coastal shark complex and established a separate quota, while also laying the framework for a quota for Smooth Dogfish (NMFS 2010). The most recent amendment established a quota for all large Hammerhead shark species separate from that of the large coastal shark complex and increased both the recreational and commercial size limit for these species to 78 inches (198 cm), and began managing Blacktip Sharks from the Gulf of Mexico separately from the large coastal shark complex in that region (NMFS 2013).

In state waters (less than 3 nm/5.56 km from shore), shark fishery management is administered by the Atlantic States Marine Fisheries Commission

(ASMFC). The Interstate FMP for Atlantic Coastal Sharks (ASMFC 2008) mostly ensured consistency between state regulations Federal regulations, with the exception of some differences in permissible gear types for state waters. The ASMFC FMP also established smooth dogfish as a separate management unit from large coastal, small coastal, and pelagic shark complexes (ASMFC 2008). Addendum I of the FMP broke with Federal regulations to allow finning of Smooth Dogfish at sea, provided the weight of fins landed did not exceed 5% of the weight of dressed carcasses landed (ASMFC 2009). Addendum II (ASMFC 2013) increased the fin:carcass weight ratio for smooth dogfish to 12% to make state regulations consistent with the Shark Conservation Act of 2010 (USA 2010).

The Shark Conservation Act of 2010 is one of several recent policy developments that have influenced shark fishery management. The Act prohibits landing of sharks caught in U.S. waters without fins naturally attached, with the exception of Smooth Dogfish (USA 2010). The Act also increases the fin:carcass weight ratio to 12% for Smooth Dogfish. Implementation of the Shark Conservation Act has put NMFS in conflict with state laws prohibiting the possession and transport of shark fins (NOAA 2013a). Shark management has also been influenced by recent petitions to list some shark species on the U.S. Endangered Species Act. In response to a petition to list Scalloped Hammerheads (*Sphryna lewini*), NMFS found that listing as Threatened or Endangered was not warranted for the Northwest Atlantic population but may be warranted for populations elsewhere in the world (Miller et al. 2013). However, in response to a petition to list the Great Hammerheads (*Sphyrna mokarran*), NMFS found that listing may be warranted and

is currently conducting an assessment of the species (NOAA 2013b). NMFS also assessing the Dusky Shark as a candidate species for listing under the Endangered Species Act (NOAA 2013c) but found that listing was not warranted due to progress made in rebuilding populations by existing management measures (McCandless et al. 2014).

The most recent stock assessments (NMFS 2012a) show mixed results with regards to shark stock health. Of the sharks assessed by NMFS, Sandbar, Dusky, Scalloped Hammerhead, Blacknose, and Porbeagle sharks are classified as overfished, with overfishing still occurring for all but Sandbar Sharks and Porbeagles. Other shark stocks are apparently responding well to fishing pressure, with non-Blacknose small coastal sharks, Gulf of Mexico Blacktip Sharks, and non-Porbeagle pelagic sharks designated as not overfished with no overfishing occurring (NMFS 2012a).

Fishery Interactions and Anthropogenic Influences on Coastal Shark Habitat

Juvenile survival is one of the most important factors regulating shark populations (Cortés et al. 2012), but the use of relatively constrained inshore and nearshore nursery habitats by many coastal shark species may place juveniles at greater risk of interactions with fisheries (Knip et al. 2010). Despite size limits and landings prohibitions, juvenile sharks are common among fishery bycatch. Juvenile sharks are incidentally captured in nearshore gillnet fisheries targeting other species such as Spanish Mackerel (*Scomberomorus maculatus*) and mixed teleosts (Thorpe and Frierson 2009, Mathers et al. 2013), longline fisheries targeting adult

coastal sharks (Morgan and Burgess 2007), and recreational fisheries (Cortés 2007). Nearshore and inshore trawl fisheries can also incidentally capture juvenile sharks, particularly species in the small coastal shark complex (Belcher and Jennings 2011).

Post-release survival after incidental capture varies by a number of factors, and some species are better able to handle capture stress and injury than others. Morgan and Burgess (2007) found that at-vessel mortality for commercial longline-captured Tiger and Sandbar Sharks was relatively low, while 81-94 % of all Scalloped Hammerhead, Great Hammerhead, Dusky, and Blacktip Sharks were dead. Capture stress responses and post-release survival can vary even among closely-related species within the same family and genus (Mandelman and Skomal 2009). Gear characteristics can also influence survival rates: gillnet capture seems to cause greater capture stress among sharks than longline capture (Hyatt et al. 2012) and time spent hooked on longline gear was the most important factor affecting mortality for three of four species assessed by Morgan and Carlson (2010). Regardless of species, juvenile age classes are more susceptible to capture mortality (Morgan and Burgess 2007, Romine et al. 2009). For especially long-lived, low-fecundity species like the Dusky Shark, incidental capture mortality of juveniles may be enough to slow or prevent population recovery (Romine et al. 2009).

Fishery interactions are not the only anthropogenic influences on shark habitats. Use of nearshore and inshore environments also exposes sharks to the impacts of coastal development (Knip et al. 2010). Habitat degradation resulting from coastal development can affect survival rate among juvenile sharks, as has been observed at a Lemon Shark (*Negaprion brevirostris*) nursery in Bimini

(Jennings et al. 2008). Conversely, juvenile Bull Sharks in the Indian River Lagoon in Florida appear to have adapted to using anthropogenic habitat modifications such as canals, but this may increase their exposure to harmful contaminants (Curtis et al. 2013).

All of these issues underscore the importance of identifying and delineating the spatial extent of shark nursery habitats. Spatial delineation of essential habitats for highly mobile species will become especially important as marine spatial planning policies move forward (Crowder and Norse 2008).

Methodology

Goals and Objectives

The goal of this study was to identify and delineate shark habitat in North Carolina waters at three spatial scales: coastal, estuarine, and microhabitat. Methods used to assess potential nursery habitat included a combination of fishery-independent sampling and social science techniques. The specific objectives were:

1. Assess nursery habitat in North Carolina waters on a coastal scale using a combination of surveys and passive acoustic telemetry to identify migratory connections to the Hatteras Bight and the environmental conditions associated with use of this area by juvenile sharks.
2. Identify and spatially delineate habitat use on an estuarine scale by sharks using data from fishery-independent gillnet and longline surveys in Pamlico Sound.

3. Describe patterns of inshore microhabitat use using a combination of relative catch data from fishery-independent survey methods and acoustic telemetry.
4. Use open-ended interviews to gather local ecological knowledge (LEK) on environmental factors associated with shark presence and abundance from fishermen who have interacted with sharks in North Carolina waters.

It is hoped that the combined results of these approaches will provide a holistic view of shark habitat associations in North Carolina. More details on methods and results are found in the chapters within in this volume.

Expected Benefits

Identification and delineation of nursery habitat may help make coastal shark fisheries more selective and sustainable by allowing fishers to avoid areas where juvenile sharks are abundant. Fishery regulations incentivize fishers to avoid incidental capture of juveniles and prohibited species, and more detailed information on migratory patterns and habitat use can aid these efforts. The results of this study may increase the precision and effectiveness of fishery management measures such as time-area closures and fishing seasons. In addition, this project will identify factors associated with shark presence and habitat use at multiple spatial scales, which may be helpful in modeling intraspecific interactions between these species and other monitored species such as sea turtles, marine mammals, and economically-important finfish. Finally, decreased fishing pressure on juveniles will help vulnerable shark populations rebuild, potentially to the point of reopening fisheries for some species.

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Chapter 1

Habitat Use by Migratory Coastal Sharks Overwintering in North Carolina Waters

Abstract

Highly migratory sharks can have ocean-scale activity areas, but often show fidelity to specific areas that represent essential habitat for these species. The area of Raleigh Bay, along coastal North Carolina between Cape Hatteras and Cape Lookout, has long been regarded as an important overwintering habitat for Sandbar Sharks (*Carcharhinus plumbeus*) and other shark species, though this has been inferred from mark-recapture and telemetry studies originating from other areas of the U.S. east coast. To directly assess the importance of Raleigh Bay and the Hatteras Bight as winter shark habitat, gillnet surveys were conducted in collaboration with commercial fishermen in February, April, and November of 2013. All sharks were identified and measured, and 15 juvenile Sandbar Sharks and two juvenile Dusky Sharks (*Carcharhinus obscurus*) were tagged with acoustic transmitters. These sharks were detected on an acoustic array deployed off of Hatteras Village and other acoustic arrays along the U.S. east coast through the Atlantic Cooperative Telemetry Network from February 2013 through October 2014. Of the tagged sharks, 12 Sandbar Sharks were detected across an area ranging from New York City to Savannah, Georgia, though the majority of tag detections occurred on the Hatteras array and arrays within Chesapeake and

Delaware Bays. Tagged sharks used the area of the Hatteras array as a winter habitat, occurring until mid-April, then returned to summer habitats in the Chesapeake and Delaware Bays where they remained until the following October. Tagged sharks were detected returning to the Hatteras array between September and March. Sensors attached to the acoustic tags showed that juvenile Sandbar Sharks occupied deeper and cooler waters off of Cape Hatteras than in their summer habitats. To more broadly delineate habitat, satellite-based sea surface temperature and chlorophyll-a concentrations recorded by the MODIS satellite were overlaid over receiver stations to identify sea surface environmental conditions during shark presence and absence at the receivers. Boosted regression tree models were used to identify the relative contribution of temperature and primary productivity to shark presence, and to identify cut points between high and low likelihood of shark presence. Mapping of the areas falling within the environmental ranges of these cut points revealed that potential juvenile Sandbar Shark habitat is restricted to an area within Raleigh Bay and near the Hatteras Bight during winter and occurs over a much broader area during other seasons. Raleigh Bay represents essential overwintering habitat for juvenile Sandbar Sharks originating from northern nurseries and should be managed carefully for the sustainability of shark populations.

Introduction

Large, highly migratory marine species can have activity spaces that range across entire ocean basins, though their distributions and migratory pathways can be bounded by factors such as water temperature and areas of high productivity (Block et al. 2011). Determining the habitat use patterns of highly migratory species is critical at multiple levels of marine conservation and management, including delineation of essential habitat (Speed et al. 2010), assessing potential fishery interactions (Queiroz et al. 2016), identifying predator-prey interactions (Lacroix and Fleming 2014), and predicting range shifts in response to climate change (Hazen et al. 2012).

Among highly migratory sharks, telemetry efforts have found that many species show site fidelity, returning regularly to specific coastal, pelagic, or estuarine habitats associated with reproduction or foraging opportunities (Hueter et al. 2005, Chapman et al. 2015). Such areas may be of disproportionate importance to sustaining populations of these species (Chapman et al. 2015). In cases where essential habitats are located near shore, sharks using them may be subjected to increased anthropogenic effects like fishing pressure and coastal development (Knip et al. 2010) and even offshore habitats where sharks congregate may spatially overlap fishing effort (Queiroz et al. 2012, 2016).

The coast of North Carolina, particularly the area of Raleigh Bay bounded by Cape Hatteras to the north and Cape Lookout to the south, appears to be especially important to several coastal shark species. Jensen and Hopkins (2001) found

evidence of overwintering habitat use by 12 shark species, including Smooth Dogfish (*Mustelus canis*), Atlantic Sharpnose Sharks (*Rhizoprionodon terraenovae*), and juveniles of species of conservation concern such as Sandbar (*Carcharhinus plumbeus*) and Dusky Sharks (*Carcharhinus obscurus*). Year-round sampling efforts documented 18 species in North Carolina nearshore waters, of which neonates were captured for 12 species (Jensen et al. 2002).

Raleigh Bay may be especially important to juvenile Sandbar Sharks, which were the second most common elasmobranch bycatch in the winter King Mackerel (*Scomberomerus cavalla*) fishery off Cape Hatteras (Jensen and Hopkins 2001) and the third most commonly documented shark species in a survey of shark nursery habitats in North Carolina waters (Jensen et al. 2002). Tag-recapture studies originating in known primary nurseries in New Jersey (Merson and Pratt, Jr. 2007), Delaware Bay (McCandless et al. 2007) and Chesapeake Bay (Grubbs et al. 2007) have identified coastal North Carolina as overwintering habitat for juveniles of this species based on the number and density of recaptures in this area during winter months. Grubbs et al. (2007) observed that while recaptures of tagged Sandbar Sharks during the summer occurred across a relatively wide latitudinal range, recaptures from November through May occurred almost exclusively in the latitudinal range of Raleigh Bay (34-36 degrees North). The majority of juvenile Sandbar Sharks tagged with pop-off satellite tags off of Virginia's Eastern Shore also showed a high concentration of tagged sharks within this area during the winter (Conrath and Musick 2008). Due to the apparent importance of coastal North Carolina to Sandbar Sharks and other species, a time-area closure to bottom longline

gear was instituted by the National Marine Fisheries Service (NMFS) that includes the majority of federal waters off of the state from January 1st through July 31st every year (NMFS 2003).

The unique biogeography of coastal North Carolina likely contributes to the diversity of shark species and the apparently extensive use of Raleigh Bay. Coastal North Carolina sits at the transition zone between the warm-temperate ecosystem to the south and cooler-temperate ecosystem to the north, with Cape Hatteras and the confluence of the Gulf Stream and Labrador Current functioning as the biogeographic break between the them (Hayden et al. 1984, Fautin et al. 2010). This transition area also represents an observable change in fish communities (Love and Chase 2007), though intrusion south of Cape Hatteras by Labrador Current water allows cool-temperate species to occur near shore in Raleigh Bay (Fautin et al. 2010). Because of its position on a biogeographic break, the fish community along North Carolina's coast may be among the first to show observable shifts in response to climate change (Parker and Dixon 1998).

Habitat selection by sharks is driven by a combination of abiotic and biotic factors (Heithaus 2007). Though abiotic factors are often more intuitive for delineating habitat (Grubbs and Musick 2007, Ward-Paige et al. 2014), satellite-based remote sensing allows observation of biotic factors such as chlorophyll-*a* concentrations, which are indicative of primary productivity (Friedland et al. 2012). In this study, the importance of Cape Hatteras in the context of known shark habitats along the U.S. east coast was assessed using a combination of acoustic

telemetry and satellite remote sensing, with the goal of identifying and spatially delineating potential habitat for sharks overwintering in North Carolina waters.

Methods

We used multiple methods to identify and assess coastal shark nursery and migratory habitat in the Hatteras Bight. Four objectives were set to gain a holistic view of the patterns of coastal shark presence off of Cape Hatteras: 1 – Survey coastal sharks near the Hatteras Bight with commercial gillnets to determine which species are present and whether there is evidence of nursery habitat, 2 – acoustically tag juvenile sharks and identify migratory connections between their overwintering habitat and other seasonal habitats, and 3 – combine satellite remote sensing data and tag detections to determine the relative influences of sea surface temperature and primary production on shark habitat selection and use these parameters to map potential seasonal habitat.

Coastal Shark Survey

Shark surveying occurred February 15-16, February 22, April 3, and November 17, 2013 aboard the F/V *Bout Time*. Sampling occurred in an area bounded by Ocracoke Inlet to the west, the point of Cape Hatteras to the east, Hatteras Island to the north, and 12.87 km from shore to the south. Sharks were captured using commercial gillnets soaked for 30 minutes at stations chosen by commercial fisherman Chris Hickman based on the potential for capturing target species. Sink gillnet gear 137.16 m in length with 15.24 cm stretched mesh was

used during February, and gillnet gear comprised of 365.76 m of 14.61 cm stretched mesh during all other sampling dates. Temperature (°C), salinity (ppt), and dissolved oxygen (mg/L) were recorded using a YSI model-85 water quality meter, and depth (m) was recorded from the onboard depth sounder.

All sharks were identified to species and sex, total length (TL, mm), fork length (FL, mm) and visible life stage characteristics were recorded. Life stage characteristics included the presence and state of umbilical scarring in juveniles, clasper length in males, and visible signs of pregnancy or recent mating in females. Age was estimated using previously published age-at-length estimates from studies on coastal shark species. Potential nursery habitat was designated by the presence of gravid females and free-swimming neonates (Castro 1993). Catch per unit effort (CPUE, sharks/gillnet set) was used to measure species abundance.

Acoustic Telemetry

Sharks in good condition and within juvenile size range for their species were chosen for acoustic tagging. Tagging procedures were similar to those developed by Heupel and Heuter (2001). During surgery, the shark was placed in a state of tonic immobility by resting it on its back on a foam operating bed. A seawater hose was placed in the shark's mouth to maintain water flow over the gills during tagging. The incision site was chosen at a location on the ventral side of the shark approximately 1/3 of the distance from the pelvic fins to the pectoral fins and the area surrounding the site was anesthetized using a 0.2 mL injection block of lidocaine. A 2-3 cm incision was made into the body cavity of the shark and a sterilized Vemco V-9TP acoustic transmitter carrying temperature and depth

sensors was inserted through the incision. The incision was then closed using an interrupted suture pattern. Acoustically-tagged sharks were marked externally using a 2-cm oval tags attached to the trailing edge of the dorsal fin. After tagging, sharks were placed in an onboard live well and allowed to recover for up to 30 minutes before release.

The acoustic transmitters carried by the tagged sharks transmitted a unique ID number, the current depth of the sensor (m), and the current temperature (°C) every 60 seconds at a 69 kHz frequency. These acoustic signals were detected and recorded on Vemco VR2W 69 kHz acoustic receivers. In order to detect tagged sharks in the vicinity of Cape Hatteras, a 12-receiver gate-style array was deployed perpendicular to the shoreline south of Hatteras Village (Figure 1). Prior field testing estimated a reliable detection range of 800 m, so receivers were placed 1.6 km apart with the line extending 19.31 km from shore. Receiver stations were anchored to the bottom using a 36.29-kg circular concrete slab secured with a #13 Danforth anchor. The receivers were suspended approximately 1.5 m from the bottom on a 1.8-m length of 0.64-cm diameter stainless steel cable held up by a hard plastic subsurface float. Receiver stations were connected to the surface by 1.9 cm diameter polypropylene rope attached to a high-flyer buoy. Detections data were downloaded from the receivers and maintenance was performed on the stations every 2-6 months as allowed by local weather.

Detections data were available from arrays outside of the Cape Hatteras area through the Atlantic Cooperative Telemetry (ACT) network, which allows researchers conducting acoustic telemetry research to easily share detections data

(Brown 2012). All ACT receivers were assumed to have a similar detection range (~ 800 m) to the receivers in the Hatteras array. Detections were plotted using ESRI ArcMap 10.1 and grouped into regions based on receiver location in reference to nearby significant geographic features. Because of the possibility of false detections and other confounding factors, tag detections were considered reliable if more than one detection was recorded in the series, or if both ID and sensor signals were recorded. Tag detections were also validated spatially and temporally by comparison with other detections of the individual shark or species; detections were considered reliable if they were consistent with movements inferred from previous detections or with the behavior of the species (i.e., not several kilometers upriver). The total number of detections and the number of individual sharks detected in each region were used to determine presence and abundance of tagged sharks, and were plotted by calendar week to combine detections from both years.

Depth and temperature data transmitted by the sensors on the acoustic tags were used to compare environmental preferences by sex, age class at the time of tagging (Young-of-year (YOY) or Age 1+, classified by length), and array region. The analysis of variance (ANOVA) procedure was used to determine whether environmental differences were statistically significant, and pairwise comparisons were made using Tukey's Honestly Significant Difference (HSD) procedure. All calculations were performed using JMP Pro 12 software.

Habitat Delineation

Juvenile shark habitat was delineated by combining tag detection data with concurrent satellite-based remote measurements of sea surface temperature (SST,

°C) and chlorophyll-a concentration (Chl-a, mg/m^3). Remote sensing data were recorded by NASA's Moderate Resolution Imaging Spectroradiometer (MODIS) Aqua satellite (Savtchenko et al. 2004). Daily images of SST and Chl-a covering the total area of tag detections (between -77.0 and -75.4 dd longitude and between 37.0 and 31.0 dd latitude) at a 1 km^2 resolution were downloaded from the NASA Ocean Color service. Whenever possible, images recorded during or within 1 day of tag detections were used. Using SeaDAS 7.3 software, sea surface layers were combined into monthly mosaics covering the date range each month during which tagged sharks were detected. For months containing no detections, images with the best coverage of the study area recorded no less than five days apart were used. The total study period encompassed February 2013 through December 2014. Months were grouped into migratory seasons (winter, spring migration, summer, fall migration) based on the location of tag detections: winter and summer were classified as the consecutive months during which most tagged sharks did not appear to move between receiver regions.

Environmental data were extracted at the locations of acoustic receivers where tagged sharks were detected using ArcGIS 10.1. All receiver locations were included for each month to ensure environmental data were extracted for locations where tagged sharks were both present and absent. Relationships between Chl-a and SST were assessed by season using ANOVA and linear regression to determine whether correlations between these factors were significant and positive or negative. Seasonal and geographic differences in mean Chl-a and SST were assessed

using ANOVA to compare seasonal means within each region and regional means within each season.

We used an approach similar to Grubbs and Musick (2007), in which areas of potential habitat are identified based on environmental conditions at which sharks were present, to delineate habitat. Boosted regression trees (BRTs) (Elith et al. 2008) were used to determine the relative contribution of either environmental factor to the presence of tagged sharks. A binary BRT model of shark presence/absence based on SST and Chl-a was generated using the script gbm.auto, which automates modeling and mapping functions of the package dismo in R (Dedman et al. 2015). In order to better identify areas of potential habitat between the arrays, cut points between high and low likelihood of detection were identified by determining which splits in SST and Chl-a measurements occurred in the majority of trees. The spatial extent of seasonal habitat for tagged sharks was delineated by using the raster calculator tool in ArcGIS 10.1 to fill in grid cells in the sea surface layers that fell within the ranges defined by cut points from the BRT model.

Results

Coastal Shark Survey

Gillnet sets were deployed at 18 stations between Ocracoke Inlet and the point of Cape Hatteras. Environmental factors varied, with significant differences in temperature (ANOVA, $F = 98.90$, $p < 0.0001$, $df = 2$), salinity (ANOVA, $F = 88.52$, $p <$

0.0001, df = 2), and dissolved oxygen (ANOVA, F = 5.07, p = 0.021, df = 2) between months. Water temperatures in February 2013 were unusually warm, while environmental measurements in April were more representative of typical late winter or early spring conditions (Table 1).

A total of 295 sharks encompassing seven species were captured during 2013 sampling, of which 196 (66.4%) were sandbar sharks (Table 2). All Sandbar Sharks were juveniles, falling within the length ranges for age 0-2 individuals (Casey et al. 1985, Sminkey and Musick 1995). Healed umbilical scars were present on 25 (12.8%) Sandbar Sharks. Based on length, young-of-year individuals made up the majority of Sandbar Sharks (108 sharks, 55.1% of total), though age-1 (60 sharks, 30.6% of total) and age-2 (28 sharks, 14.3% of total) individuals were also present. Both sexes were nearly equally represented (50.5% female, 49.5% male). Sandbar Sharks were only captured in February but were extremely abundant, with a mean CPUE of 17.81 sharks/set.

Dusky and Thresher (*Alopias vulpinus*) Sharks within juvenile size ranges were present during gillnet sampling. Three Dusky Sharks were captured and all were within the young-of-year length range for the species (Natanson et al. 1995, 2013). Two females were caught in February, and one male was caught in November. Both Thresher Sharks were females and captured during April sampling. One Thresher Shark fell within the published length range for young-of-year sharks, while the other was likely an age-1 individual (Natanson 2002).

Smooth Dogfish, Atlantic Sharpnose Sharks, Scalloped Hammerhead Sharks (*Sphyrna lewini*), and Spiny Dogfish (*Squalus acanthias*) were all present during

gillnet sampling. Based on published length-at-age estimates, only adult-sized individuals were captured for these species (Nammack et al. 1985, Conrath and Musick 2002, Loefer and Sedberry 2003, Piercy et al. 2007, Bubley et al. 2012). Smooth Dogfish were the only species that was present during all sampling months (Figure 2). In contrast, Spiny Dogfish were only captured in April, but were highly abundant (mean CPUE = 15.57 sharks/set). Atlantic Sharpnose Sharks were captured during warm water sets in February and November. Only one Scalloped Hammerhead was caught, an adult male capture during February sampling. All Smooth and Spiny Dogfish were females, and only two of 16 Atlantic Sharpnose Sharks were males. Eight Smooth Dogfish and one Atlantic Sharpnose Shark caught in November were visibly pregnant, but pregnancy stage was not confirmed.

Acoustic Telemetry

During 2013 sampling, 15 juvenile Sandbar Sharks and two juvenile Dusky Sharks were tagged with acoustic transmitters. All but three Sandbar Sharks fell within young-of-year length range, while the remaining three were within the year-1 length range (Casey et al. 1985, Sminkey and Musick 1995). Both Dusky Sharks fell within the upper range of length measurements for young-of-year individuals (Natanson et al. 1995, 2013). The sex ratio of tagged sharks was roughly even for both species, but all Age 1+ sandbar sharks were males. All Sandbar Sharks and one Dusky Shark were tagged during February sampling, and the second Dusky Shark was tagged in November (Table 3). All tagged sharks were captured and released in the sampling area between Ocracoke Inlet and Cape Hatteras (Figure 3).

No Dusky Shark tag detections were recorded on the Hatteras array or any other arrays in the ACT network. Among the Sandbar Sharks, 12 of the 15 transmitters were detected along the U.S. east coast. A total of 560 tag detections were recorded on 68 acoustic receivers within the ACT Network covering an area from New York to Georgia. The majority of Sandbar Shark detections ranged from Cape Hatteras to Cape May, New Jersey, though a single detection was recorded near the mouth of the Hudson River and another was detected in a tidal creek within the Herb River near Savannah, Georgia (Figure 4). Most sharks were detected on stationary arrays of Vemco VR2W receivers maintained in the Chesapeake Bay and surrounding waters (Carter Waterson, U.S. Navy, personal communication), Delaware Bay (Dewayne Fox and Lori Brown, Delaware State University, personal communication), coastal New Jersey (Heather Corbett, NJ Division of Fish and Wildlife, personal communication) and the Hudson River (Dewayne Fox and Lori Brown, Delaware State University, personal communication). Two sharks were detected by a Vemco Mobile Transciever (VMT) mounted on a freely-roaming Sand Tiger Shark (*Carcharias taurus*) (Haulsee et al. 2016), though exact locations of tag detections could not be determined (Danielle Haulsee, University of Delaware, personal communication).

Detections plotted by date and array region revealed seasonal movements between array regions (Figure 5). No Sandbar Sharks were detected on the Hatteras array after April 21, 2013. The first detection on the Chesapeake array occurred on May 12, 2013, and six of the tagged sharks were detected in the area until June 9, 2013, after which none of the tagged sharks were detected on the Chesapeake array

until July 30, 2013. One individual was detected repeatedly within Delaware Bay and near the coast of Cape May from June 15 – September 16, 2013. Tagged sharks returned to the Chesapeake region July 30, 2013 and six individuals were detected within that array until October 21, 2013. Despite the loss of eight of the 12 receivers over the course of the study period, the Hatteras array recorded three individual Sandbar Sharks in the fall, winter, and spring, with detections made September 30, December 4, 2013, and March 13, 2014. In 2014, juvenile Sandbar Sharks returned to the Chesapeake region May 25 and were detected within the array through October 16. No reliable tag detections were recorded in the Delaware region in 2014.

Some of the tagged sharks did not appear to follow the same movement patterns. Three sharks recorded on the Hatteras array in 2013 were never detected on the Chesapeake or Delaware Bay arrays, and one of these sharks returned to the Hatteras region in September (Figure 5). The shark that returned (shark 10847) was detected in the Herb River, tidal estuary east of Savannah, Georgia, on August 24 the following summer. One individual (shark 10848) appeared to move north into the Chesapeake region consistently with other tagged sharks, but after May 14, 2013 the movement pattern of this shark changed. Most detections of this tag after this date recorded only the depth sensor, though the temperature sensor was detected once. Between-region movements inferred from the detections data were much faster than for the other juvenile Sandbar Sharks, with the tag detected in the Chesapeake array on May 14 before being redetected along the Delaware coast on May 18, nearly a month before any other tag detections in the Delaware region, and

appearing to reach the Hudson River by June 16, 2013. Because the aberrant behavior of sharks 10847 and 10848 could not be compared with any other tagged sharks, detections of shark 10847 in the Savannah region and detections of shark 10848 after May 14, 2013 were excluded from further analysis.

Generally, the Chesapeake region recorded both the highest number of tag detections and the highest number of individual sharks (Figure 6). Detections within the Chesapeake array peaked in June and late August with a dip in both total detections and number of sharks from mid-July to mid-August. The Hatteras region detected the most sharks during the winter months, though damage to the array likely resulted in fewer detections recorded later in the year. Detections in the Delaware region overlapped with the timing of detections in the Chesapeake array, but tagged sharks appeared to arrive later and leave earlier in the year in the Delaware region. Only one individual shark was detected in the Delaware region.

The sensors attached to the acoustic tags provided 254 reliable depth recordings and 245 reliable temperature recordings. Overall, juvenile Sandbar Sharks were detected at depths between 1.32 and 29.02 m and between 13.04 and 27.95 °C water temperature. When sensor data were plotted by week, juvenile Sandbar Sharks appeared to occupy shallower depths and higher temperatures during the summer and fall (Figure 7).

Depth and temperature data transmitted by sensors did not show significant differences in these environmental factors between Age 1+ and YOY Sandbar Sharks, but females were detected at significantly deeper depths than males (Table 4). Tagged sharks were detected at significantly different depths in all three regions,

occurring at the shallowest depths in the Delaware region and the deepest depths in the Hatteras region (Table 5). Temperatures at which tagged sharks were detected were not significantly different between the Chesapeake and Delaware array regions, but were significantly lower than all other regions in the Hatteras array (Table 5).

Habitat Delineation

Based on the timing of tag detections in different regions, the months of January through March were classified as Winter, April through June as Spring Migration, July through September as Summer, and October through December as Fall Migration. Correlations between Chl-a and SST were negative during all seasons except summer, though the relationship was only statistically significant during winter (Table 6). The Hatteras region consistently showed the highest mean SST and lowest mean Chl-a regardless of season, but the highest Chl-a measurements within the Hatteras region occurred during winter (Table 7).

The best-fitting binary BRT model showed that SST contributed a slightly greater percentage of tree splits to the model than Chl-a (Table 8). The effect of SST on the probability of detecting a tagged Sandbar Shark increased in a stepwise fashion and comparison with detection data showed that each step represented a minimum cut point for a different season (Figure 8). Chl-a had a more consistent effect on Sandbar Shark detection probability across seasons, and the marginal effect plot showed minimum and maximum cut points associated with elevated tag detection probability (Figure 8).

Projected habitat maps showed that potential juvenile Sandbar Shark habitat was spatially restricted during winter to near shore Raleigh Bay, specifically the area between Ocracoke Inlet and Diamond Shoals off of Cape Hatteras (Figure 9). Potential spring and fall habitats covered the greatest spatial extent, but all projected habitats fell relatively near the coast. Summer habitat appeared to be mostly contained within estuaries and near shore Mid-Atlantic waters between Virginia Beach and Long Island, New York. During all seasons, potential habitat was projected near shore south of Cape Fear into South Carolina waters. Habitat was also projected within Pamlico Sound and other North Carolina estuaries during all seasons. The spatial extent of juvenile Sandbar Shark habitat fell within northern and near shore portions of the Mid-Atlantic shark closed area during winter, spring, and fall (Figure 9).

Discussion

The results of this study confirm that Raleigh Bay, particularly the northern extent between Ocracoke Inlet and Diamond Shoals, is important overwintering habitat for juvenile Sandbar Sharks. In addition, species of conservation concern (Dusky Shark, Scalloped Hammerhead, Thresher Shark) and important to local fisheries (Atlantic Sharpnose Shark, Smooth Dogfish, Spiny Dogfish) were present during the winter study period. All tagged juvenile Sandbar Sharks were using the area within detection range of the Hatteras array as overwintering habitat, and most tagged sharks returned to Chesapeake Bay, though one was also detected in

Delaware Bay and another appeared to travel south. Sensor tag data showed evidence for difference in habitat use patterns between winter and summer habitats, with tagged sharks occupying significantly cooler and deeper water in the Hatteras region. A combination of relatively warm temperature and high productivity appears to concentrate potential juvenile Sandbar Shark habitat within Raleigh Bay during the winter, which may make this area essential habitat for the species.

All coastal sharks captured during gillnet sampling were members of the winter shark assemblage observed by Jensen and Hopkins (2001) off of Cape Hatteras. Among the species documented within Raleigh Bay are Scalloped Hammerhead and Dusky Sharks, which recently have been discussed for protection under the U.S. Endangered Species Act (Miller et al. 2013, McCandless et al. 2014). The NMFS coastal shark bottom longline survey has consistently captured greater numbers of Dusky Sharks within the Mid-Atlantic Shark Closure Area along coastal North Carolina (NMFS 2003), particularly within or adjacent to Raleigh Bay, than other areas along the U.S. southeastern coast (McCandless et al. 2014). Other species encountered during gillnet sampling are important to fisheries: Atlantic Sharpnose Sharks are the most commonly-landed species in longline and gillnet fisheries targeting coastal sharks (NMFS 2015a) and Spiny and Smooth Dogfish support the highest-volume shark fisheries in the United States (NMFS 2015b). The Smooth Dogfish gillnet fishery is particularly active in North Carolina waters, which has consistently been one of the states with the highest landings in this fishery (SEDAR 2015). The overlap of species of high conservation concern with species

targeted by fisheries may create complications for the management of coastal shark and other local fisheries.

Though other efforts to track juvenile Sandbar Sharks have established the importance of coastal North Carolina as overwintering habitat (Grubbs et al. 2007, McCandless et al. 2007, Conrath and Musick 2008), this study is the first to use acoustic telemetry to track this species from its overwintering habitat to their summer nurseries. Tagging effort on Sandbar Sharks occurred only over the course of one day and a relatively low number were tagged within a small spatial area. Despite this, two estuaries known to function as summer habitat were represented among the tagged sharks, with another shark potentially associated with nursery habitats south of Cape Fear. This finding, paired with the unexpectedly high CPUE during February sampling, suggests that juvenile Sandbar Sharks originating from multiple summer nurseries concentrate within the same area south of Cape Hatteras during the winter. This may also explain observations by local fishermen of high Sandbar Shark abundance specifically associated with Diamond Shoals and the Hatteras Bight (Chapter 4, this volume).

Habitat use patterns inferred from sensor tags corroborate those observed by Conrath and Musick (2008), with tagged Sandbar Sharks occurring at greater depths and lower temperatures within overwintering habitat. Occurring at lower temperatures is expected during winter, and preference for deeper habitats may be represent selection the warmest part of the water column in an area with vertical thermal stratification. This potential explanation is supported by the fact that SST was generally lower than the water temperatures recorded by acoustic sensor tags.

Selection of specific parts of the water column is common among pelagic sharks, perhaps best exemplified by the Blue Shark (*Prionace glauca*), which moves vertically between surface and bathypelagic depths following prey and temperature fronts (Queiroz et al. 2012). The only significant difference in habitat use between demographic groups was an apparent preference for shallower depths among males, though this may be a result of a single male shark making up the majority of tag detections in the Delaware region.

Remote sensing data provided a potential explanation for why Raleigh Bay concentrates juvenile sandbar sharks from multiple summer habitats (Merson and Pratt, Jr. 2001, 2007, Grubbs et al. 2007, McCandless et al. 2007, Conrath and Musick 2008) as well as several other shark species (Jensen and Hopkins 2001, Jensen et al. 2002). Model results showed that juvenile sandbar shark presence could be predicted using SST and Chl-a measurements in combination, with sharks occurring in relatively warm water temperatures with high productivity. During winter, the significant negative correlation between SST and Chl-a may require trade-offs in habitat selection for juvenile Sandbar Sharks between water temperatures within thermal tolerance range and high productivity. However, though Chl-a measurements were still lower during winter in the Hatteras region than the other array regions, the highest Chl-a concentrations recorded in the Hatteras region occurred during winter. Spatial habitat delineation showed that potential juvenile Sandbar Shark winter habitat occurred within a relatively small spatial area associated with Cape Hatteras where SST measurements and Chl-a measurements over their respective minimum winter cut points overlapped. Near Cape Hatteras,

the proximity of the shelf break and Gulf Stream combine with wind direction to create areas of high productivity south of the Hatteras Bight during the winter (Lohrenz et al. 2002). Therefore the unique geography of Cape Hatteras allows overwintering juvenile Sandbar Sharks to mitigate the trade-off between temperature and productivity.

Habitat delineation also revealed a broad area within Onslow Bay between Cape Lookout and Cape Fear where potential habitat was consistently absent. Sandbar Sharks are known to use estuaries along the South Carolina coast as primary nurseries (Abel et al. 2007, Ulrich et al. 2007) and have been observed pupping within North Carolina waters (Jensen et al. 2002) but Sandbar Sharks from southern nurseries are not known to use Raleigh Bay as overwintering habitat. Only three Sandbar Sharks tagged in South Carolina waters have been recaptured off of North Carolina, all of which were captured during spring or fall months, and of these only one occurred near Cape Hatteras (Brian Frazier, SC Department of Natural Resources, personal communication). Some sharks tagged in northern summer habitats have been recaptured during summer months in areas south of Cape Fear, indicating that some juvenile Sandbar Sharks may remain in southern waters without migrating back to their primary nurseries (McCandless et al. 2007). Detection of one of this study's tagged sharks in the Herb River near Savannah, Georgia suggests that this may have occurred, though the removal of the Hatteras array following severe weather damage in May 2014 prevented confirmation of that shark's return to Hatteras the following winter. Habitat delineation results paired with evidence of a lack of a regular migratory connection from previous tagging

studies suggests that the broad area of presumably unsuitable habitat within Onslow Bay may isolate juvenile Sandbar Sharks overwintering off of Cape Hatteras from those using coastal South Carolina as primary nursery habitat. The possibility of resident juvenile Sandbar Sharks is also plausible, based on the delineation of potential habitat within Pamlico Sound and the presence of juveniles within estuarine North Carolina waters during summer months (Jensen et al. 2002, Chapters 2 and 3, this volume).

The choice of environmental parameters used to delineate juvenile Sandbar Shark habitat was driven by the availability of equipment as well as standard measures used in other studies on highly migratory marine species (e.g. Block et al. 2011). While cholorphyll-a concentrations are more directly associated with planktivorous species (Curtis et al. 2014), satellite telemetry data have also shown that high trophic level predators are often associated with this feature (Block et al. 2011). Chlorophyll-a concentrations are indicative of high primary productivity and phytoplankton abundance, which can support higher trophic levels and ultimately provide food sources for predators (Block et al. 2011, Friedland et al. 2012). Delineating habitat based solely on SST and Chl-a proved to be effective and provided realistic and intuitive results, but other environmental factors likely play an important role. Salinity can be an especially important component of habitat selection by sharks in near shore and estuarine environments (Abel et al. 2007, Grubbs and Musick 2007, Froeschke et al. 2010, Chapter 2, this volume), but satellite-based remote sensing of sea surface salinity has only recently become available (Reul et al. 2013). The indirect effect of salinity may be observable

through the maximum Chl-a cut point, as the highest Chl-a concentrations tended to occur in the upper reaches of estuaries and estuarine Sandbar Shark habitat is limited by salinity (Grubbs and Musick 2007, see also Chapter 2, this volume).

All of these environmental factors serve to concentrate juvenile Sandbar Sharks within winter habitat near Cape Hatteras, with important implications for area fisheries. Fishermen operating in this area report that juvenile Sandbar Sharks can be abundant to the point of becoming a nuisance, and because this species is prohibited outside of the limited-access research fishery, encounters with large numbers of juvenile Sandbar Sharks represent lost time (Chapter 4, this volume). In addition, the Mid-Atlantic shark closed area effectively prohibits the use of bottom longline gear in North Carolina ocean waters for half the year (NMFS 2003). Potential habitat was delineated near shore and confined to the northern portions of the closed area, suggesting that portions of the bottom longline closed area south of Cape Lookout may not contribute as much protection for juvenile Sandbar Sharks. However, the extent of essential habitat for other species of concern such as Dusky and Scalloped Hammerhead Sharks should be assessed before any changes to the Mid-Atlantic shark closed are made.

Boosted regression tree models have a number of advantages in ecological applications, including the ability to handle multiple data types and distributions, the ability to provide both explanatory and predictive results, and greater predictive power through the fitting of multiple trees (Elith et al. 2008). Regression tree analysis was used by Grubbs and Musick (2007) to delineate juvenile Sandbar Shark habitat within Chesapeake Bay based on environmental cut points between high and

low shark CPUE. Boosted trees have been used to identify habitat for coastal sharks within Texas estuaries (Froeschke et al. 2010) and skates in the Irish Sea (Dedman et al. 2015) by interpolating predicted presence or abundance from the model results across the study area. Due to the limited spatial coverage by the acoustic arrays used to detect tag sharks and the desire to identify potential habitat outside of the coverage area of the arrays, these approaches were combined by using cut points from the boosted trees to fill in areas of habitat falling within environmental ranges of high likelihood of shark presence. This method provided the broadest coverage for assessing potential habitat but was also dependent on the accuracy and completeness of the satellite data layers. If shark presence/abundance data points have a more even spatial distribution it may be more appropriate to interpolate predictions of shark presence or abundance rather than rely on the resolution of the environmental data.

An important caveat is that shark habitat was delineated based on tag detections on arrays that were primarily distributed north of Cape Hatteras. Only one array south of Cape Hatteras detected any tagged sharks. Delineated habitat may represent the preferred habitat ranges for Sandbar Sharks originating from northern primary nurseries and may not reflect environmental ranges preferred by sharks in southern nurseries. Acoustic tagging of juvenile Sandbar Sharks from nurseries in South Carolina and Georgia could be used to illuminate the full extent of their migrations and identify differences in environmental preferences between juveniles from primary nurseries north and south of Cape Hatteras.

In the open ocean, predator movements are often driven by trade-offs between temperature and productivity (Block et al. 2011, Queiroz et al. 2012, Hazen et al. 2012) and our results suggest similar motivations for highly migratory coastal species such as Sandbar Sharks. The spatial extent of potential habitat for juvenile Sandbar Sharks seems to be restricted to an area between Ocracoke Inlet and the Hatteras Bight during the winter, which means sharks in this area are likely more concentrated and possibly more vulnerable to exploitation or environmental degradation (Knip et al. 2010). These results reinforce the importance of northern Raleigh Bay and Cape Hatteras as overwintering habitat for juvenile Sandbar Sharks, and potentially other species as well. The potential effects of fishery management and marine spatial planning policies on shark populations overwintering in this area should be carefully considered.

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Tables

Table 1-1. Mean environmental conditions by month during 2013 gillnet sampling between Cape Hatteras and Ocracoke Inlet, North Carolina.

	Mean ± SD		
Environmental Variable	February	April	November
N sets	10	4	4
Temperature (°C)	14.66 ± 0.99	10.63 ± 0.22	18.46 ± 0.44
Salinity (ppt)	34.79 ± 0.51	31.36 ± 0.19	33.39 ± 0.37
Dissolved oxygen (mg/L)	13.22 ± 3.51	11.35 ± 1.10	8.03 ± 0.16
Depth (m)	18.65 ± 2.74*	13.30 ± 5.03	19.66 ± 4.48

* Depth only recorded at 7 stations in February

Table 1-2. Number (N), total length (TL), and environmental measurements (mean ± SD) for shark species captured during 2013 gillnet sampling near Cape Hatteras.

Species	N	TL (mm)	Temp (°C)	Salinity (ppt)	DO (mg/L)	Depth (m)
Atlantic Sharpnose Shark	16	959.63 ± 30.27	17.32 ± 1.85	34.31 ± 1.04	11.06 ± 4.66	22.20 ± 1.46
Dusky Shark	3	1213.33 ± 72.34	16.17 ± 2.15	34.53 ± 0.57	11.72 ± 4.77	19.48 ± 5.65
Sandbar Shark	196	808.54 ± 77.73	14.88 ± 0.70	35.08 ± 0.64	16.73 ± 2.59	19.21 ± 3.32
Scalloped Hammerhead	1	1854.00	15.70	35.90	18.70	
Smooth Dogfish	11	1074.09 ± 63.60	16.80 ± 3.27	33.08 ± 0.96	9.17 ± 2.81	18.10 ± 2.46
Spiny Dogfish	63	870.63 ± 41.00	10.51 ± 0.18	31.23 ± 0.16	10.78 ± 0.73	10.10 ± 4.87
Thresher Shark	2	1803.50 ± 215.67	10.70 ± 0.28	31.35 ± 0.07	10.24 ± 0.01	15.39 ±1.07

Table 1-3. Transmitter ID number, species, tagging date, sex, and total length (TL) for coastal sharks tagged with acoustic transmitters during 2013 gillnet sampling near Cape Hatteras.

Transmitter ID	Species	Tagging date	Sex	TL (mm)
10849	Dusky shark	2/15/13	F	1250
10831	Dusky shark	11/17/13	M	1260
10853	Sandbar shark	2/15/13	M	879
10851	Sandbar shark	2/15/13	M	869
10852	Sandbar shark	2/15/13	M	725
10854	Sandbar shark	2/15/13	F	792
10850	Sandbar shark	2/15/13	M	907
10847	Sandbar shark	2/15/13	F	780
10848	Sandbar shark	2/15/13	F	756
10840	Sandbar shark	2/15/13	M	705
10841	Sandbar shark	2/15/13	M	741
10832	Sandbar shark	2/15/13	M	735
10833	Sandbar shark	2/15/13	F	749
10835	Sandbar shark	2/15/13	F	610
10834	Sandbar shark	2/15/13	F	712
10836	Sandbar shark	2/15/13	M	721
10827	Sandbar shark	2/15/13	F	772

Table 1-4. ANOVA results comparing mean depth and temperature recorded by sensor tags between Age 1+ and YOY and between female and male juvenile Sandbar Sharks.

	Mean ± SD		ANOVA	
Age Class	Age 1+	YOY	*F*	*p*
N	27	227		
Depth (m)	11.58 ± 5.93	13.07 ± 5.37	1.81	0.18
N	29	216		
Temperature (°C)	23.38 ± 2.49	22.85 ± 2.92	0.86	0.35
Sex	Female	Male	*F*	*p*
N	197	57		
Depth (m)	13.94 ± 4.81	9.36 ± 6.00	35.66	<0.0001
N	198	47		
Temperature (°C)	22.91 ± 2.71	22.95 ± 3.52	0.01	0.92

Table 1-5. ANOVA and Tukey's Honestly Significant Difference results comparing depth and temperature data recorded by sensors attached to acoustic tags carried by juvenile Sandbar Sharks.

		Depth (m)			Temperature (°C)	
Region	N	Mean ± SD	Connecting Letters	N	Mean ± SD	Connecting Letters
Delaware	14	4.52 ± 2.31	C	12	23.15 ± 4.78	A
Chesapeake	231	13.06 ± 4.91	B	223	23.22 ± 2.32	A
Hatteras	9	21.98 ± 4.20	A	10	15.85 ± 2.45	B
F		37.77			42.03	
p		<0.0001			<0.0001	

Table 1-6. ANOVA and linear regression results determining significance and direction of seasonal relationships between chlorophyll-a concentrations (Chl-a) and sea surface temperature (SST) recorded by the MODIS satellite at ACT Network receiver sites along the U.S. East Coast in 2013-2014.

Chl-a vs. SST	ANOVA		Regression	
Season	*F*	*p*	Equation	r^2
Winter	27.40	<0.0001	Chl-a = 10.58 – 0.54(SST)	0.085
Spring Migration	1.25	0.26	Chl-a = 13.43 - 0.11(SST)	0.004
Summer	0.17	0.68	Chl-a = 9.37 + 0.07(SST)	0.0006
Fall Migration	0.22	0.64	Chl-a = 7.25 – 0.04(SST)	0.002

Table 1-7. Mean temperature (SST) and chlorophyll-a (Chl-a) recorded by MODIS within each array region by season, with ANOVA results comparing mean values between regions and seasons.

	SST (Mean ± SD)			ANOVA (Region)	
Season	Chesapeake	Delaware	Hatteras	*F*	*p*
Winter	6.34 ± 2.43	4.21 ± 2.32	10.87 ± 3.15	59.41	<0.0001
Spring Migration	16.94 ± 4.37	13.99 ± 4.86	18.90 ± 3.15	18.29	<0.0001
Summer	21.59 ± 4.03	20.60 ± 4.80	25.13 ± 2.52	9.58	<0.0001
Fall Migration	19.79 ± 3.60	15.83 ± 6.12	16.79 ± 3.28	9.68	0.0001
F (Season)	681.26	173.69	76.25		
p (Season)	<0.0001	<0.0001	<0.0001		
	Chl-a (Mean ± SD				
Winter	6.93 ± 4.68	9.69 ± 6.54	2.72 ± 0.73	16.14	<0.0001
Spring Migration	11.08 ± 5.48	16.62 ± 11.52	1.26 ±0.81	44.92	<0.0001
Summer	10.07 ± 5.19	15.66 ± 16.60	0.74 ± 0.41	24.10	<0.0001
Fall Migration	5.60 ± 2.37	10.48 ± 6.40	1.44 ± 0.17	25.79	<0.0001
F (Season)	42.46	5.59	35.87		
p (Season)	<0.0001	0.001	<0.0001		

Table 1-8. Boosted Regression Tree results assessing the contribution of Chlorophyll-a concentrations (Chl-a) and sea surface temperature (SST) on the presence of tagged juvenile Sandbar Sharks by season, including learning rate (lr), bag fraction (bf), and percentage of tree splits contributed by each environmental factor.

Parameter	Value
lr	0.001
bf	0.5
N trees	6550
SST contribution (% splits)	58.57
Chl-a contribution (% splits)	41.63

Figures

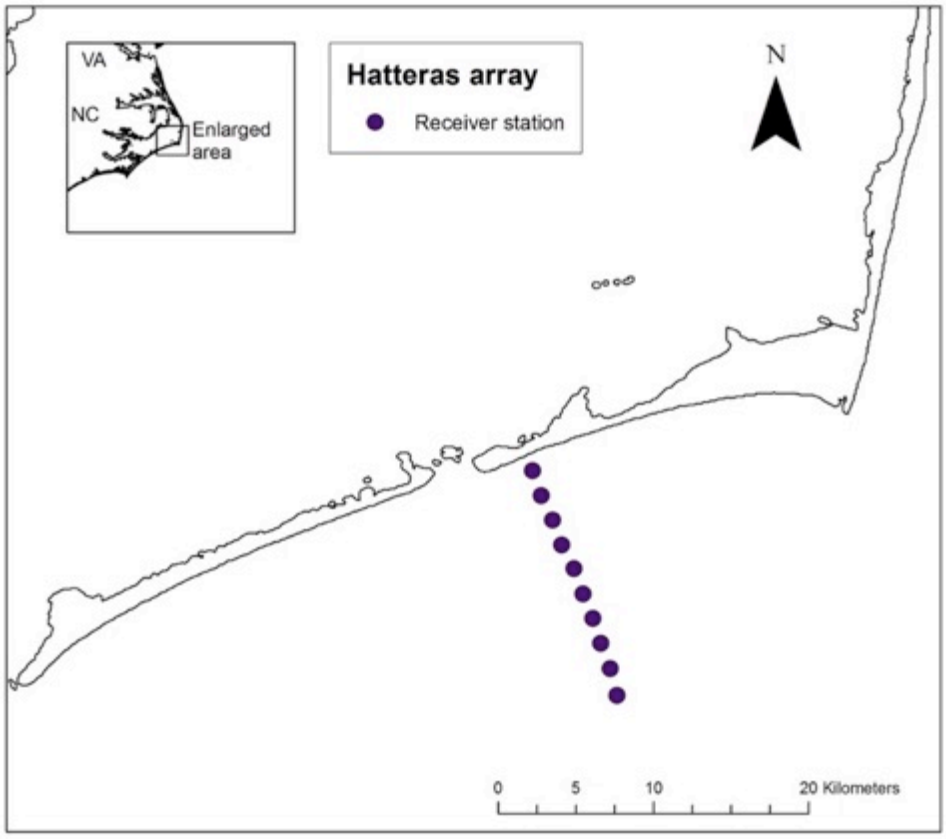

Figure 1-1. Acoustic receiver locations in the Cape Hatteras Array.

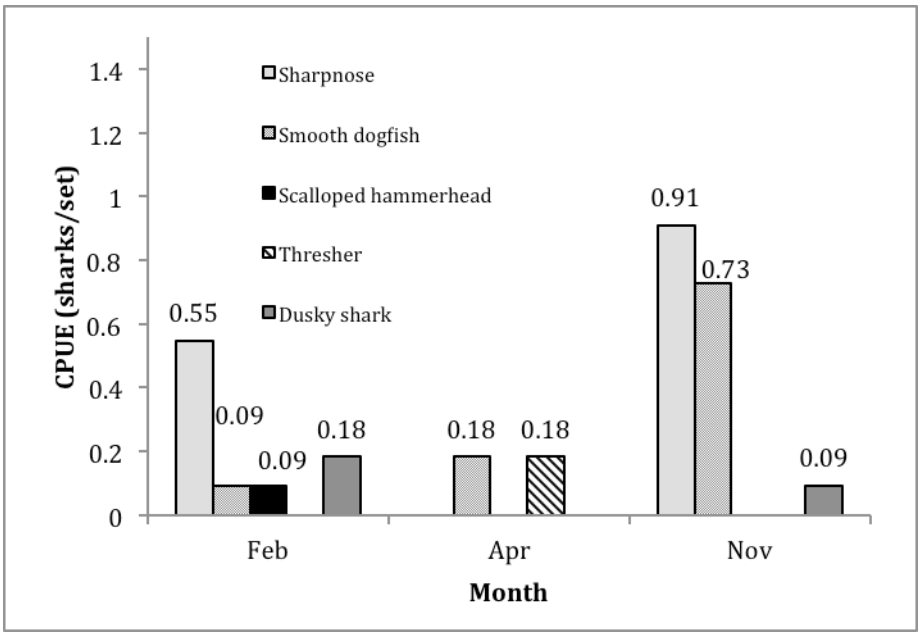

Figure 1-2. Catch per unit effort (CPUE, sharks/set) by month for shark species other than Sandbar Shark (CPUE = 17.81, February only) and Spiny Dogfish (CPUE = 15.57, April only) captured during 2013 gillnet sampling near Cape Hatteras.

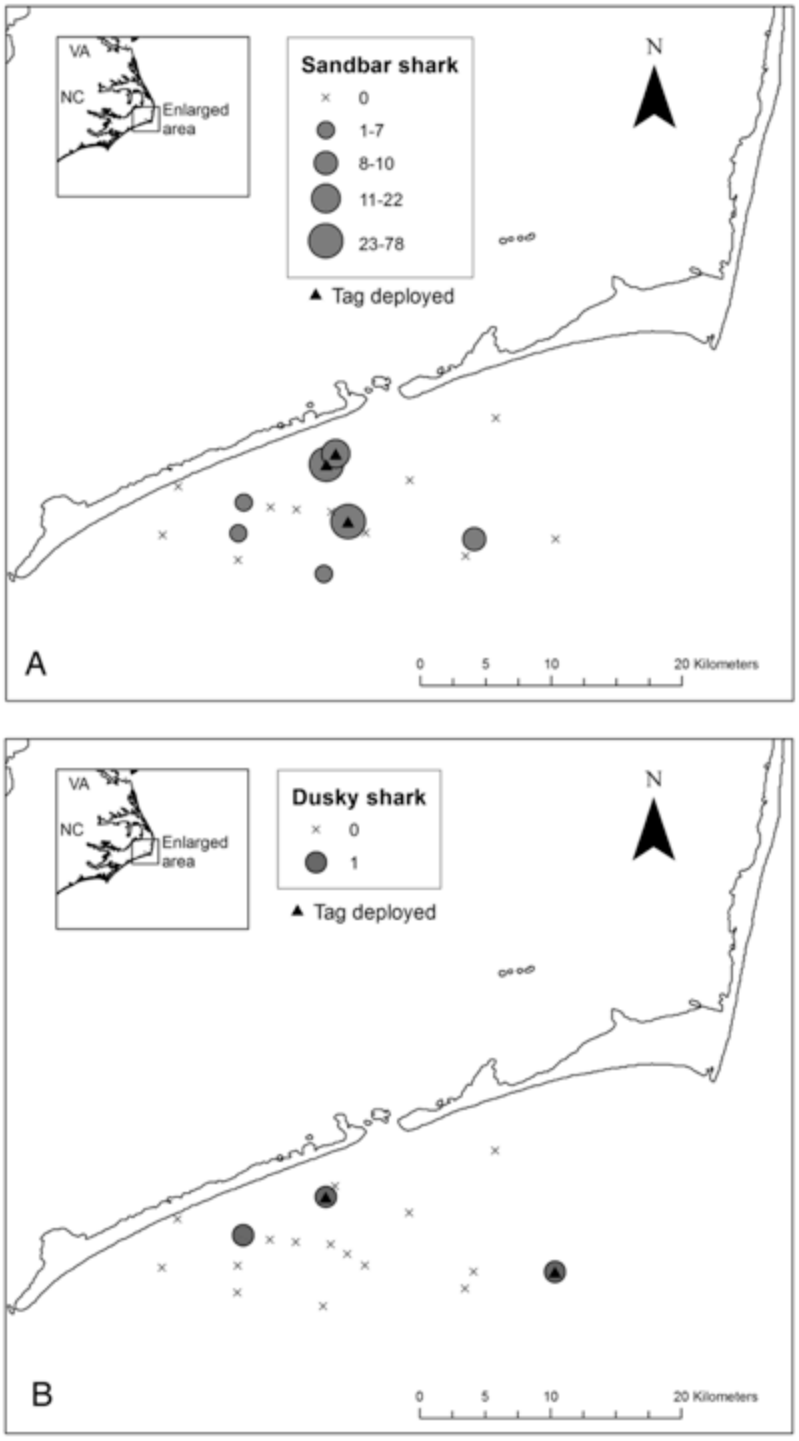

Figure 1-3. Capture and tag release locations for A.) Sandbar and B.) Dusky Sharks captured during 2013 gillnet sampling near Cape Hatteras.

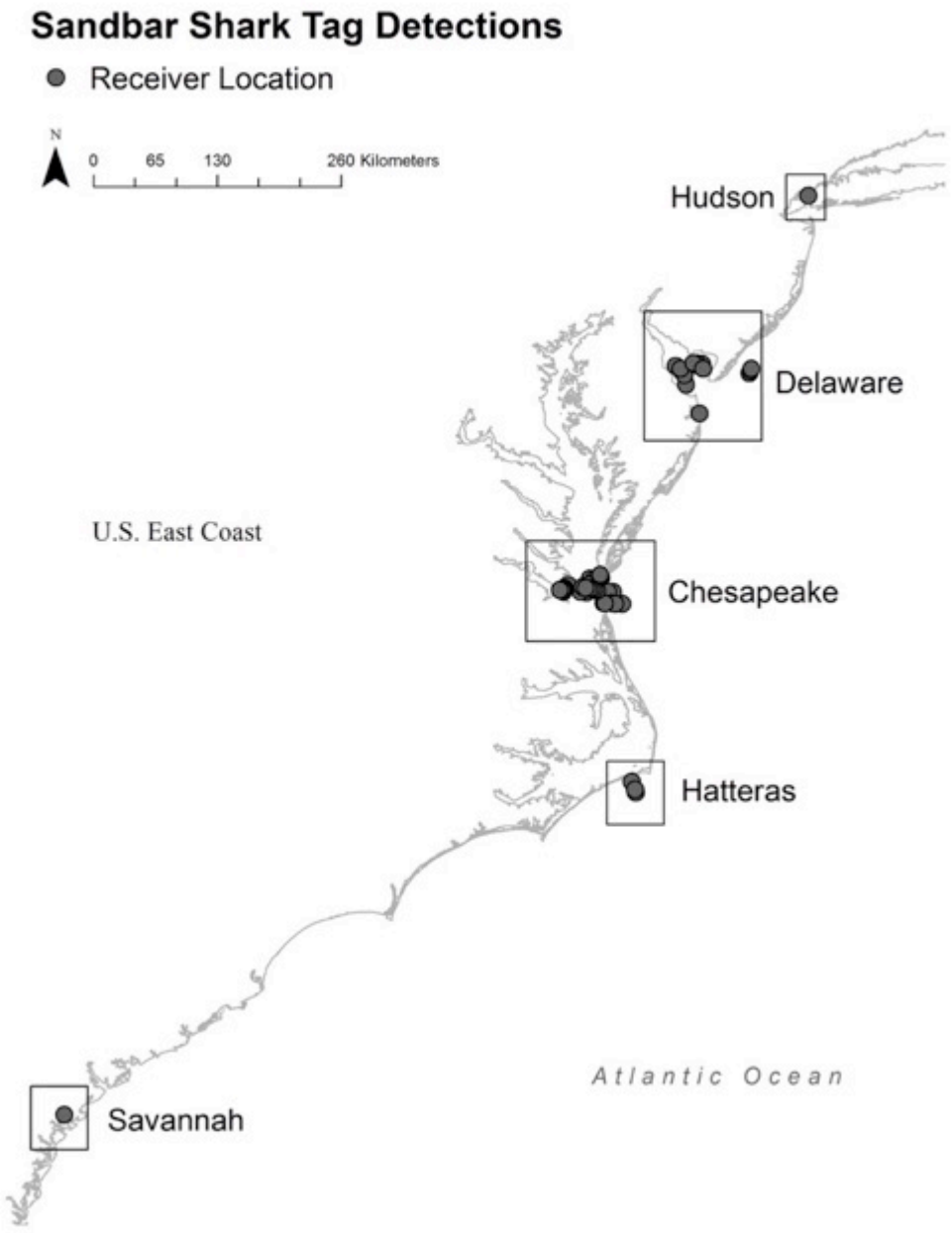

Figure 1-4. Tag detection locations of juvenile Sandbar Sharks tagged off of Cape Hatteras in February 2013 and detected between February 2013 and October 2014, with corresponding array regions.

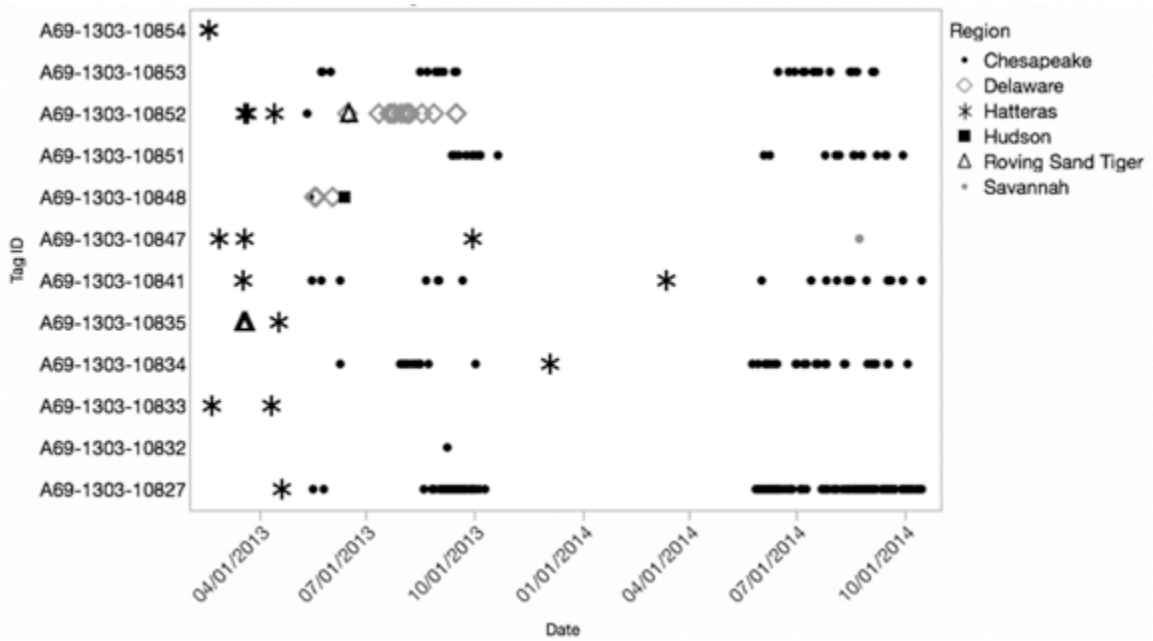

Figure 1-5. Detections of individual tagged juvenile Sandbar Sharks by date and array region.

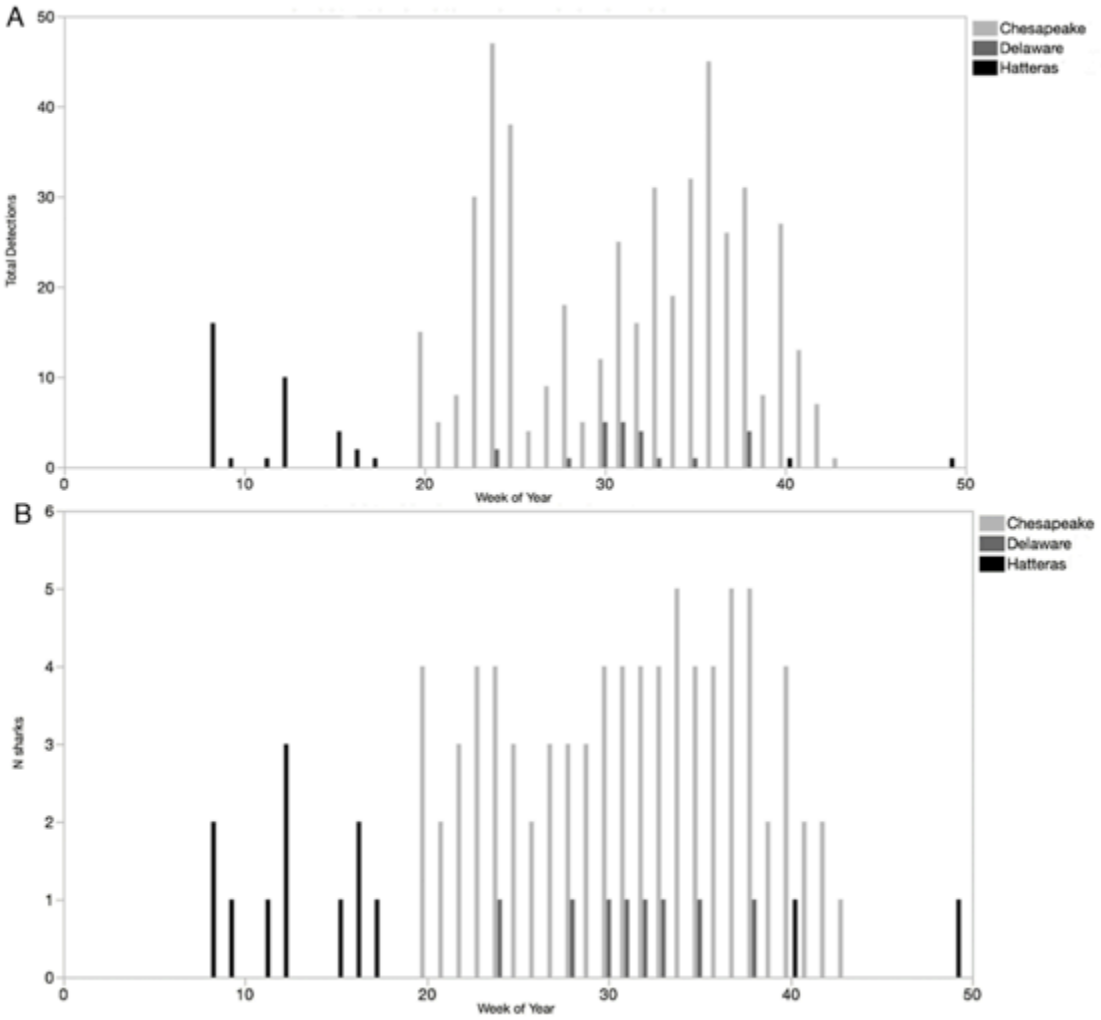

Figure 1-6. A.) Total number of detections and B.) total number of individual tagged Sandbar Sharks in the Chesapeake, Delaware, and Hatteras array regions by week of the year.

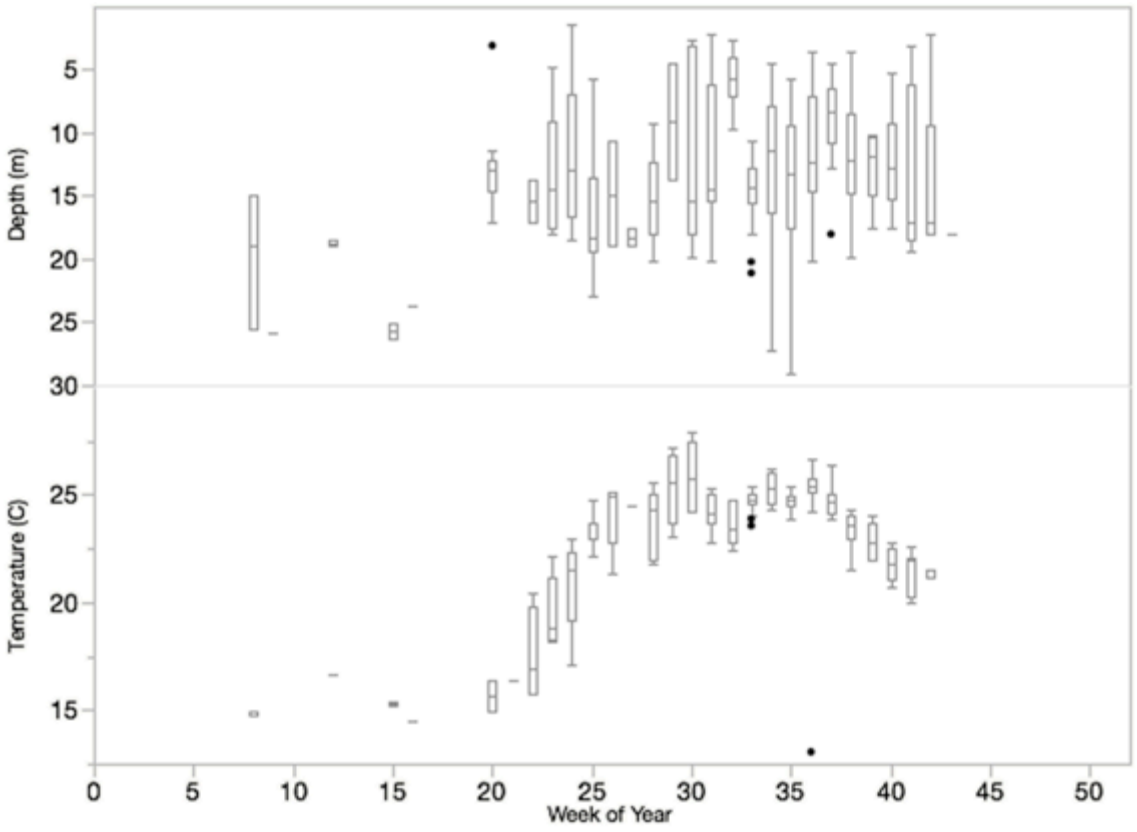

Figure 1-7. Box plots of depth (m) and temperature (°C) data from pressure and temperature sensors attached to acoustic tags deployed on juvenile Sandbar Sharks.

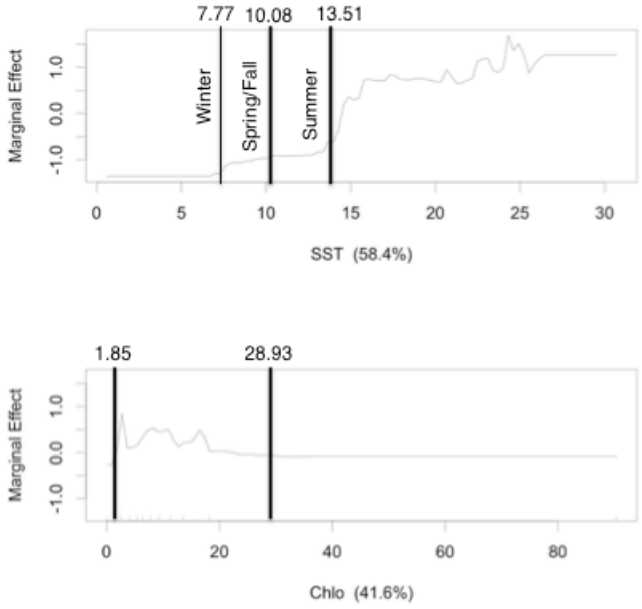

Figure 1-8. Marginal effect plots showing the effect of sea surface temperature (SST, °C) and chlorophyll a concentrations (Chl-a, mg/m^3) on the probability of detecting a tagged Sandbar Shark with cut point locations and values.

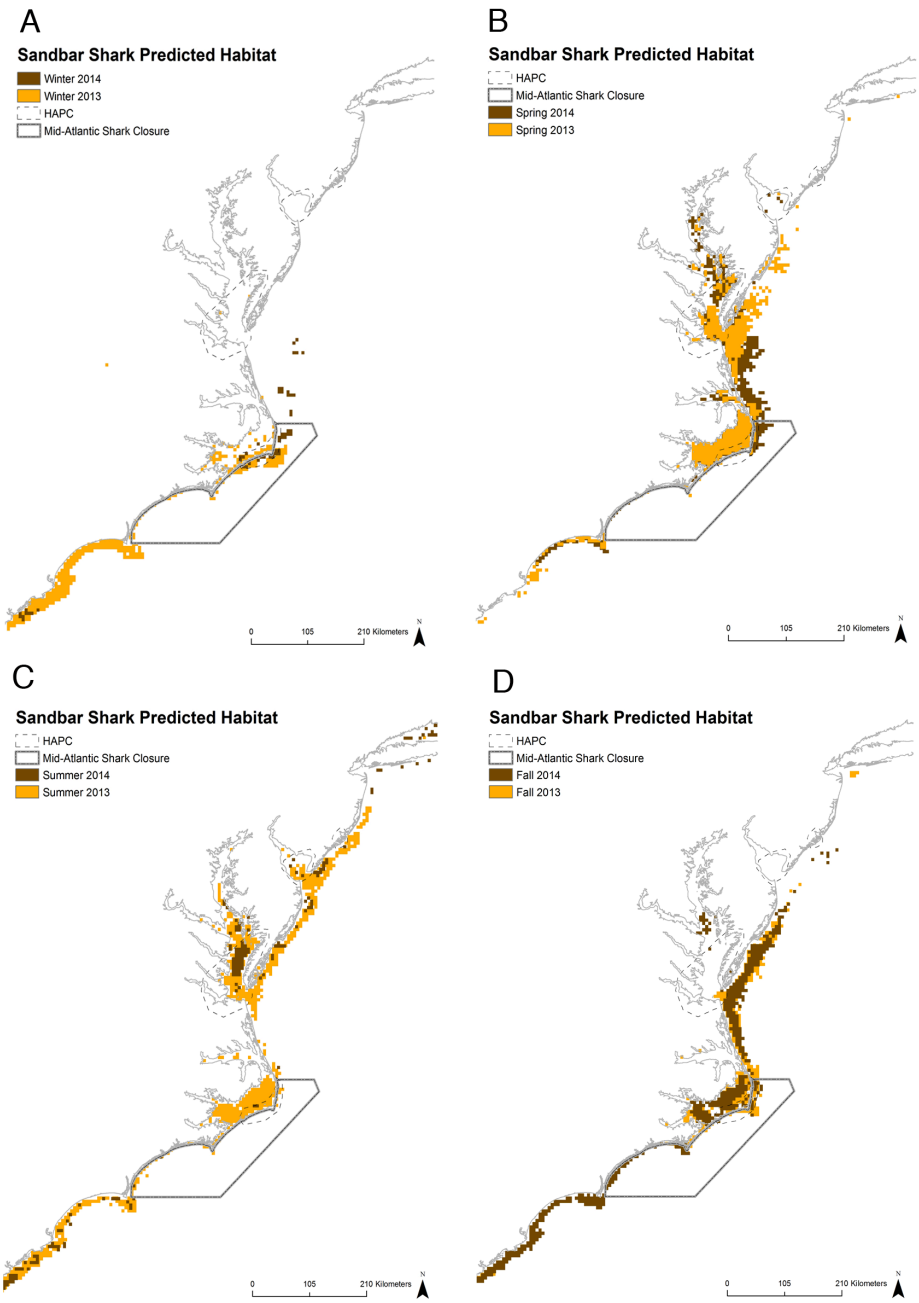

Figure 1-9. Projected juvenile Sandbar Shark habitat based on BRT cut points of chlorophyll-a concentration (Chl-a, mg/m^3) and sea surface temperature (SST, °C) between high and low probability of shark presence during A.) winter, B.) spring migration, C.) summer, and D.) fall migration.

Chapter 2

Delineation and Mapping of Coastal Shark Habitat within Pamlico Sound, North Carolina

Abstract

Estuaries function as important nursery and foraging habitat for many coastal species, including highly migratory sharks. Pamlico Sound, North Carolina, is one of the largest estuaries in the continental United States and potentially provides a variety of potential habitats for sharks. In order to identify and spatially delineate shark habitat within Pamlico Sound, shark catch and environmental data from the 2007-2014 North Carolina Division of Marine Fisheries gillnet and longline surveys within the estuary were analyzed. Principle species in the Pamlico Sound shark assemblage were identified and environmental data recorded at survey sites (depth, temperature, salinity, dissolved oxygen, SAV distance, and inlet distance) were interpolated across Pamlico Sound to create seasonal environmental grids with a 90-m^2 cell size. Boosted regression tree analysis was used to identify the most important environmental factors and ranges associated with presence and abundance of each principle species, and the resulting models were used to predict shark abundance based on the values within the environmental grid cells. The Atlantic Sharpnose Shark (*Rhizoprionodon terraenovae*), Blacktip Shark (*Carcharhinus limbatus*), Bull Shark (*Carcharhinus leucas*), Sandbar Shark (*Carcharhinus plumbeus*), Smooth Dogfish (*Mustelus canis*), and Spiny Dogfish

(*Squalus acanthias*) were the principle species in Pamlico Sound. Most species were associated with proximity to the inlet or high salinity and warm temperatures, but the Bull Shark preferred greater inlet distances and the Spiny Dogfish preferred lower temperatures than the other species. Extensive Smooth Dogfish habitat overlap with seagrass beds suggests that this habitat type may be a critical part of nursery habitat for this species. Spatial delineation of shark habitat within the estuary will allow for better assessment of potential overlap and interactions with other species.

Introduction

Estuaries often function as important habitat for marine species, though the dynamic nature of the transition from freshwater to saltwater has a significant affect on habitat use and availability (Attrill and Rundle 2002). Within these environments, habitat selection and use patterns are influenced by abiotic factors such as temperature and salinity (Dunson and Travis 1991) and biotic factors such as food availability and predation risk (Lipcius and Hines 1986, Main 1987). Often abiotic and biotic factors interact, such as in cases where a species may select habitat outside the preferred environmental ranges of its predators (Paterson and Whitfield 2000, Ross 2003) or competitors (Ross and Url 1986).

Though often associated with juvenile teleosts and invertebrates, estuaries also function as nursery and foraging habitats for coastal sharks (McCandless et al. 2007). Like other species, sharks likely select estuarine habitats based on a

combination of abiotic environmental tolerances and biotic factors such as prey availability, predator avoidance, and competition (Heithaus 2007). Generally sharks appear to select habitat that minimizes predation risk or competition (Heupel and Simpfendorfer 2011, Guttridge et al. 2012) though this can come at the expense of foraging opportunities (Bush and Holland 2002, Heupel and Hueter 2002). However, other species occur in habitats with far greater predation risk, seeming to prioritize feeding over protection (Carlson et al. 2008). Most studies on shark habitat use within estuaries have focused on juveniles or small-bodied species, but the ecological role of sharks can change dramatically with increasing size (Heupel et al. 2014). Large, apex predatory sharks can exert direct and indirect top-down affects that may have observable landscape effects on estuarine habitats (Heithaus et al. 2012, Burkholder et al. 2013) and influence the habitat use patterns of other predators, including other elasmobranchs (Heithaus 2001, Vaudo and Heithaus 2013). Even juvenile or small-bodied sharks can occupy similar trophic niches to high-trophic level teleosts and other estuarine predators, possibly functioning as competitors or predators of species of value to fisheries (Buchheister and Latour 2015, Shaw et al. 2016).

Identification of important shark habitat is complicated by their generally highly mobile nature (Speed et al. 2010). However, recurrent site fidelity to specific areas or habitats is widespread even among highly migratory shark species (Hueter et al. 2005, Chapman et al. 2015). Fidelity to nearshore or estuarine habitats can concentrate otherwise wide-ranging sharks into areas with an increased exposure to anthropogenic affects such as fishing pressure, pollution, and coastal

development (Hueter et al. 2005, Knip et al. 2010). Seasonal or regular presence of sharks in an estuary can also cause changes in the behavior and distribution of other species within the system with possible cascading ecological effects (Dill et al. 2003). Therefore identifying and delineating estuarine shark habitat is important to both managing shark populations and broader ecosystem-based management.

Pamlico Sound, North Carolina's largest estuary and the largest barrier island lagoon system in the United States, potentially includes a wide variety of potential shark habitats. Pamlico Sound has the greatest proportion of open water of any U.S. barrier island estuary, though average depth is relatively shallow in comparison to other large estuaries (Hayden and Dolan 1979). High salinity water enters the system from the southeast through Hatteras Inlet, Ocracoke Inlet, and Core Sound and exits in the northern extent of the estuary through Oregon Inlet (Roelofs and Bumpus 1953). Fresh water flows into the estuary from the west through the Neuse and Pamlico Rivers, and also enters from Albemarle Sound north of Oregon Inlet. This creates a horizontal salinity ecocline with salinity increasing west to east (Roelofs and Bumpus 1953). Pamlico Sound is situated at a biogeographic break, delineated at Cape Hatteras, between temperate and subtropical marine ecosystems (Hayden et al. 1984, Fautin et al. 2010). Due to its biogeographic position, Pamlico Sound encompasses the majority of the range overlap between the temperate seagrass *Zostera marina* and the subtropical seagrass *Halodule wrightii*, which combine to give the estuary one of the greatest areas of seagrass habitat on the U.S. east coast (Ferguson et al. 1993, Fonseca and Uhrin 2009). Pamlico Sound is

uniquely situated to support a diverse shark community that varies both spatially and seasonally.

Though biotic factors such as prey availability and competitive exclusion can be important in determining the distribution and habitat use behavior of sharks in an estuary, delineating habitat based on spatial and abiotic environmental factors is likely more tractable and more easily applied to management applications (Grubbs and Musick 2007). Here we use fishery-independent survey data to describe the coastal shark community within Pamlico Sound, determine the environmental preferences of the principle species in this community, and delineate the spatial extent of potential estuarine habitat.

Methods

Shark catch and environmental data were obtained from the North Carolina Division of Marine Fisheries (NCDMF) fishery-independent gillnet and longline surveys conducted within Pamlico Sound. The study period ranged from 2007 through 2014 for both surveys. The gillnet survey has been conducted since 2001, but only 2007-2014 data were used to match temporal coverage with the longline survey. The total combined area of the surveys covered the east side of the sound from Oregon Inlet to the entrance of Core Sound near Portsmouth Island, and the west side of the sound from Stumpy Point to the entrance of Core Sound near Cedar Island including the estuarine portions of the Neuse and Pamlico Rivers (Figure 1). Set locations were selected using a stratified-random sampling design in which the

total study area from the shoreline to approximately the 2 m depth contour was divided into eight substrata, then further divided into 1.85 by 1.85-km cells that were chosen at random before each month of sampling. Effort was composed of 619-624 sets per year for the gillnet survey and 72-137 sets per year for the longline survey, though effort was standardized at 72 sets per year starting in 2011 for the longline survey. Gillnet sampling was conducted February-December of each year, while longline sampling was conducted July-November. Gillnet gear was composed of a 219.46-m sink gillnet made up of eight 27.43-m sections of 7.62, 8.89, 10.16, 11.43, 12.70, 13.97, 15.24, and 16.51-cm stretched mesh soaked for 12 hours from sunset to sunrise. Longline gear consisted of a 1500-m mainline with 100 gangions attached at 15-m intervals, each of which was made up of 0.7 m of 91 kg test monofilament and a 15/0 circle hook baited with locally available bait fish, which was allowed to soak for 30 minutes per set.

All captured sharks were identified to species and total length (TL, mm) and sex were recorded. At each station, depth (m), water temperature (°C), salinity (ppt), and dissolved oxygen concentration (mg/L) were recorded during prior to setting gear. Straight-line distances from the nearest inlet (km) and nearest seagrass bed (m) were measured for each set location using the spatial join function in ArcGIS 10.1 (ESRI). Locations and spatial extents of seagrass beds were taken from maps generated using data collected from aerial and boat-based surveys conducted by the Albemarle-Pamlico National Estuarine Partnership (APNEP) (Kenworthy et al. 2012).

All species with more than 40 individuals captured during the survey period were designated as principle species for further analysis. Both sets of catch data combined were assumed to provide equal catchability for each species across Pamlico Sound. Shark catch per unit effort (CPUE) was standardized as sharks per set (sharks/set) and plotted by year, month, and season. Seasons were designated by calendar date: December 1-February 29 was classified as Winter, March 1-April 15 as Early Spring, April 16-May 31 as Late Spring, June 1-August 30 as Summer, September 1-October 15 as Early Fall, and October 16-November 31 as Late Fall. Seasons with the highest CPUE for each species were noted for habitat mapping. In order to determine the possibility of habitat overlap, discriminant function analysis (DFA) was used to classify the principle species using the six environmental factors (depth, temperature, salinity, dissolved oxygen, seagrass distance, and inlet distance). Species either in close proximity on the canonical plot or frequently misclassified as each other were considered to have overlapping habitat preferences.

Habitat delineation followed the approach used by Froeschke et al. (2010) and Dedman et al. (2015), in which boosted regression tree (BRT) analysis was used to predict presence and abundance based on environmental factors, which was then mapped spatially. BRT analysis was used because it provides a number of advantages in modeling habitat in data-poor situations such as highly zero-inflated CPUE data common in elasmobranch surveys, while reducing the variance that may bias habitat delineation using single regression trees (Elith et al. 2008, Dedman et al. 2015). Habitat delineation proceeded in two stages: first, relationships between

environmental factors and species presence/abundance were identified using BRTs, then these relationships were used to predict potential abundance throughout the entire area of Pamlico Sound covered by NCDMF surveys. To accomplish this, the function "gbm.auto" was used to automate several functions of the package "dismo" in R.

Prior to BRT analysis, temperature, salinity, dissolved oxygen, SAV distance, and inlet distance measurements at NCDMF gillnet and longline stations were interpolated across Pamlico Sound using Bayesian Empirical Kriging in ArcGIS 10.1. Temperature, salinity, and dissolved oxygen were expected to vary by season so these environmental layers were interpolated for each season. These layers were then converted to raster grids with a 90-m^2 cell size. Depth was represented by a raster of bathymetry data within Pamlico Sound and the lower Neuse and Pamlico Rivers made from data collected by the U.S. Geological Survey (USGS). Data from environmental rasters were extracted and combined into seasonal .csv files for import into R during BRT modeling. At this stage multiple linear correlation analysis was used to identify any strong autocorrelations between environmental factors.

In R, the "gbm.auto" function automatically generated trees using machine learning to reduce variance by selecting the most common splits between presence/absence and low/high abundance. Binary presence/absence trees used data from all sets to model the likelihood of at the species being captured, while Gaussian abundance trees used log-transformed abundance data from sets in which the species was present. Abundance predictions were then back-transformed and

multiplied by the predicted presence likelihoods to give predicted abundance values that incorporated the likelihood of the species being present. In addition, the function generated marginal effect plots for each environmental factor showing the positive or negative effect of that factor on the presence or abundance of the species, as well as the contribution of that factor to the overall model based on the percentage of tree splits associated with it. Often it was necessary to repeat this procedure multiple times for each species to determine the learning rate (lr) and bag fraction (bf) that would allow both the binary and Gaussian models to proceed.

After BRT analysis, the predictor model was applied to the environmental raster grid data from the season in which the species was most abundant, which created predicted abundance values based on the environmental factors in each grid cell. These data were then imported back into ArcGIS and interpolated using Bayesian Empirical Kriging to depict the predicted abundance for each principle species within Pamlico Sound.

Results

A total of 2,408 sharks were captured from 2007-2014 in NCDMF surveys within Pamlico Sound. In all, 11 species were represented with an additional 44 individuals classified as either unknown or misidentified Carcharhinidae (Table 1). Misidentified sharks were designated based on mismatches between the measured TL and published size ranges for the recorded species name. Individuals within neonatal size range were present among Atlantic Sharpnose Sharks (*Rhizoprionodon*

terraenovae), Bull Sharks (*Carcharhinus leucas*), Finetooth Sharks (*Carcharhinus isodon*), Sandbar Sharks (*Carcharhinus plumbeus*), Smooth Dogfish (*Mustelus canis*), and Spinner Sharks (*Carcharhinus brevipinna*). The Atlantic Sharpnose Shark, Blacktip Shark (*Carcharhinus limbatus*), Bull Shark, Sandbar Shark, Smooth Dogfish, and Spiny Dogfish (*Squalus acanthias*) were designated as the principle species for further analysis (Table 1).

Highest overall CPUE was found for the Smooth Dogfish, which was also the only species recorded during all seasons (Figure 2). The Spiny Dogfish was the most abundant species during winter and early spring months, while peak Smooth Dogfish CPUE peaked in early spring and summer. All other shark species were absent during the winter and most abundant during the summer with the exception of the Sandbar Shark, which was captured most often during early and late fall. Catches of Smooth Dogfish and Atlantic Sharpnose Sharks were higher during the first half of the survey period, while the majority of Bull Sharks were captured after 2011 (Figure 2).

Results of the DFA showed 80.53 % correct classification for the six principle shark species based on environmental factors (Wilks' Lambda = 0.163, $F = 74.87$, df = 66, $p < 0.0001$). Canonicals 1 and 2 explained 94.1 % of the variation (Table 2). Canonical 1 showed strong positive correlations with temperature and salinity and a strong negative correlation with dissolved oxygen, while canonical 2 showed a strong positive correlation with SAV distance (Table 2). The canonical plot showed an obvious preference for lower temperatures among Spiny Dogfish and greater SAV distance among Bull Sharks, while the other species appeared to cluster

together (Figure 3). Spiny Dogfish showed the highest percentage of correct classification (92.2 %), followed by Bull Sharks (91.7 %) and Smooth Dogfish (82.3 %) (Table 3). Exactly half of the Blacktip Sharks were correctly classified, while Atlantic Sharpnose Sharks and Sandbar Sharks were incorrectly classified as Smooth Dogfish more often than they were correctly classified (Table 3).

Multiple linear correlation analysis showed that all correlations between environmental factors were statistically significant ($p < 0.05$). Strong negative correlations ($r < -0.5$) were found between temperature and dissolved oxygen and between inlet distance and salinity (Table 4). Because Spiny Dogfish were most abundant during winter and all other principle species were present during summer, the temperature, salinity, and dissolved oxygen measurements recorded during these seasons were used to create environmental grids within Pamlico Sound. Environmental grids showed differences in the spatial distribution of temperature, salinity, and dissolved oxygen measurements, though warmer temperatures, higher salinity, and higher dissolved oxygen were consistently found in the southern and eastern portions of Pamlico Sound (Figure 4).

Binary and Gaussian BRT models were generated for all species except the Blacktip Shark, which occurred in too few sets to consistently resolve a Gaussian abundance model so only binary presence/absence model was used for this species. Environmental factors contributing the most tree splits for each species differed between the binary and Gaussian BRT models (Table 5). Inlet distance was among the three contributors to the binary model for all species, and temperature as included among the top three contributing environmental factors for five of the six

principle species. Salinity was among the three environmental factors contributing the most to Gaussian abundance models for all five species (Table 5).

Marginal effect plots showed consistencies and differences between species in the effect of the top three environmental factors on presence (Figure 5). Positive effects on presence were associated with relatively short inlet distance (< 40 km) for all species except the Bull Shark, and temperatures greater than 15 °C were positively associated with presence for all species except the Spiny Dogfish. For species with salinity as one of the three environmental factors contributing most to the binary model, salinities greater than 20 ppt had a positive effect on presence (Figure 5).

Marginal effects of environmental factors on Gaussian BRT models of abundance suggested more complex relationships than those models for presence (Figure 6). Salinity was included within the top three contributing environmental factors for all species, and three of the five species showed a pronounced positive relationship between abundance and salinities greater than 25-27 ppt. The two exceptions were Bull Sharks, which showed a positive relationship beginning at a lower threshold around 17 ppt, and Spiny Dogfish, which showed a peak in positive association with abundance between approximately 17 and 25 ppt. Complex, nonlinear relationships were also observed involving dissolved oxygen with Sandbar Sharks and Smooth Dogfish abundance, and with temperature and Spiny Dogfish abundance (Figure 6).

Predicted abundance maps explicitly showed the importance of inlet distance, with four of the six principle shark species predicted to occur within a

relatively well-defined distance from the inlets (Figure 7). While predicted abundances of Atlantic Sharpnose and Blacktip Sharks were strongly associated with the inlets, Sandbar Shark and Spiny Dogfish abundance also showed more complex associations with depth contours. The models predicted high Smooth Dogfish abundance primarily along the eastern Pamlico Sound in an area coinciding with extensive seagrass beds adjacent to the Outer Banks. The Bull Shark was the only species with areas of high predicted abundance in the western portions of the estuary, with two distinct "hot spots" at the Long Shoal River between the Pamlico River and Albemarle Sound and east of Goose Creek Island near the mouth of the Pamlico River (Figure 7).

Discussion

The results of this study show that Pamlico Sound provides habitat for a diverse and dynamic assemblage of shark species, with principle species known to occur in estuarine or nearshore environments in both temperate waters to the north (Sandbar Shark, Smooth Dogfish, Spiny Dogfish) and subtropical systems to the south (Atlantic Sharpnose Shark, Blacktip Shark, Bull Shark) (Castro 2011). The highest shark diversity occurred during the summer but sharks were also present during cooler months, with Spiny Dogfish most abundant during winter and Sandbar Sharks primarily occurring in fall months. Size data suggest that Pamlico Sound may provide primary nursery habitat for Atlantic Sharpnose Sharks, Bull Sharks, Sandbar Sharks, and Smooth Dogfish, and likely serves as secondary nursery

or foraging habitat for the other principle species. Shark presence within the estuary was primarily associated with distance from the inlet, and temperature and abundance appeared to be associated with salinity, though other environmental factors were of importance on a species-specific basis. BRT models identified the inlets as important landmarks in delineating shark habitat for most of the principle species, though Bull Sharks appeared to associate with river mouths and areas of high Smooth Dogfish abundance closely overlapped seagrass beds on the sound side of the Outer Banks. Bull Sharks and Smooth Dogfish showed the most unique habitat preferences while Spiny Dogfish habitat spatially overlapped many of the same areas used by Atlantic Sharpnose, Blacktip, and Sandbar Sharks but differed from these species based on seasonal and temperature preferences.

The assemblage of shark species within Pamlico Sound is made up of species known to occur in other nearshore and estuarine habitats in the Mid-Atlantic and Southeastern regions of the U.S. east coast (McCandless et al. 2002, 2007) and the seasonal and monthly occurrence of the principle species follows the timing of seasonal migrations. Blacktip Sharks undergo large-scale seasonal migrations, with peak abundance in North and South Carolina waters during the summer and southeastern Florida during the winter (Castro 1996, Kajiura and Tellman 2016). Conversely, Spiny Dogfish peak abundance occurred during winter, when the distribution of this species extends south of Cape Hatteras (Stehlik 2007, Sagarese et al. 2014b). Perhaps the best-known example of the importance of North Carolina waters as overwintering habitat for a shark species is the migration of Sandbar Sharks into an area south of Cape Hatteras during winter (Jensen and Hopkins 2001,

Merson and Pratt, Jr. 2001, Grubbs et al. 2007, Conrath and Musick 2008, see also Chapter 1). However, Sandbar Sharks were highly abundant during the early and late fall but completely absent during winter, suggesting that this species may enter the sounds as part of their migration from summer habitats but may make use of nearshore oceanic waters in the peak of winter to increase access to warmer temperatures and higher primary productivity (Chapter 1, this volume).

Based on the predominance of juvenile size classes within the Pamlico Sound shark assemblage, this estuary may serve as nursery habitat for several species, particularly the Smooth Dogfish. The size distribution of this species was strongly skewed towards neonate and early juvenile sizes (Conrath and Musick 2002) and peak abundance in the late spring and summer coincided with parturition timing and nursery habitat use in other estuaries (Rountree and Able 1996). Given the high abundance of Smooth Dogfish in NCDMF surveys alone, Pamlico Sound may be worthy of consideration as essential habitat for this species. The presence of neonate Bull Sharks is noteworthy due to a lack of evidence for nursery habitat for this species within Pamlico Sound prior to 2011 (Schwartz 2012). Neonate Bull Sharks occurred within the time frame of parturition in Indian River Lagoon, a confirmed primary nursery (Curtis et al. 2011), suggesting that these individuals were born within the estuary and did not migrate from elsewhere. The use of Pamlico Sound as a primary nursery by Bull Sharks may be a recent phenomenon associated with rising water temperatures within the estuary (Bangley et al., in review). Neonate Sandbar Sharks are likely migrants from primary nurseries in the Chesapeake and Delaware Bays (Merson and Pratt, Jr. 2001, Grubbs et al. 2007) but

there is evidence for limited use of North Carolina waters as a primary nursery for resident sharks (Jensen et al. 2002).

Discriminant function analysis provided evidence for little habitat overlap between Bull Shark or Spiny Dogfish and other shark species in Pamlico Sound. The main drivers of these habitat differences appeared to be water temperature for the Spiny Dogfish and inlet distance or salinity for the Bull Shark. These results are in keeping with preferences for low temperatures among Spiny Dogfish (Ulrich et al. 2007, Sagarese et al. 2014b) and comparatively low salinities and greater inlet distances among Bull Sharks (Wiley and Simpfendorfer 2007, Heupel and Simpfendorfer 2008, Froeschke et al. 2010) documented in other systems. The majority of misclassifications were other species classified at Smooth Dogfish, likely a result of the abundance of this species within the survey catches and its presence during all seasons. Though Smooth Dogfish were the third most correctly-classified species, this may be a function of the large sample size reducing uncertainty for this species as it was classified within the same canonical biplot space as the other species that proved to be difficult to classify based on environmental factors. The low classification success among the remaining species may be an indication of similar or overlapping habitat preferences among Atlantic Sharpnose, Blacktip, and Sandbar Sharks in Pamlico Sound. High misclassification rates between particular species may also represent seasonal overlap. For example, there was high misclassification between Atlantic Sharpnose and Blacktip Sharks, two species that were most abundant during summer months. The Sandbar Shark was less associated with warm temperatures than the other Carcharhinid species and

showed nearly equal misclassification as the Blacktip Shark and Spiny Dogfish, which were almost exclusively warm and cold temperature species, respectively. This may reflect a broad temperature range for the Sandbar Shark, leading to a broader seasonal presence and the potential for habitat overlap with species in both the warm and cold-water shark assemblages.

Model results and the resulting predicted abundance maps identified the most important environmental factors structuring the Pamlico Sound shark assemblage and confirmed many of the habitat associations found during discriminant function analysis. Inlet distance and temperature were strongly associated with presence for the majority of species and salinity was strongly associated with abundance of all species, suggesting that these may be the main drivers of shark presence and abundance within Pamlico Sound. Given the strong correlation between inlet distance and salinity, it is likely that inlet distance preferences are directly related to salinity preferences, and the influence of these two factors may be interchangeable. Other studies of estuarine shark habitat use have found that inlet distance can serve as a proxy for salinity preference when delineating habitat and may actually be more useful from a management perspective (Grubbs and Musick 2007, Froeschke et al. 2010). Both temperature and salinity are major influences on shark habitat selection: temperature-associated movements are widespread among shark species and the majority of sharks tolerate a relatively narrow range of high salinities (Schlaff et al. 2014). Broadly, seasonal shark presence within Pamlico Sound is likely driven by temperature while the spatial extent of habitat available within the estuary is defined by salinity.

However, the influence of other environmental factors may be important in defining habitat on a more species-specific basis.

The effects of temperature and salinity on predicted abundance matched the unique habitat preferences for Spiny Dogfish and Bull Sharks found using discriminant function analysis. Increasing temperature had a positive effect on presence for all species except the Spiny Dogfish, while increasing salinity or decreasing inlet distance had a positive effect on the presence of all species except the Bull Shark. However, relationships between abundance and salinity differed from expectations for these species. Bull Shark abundance was positively associated with increasing salinity, though the threshold at which salinity began to have a positive effect was lower for this species than the others. The ability of Bull Sharks to utilize a broader salinity range than other species has been noted in several studies of multispecies shark assemblages (Wiley and Simpfendorfer 2007, Froeschke et al. 2010, Bethea et al. 2014). This adaptation may allow juvenile Bull Sharks to occupy portions of estuarine nursery areas that are inaccessible to other shark species, allowing them to reduce competition and predation risk (Heupel and Simpfendorfer 2011). Effects of temperature and salinity on Spiny Dogfish abundance are interesting. Temperature preferences reflect seasonal presence, as Spiny Dogfish migrate into North Carolina waters during the winter (Stehlik 2007) when most other species were absent from Pamlico Sound. However, other shark species are known to occur within the coastal oceanic waters just outside the estuary during the winter, including large numbers of juvenile Sandbar and Dusky (*Carcharhinus obscurus*) Sharks (Jensen and Hopkins 2001). The increased

abundance of Spiny Dogfish at mid-range salinities suggests that this species enters the estuary to avoid competition with other sharks, though observations from local fishermen also suggest the sound may represent a cool water refuge during periods when the Gulf Stream is in close proximity to shore (Chapter 4, this volume).

The importance of inlet distance for four of the six principle shark species was obvious from maps of predicted abundance. Predicted abundances of Atlantic Sharpnose Sharks, Blacktip Sharks, Sandbar Sharks, and Spiny Dogfish were above the minimum only within a certain radius around Oregon, Hatteras, and Ocracoke Inlets. These species are primarily coastal species associated with nearshore or lower estuarine environments (Castro 1993, Ulrich et al. 2007, Sagarese et al. 2014b, Bethea et al. 2014) and are likely transient within Pamlico Sound, making short foraging trips into the estuary from the ocean. Blacktip Sharks in Texas estuaries were predicted to occur exclusively near tidal inlets (Froeschke et al. 2010) and a study of shark habitat in the Florida panhandle that included both estuarine and nearshore areas predicted the highest rates of occurrence for Atlantic Sharpnose and Blacktip Sharks at nearshore areas open to the Gulf of Mexico (Ward-Paige et al. 2014). Potential juvenile Sandbar Shark habitat in the Chesapeake Bay was found to be limited to within 34.5 km of the estuary mouth (Grubbs and Musick 2007). Though salinity preferences prevent these shark species from penetrating far into the estuary, the extremely limited access through the inlets creates geographic bottlenecks that may facilitate important ecological interactions between these sharks and any migratory species transiting between the ocean and Pamlico Sound.

Mapping predicted shark abundances may have also identified important geographic features for the Bull Shark and Smooth Dogfish, the two principle species making more extensive use of Pamlico Sound. Bull Shark predicted abundance identified two potential "hot spots" of habitat for this species in relatively close proximity to sources of freshwater inflow: one at the Long Shoal River approximately equidistant from the mouth of the Pamlico River and the entrance to the Albemarle Sound, and one east of Goose Creek Island between the mouths of the Pamlico and Neuse Rivers. As mentioned above, lower-salinity habitats may provide a low-mortality environment for juvenile Bull Sharks by reducing the likelihood of interacting with other sharks (Heupel and Simpfendorfer 2011). Also, habitat near freshwater outflows may allow the juvenile Bull Sharks access to pulses of prey from freshwater sources (Matich and Heithaus 2014). The majority of the potential high-abundance area for Smooth Dogfish occurred within areas of extensive seagrass coverage along the sound side of the Outer Banks. Though juvenile Smooth Dogfish are unlikely to be small enough to take shelter within seagrass, these habitats may provide increased foraging opportunities. Smooth Dogfish primarily feed upon crustaceans such as crabs and shrimp (Rountree and Able 1996, Gelsleichter et al. 1999), and many crustacean species make extensive use of seagrass habitat (Main 1987, Baillie et al. 2014). Other mobile predators such as Red Drum (*Sciaenops ocellatus*) and Bonnethead Sharks (*Sphyrna tiburo*) are known to associate with seagrass habitat (Heupel et al. 2006, Fodrie et al. 2015). Therefore the extensive seagrass beds in the eastern portions of Pamlico Sound may be critical foraging habitat for juvenile Smooth Dogfish, and populations of this

species may be impacted by degradation or restoration of seagrass habitat. However, habitat preferences based on foraging opportunities cannot be confirmed without data on prey distributions, and habitat selection may not match prey distributions if predator avoidance is prioritized over foraging opportunities (Heupel and Hueter 2002).

This study is one of relatively few to define and predict elasmobranch habitat using environmental and spatial factors. An early example was Grubbs and Musick (2007), who used regression tree analysis to spatially delineate juvenile Sandbar Shark habitat within the lower Chesapeake Bay. Froeschke et al. (2010) followed this example by using boosted regression tree analysis to create predicted abundance models for three shark species in Texas estuaries, then Dedman et al. (2015) developed the gbm.auto script for R to automate the BRT analysis and mapping processes while identifying habitat for species in the Irish Sea skate complex. Other modeling approaches used to predict spatial habitat extent for elasmobranchs include the use of generalized additive models to identify Spiny Dogfish habitat in the northwest Atlantic (Sagarese et al. 2014a) and generalized linear mixed models to spatially delineate habitat for six shark species in the northeastern Gulf of Mexico (Ward-Paige et al. 2014). The boosted regression tree approach seemed most appropriate for the data used in this study due to its flexibility and statistical power in situations with zero-inflated distributions and relatively low sample sizes (Elith et al. 2008). However, the low number of sets with positive presence required extensive trial-and-error to determine the combination of learning rate and bag fraction that would allow Gaussian abundance models to

consistently resolve for some species. In similarly data-poor situations, which are common in elasmobranch research, it may be most appropriate to model binary presence/absence only, and this was the approach that ultimately needed to be taken for the Blacktip Shark. Also, my study could benefit from post-hoc validation, which was accomplished by Grubbs and Musick (2007) using subsequent catches and tag returns within the predicted habitat areas. This would likely be a simple matter of mapping NCDMF survey catches from years after 2014 to determine if they fall within the areas of high predicted abundance, but at the time of writing 2015 gillnet and longline survey data were not yet available. The final caveat is the lack of sets beyond the 2-m depth contour within Pamlico Sound, which lead to undersampling of much of the interior, deep water area of the estuary. Because of this, the habitat models generated during this study may not accurately reflect the influence of depth or habitat types found in deep areas of Pamlico Sound.

Shark diversity within Pamlico Sound is as diverse as any estuarine assemblage on the U.S. east coast (McCandless et al. 2002, 2007) and the principle species represent both warm and cold-water shark communities, which means sharks are present within the estuary year-round. Species presence within Pamlico Sound appears to be largely temperature-driven, while spatial habitat extent is a function of salinity in combination with other environmental factors. Sharks are clearly a part of the Pamlico Sound estuarine community and patterns of habitat use within the estuary may reflect or facilitate interactions with other species, including economically-important teleost and crustacean species. With the identification of areas regularly used by sharks, further work should assess potential habitat overlap

with other species and the nature and extent of interactions involving sharks in Pamlico Sound.

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Tables

Table 2-1. Total number captured and mean ± standard deviation total length (TL) and environmental factor measurements for each shark species captured during NCDMF gillnet and longline surveys within Pamlico Sound. Species denoted with an asterisk were designated principle species.

Species	N	TL (mm) (Min-Max)	Depth (m) (Min-Max)	Temp (°C) (Min-Max)	Sal (ppt) (Min-Max)	DO (mg/L) (Min-Max)	SAV Dist (m) (Min-Max)	Inlet Dist (km) (Min-Max)
*Atlantic Sharpnose Shark *Rhizoprionodon terraenovae*	98	722.9 ± 209.1 (338-1040)	1.7 ± 0.7 (0.6-4.0)	26.1 ± 2.6 (18.3-30.6)	28.2 ± 5.0 (17.1-35.6)	7.1 ± 0.9 (5.3-11.6)	639.6 ± 732.9 (0.0-2773.5)	8.3 ± 5.6 (0.2-39.7)
*Blacktip Shark *Carcharhinus limbatus*	52	1305.7 ± 108.5 (819-1638)	2.1 ± 0.7 (0.4-3.4)	28.6 ± 1.6 (24.4-32.0)	24.9 ± 5.0 (12.4-35.6)	6.9 ± 1.1 (3.5-10.4)	1038.5 ± 1138.9 (0.0-5373.2)	7.8 ± 3.8 (0.0-21.7)
Bonnethead Shark *Sphyrna tiburo*	21	909.7 ± 86.1 (748-1104)	1.6 ± 0.6 (0.6-2.3)	28.3 ± 1.8 (25.6-31.4)	26.7 ± 3.5 (19.8-31.2)	7.6 ± 1.3 (5.6-9.3)	40.3 ± 54.2 (0.0-203.9)	13.7 ± 10.0 (3.6-32.0)
*Bull Shark *Carcharhinus leucas*	48	849.6 ± 230.1 (598-1829)	1.5 ± 0.6 (0.6-3.4)	27.9 ± 2.6 (19.7-32.9)	19.6 ± 4.0 (6.0-27.6)	6.8 ± 1.1 (4.0-9.5)	1332.2 ± 1372.8 (0.0-5698.1)	36.6 ± 6.3 (11.1-48.2)
Finetooth Shark *Carcharhinus isodon*	9	671.0 ± 288.3 (493-1435)	2.0 ± 0.2 (1.5-2.4)	26.8 ± 1.2 (26.3-29.9)	26.6 ± 1.7 (25.9-31.1)	6.4 ± 0.3 (6.0-7.1)	40.8 ± 122.4 (0.0-367.2)	8.3 ± 2.3 (3.4-12.7)
Unknown/Misidentified *Carcharhinus* sp.	44	457.4 ± 64.0 (320-582)	2.2 ± 0.5 (0.8-2.4)	26.8 ± 1.1 (22.8-28.6)	27.4 ± 3.0 (20.8-29.8)	7.4 ± 0.5 (6.5-8.9)	260.8 ± 175.9 (0.0-1048.9)	11.3 ± 9.3 (6.1-40.6)
Sand Tiger Shark *Carcharias taurus*	2	1003.5 ± 157.7 (829-1115)	3.1 ± 1.3 (2.1-4.0)	20.3 ± 7.7 (14.8-25.8)	21.4 ± 2.6 (19.5-23.2)	7.6 ± 2.4 (5.9-9.3)	1378.4 ± 1797.6 (107.3-2649.4)	21.2 ± 21.4 (6.1-36.3)
*Sandbar Shark *Carcharhinus plumbeus*	49	875.4 ± 269.1 (597-2033)	2.2 ± 0.8 (0.6-3.7)	21.8 ± 4.5 (12.3-27.5)	25.9 ± 3.5 (18.0-32.3)	7.8 ± 1.2 (5.3-11.6)	491.0 ± 679.7 (0.0-2925.0)	12.3 ± 6.7 (2.7-32.3)
Scalloped Hammerhead *Sphyrna lewini*	1	1189	2.1	27	25.3	7.9	273.5	4.4
*Smooth Dogfish *Mustelus canis*	1582	674.2 ± 112.3 (332-1228)	1.6 ± 0.6 (0.5-4.3)	25.4 ± 4.4 (10.6-32.4)	27.5 ± 2.7 (16.8-34.7)	7.7 ± 1.6 (2.0-15.6)	90.7 ± 179.4 (0.0-1880.3)	14.4 ± 8.6 (1.3-35.5)
Spinner Shark *Carcharhinus brevipinna*	3	504.0 ± 16.7 (489-552)	2.4 ± 0.6 (1.8-3.0)	28.8 ± 1.3 (27.5-30.1)	29.7 ± 0.7 (28.4-29.8)	5.2 ± 0.8 (4.6-6.1)	1131.4 ± 1079.3 (477.5-2377.2)	9.0 ± 0.6 (8.5-9.8)
*Spiny Dogfish *Squalus acanthias*	499	855.6 ± 70.4 (511-1010)	1.8 ± 0.5 (0.5-2.9)	12.9 ± 3.1 (3.4-29.9)	21.2 ± 4.8 (6.7-34.7)	9.9 ± 1.8 (6.0-14.3)	61.6 ± 124.1 (0.0-1336.0)	13.4 ± 9.1 (2.8-34.1)

Table 2-2. Canonical eigenvalues, cumulative percent of variation explained, and canonical structure from discriminant function analysis classifying principle shark species by environmental factors.

			Canonical Structure					
Canonical	Eigenvalue	Cumulative %	Depth	Temp	Sal	DO	SAV Dist	Inlet Dist
1	2.11	71.5	-0.13	0.96	0.67	-0.64	0.18	0.04
2	0.67	94.1	0.1	0.06	-0.43	-0.11	0.86	0.34
3	0.15	99.4	0.35	-0.1	0.15	0.01	0.38	-0.9
4	0.01	99.8	-0.47	-0.18	0.52	-0.15	0.18	-0.01
5	0.01	100	0.79	-0.18	0.27	-0.25	0.13	0.23

Table 2-3. Percent of individuals of principle shark species classified as each species based on environmental factors by discriminant function analysis. Correct classifications are in bold.

Species	Predicted					
Actual	Atl Sharpnose Shark	Blacktip Shark	Bull Shark	Sandbar Shark	Smooth Dogfish	Spiny Dogfish
Atl Sharpnose Shark	**28.4**	16.8	1.1	4.2	45.3	4.2
Blacktip Shark	7.7	**50.0**	9.6	0.0	32.7	0.0
Bull Shark	0.0	2.1	**91.7**	2.1	2.1	2.1
Sandbar Shark	4.7	25.6	2.3	**18.6**	27.9	20.9
Smooth Dogfish	0.3	1.2	0.0	9.8	**82.3**	6.4
Spiny Dogfish	0.2	0.0	0.0	4.4	3.2	**92.2**

Table 2-4. Linear correlations between environmental factors recorded during 2007-2014 NCDMF gillnet and longline surveys in Pamlico Sound.

	Depth	Temp	Sal	DO	SAV Dist	Inlet Dist
Depth	1.00					
Temp	0.11	1.00				
Sal	0.15	0.07	1.00			
DO	-0.13	-0.54	-0.03	1.00		
SAV Dist	0.24	0.10	-0.04	-0.10	1.00	
Inlet Dist	-0.14	-0.04	-0.67	-0.03	0.06	1.00

Table 2-5. Parameters (lr=learning rate, bf=bag fraction) and contribution (% of tree splits) for binary presence/absence and Gaussian abundance BRT models predicting abundance of principle shark species in Pamlico Sound. Bold text denotes the three environmental factors contributing the most tree splits for each species.

	Binary Model			% Contribution					
Species	lr	bf	N Trees	Depth	Temp	Sal	DO	SAV Dist	Inlet Dist
Atl Sharpnose Shark	0.001	0.55	1950	3.56	**20.26**	14.27	**21.39**	16.32	**24.20**
Blacktip Shark	0.001	0.5	1650	2.72	**23.36**	15.08	10.22	**26.79**	**21.85**
Bull Shark	0.00044	0.78	4050	3.27	**21.94**	13.07	13.34	**17.61**	**30.78**
Sandbar Shark	0.00015	0.8	2415	12.73	14.60	**16.71**	**31.72**	7.62	**16.62**
Smooth Dogfish	0.001	0.5	7200	4.49	**17.21**	**33.62**	8.95	16.86	**18.86**
Spiny Dogfish	0.001	0.5	4850	8.57	**30.15**	**12.94**	11.35	11.68	**25.31**
	Gaussian Model			% Contribution					
Species	lr	bf	N Trees	Depth	Temp	Sal	DO	SAV Dist	Inlet Dist
Atl Sharpnose Shark	0.001	0.55	3550	**21.99**	11.07	**20.93**	17.14	**23.07**	5.79
Bull Shark	0.00044	0.78	3500	**28.06**	3.94	**24.92**	5.02	14.86	**23.20**
Sandbar Shark	0.00015	0.8	105	12.21	0.48	**33.01**	**31.58**	**22.21**	0.00
Smooth Dogfish	0.001	0.5	1150	4.85	15.66	**26.68**	**21.81**	8.63	**22.37**
Spiny Dogfish	0.001	0.5	2600	7.64	**19.02**	**30.14**	14.48	9.46	**19.26**

Figures

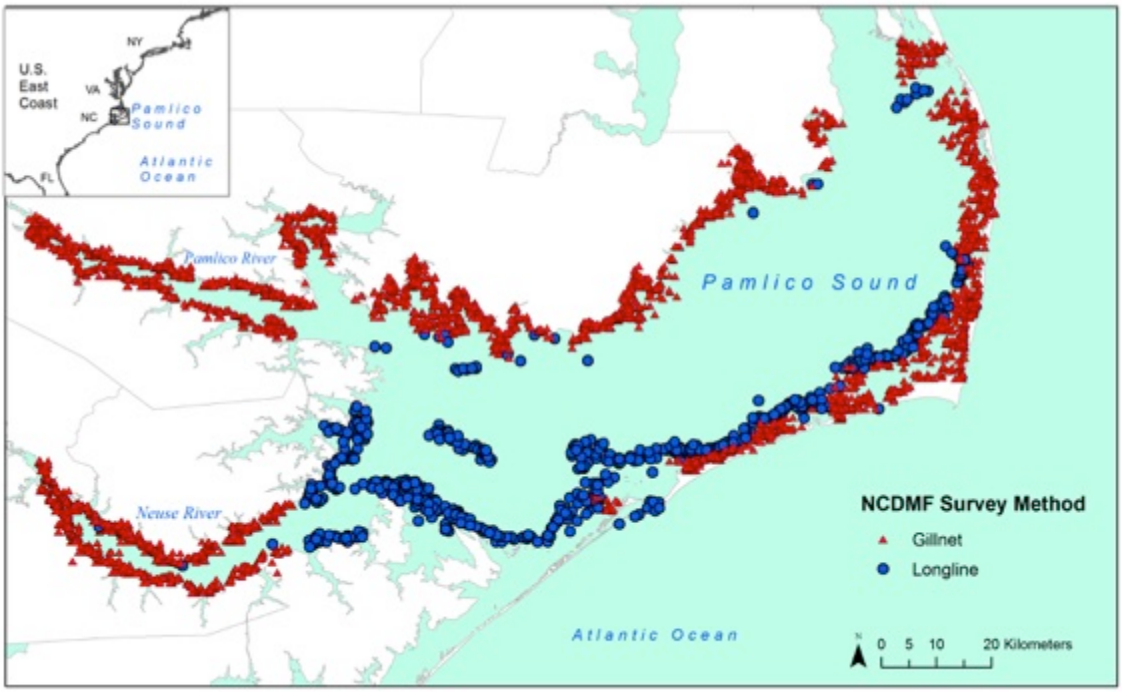

Figure 2-1. North Carolina Division of Marine Fisheries (NCDMF) gillnet (red triangeles) and longline (blue circles) survey stations within Pamlico Sound from 2007-2014.

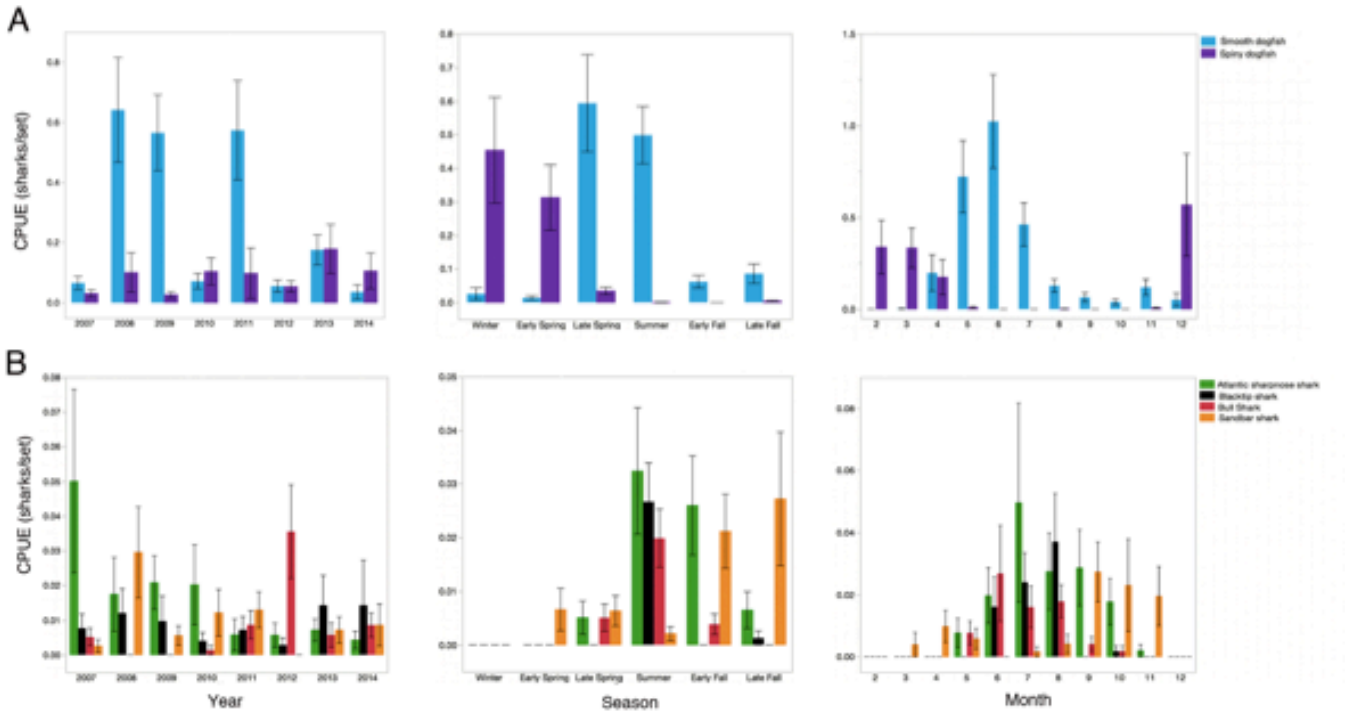

Figure 2-2. Mean CPUE (sharks/set) by year, season, and month for A. Smooth and Spiny Dogfish and B. Atlantic Sharpnose, Blacktip, Bull, and Sandbar Sharks captured in Pamlico Sound from 2007-2014.

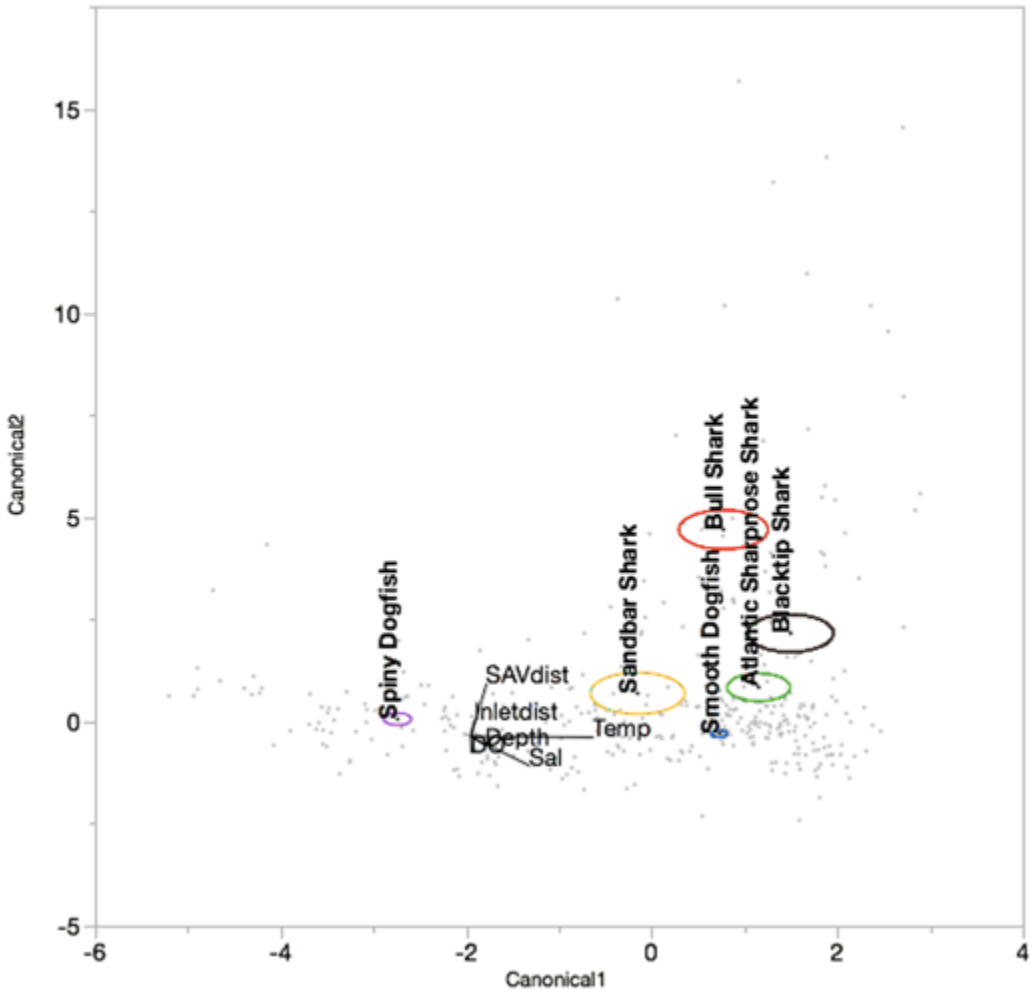

Figure 2-3. Discriminant function analysis canonical correspondence plot showing classification of principle shark species based on canonicals 1 and 2. Correlations between canonicals and environmental factors are shown in the biplot rays, and ellipses represent 95 % confidence intervals.

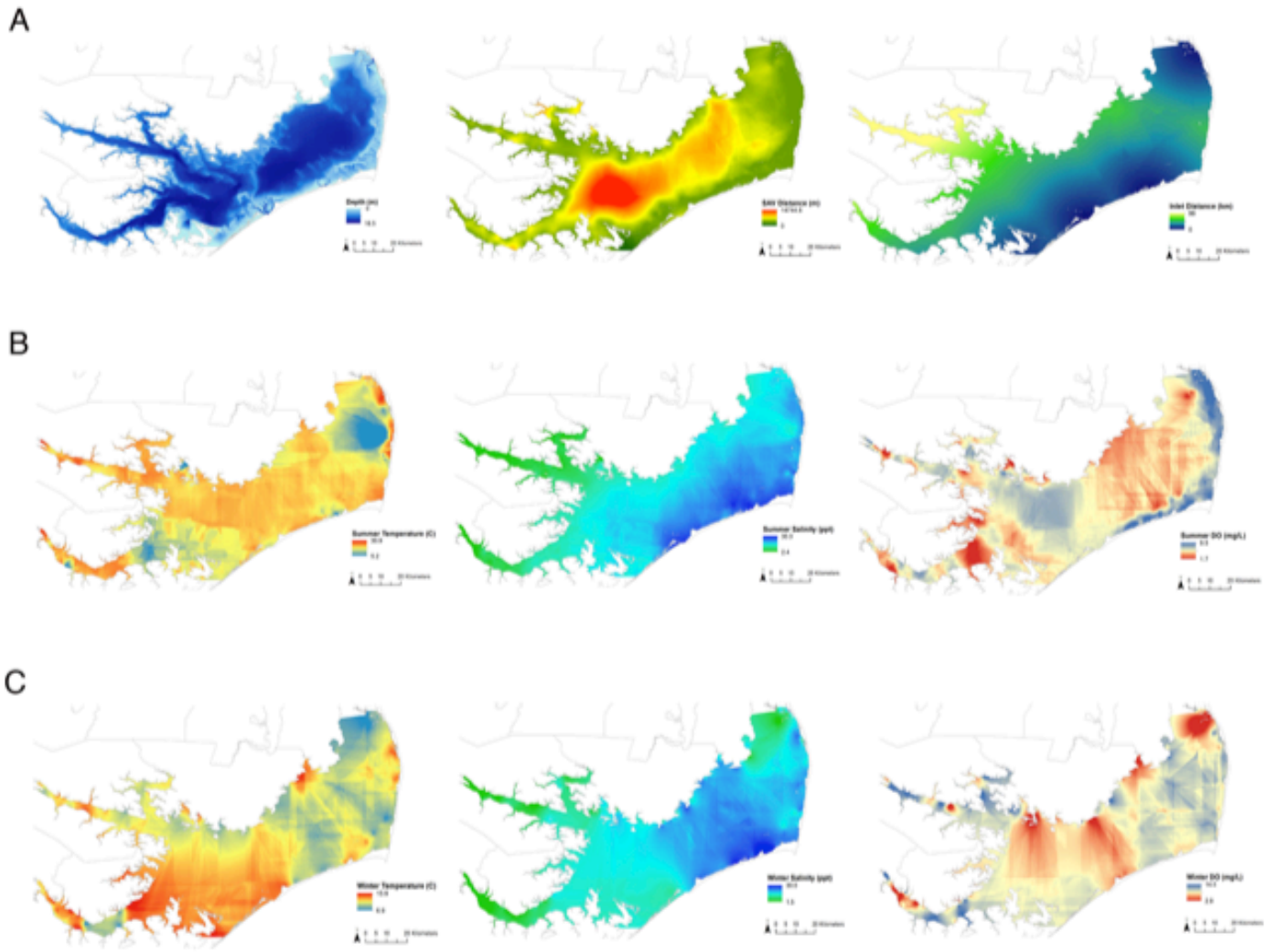

Figure 2-4. Environmental grids for Pamlico Sound representing A. static spatial features (depth, SAV distance, and inlet distance) and temperature, salinity, and dissolved oxygen during B. summer and C. winter.

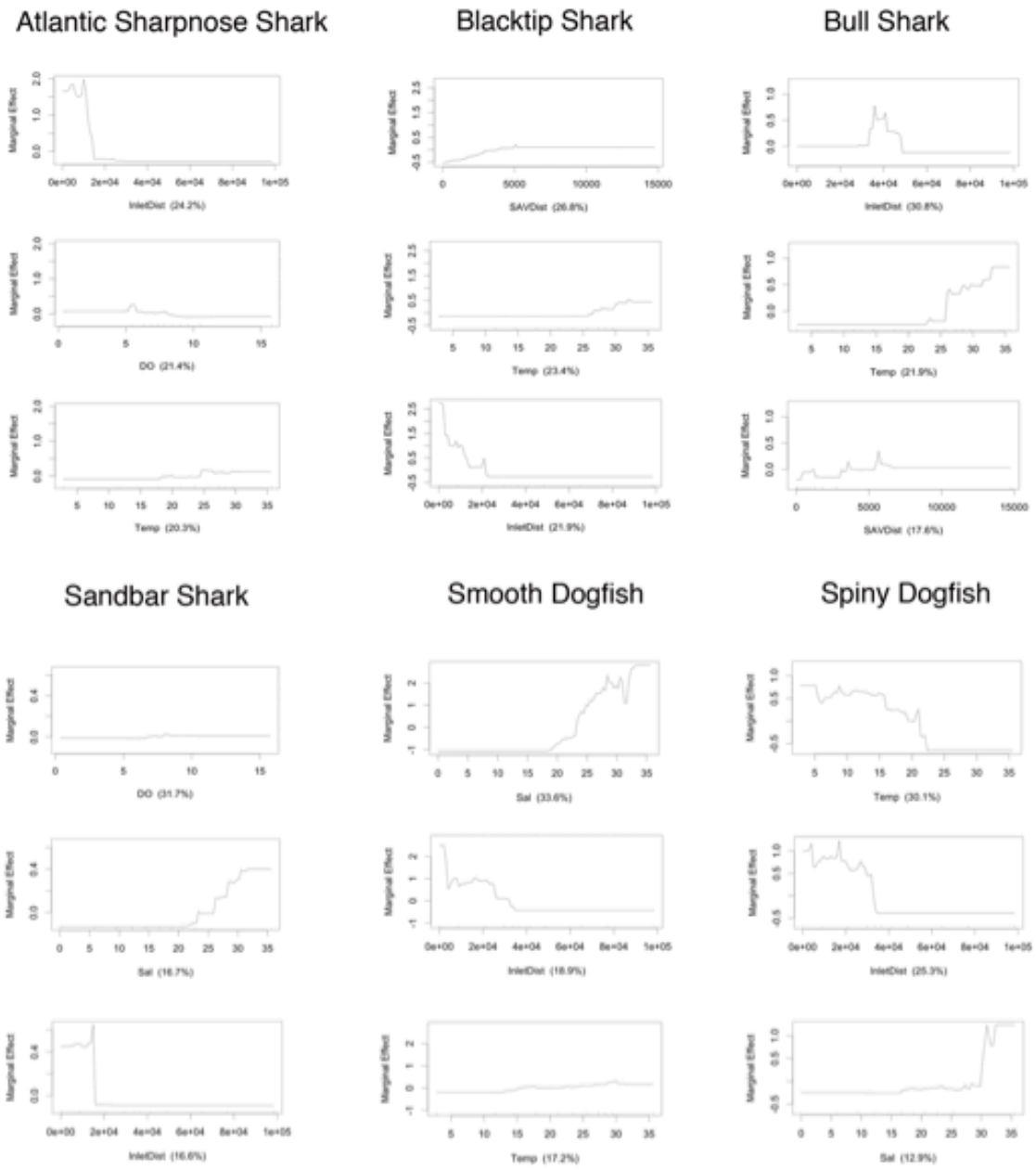

Figure 2-5. Marginal effect plots showing the influence of the three environmental factors that contributed most to the binary BRT model in predicting presence of the six principle shark species within Pamlico Sound.

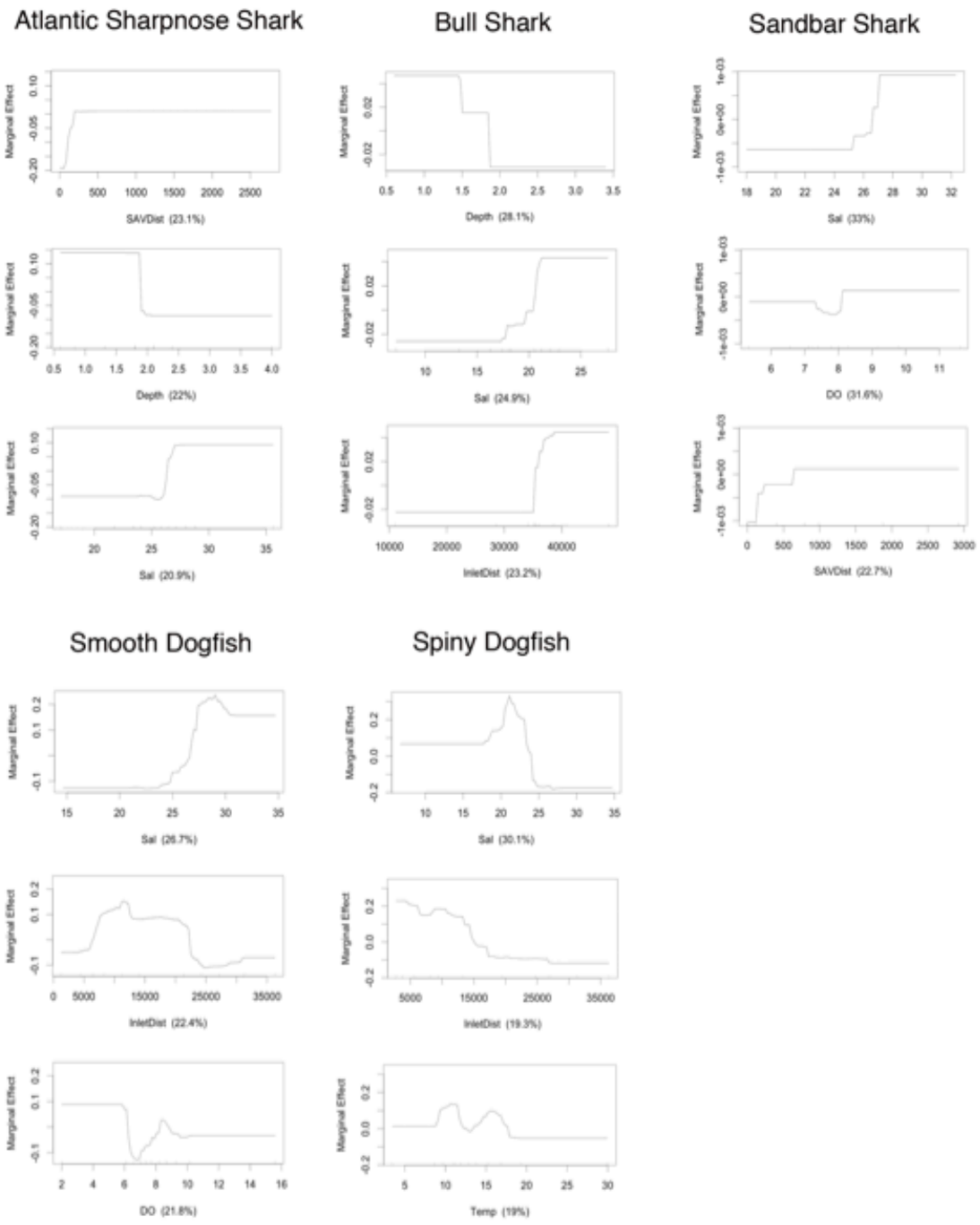

Figure 2-6. Marginal effect plots showing the influence of the three environmental factors contributing the most to the Gaussian BRT model on abundance for five of the principle shark species in Pamlico Sound.

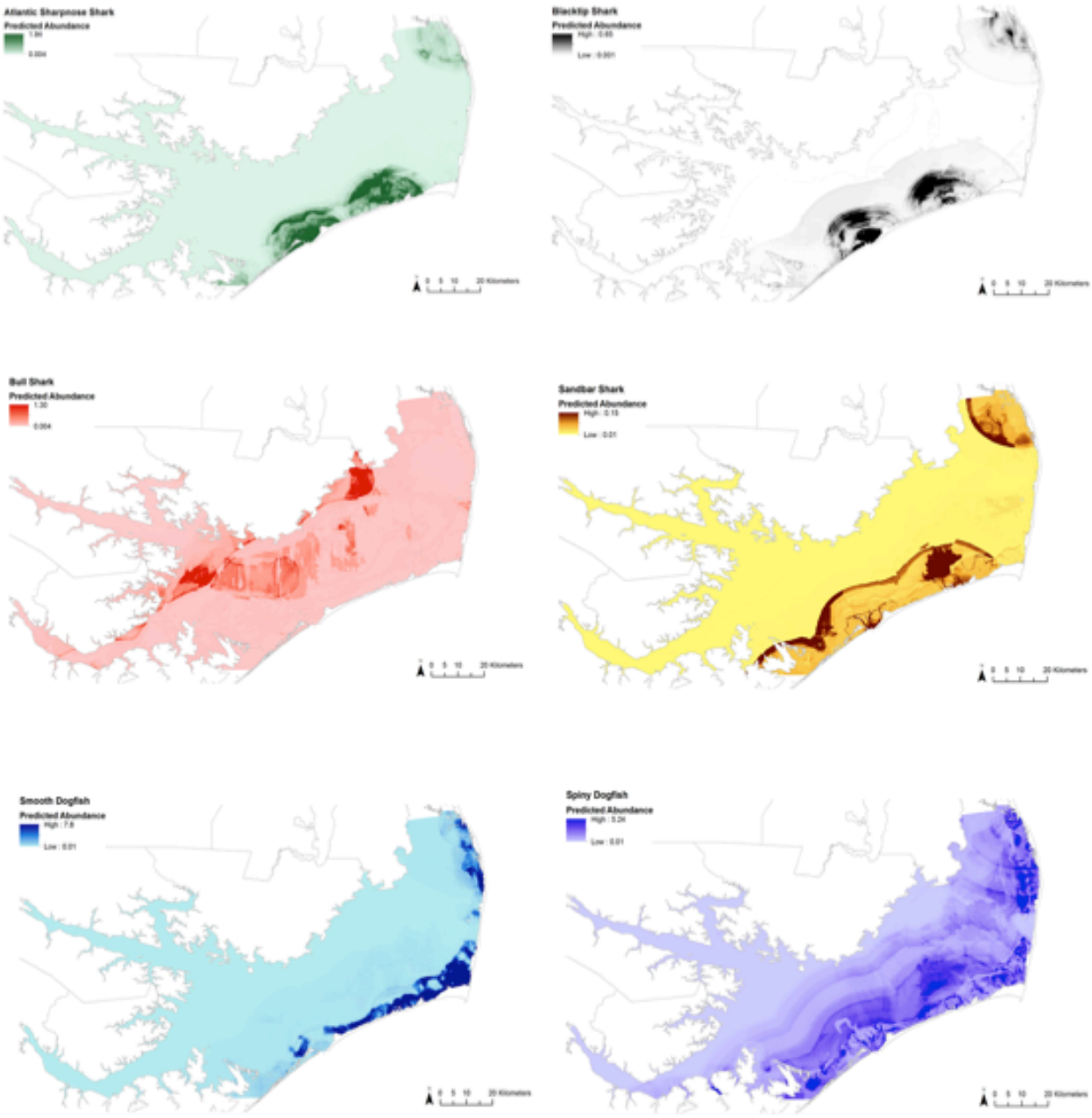

Figure 2-7. Predicted abundance maps for the six principle shark species within Pamlico Sound based on combined presence/absence and abundance BRT results. Predicted Blacktip Shark abundance is based only on the presence/absence model.

Chapter 3

Nursery Habitat and Resource Partitioning in the Elasmobranch Community of a North Carolina Estuary

Abstract

In marine communities, resource partitioning can be as important as abiotic environmental preferences in determining habitat use patterns. We attempted to identify patterns of habitat use and determine the influence of resource partitioning among the elasmobranch community in Back and Core Sounds, North Carolina using a combination of fishery-independent survey and acoustic telemetry methods. Gillnet, longline, drumline, and rod and reel sampling captured a total of 160 elasmobranchs representing 12 species within the estuary, and differences between seven principle species were assessed in terms of temporal, environmental, and spatial habitat factors. Three Blacknose Sharks (*Carcharhinus acronotus*) and one Blacktip Shark (*Carcharhinus limbatus*) were tagged with acoustic transmitters, and estuarine microhabitat preferences of these and other previously-tagged predators were recorded using acoustic receivers deployed at deep channel, seagrass, sand flat, and oyster reef sites. Back and Core Sounds may function as primary nursery habitat for Atlantic Sharpnose Sharks (*Rhizoprionodon terraenovae*), Cownose Rays (*Rhinoptera bonasus*), and Smooth Dogfish (*Mustelus canis*), and as secondary nursery and foraging habitat for Blacktip Sharks. The elasmobranch community was broadly divided into cool and warm temperature communities. Blacknose and

Blacktip Sharks showed evidence of spatial resource partitioning based on distance from the nearest inlet. Four elasmobranch species were detected by acoustic receivers and were primarily detected at night. One large Bull Shark (*Carcharhinus leucas*) repeatedly visited receiver stations near oyster reef habitat. Generally, larger sharks occurred in the estuary at night, suggesting elevated predation risk for small and juvenile elasmobranchs as well as other estuarine species at these times.

Introduction

Marine predators can significantly influence marine communities through direct and indirect interspecific interactions (Heithaus et al. 2012). Elasmobranchs typically occupy high trophic levels within marine communities, and some species function as apex predators in these ecosystems (Cortés 1999, Heithaus et al. 2010, Hussey et al. 2015b). The habitat use patterns of elasmobranchs, particularly apex predator shark species, can have top-down ecosystem effects throughout the food web to the level of primary producers (Vaudo and Heithaus 2013, Burkholder et al. 2013, Heithaus et al. 2014). Therefore, knowledge of habitat use by elasmobranchs may be an important factor in measuring ecosystem health and productivity. This may be especially important in estuaries, where many processes are driven by top-down trophic effects (Heck and Valentine 2007).

Habitat use by sharks is likely driven by a combination of abiotic factors, in the form of environmental preferences, and biotic factors, in the form of interspecific relationships with prey, competitors, or predators (Heithaus 2007).

Depending on body size, sharks can function as apex predators or mesopredators, though local environmental conditions may determine the trophic role of a given species in a given habitat (Heupel et al. 2014). Sharks often use estuarine habitats as nurseries, which can be classified as primary nurseries where parturition occurs or secondary nurseries functioning as foraging habitat or shelter for juveniles (Bass 1978). In near shore oceanic and estuarine environments, multiple shark species and size classes may coexist (Simpfendorfer and Milward 1993) and competition may lead to resource partitioning among sympatric elasmobranchs and other high tropic-level predators (Heithaus and Vaudo 2012). Resource partitioning is widespread among fishes and can be expressed as trophic, spatial, or temporal differences in habitat use between species occupying the same system (Ross and Url 1986). Different types of resource partitioning have been observed among elasmobranchs: species with similar prey preferences showed differences in spatial habitat use within Apalachicola Bay, Florida (Bethea et al. 2004), while different species occupying the same spatial area within Cleveland Bay showed evidence of trophic resource partitioning (Kinney et al. 2011). Few studies have assessed diel habitat use by elasmobranchs, but four of six Carcharhinid shark species captured in longline surveys conducted along the southeastern coast of the United States were primarily caught at night (Driggers et al. 2012).

A variety of methods can be used to investigate habitat use by elasmobranchs, and each approach has its own unique set of requirements and limitations (Simpfendorfer and Heupel 2012). While catch rates from fishery-independent surveys can cover a broad spatial area, acoustic telemetry can provide

24-hour tracking of multiple species at finer spatial scales (Simpfendorfer and Heupel 2012, Donaldson et al. 2014). In this study, we use a combination of fishery-independent survey data and acoustic telemetry to identify estuary- and microhabitat-scale patterns of habitat use and to determine whether resource partitioning is occurring between elasmobranchs in a warm-temperate estuarine ecosystem.

Methods

Fishery-Independent Survey

Elasmobranchs were captured during a shark survey conducted in Back and Core Sounds, North Carolina, using gillnet, longline, and drumline gear (Figure 1). Sampling occurred from March 21 to November 16 in 2014 and from April 13 to October 15 in 2015. Most sampling occurred between 1200 and 2200 hours in order to ensure sampling before and after sunset, though some opportunistic sampling occurred outside this time frame. Sampling sites were chosen using a stratified-random strategy in which the area from lower Newport River to Jarrett Bay was divided into five major strata based on expected environmental differences, which were then further divided into 6-9 numbered substrata that were randomly chosen before each sampling date. Gillnet gear measured 45 m in length and 2 m in height, and was comprised of five 9-m panels ranging from 2.5- to 14-cm stretched mesh. Longline gear consisted of a 274-m mainline with 20-30 gangions constructed of 1 m of 136 kg-test monofilament line and a 12/0 circle hook.

Drumline gear consisted of a single 15-m long, 408.23 kg-test monofilament leader with a 15/0 circle hook mounted on a 18.14-kg weight. Longline hooks were baited with cut Atlantic Mackerel (*Scomber scombrus*) supplemented by locally available baitfish, and the drumline was baited with cut sections of Spiny Dogfish (*Squalus acanthias*), Atlantic Sharpnose Shark (*Rhizoprionodon terraenovae*), or Striped bass (*Morone saxatilis*). When possible, a combination of multiple gear types was deployed simultaneously within 100 m of each other. Soak time was limited to 30 minutes for all gears. Elasmobranch catches were occasionally supplemented using rod and reel gear deployed during soak times.

At each station, depth (m) was recorded using an onboard depth sounder and temperature (°C), salinity (ppt), and dissolved oxygen (mg/L) were recorded from the middle of the water column using a YSI Pro 2030 water quality meter. Set time (hours, 24 hour cycle) was recorded at time of gear deployment. All sampling locations were plotted in ArcGIS 10.1, and straight-line distance from the nearest inlet (km) and the nearest seagrass bed (m) were calculated using the spatial join function. Inlet locations were represented by lines drawn across the entrances to Beaufort and Barden's Inlets in ArcGIS, while seagrass bed locations were taken from layers generated by the Albemarle-Pamlico National Estuarine Partnership's submerged aquatic vegetation (SAV) mapping data (Kenworthy et al. 2012) In the remainder of the manuscript all of these temporal, spatial, and environmental factors are referred to collectively as habitat variables.

All captured elasmobranchs were identified to species and sex was recorded. Total length (TL, mm) and fork length (FL, mm) were recorded for all sharks, and

disc width (DW, mm) was recorded for all batoids. For each species mean habitat variables were calculated. Species with catches greater than 10 individuals in the survey were considered principle species for further analysis, with the exception of the blacktip shark (*Carcharhinus limbatus*), which was a target species in the survey and included in further analysis in spite of a lower sample size. Spatial overlap between principle species was expressed as the percentage of sets in which a given species was captured and which also contained the other species. Discriminant function analysis (DFA) was used to determine whether the principle species could be correctly classified based on habitat variables.

Prior to comparisons between species, overall set time was checked for a VonMises distribution using Watson's U^2 test and for a bias in time distribution using Rayleigh's test of uniformity (Fisher 1993, Zar 1999). All other variables were checked for a normal distribution using histogram and qq plot analyses. Watson's two-sample test was used for comparisons between mean set time for each species and overall set time, and for pairwise comparisons between species. Oneway ANOVA procedure and Tukey's Honestly Significant Difference analysis were used to make pairwise comparisons in other habitat variables between species. Statistics involving set time were calculated using the package "circular" in R to account for the circular nature of time data, while calculations involving all other variables were performed in JMP Pro 12.

Acoustic Telemetry

Acoustic telemetry occurred during the 2015 survey season and was used to assess fine-scale habitat preferences. Four species were targeted for acoustic

tagging: the Blacknose Shark (*Carcharhinus acronotus*), Bull Shark (*Carcharhinus leucas*), Blacktip Shark, and Bonnethead Shark (*Sphyrna tiburo*). Condition was assessed using signs of stress such as coloration and responsiveness to stimuli, and sharks in good condition were selected for internal acoustic tagging. Sharks selected for tagging were placed in a state of tonic immobility and gills were irrigated with flowing seawater from a hose inserted into the mouth. Prior to surgery, an incision site was chosen on the left ventral side of the shark approximately 2/3 of the distance from the mouth to the pelvic fins. The incision site was disinfected by swabbing with Novalsan and locally anesthetized using a 0.2-mL lidocaine injection, then an approximately 4-5.5 cm incision was made through the skin and musculature into the body cavity. A sterilized Vemco V16 69-kHz transmitter was inserted into the body cavity through the incision, which was then closed using an interrupted stitching pattern. After surgery, sharks were returned to the water and held alongside the boat until normal swimming behavior resumed and then released. All surgical and handling procedures were approved by ECU's Institutional Animal Care and Use Committee (AUP # D306).

Transmitters were detected by Vemco VR2W 69 kHz receivers deployed in the area surrounding Middle Marsh, an isolated shallow salt marsh habitat in the center of Back Sound, with the goal of determining habitat preferences of the tagged sharks (Figure 1). This site was chosen due to the variety of discrete subtidal habitat types around the perimeter of the marsh and its location between the two main channels running through the estuary, with the assumption that sharks would use these channels to move in and out of Back Sound. Receivers were housed in PVC

tubes mounted inside half-cinderblocks and connected to the surface by a single polypropylene line tied to a foam crab pot float or "poly ball" buoy. Deployment sites were chosen to ensure that a single subtidal habitat type was spatially dominant within the receiver's detection range. Four habitat types were represented: deep (> 2 m) channels, shallow sand flats, oyster reefs, and seagrass beds. Receivers were deployed at sites both relatively near and far from Beaufort Inlet, the nearest opening to the Atlantic Ocean. Deployment sites were given a two-letter designation based on the habitat type and distance from the inlet: channel (C), sand flat (S), oyster reef (O), and seagrass (G), followed by near (N) or far (F) from the inlet (e.g., GN would designate the seagrass near inlet site). Receivers were deployed at seagrass and sand flat sites on May 28 and an additional four receivers loaned by the Ocean Tracking Network (OTN) were deployed at deep channel and oyster reef sites on September 30, 2015. Receivers remained in the water until retrieval and data download on November 11-12, 2015. Prior to retrieval, receiver range was tested by lowering a test tag approximately 1 m under the surface for 5 min at increasing distances from the receiver, after which exact distance was calculated using ArcGIS. Detection efficiency was calculated as the proportion of detected transmissions out of expected transmissions over distance.

Transmitter numbers that did not correspond to any of the sharks tagged in this study were identified through the tag database maintained by the Atlantic Cooperative Telemetry (ACT) Network (Brown 2012, www.theactnetwork.com) and were included in habitat use analysis with the permission of the researchers responsible for tagging. Habitat preference was assessed for each species by

observing the number of detections and number of visits, defined as discrete periods of sustained detection. Temporal patterns of habitat use were also assessed by date and time of day, which was classified by dividing hours of the day into Day (0730-1800 hours), Night (2000-0500 hours) and Twilight (0500-0730 or 1800-2000 hours). For species with a sufficient number of detections, the mean hour of presence in the Middle Marsh array and at each receiver were calculated. Rayleigh's test of uniformity was used to determine whether detections occurred over a statistically significant time period.

Results

Fishery-Independent Survey

Sampling covered a time frame between 0700 and 0200 hours the following day, though most survey sets occurred between 1100 and 2100 hours. Mean set time (± standard deviation) was 1707 ± 055. Watson's test of uniformity showed that set time data fit a VonMises distribution ($U^2 = 0.1633$, $p < 0.01$) and Rayleigh's test of uniformity showed that the time covered by the survey fit a definite temporal range ($Z = 0.656$, $p < 0.0001$). All other factors fit a normal distribution.

Over both sampling years, 160 elasmobranchs of 12 positively identified species were captured (Table 1). In addition, two sharks visually identified as members of the genus *Carcharhinus* broke out of the gear before they could be brought onboard and processed. The most common elasmobranchs captured were spiny dogfish, Atlantic Sharpnose shark, and Southern Stingray (*Dasyatis*

americana). Length measurements for the Atlantic Sharpnose Shark covered neonate to mature adult size ranges (Loefer and Sedberry 2003), though no individuals between young-of-year and mature size ranges were captured (Figure 2). Back Sound was further confirmed as a primary nursery for Atlantic Sharpnose Sharks by the capture of a pregnant female that gave birth to fully-developed neonates onboard the research vessel during processing. Cownose Rays (*Rhinoptera bonasus*) and Smooth Dogfish (*Mustelus canis*) showed a similar length-fequency pattern, with individuals of age 0-1 and mature size ranges present (Smith and Merriner 1987, Conrath and Musick 2002) but older juveniles and subadults absent. All Blacktip Sharks fell within age 1+ juvenile size ranges (Barry et al. 2008), and juveniles also were present among Bluntnose Stingrays (*Dasyatis say*), Sandbar Sharks (*Carcharhinus plumbeus*), and Southern Stingrays. Only subadult or adult size classes of Blacknose Sharks, Bull sharks, Bonnetheads, and Spiny Dogfish were captured (Table 1, Figure 2).

Mean time of capture for the majority of species was in mid-afternoon (1530-1730 hours), though Blacknose and Bull Sharks appeared to be more nocturnal and Bonnetheads were captured during morning hours (Table 2). Spiny and Smooth Dogfish were captured at the lowest mean temperatures of any species. Bonnethead, *Carcharhinus* sp., and Cownose Rays occurred at the lowest salinities, while all species were captured in relatively normoxic conditions. Bull and Blacknose Sharks occurred closest to inlets, and Bullnose Rays and Bonnetheads were captured at the shortest distances from seagrass beds (Table 2). The seven most abundant species were selected as the principle species for further analysis:

Atlantic Sharpnose Shark, Blacknose Shark, Blacktip Shark, Cownose Ray, Smooth Dogfish, Southern Stingray, and Spiny Dogfish.

Spatial overlap between the principle species was generally relatively low, though overlaps between Blacktip Sharks and Southern Stingrays and between Smooth Dogfish and Atlantic Sharpnose Sharks approached 50 % (Figure 3). Atlantic Sharpnose Sharks showed the highest degree of spatial overlap, co-occurring with all species except Cownose Rays. Southern Stingrays also were relatively ubiquitous, co-occurring with all species except Spiny Dogfish and Cownose Rays, while Smooth and Spiny Dogfish co-occurred with three species each. Blacknose and blacktip sharks only co-occurred with Atlantic Sharpnose Sharks and Southern Stingrays. Of the seven principle species, only Spiny Dogfish co-occurred with Cownose Rays (Figure 3).

Discriminant function analysis correctly classified 59.15 % of sharks to species based on habitat variables (Wilks' Lambda = 0.135, $p < 0.0001$). Canonicals 1 and 2 explained 91.9 % of the variance and were driven primarily by temperature and dissolved oxygen, respectively (Table 3). Spiny and Smooth Dogfish appeared to be isolated from the other species by temperature, while Atlantic Sharpnose and Blacknose sharks overlapped completely (Figure 4). Spiny Dogfish had the highest percentage of correct classification (95.00 %), followed by Blacktip Sharks (71.43 %) (Table 3). Approximately half of Blacknose Sharks (53.85 %) and Smooth Dogfish (50.00 %) were correctly classified, and fewer than half of the individuals of all other species were correctly classified based on habitat variables (Table 3).

Watson's two-sample test results showed that differences between mean set time and overall set time were significant for Spiny Dogfish and marginally significant for Smooth Dogfish (Table 5). Mean set time for Spiny Dogfish differed significantly from all other principle species (Table 6). A significant difference in set time was also found between Cownose Rays and Smooth Dogfish, and a weakly significant difference between Blacknose Sharks and Smooth Dogfish (Table 6). Significant differences between principle species were found for all factors except depth (Table 7). Generally Smooth and Spiny Dogfish showed similar environmental ranges (signified by overlapping letters in Tukey's HSD analysis) while Atlantic Sharpnose Sharks, Blacktip Shark, Blacknose Sharks, and Southern Stingrays showed overlapping ranges for temperature, salinity, and dissolved oxygen. The only species with no overlapping letters in inlet distance were Blacknose and Blacktip sharks (Table 7).

Acoustic Telemetry

A total of four sharks were acoustically tagged: one Blacktip shark and three Blacknose sharks (Table 8). All tagged sharks were male. The Blacktip Shark fell within immature size range for the species, while all tagged Blacknose sharks were over the size at maturity. No Bonnethead sharks were captured, and available tagging equipment was insufficient to penetrate the skin of one 2.5-m TL Bull Shark caught during the 2015 survey. Inclement weather forced the release of other Blacktip and Blacknose Sharks without tagging.

Range testing showed that detection was possible up to 500 m from receivers at deep channel sites, but detection probability dropped sharply in shallow habitat

areas, with maximum detection range estimated at 50 m into seagrass, sand flat, or oyster reef habitats. More detailed analysis of detection probability was prevented by a sudden onset of high wind during range testing.

Of the tagged sharks, two Blacknose sharks were detected on the array. Blacknose shark 1488 was detected continuously on the GN and ON receivers from August 10, one day after tagging, until November 11 when both receivers were recovered. This suggests that this shark suffered post-release mortality; its transmitter settled on the bottom within detection range of both receivers. Shark 1483 was detected briefly on the SN receiver approximately two hours after tagging and was not detected again for the rest of the study (Figure 5).

Three other transmitters were detected during the study period, corresponding with a Bull Shark, a Finetooth Shark (*Carcharhinus isodon*), and a Cownose Ray, each tagged by other researchers within the ACT Network (Table 9). Of these animals, the bull shark, finetooth shark, and weakfish made multiple visits to receiver sites (Figure 5).

Tagged animals were detected on all receivers with the exception of GN, though this receiver did detect the presumed stationary transmitter deployed on Blacknose Shark 1488 (Figure 6). The Bull Shark was detected on all receivers except those in seagrass habitats. The SF receiver made up a slight majority of Bull Shark detections, but was followed closely by the two receivers in oyster reef habitats. An equal number of Finetooth Shark detections were recorded on the GF and SF receivers. The Cownose Ray was detected exclusively at oyster reef sites and the Blacknose Shark was only detected at the SN receiver (Figure 6).

The majority of detections for all species were recorded at night, with the exception of the Finetooth Shark, which was recorded an equal number of times during day and night hours (Figure 6). The Bull Shark was the only species detected during twilight hours. Both the Blacknose Shark and Cownose Ray were only detected at night. Detection locations varied by time of day for both the Bull and Finetooth Sharks. The Bull Shark was detected more often at near-inlet sites during twilight hours than at night, though the only daytime detection of this shark occurred at a site far from the inlet. The majority of nighttime Bull Shark detections occurred at the sand flat and oyster reef sites far from the inlet. The Finetooth Shark was only detected at stations far from the inlet, and occurred at a seagrass site during daytime hours and a sand flat at night. All other tagged animals were detected only at one station and strictly occurred at night (Figure 6).

Of all the tagged animals detected at the Middle Marsh receiver sites, the Bull Shark was detected most often and was the only species to be detected at multiple receivers in the same day, allowing for more detailed analysis of temporal habitat use patterns. The Bull Shark appeared to be mostly nocturnal, with time of detection ranging between 2046 and 0739 hours. The mean hour of detection was 0236 (± SD 102) hours and Rayleigh's test results showed this time distribution to be significant ($Z = 0.5883$, $p = 0.003$).

The low sample size of detections precluded statistical analysis of detection time on individual receivers. The CF, ON, OF, and SF receivers were visited on multiple days during the time of receiver deployment, and on October 8 the Bull Shark was detected over a continuous 20-minute period at receiver SF followed by a

12-minute period at receiver ON. Though receiver SF accounted for the most Bull Shark detections at a single site, the two oyster reef sites combined had more Bull Shark detections than the sand flat sites.

Discussion

Catch data from the fishery-independent survey show evidence of spatial resource partitioning between some of the seven principle species, with some potential evidence for temporal resource partitioning on a seasonal scale. Acoustic telemetry data were limited but provided evidence of diel and habitat type preferences for Bull Sharks. Though it is clear that more data are needed, the results from this study provide basic data on elasmobranch habitat use in this estuary that can be used as a baseline for future studies.

Based on the presence of neonate and age-0 size classes and direct observation of parturition, Back and Core Sounds function as primary nurseries for Atlantic Sharpnose Sharks, Cownose Rays, and Smooth Dogfish (Smith and Merriner 1987, Conrath and Musick 2002, Loefer and Sedberry 2003). Adults of both Atlantic Sharpnose Sharks and Smooth Dogfish were captured over relatively short periods of time (late April-May for Smooth dogfish, May-June for Atlantic Sharpnose Sharks) but both males and females were present, possibly indicating that adults of these species enter Back and Core Sounds to give birth and mate before returning to near shore oceanic habitats. Older juveniles and subadults were not documented for any of these species, suggesting that immature size classes may make use of near shore

oceanic or estuarine habitats not covered by this survey. Juvenile Atlantic Sharpnose Sharks greater than the age-0 size class primarily occur in coastal nearshore habitats along the South Carolina coast (Ulrich et al. 2007), and similar behavior by this species in North Carolina waters may explain the lack of older juveniles in this survey. The appearance of both adult and juvenile Smooth Dogfish coincides with the timing of parturition in confirmed nurseries to the north (Rountree and Able 1996) and could indicate that Back and Core Sounds function as a primary nursery near the southern extent of their distribution. However, juvenile Smooth Dogfish were rare in comparison with their ubiquity in eastern Pamlico Sound (Chapter 2, this volume), perhaps indicating that the Back/Core Sound system is not a major nursery. Captures of age-0 and adult Cownose Rays coincided with observed migratory patterns through the North Carolina sounds (Smith and Merriner 1987, Goodman et al. 2010), though captures of juveniles in August may indicate that some individuals remain in Back Sound or Newport River for the summer. All Blacktip Sharks were juveniles over the age-0 size range (Barry et al. 2008) and none were captured prior to June. A similar size range and temporal pattern was observed in Pamlico Sound (Chapter 2, this volume). Blacktip Sharks are known to give birth in South Carolina estuaries (Castro 1996, Ulrich et al. 2007) and may infrequently use the North Carolina coast south of Cape Fear as a primary nursery (Thorpe et al. 2004) but Back and Core Sounds likely represent a secondary nursery or foraging habitat for older juveniles born in primary nurseries to the south.

Generally, all analyses showed similar results for habitat use comparisons between the seven principle species. The primary difference between most species was temperature, with a cold-temperature group that included the Spiny and Smooth Dogfishes as well as the Cownose Ray, and a warm-temperature group that included the Blacknose and Blacktip Sharks and the Southern Stingray. Spiny Dogfish had the highest affinity for cooler temperatures and occurred primarily in the first half of the survey period. This behavior is somewhat consistent with the seasonal presence of this species in the southeastern U.S. (Stehlik 2007, Ulrich et al. 2007, see also Chapters 1 and 2, this volume), though Spiny Dogfish in Back and Core Sounds occurred at higher temperatures and later in the year than previously recorded (Bangley and Rulifson 2014). Smooth Dogfish also occurred primarily at lower temperatures, though juveniles were sporadically captured throughout the survey season. This closely matches observations from Bulls Bay, South Carolina, where adult females leave the estuary as the water warms but may give birth before migrating north (Castro 1993). These two species, particularly the Spiny Dogfish, are likely the dominant shark species in Back and Core Sounds during cooler months.

The most striking example of resource partitioning in the fishery-independent survey was between Blacknose and Blacktip Sharks, which never co-occurred in survey sets but had no significant differences in habitat variables other than inlet distance. Blacknose Sharks were captured much closer to inlets than Blacktip Sharks, which is consistent with shark survey results from South Carolina, where Blacknose Sharks within estuarine waters were only captured in close

proximity to inlets (Ulrich et al. 2007). However, Blacktip Sharks show a preference for proximity to inlets in other estuaries, particularly those with a wide salinity range (Froeschke et al. 2010, Chapter 2, this volume). Back and Core Sounds have relatively low freshwater input and most salinity measurements taken during sampling were within the ranges in which both species have been observed in South Carolina waters (Ulrich et al. 2007), which may mean that sharks are not spatially constrained by salinity in this system. The difference in inlet distance between the two species may represent spatial resource partitioning between similarly-sized adult Blacknose Sharks and juvenile Blacktip Sharks. Though diet studies specific to North Carolina waters are lacking, data from other locations suggest a relatively high trophic overlap between Blacknose Sharks and juvenile Blacktip Sharks, with an emphasis on sciaenid and clupeid fishes in the diet of both species (Castro 1996, Bethea et al. 2004, Ford 2012).

The Atlantic Sharpnose Shark proved to be a generalist with habitat preferences that overlapped all of the principle species, and was captured concurrently at least once with every species except the Cownose Ray. Atlantic Sharpnose Sharks co-occurred with Blacknose, Blacktip, and Sandbar Sharks large enough to be a predatory threat, and one Blacktip Shark was captured while attempting to prey on a small Atlantic Sharpnose Shark. The projected habitat area for this species in Pamlico sound overlapped habitat for Blacktip and Sandbar Sharks as well (Chapter 2, this volume). Generally, juvenile sharks are thought to prioritize predator avoidance over foraging opportunities in estuarine habitats (Bush and Holland 2002, Heupel and Hueter 2002), which suggests that Atlantic

Sharpnose Sharks, particularly the young-of-year juveniles that were nearly ubiquitous in the estuary during the summer months, should select habitat that minimizes their potential to encounter larger shark species. This apparent mismatch may be explained by the relationship between life history and risk tolerance that occurs in some teleosts, in which shorter-lived, faster-growing species are more likely to risk predation to increase foraging opportunities (Frid et al. 2012). Atlantic Sharpnose Sharks grow relatively rapidly compared to other most of the other shark species present in this estuary (Loefer and Sedberry 2003) and habitat use patterns observed in the northern Gulf of Mexico show a similar lack of predator avoidance (Carlson et al. 2008). The Atlantic Sharpnose Sharks present in Back and Core Sounds appear to follow a similar strategy, occurring across a wide variety of habitats in order to increase foraging opportunities.

Few studies on elasmobranch habitat use include detailed information on batoids. Cownose Rays appeared to select for mid-range temperatures that primarily occurred during the spring and fall, which matches observations of mass migrations of this species through Back and Core Sounds during these seasons (Peterson et al. 2001, Goodman et al. 2010). Though Cownose Rays had little habitat or spatial overlap with the other principle species, Bullnose Rays (*Myliobatis freminvelli* Lesueur, 1824) were often captured concurrently with them. Southern Stingrays, like Atlantic Sharpnose Sharks, showed highly generalist habitat preferences, though captures of this species primarily occurred at warmer temperatures. Other studies of Southern Stingray habitat use show that this species tends to move between habitat patches (Tilley et al. 2013) and their foraging habits

may be more associated with tidal cycles than temporal or environmental factors (Gilliam and Sullivan 1993). Patterns of habitat use by other ray species with similar morphology and ecology to Southern Stingrays are influenced by the presence of predators, particularly large sharks (Vaudo and Heithaus 2013), and the abundance of the Southern Stingray in Back and Core Sounds may make it an ideal model species for studying habitat use changes in response to predation risk in this system.

Data on diel temporal habitat partitioning from fishery-independent sampling were inconclusive with the exception of the Spiny Dogfish, which showed a strong affinity for mid-afternoon. This species is known to form aggregations (Stehlik 2007), and significant differences in capture time from other species may be a result of high catches in individual sets during mid-afternoon hours. The Blacknose Shark appeared to be more nocturnal than the other principle species, though set time only differed significantly from Smooth and Spiny dogfish, which were both primarily captured in mid-afternoon. Most sampling occurred during afternoon and evening hours, which may have under-sampled species that appeared to be more active before noon or after midnight such as Bonnethead Sharks, Bullnose Rays, and Bull Sharks. Future efforts should attempt to sample equally during the entire 24-hour diel cycle.

It is impossible to generalize habitat use patterns from single individuals, but acoustic telemetry results did generate some interesting observations. Most tagged animals were detected at night, though the Bull Shark was also detected during twilight hours and the Finetooth Shark occurred equally during both day and night

hours. Other studies in estuarine habitats have found mobile predators to be more abundant and active at night (Grabowski et al. 2005, Hammerschlag et al. 2010). Tag detections were greater in shallow, more complex habitats than deep channels despite a much greater detection range in deeper habitats, with oyster reefs as the preferred or exclusive habitat for most of the animals detected on the Middle Marsh array. The relationship between structured habitat and predators is complex: while habitat such as oyster reefs may serve as a refuge for prey species, the increased abundance of prey in such habitats may also attract predators, enhancing predation risk at habitat edges (Gorman et al. 2009, Hammerschlag et al. 2010). In North Carolina waters, oyster reefs have been found to enhance the local presence and abundance of some mobile predator species, including elasmobranchs, while reducing the abundance of others (Grabowski et al. 2005, Pierson and Eggleston 2014).

The most comprehensive tag detection data were gathered for a single Bull Shark tagged outside of the study area that made repeated visits to various sites within the Middle Marsh array from late September through mid-October. This shark was 2.13 m TL at the time of tagging in 2010 (Debra Abercrombie, Stony Brook University, personal communication), and likely functions as an apex predator within the estuary. It was detected almost exclusively between sunset and sunrise, which is consistent with the nocturnal feeding habits documented for this species (Driggers et al. 2012). Bull Sharks are known to prey on large teleosts and other sharks (Snelson et al. 1984, Hammerschlag et al. 2012), making this individual a predatory threat to most of the other species documented during the study. The

timing of this shark's presence at Middle Marsh overlaps the diel temporal range of the largest sharks captured during fishery-independent sampling, including another adult-sized bull shark and relatively large Blacknose and Sandbar Sharks, suggesting that risk of predation by large sharks in Back and Core Sounds may be elevated at night. Large sharks are usually thought to occur in deeper water within estuaries, but this shark made frequent visits to shallow habitats, at least two of which extended longer than 10 minutes. This may be explained by the effects of habitat on prey: shallow water may reduce the maneuverability of a large shark, but it also may impede escape for prey confined to the same habitat, making foraging or pursuit of prey into shallow habitats an effective hunting tactic (Heithaus et al. 2009).

Though acoustic receiver sites were chosen to represent the specific habitat type that was visually dominant during deployment, habitat was likely a mosaic of several substrate or structure types at each site. This limits the ability to positively define associations between tagged animals and particular habitat types due to likely complex relationships between habitats (Grabowski et al. 2005). For example, the Bull Shark detected at oyster reef receivers may have actually been foraging in adjacent sand flat areas within detection range. Habitat types within detection range should be surveyed thoroughly to determine the combination of habitats and the relative area taken up by each.

Overall, elasmobranchs in Back and Core Sounds were spatial and temporal generalists, occurring throughout the estuary wherever and whenever environmental conditions allowed, with strong evidence for spatial resource partitioning only found between similarly-sized Blacknose and Blacktip Sharks.

This suggests that elasmobranchs in this system are highly mobile and likely utilize multiple habitat types and spatial regions within the estuary. Use of multiple habitat types over a broad spatial area is a common tactic for mobile estuarine predators and has been previously documented for some of the elasmobranch species in this study (Heupel and Hueter 2002, Collins et al. 2007, Carlson et al. 2008, Tilley et al. 2013, Drymon et al. 2014) as well as other mobile species such as Red Drum (*Sciaenops ocellatus*) (Fodrie et al. 2015).

Though combining fishery-independent sampling with acoustic telemetry can provide a more comprehensive assessment of habitat use within an estuary than either method alone, conflicts between the strategies of the two surveys limited the study. The stratified-random sampling strategy employed during sampling reduced the probability of encountering target species, ultimately reducing the number of tags deployed. The ability to coordinate with other researchers is an advantage of involvement in a network of acoustic arrays (Donaldson et al. 2014, Hussey et al. 2015a) and allowed us to make observations on habitat use by mobile predators despite the unexpected limitations of our own tagging efforts. Further efforts to combine fishery-independent survey and acoustic telemetry methods should better balance the needs of the two techniques, and information on habitat preferences gathered by this study can be used to inform future sampling targeting particular species. With further refining of sampling and tagging strategies, these combined methods will be effective in answering questions about estuarine habitat use by mobile predators such as sharks.

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Tables

Table 3-1. Total catch, catch by gear (DL=drumline, GN=gillnet, LL=longline, RR=rod and reel), and minimum and maximum total length and fork length or disc width (FL/DW, mm) for species captured during 2014-2015 elasmobranch sampling in Back and Core Sounds, North Carolina. Species marked with an asterisk were considered principle species for further analysis.

		Catch by Gear					
Species	Total Catch	DL	GN	LL	RR	Min-Max TL (mm)	Min-Max FL/DW (mm)
Atlantic sharpnose shark*	33	0	6	26	1	326-1000	272-826
Blacknose shark*	13	1	0	12	0	990-1320	798-1115
Blacktip shark*	7	0	0	7	0	1112-1295	885-1030
Bluntnose stingray	5	0	5	0	0	-	150-450
Bonnethead	3	0	3	0	0	955-1080	762-870
Bull shark	1	1	0	0	0	2500	2090
Bullnose ray	4	0	4	0	0	-	436-458
Carcharhinus sp.	2	0	2	0	0	-	-
Cownose ray*	12	0	9	3	0	-	414-1016
Sandbar shark	3	0	0	3	0	1219-1600	991-1195
Smooth dogfish*	10	0	4	6	0	605-1265	512-1065
Southern stingray*	27	0	3	24	0	-	405-960
Spiny dogfish*	40	0	5	32	3	790-970	698-860

Table 3-2. Mean ± standard deviation (SD) temporal, environmental, and spatial factors for each elasmobranch species captured during 2014-2015 sampling in Back and Core Sounds, North Carolina.

	Mean ± SD						
Species	Set Time (hours)	Depth (m)	Temp (°C)	Salinity (ppt)	DO (mg/L)	Inlet Distance (km)	SAV Distance (m)
Atlantic sharpnose shark	1632 ± 059	2.55 ± 1.23	26.56 ± 3.14	32.28 ± 3.36	7.95 ± 1.46	6.75 ± 5.02	569.73 ± 420.41
Blacknose shark	1908 ± 042	3.17 ± 1.82	25.98 ± 2.79	34.47 ± 2.89	8.39 ± 1.44	3.53 ± 2.20	791.46 ± 639.24
Blacktip shark	1554 ± 059	2.85 ± 1.87	29.31 ± 0.90	31.56 ± 3.47	6.75 ± 1.48	9.48 ± 5.27	559.20 ± 637.74
Bluntnose stingray	1551 ± 036	1.40 ± 0.82	26.32 ± 3.80	32.72 ± 1.03	7.92 ± 0.74	5.37 ± 1.58	33.91 ± 38.14
Bonnethead	1029 ± 017	1.08 ± 0.65	25.93 ± 1.62	27.87 ± 0.98	6.76 ± 0.24	6.35 ± 1.99	40.50 ± 10.32
Bull shark	1936 ± 0	3.81 ± 0	29.00 ± 0	38.60 ± 0	8.38 ± 0	1.17 ± 0	490.27 ± 0
Bullnose ray	1310 ± 029	2.17 ± 0.42	24.08 ± 1.04	33.40 ± 0.49	7.61 ± 0.66	11.03 ± 5.29	179.84 ± 145.56
Carcharhinus sp.	1105 ± 0	2.44 ± 0	22.80 ± 0	28.50 ± 0	7.30 ± 0	15.48 ± 0	228.77 ± 0
Cownose ray	1532 ± 044	2.11 ± 0.50	25.52 ± 3.37	29.98 ± 2.44	8.81 ± 1.38	7.87 ± 5.18	245.99 ± 143.17
Sandbar shark	1944 ± 033	3.40 ± 1.20	24.30 ± 4.69	34.50 ± 2.69	8.36 ± 1.37	5.33 ± 4.27	389.92 ± 494.23
Smooth dogfish	1738 ± 041	2.74 ± 0.71	21.51 ± 3.79	34.18 ± 2.42	8.24 ± 0.69	7.66 ± 5.45	489.40 ± 385.92
Southern stingray	1706 ± 052	3.24 ± 1.53	28.03 ± 1.31	32.21 ± 4.70	9.77 ± 2.58	4.81 ± 2.76	665.84 ± 525.54
Spiny dogfish	1723 ± 028	2.92 ± 0.71	20.07 ± 1.64	35.43 ± 1.04	8.25 ± 0.44	5.65 ± 2.70	219.32 ± 200.35

Table 3-3. Canonical values, cumulative percent of variance explained, and correlations with environmental factors for canonicals generated by discriminant function analysis.

			Canonical Structure					
Canonical	Eigenvalue	Cumulative %	Set Time	Temp	Sal	DO	SAV Dist	Inlet Dist
1	3.29	84.45	-0.18	0.92	-0.44	0.12	0.43	0.01
2	0.29	91.95	-0.11	0.01	-0.17	0.89	-0.09	-0.32
3	0.24	98.09	0.43	-0.08	0.68	0.23	0.47	-0.70
4	0.04	99.14	0.40	-0.23	0.09	0.27	0.59	0.17
5	0.03	99.80	0.78	0.10	-0.27	-0.05	-0.16	-0.28
6	0.01	100.00	0.04	0.29	0.48	0.27	-0.46	0.54

Table 3-4. Percentage (%) of individual sharks classified as each species based on temporal, environmental, and spatial factors using discriminant function analysis. Correct classifications are in bold.

Species	Atl sharpnose shark	Blacknose shark	Blacktip shark	Cownose ray	Smooth dogfish	Southern stingray	Spiny dogfish
Atl sharpnose shark	**33.33**	21.21	9.09	0.00	6.06	27.27	3.03
Blacknose shark	0.00	**53.85**	0.00	0.00	15.38	30.77	0.00
Blacktip shark	0.00	14.29	**71.43**	14.29	0.00	0.00	0.00
Cownose ray	8.33	0.00	0.00	**41.67**	16.67	25.00	8.33
Smooth dogfish	20.00	0.00	0.00	10.00	**50.00**	0.00	20.00
Southern stingray	14.81	7.41	11.11	18.52	0.00	**48.15**	0.00
Spiny dogfish	0.00	0.00	0.00	0.00	5.00	0.00	**95.00**

Table 3-5. Watson's two sample test results comparing mean set time (hours) for principle elasmobranch species with overall mean sampling time in Back and Core Sounds.

Species	Mean Set Time	U^2	p
Atlantic Sharpnose Shark	1632 ± 059	0.1	> 0.10
Blacknose Shark	1908 ± 042	0.12	> 0.10
Blacktip Shark	1554 ± 059	0.06	> 0.10
Cownose Ray	1532 ± 044	0.12	> 0.10
Smooth Dogfish	1738 ± 041	0.17	< 0.10
Southern Stingray	1706 ± 052	0.06	> 0.10
Spiny Dogfish	1723 ± 028	0.86	< 0.001

Table 3-6. Watson's two-sample test of heterogeneity results (U^2) determining difference in mean set time between the seven principle species captured in Back and Core Sounds, North Carolina.

Species	Atl sharpnose shark	Blacknose shark	Blacktip shark	Cownose ray	Smooth dogfish	Southern stingray	Spiny dogfish
Atl sharpnose shark	-						
Blacknose shark	0.092	-					
Blacktip shark	0.062	0.078	-				
Cownose ray	0.097	0.1	0.1	-			
Smooth dogfish	0.117	0.155*	0.136	0.262***	-		
Southern stingray	0.067	0.085	0.077	0.107	0.135	-	
Spiny dogfish	0.485****	0.324**	0.270**	0.400****	0.403****	0.469****	-

*< 0.10, **< 0.01 ***< 0.05 ****<0.001

Table 3-7. ANOVA results showing significant differences in environmental and spatial habitat variables between principle elasmobranch species sampled in Back Sound, North Carolina, with Tukey's Honestly Significant Difference grouping letters.

ANOVA/Tukey Results	Depth	Temperature	Salinity	DO	Inlet Distance	SAV Distance
F	1.769	42.604	7.56	5.72	3.187	5.672
p	0.11	<0.0001	<0.0001	<0.0001	0.006	<0.0001
Atlantic sharpnose shark	A	AB	BC	B	AB	AB
Blacknose shark	A	AB	AB	AB	B	A
Blacktip shark	A	A	BC	B	A	ABC
Cownose ray	A	B	C	AB	AB	BC
Smooth dogfish	A	C	AB	AB	AB	ABC
Southern stingray	A	AB	BC	A	AB	AB
Spiny dogfish	A	C	A	B	AB	C

Table 3-8. Information for Blacktip and Blacknose Sharks acoustically tagged and released Back Sound, NC during 2015 sampling.

Shark ID	Species	Tagging Date	TL (mm)	Sex
1490	Blacktip shark	6/14/15	1295	M
1491	Blacknose shark	6/29/15	1280	M
1488	Blacknose shark	8/9/15	1162	M
1483	Blacknose shark	9/19/15	1005	M

Table 10. Species not tagged during 2015 sampling detected on receivers placed around Middle Marsh, with researchers and institutions responsible for tagging.

Species	Tagging Researchers	Institution
Bull shark	Debra Abercrombie	Stony Brook University
Finetooth shark	Eric Reyier	Kennedy Space Center Ecological Program
	Joseph Iafrate	Naval Undersea Warfare Center
	Stephanie Watwood	Naval Undersea Warfare Center
Cownose ray	Matthew Ogburn	Smithsonian Environmental Research Center
	Robert Fisher	Virginia Institute of Marine Science

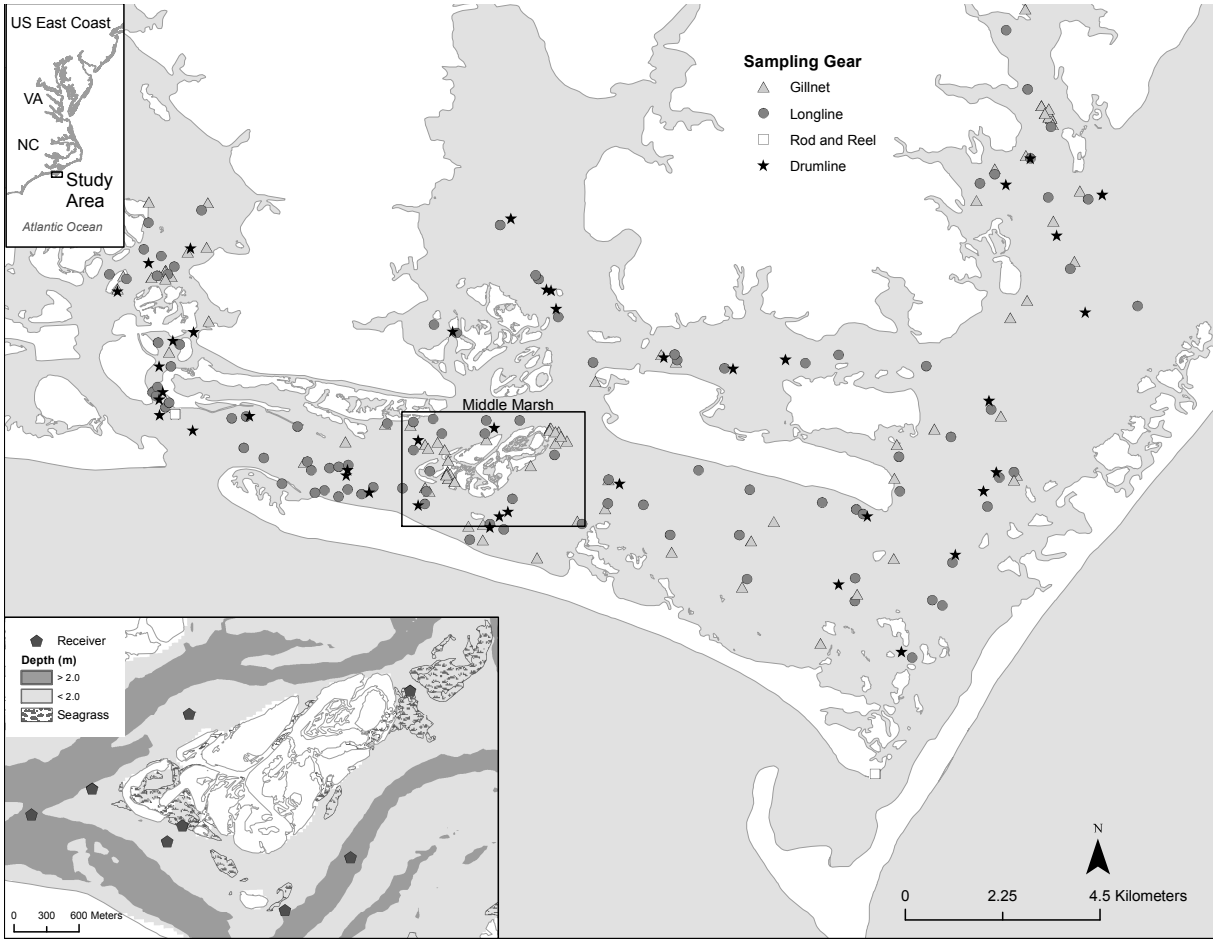

Figure 3-1. Fishery-independent gillnet, longline, rod and reel, and drumline sampling locations from 2014-2015 within Back and Core Sounds, North Carolina and locations of acoustic receivers deployed in 2015 in the vicinity of Middle Marsh (inset map).

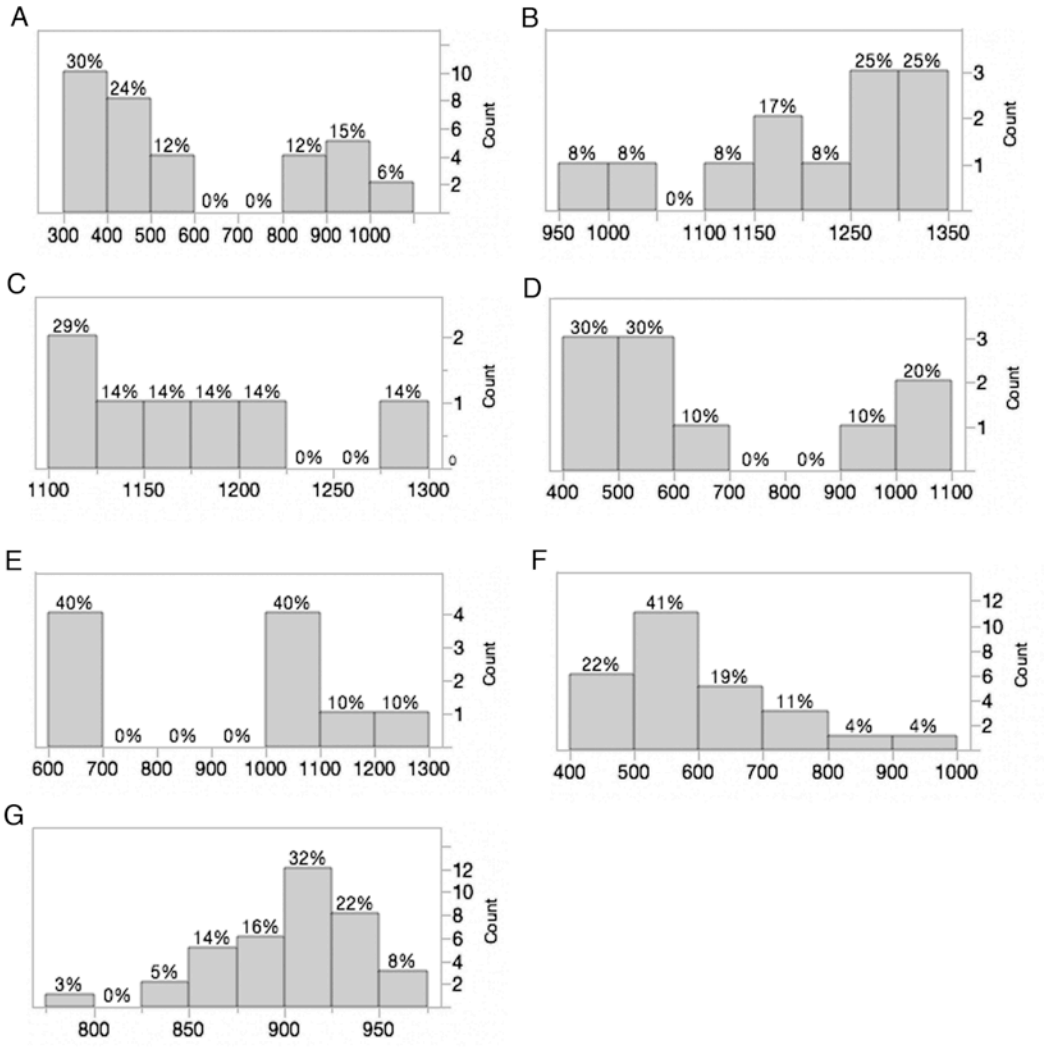

Figure 3-2. Length-frequency histograms with count and percentage of total population for A. Atlantic Sharpnose Sharks, B. Blacknose Sharks, C. Blacktip Sharks, D. Cownose Rays, E. Smooth Dogfish, F. Southern Stingrays, and G. Spiny Dogfish catpured in Back and Core Sounds during 2014-2015 sampling. Lengths are total length (mm) for sharks and disc width (mm) for rays.

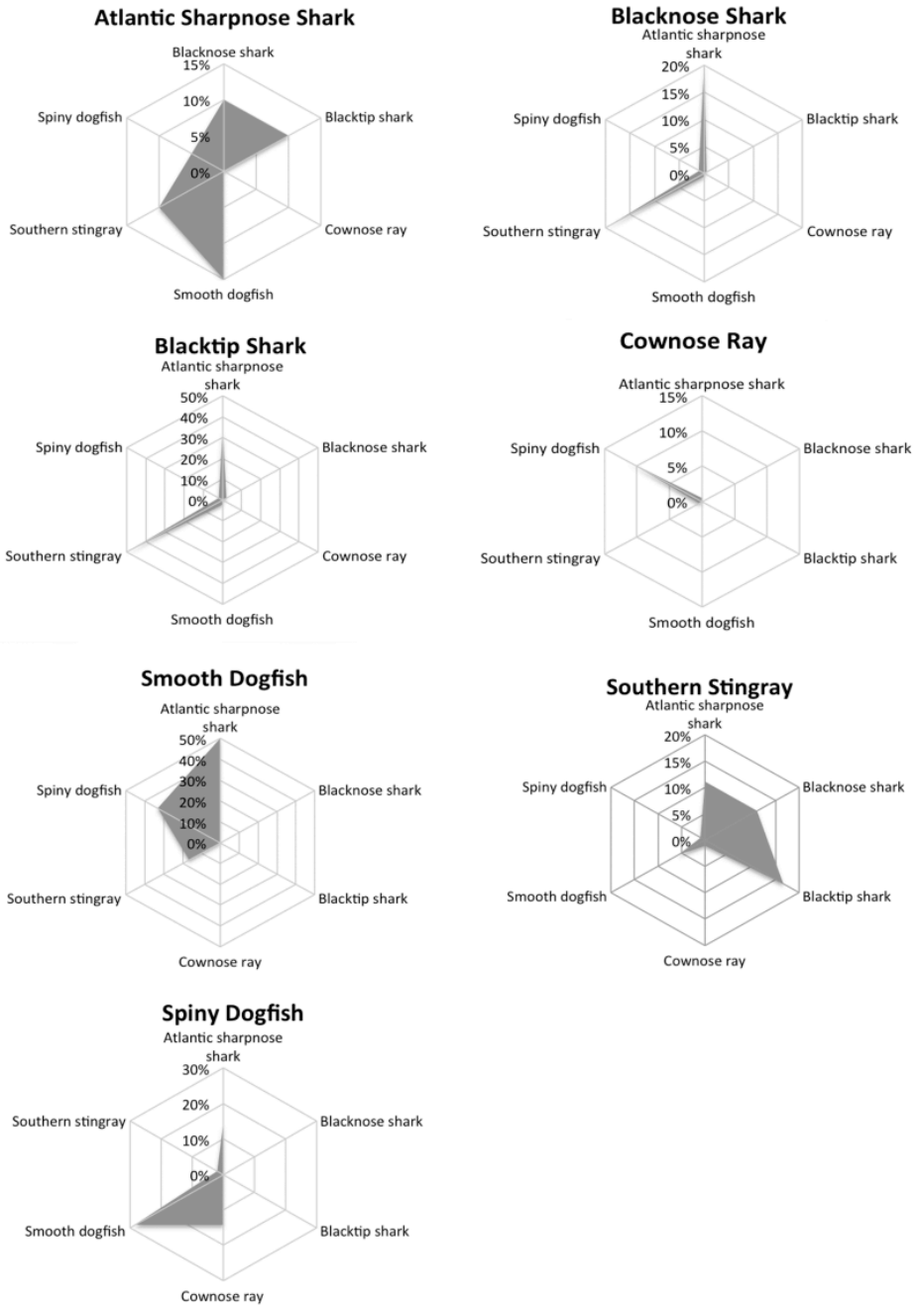

Figure 3-3. Radio graphs depicting spatial overlap between each principle elasmobranch species and the other principle species, expressed as the percent of sets in which the given species was caught with the other.

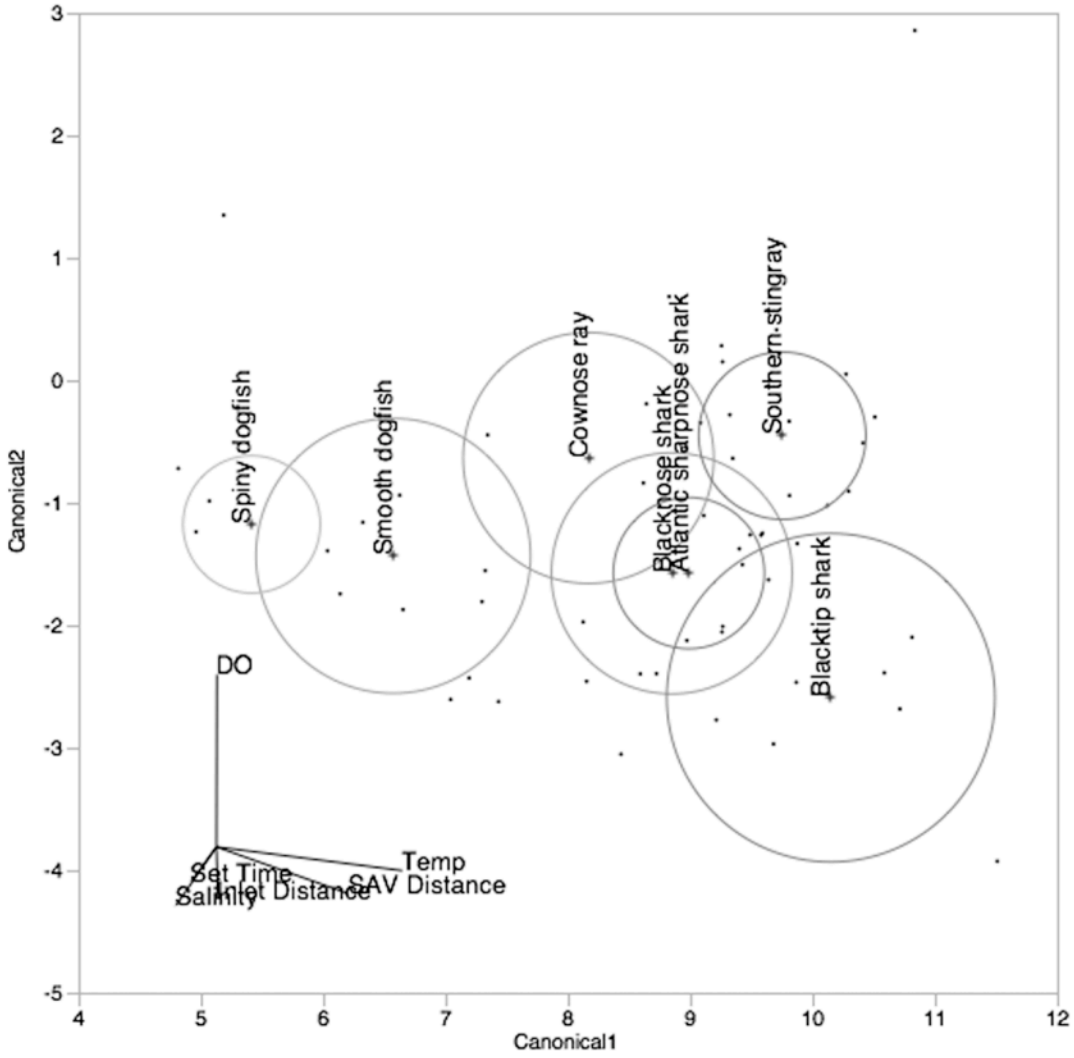

Figure 3-4. Discriminant function analysis plot classifying principle elasmobranch species based on temporal, environmental, and spatial factors. Ellipses represent 95 % confidence intervals. Biplot rays are placed off-center to improve legibility.

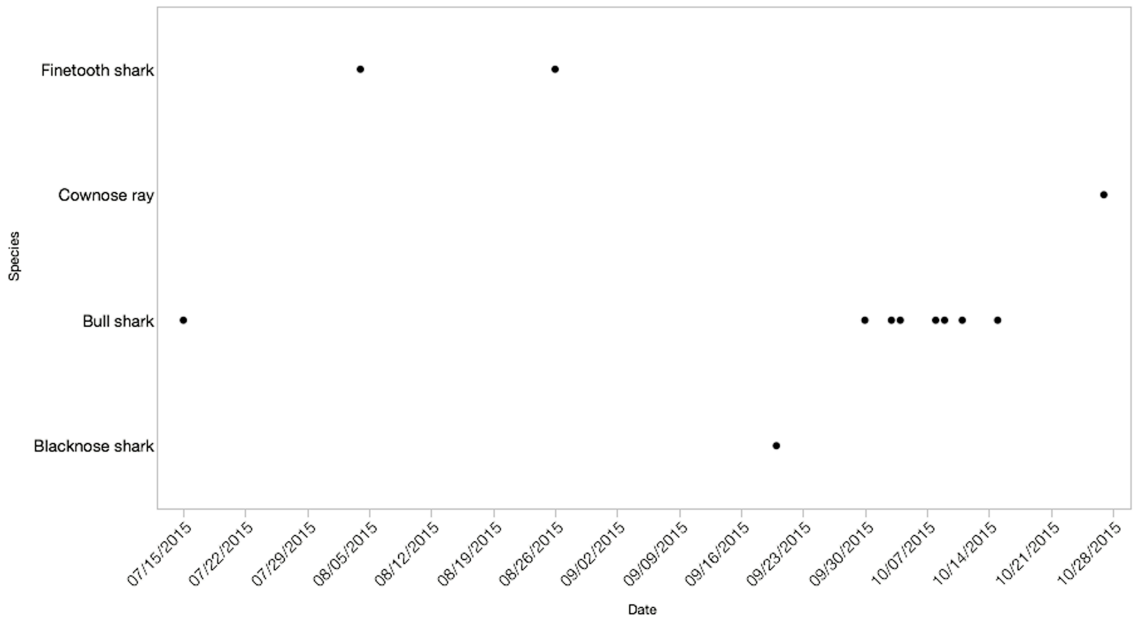

Figure 3-5. Dates of detection for each species recorded by acoustic receivers deployed in the vicinity of Middle Marsh, North Carolina.

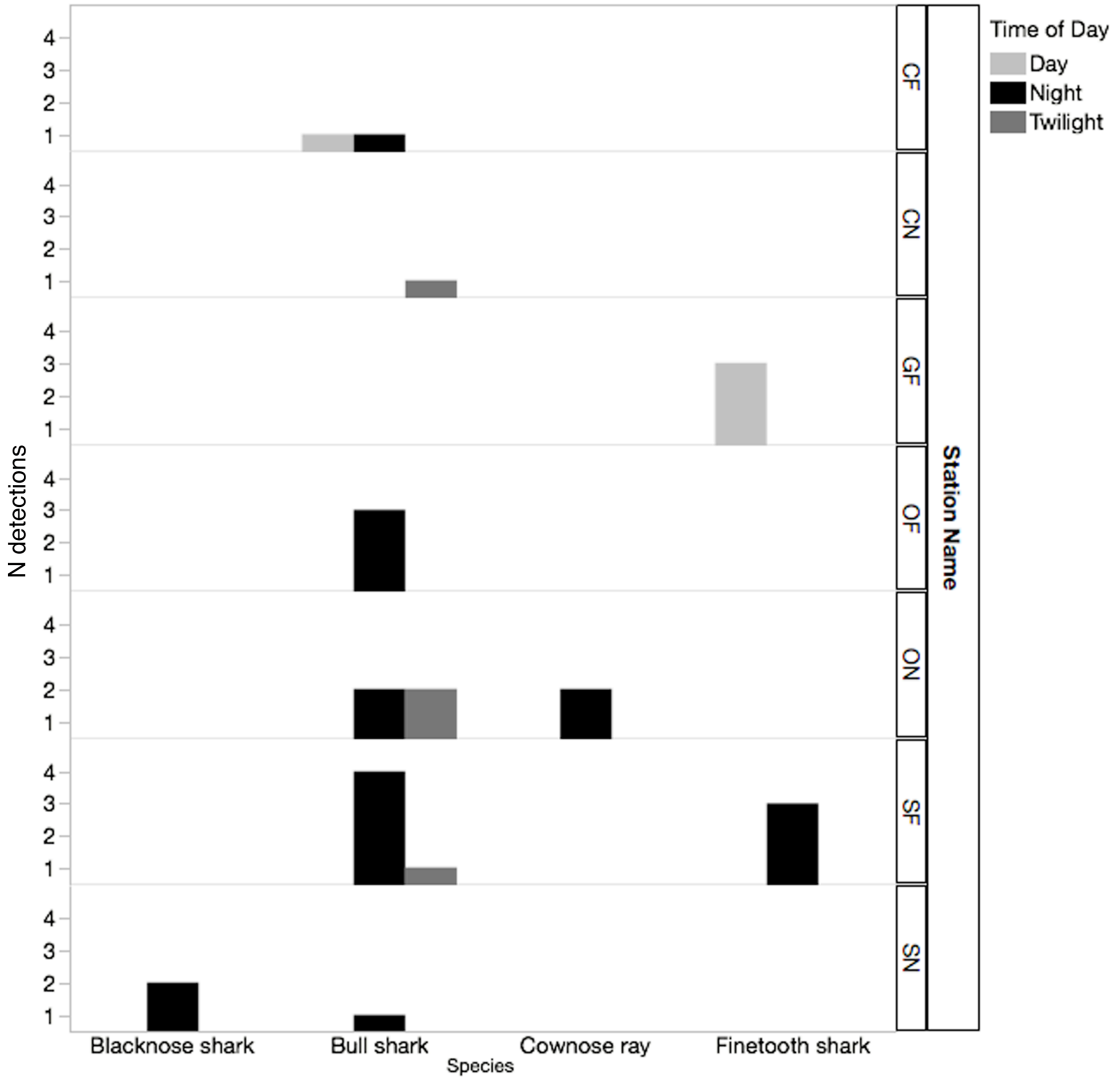

Figure 3-6. Number of detections by time period at each Middle Marsh acoustic receiver site for each species recorded within the array.

Chapter 4

Inferring Coastal Shark Habitat Use Patterns from the Local Ecological Knowledge of North Carolina Fishermen

Abstract

Local ecological knowledge can be useful in assessing the limits of fishery-independent sampling and identifying avenues for future research, but efforts to verify observations with external information should be included as a way to measure confidence. In this study, structured open-ended interviews were conducted with North Carolina fishermen experienced in either targeting or encountering coastal sharks. Subjects selected for interviews were chosen by first interviewing a single contact in each of three sampling locations: Wanchese, Hatteras, and Cape Lookout. Further interview subjects were chosen using a snowball sampling strategy in which fishermen referred by a previously interviewed fisherman were contacted. This sampling strategy resulted in interviews with eight fishermen spread across all sampling locations. Interview responses were compiled and similar observations made by at least 50 % of interviewed fishermen were considered hypotheses and tested against information available from surveys conducted during this dissertation research, other fishery-independent shark surveys, and primary literature. All hypotheses generated by fisherman observations were supported or could be considered plausible based on comparisons with external data. Key observations included increasing shark populations, migrations associated with movements and behavior of prey species,

and the importance of Blacktip Sharks (*Carcharhinus limbatus*) and Sandbar Sharks (*Carcharhinus plumbeus*) in North Carolina fisheries. Plausible hypotheses worthy of future study included specific habitat characteristics identified for Sandbar Sharks, Dusky Sharks (*Carcharhinus obscurus*), Hammerheads (*Sphyrna* sp.), and Tiger Sharks (*Galeocerdo cuvier*).

Introduction

Management and conservation of highly migratory species such as coastal sharks can be complicated by the wide-ranging activity areas of these species and their ability to cross jurisdictional boundaries (Speed et al. 2010). However, many shark species show predictable movements and site fidelity to particular areas that may be of greater importance to survival than other areas within activity range (Hueter et al. 2005, Chapman et al. 2015). Identification of these areas is a crucial component of management and conservation areas for these species (Hueter et al. 2005) but habitat delineation efforts are limited by the ability of researchers to cover a sufficient spatial and temporal sampling range. The advancement of telemetry methods has allowed researchers to expand the spatial and temporal range of observations of marine species (Donaldson et al. 2014, Hussey et al. 2015) but initial baseline data are still needed to efficiently deploy tracking devices and receivers (Heupel and Heuter 2001).

Incorporating the local ecological knowledge (LEK) of fishermen can allow researchers to fill gaps in knowledge and can be effective in informing sampling

efforts (Johannes et al. 2000). With proper planning the use of ethnographic methods such as free lists and interviews can generate data that can be analyzed using the same methods as fishery-independent field data (Garcia-Quijano 2007). Fishermen often make use of marine environments at different spatial and temporal scales than standardized scientific surveys, and differences between their observations and data collected by existing surveys can lead to empirically-testable hypotheses (Davis et al. 2004). Observations can be compared with existing or concurrently-collected fishery-independent data to assess the likelihood that they represent valid hypotheses (Silvano and Valbo-Jørgensen 2008). When LEK observations can be verified with independently-collected data, novel observations from the same sources can be considered valid in informing management decisions and further research (Gilchrist et al. 2005, Davis and Ruddle 2010). This approach has identified causes and solutions to fishery management issues that may not have been found through the use of standardized field sampling alone (Johannes et al. 2000).

Though ethnographic methods focusing on sharks primarily have been used to characterize fisheries (e.g. Fisher and Ditton 1993), these methods may also be helpful in identifying aspects of coastal shark ecology. Maynou et al. (2011) used LEK to assess population declines among sharks and other marine megafauna in the Mediterranean Sea that matched those observed in scientific surveys. This approach has also been used to identify potential areas of estuarine and riverine shark habitat in remote parts of Fiji (Rasalato et al. 2010). Sharks provide some advantages for conducting LEK surveys: their presence is generally obvious, species

can be readily identified by locals, and they can be culturally significant in some areas (Rasalato et al. 2010).

The goal of this study was to assess the use of LEK in studies of shark habitat use within North Carolina waters. The validity of LEK data was determined by comparing the observations of local fishermen with independently collected data from recent surveys of coastal shark habitat, including surveys undertaken as part of this dissertation and other fishery-independent surveys. If LEK was determined to be a valid source of information on shark habitat use patterns, then novel hypotheses were identified and potential methods for testing them were discussed.

Methods

Sampling effort was focused the North Carolina coast between Beaufort Inlet and Wanchese, encompassing the area of Raleigh Bay and the northern Outer Banks. Three main locations were chosen as starting points for sampling: Cape Lookout (including Atlantic Beach, Beaufort, and Down East communities), Hatteras Village, and Wanchese.

Fishermen were selected as interview subjects using a combination of convenience and snowball sampling (Goodman 1961). Initial contacts in each starting location were fishermen who have previously worked with the author on research involving sharks and who were considered well-informed about sharks in North Carolina waters. Structured open-ended interviews were conducted with the initial contacts, who were then asked to name and provide contact information for

other fishermen within their communities that they felt would provide high-quality information on shark habitat use. These fishermen were contacted for the next round of interviews and also asked to recommend further community members who they considered well-informed about sharks. This process was continued until attempts to contact the next round of fishermen were unsuccessful, or further recommendations only included fishermen who had already been interviewed or contacted. In an attempt to reduce bias, fishermen were not given any information on others who had been recommended for or participated in interviews, with the exception of naming at least one previous fisherman who recommended them during introduction.

Interviews were conducted opportunistically either in-person or over the phone and consisted of a series of standardized questions to which fishermen were allowed to provide open-ended answers. The questions were grouped into three parts (Table 1). Part 1 was comprised of questions establishing the fisherman's home port, fishing experience, and participation in targeted shark fisheries. For the purposes of this study Spiny Dogfish (*Squalus acanthias*) and Smoothhound (*Mustelus* sp.) fisheries were classified as targeted shark fisheries. If no participation in targeted shark fisheries was mentioned, the interview proceeded to Part 2, which was made up of questions about sharks encountered by the fisherman while targeting other species. If participation in targeted shark fisheries was mentioned, the interview proceeded to Part 3, which included more specific questions about species targeted and encountered while shark fishing. Fishermen responding to questions in this section were asked to specify whether the shark

species they mentioned were targeted or incidentally encountered. Part 3 also included a list of environmental factors with the potential to influence shark presence or abundance, to which fishermen were asked to answer "yes" or "no" regarding their perception of the importance of these factors to the likelihood of encountering sharks. All fishermen were asked all questions in Part 1 and either Part 2 or 3 in the same order, and were given the option to refuse to answer any individual question. Exact wording of interview questions was occasionally changed for clarity if the respondent indicated that they did not understand the question. Responses to interview questions were recorded by hand during the interview, and the name of the fisherman was not included on the sheet of responses.

Notes taken on responses were coded into standardized terms (Table 2). A fisherman's home port was defined as either where the fisherman moored their vessel or where they made most of their landings, depending on which of these options was emphasized. Responses regarding participation in fisheries were grouped into common themes (see Table 2 for examples). Responses directly referencing a particular shark species and/or environmental factor were also grouped accordingly. The percentage of fishermen directly referencing specific shark species, prey species, or environmental factors was calculated and plotted to identify species and environmental conditions that may be of particular importance to shark interactions with North Carolina fisheries.

Similar or identical observations made by 50 % or more of interviewed fishermen were considered potential hypotheses and were assessed using a modified version of the methodology of Silvano and Valbo-Jørgensen (2008).

Hypotheses from interview responses were generated by both general and species-specific observations mentioned by 50 % or more of the interviewed fishermen. Species-specific observations by 50 % or more fishermen mentioning a given species were used to generate species-specific hypotheses. Hypotheses were compared with data available from external sources. External sources included data collected while preparing other chapters of this dissertation, fishery-independent surveys conducted within North Carolina waters from state and federal fishery management agencies and universities, and information available from primary literature sources. Primary literature sources were found by searching for papers containing terms or concepts matching LEK hypotheses using Google Scholar.

Hypotheses were given one of four potential classifications based on the results of consultations of external data. Hypotheses based on observations that closely matched observations reported by external sources on the exact species, and within close geographic proximity to North Carolina, were classified as "Supported". Observations were classified as "Plausible" if matching or similar observations were reported by external sources on similar species or from geographic locations farther from the study area than adjacent state or federal waters, or if a general hypothesis was only supported on a species-specific basis. "Implausible" hypotheses were those that did not match any reported observations from external sources but were also not directly contradicted by them. Hypotheses were considered "Contradicted" if LEK observations were the opposite of observations from external sources. The observations of fishermen were considered a valid source of testable hypotheses if the majority of observations were classified as Supported.

Results

Snowball sampling did not proceed beyond the second round in any sampling locations. A total of eight fishermen were interviewed, equivalent to approximately 42.1 % of fishermen holding directed or incidental shark permits in the area of North Carolina between Atlantic Beach and Wanchese (19 federal directed or incidental shark permits) (NMFS 2016). All three main locations in the survey (Cape Lookout, Hatteras Village, and Wanchese) were represented in the sample. Areas identified as home ports and fishing areas encompassed the entire study area from Beaufort Inlet to Oregon Inlet (Figure 1). Identified fishing areas outside the study area included the continental shelf from Montauk, NY to Florida, the Eastern Shore region of Virginia, and southeastern North Carolina to Holden Beach. Experience ranged from 12-39 years of fishing with a mean (± standard deviation) of 25.1 ± 10.1 years. Five respondents were primarily commercial fishermen and three primarily participated in for-hire charter or guide services. Seven of the eight interviewed fishermen included sharks among their usual target species.

Responses to questions about fishery participation indicated that interviews covered a broad cross-section of fishermen with experience encountering sharks. Reasons for entering the fishery were relatively evenly distributed across respondents, with a majority (62.5 %) indicating reasons related to increased opportunity such as the availability of sharks when other target species were scarce

or encouragement from government agencies (Figure 2A). Gillnets were used by 62.5 % of interviewed fishermen, followed by rod and reel gear (50 %), bottom longline (37.5 %) and pelagic longline (12.5 %) (Figure 2B). Interviewed fishermen targeted a variety of species, with the highest percentage (50 %) targeting Tuna species (including Bluefin Tuna *Thunuus thyunnus*, Yellowfin Tuna *Thunnus albacares*, Blackfin Tuna *Thunnus atlanticus*, and False Albacore *Euthyunnus alletteratus*) followed by Bluefish (*Pomatomus saltatrix*), King Mackerel (*Scomberomorus cavalla*), and Spanish Mackerel (*Scomberomorus maculatus*) with 37.5 % of respondents each (Figure 2C). Fishing locations named or referenced by respondents included areas outside of North Carolina state ocean waters (62.5 % of respondents), within state ocean waters (87.5 % of respondents), and within estuarine waters (37.5 % of respondents) (Figure 2D).

A total of 18 shark species were named as either being targeted or encountered by fishermen (Table 3). Two species were targeted by a majority (71.4 %) of fishermen participating in directed shark fisheries: the Blacktip Shark (*Carcharhinus limbatus*) and Smooth Dogfish (*Mustelus canis*) (Figure 3A). The Dusky Shark (*Carcharhinus obscurus*) was reported as being encountered by the most respondents (62.5 %) and half of respondents included Hammerheads (*Sphyrna* sp.), Sand Tiger Sharks (*Carcharias taurus*), Sandbar Sharks (*Carcharhinus plumbeus*), and Tiger Sharks (*Galeocerdo cuvier*) among species they encountered (Figure 3B). When mentions of species in both targeted and encountered contexts were combined, Blacktip Sharks and Sandbar Sharks were the most commonly

mentioned species (87.5 % of respondents), followed by Dusky Sharks, Smooth Dogfish, and Spiny Dogfish (75 % of respondents) (Figure 3C).

Prey availability (100 % of respondents) and temperature (87.5 % of respondents) were most commonly identified as important environmental related to likelihood of encountering coastal sharks (Figure 4A). Menhaden (likely Atlantic Menhaden *Brevoortia tyrannus*) were the prey species most commonly named in association with shark movements and presence (65.2 % of respondents) followed by Bluefish and discarded shrimp trawl bycatch (Figure 4B).

Six hypotheses that could be applied to coastal sharks in general were generated by combining similar interview responses from at least 50 % of respondents. Of these, four were considered Supported based on robust support from primary literature and other chapters in this volume (Table 4). The other two hypotheses were considered Plausible due to limited or indirect support (in the case of migrations following prey availability) or support only on a species-specific basis (as was the case for increased size with depth).

All species-specific LEK hypotheses were classified as either Supported or Plausible after consulting external data sources (Table 5). Among species with more than one LEK hypothesis, the majority of hypotheses related to Atlantic Sharpnose Sharks, Blacktip Sharks, Sandbar Sharks, Smooth Dogfish, and Spiny Dogfish were Supported, while the majority of hypotheses regarding Dusky Sharks and Tiger Sharks were considered Plausible based on the results of studies conducted in locations far from North Carolina waters or studies with limited sample sizes.

Discussion

There were no hypotheses based on the observations of at least 50 % of interviewed fishermen that could be considered implausible or contrary to external data sources, suggesting that LEK could be useful for filling knowledge gaps and directing future efforts for shark surveys. Observations included most species considered to have a regular presence in North Carolina waters and identified specific habitat characteristics, interspecific interactions, and fishery interactions that could be empirically tested using fishery-independent methods.

The sample population was the equivalent of nearly half of the total number of north/central North Carolina fishermen holding directed or incidental shark permits (NMFS 2016). The inability to continue snowball sampling beyond the second round of interviews in any particular community supports the assumption that the sample size is representative of a relatively small, elite community of fishermen in North Carolina. Though the interviewed population included one individual who did not identify sharks among their primary target species, most fishermen identified during snowball sampling were shark fishermen, and the fact that the questions focused on coastal sharks likely caused interview respondents to recommend shark fishermen. No named home port or fishing area was shared between more than 50 % of interviewed fishermen, decreasing potential response bias from any particular geographic location within the overall study area. Most gear types used in commercial and recreational dogfish or coastal shark fisheries were included, though most fishermen utilized more than one gear type. Other targeted species named by fishermen are captured in fisheries known to have a high

shark interaction and bycatch rates (Jensen and Hopkins 2001, Thorpe and Frierson 2009, Mathers et al. 2013, Enzenauer et al. 2015).

Most general LEK hypotheses applying to all coastal sharks were consistent with external information. There was total consensus among interviewed fishermen that shark populations have shown recent increases and that migrations are associated with prey movements. Recent population increases have been noted for species identified by North Carolina fishermen, including Bull Sharks, Tiger Sharks, Dusky Sharks, and Spiny Dogfish (Rago and Sosebee 2010, Carlson et al. 2012, McCandless et al. 2014). General increases in coastal shark populations have been observed by the NMFS fishery-independent shark longline survey (Jones 2015) and in the form of increased shark landings in the United States despite a continuing decreasing trend worldwide (Davidson et al. 2016). These increases have been attributed to shark fishery management policies in United States waters (McCandless et al. 2014, Davidson et al. 2016), an opinion shared by many of the interviewed fishermen. The hypothesis that coastal shark migrations follow migrations of prey species has some indirect support from observations of migration timing coinciding with prey spawning activity (Kajiura and Tellman 2016) and associations between pelagic shark movements and high-productivity areas (Block et al. 2011, Queiroz et al. 2016). Menhaden species (*Brevoortia* sp.) have been found to make up a significant portion of the diets of several coastal shark species occurring in North Carolina (Castro 1996, Bethea et al. 2004, Ellis and Musick 2007, Bangley and Rulifson 2014b) and associations with particular temperature ranges are widespread among coastal sharks (see Schlaff et al. 2014 for

a review, also Chapters 1-3, this volume). Though selection of shallow habitats by juvenile coastal sharks is well-documented (Heithaus 2007, Grubbs 2010, Guttridge et al. 2012), increasing size with depth was only supported by external data on a species-specific basis. Sandbar Sharks show an ontogenetic shift towards deeper habitats with increasing size (Grubbs et al. 2007, Grubbs 2010) and catch and satellite telemetry data suggest that Dusky Shark juveniles occur closer to shore than adults (Jensen and Hopkins 2001, Carlson and Gulak 2012, Hoffmayer et al. 2014). However, large adult Tiger and Great Hammerhead Sharks (*Sphyrna mokarran*) have been observed foraging in shallow nearshore or estuarine habitats (Heithaus et al. 2002, Roemer et al. 2016).

Species referenced specifically by fishermen were generally the most common species in fishery-independent shark surveys and comprised significant portions of fishery landings or bycatch (Mathers et al. 2013, Schwartz et al. 2013, Enzenauer et al. 2015, Jones 2015, see also Chapters 1-3, this volume). Blacktip and Sandbar Sharks appeared to be of particular importance to North Carolina fishermen. Blacktip Sharks are targeted in both commercial and recreational shark fisheries (NMFS 2012, 2015, Enzenauer et al. 2015). Sandbar Sharks can only be landed by participants in a limited-access research fishery (NMFS 2007) but can be a highly abundant bycatch in gillnet fisheries (Jensen and Hopkins 2001, Thorpe and Frierson 2009). A time-area closure put in place to protect juveniles Sandbar Sharks effectively closes bottom longline fisheries in North Carolina waters for half the year (NMFS 2003), giving this species considerable influence over other fisheries in the area. Tiger Sharks and Hammerhead species were mentioned relatively often

compared to their importance in fishery landings (NMFS 2015) but are easily-identified charismatic species that may be more memorable for fishermen. Recent fishery management policies place Hammerheads under a low quota linked to the large coastal shark fishery (NMFS 2013) so this group may receive more attention from fishermen due to their potential to influence the length of the coastal shark season.

Most species-specific LEK hypotheses were supported by external data, but interview responses suggest that more information about habitat use by Sandbar Sharks, Dusky Sharks, Hammerheads, and Tiger Sharks in North Carolina waters is needed. Specific habitat characteristics were mentioned for each of these species that could be key to avoiding or more efficiently targeting them. Differences in juvenile and adult habitat use by Sandbar and Dusky Sharks could influence the size and duration of the Mid-Atlantic shark closure (NMFS 2003) and could be empirically assessed using fishery-independent surveys stratified by depth, temperature, and season. Interview responses also suggested intriguing associations with particular habitat features among Hammerhead species and Tiger Sharks that are plausible based on external data. Vertical movements of Scalloped Hammerhead Sharks (*Sphyrna lewini*) have been associated with thermoclines (Hoffmayer et al. 2013, Ketchum et al. 2014), so it may be possible that Hammerheads associate with horizontal temperature changes resulting from the proximity of the Gulf Stream to Cape Hatteras. Tiger Sharks are known to show site fidelity to particular foraging habitats (Meyer et al. 2010, Werry et al. 2014) and have long been documented near Cape Lookout during the summer (Gudger 1948).

For both Hammerhead species and Tiger Sharks, associations with particular habitats could be assessed using telemetry methods (see Chapters 1 and 3, this volume, for examples).

The results of this study show that LEK observations can be a valid source of quantifiable data that can be used to develop studies to answer fishery management questions (Neis et al. 1999, Johannes et al. 2000). Due to the limited level of participating in shark fisheries in north/central North Carolina, interviews ultimately focused on a small, elite group of shark fishermen within each sampling location. Interviews with members of elite groups can provide large amounts of data that may be otherwise inaccessible or obscured from surveys of the general population, but sampling elite populations also requires a considerable time commitment in making contacts and preparing for interviews (Goldstein 2003). Using referrals from previously interviewed fishermen and standardizing interview questions allowed access into this small, geographically widespread community while assuring that data could be compared across individual fishermen and sampling locations. Use of external data to verify LEK observations also allowed for an assessment of the accuracy of interview responses, increasing confidence that interview responses represented valid observations (Gilchrist et al. 2005, Davis and Ruddle 2010).

In conclusion, the observations of fishermen targeting highly migratory species such as coastal sharks can be considered a valid source of information on the local habitat use patterns of those species. Therefore LEK, provided it is sourced from a plurality of experts, can be used to identify and answer questions not

covered by existing fishery-independent efforts. In this example, the accurate observations of North Carolina fishermen provided several empirically testable hypotheses with important implications to the area's fisheries. In particular, hypotheses regarding habitat differences between juvenile and adult Sandbar and Dusky Sharks and potential aggregation areas for Hammerheads can be tested using established methods for assessing shark habitat use, and could directly affect the ability of fishermen to avoid these species while more efficiently pursuing target species.

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Tables

Table 4-1. Standardized interview questions used to gather local ecological knowledge of shark habitat use patterns from North Carolina fishermen.

Part 1 – Background Information
1. What is your home port?
2. How many years of fishing experience do you have?
3. Where did you learn to fish?
4. What species do you typically target?
Part 2 – Not Targeting Sharks
1. How did you get into your target fisheries?
2. What kind of gear do you usually use?
3. When do you start to encounter juvenile or adult sharks?
4. What species do you encounter?
5. Do you encounter more juvenile or adult sharks?
6. Do you find juvenile and adult sharks in different locations or conditions?
7. Are there specific areas or conditions you avoid because of shark encounters?
8. Under what conditions do you see or hear about large numbers of sharks near the beach?
9. Under what conditions do you see or hear about large numbers of sharks in deeper waters?
10. Under what conditions do you see or hear about large numbers of sharks migrating through the local area?
11. What is the farthest north/south/inshore/offshore that you have encountered sharks?
12. When sharks migrate through the area, do they associate with any landmarks such as the beach, shoals, currents, etc?
Part 3 – Targeting Sharks
1. How did you get into shark fisheries?
2. What kind of gear do you usually use?
3. What species do you normally target or encounter?
4. How do you know where to go to catch sharks?
5. Where have you seen large numbers of juvenile or adult sharks?
6. When do you see large numbers of juvenile or adult sharks?
7. State "yes" or "no" regarding whether the following environmental factors are an important influence on the likelihood of catching or encountering sharks. Feel free to go into further detail. Factors: depth, water temperature, bottom type, wind direction, wave height, weather/front systems, climate change, salinity, ocean currents, prey availability and behavior, tides.
8. Are there certain prey species that sharks follow more than others?
9. Under what conditions do you see sharks close to the beach?
10. Under what conditions do you see sharks in deeper water?
11. Under what conditions do you see sharks migrating through the area?
12. What time of year do you encounter adult or juvenile sharks in your gear?
13. What is the farthest north/south/inshore/offshore you have fished for sharks?
14. How has the abundance of sharks changed over your years of fishing?
15. Are there certain areas or landmarks you regularly fish due to a high likelihood of catching sharks?

Table 4-2. Example responses to the question about entering fisheries grouped into standardized response categories.

Question	Responses	Response Category
How did you get into your target/shark fishery?	"Taking advantage of an abundant species."	Increased Opportunity
	"Species available during off-season."	
	"Encouraged by government or NMFS."	
	"Taking advantage of an underutilized species."	Underutilized Species
	"Grew up fishing."	Social
	"Moved to the area."	
	"Learned to fish from family members."	
	"Market prices."	Market
	"Good market value with low expenditure"	
	"Demand from clients."	
	"Experience on other boats."	Experience/Gear
	"Trying out a new gear type."	

Table 4-3. Common names, scientific names, and local common names for shark species specifically mentioned by interviewed North Carolina fishermen.

Common Name	Scientific Name	Local Common Names
Atlantic Sharpnose Shark	*Rhizoprionodon terraenovae*	White Dotter, Hot Dog
Blacknose Shark	*Carcharhinus acronotus*	
Blacktip Shark	*Carcharhinus limbatus*	
Bonnethead Shark	*Sphyrna tiburo*	
Bull Shark	*Carcharhinus leucas*	
Dusky Shark	*Carcharhinus obscurus*	
Finetooth Shark	*Carcharhinus isodon*	
Hammerhead	*Sphyrna* sp.	
Lemon Shark	*Negaprion brevirostris*	
Sand Tiger Shark	*Carcharias taurus*	Garbage Mouth
Sandbar Shark	*Carcharhinus plumbeus*	Biter, Elbow Biter
Shortfin Mako	*Isurus oxyrinchus*	
Smooth Dogfish	*Mustelus canis*	Screw Dog, Mamie
Spinner Shark	*Carcharhinus brevipinna*	
Spiny Dogfish	*Squalus acanthias*	Dog
Thresher Shark	*Alopias vulpinus*	
Tiger Shark	*Galeocerdo cuvier*	
White Shark	*Carcharodon carcharias*	

Table 4-4. General hypotheses applied to all coastal shark species in North Carolina compiled from similar interview responses from at least 50 % of fishermen.

Hypothesis	% Respondents	Review Status	References
Increasing shark populations	100	Supported	Carlson et al. (2012), McCandless et al. (2014), Davidson et al. (2016)
Migrations follow prey availability	100	Plausible	Chapter 3, Block et al. (2011), Kajiura and Tellman (2016)
Importance of Menhaden prey	62.5	Supported	Castro (1996), Bethea et al. (2004), Ellis and Musick (2007), Bangley and Rulifson (2014)
Importance of temperature	87.5	Supported	Chapter 1, Chapter 2, Chapter 3, Schlaff et al. (2014)
Increase in size with depth	50	Plausible	Grubbs et al. (2007), Grubbs (2010), Guttridge et al. (2012), Heithaus et al. (2002), Roemer et al. (2016)
Reduced juvenile abundance with depth	50	Supported	Heithaus (2007), Guttridge et al. (2012), Grubbs (2010)

Table 4-5. Species-specific hypotheses compiled from similar interview responses from at least 50 % of fishermen who directly referenced the particular species.

Hypothesis	% Respondents	Review Status	References
Atlantic Sharpnose Shark			
Temperature range 15-29 °C	100	Supported	Chapter 1, Chapter 2, Chapter 3, Ulrich et al. (2007)
Prey upon Menhaden	50	Plausible	Bethea et al. (2004, 2006)
Prefer higher salinity	50	Supported	Chapter 2, Abel et al. (2007)
Adults present earlier in spring than juveniles	50	Supported	Chapter 3, Ulrich et al. (2007)
Arrive earlier in year than other coastal sharks	50	Supported	Chapter 2, Chapter 3, Ulrich et al. (2007)
Blacktip Shark			
Occur at shallow depths	50	Supported	Chapter 2, Chapter 3, Grabowski et al. (2005)
Temperature range 15-29 °C	50	Supported	Chapter 2, Chapter 3, Ulrich et al. (2007)
Prefer higher salinity	50	Supported	Chapter 2, Froeschke et al. (2010)
Prey on Menhaden	66.7	Supported	Castro (1996), Bethea et al. (2004)
Present May-October	83.3	Supported	Chapter 2, Chapter 3, Ulrich et al. (2007)
Follow shrimp trawl activity	50	Supported	Castro (1996)
Bonnethead Shark			
Occur within estuaries	66.7	Supported	Chapter 2, Chapter 3, Castro (1993), Heupel et al. (2006)
Bull Shark			
Occur within estuaries	100	Supported	Chapter 2, Chapter 3, Curtis et al. (2011), Schwartz (2012)
Dusky Shark			
Prey on hooked fish	50	Plausible	Raby et al. (2014)
Juveniles closer to shore	50	Plausible	Chapter 1, Jensen and Hopkins (2001), Carlson and Gulak (2012), Hoffmayer et al. (2014)
Juveniles overwinter near Cape Hatteras	50	Supported	Chapter 1, Jensen and Hopkins (2001), Jensen et al. (2002)
Hammerheads			
Aggregate near temperature changes (11-24 °C)	50	Plausible	Hoffmayer et al. (2013), Ketchum et al. (2014)
Occur close to shore during winter	50	Supported	Chapter 1, Jensen and Hopkins (2001)
Sand Tiger Shark			
Prey on Menhaden	66.7	Supported	Gelsleichter et al. (1999)
Sandbar Shark			

Juveniles closer to shore	60	Supported	Chapter 1, Grubbs et al. (2007), Grubbs (2010)
Adult depth range 18-64 m	60	Plausible	Grubbs et al. (2007, Grubbs (2010)
Juveniles at lower temperatures	60	Plausible	Grubbs (2010)
Juveniles overwinter near Cape Hatteras	100	Supported	Chapter 1, Merson and Pratt, Jr. (2001), Grubbs et al. (2007), Conrath and Musick (2008)
Prey on Menhaden	60	Supported	Ellis and Musick (2007)
Smooth Dogfish			
Aggregations of juveniles in spring	50	Supported	Chapter 2, Castro (1993), Rountree and Able (1996), Schwartz (2000), Jensen et al. (2002)
Juveniles closer to shore	75	Supported	Castro (1993), Rountree and Able (1996), Schwartz (2000)
Pregnant females present in fall	50	Supported	Chapter 1, Jensen and Hopkins (2001)
Spinner Shark			
Prey on Menhaden	80	Plausible	Bethea et al. (2004)
Present May-November	60	Supported	Jensen et al. (2002)
Spiny Dogfish			
Prefer cooler temperatures than other species	60	Supported	Chapter 1, Chapter 2, Ulrich et al. (2007)
Occur within estuaries	80	Supported	Chapter 2, Chapter 3, Bangley and Rulifson (2014a)
Tiger Shark			
Temperature range 15-29 °C	50	Plausible	Gudger (1948), Jensen et al. (2002)
Prefer higher salinity	50	Supported	Hueter and Tyminski (2007), Ulrich et al. (2007)
Occur over live bottom structure	50	Plausible	Gudger (1948), Meyer et al. (2010), Werry et al. (2014)

Figures

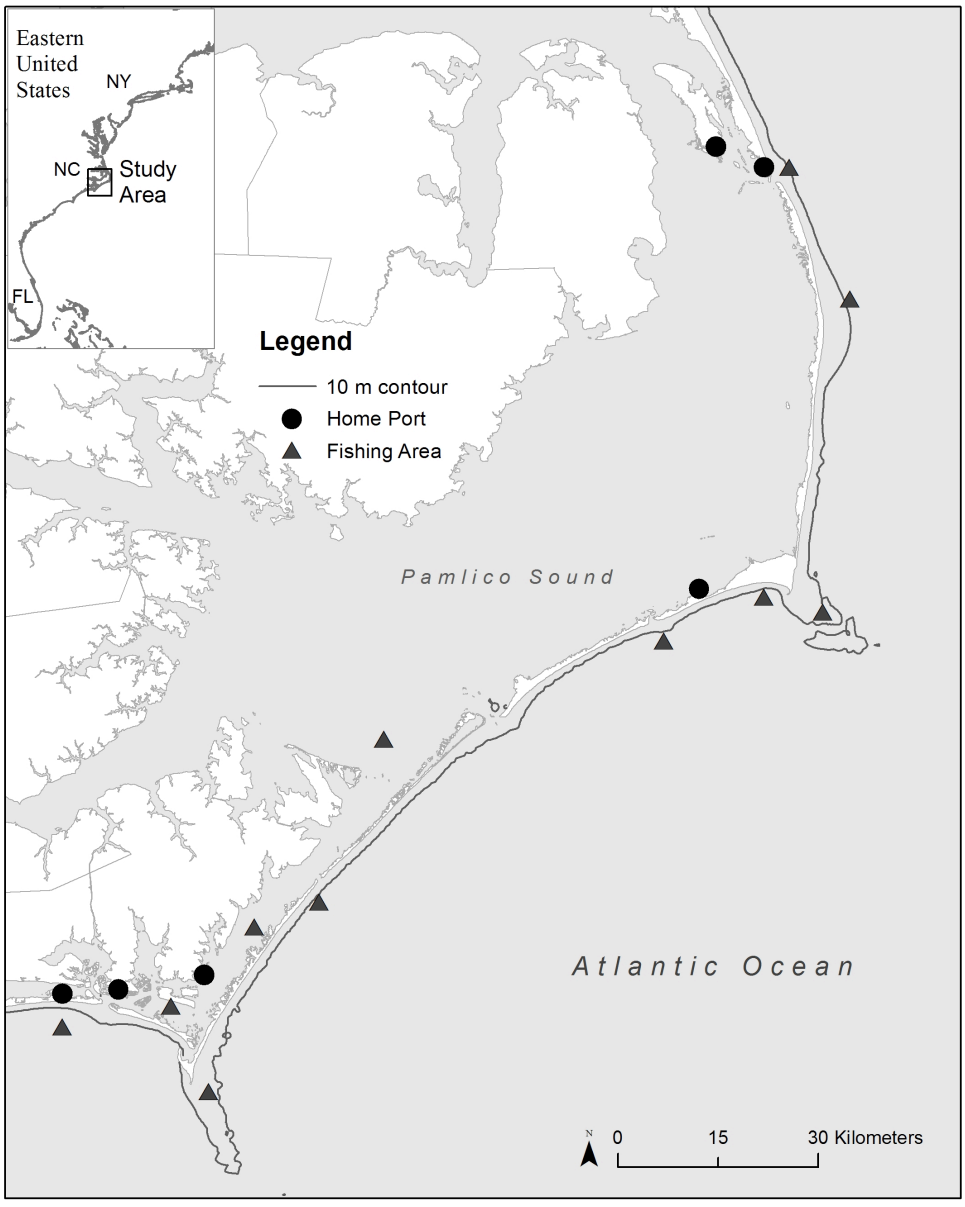

Figure 4-1. Home ports and fishing areas between Beaufort Inlet and the northern Outer Banks identified by interviewed fishermen.

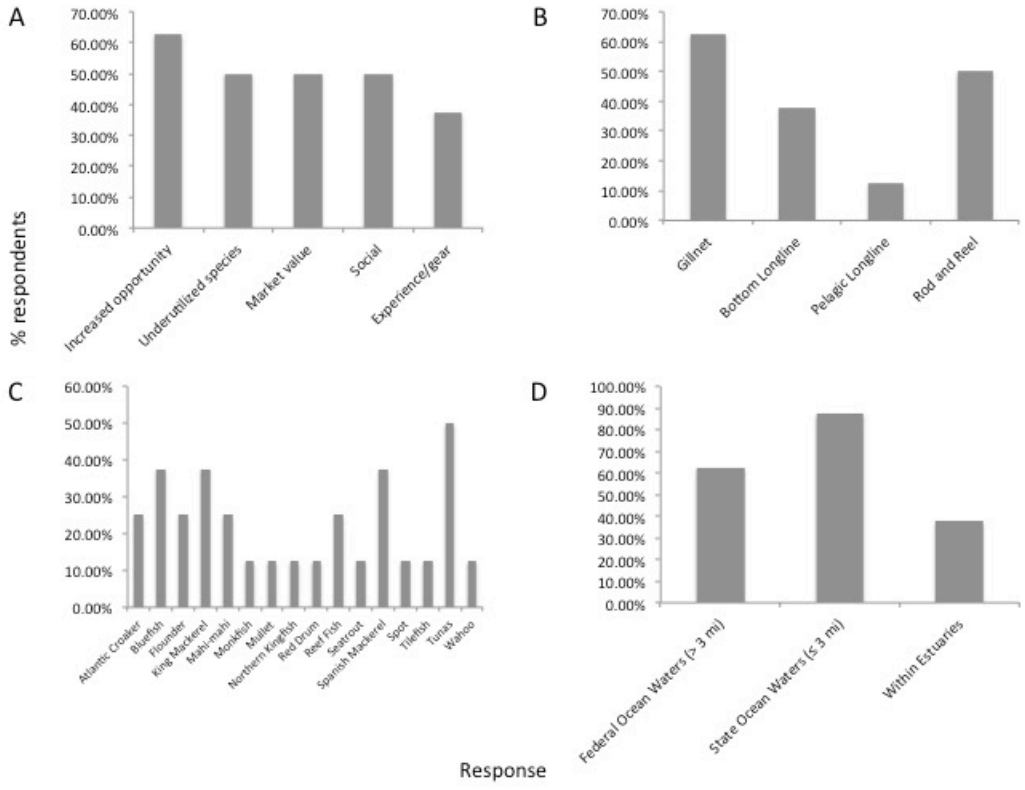

Figure 4-2. Responses from interviewed fishermen to questions related to fishery participation: A. reason for entering fishery, B. typically-used gear, C. non-shark target species, and D. fishing areas. Percentage of responses may add to greater than 100 % due to multiple different responses from the same fisherman.

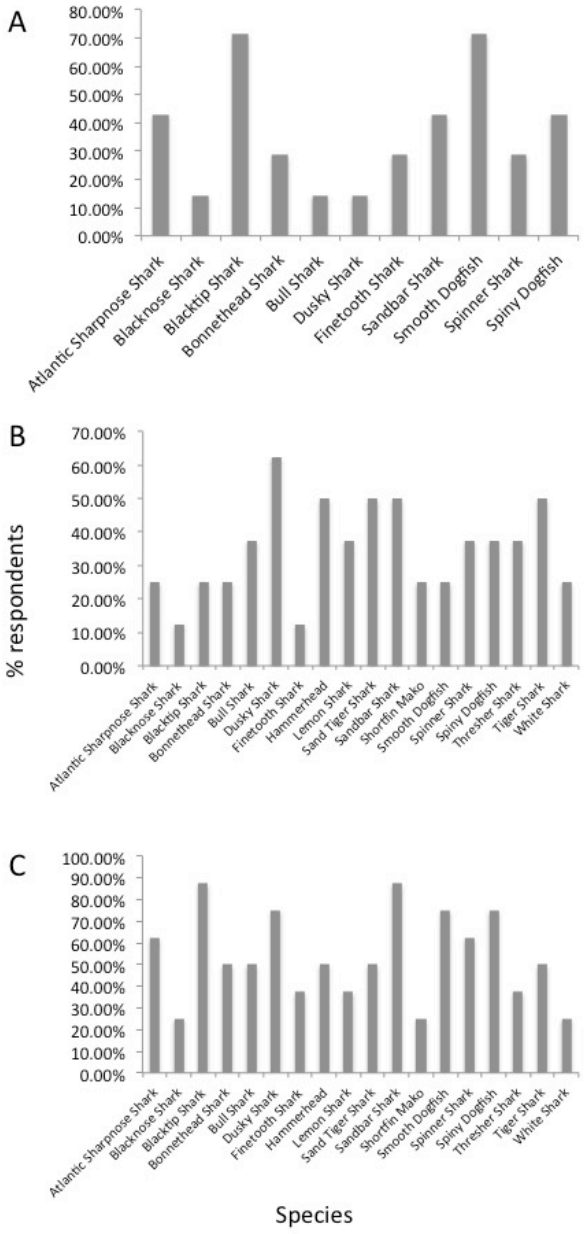

Figure 4-3. Species mentioned by interviewed fishermen in the context of A. active fishery targets, B. incidentally-encountered species, and C. both targeted and encountered species combined. Only fishermen participating in shark fisheries counted towards percentages in A.

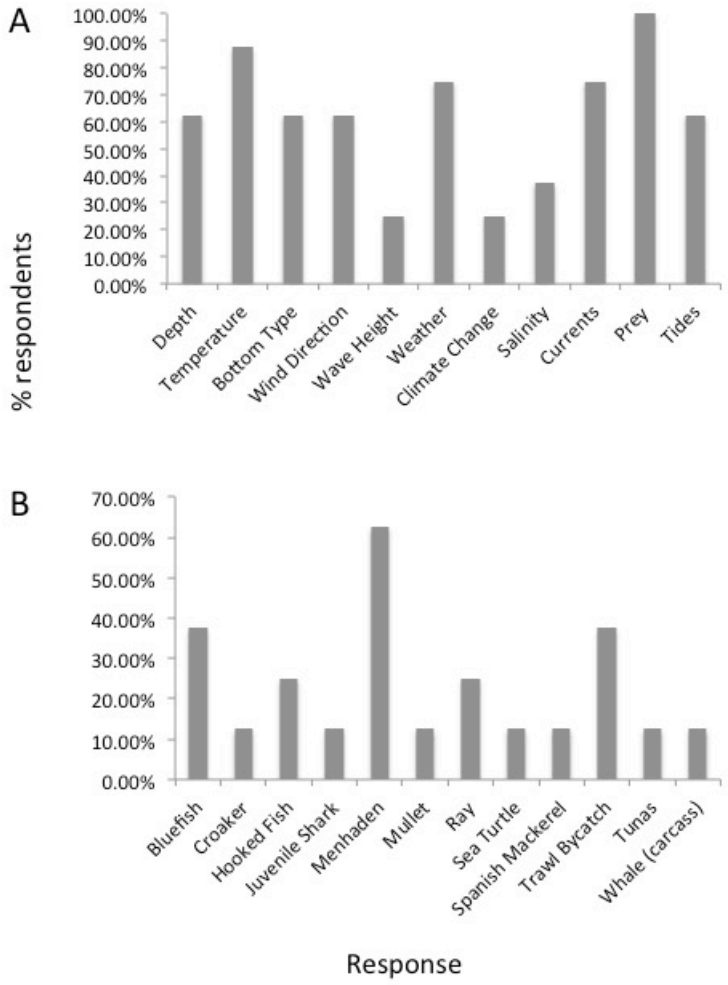

Figure 4-4. Responses from interviewed fishermen regarding A. environmental factors affecting shark presence or abundance and B. prey species or food sources associated with shark presence or abundance.

Conclusions

Review and Management Implications of Coastal Shark Habitat Use Patterns in North Carolina Waters

The preceding chapters represent a multi-year effort to identify and delineate coastal shark habitat in North Carolina waters. This effort involved both fishery-dependent and -independent surveys and encompassed both estuarine and nearshore coastal waters. A variety of capture, acoustic telemetry, geospatial, and anthropological methods were used in order to document the widest possible array of species and environments. Below are general observations, followed by species-specific profiles compiling data from all parts of this study.

General Observations

Sampling efforts ultimately focused on the coastal and estuarine waters of north/central North Carolina, defined as the area from Beaufort Inlet and Cape Lookout to Oregon Inlet and the northern portion of Pamlico Sound. The elasmobranch assemblage in this region has received comparatively little attention compared to the lower Chesapeake Bay (e.g., Grubbs and Musick 2007), southeastern North Carolina near Cape Fear (Thorpe et al. 2004), or coastal South Carolina (e.g., Ulrich et al. 2007). Notable exceptions are the statewide efforts of Jensen et al. (2002) and the long-running but spatially limited University of North Carolina (UNC) coastal shark survey near Cape Lookout (Schwartz et al. 2013).

Aside from the UNC survey, fishery-independent gillnet and longline surveys conducted by the North Carolina Division of Marine Fisheries (NCDMF) in Pamlico Sound (Paramore 2009, 2011) are the only sources of long-term catch data from elasmobranchs in north/central North Carolina.

Due to the position of Cape Hatteras and Cape Lookout at the transition between major marine biogeographic regions (Hayden et al. 1984, Fautin et al. 2010), coastal and estuarine North Carolina represents a unique environment where cool-temperate and subtropical species ranges converge (Love and Chase 2007). All surveys in my study found evidence that this is true among the local assemblage of sharks. Species were documented during winter off of the Outer Banks, particularly in the vicinity of Cape Hatteras, that are typically present during the summer even in areas south of North Carolina (Chapters 1 and 4, Grubbs et al. 2007, Ulrich et al. 2007). Estuarine sampling also documented species considered to be winter migrants lingering long into the spring or even occurring year-round (Chapters 2 and 3, Bangley and Rulifson 2014). The results of this study suggest that the biogeography of the North Carolina coast may create opportunities for unique habitat use and migratory behaviors among local coastal shark populations.

North Carolina's position at the confluence of the Gulf Stream and Labrador Currents (Roelofs and Bumpus 1953, Hayden et al. 1984) may also make it one of the first areas to show evidence of species shifts in response to large-scale climate change (Parker and Dixon 1998). The presence and abundance of many of the shark species documented in this study were driven by spatially variable features like temperature, salinity, and high primary productivity (Chapters 1-4, Grubbs and

Musick 2007, Froeschke et al. 2010, Block et al. 2011, Queiroz et al. 2016). Climate change can lead to perturbations in the extent and availability of these habitat features, which can in turn lead to changes in the distribution of highly migratory species such as sharks (Hazen et al. 2012, Pinsky et al. 2013). A vulnerability assessment predicted relatively neutral effects of climate change on the distribution and population of highly mobile shark species in the Northwest Atlantic Ocean (Hare et al. 2016) but long-term data sets in the North Sea show evidence of climate-driven distribution shifts among demersal shark and skate species in that region (Sguotti et al. 2016). In my study, habitat areas delineated by temperature and primary productivity revealed that some shark species in North Carolina are confined to small habitat ranges during certain times of the year (Chapter 1) or may be utilizing habitat areas that have only recently become accessible due to temperature changes (Chapter 2, Bangley et al. in review). Identification of spatially-variable environmental factors defining habitat will allow researchers and fishery managers to better predict potential shifts in distribution or loss of habitat due to climate change.

Evidence for fidelity to certain habitat types was also found during this study. Shark distribution in relation to seagrass habitat was directly assessed during surveys within estuarine waters (Chapters 2 and 3) and fine-scale acoustic telemetry revealed a possible association with oyster reefs by an apex predator species (Chapter 3). In coastal waters, local ecological knowledge provided evidence for shallow shoals and live bottom reef habitats to migratory coastal sharks (Chapter 4). Some shark species are known to return to the same individual

foraging or nursery habitats on a regular basis during their long-distance migrations (Hueter et al. 2005, Chapman et al. 2015) and may show site fidelity to specific habitat features such as reefs (Meyer et al. 2010). Declines in seagrass and oyster reef habitats (Waycott et al. 2009, Beck et al. 2011) may have distribution and ultimately population effects on sharks that depend on these habitats, and the potential for damage to oceanic habitats should be a consideration for coastal and offshore energy development (Crowder and Norse 2008). Conversely, ecosystem interactions involving sharks may serve to structure and maintain certain habitats (Burkholder et al. 2013, Heithaus et al. 2014).

Interspecific interactions may also factor into shark habitat use patterns in North Carolina waters, but were only indirectly assessed in this study. Habitat use by juvenile or small coastal shark species is thought to be driven by a combination of accessibility to prey and refuge from predators or competitors (Heithaus 2007, Heithaus and Vaudo 2012). The trophic role of sharks in an ecosystem may change with body size, with only the largest individuals or species functioning as true apex predators (Heupel et al. 2014). Local fishermen assumed that migratory presence of coastal sharks in North Carolina waters was driven primarily by migrations of prey species (Chapter 4). It is difficult to determine whether associations between marine predators and prey are intentional or coincidental, but mass migrations of some species coincide with the spawning seasons of their primary prey (Kajiura and Tellman 2016) and the presence of some species in Back Sound overlapped the timing of the spawning season of potential prey species (Chapter 3, Luczkovich et al. 2008). Catch data from estuarine surveys also suggested habitat selection by some

species as a way to avoid overlap with other juvenile sharks that may serve as potential competitors (Chapters 2 and 3, Heupel and Simpfendorfer 2011). Associations between sharks and other species may lead to interactions with fisheries (Chapter 4) and can have significant local impacts on the abundance (Beamish et al. 1992, Lacroix and Fleming 2014) and behavior (Dill et al. 2003) of prey species. Comparisons between locations and spatial extent of shark and prey, predator, or competitor species habitat can be used to assess the potential impact of these interactions.

Whether directly targeted or encountered in the process of fishing for other species, sharks may have a strong influence over North Carolina's marine fisheries. North Carolina is one of the top five states in terms of shark fishery landings (NMFS 2012). From January 1st through July 31st, most of the state's waters fall within the Mid-Atlantic shark closed area (NMFS 2003), which prohibits bottom longline gear to protect juvenile Sandbar Sharks (*Carcharhinus plumbeus*) and Dusky Sharks (*Carcharhinus obscurus*). Fishermen also report an abundance of Hammerheads (*Sphyrna* sp., likely Scalloped Hammerhead *Sphyrna lewini*), particularly during winter months (Chapters 1 and 4). Since 2013 Hammerheads have been managed under low quotas linked to the large coastal shark fishery quotas, which causes the large coastal shark fishery to close when the Hammerhead quota has been reached (NMFS 2013). This makes large Hammerheads a so-called "choke species" in the large coastal shark fishery, and high interaction rates can limit opportunities in this fishery. Fishermen also reported lost catches to depredation by sharks, with some species described as a nuisance to charter fisheries (Chapter 4). The habitat areas

delineated by this study may allow fishermen to better avoid prohibited or nuisance species while more efficiently targeting other shark or teleost species. Habitat delineation may have other implications for shark fishery management, such as defining the true extent of essential habitat within the Mid-Atlantic shark closed area (Chapter 1).

Species Profiles

Atlantic Sharpnose Shark – *Rhizopronodon terraenovae*

Atlantic Sharpnose Sharks (*Rhizoprionodon terraenovae*) were documented during all coastal nearshore and estuarine surveys (Chapters 1-3) and among the species most often referenced by fishermen (Chapter 4). Individuals captured in nearshore waters were mature adults of both sexes (Chapter 1) and in estuarine waters adult males were primarily captured in mid-spring (April-May,) preceding the presence of neonate and young-of-year juveniles in mid-late May (Chapters 2 and 3). These patterns of coastal nearshore and estuarine habitat use are consistent with observations of this species in South Carolina (Ulrich et al. 2007) and Florida Gulf coast waters (Carlson et al. 2008). However, an adult female was captured and gave birth to fully-formed pups within Beaufort Inlet in Back Sound (Chapter 3), which contradicts the observation made by Ulrich et al. (2007) that parturition occurs in nearshore environments, after which juvenile Atlantic Sharpnose Sharks enter estuaries. Estuarine habitat was limited by salinity and distance from the nearest inlet in Pamlico Sound (Chapter 2) but significant habitat preferences could

not be defined for the species in Back Sound, a higher-salinity estuary (Chapter 3). Spatiotemporal overlap was high between Atlantic Sharpnose Sharks and potential predators including larger juvenile Blacktip Sharks (*Carcharhinus limbatus*) (Chapters 2 and 3, Castro (1996). A similar lack of refuging behavior was found in the northern Gulf of Mexico by Carlson et al. (2008). This behavior coupled with a higher fecundity and growth rate relative to other shark species (Loefer and Sedberry 2003) suggest that Atlantic Sharpnose Shark habitat selection may prioritize foraging opportunities fueling rapid growth over protection from predators.

Blacknose Shark – *Carcharhinus acronotus*

Blacknose Sharks (*Carcharhinus acronotus*) were among the most common sharks in Back Sound (Chapter 3), but were absent in NCDMF surveys of Pamlico Sound (Chapter 2) and were not often mentioned by fishermen with home ports north of Core Sound (Chapter 4). This species is likely only an uncommon catch north of Cape Lookout and a rare transient north of Cape Hatteras (Schwartz 2003, Castro 2011) but can be highly abundant in the vicinity of Cape Lookout (Schwartz 1984). Blacknose sharks were exclusively captured in close proximity to inlets in Back and lower Core Sounds (Chapter 3), which is consistent with their distribution in nearshore and estuarine South Carolina (Ulrich et al. 2007). Evidence was found for potential spatial resource partitioning between Blacknose Sharks and similarly-sized juvenile Blacktip Sharks in Back and Core Sounds, with Blacktip Sharks occurring farther from inlets (Chapter 3). Both Blacknose and Blacktip Sharks are

ecologically similar (Castro 1996, Ford 2012) and spatial resource partitioning may mitigate competition between these species. Blacknose Sharks also appeared to be more nocturnal than other elasmobranchs occurring within Back and Core Sounds (Chapter 3).

Blacktip Shark – *Carcharhinus limbatus*

Blacktip Sharks were among the most common shark species in Pamlico Sound and the Back/Core Sound system during the summer (Chapters 2 and 3). This species was also one of the two shark species most often mentioned by interviewed fishermen (Chapter 4). Blacktip Sharks are one of the primary target species in the commercial large coastal shark fishery and are also specifically targeted by recreational catch-and-release anglers (Chapter 4, NMFS 2015), giving them a greater cultural importance than other species in the North Carolina shark fishing community. Blacktip Shark presence in estuarine waters coincided with the warmest temperatures (Chapters 2 and 3), and the first individual captured in 2015 sampling in Back Sound was caught two weeks prior to when the first individual was captured in 2014 (Chapter 3). This suggests that temperature cues Blacktip Shark presence in North Carolina waters more than other potential cues such as day length. Potential habitat for this species was constrained by salinity in Pamlico Sound (Chapter 2) but not in Back and Core Sounds (Chapter 3). Blacktip Shark habitat in other nearshore or estuarine habitats is strongly associated with salinity or distance from the nearest inlet (Froeschke et al. 2010, Ward-Paige et al. 2014). The generally high salinity within Back and Core Sounds may have allowed juvenile

Blacktip Sharks to penetrate farther into the estuary and may have also facilitated spatial habitat partitioning between this species and Blacknose Sharks based on inlet distance (Chapter 3). A Blacktip Shark was captured in the process of feeding on a hooked juvenile Atlantic Sharpnose Shark (Chapter 3) and smaller elasmobranchs including batoids and juvenile Bonnethead Sharks (*Sphyrna tiburo*) have been found in the stomach contents of this species (Castro 1996), suggesting that juvenile Blacktip Sharks may be a predatory threat to other high trophic-level species sharing their estuarine habitat (Shaw et al. 2016).

Bonnethead Shark – *Sphyrna tiburo*

Bonnethead Sharks were expected to be more common in this study, but only limited catches were recorded exclusively in gillnet sets in Pamlico Sound (Chapter 2) and Back and Core Sounds (Chapter 3). The catches that were recorded were among the closest proximity to seagrass beds, which is consistent with habitat use patterns noted in estuaries on the Gulf coast of Florida (Heupel et al. 2006). Bonnethead Sharks feed almost exclusively on benthic crustaceans such as crabs and shrimp (Bethea et al. 2011), which may significantly limit their catchability using longline, drumline, or rod and reel gear baited with fish (Ulrich et al. 2007). Bonnethead Sharks were also captured at relatively high temperatures, and their presence may be dependent on certain high temperature thresholds. Bonnetheads are among the most common species in North Carolina nearshore waters south of Cape Fear (Thorpe et al. 2004) and in estuarine South Carolina waters (Ulrich et al. 2007).

Bull Shark – *Carcharhinus leucas*

Bull Sharks were the largest species directly encountered in estuarine surveys during this study (Chapters 2 and 3) and sampling in Pamlico and Back Sounds provided opportunities to identify habitat for both juveniles and adults. Juvenile Bull Sharks captured in NCDMF surveys fell within neonate, young-of-year, and older juvenile size ranges, with two larger subadults captured in southeastern Pamlico Sound (Chapter 2). The majority of juvenile Bull Sharks were captured in a discrete area along the northwestern shore of Pamlico Sound, and habitat was characterized by mid-range salinity, warm temperature, and high inlet distance (Chapter 2). Potential Bull Shark habitat delineated within Pamlico Sound was mostly beyond the inlet distances of the other shark species (Chapter 2). Bull Sharks are more tolerant of lower salinities than other shark species and can enter fresh water, which allows juveniles to select habitats unavailable to other elasmobranchs (Heupel and Simpfendorfer 2011). Bull Shark nursery habitats have more specific features than other coastal shark nurseries, and are typically located in upper estuarine areas with high freshwater inflow and limited access to the ocean (Hueter and Tyminski 2007, Curtis et al. 2011, Drymon et al. 2014). Potential Bull Shark habitat delineated within Pamlico Sound was mostly beyond the inlet distances of the other shark species (Chapter 2). Interestingly, juvenile Bull Sharks were rarely caught in Pamlico Sound prior to 2011 (Chapter 2, Schwartz 2012), and the newly-consistent presence of this species in the estuary may be related to

increasing water temperatures during the late spring, making this a potential model species for climate-driven distribution shifts (Bangley et al. in review).

Two adult male Bull Sharks were documented in Back Sound. A 2.5 m total length individual was captured by drumline in the vicinity of Radio Island in July 2015 and another large individual carrying an acoustic transmitter was detected repeatedly on receivers deployed in the Middle Marsh array during the following October (Chapter 3). Most tag detections occurred during nighttime hours on receivers placed at oyster reef habitats (Chapter 3). Though it is impossible to draw definitive conclusions from two individuals, the presence of large mature Bull Sharks is confirmed in North Carolina estuarine waters, where they likely function as apex predators.

Dusky Shark – *Carcharhinus obscurus*

Like Sandbar Sharks, Dusky Shark juveniles overwinter in North Carolina nearshore waters (Jensen and Hopkins 2001, Jensen et al. 2002). Three juveniles were captured in February and November during tagging efforts off of Cape Hatteras and two were acoustically tagged, though none were subsequently detected (Chapter 1). This species is commonly referenced by local fishermen and is implicated in attacking hooked fish during commercial longlining and charter fishing (Chapter 4). Differences between adult and juvenile habitat could not be assessed by this study but limited satellite telemetry results show that adult Dusky Sharks tend to follow the outer edge of the continental shelf (Carlson and Gulak 2012, Hoffmayer et al. 2014). This is one of the species protected by the Mid-

Atlantic shark closed area (NMFS 2003) and a better understanding of juvenile habitat use patterns could have a considerable impact on fishing opportunities for North Carolina fishermen.

Finetooth Shark – *Carcharhinus isodon*

The Finetooth Shark (*Carcarhinus isodon*) was sporadically captured in NCDMF surveys in Pamlico Sound (Chapter 2) and a single individual carrying an acoustic transmitter was detected by receivers placed at seagrass and shallow sand flat habitats in Back Sound (Chapter 3). This species is considered a sporadic visitor to North Carolina waters (Schwartz 2003) but becomes more common south of Cape Fear (Thorpe et al. 2004, Ulrich et al. 2007).

Lemon Shark – *Negaprion brevirostris*

No Lemon Sharks (*Negaprion brevirostris*) were captured in estuarine or nearshore surveys, but large adults were mentioned being occasionally captured in estuarine waters by interviewed fishermen (Chapter 4). This species is considered a rare visitor to North Carolina waters (Schwartz 2003, Castro 2011).

Sand Tiger Shark – *Carcharias taurus*

A pair of juvenile Sand Tiger Sharks (*Carcharhias taurus*) was captured during NCDMF sampling in Pamlico Sound (Chapter 2) and the species was mentioned as an incidentally-encountered species by interviewed fishermen (Chapter 4). Sand Tiger Sharks carrying acoustic transmitters were one of the most

abundant and consistently-detected species recorded by an acoustic array maintained off of Hatteras Village, where the species was detected year round but abundance peaked during migratory periods in the spring and fall (Rulifson et al. in prep). Sand Tiger Sharks overwinter in North and South Carolina waters and migrate through the Cape Hatteras area in large numbers (Kneebone et al. 2014).

Sandbar Shark – *Carcharhinus plumbeus*

The Sandbar Shark is likely one of the most ecologically and culturally significant shark species in North Carolina waters. Nearshore winter sampling (Chapter 1), year-round sampling in Pamlico Sound by NCDMF (Chapter 2), and April-November sampling in Back and Core Sounds (Chapter 3) all documented Sandbar Sharks. Combined data suggest that this species is present in North Carolina waters year-round. Interviewed fishermen referenced the Sandbar Shark more often than any other species except the Blacktip Shark, and reported very specific habitat characteristics for this species (Chapter 4). Habitat delineation based on temperature and primary productivity at tag detection locations predicted an extremely narrow area of winter habitat surrounding Cape Hatteras and the northern half of Raleigh Bay, providing a potential explanation for the high concentrations of juveniles observed in this area (Chapter 1, Jensen and Hopkins 2001, Merson and Pratt, Jr. 2001, Grubbs et al. 2007, Conrath and Musick 2008). Though no tagged Sandbar Sharks were detected by acoustic receivers deployed at Cape Hatteras between mid-April and late September, catches in Pamlico and Back Sounds (Chapters 2 and 3) provide evidence for limited resident or summer nursery

habitat in North Carolina waters. Sandbar Shark habitat was limited by salinity and inlet distance in Pamlico Sound (Chapter 2) but individuals were captured at sites relatively far up the estuary in Back Sound (Chapter 3), suggesting that Sandbar Sharks are able to penetrate far into an estuary if not limited by salinity.

Landing Sandbar Sharks is prohibited in United States waters except for fishermen participating in a limited-access research fishery for the species, and the Mid-Atlantic shark closed area was put in place to protect juveniles of this species (NMFS 2003, 2007). Despite historical population declines, juvenile Sandbar Sharks can be extremely abundant near Cape Hatteras during winter (Chapters 1 and 4). Avoidance of juvenile Sandbar Shark aggregations is key to successful fishing near Cape Hatteras (Chapter 4). Juvenile Sandbar Shark habitat appears to only encompass the nearshore, northern portions of the Mid-Atlantic shark closed area (Chapter 1), which corroborates the observations of fishermen (Chapter 4). Further research including a broader size range of Sandbar Sharks should be conducted to identify the most important areas needed to protect juveniles while still allowing opportunities for fishing activity.

Scalloped Hammerhead Shark – *Sphyrna lewini*

A single mature male Scalloped Hammerhead Shark was captured during winter sampling off of Cape Hatteras (Chapter 1) and a juvenile was caught during NCDMF gillnet sampling in Pamlico Sound (Chapter 2). Overwintering behavior by aggregations of Scalloped Hammerheads was observed by local fishermen (Chapter 4), but is not well-documented by fishery-independent surveys. Scalloped

Hammerheads are among the most severely depleted shark species (Hayes et al. 2009, Baum and Blanchard 2010). As such, they have been discussed for listing under the U.S. Endangered Species Act (Miller et al. 2013). Currently Scalloped Hammerheads and other large Hammerhead species are managed under a low quota linked to the large coastal shark fishery, and reaching the Hammerhead quota closes the entire large coastal shark fishery in an effort to avoid further bycatch mortality (NMFS 2013). Therefore fishermen are incentivized to avoid interactions with Scalloped Hammerheads, and further attempts to identify habitat for this species will likely be of assistance in that goal.

Shortfin Mako – *Isurus oxyrinchus*

The Shortfin Mako (*Isurus oxyrinchus*) is a pelagic species mentioned among the species encountered by North Carolina fishermen (Chapter 4). Shortfin Makos are encountered while fishing for Tuna species and may occur closer to shore during periods of Gulf Stream encroachment near shore (Schwartz 2003, Castro 2011).

Smooth Dogfish – *Mustelus canis*

The Smooth Dogfish (*Mustelus canis*) is one of the most high-volume shark fisheries in the United States (NMFS 2015b) and is commonly targeted by North Carolina fishermen (Chapter 4). Adult, possibly pregnant Smooth Dogfish were captured in November off of Cape Hatteras (Chapter 1). Adults and juveniles were caught during all seasons in NCDMF sampling within Pamlico Sound, where they were the most abundant shark species (Chapter 2). In comparison to Pamlico

Sound, Smooth Dogfish were less common in Back and Core Sounds (Chapter 3). The high abundance of juveniles and the fact that adult and juvenile presence coincides with the timing of parturition in other estuaries (Rountree and Able 1996, Conrath and Musick 2002) provide evidence that Pamlico Sound may function as a productive primary nursery habitat for this species. Potential Smooth Dogfish habitat in Pamlico Sound showed high spatial overlap with known seagrass beds, suggesting that seagrass habitat may be important for this species (Chapter 2). Smooth Dogfish feed primarily on crustacean prey, which may be more available in seagrass habitat (Rountree and Able 1996, Gelsleichter et al. 1999). The large spatial extent of seagrasss habitat in eastern Pamlico Sound may make this estuary an ideal nursery habitat for Smooth Dogfish. Though juveniles were captured in Back and Core Sounds (Chapter 3) and nurseries have been identified as far south as Bulls Bay, South Carolina (Castro 1993), Pamlico Sound likely represents the southernmost major nursery for the Smooth Dogfish.

Spinner Shark – *Carcharhinus brevipinna*

Spinner Sharks were referenced by half of the interviewed fishermen (Chapter 4) and juveniles were a rare catch in NCDMF surveys in Pamlico Sound (Chapter 2). This species was described as abundant from May through October by interviewed fishermen (Chapter 4) but it appears to prefer nearshore habitat over estuarine habitat and is only rarely captured within estuaries (Ulrich et al. 2007). Large numbers of neonates have been caught during the spring and summer near the inlets of the Outer Banks (Jensen et al. 2002) and may be more abundant than

Blacktip Sharks in North Carolina waters south of Cape Fear (Thorpe et al. 2004). It is likely that if nearshore sampling had occurred during the spring or summer that this species would have been well-represented in the catch.

Spiny Dogfish – *Squalus acanthias*

The Spiny Dogfish is undoubtedly the most abundant shark species in North Carolina waters during the winter, though juvenile Sandbar Sharks may be more locally abundant. Spiny Dogfish were captured during nearshore winter sampling (Chapter 1), occurred in large numbers during the winter and early spring in Pamlico Sound (Chapter 2), and aggregations were encountered in Back and Core Sounds until June (Chapter 3). As expected, Spiny Dogfish were identified as a targeted and commonly-encountered species by fishermen (Chapter 4). Nearly all Spiny Dogfish were adult females, which is consistent with migratory and sexual segregation behavior documented for the species (Stehlik 2007, Sagarese et al. 2014). One unexpected result of this study was the discovery of delayed migration or possibly resident behavior among Spiny Dogfish in Back and Core Sounds, which persisted in the system long after date and temperature thresholds associated with northern migrations (Schwartz 2003, Stehlik 2007, Bangley and Rulifson 2014). This behavior was not observed among Spiny Dogfish in Pamlico Sound or nearshore, and proves that there is still much to be learned about the ecology of this well-studied species.

Thresher Shark – *Alopias vulpinus*

Juvenile Thresher Sharks (*Alopia vulpinus*) were captured during winter sampling off of Cape Hatteras (Chapter 1) and were listed among the species encountered by North Carolina fishermen (Chapter 4). Thresher Sharks captured during nearshore sampling were found in low-temperature water associated with catches of Spiny Dogfish and Smooth Dogfish but not in higher-temperature areas where Sandbar, Dusky, and Atlantic Sharpnose Sharks were caught (Chapter 1). Thresher Sharks may be a part of the winter shark assemblage in North Carolina waters and may select nearshore habitat bounded by lower temperatures. Adult Thresher Sharks are typically pelagic, but juveniles have been documented in nearshore North Carolina waters, which may serve as a nursery habitat for this species (Jensen and Hopkins 2001, Jensen et al. 2002, Thorpe et al. 2004).

Tiger Shark **–** *Galeocerdo cuvier*

Tiger Sharks were not documented during nearshore or estuarine sampling, but were referenced often by fishermen (Chapter 4). Based on their observations, Tiger Sharks are locally abundant during summer months over live bottom and other nearshore structured habitats, particularly in the vicinity of Cape Lookout. Historically this species has been recorded at Cape Lookout, where it was thought the geography of the barrier islands acted as a "natural fish trap" funneling prey species and ultimately large Tiger Sharks into the area (Gudger 1948). More recently, several Tiger Sharks fitted with satellite tags near Port Royal Sound, South Carolina have been tracked near Cape Lookout and the associated shoals, and in

2014 one individual appeared to remain resident there for most of the summer (OCEARCH 2016). Tiger Sharks may have been undersampled by this study due to a lack of nearshore sampling during summer.

White Shark – *Carcharodon carcharias*

The White Shark (*Carcharodon carcharias*) was among the species encountered by North Carolina fishermen (Chapter 4). North Carolina is known to be part of the overwintering range of this species (Curtis et al. 2014) and several individuals carrying acoustic transmitters were detected migrating past Cape Hatteras during the spring and fall (Rulifson et al. in prep). Many of the white sharks tagged with satellite transmitters in the Northwest Atlantic Ocean have moved through or maintained short resident periods within North Carolina waters, generally in association with the shoals off of Cape Hatteras and Cape Lookout, and two sharks have been tracked apparently entering and leaving Pamlico Sound (OCEARCH 2016).

Batoids

Several batoid species were captured during sampling in Back and Core Sounds, of which Southern Stingrays (*Dasyatis americana*) and Cownose Rays (*Rhinoptera bonasus*) were the most common (Chapter 3). Rays have received little attention in ecosystem surveys in North Carolina estuaries, but may be an important component of the elasmobranch community. Cownose Ray habits have received some attention due to their part in a suspected trophic cascade that may have

contributed to low scallop fishery catches (Peterson et al. 2001, Myers et al. 2007) though subsequent analysis found little support for these trophic relationships (Bade et al. 2014, Grubbs et al. 2016). The timing of Cownose Ray catches in Back and Core Sounds was consistent with known migratory patterns through North Carolina estuaries (Goodman et al. 2010). Southern Stingrays showed generalist habitat preferences based on catch data, but their abundance suggests that they may be ecologically important in this estuarine system (Chapter 3). Though ecological interactions involving Southern Stingrays have not received as much attention as those involving sharks or Cownose Rays, the feeding behavior of this species can affect seagrass growth and spatial extent through bioturbation, though these effects may vary by seagrass species (Valentine et al. 1994). Southern Stingrays feed on large numbers of crabs and shrimp (Gilliam and Sullivan 1993), but any potential affects on economically-important crustacean species in North Carolina have not been assessed. Batoids make up an important component of the North Carolina elasmobranch assemblage and are deserving of further research.

Conclusion

This study provides compiled data on shark habitat use patterns in North Carolina waters from several sources, and should be used as a baseline for future studies in the area. All surveys combined documented a total of 18 shark species within the nearshore and estuarine waters of North Carolina. For some species, specific habitat features, locations, and spatial extents were identified and

delineated, and this information should be used in future coastal development and fishery management decisions affecting these areas. The results of this study show that areas within North Carolina's coastal and estuarine waters are critical habitat for species of both economic interest and conservation concern, and illuminated the important roles sharks play in the fisheries of this highly productive ecosystem. However, there is still likely much to be learned. It is my hope that further efforts to define shark habitat and habitat use behavior in an ecosystem context will illuminate the ecosystem role and cultural importance of these species.

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Appendix

IACUC and IRB protocols

EAST CAROLINA UNIVERSITY
ANIMAL USE PROTOCOL (AUP) FORM
LATEST REVISION NOVEMBER, 2013

Project Title:

Identification of Juvenile Spiny Dogfish Habitats in North Carolina Coastal Waters

	Principal Investigator	Secondary Contact
Name	Roger A. Rulifson	Charles Bangley
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For IACUC Use Only

AUP #			
New/Renewal			
Full Review/Date	DR/Date		
Approval Date			
Study Type			
Pain/Distress Category			
Surgery	Survival	Multiple	
Prolonged Restraint			
Food/Fluid Regulation			
Other			
Hazard Approval/Dates	Rad	IBC	EHS
OHP Enrollment			
Mandatory Training			
Amendments Approved			

I. Personnel

A. Principal Investigator(s):

Click here to enter text.

B. Department(s):

Click here to enter text.

C. List all personnel (PI's, co-investigators, technicians, students) that will be working with live animals and describe their qualifications and experience with these specific procedures. If people are to be trained, indicate by whom:

Name/Degree/Certification	Position/Role(s)/ Responsibilities in this Project	Required Online IACUC Training (Yes/No)	Relevant Animal Experience/Training (include species, procedures, number of years, etc.)
Roger Rulifson	Principle investigator	Yes	Senior Scientist – Institute for Coastal Science and Policy Professor – Department of Biology Online training course (2013)
Charles Bangley	Investigator, animal capture, field procedures, data analysis	Yes	Online Training Course (2010, 2013) Animal Handling (2010, 2013) Animal Survival Surgery (2011) PhD student – Coastal Resource Management M.S. – Biology – East Carolina University B.S. – Marine Biology – University of Rhode Island Fisheries science experience – trawl, gillnet, longline, and beach seine surveys, species identification and live release techniques, assistance with surgical implantation of acoustic receivers Species experience: Coastal sharks including spiny dogfish, smooth dogfish, sandbar shark, dusky shark, Atlantic sharpnose shark
Andrea Dell'Apa	Investigator, animal capture, field procedures, data	Yes	Online Training Course (2010, 2013)

	analysis		Animal Handling (2010) Animal Survival Surgery (2010) Ph.D student in Coastal Resources Management. Seven years of fisheries and marine field experience, including 2 months as a field technician conducting acoustic telemetry tracking studies on Great White sharks in South Africa, 1 month of volunteer experience with the ECU spiny dogfish program (Sea Grant Fund #08-FEG-11), 9 months of acoustic telemetry research on the spiny dogfish shark (CFRC project), including acoustic transmitter surgeries. M.Sc. in Marine Biology and Oceanography – Università Politecnica delle Marche (Ancona – Italy)
Jennifer Cudney-Burch	Investigator, animal capture, field procedures, data analysis	Yes	Online Training Course (2008, 2010) Animal Handling (2008) Animal Survival Surgery (2008) Ph.D student in Coastal Resources Management. Nine years of fisheries field experience, 2 years as a research technician, including acoustic transmitter surgeries, learned aseptic field surgeries, and 3 years of acoustic telemetry research on the spiny dogfish (Sea Grant Fund #08-FFEG-11, and CFRC project) M.Sc. Biology – East Carolina University

			B.Sc. – Ohio Northern University
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II. Regulatory Compliance

A. Non-Technical Summary

Using language a non-scientist would understand, please provide a clear, concise, and sequential description of animal use. Additionally, explain the overall study objectives and benefits of proposed research or teaching activity to the advancement of knowledge, human or animal health, or good of society. (More detailed procedures are requested later in the AUP.)

Do not cut and paste the grant abstract.

Cape Hatteras, North Carolina, has been identified as an important nursery and foraging ground for several coastal shark species. Juveniles and neonates of species both important to fisheries (blacktip shark, sandbar shark, smooth and spiny dogfish) and of conservation concern (dusky shark, hammerhead species) have been observed near the Hatteras Bight by North Carolina watermen and researchers. Though the potential for shark nursery habitat in the Hatteras Bight has been considered for some species (dusky and sandbar sharks), for others these observations are unrecognized in the scientific literature and are nominally considered as "anecdotal evidence" by managers. For this reason current management measures for the spiny dogfish and coastal shark fisheries are controversial among North Carolina fishermen. Juveniles and neonates of other shark species have been common bycatch in the dogfish surveys, which may be an effective platform for gathering data on these other sharks. Therefore, we propose to explore the Hatteras Bight as a potential nursery ground for spiny dogfish and other coastal sharks using gillnet survey techniques and acoustic tagging methods.

B. Ethics and Animal Use

B.1. Duplication

Does this study duplicate existing research? Yes

If yes, why is it necessary? (note: teaching by definition is duplicative)

This study continues and expands on previous acoustic telemetry work by the Rulifson lab. Information from more tagged animals will be needed to truly define migration pathways, nursery habitat, and other areas of high activity.

B.2. Alternatives to the Use of Live Animals

Are there less invasive procedures, other less sentient species, isolated organ preparation, cell or tissue culture, or computer simulation that can be used in place of the live vertebrate species proposed here? No

If yes, please explain why you cannot use these alternatives.

Click here to enter text.

B.3. Consideration of Alternatives to Painful/Distressful Procedures

a. Include a literature search to ensure that alternatives to all procedures that may cause more than momentary or slight pain or distress to the animals have been considered.

1. Please list all of the potentially painful or distressful procedures in the protocol:

Capture, acoustic transmitter deployment.

2. For the procedures listed above, provide the following information (please do not submit search results but retain them for your records):

Date Search was performed:	06/19/13
Database(s) searched:	Google Scholar
Time period covered by the search (i.e. 1975-2013):	2001-2013
Search strategy (including scientifically relevant terminology):	shark longline mortality, shark gillnet mortality, shark capture survivability, shark surgery, fish surgery, acoustic telemetry

Other sources consulted:	Conversations with researchers familiar with longline and gillnet shark surveys, assistance with surgical procedures on spiny dogfish, observations made while assisting with shark longline and gillnet surveys, ECU IACUC animal survival surgery workshop

3. In a few sentences, please provide a brief narrative indicating the results of the search(es) to determine the availability of alternatives and explain why these alternatives were not chosen. Also, please address the 3 Rs of refinement, reduction, and replacement in your response. Refinement refers to modification of husbandry or experimental procedures to enhance animal well-being and minimize or eliminate pain and distress. Replacement refers to absolute (i.e. replacing animals with an inanimate system) or relative (i.e. using less sentient species) replacement. Reduction involves strategies such as experimental design analysis, application of newer technologies, use of appropriate statistical methods, etc., to use the fewest animals or maximize information without increasing animal pain or distress.

Survival rate of sharks captured in longline and gillnet gear varies by species, but generally is minimized by limiting soak time (Morgan and Carlson 2010). Most traditional mark-recapture studies on sharks have a less than 5% recapture rate, requiring the capture and tagging of thousands of animals in order to gather sufficient data (Kohler and Turner 2001). Telemetry methods require far fewer individuals and provide fine-scale, short-term data that is lacking in mark-recapture studies (Sims 2010). In fishes, surgically implanted acoustic transmitters are preferable to externally mounted transmitters because they are less likely to interfere with swimming behavior, cause infection, or be shed by the animal, but aseptic techniques must be used to minimize infection or other complications from the surgery (Mulcahy 2003). In sharks, the standard practice is to make the incision and insert the transmitter anterior to the pelvic fins, and allow the shark to recover in flowing seawater, assessing condition before release (Heupel and Heuter 2001). If best practices are followed, mortality from surgical procedures is very low even for species susceptible to stress: in a study on juvenile blacktip sharks (*Carcharhinus limbatus*), only five of 92 sharks surgically implanted with acoustic transmitters died within 24 hours of surgery, and two of these were a result of immediate post-release predation (Heupel and Simpfendorfer 2002). This type of research cannot be completed with a non-animal model.

C. Hazardous Agents

1. Protocol related hazards (chemical, biological, or radiological): Please indicate if any of the following are used in animals and the status of review/approval by the referenced committees:

HAZARDS	Oversight Committee	Status (Approved, Pending, Submitted)/Date	AUP Appendix I Completed?
Radioisotopes	**Radiation**	Click here to enter text.	Choose an item.
Ionizing radiation	**Radiation**	Click here to enter text.	Choose an item.
Infectious agents (bacteria, viruses, rickettsia, prions, etc.)	**IBC**	Click here to enter text.	Choose an item.
Toxins of biological origins (venoms, plant toxins, etc.)	**IBC**	Click here to enter text.	Choose an item.
Transgenic, Knock In, Knock Out Animals---breeding, cross breeding or any use of live animals or tissues	**IBC**	Click here to enter text.	Choose an item.
Human tissues, cells, body fluids, cell lines	**IBC**	Click here to enter text.	Choose an item.
Viral/Plasmid Vectors/Recombinant DNA or recombinant techniques	**IBC**	Click here to enter text.	Choose an item.
Oncogenic/toxic/mutagenic chemical agents	**EH&S**	Click here to enter text.	Choose an item.
Nanoparticles	**EH&S**	Click here to enter text.	Choose an item.
Cell lines, tissues or other biological products injected or implanted in animals	**DCM**	Click here to enter text.	Choose an item.
Other agents		Click here to enter text.	Choose an item.

2. Incidental hazards

Will personnel be exposed to any incidental zoonotic diseases or hazards during the study (field studies, primate work, etc)? If so, please identify each and explain steps taken to mitigate risk:

Dr Rulifson, Charles Bangley, Andrea Dell'Apa, and Jennifer Cudney-Burch have each had extensive boat safety and hazards training. Dr Rulifson co-taught a class on boating safety in conjunction with the NC Coast Guard Auxiliary for several years. Jennifer has had CPR/First Aid training and renewal since 2005.

Andrea received training in Italy for boat safety, as part of his driving boat license course (2009), and received additional MOCC training at ECU (2010). Charles is a former lifeguard and has current DAN CPR and First Aid certifications, as well as MOCC boat-handling certification. All participants have experience working aboard large and small research vessels in all types of weather, and are familiar with the hazards associated with working aboard boats in inclement conditions. Suitable personal equipment (life jackets, rain gear, float coats, insulated work boots, mustang survival suits) are available for protection against marine hazards. The potential exists to encounter hazardous marine life, and this is mitigated by the use of protective equipment such as gloves and foul-weather gear. All participants are experienced in identifying potentially hazardous marine organisms. Risk of injury is mitigated through protective equipment (gloves) and proper handling. The sharks will be anesthetized and inverted to induce tonic immobility, reducing panic response in the animals. Exposure to parasitic, viral, or bacterial diseases from contact with marine life will be minimized by wearing gloves when handling sharks and washing hands after contact.

III. Animals and Housing

A. Species and strains:

Principal species - *Carcharhinus limbatus*, *C. plumbeus*, *C. obscurus*, *Sphyrna lewini, Rhizoprionodon terraenovae*, *Mustelus canis*

B. Weight, sex and/or age:

Neonate to juvenile, 30-150 cm in length

C. Animal numbers:

1. Please complete the following table:

Total number of animals in treatment and control groups	Additional animals (Breeders, substitute animals)	Total number of animals used for this project
60 individuals total: external and internal tagging performed opportunistically on sharks in the principal species group (spiny dogfish, smooth dogfish, sandbar shark, dusky shark, blacktip shark, Atlantic sharpnose shark, scalloped hammerhead).	+5 (surgical mortality)	=65

2. Justify the species and number (use statistical justification when possible) of animals requested:

The principal species are the target of this research and cannot be replaced with others. We need to tag a sufficient number of animals to provide reliable outcomes on juvenile time and space distribution in the study area, and especially on environment parameters such as water temperature and depth experienced by tagged animals during the study.

3. Justify the number and use of any additional animals needed for this study:

Additional animals will be used only if a shark fails to recover from surgery within a half hour. A survival surgery rate of 100% has been obtained by this project team during previous and on-going research study on these species. Short-term (24-hr) mortality of gillnet-caught sharks was found to be 17% (Rulifson, 2007). Gillnets will be deployed for no more than half-hour soaks. A ratio comparison (e.g., 17% in 24 hrs vs. x% in 2 hrs) suggests an immediate 1.4% mortality for gillnets.

a. For unforeseen outcomes/complications:

While survivorship is expected to be high and all tagged sharks have been released alive, there is always the possibility of mortality during surgery. Five extra sharks should be sufficient to account for this.

b. For refining techniques:

Some species may require modifications to equipment (i.e. larger surgical needles). Five extra sharks should allow us to have back-up animals for this purpose.

c. For breeding situations, briefly justify breeding configurations and offspring expected:

Click here to enter text.

d. Indicate if following IACUC tail snip guidelines: Choose an item.

(if no, describe and justify)

Click here to enter text.

4. Will the phenotype of mutant, transgenic or knockout animals predispose them to any health, behavioral, physical abnormalities, or cause debilitating effects in experimental manipulations? No **(if yes, describe)**

Click here to enter text.

5. Are there any deviations from standard husbandry practices? No **If yes, then describe conditions and justify the exceptions to standard housing (temperature, light cycles, sterile cages, special feed, prolonged weaning times, wire-bottom cages, etc.):**

Click here to enter text.

6. The default housing method for social species is pair or group housing (including mice, rats, guinea pigs, rabbits, dogs, pigs, monkeys). Is it necessary for animals to be singly housed at any time during the study?

Choose an item. **(If yes, describe housing and justify the need to singly house social species):**
Click here to enter text.

7. Are there experimental or scientific reasons why routine environmental enrichment should not be provided? Choose an item.

(If yes, describe and justify the need to withhold enrichment)
Click here to enter text.

8. If wild animals will be captured or used, provide permissions (collection permit # or other required information):
Lab and field activities conducted in NC are supported under NC DMF permit # 706671 ("Scientific or educational collection permit") which is renewed annually by the Department of Biology at ECU.

9. List all laboratories or locations outside the animal facility where animals will be used. Note that animals may not stay in areas outside the animal facilities for more than 12 hours without prior IACUC approval. For field studies, list location of work/study site.
Animals will be retained aboard commercial fishing vessel based out of Cape Hatteras, NC, for approximately ½ hour in holding tanks. Animals will be collected in shallow coastal environments (<150 ft) to minimize barotraumas, and returned within the same capture region. Surgical animals will be observed for 10 minutes to ½ hour after surgery until sustained normal swimming activity is noted for 5 or more minutes.

IV. Animal Procedures

A. Outline the Experimental Design including all treatment and control groups and the number of animals in each. Tables or flow charts are particularly useful to communicate your design. Briefly state surgical plans in this section. Surgical procedures can be described in detail in IV.S.
60 juvenile sharks will be collected using gillnets and tagged both with external Floy tags and internal acoustic tags. Animals will be released approximately in the same location where captured. These animals will be tracked on currently-existing acoustic arrays off of Cape Hatteras and elsewhere on the U.S. east coast.

In sections IV.B-IV.S below, please respond to all items relating to your proposed animal procedures. If a section does not apply to your experimental plans, please leave it blank.

Please refer to DCM and IACUC websites for relevant guidelines and SOPs.

B. <u>Anesthesia/Analgesia/Tranquilization/Pain/Distress Management For Procedures Other than Surgery:</u>

Adequate records describing anesthetic monitoring and recovery must be maintained for all species.

If anesthesia/analgesia must be withheld for scientific reasons, please provide compelling scientific justification as to why this is necessary:

Click here to enter text.

1. Describe the pre-procedural preparation of the animals:

a. Food restricted for Click here to enter text. **hours**

b. Food restriction is not recommended for rodents and rabbits and must be justified:

Click here to enter text.

c. Water restricted for Click here to enter text. **hours**

d. Water restriction is not recommended in any species for routine pre-op prep and must be justified:

Click here to enter text.

2. Anesthesia/Analgesia *for Procedures Other than Surgery*

	Agent	Concentration	Dose (mg/kg)	Max Volume	Route	Frequency	Number of days administered
Pre-procedure analgesic	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.
Pre-anesthetic	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.
Anesthetic	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.

Post procedure analgesic	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.
Other	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.

3. Reason for administering agent(s):
Click here to enter text.
4. For which procedure(s):
Click here to enter text.
5. Methods for monitoring anesthetic depth:
Click here to enter text.
6. Methods of physiologic support during anesthesia and recovery:
Click here to enter text.
7. Duration of recovery:
Click here to enter text.
8. Frequency of recovering monitoring:
Click here to enter text.
9. Specifically what will be monitored?
Click here to enter text.
10. When will animals be returned to their home environment?
Click here to enter text.
11. Describe any behavioral or husbandry manipulations that will be used to alleviate pain, distress, and/or discomfort:
Click here to enter text.

C. Use of Paralytics

1. Will paralyzing drugs be used? Choose an item
2. For what purpose:
Click here to enter text.
3. Please provide scientific justification for paralytic use:
Click here to enter text.
4. Paralytic drug:
Click here to enter text.

5. Dose:
Click here to enter text.
6. Method of ensuring appropriate analgesia during paralysis:
Click here to enter text.

D. Blood or Body Fluid Collection

1. Please fill out appropriate sections of the chart below:

	Location on animal	Needle/catheter size	Volume collected	Frequency of procedure	Time interval between collections
Blood Collection	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.
Body Fluid Collection	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.
Other	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.

E. Injections, Gavage, & Other Substance Administration

1. Please fill out appropriate sections of the chart below:

	Compound	Location & Route of admin	Needle/catheter/gavage size	Max volume admin	Freq of admin (ie two times per day)	Number of days admin (ie for 5 days)	Max dosages (mg/kg)
Injection / Infusion	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.
Gavage	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.
Other	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.

3. **Pharmaceutical grade drugs, biologics, reagents, and compounds are defined as agents approved by the Food and Drug Administration (FDA) or for which a chemical purity standard has been written/established by any recognized pharmacopeia such as USP, NF, BP, etc. These standards are used by manufacturers to help ensure that the products are of the appropriate chemical purity and quality, in the appropriate solution or compound, to ensure stability, safety, and efficacy. For all injections and infusions for CLINICAL USE, PHARMACEUTICAL GRADE compounds must be used whenever possible. Pharmaceutical grade injections and infusions for research test articles are preferred when available. If pharmaceutical grade compounds are not available and non-pharmaceutical grade agents must be used, then the following information is necessary:**

a. **Please provide a scientific justification for the use of ALL non-pharmaceutical grade compounds. This may include pharmaceutical-grade compound(s) that are not available in the appropriate concentration or formulation, or the appropriate vehicle control is unavailable.**
b. **Indicate the method of preparation, addressing items such as purity, sterility, pH, osmolality, pyrogenicity, adverse reactions, etc. (please refer to ECU IACUC guidelines for non-pharmaceutical grade compound use), labeling (i.e. preparation and use-by dates), administration and storage of each formulation that maintains stability and quality/sterility of the compound(s).**

Click here to enter text.

F. Prolonged restraint with mechanical devices

Prolonged restraint in this context means *beyond routine care and use procedures* for rodent and rabbit restrainers, and large animal stocks. Prolonged restraint also includes *any* use of slings, tethers, metabolic crates, inhalation chambers, primate chairs and radiation exposure restraint devices.

1. **For what procedure(s):**

Click here to enter text.

2. **Explain why non-restraint alternatives cannot be utilized:**

Click here to enter text.

3. Restraint device(s):

Click here to enter text.

4. Duration of restraint:

Click here to enter text.

5. Frequency of observations during restraint/person responsible:

Click here to enter text.

6. Frequency and total number of restraints:

Click here to enter text.

7. Conditioning procedures:

Click here to enter text.

8. Steps to assure comfort and well-being:

Click here to enter text.

9. Describe potential adverse effects of prolonged restraint and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):

Click here to enter text.

G. Tumor Studies, Disease Models, Toxicity Testing, Vaccine Studies, Trauma Studies, Pain Studies, Organ or System Failure Studies, Shock Models, etc.

1. **Describe methodology:**

Click here to enter text.

2. **Expected model and/or clinical/pathological manifestations:**

Click here to enter text.

3. **Signs of pain/discomfort:**

Click here to enter text.

4. **Frequency of observations:**

Click here to enter text.

5. **Describe potential adverse side effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):**

Click here to enter text.

H. Treadmills/Swimming/Forced Exercise

1. Describe aversive stimulus (if used):

Click here to enter text.

2. Conditioning:

Click here to enter text.

3. Safeguards to protect animal:

Click here to enter text.

4. Duration:

Click here to enter text.

5. Frequency:

Click here to enter text.

6. Total number of sessions:

Click here to enter text.

7. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):

Click here to enter text.

I. Projects Involving Food and Water Regulation or Dietary Manipulation

(*Routine pre-surgical fasting not relevant for this section)*

1. Food Regulation

a. Amount regulated and rationale:

Click here to enter text.

b. Frequency and duration of regulation (hours for short term/weeks or months for long term):

Click here to enter text.

c. Frequency of observation/parameters documented (i.e. recording body weight, body condition, etc.):

Click here to enter text.

d. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):

Click here to enter text.

2. Fluid Regulation

a. Amount regulated and rationale:

Click here to enter text.

b. Frequency and duration of regulation (hours for short term/weeks or months for long term):

Click here to enter text.

c. Frequency of observation/parameters documented (body weight, hydration status, etc.):

Click here to enter text.

d. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):
Click here to enter text.

3. Dietary Manipulations

a. Compound supplemented/deleted and amount:
Click here to enter text.

b. Frequency and duration (hours for short term/week or month for long term):
Click here to enter text.

c. Frequency of observation/parameters documented:
Click here to enter text.

d. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):
Click here to enter text.

J. Endoscopy, Fluoroscopy, X-Ray, Ultrasound, MRI, CT, PET, Other Imaging

1. Describe animal methodology:
Click here to enter text.

2. Duration of procedure:
Click here to enter text.

3. Frequency of observations during procedure:
Click here to enter text.

4. Frequency/total number of procedures:
Click here to enter text.

5. Method of transport to/from procedure area:
Click here to enter text.

6. Describe potential adverse side effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):
Click here to enter text.

7. Please provide or attach appropriate permissions/procedures for animal use on human equipment:
Click here to enter text.

K. Polyclonal Antibody Production

1. **Antigen/adjuvant used and justification for adjuvant choice:**
   Click here to enter text.
2. **Needle size:**
   Click here to enter text.
3. **Route of injection:**
   Click here to enter text.
4. **Site of injection:**
   Click here to enter text.
5. **Volume of injection:**
   Click here to enter text.
6. **Total number of injection sites:**
   Click here to enter text.
7. **Frequency and total number of boosts:**
   Click here to enter text.
8. **What will be done to minimize pain/distress:**
   Click here to enter text.
9. **Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):**
   Click here to enter text.

L. Monoclonal Antibody Production

1. Describe methodology:
Click here to enter text.

2. Is pristane used: Choose an item.

Volume of pristane:
Click here to enter text.

3. Will ascites be generated: Choose an item.

i. **Criteria/signs that will dictate ascites harvest:**
   Click here to enter text.
ii. **Size of needle for taps:**
   Click here to enter text.
iii. **Total number of taps:**
   Click here to enter text.
iv. **How will animals be monitored/cared for following taps:**
   Click here to enter text.

4. What will be done to minimize pain/distress:
Click here to enter text.

5. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):

Click here to enter text.

M. Temperature/Light/Environmental Manipulations

1. Describe manipulation(s):
Click here to enter text.

2. Duration:
Click here to enter text.

3. Intensity:
Click here to enter text.

4. Frequency:
Click here to enter text.

5. Frequency of observations/parameters documented:
Click here to enter text.

6. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):
Click here to enter text.

N. Behavioral Studies

1. Describe methodology/test(s) used:
Click here to enter text.

2. Will conditioning occur? If so, describe:
Click here to enter text.

3. If aversive stimulus used, frequency, intensity and duration:
Click here to enter text.

4. Length of time in test apparatus/test situation: *(i.e., each test is ~10 mins)*
Click here to enter text.

5. Frequency of testing and duration of study: *(i.e., 5 tests/week for 6 months)*
Click here to enter text.

6. Frequency of observation/monitoring during test:
Click here to enter text.

7. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):
Click here to enter text.

O. Capture with Mechanical Devices/Traps/Nets

1. Description of capture device/method:

Small mesh gillnets will be deployed to capture individuals, for a maximum period of 30 minutes. The first set will be just after dawn and will continue with sets throughout the day until early afternoon.

2. Maximum time animal will be in capture device:

30 minutes

3. Frequency of checking capture device:

Gillnets will be retrieved after 30 minutes.

4. Methods to ensure well-being of animals in capture device:

Short soak times, gentle removal from gear (commercial fishermen will be instructed to not rip sharks out of or from gear). Also, retrieval will be slow due to fishing processing, which will allow acclimation to depth.

5. Methods to avoid non-target species capture:

Short soak time and immediate removal from gear if a non-target animal is captured.

6. Method of transport to laboratory/field station/processing site and duration of transport:

Sharks will be tagged and released at the same location.

7. Methods to ensure animal well-being during transport:

Sharks will be placed in holding tanks with circulating seawater onboard vessel to minimize stress. During surgery, sharks will have seawater flushed over the gills

8. Expected mortality rates:

We do not expect any short-term mortality of sharks

9. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):

Surgically implanted animals that do not display normal swimming activity after ½ hour will be anesthetized, euthanized, and held on ice until they may be properly disposed. Sharks will be anesthetized with 1 g/L MS-222 and then euthanized by severing the spinal cord

P. Manipulation of Wild-Caught Animals in the Field or Laboratory

1. **Parameters to be measured/collected:**

   All animals captured in the gear will be measured, sexed, and observed for general condition, previous tagging efforts, and extreme short-term mortality.

Animals selected to receive surgery will be anesthetized using tonic immobility, measured, sexed, subject to surgical procedure, observed for 10 minutes to ½ hour, tagged with an external tag and released immediately

2. **Approximate time required for data collection per animal:**

It takes approximately 2 minutes to measure an individual. External tagging takes 2 minutes. Surgery takes approximately 10-15 minutes

3. Method of restraint for data collection:

A surgical table with foam padding will be used to cushion the sharks and minimize patient movement or movements induced by the pitch and roll of the vessel. An assistant will help in holding the animal up-side down in place.

4. Methods to ensure animal well-being during processing:

Sharks will be kept in holding tanks until processing and have water flushed over the gills during surgery

5. Disposition of animals post-processing:

Sharks will be allowed to recover in live well tanks and gently returned to the water

6. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):

If the animal does not recover from surgery within a half hour, it will be euthanized by anesthetizing with MS-222 and severing the spinal cord

Q. Wildlife Telemetry/Other Marking Methods

1. Describe methodology (including description of device):

Sharks that are surgically implanted with compatible size tags will be returned to the water after a successful recovery period and will resume normal movement behaviors. Compatible receivers will be deployed in the study area, and we will receive acoustic detection data from individuals swimming within receivers range.

External Floy tags has a steel core and a barb on one side, designed to catch within the muscle tissue of the shark. The tag is inserted into the musculature using a canula, which is a sharpened, large gauge needle into which the tag is placed. After being pushed into the muscle tissue, the canula is gently slid out, the barb on the tag lodges into the muscle, and the tag is left behind.

2. Will telemetry device/tags/etc. be removed? Choose an item. If so, describe:

Tags will not be removed unless the animal does not survive surgery. If this is the case, the tag will be reinitialized, sterilized, and used in another surgery.

3. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):

We are actively advertising this study via a website, and by contacting agencies and departments that may encounter our tagged sharks. Sharks are easily identifiable as a study animal due to the bright external tag by the dorsal fin, and the surgical scars on the abdomen. We expect both recreational and commercial fishermen to encounter our animals. We ask that sharks bearing our tags be returned to the water to allow the study to continue. Sharks captured by fishermen will likely be released, due to measuring less than regulated size limits.

R. Other Animal Manipulations

1. Describe methodology:
Click here to enter text.

2. Describe methods to ensure animal comfort and well-being:
Click here to enter text.

3. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):
Click here to enter text.

S. Surgical Procedures

All survival surgical procedures must be done aseptically, regardless of species or location of surgery. Adequate records describing surgical procedures, anesthetic monitoring and postoperative care must be maintained for all species.

1. Location of Surgery (Building & Room #):
Onboard commercial fishing vessel

2. Type of Surgery (check all that are appropriate):
Click here to enter text.

☐ **Non-survival surgery (animals euthanized without regaining consciousness)**
☒ **Major survival surgery (major surgery penetrates and exposes a body cavity or produces substantial impairment of physical or physiologic function)**
☐ **Minor survival surgery**
☐ **Multiple survival surgery**
If yes, provide scientific justification for multiple survival surgical procedures:
Click here to enter text.

3. Describe the pre-op preparation of the animals:

a. **Food restricted for** Click here to enter text. **hours**
b. **Food restricted is not recommended for rodents and rabbits and must be justified:**

Click here to enter text.

c. Water restricted for Click here to enter text. **hours**

d. Water restriction is not recommended in any species for routine pre-op prep and be justified:

Click here to enter text.

4. Minimal sterile techniques will include (check all that apply):

Please refer to DCM Guidelines for Aseptic Surgery for specific information on what is required for each species and type of surgery (survival vs. non-survival).

☒ **Sterile instruments**

How will instruments be sterilized?

Instruments will be autoclaved by surgery staff and stored in sterile packaging

If serial surgeries are done, how will instruments be sterilized between surgeries:

Instruments will be placed in Nolvasan disinfectant, a sterile wash, and then placed in a glass bead sterilizer. Each surgical pack will be sterilized between each surgery. One surgical assistant will be responsible for continuously sterilizing instruments via cold sterilization while the other provides assistance directly to the surgeon. Each surgical pack will be used 2 times a day.

☒ **Sterile gloves**

☐ **Mask**

☐ **Cap**

☐ **Sterile gown**

☐ **Sanitized operating area**

☐ **Clipping or plucking of hair or feathers**

☒ **Skin preparation with a sterilant such as betadine**

☒ **Practices to maintain sterility of instruments during surgery**

☐ **Non-survival (clean gloves, clean instruments, etc.)**

5. Describe all surgical procedures:

a. Skin incision size and site on the animal:

Incision will be approximately 1-2 cm long, and be located on the abdomen 2/3 of the way between the pectoral and pelvic fin. The incision will be made on the midline of the shark where the muscle layers are thinnest

b. Describe surgery in detail (include size of implant if applicable):

The sharks will be placed into holding tanks that will have fresh seawater pumped in. Surgeries will start after tonic immobility is reached (the shark has stopped reacting to stimuli and shows minimal outward signs of stress). Sharks will be placed onto foam padding surgery table. Sea water will be continuously flushed over gills. The incision site will be swabbed with a Q tip soaked on Nolvasan disinfectant. A small incision will be made in the abdomen at roughly 2/3 of the distance between the pectoral fins and the cloaca, on the midline of the sharks. A sterilized transmitter (36 mm X 9 mm X 2.9 g in water) will be inserted into the abdomen and gently pushed anterior the incision. The incision will be closed using either a simple continuous or simple interrupted surgical suture pattern. The shark will be placed into a recovery tank and observed for up to ½ hour prior to release.

c. Method of wound closure:

Click here to enter text.

i. Number of layers

Click here to enter text.

ii. Type of wound closure and suture pattern:

Simple continuous or simple interrupted suture pattern

iii. Suture type/size/wound clips/tissue glue:

3-0 absorbable monofilament suture

iv. Plan for removing of skin sutures/wound clip/etc:

We will not remove the sutures

6. Anesthetic Protocol:

a. If anesthesia/analgesia must be withheld for scientific reasons, please provide compelling scientific justification as to why this is necessary:

lidocaine provides longer residual analgesia.

b. Anesthesia/Analgesia For Surgical Procedures

	Agent	Dose (mg/kg or %)	Volume	Route	Frequency	Number of days administered
Pre-operative analgesic	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.
Pre-anesthetic	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.
Anesthetic	Lidocaine	2 %	2 mL	Injection block	Once	Click here to enter text.

Post-operative Analgesic	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.
Other	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.

c. Methods that will be used to monitor anesthetic depth (include extra measures employed when paralyzing agents are used):

Sharks will be gently held up-side down on a surgical table (with fresh circulating seawater flushed over the gills). Surgery will not start until after the shark ceases to move

d. Methods of physiologic support during anesthesia and immediate post-op period (fluids, warming, etc.):

Flushing of fresh seawater across gills, tonic immersion, recovery in fresh seawater

e. List what parameters are monitored during immediate post-op period. Provide the frequency and duration:

10 -30 minutes

f. Describe any other manipulations that will be used to alleviate pain, distress, and/or discomfort during the immediate post-op period (soft bedding, long sipper tubes, food on floor, dough diet, etc.):

We will ensure that gill activity continues throughout surgery

g. List criteria used to determine when animals are adequately recovered from anesthesia and when the animals can be returned to their home environment:

After observed swimming normally in recovery holding tanks for a period of 10 minutes or longer

7. Recovery from Surgical Manipulations (after animal regains consciousness and is returned to its home environment)

Click here to enter text.

a. What parameters (behavior, appetite, mobility, wound healing, etc.) will be monitored:

Transmitters pings (impulses). Rapid changes in impulse directionality, provide a clue of animal recovery from surgery

b. How frequently (times per day) will animals be monitored:

Sharks will be checked for proper transmitter functioning while in the recovery tank. We will deploy moored receiver that will track sharks movements while in the study area. Other moored receivers are located

along the coast and will also track the sharks as they migrate between North Carolina and Canada

c. How long post-operatively (days) will animals be monitored:

Up to 3 years

8. Surgical Manipulations Affecting Animals

a. Describe any signs of pain/discomfort/functional deficits resulting from the surgical procedure:

Thrashing during surgery

b. What will be done to manage any signs of pain or discomfort (include pharmacologic and non-pharmacologic interventions):

Surgery procedures will cease until shark stop moving (with the wound held shut by the surgeon)

c. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):

Buoyancy/orientation issues may occur because the open air surgery allows air into the body cavity. Therefore, sharks will be allowed a half hour recovery time with assisted swimming (gently grasping the caudal peduncle and moving the animal back and forth in the water) as necessary. If the animal dies or shows an inability to recover from surgery within a half hour, it will be humanely euthanized

V. Euthanasia

Please refer to the AVMA Guidelines for the Euthanasia of Animals: 2013 Edition and DCM Guidelines to determine appropriate euthanasia methods.

A. Euthanasia Procedure. ***All investigators, even those conducting non-terminal studies, must complete this section in case euthanasia is required for humane reasons.***

1. Physical Method- If a physical method is used, the animal should be first sedated/anesthetized with CO_2 or other anesthetic agent. If prior sedation is not possible, a scientific justification must be provided:

Sharks will be dosed with buffered MS-222 (1 g/L) and then the spinal cord will be severed

2. Inhalant Method- Choose an item.
(if other, describe the agent and delivery method)
Click here to enter text.

3. Non-Inhalant Pharmaceutical Method (injectables, MS-222, etc.)- Please provide the following:

a. Agent:
MS-222

b. Dose or concentration:
1 g/L

c. Route:
In holding tank water

B. Method of ensuring death (can be physical method, such as pneumothorax or decapitation for small species and assessment method such as auscultation for large animals):

Freezer flowing euthanasia will be used as a means to confirm death

C. Describe disposition of carcass following euthanasia:

Carcasses will be returned to the university or an appropriate lab facility for examination, dissection, and disposal. They will be transported on ice in coolers until reaching a facility that is capable of disposing the remains. Carcasses will be placed in red biohazard bags and frozen until pick-up. Contents of biohazard bags are incinerated.

I acknowledge that humane care and use of animals in research, teaching and testing is of paramount importance, and agree to conduct animal studies with professionalism, using ethical principles of sound animal stewardship. I further acknowledge that I will perform only those procedures that are described in this AUP and that my use of animals must conform to the standards described in the Animal Welfare Act, the Public Health Service Policy, The Guide For the Care and Use of Laboratory Animals, the Association for the Assessment and Accreditation of Laboratory Animal Care, and East Carolina University.

Please submit the completed animal use protocol form via e-mail attachment to iacuc@ecu.edu. You must also carbon copy your Department Chair.

PI Signature: _____ Date: _____

Veterinarian:_____Date:_____

IACUC Chair: _____Date:_____

APPENDIX 1-HAZARDOUS AGENTS			
Principal Investigator:	Campus Phone:		Home Phone:
IACUC Protocol Number:	Department:		E-Mail:
Secondary Contact: Department:	Campus Phone:	Home Phone:	E-Mail:
Chemical Agents used:		Radioisotopes used:	
Biohazardous Agents used:	Animal Biosafety Level:		Infectious to humans?
PERSONAL PROTECTIVE EQUIPMENT REQUIRED:			
Route of Excretion:			
Precautions for Handling Live or Dead Animals:			
Animal Disposal:			
Bedding/Waste Disposal:			
Cage Decontamination:			
Additional Precautions to Protect Personnel, Adjacent Research Projects including Animals and the Environment:			
Initial Approval Safety/Subject Matter Expert Signature & Date _____			

East Carolina University

Animal Care and
Use Commitee
212 Ed Warren Life
Sciences Building
East Carolina University
Greenville, NC 27834

252-744-2436 office
252-744-2355 fax

May 16, 2012

Roger Rulifson, Ph.D.
Department of ICSP/Biology
Howell Science Complex
East Carolina University

Dear Dr. Rulifson:

Your Animal Use Protocol entitled, "Identifying Nursery Habitats for Coastal Sharks within Pamlico Sound, NC" (AUP #D276) was reviewed by this institution's Animal Care and Use Committee on 5/16/12. The following action was taken by the Committee:

"Approved as submitted"

A copy is enclosed for your laboratory files. Please be reminded that all animal procedures must be conducted as described in the approved Animal Use Protocol. Modifications of these procedures cannot be performed without prior approval of the ACUC. The Animal Welfare Act and Public Health Service Guidelines require the ACUC to suspend activities not in accordance with approved procedures and report such activities to the responsible University Official (Vice Chancellor for Health Sciences or Vice Chancellor for Academic Affairs) and appropriate federal Agencies.

Sincerely yours,

Scott E. Gordon, Ph.D.
Chairman, Animal Care and Use Committee

SEG/jd

enclosure

East Carolina University is a constituent institution of the University of North Carolina. An equal opportunity university.

EAST CAROLINA UNIVERSITY
ANIMAL USE PROTOCOL (AUP) FORM
LATEST REVISION NOVEMBER, 2013

Project Title:

Habitat use by juvenile coastal sharks in Back and Core Sounds

	Principal Investigator	Secondary Contact
Name	Dr. Roger A. Rulifson	Charles W. Bangley
Dept.	Institute for Coastal Science and Policy	Institute for Coastal Science and Policy
Office Ph #	252-328-9400	252-328-9407
Cell Ph #	252-412-4411	401-829-0782
Pager #	Click here to enter text.	Click here to enter text.
Home Ph #	Click here to enter text.	Click here to enter text.
Email	rulifsonr@ecu.edu	bangleyc09@students.ecu.edu

For IACUC Use Only

AUP #			
New/Renewal			
Full Review/Date	DR/Date		
Approval Date			
Study Type			
Pain/Distress Category			
Surgery	Survival	Multiple	
Prolonged Restraint			
Food/Fluid Regulation			
Other			
Hazard Approval/Dates	Rad	IBC	EHS
OHP Enrollment			
Mandatory Training			
Amendments Approved			

II. Personnel

D. Principal Investigator(s):

Roger A. Rulifson

E. Department(s):

Institute for Coastal Science and Policy

F. List all personnel (PI's, co-investigators, technicians, students) that will be working with live animals and describe their qualifications and experience with these specific procedures. If people are to be trained, indicate by whom:

Name/Degree/Certification	Position/Role(s)/ Responsibilities in this Project	Required Online IACUC Training (Yes/No)	Relevant Animal Experience/Training (include species, procedures, number of years, etc.)
Roger A. Rulifson	PI, administration	Yes	Senior Scientist – Institute for Coastal Science and Policy Professor – Department of Biology Online Training Course (2013)
Charles W. Bangley	Investigator, animal capture, conducting field and laboratory procedures	Yes	Online Training Course (2010, 2013) Animal Handling (2010, 2013) Animal Survival Surgery (2011) PhD student – Coastal Resource Management M.S. – Biology – East Carolina University B.S. – Marine Biology – University of Rhode Island Fisheries science experience – trawl, gillnet, longline, and beach seine surveys, species identification and live release techniques, assistance with surgical implantation of acoustic receivers – spiny dogfish, smooth dogfish, sandbar shark, dusky shark,

			Atlantic sharpnose shark
Click here to enter text.	Click here to enter text.	Choose an item.	Click here to enter text.
Click here to enter text.	Click here to enter text.	Choose an item.	Click here to enter text.
Click here to enter text.	Click here to enter text.	Choose an item.	Click here to enter text.
Click here to enter text.	Click here to enter text.	Choose an item.	Click here to enter text.
Click here to enter text.	Click here to enter text.	Choose an item.	Click here to enter text.
Click here to enter text.	Click here to enter text.	Choose an item.	Click here to enter text.
Click here to enter text.	Click here to enter text.	Choose an item.	Click here to enter text.
Click here to enter text.	Click here to enter text.	Choose an item.	Click here to enter text.

II. Regulatory Compliance

B. Non-Technical Summary

Using language a non-scientist would understand, please provide a clear, concise, and sequential description of animal use. Additionally, explain the overall study objectives and benefits of proposed research or teaching activity to the advancement of knowledge, human or animal health, or good of society. (More detailed procedures are requested later in the AUP.)

Do not cut and paste the grant abstract.

Back and Core Sounds, NC, are potentially contains important nursery habitat for coastal sharks, and contain a wide variety of microhabitat such as oyster reefs, sand flats, and seagrass beds. To determine which areas of the sounds are used by coastal sharks, a combination of fishery-independent survey techniques and passive acoustic telemetry will be used. Sharks will be captured by rod and reel, drumline, gillnet, and longline gear, deployed at areas adjacent to or far from important habitat types within Back and Core Sound. Species, length, sex, and externally visible life history parameters will be recorded for all captured sharks. Stomach contents will be collected non-lethally using gastric lavage from no more than 10 sharks of each species at each station. Select sharks in good condition will be chosen for acoustic telemetry analysis. Acoustic transmitters will be surgically implanted in the body cavity of the chosen sharks, and receivers will be deployed at locations within different habitat types. Sampling will occur 2-3 times per month from March-November, and acoustic receivers will be maintained from July-

November. Relative catch rates and acoustic detections will be used to determine which habitat types are used most often, and stomach contents will be compared with prey species available in each habitat type.

B. Ethics and Animal Use

B.1. Duplication

Does this study duplicate existing research? Yes.

If yes, why is it necessary? (note: teaching by definition is duplicative)

Juvenile shark surveys are conducted regularly in Virginia and South Carolina waters. Juvenile sharks are also documented in NCDMF longline and gillnet surveys in Pamlico Sound. However, no survey specifically targeting coastal sharks has been conducted in Back and Core Sounds, and no nearby shark surveys incorporate stomach content analysis or acoustic telemetry to assess habitat selectivity.

B.2. Alternatives to the Use of Live Animals

Are there less invasive procedures, other less sentient species, isolated organ preparation, cell or tissue culture, or computer simulation that can be used in place of the live vertebrate species proposed here? No.

If yes, please explain why you cannot use these alternatives.

Click here to enter text.

B.3. Consideration of Alternatives to Painful/Distressful Procedures

a. Include a literature search to ensure that alternatives to all procedures that may cause more than momentary or slight pain or distress to the animals have been considered.

1. Please list all of the potentially painful or distressful procedures in the protocol:

Capture, stomach content collection, acoustic transmitter deployment.

2. For the procedures listed above, provide the following information (please do not submit search results but retain them for your records):

Date Search was performed:	02/25/14

Database(s) searched:	Google Scholar
Time period covered by the search (i.e. 1975-2013):	1990-2013
Search strategy (including scientifically relevant terminology):	shark longline mortality, shark gillnet mortality, shark capture survivability, shark surgery, fish surgery, acoustic telemetry
Other sources consulted:	Conversations with researchers familiar with coastal shark surveys, experience with surgical and gastric lavage procedures on spiny dogfish and coastal sharks, observations made while assisting with shark longline and gillnet surveys, ECU IACUC animal survival surgery workshop

3. In a few sentences, please provide a brief narrative indicating the results of the search(es) to determine the availability of alternatives and explain why these alternatives were not chosen. Also, please address the 3 Rs of refinement, reduction, and replacement in your response. Refinement refers to modification of husbandry or experimental procedures to enhance animal well-being and minimize or eliminate pain and distress. Replacement refers to absolute (i.e. replacing animals with an inanimate system) or relative (i.e. using less sentient species) replacement. Reduction involves strategies such as experimental design analysis, application of newer technologies, use of appropriate statistical methods, etc., to use the fewest animals or maximize information without increasing animal pain or distress.

Survival rate of sharks captured in commercial fishing and scientific sampling gear varies by species, but generally is minimized by limiting soak time (Morgan and Carlson 2010). In order to balance soak time necessary to adequately sample the site and shark survival, our sets will be limited to soak times of 30 minutes (gillnet and longline) or 1 hour (drumline), which allow for high survival in nearly all sampled species (Morgan and Carlson 2010). In sharks, physiological stress response increases with fight time (Skomal and Bernal 2010), so efforts will be made to minimize fight time in hook and line sets. Stomach tube gastric lavage is non-lethal and effective, consistently recovering over 90% of stomach contents with a 100% survival rate for spiny dogfish sharks (Bangley et al. 2013, Bangley and Rulifson In Review). This method will be used to collect stomach content data without sacrificing the animals. For stomach content analysis, the general rule is "the more the better," and the number of samples necessary to adequately describe feeding habits can

vary both by the study area and species. In a multispecies study of adult-sized sharks in nearshore Virginia waters, Gelscleichter et al. (1999) found that only 64 stomach content samples were needed to describe the diet of the crustacean specialist smooth dogfish, but 153 samples inadequately described the diet of the generalist dusky shark. Dietary breadth is usually constrained in juvenile sharks by both their smaller size and activity areas relative to adults (Grubbs 2010). Because of this we should require fewer samples than are needed for adult sharks in more open study areas. Most traditional mark-recapture studies on sharks have a less than 5% recapture rate, requiring the capture and tagging of thousands of animals in order to gather sufficient data (Kohler and Turner 2001). Acoustic telemetry methods require the capture and handling of far fewer individuals and provide fine-scale, short-term data that is lacking in mark-recapture studies, with the added benefit of multiple redetections for individual animals. For example, our previous work off of Hatteras shows a redetection rate of 73% with only 15 sharks tagged, and individuals were detected multiple times over the course of 9 months. Heupel and Simpfendorfer (2002) tagged 92 sharks over the course of three years in a semi-enclosed bay and attained a 100% detection rate. In addition, acoustic detections of relatively few tagged sharks in different habitat types provide better data on habitat preferences than relative catch rates of high numbers of sharks near or in those habitats (Sims 2010). In fishes, surgically implanted acoustic transmitters are preferable to externally mounted transmitters because they are less likely to interfere with swimming behavior, cause infection, or be shed by the animal, but aseptic techniques must be used to minimize infection or other complications from the surgery (Mulcahy 2003). In sharks, the standard practice is to put the shark in a state of tonic immobility, make the incision and insert the transmitter anterior to the pelvic fins, and allow the shark to recover in flowing seawater, assessing condition before release (Heupel and Heuter 2001). If best practices are followed, mortality from surgical procedures is very low even for species susceptible to stress: in a study on juvenile blacktip sharks (*Carcharhinus limbatus*), only five of 92 sharks surgically implanted with acoustic transmitters died within 24 hours of surgery, and two of these were a result of immediate post-release predation (Heupel and Simpfendorfer 2002). This type of research cannot be completed with a non-animal model, and requires working with specific species.

C. Hazardous Agents

4. Protocol related hazards (chemical, biological, or radiological): Please indicate if any of the following are used in animals and the status of review/approval by the referenced committees:

HAZARDS	Oversight Committee	Status (Approved, Pending, Submitted)/Date	AUP Appendix I Completed?
Radioisotopes	**Radiation**	Click here to enter text.	Choose an item.

Ionizing radiation	**Radiation**	Click here to enter text.	Choose an item.
Infectious agents (bacteria, viruses, rickettsia, prions, etc.)	**IBC**	Click here to enter text.	Choose an item.
Toxins of biological origins (venoms, plant toxins, etc.)	**IBC**	Click here to enter text.	Choose an item.
Transgenic, Knock In, Knock Out Animals---breeding, cross breeding or any use of live animals or tissues	**IBC**	Click here to enter text.	Choose an item.
Human tissues, cells, body fluids, cell lines	**IBC**	Click here to enter text.	Choose an item.
Viral/Plasmid Vectors/Recombinant DNA or recombinant techniques	**IBC**	Click here to enter text.	Choose an item.
Oncogenic/toxic/mutagenic chemical agents	**EH&S**	Click here to enter text.	Choose an item.
Nanoparticles	**EH&S**	Click here to enter text.	Choose an item.
Cell lines, tissues or other biological products injected or implanted in animals	**DCM**	Click here to enter text.	Choose an item.
Other agents		Click here to enter text.	Choose an item.

5. Incidental hazards

Will personnel be exposed to any incidental zoonotic diseases or hazards during the study (field studies, primate work, etc)? If so, please identify each and explain steps taken to mitigate risk:

Dr Rulifson and Charles Bangley have each had extensive boat safety and hazards training. Dr Rulifson co-taught a class on boating safety in conjunction with the NC Coast Guard Auxiliary for several years. Charles is a former lifeguard and has current DAN CPR and First Aid certifications, as well as MOCC boat-handling certification. All participants have experience working aboard large and small research vessels in all types of weather, and are familiar with the hazards associated with working aboard boats in inclement conditions. Suitable personal equipment (life jackets, rain gear, float coats, insulated work boots, mustang survival suits) will be available for protection against marine hazards. The potential exists to encounter hazardous marine life, and this is mitigated by the use of protective equipment such as gloves and foul-weather gear. All participants are experienced in identifying potentially hazardous marine organisms. Risk of injury is mitigated through protective equipment (gloves)

and proper handling. The sharks will be anesthetized and inverted to induce tonic immobility, reducing panic response in the animals. Exposure to parasitic, viral, or bacterial diseases from contact with marine life will be minimized by wearing gloves when handling sharks and washing hands after contact.

III. Animals and Housing

C. Species and strains:

Principal species - *Carcharhinus limbatus*, *C. leucas, S. tiburo, Rhizoprionodon terraenovae*, *Mustelus canis*

D. Weight, sex and/or age:

Click here to enter text.

C. Animal numbers:

1. Please complete the following table:

Total number of animals in treatment and control groups	Additional animals (Breeders, substitute animals)	Total number of animals used for this project
50 individuals total: internal tagging performed opportunistically on sharks in the principal species group. Stomach contents will be collected from a maximum of 10 individuals per station with a goal of 30 samples for each principle species (at least 150 individuals).	+10 (surgical mortality or back-up animasl)	=210

2. Justify the species and number (use statistical justification when possible) of animals requested:

The principal species are the most reliably encountered, ecologically important, and economically important species in the study area, and cannot be replaced by other species. We need to tag a sufficient number of animals to provide a reliable probability of detecting the animals within the estuary. Our prior research off of Cape Hatteras shows that 10-15 sharks per species should be sufficient to expect a redetection of 70-80% of tagged sharks in an open coastal environment. Working in a Florida coastal lagoon that shares features with Back Sound, Heupel and Simpfendorfer (2002) tagged 18-44 juvenile blacktip sharks per year over three years and redetected every individual. Usually, a high number of stomach content samples are necessary to ensure a representative sample covering the breadth of the diet. The exact number needed can vary, with relatively fewer needed for

specialist feeders and higher numbers needed for dietary generalists. Dietary breadth can also be limited by the geographic constraints of the study area. Cumulative prey curve analysis shows 64 samples adequately described the diet of a specialist shark species in an open nearshore study area (Gelsleichter et al. 1999). Within the limited geographic range of this study, we conservatively estimate that 30 stomach content samples per species may sufficiently account for common prey species.

3. Justify the number and use of any additional animals needed for this study:

Additional animals will be used only if a shark fails to recover from surgery within a half hour. A survival surgery rate of 100% has been obtained by this project team during previous and on-going research study on these species.

a. For unforeseen outcomes/complications:

While survivorship is expected to be high and all tagged sharks have been released alive, there is always the possibility of mortality during surgery. Five extra sharks should be sufficient to account for this.

c. For refining techniques:

Some species may require modifications to equipment (i.e. larger surgical needles). Five extra sharks should allow us to have back-up animals for this purpose.

c. For breeding situations, briefly justify breeding configurations and offspring expected:

Click here to enter text.

d. Indicate if following IACUC tail snip guidelines: Choose an item.

(if no, describe and justify)

Click here to enter text.

4. Will the phenotype of mutant, transgenic or knockout animals predispose them to any health, behavioral, physical abnormalities, or cause debilitating effects in experimental manipulations? No. **(if yes, describe)**

Click here to enter text.

5. Are there any deviations from standard husbandry practices? Choose an item. **If yes, then describe conditions and justify the exceptions to standard housing (temperature, light cycles, sterile cages, special feed, prolonged weaning times, wire-bottom cages, etc.):**

Click here to enter text.

6. The default housing method for social species is pair or group housing (including mice, rats, guinea pigs, rabbits, dogs, pigs,

monkeys). Is it necessary for animals to be singly housed at any time during the study?

Choose an item. **(If yes, describe housing and justify the need to singly house social species):**

Click here to enter text.

7. Are there experimental or scientific reasons why routine environmental enrichment should not be provided? Choose an item.

(If yes, describe and justify the need to withhold enrichment)

Click here to enter text.

8. If wild animals will be captured or used, provide permissions (collection permit # or other required information):

Lab and field activities conducted in NC are supported under NC DMF permit # 706671 ("Scientific or educational collection permit") which is renewed annually by the Department of Biology at ECU.

9. List all laboratories or locations outside the animal facility where animals will be used. Note that animals may not stay in areas outside the animal facilities for more than 12 hours without prior IACUC approval. For field studies, list location of work/study site.

Sharks will be captured, stomach contents collected, and surgical procedures performed aboard small (>24 feet in length) research vessels on Back and Core Sounds in the vicinity of Beaufort, Davis, and Cedar Island, NC.

IV. Animal Procedures

B. Outline the Experimental Design including all treatment and control groups and the number of animals in each. Tables or flow charts are particularly useful to communicate your design. Briefly state surgical plans in this section. Surgical procedures can be described in detail in IV.S.

Juvenile sharks will be captured using rod and reel, drumline, gillnet, and longlinge gear. Stomach contents will be collected from 10 individual sharks per station using stomach tube gastric lavage. During lavage procedures, a 20-50 mm diameter acrylic tube (tube diameter will depend on the mouth width of the shark) will be inserted into the stomach through the esophagus, and flushed with salt water until three consecutive flushes return no stomach contents. Sharks that have undergone gastric lavage will be supplied with salt water over their gills before release. Over the course of the study period, 50 select individuals in good condition will be tagged both with external Floy tags and internal acoustic tags. Stomach contents will not be collected from sharks chosen for acoustic telemetry.

Animals will be released approximately in the same location where captured. These animals will be tracked on acoustic receivers to be deployed within Back Sound from July-November 2014.

In sections IV.B-IV.S below, please respond to all items relating to your proposed animal procedures. If a section does not apply to your experimental plans, please leave it blank.

Please refer to DCM and IACUC websites for relevant guidelines and SOPs.

B. Anesthesia/Analgesia/Tranquilization/Pain/Distress Management For Procedures Other than Surgery:

Adequate records describing anesthetic monitoring and recovery must be maintained for all species.

If anesthesia/analgesia must be withheld for scientific reasons, please provide compelling scientific justification as to why this is necessary:

Tonic immobility is used in place of anesthesia/analgesia. Tonic immobility can be induced in less than 40 seconds for most species and produces a deep enough response to allow safe handling and surgical procedures (Henningsen 1994). It is also preferable to anesthesia because sharks recover from tonic immobility immediately upon being righted (Whitman et al. 1986). MS-222 can have a half-life of up to 56 minutes in sharks, during which the shark may still feel the effects (Stenger and Maren 1974). Also, the sharks in this study are targeted by fisheries, and MS-222 requires at least a 21-day withdrawal period before fish subjected to it can be safely consumed (Marking and Meyer 1985). It is impossible to know whether the released sharks will be captured by fisheries and how long after release this may occur, so the only way to verify that the sharks would be safe for human consumption would be to keep them in captivity until total MS-222 withdrawal is confirmed.

1. Describe the pre-procedural preparation of the animals:

c. Food restricted for Click here to enter text. **hours**

d. Food restriction is not recommended for rodents and rabbits and must be justified:

Click here to enter text.

c. Water restricted for Click here to enter text. **hours**
d. Water restriction is not recommended in any species for routine pre-op prep and must be justified:
Click here to enter text.

2. Anesthesia/Analgesia *for Procedures Other than Surgery*

	Agent	Concentration	Dose (mg/kg)	Max Volume	Route	Frequency	Number of days administered
Pre-procedure analgesic	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.
Pre-anesthetic	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.
Anesthetic	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.
Post procedure analgesic	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.
Other	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.

3. Reason for administering agent(s):
Click here to enter text.
4. For which procedure(s):
Click here to enter text.
5. Methods for monitoring anesthetic depth:
Click here to enter text.
6. Methods of physiologic support during anesthesia and recovery:
Click here to enter text.
7. Duration of recovery:
Click here to enter text.

8. Frequency of recovering monitoring:
Click here to enter text.
9. Specifically what will be monitored?
Click here to enter text.
10. When will animals be returned to their home environment?
Click here to enter text.
11. Describe any behavioral or husbandry manipulations that will be used to alleviate pain, distress, and/or discomfort:
Click here to enter text.

C. Use of Paralytics

1. Will paralyzing drugs be used? Choose an item
2. For what purpose:
Click here to enter text.
3. Please provide scientific justification for paralytic use:
Click here to enter text.
4. Paralytic drug:
Click here to enter text.
5. Dose:
Click here to enter text.
6. Method of ensuring appropriate analgesia during paralysis:
Click here to enter text.

D. Blood or Body Fluid Collection

1. Please fill out appropriate sections of the chart below:

	Location on animal	Needle/catheter size	Volume collected	Frequency of procedure	Time interval between collections
Blood Collection	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.
Body Fluid Collection	Stomach, esophagus	Varies by shark, 20-50 mm diameter	Full volume of stomach contents (0-	Once.	N/A

			0.5L)		
Other	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.

E. Injections, Gavage, & Other Substance Administration

1. Please fill out appropriate sections of the chart below:

	Compound	Location & Route of admin	Needle/catheter/gavage size	Max volume admin	Freq of admin (ie two times per day)	Number of days admin (ie for 5 days)	Max dosages (mg/kg)
Injection / Infusion	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.
Gavage	Salt water	Stomach, esophagus	Varies by shark, 20-50 mm diameter	Full volume of stomach contents removed (0-0.5L), up to three flushes, nothing added	Once.	N/A	N/A
Other	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.

6. **Pharmaceutical grade drugs, biologics, reagents, and compounds are defined as agents approved by the Food and Drug Administration (FDA) or for which a chemical purity standard has been written/established by any recognized pharmacopeia such as USP, NF, BP, etc. These standards are used by manufacturers to help ensure that the products are of the appropriate chemical purity and quality, in the appropriate solution or compound, to ensure stability, safety, and efficacy. For all injections and infusions for CLINICAL USE, PHARMACEUTICAL GRADE compounds must be used whenever possible. Pharmaceutical grade injections and infusions**

for research test articles are preferred when available. If pharmaceutical grade compounds are not available and non-pharmaceutical grade agents must be used, then the following information is necessary:

c. **Please provide a scientific justification for the use of ALL non-pharmaceutical grade compounds. This may include pharmaceutical-grade compound(s) that are not available in the appropriate concentration or formulation, or the appropriate vehicle control is unavailable.**
d. **Indicate the method of preparation, addressing items such as purity, sterility, pH, osmolality, pyrogenicity, adverse reactions, etc. (please refer to ECU IACUC guidelines for non-pharmaceutical grade compound use), labeling (i.e. preparation and use-by dates), administration and storage of each formulation that maintains stability and quality/sterility of the compound(s).**

Click here to enter text.

F. Prolonged restraint with mechanical devices

Prolonged restraint in this context means *beyond routine care and use procedures* for rodent and rabbit restrainers, and large animal stocks. Prolonged restraint also includes *any* use of slings, tethers, metabolic crates, inhalation chambers, primate chairs and radiation exposure restraint devices.

1. For what procedure(s):
Click here to enter text.
2. Explain why non-restraint alternatives cannot be utilized:
Click here to enter text.
3. Restraint device(s):
Click here to enter text.
4. Duration of restraint:
Click here to enter text.
5. Frequency of observations during restraint/person responsible:
Click here to enter text.
6. Frequency and total number of restraints:
Click here to enter text.
7. Conditioning procedures:
Click here to enter text.

8. Steps to assure comfort and well-being:
Click here to enter text.

9. Describe potential adverse effects of prolonged restraint and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):
Click here to enter text.

G. Tumor Studies, Disease Models, Toxicity Testing, Vaccine Studies, Trauma Studies, Pain Studies, Organ or System Failure Studies, Shock Models, etc.

1. Describe methodology:
Click here to enter text.

2. Expected model and/or clinical/pathological manifestations:
Click here to enter text.

3. Signs of pain/discomfort:
Click here to enter text.

4. Frequency of observations:
Click here to enter text.

5. Describe potential adverse side effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):
Click here to enter text.

H. Treadmills/Swimming/Forced Exercise

1. Describe aversive stimulus (if used):
Click here to enter text.

2. Conditioning:
Click here to enter text.

3. Safeguards to protect animal:
Click here to enter text.

4. Duration:
Click here to enter text.

5. Frequency:
Click here to enter text.

6. Total number of sessions:
Click here to enter text.

7. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):

Click here to enter text.

I. Projects Involving Food and Water Regulation or Dietary Manipulation

(*Routine pre-surgical fasting not relevant for this section)*

1. Food Regulation

b. Amount regulated and rationale:
Click here to enter text.

b. Frequency and duration of regulation (hours for short term/weeks or months for long term):
Click here to enter text.

c. Frequency of observation/parameters documented (i.e. recording body weight, body condition, etc.):
Click here to enter text.

d. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):
Click here to enter text.

2. Fluid Regulation

a. Amount regulated and rationale:
Click here to enter text.

b. Frequency and duration of regulation (hours for short term/weeks or months for long term):
Click here to enter text.

c. Frequency of observation/parameters documented (body weight, hydration status, etc.):
Click here to enter text.

d. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):
Click here to enter text.

3. Dietary Manipulations

c. Compound supplemented/deleted and amount:
For sharks undergoing gastric lavage, the full volume of stomach contents will be removed.

d. Frequency and duration (hours for short term/week or month for long term):
Once per shark.

c. Frequency of observation/parameters documented:

Stomach contents will be collected once, then sharks will be released. Sharks that are not moving or swimming vigorously immediately prior to release will be kept in holding tank and observed for up to 10 minutes.

d. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):

Lavage procedures are expected to be non-lethal, but injury and adverse affects from capture stress are possible. If sharks are too injured or unhealthy for release, they will be anesthetized with 1 g/L MS-222 and euthanized by severing the spinal column.

J. Endoscopy, Fluoroscopy, X-Ray, Ultrasound, MRI, CT, PET, Other Imaging

1. Describe animal methodology:

Click here to enter text.

2. Duration of procedure:

Click here to enter text.

3. Frequency of observations during procedure:

Click here to enter text.

4. Frequency/total number of procedures:

Click here to enter text.

5. Method of transport to/from procedure area:

Click here to enter text.

6. Describe potential adverse side effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):

Click here to enter text.

7. Please provide or attach appropriate permissions/procedures for animal use on human equipment:

Click here to enter text.

K. Polyclonal Antibody Production

5. Antigen/adjuvant used and justification for adjuvant choice:

Click here to enter text.

6. Needle size:

Click here to enter text.

7. Route of injection:
Click here to enter text.

8. Site of injection:
Click here to enter text.

5. Volume of injection:
Click here to enter text.

6. Total number of injection sites:
Click here to enter text.

7. Frequency and total number of boosts:
Click here to enter text.

8. What will be done to minimize pain/distress:
Click here to enter text.

9. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):
Click here to enter text.

L. Monoclonal Antibody Production

1. Describe methodology:
Click here to enter text.

2. Is pristane used: Choose an item.

Volume of pristane:
Click here to enter text.

3. Will ascites be generated: Choose an item.

ii. Criteria/signs that will dictate ascites harvest:
Click here to enter text.

ii. Size of needle for taps:
Click here to enter text.

iii. Total number of taps:
Click here to enter text.

iv. How will animals be monitored/cared for following taps:
Click here to enter text.

4. What will be done to minimize pain/distress:
Click here to enter text.

5. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):
Click here to enter text.

M. Temperature/Light/Environmental Manipulations

1. Describe manipulation(s):
Click here to enter text.

2. Duration:
Click here to enter text.
3. Intensity:
Click here to enter text.
4. Frequency:
Click here to enter text.
5. Frequency of observations/parameters documented:
Click here to enter text.
6. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):
Click here to enter text.

N. Behavioral Studies

3. Describe methodology/test(s) used:
Click here to enter text.
4. Will conditioning occur? If so, describe:
Click here to enter text.
3. If aversive stimulus used, frequency, intensity and duration:
Click here to enter text.
4. Length of time in test apparatus/test situation: *(i.e., each test is ~10 mins)*
Click here to enter text.
5. Frequency of testing and duration of study: *(i.e., 5 tests/week for 6 months)*
Click here to enter text.
6. Frequency of observation/monitoring during test:
Click here to enter text.
7. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):
Click here to enter text.

O. Capture with Mechanical Devices/Traps/Nets

2. Description of capture device/method:
Four gear types will be used to capture sharks. Rod and reel gear will have terminal tackle consisting of a 2-foot wire leader and size 8/0 circle hook. Drumlines will each consist of a 40 lb anchor connected to a swivel-mounted leader consisting of 100 feet of 900 lb-test monofilament line and a size 12/0 circle hook. Gillnet gear will be 50 yards in length with stretched mesh ranging

from 1-5 inches. Longline gear will consist of a 300 yard mainline mounted with 50 gangions, which will be made up of 3 feet of 300 lb-test monofilament line and a size 10/0 circle hook connected to the mainline by a longline clip with a swivel.

2. Maximum time animal will be in capture device:

Soak times will be limited to 30 minutes for gillnet and longline gear and 1 hour for drumlines. Rod and reel gear will soak until the hook is taken, and captured sharks will be brought to the boat as quickly as possible.

3. Frequency of checking capture device:

Gear will be checked every 30 minutes (gillnet and longline) or hour (drumline). Rod and reel gear will be constantly monitored.

4. Methods to ensure well-being of animals in capture device:

Short soak times and gentle removal from gear. Drumline and longline gears incorporate swivel mountings to allow a greater range of motion for captured sharks.

5. Methods to avoid non-target species capture:

Short soak time and immediate removal from gear if a non-target animal is captured.

6. Method of transport to laboratory/field station/processing site and duration of transport:

Sharks will be captured and released at the same location.

7. Methods to ensure animal well-being during transport:

During surgery, sharks will have seawater flushed over the gills.

8. Expected mortality rates:

We do not expect any short-term mortality of sharks.

9. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):

Surgically implanted animals that do not display normal swimming activity after ½ hour will be anesthetized, euthanized, and held on ice until they may be properly disposed. Sharks will be anesthetized with 1 g/L MS-222 and then euthanized by severing the spinal cord

P. Manipulation of Wild-Caught Animals in the Field or Laboratory

6. Parameters to be measured/collected:

Species, sex, total length, fork length, and externally visible signs of life stage (i.e. umbilical scarring, pregnancy) will be recorded for each shark.

7. Approximate time required for data collection per animal:

It takes approximately 2 minutes to measure an individual. External tagging takes 2 minutes. Surgery takes approximately 10-15 minutes

8. Method of restraint for data collection:

A surgical table with foam padding will be used to cushion the sharks and minimize patient movement or movements induced by the pitch and roll of the vessel. An assistant will help in holding the animal up-side down in place.

9. Methods to ensure animal well-being during processing:

Sharks will be kept in holding tanks until processing and have water flushed over the gills during surgery.

10. Disposition of animals post-processing:

Sharks will be allowed to recover in live well tanks and gently returned to the water.

6. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):

If the animal does not recover from surgery within a half hour, it will be euthanized by anesthetizing with MS-222 and severing the spinal cord

Q. Wildlife Telemetry/Other Marking Methods

1. Describe methodology (including description of device):

Sharks that are surgically implanted with compatible size tags will be returned to the water after a successful recovery period and will resume normal movement behaviors. Compatible receivers will be deployed in the study area, and we will receive acoustic detection data from individuals swimming within receivers range. External Floy tags has a steel core and a barb on one side, designed to catch within the muscle tissue of the shark. The tag is inserted into the musculature using a canula, which is a sharpened, large gauge needle into which the tag is placed. After being pushed into the muscle tissue, the canula is gently slid out, the barb on the tag lodges into the muscle, and the tag is left behind.

2. Will telemetry device/tags/etc. be removed? No. **If so, describe:**

Tags will not be removed unless the animal does not survive surgery. If this is the case, the tag will be reinitialized, sterilized, and used in another surgery.

3. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):

We are actively advertising this study via a website, and by contacting agencies and departments that may encounter our tagged sharks. Sharks are easily identifiable as a study animal due to the bright external tag by the dorsal fin, and the surgical scars on the abdomen. We expect both recreational and commercial fishermen to encounter our animals. We ask that sharks bearing our tags be returned to the water to allow the study to continue. Sharks captured by fishermen will likely be released, due to measuring less than regulated size limits.

R. Other Animal Manipulations

2. Describe methodology:

Click here to enter text.

2. Describe methods to ensure animal comfort and well-being:

Click here to enter text.

3. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):

Click here to enter text.

S. Surgical Procedures

All survival surgical procedures must be done aseptically, regardless of species or location of surgery. Adequate records describing surgical procedures, anesthetic monitoring and postoperative care must be maintained for all species.

1. Location of Surgery (Building & Room #):

Aboard research vessel.

2. Type of Surgery (check all that are appropriate):

Click here to enter text.

☐ **Non-survival surgery (animals euthanized without regaining consciousness)**

☒ **Major survival surgery (major surgery penetrates and exposes a body cavity or produces substantial impairment of physical or physiologic function)**

☐ **Minor survival surgery**

☐ **Multiple survival surgery**

If yes, provide scientific justification for multiple survival surgical procedures:

Click here to enter text.

3. Describe the pre-op preparation of the animals:

c. **Food restricted for** Click here to enter text. **hours**

d. **Food restricted is not recommended for rodents and rabbits and must be justified:**

Click here to enter text.

c. Water restricted for Click here to enter text. **hours**

d. Water restriction is not recommended in any species for routine pre-op prep and be justified:

Click here to enter text.

4. Minimal sterile techniques will include (check all that apply):

Please refer to DCM Guidelines for Aseptic Surgery for specific information on what is required for each species and type of surgery (survival vs. non-survival).

☒ **Sterile instruments**

How will instruments be sterilized?

Instruments will be autoclaved by surgery staff and stored in sterile packaging.

If serial surgeries are done, how will instruments be sterilized between surgeries:

Instruments will be placed in a glass bead sterilizer. Each surgical pack will be sterilized between each surgery. One surgical assistant will be responsible for continuously sterilizing instruments while the other provides assistance directly to the surgeon. Each surgical pack will be used no more than 2 times a day.

☒ **Sterile gloves**

☐ **Mask**

☐ **Cap**

☐ **Sterile gown**

☐ **Sanitized operating area**

☐ **Clipping or plucking of hair or feathers**

☒ **Skin preparation with a sterilant such as betadine**

☒ **Practices to maintain sterility of instruments during surgery**

☐ **Non-survival (clean gloves, clean instruments, etc.)**

5. Describe all surgical procedures:

a. Skin incision size and site on the animal:

Incision will be approximately 1-2 cm long, and be located on the abdomen 2/3 of the way between the pectoral and pelvic fin. The incision will be made on the midline of the shark where the muscle layers are thinnest.

b. Describe surgery in detail (include size of implant if applicable):

The sharks will be placed into holding tanks that will have fresh seawater pumped in. Surgeries will start after tonic immobility is reached (the shark has stopped reacting to stimuli and shows minimal outward signs of stress). Sharks will be placed onto foam padding surgery table. Sea water will be continuously flushed over gills. The incision site will be swabbed with a Q tip soaked on Nolvasan disinfectant. A small incision will be made in the abdomen at roughly 2/3 of the distance between the pectoral fins and the cloaca, on the midline of the sharks. A sterilized transmitter (36 mm X 9 mm X 2.9 g in water) will be inserted into the abdomen and gently pushed anterior the incision. The incision will be closed using either a simple continuous or simple interrupted surgical suture pattern. The shark will be placed into a recovery tank and observed for up to ½ hour prior to release.

c. Method of wound closure:

Sutures.

i. Number of layers

Click here to enter text.

ii. Type of wound closure and suture pattern:

Simple continuous or simple interrupted suture pattern.

iii. Suture type/size/wound clips/tissue glue:

3-0 absorbable monofilament suture.

iv. Plan for removing of skin sutures/wound clip/etc:

We will not remove the sutures.

6. Anesthetic Protocol:

a. If anesthesia/analgesia must be withheld for scientific reasons, please provide compelling scientific justification as to why this is necessary:

Click here to enter text.

b. Anesthesia/Analgesia For Surgical Procedures

	Agent	Dose (mg/kg or %)	Volume	Route	Frequency	Number of days administered
Pre-operative analgesic	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.
Pre-anesthetic	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.
Anesthetic	Lidocaine	2 %	2 mL	Injection block	Once	Click here to enter text.
Post-operative	Click here to enter text.	Click here to enter text.	Click here to enter	Click here to enter	Click here to enter	Click here to enter text.

Analgesic			text.	text.	text.	
Other	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.

c. Methods that will be used to monitor anesthetic depth (include extra measures employed when paralyzing agents are used):

Sharks will be gently held up-side down on a surgical table (with fresh circulating seawater flushed over the gills). Surgery will not start until after the shark ceases to move.

d. Methods of physiologic support during anesthesia and immediate post-op period (fluids, warming, etc.):

Flushing of fresh seawater across gills, tonic immersion, recovery in fresh seawater.

e. List what parameters are monitored during immediate post-op period. Provide the frequency and duration:

Responsiveness after being turned right-side up or placed in holding tank. 5-10 minutes.

f. Describe any other manipulations that will be used to alleviate pain, distress, and/or discomfort during the immediate post-op period (soft bedding, long sipper tubes, food on floor, dough diet, etc.):

We will ensure that gill activity continues throughout surgery. A lidocaine block will be given at the beginning of surgical procedures to provide pain relief during and after surgery.

g. List criteria used to determine when animals are adequately recovered from anesthesia and when the animals can be returned to their home environment:

After observed swimming normally in recovery holding tanks for a period of 5 minutes or longer.

7. Recovery from Surgical Manipulations (after animal regains consciousness and is returned to its home environment)

Click here to enter text.

a. What parameters (behavior, appetite, mobility, wound healing, etc.) will be monitored:

Transmitters pings (impulses). Rapid changes in impulse directionality, provide a clue of animal recovery from surgery.

b. How frequently (times per day) will animals be monitored:

Sharks will be checked for proper transmitter functioning while in the recovery tank. We will deploy moored receivers that will detect sharks in the study area. Other moored receivers are located along the coast and will also track the sharks as they migrate out of the study area.

c. How long post-operatively (days) will animals be monitored:

Up to 3 years.

8. Surgical Manipulations Affecting Animals

a. Describe any signs of pain/discomfort/functional deficits resulting from the surgical procedure:

Thrashing during surgery.

b. What will be done to manage any signs of pain or discomfort (include pharmacologic and non-pharmacologic interventions):

Surgery procedures will cease until shark stop moving (with the wound held shut by the surgeon).

c. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):

Buoyancy/orientation issues may occur because the open air surgery allows air into the body cavity. Therefore, sharks will be allowed a 10-minute recovery time with assisted swimming (gently grasping the caudal peduncle and moving the animal back and forth in the water) as necessary. If the animal shows an inability to recover from surgery within a half hour, it will be humanely euthanized.

V. <u>Euthanasia</u>

Please refer to the AVMA Guidelines for the Euthanasia of Animals: 2013 Edition and DCM Guidelines to determine appropriate euthanasia methods.

B. Euthanasia Procedure. ***All investigators, even those conducting non-terminal studies, must complete this section in case euthanasia is required for humane reasons.***

1. Physical Method- If a physical method is used, the animal should be first sedated/anesthetized with CO_2 or other anesthetic agent. If prior sedation is not possible, a scientific justification must be provided:

Sharks will be dosed with buffered MS-222 (1 g/L) and then the spinal cord will be severed.

2. Inhalant Method- Choose an item.
(if other, describe the agent and delivery method)
Click here to enter text.

3. Non-Inhalant Pharmaceutical Method (injectables, MS-222, etc.)- Please provide the following:

a. Agent:
MS-222

b. Dose or concentration:
1 g/L

c. Route:
In holding tank water.

B. Method of ensuring death (can be physical method, such as pneumothorax or decapitation for small species and assessment method such as auscultation for large animals):

Freezing flowing euthanasia will be used as a means to confirm death.

C. Describe disposition of carcass following euthanasia:

Carcasses will be returned to the university or an appropriate lab facility for examination, dissection, and disposal. They will be transported on ice in coolers until reaching a facility that is capable of disposing the remains. Carcasses will be placed in red biohazard bags and frozen until pick-up. Contents of biohazard bags are incinerated.

I acknowledge that humane care and use of animals in research, teaching and testing is of paramount importance, and agree to conduct animal studies with professionalism, using ethical principles of sound animal stewardship. I further acknowledge that I will perform only those procedures that are described in this AUP and that my use of animals must conform to the standards described in the Animal Welfare Act, the Public Health Service Policy, The Guide For the Care and Use of Laboratory Animals, the Association for the Assessment and Accreditation of Laboratory Animal Care, and East Carolina University.

Please submit the completed animal use protocol form via e-mail attachment to iacuc@ecu.edu. You must also carbon copy your Department Chair.

PI Signature: _____ Date: _____

Veterinarian:_____Date:_____

IACUC Chair: _____Date:_____

APPENDIX 1-HAZARDOUS AGENTS			
Principal Investigator:	Campus Phone:		Home Phone:
IACUC Protocol Number:	Department:		E-Mail:
Secondary Contact: Department:	Campus Phone:	Home Phone:	E-Mail:
Chemical Agents used:		Radioisotopes used:	
Biohazardous Agents used:	Animal Biosafety Level:		Infectious to humans?
PERSONAL PROTECTIVE EQUIPMENT REQUIRED:			
Route of Excretion:			
Precautions for Handling Live or Dead Animals:			
Animal Disposal:			
Bedding/Waste Disposal:			
Cage Decontamination:			
Additional Precautions to Protect Personnel, Adjacent Research Projects including Animals and the Environment:			
Initial Approval Safety/Subject Matter Expert Signature & Date _____			

East Carolina University

Animal Care and Use Commitee
212 Ed Warren Life Sciences Building
East Carolina University
Greenville, NC 27834

252-744-2436 office
252-744-2355 fax

March 24, 2014

Roger Rulifson, Ph.D.
Department of ICSP/Biology
Howell Science Complex
East Carolina University

Dear Dr. Rulifson:

Your Animal Use Protocol entitled, "Habitat Use by Juvenile Coastal Sharks in Back and Core Sounds" (AUP #D306) was reviewed by this institution's Animal Care and Use Committee on 3/24/14. The following action was taken by the Committee:

"Approved as submitted"

A copy is enclosed for your laboratory files. Please be reminded that all animal procedures must be conducted as described in the approved Animal Use Protocol. Modifications of these procedures cannot be performed without prior approval of the ACUC. The Animal Welfare Act and Public Health Service Guidelines require the ACUC to suspend activities not in accordance with approved procedures and report such activities to the responsible University Official (Vice Chancellor for Health Sciences or Vice Chancellor for Academic Affairs) and appropriate federal Agencies. **Please ensure that all personnel associated with this protocol have access to this approved copy of the AUP and are familiar with its contents.**

Sincerely yours,

Susan McRae, Ph.D.
Chair, Animal Care and Use Committee

SM/jd

Enclosure

East Carolina University is a constituent institution of the University of North Carolina. An equal opportunity university

East Carolina University Animal Use Protocol (AUP) Amendment Form
Latest Revision, February 2014

FOR IACUC USE ONLY	
AUP #	
Date received:	
Full Review and date:	Designated Reviewer and date:
Approval date:	
Pain Category:	
Amendments approved:	
Minor Amendment:	
Significant Amendment:	If so, number?

Please fill out completely and email to davenportp@ecu.edu or iacuc@ecu.edu

PROJECT INFORMATION: Please list AUP Number and Title
Habitat use by juvenile coastal sharks in Back and Core Sounds

Principal Investigator:
Roger Rulifson

1. What is the purpose or rationale for the protocol amendment?
Briefly explain in simple, non-technical language the reason(s) for amending the project.
Blacknose sharks (*Carcharhinus acronotus*) have proven to be unexpectedly common in the study area and are a species of interest for acoustic tagging. Genetic sampling procedures for bonnethead sharks are added as part of a collaboration with NC Aquariums and NC DMF.

2. Will different people use the animals or are new personnel being added to the AUP? Will previously approved personnel be assuming new roles or responsibilities?
Choose an item.
If so, list qualifications and training.
Click here to enter text.

3. Have protocol related hazards (transgenic animals, infectious, chemical or biologic agents) changed with this amendment? Choose an item.
If so, please describe the hazard and the oversight committee associated with this hazard (see AUP form II.E.1). If any hazardous agents have changed since the original AUP, please fill out the attached Hazardous Agents Form (Appendix 1). Oversight committee approval is required before the amendment can be approved by IACUC.
Click here to enter text.

4. Please indicate changes to the animals or animal numbers.
a. Will the strain or sex of the animals change? Choose an item.
If so, please list changes (use complete strain nomenclature and a brief description of the line/strain when possible).

b. Will the phenotype of mutant, transgenic or knockout animals predispose them to any health, behavioral, physical abnormalities, or cause debilitation effects in experimental manipulations? (if yes, describe)
Click here to enter text.

c. Will additional animals be needed? Choose an item.
If so, please justify the increase in animal numbers, using statistical justification whenever possible.
Blacknose sharks have proven to be abundant in the study area and are a good candidate for acoustic tagging. They are also a species of interest to NC DMF and NMFS. An addition of 30 individuals of this species provides an equivalent sample size to the other principle species.

5. Are there any unusual husbandry and environmental conditions required, including single housing? Social housing is now the default housing for social species.
Choose an item.

If yes, then describe conditions and justify the exceptions to standard housing. (see AUP form III.C.5-7)
Click here to enter text.

6. Will there be new non-surgical procedures involving the animals? Choose an item.
Please describe these procedural changes with the same level of detail as is required in a new protocol (see AUP form IV. B-R). Addition of or changes to surgical procedures are addressed in question 12.
Click here to enter text.

7. Will additional sampling of body fluids or tissues collections occur? Choose an item.
Will additional administration of substances occur? Choose an item.
If so, please fill out table(s) below and address non-pharmaceutical grade substances in questions 'a' and 'b' below the table:

Blood or Body Fluid Collection

	Location on animal	Needle/catheter size	Volume collected	Frequency of procedure	Time interval between collections
Blood Collection	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.
Body Fluid Collection	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.
Other	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.

Injections, Gavage, & Other Substance Administration

	Compound	Location & Route of admin	Needle/catheter/gavage size	Max volume admin	Freq of admin (i.e. two times per day)	Number of days admin (i.e. for 5 days)	Max dosage (mg/kg
Injection / Infusion	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.
Gavage	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.
Other	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.	Click here to enter text.

Pharmaceutical grade drugs, biologics, reagents, and compounds are defined as agents approved by the Food and Drug Administration (FDA) or for which a chemical purity standard has been written/established by any recognized pharmacopeia such as USP, NF, BP, etc. These standards are used by manufacturers to help ensure that the products are of the appropriate chemical purity and quality, in the appropriate solution or compound, to ensure stability, safety, and efficacy. For all injections and infusions for CLINICAL USE, PHARMACEUTICAL GRADE compounds must be used whenever possible. Pharmaceutical grade injections and infusions for research test articles are preferred when available. If pharmaceutical grade compounds are not available and non-pharmaceutical grade agents must be used, then the following information is necessary:

e. **Please provide a scientific justification for the use of ALL non-pharmaceutical grade compounds. This may include pharmaceutical-grade compound(s) that are not available in the appropriate concentration or formulation, or the appropriate vehicle control is unavailable.**

f. **Indicate the method of preparation, addressing items such as purity, sterility, pH, osmolality, pyrogenicity, adverse reactions, etc. (please refer to ECU IACUC guidelines for non-pharmaceutical grade compound use), labeling (i.e. preparation and use-by dates), administration and storage of each formulation that maintains stability and quality/sterility of the compound(s).**

Click here to enter text.

8. Are animals expected to experience more clinical illness, pain, or distress as a result of the procedures proposed in this amendment? Choose an item.
If yes, please complete literature search for alternatives to the use of potentially painful/distressful procedures (see below).
Click here to enter text.

How will any pain or distress from this procedure be minimized?
Click here to enter text.

a. Include a literature search to ensure that alternatives to all procedures that may cause more than momentary or slight pain or distress to the animals have been considered.

1. Please list all of the potentially painful or distressful procedures in the protocol:
Click here to enter text.

2. For the procedures listed above, provide the following information (please do not submit search results but retain them for your records):

Date Search was performed:	Click here to enter text.
Database(s) searched:	Click here to enter text.
Time period covered by the search (i.e. 1975-2013):	Click here to enter text.
Search strategy (including scientifically relevant terminology):	Click here to enter text.
Other sources consulted:	Click here to enter text.

3. In a few sentences, please provide a brief narrative indicating the results of the search(es) to determine the availability of alternatives and explain why these alternatives were not chosen. Also, please address the 3 Rs of refinement, reduction, and replacement in your response. Refinement refers to modification of husbandry or experimental procedures to enhance animal well-being and minimize or eliminate pain and distress. Replacement refers to absolute (i.e. replacing animals with an inanimate system) or relative (i.e. using less sentient species) replacement. Reduction involves strategies such as experimental design analysis, application of newer technologies, use of appropriate statistical methods, etc., to use the fewest animals or maximize information without increasing animal pain or distress.

Click here to enter text.

9. Will there be addition of or change in the method of anesthesia, analgesia, or euthanasia? Choose an item.
If so, describe in detail (see AUP form IV. B, S, and V.).
Click here to enter text.

10. Will prolonged restraint of conscious animals be required? Choose an item.
If so, describe in detail (see AUP form IV. F).
Click here to enter text.

11. Will surgical procedures be added or surgical plans from the original protocol change? (major, minor, multiple survival, additional procedures). Choose an item.
If so, describe in detail (see AUP form IV.S).
Click here to enter text.

PI Signature: _____ Date:_____

Veterinarian: _____ Date:_____

IACUC Chair: _____ Date:_____

<table>
<tr><td colspan="5">APPENDIX 1-HAZARDOUS AGENTS</td></tr>
<tr><td colspan="2">Principal Investigator:</td><td colspan="2">Campus Phone:</td><td>Home Phone:</td></tr>
<tr><td colspan="2">IACUC Protocol Number:</td><td colspan="2">Department:</td><td>E-Mail:</td></tr>
<tr><td colspan="2">Secondary Contact:

Department:</td><td>Campus Phone:</td><td>Home Phone:</td><td>E-Mail:</td></tr>
<tr><td colspan="3">Chemical Agents Used:</td><td colspan="2">Radioisotopes Used:</td></tr>
<tr><td colspan="3">Biohazardous Agents Used:</td><td>Animal Biosafety Level:</td><td>Infectious to humans?</td></tr>
<tr><td colspan="5">PERSONAL PROTECTIVE EQUIPMENT REQUIRED:</td></tr>
<tr><td colspan="5">Route of Excretion:</td></tr>
<tr><td colspan="5">Precautions for Handling Live or Dead Animals:</td></tr>
<tr><td colspan="5">Animal Disposal:</td></tr>
<tr><td colspan="5">Bedding/Waste Disposal:</td></tr>
<tr><td colspan="5">Cage Decontamination:</td></tr>
<tr><td colspan="5">Additional Precautions to Protect Personnel, Adjacent Research Projects including Animals and the Environment:</td></tr>
<tr><td colspan="3">Initial Approval
Safety/Subject Matter Expert Signature & Date

_____</td><td colspan="2"></td></tr>
</table>

East Carolina University

Animal Care and Use Commitee
212 Ed Warren Life Sciences Building
East Carolina University
Greenville, NC 27834

252-744-2436 office
252-744-2355 fax

June 9, 2015

Roger Rulifson, Ph.D.
Department of ICSP/Biology
Flanagan Building
East Carolina University

Dear Dr. Rulifson:

The Amendment to your Animal Use Protocol entitled, "Habitat Use by Juvenile Coastal Sharks in Back and Core Sounds" (AUP #D306) was reviewed by this institution's Animal Care and Use Committee on June 8, 2015. The following action was taken by the Committee:

"Approved as submitted"

Please contact Dale Aycock at 744-2997 prior to hazard use

A copy is enclosed for your laboratory files. Please be reminded that all animal procedures must be conducted as described in the approved Animal Use Protocol. Modifications of these procedures cannot be performed without prior approval of the ACUC. The Animal Welfare Act and Public Health Service Guidelines require the ACUC to suspend activities not in accordance with approved procedures and report such activities to the responsible University Official (Vice Chancellor for Health Sciences or Vice Chancellor for Academic Affairs) and appropriate federal Agencies. **Please ensure that all personnel associated with this protocol have access to this approved copy of the AUP and are familiar with its contents.**

Sincerely yours,

Susan McRae, Ph.D.
Chair, Animal Care and Use Committee

SM/jd

enclosure

East Carolina University is a constituent institution of the University of North Carolina. An equal opportunity university

Assessing local ecological knowledge of coastal shark and dogfish movements among North Carolina commercial fishers

Charles Bangley
IRB Protocol

Introduction

There is often perceived disagreement between the observations of commercial fishermen and the fishery-independent ecological surveys that inform fisheries management. However, trends observed by fishers often agree well with ecological surveys, and these observations may capture recent ecological changes more readily than fishery-independent surveys (Rochet et al. 2008). Fishers generally spend more time on the water than researchers, and the observations of experienced fishers can fill gaps in fisheries science (Johannes et al. 2000). Interviews with fishers have proven effective at documenting population trends in even long-lived species such as sharks and marine mammals (Maynou et al. 2011).

Recently, stringent regulations on coastal shark and dogfish fisheries have provoked outrage from commercial fishermen who claim that these species are abundant to the point of being pests. The goal of this study is to assess the ecological knowledge of North Carolina commercial fishers with regards to trends in abundance and habitat preferences of coastal and dogfish sharks in the vicinity of Cape Hatteras and the Outer Banks. The results of this study will be compared with trends found by fishery-independent surveys in North Carolina waters, and will be used to inform sampling station locations for future coastal shark surveys.

Protocol

Information on coastal shark and dogfish presence will be collected through open-ended, semi-structured interviews with 5-15 commercial fishermen from the communities of Hatteras and Wanchese. Potential participants will be introduced to the interviewer by Chris Hickman, a fisherman from Hatteras who has both an established relationship with the Rulifson lab and familiarity with the Outer Banks fishing community. Potential participants will be informed of the goals of the study and confidentiality policies, and participation in interviews will be totally voluntary.

Interview location and time will be agreed upon between the interviewer and participant. Total interview time will be structured to take approximately one hour. The interviews will consist of a series of open-ended questions consisting of three sections. All participants will be asked questions from Section 1, which establishes commercial fishing background and determines whether coastal sharks and/or dogfish are targeted directly. If not, questions will be drawn from Section 2, which concern observations made while encountering sharks during normal fishing operation. If the participant is involved in commercial fisheries for sharks or dogfish, then questions will be drawn from Section 3, which are more specific to species commonly targeted by the fishery. If early questions indicate that any particular shark species is either encountered or targeted more often than others, later questions will be tailored to gather information on that species.

Confidentiality

Before choosing whether to participate in interviews, all potential participants will be informed about the goals of this study and confidentiality policies. Interview responses will be recorded by hand. Data gathered during interviews will be reported anonymously and will be formatted to present results as general trends rather than individual responses (i.e. "25% of fishers reported blacktip sharks in the fall"). All questions on personal background concern only commercial fishing, and any information that may potentially reveal a participant's identity will be omitted from reported results. Participants will be allowed to decline to answer any questions or terminate the interview at any time if they are uncomfortable with revealing personal information.

References

Johannes, R. E., M. M. R. Freeman, and R. J. Hamilton. 2000. Ignore fishers' knowledge and miss the boat. Fish and Fisheries 1: 257-271.

Maynou, F., M. Sbrana, P. Sartor, C. Maravelias, S. Kavadas, D. Damalas, J. E. Cartes, and G. Osio. 2011. Estimating trends of population decline in long-lived marine species in the Mediterranean Sea based on fishers' perceptions. PLOS One 6: e21818.

Rochet, M. J., M. Prigent, J. A. Bertrand, A. Carpentier, F. Coppin, J. Delpech, G. Fontenelle, E. Foucher, K. Mahe, E. Rostiaux, and V. M. Trenkel. 2008. Ecosystem trends: evidence for agreement between fishers' perceptions and scientific information. ICES Journal of Marine Science 65: 1057-1068.

RX: Your Exempt study has been approved

umcirb@ecu.edu

Fri 12/13/2013 1:37 PM

To:Bangley, Charles W <BANGLEYC09@students.ecu.edu>;

EAST CAROLINA UNIVERSITY
University & Medical Center Institutional Review Board Office
4N-70 Brody Medical Sciences Building· Mail Stop 682
600 Moye Boulevard · Greenville, NC 27834
Office 252-744-2914 · Fax 252-744-2284 · www.ecu.edu/irb

Notification of Exempt Certification

From: Social/Behavioral IRB
To: Charles Bangley
CC:
Hans Vogelsong
Date: 12/13/2013
Re: UMCIRB 13-002313
Assessing local ecological knowledge of coastal shark and dogfish movements among North Carolina commercial fishers.

I am pleased to inform you that your research submission has been certified as exempt on 12/6/2013 . This study is eligible for Exempt Certification under category #2&5 .

It is your responsibility to ensure that this research is conducted in the manner reported in your application and/or protocol, as well as being consistent with the ethical principles of the Belmont Report and your profession.

This research study does not require any additional interaction with the UMCIRB unless there are proposed changes to this study. Any change, prior to implementing that change, must be submitted to the UMCIRB for review and approval. The UMCIRB will determine if the change impacts the eligibility of the research for exempt status. If more substantive review is required, you will be notified within five business days.

The UMCIRB office will hold your exemption application for a period of five years from the date of this letter. If you wish to continue this protocol beyond this period, you will need to submit an Exemption Certification request at least 30 days before the end of the five year period.

The Chairperson (or designee) does not have a potential for conflict of interest on this study.