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A TANGLE NET STUDY ON RELATIVE ABUNDANCE AND HABITAT PREFERENCE OF ELASMOBRANCHS IN NORTH INLET ESTUARY, SOUTH CAROLINA

by

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Submitted in Partial Fulfillment of the

Requirements for the Degree of Master of Science in

Coastal Marine and Wetland Studies in the

College of Natural and Applied Sciences

Coastal Carolina University

2012

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Acknowledgements

I would like to thank my committee members Dr. Dan Abel, Dr. Rob Young, and Dr. Dennis Allen for all of their help, guidance, and most especially for their time. Dr. Abel was a constant source of encouragement and a wonderful mentor in the process of writing this thesis. Dr. Young was very helpful in the planning process of this research project and always had helpful hints and suggestions for both my field and lab work. Dr. Allen was a wealth of knowledge about North Inlet estuary and was very helpful in designing my lab work. I would like to thank the Baruch Foundation for the use of property and equipment during my tank trials. I thank Coastal Carolina University for the use of boats, vehicles, and equipment throughout this research project. Finally, I must thank my husband Alan, who was my rock and my strength throughout graduate school, always encouraging me and helping me in any way possible.

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INTRODUCTION

Elasmobranchs (sharks, rays, and skates) are common residents of estuaries in the southeastern U.S. Ecologically, most large sharks are top predators while rays and smaller sharks are mesopredators that connect upper and lower trophic levels (Baum and Worm 2009, Vaudo 2011, Vaudo and Heithaus 2011). Despite the importance of sharks and rays in estuaries and other marine ecosystems, the variables important to how they select habitats remain largely unknown except for a few species. Studies of habitat selection in elasmobranchs have focused on pelagic sharks in shallow systems, whose habitat preference is based on factors that include predator avoidance (e.g. Morrissey and Gruber 1993), prey abundance (e.g. Heithaus et al. 2002), depth (e.g. Rechisky and Wetherbee 2003), and tidal currents (e.g. Steiner 2007). Heupel et al. (2006) included bottom type in a comprehensive study of habitat selection on bonnethead sharks, Sphyrna *tiburo*; however, they examined only grassy and non-grassy bottom types and did not include sediment. In general, no study on habitat selection in elasmobranchs has included sediment preference. For sharks, sediment preference may contribute to habitat selection in estuarine systems because the shallow depths of these systems result in more frequent interactions with the benthic environment (e.g. for feeding or movement between habitats). Sediment characteristics may be more important to rays, many of which bury and/or forage in sediment.

Studies on the ecology of elasmobranch fishes in northeastern South Carolina estuarine and near-shore waters have included habitat factors. These shark studies researched: habitat partitioning and the effect of creek size and tidal phases on shark distribution during the nursery season in North Inlet (Yednock 2005); distribution and

site fidelity of *Rhizoprionodon terraenovae* in North Inlet (Maxwell 2008); elasmobranch populations in human-impacted and pristine estuaries (Murrells Inlet and North Inlet, respectively; McDonough 2008); distribution of sharks in Winyah Bay based on temperature, salinity, depth, and month (Abel *et al.* 2007, Gary 2009); and residence time of *Carcharhinus limbatus* in North Santee estuary (Schreer 2010). A single study focused on batoid rays, specifically the distribution of *Dasyatis americana* and *Dasyatis sabina*, in Winyah Bay (Klein-Majors 2006).

In the summer of 2011, I undertook a study of the relative abundance, distribution, and sediment preference of elasmobranchs in North Inlet estuary, South Carolina using tangle nets, a method previously unused in this area. Further, I assessed sediment preference in the two most numerous elasmobranchs captured, bluntnose rays, *Dasyatis sayi,* and bonnethead sharks, *Sphyrna tiburo*. In addition, the ray was employed in *ex situ* sediment choice experiments.

The presence of substantial and relatively unstudied populations of both *D. sayi* and *S. tiburo* in North Inlet where a variety of bottom and sediment types exist, offered an opportunity to test if sediment types are important to these species. We set tangle nets over two sediment types (mud and sand) and an *ex situ* choice experiment was conducted on captured rays to aid in determining the role of sediment in habitat selection of this group.

Questions and Hypotheses

My main question during this study was: do elasmobranchs exhibit a sediment preference between mud and sand habitats? I hypothesized that both *S. tiburo* and *D.*

sayi had a sediment preference, since both of these species forage in the sediment, and since *D. sayi* rests on or buries in the sediment. I also questioned whether or not salinity, temperature, turbidity, or dissolved oxygen play a role in elasmobranch habitat preference; however, I hypothesized that these environmental factors would not affect habitat preference for either *D. sayi* or *S. tiburo* since North Inlet is a well-mixed estuary and the range of water variables would be expected to overlap on a macro scale between mud and sand habitats. I also conducted choice tank trials asking the question: do *D. sayi* show a sediment preference in a contained environment with a choice of sand or mud sediment, and do individual *D. sayi* select the same sediment in the tank as the sediment it was captured over? I hypothesized that rays would show a sediment preference in the tank trials and that their choice tank preference would correspond with the sediment they were captured over.

METHODS

Study Sites

North Inlet, (Figure 1) South Carolina is a relatively unimpacted estuary north of Winyah Bay with limited freshwater input from several small creeks (Dame *et al.* 1986). North Inlet is a well-mixed estuary with salinities that typically range between 31 and 34 psu. Up to 55% of the water is exchanged with the ocean on each tidal cycle. The average tidal range is 1.4 m, and tidal current velocities in the mouth of the inlet are on the order of 1.4 m sec⁻¹. Two shallow subtidal channels at the southern edge of North Inlet estuary connect to the Mud Bay region of Winyah Bay; during periods of major freshwater inflow into Winyah Bay, brackish water can penetrate the southern and central portion of North Inlet for short periods. Summer water temperatures in the main channels can reach 31°C (Dame *et al.* 1986).

Areas of mud and sand were mapped in North Inlet with the objective of finding large areas of sediment classified as either sand or mud. Surface grabs were taken with a minimum of 40 mL of sediment for analysis. Large oyster reefs are present in North Inlet, but mud and sand make up a much larger percentage of the bottom coverage (Potthoff and Allen 2003). Sampling sites were chosen in areas where sharks and rays would likely be able to bury or forage in the sediment, which excluded oyster reef habitat where the sediment is not exposed. Moreover, fishing on or near oyster reefs could damage the nets. The depth of North Inlet is 15 m at its deepest point, but mean depth in the main waterways is closer to 3 m at high tide. Fishing sites were restricted to a maximum depth of 2 m because of the net height. Sample sites were divided into 4 width

categories, < 20 m, 20 - 30 m, 30 - 50 m, and > 50 m to determine if creek width had an effect on CPUE.

Sediment Classification and Site Selection

Sediment and site classification was based on > 50% of the sample containing either mud (Φ > 4, < 63 µm) or sand (Φ -1 to 4, 63-500 µm) particle sizes (Blott and Pye 2001). During a preliminary study, surface grab samples were taken with an Ekman bottom grabber from the bottom at approximately the center and each side of the creek. These 3 samples per site were compared to ensure that the cross section of the creek was all either sand or mud. I then sampled the sediment as far up the creek as possible to ensure that the area upstream from the site was the same sediment classification as the site itself.

From each sediment sample, 40 mL of sediment was placed in a beaker, dried at 60° C, weighed, sieved through a 2 mm sieve and a 63 µm sieve, dried again at 60° C, and reweighed. Salt and mud were washed out of the sample during sieving and the percentage of mud, sand, and shell gravel in the sample was determined by weight. In the case that a sample did not contain at least 50% sand or mud, that site was not used in the study.

Thirty-six sites were chosen based on creek size and lack of visible oyster reefs and were not randomized. These sites were tested for sand or mud bottom and 24 sites were chosen based on sediment composition, depth, and proximity to large oyster reefs. Two of the 36 sites were not at least 50% mud or sand, 3 sites were only >50% sand or mud by 1%, 2 sites were deeper than 2.1 m, and 5 sites were too close to oyster reefs to be able to set the nets without snagging on the reefs; these sites were not used. Of the 24 suitable sites, 5 had a mud bottom and 19 had a sandy bottom, which is representative of the availability of each habitat in North Inlet (Potthoff and Allen 2003). Each sampling location is described in Table 1.

Net fishing

All sampling took place May 26, 2011 through September 18, 2011. Mud and sand sites were fished on alternating but sequential days (47 sampling days) to ensure consistency between the two treatments with the time of day each treatment was fished. Five or six days at a time were skipped when slack high tide occurred too late in the day for daylight fishing. Since only mud or sand sites were sampled on a given day, tidal stage was consistent between these two treatments. Because more sandy bottom habitat was available than muddy bottom habitat within the inlet, proportionately more samples were collected over sand than mud bottoms.

A braided nylon (Millner 1985, Snelson *et al.* 1989) tangle net with a 203 mm square mesh size (Millner1985) was used to collect elasmobranchs. Similar nets have been used in other ray and shark studies where bait would not be appropriate as an attracting factor (Millner 1985, Snelson *et al.* 1989). The net had a weighted bottom line and a floating top line to keep it vertical in the water column. The net was 30.48 m long and rose no more than 1.82 m from the substrate with the bottom line on the substrate. Most fishing sites were close to a 1.82 m depth, allowing the entire water column to be fished.

Each end of the top line was marked with a buoy, and each end of the bottom line was held in place by an anchor. In wide channels (> 30 m), one end of the net was placed 1 m on shore, and the other end of the net was set upcurrent and away from shore, creating a funnel shape. In narrow channels or at the mouth of a small tidal creek (< 30 m), both ends of the net were placed on opposite shores (1 m onshore) to completely span the creek. Both of these fishing methods are illustrated in Figure 2.

When a stingray encountered the net and turned away from it, the barb of the ray snagged on the netting, causing the ray to struggle and to become entangled in the net (Millner 1985). Rays that did not have barbs could still be caught when the net wrapped around them as they hit it. Compared to other fishing methods such as longlining and trawling, tangle nets seem to lessen if not eliminate capture mortality of rays; Millner (1985) had zero mortality in his study and Snelson *et al.* (1989) reported that the rays were always alive and in good condition when recovered from the net even after an overnight set. When a shark encountered the net, its head appeared to penetrate the mesh, and as it struggled it became entangled. Nets were continuously monitored and sharks were removed from the nets immediately to reduce capture mortality.

During a preliminary study, 10 nets were fished from slack high to slack low tide and 10 from slack low to slack high tide. Elasmobranchs were captured only during the first 4 hours of ebb tide, as they moved in the direction of the tide. Therefore, each net was set during ebb tide and was allowed to soak for 30 minutes. The 30 minute soak time was determined by previous longline studies conducted in this system and nearby in which this amount of time was shown to minimize shark mortality (Abel *et al.* 2007, Belcher and Jennings 2010). Elasmobranchs are known to move with the tidal current

(Medved and Marshall 1983, Ackerman *et al.* 2000). *S. tiburo* swims continuously and were caught as they left small tidal creeks and moved out with the tide, allowing us to determine which habitat they were over during slack tide (shortly before capture). During ebb tide, small tidal creeks were completely blocked by the nets and elasmobranchs following the tide out of the creek were caught (Figure 2).

Site environmental factors

A YSI multimeter was used to measure water temperature, salinity, and dissolved oxygen at each sampling site in the bottom half of the water column. Turbidity readings were taken from one of three water quality monitoring stations in North Inlet (http://cdmo.baruch.sc.edu) nearest to the site being fished.

Animal processing

Species, sex, total length, fork length, and precaudal length were determined for each shark (Abel *et al.* 2007). Relative age was categorized as neonate (umbilical scar open), young-of-the-year (umbilical scar healed but visible) or mature or immature (based on clasper calcification in males and by size in females) (Steiner 2007). Sharks were tagged through the first dorsal fin using a curved stainless steel #2 needle. SpiderwireTM monofilament fishing line (thin line at high test strength) was sutured through the dorsal fin and a FloyTM vinyl laminated disc identification tag with a contact phone number was tied onto the dorsal fin. Any shark that died during processing was dissected to analyze stomach contents and to determine if female sharks were pregnant.

All rays were processed by determining species and sex, measuring disk width, and determining relative age both by the presence or absence of an umbilical scar and by disk size (Snelson *et al.* 1989). All rays were tagged using a suturing method at the base of the tail modified from the procedure described by LePort *et al.* (2008). First, the barb was cut with tinsnips to release the ray from the net. Then the tail was secured by wrapping a towel around it to protect both the ray and handlers from injury. A curved stainless steel #2 needle was used to thread 30 lb. Spiderwire[™] monofilament fishing line dorso-laterally through one side of the tail, through muscle, and exiting ventrally. The Spiderwire[™] was looped through a Floy[™] vinyl laminated disc identification tag with a contact phone number and the Spiderwire[™] was tied to hold the tag in place. Tagging was conducted to allow monitoring of animal movements within a system and to allow the determination of site fidelity. Any ray that died during this study was dissected to analyze stomach contents and to determine if female rays were pregnant.

Net Statistics

Catch-per-unit-effort (CPUE) was defined as number of animals captured per net (e.g. 3 sharks / 1 net = 3 CPUE). Mean CPUE was also used for certain categories and was defined as the number of animals captured per number of nets fished for that category (e.g. 49 rays caught / 187 nets fished = 0.262 CPUE). Mean CPUE was compared between sand and mud habitats for 3 categories: each species, each sex within a species, and stages of maturity within a species. To determine if rays or sharks exhibited a sediment preference, the CPUE of each net over sand and mud sites (independent samples or sites) were compared using the Mann-Whitney U nonparametric test. The data were transformed to eliminate the zero CPUE numbers by adding 0.5 to all data points. The same procedure was used to compare the CPUE between small and large creeks. A linear regression analysis was conducted for *D. sayi*

and *S. tiburo* over the range of mud and sand percent composition and over the range of dissolved oxygen, salinity, temperature, and turbidity. To further examine sediment preference for both *D. sayi* and *S. tiburo*, the results were analyzed by X² to determine if the observed catch significantly differed from the expected catch based on the fishing effort over each sediment category.

Choice experiment

Choice tank experiments were conducted on *D. sayi* to examine sediment preference in a controlled environment and to determine if the ray would choose the same sediment that it was captured over. Sharks could not be used in the tank trials because a large enough transport tank and a large enough choice tank were not available for the sharks to survive in captivity. If rays preferred the same sediment in the tank, then the hypothesis that elasmobranchs in this system exhibit a sediment preference would be supported. The tank conditions were similar to slack tide conditions, since there was little water movement in the tank.

Two circular flow-through tanks 2.43 m in diameter were divided in half, with either sand or mud at least 15 cm deep covering the bottom. A standpipe in the center of the tank maintained the water depth at 0.6 m and a wooden 2x4 section was placed vertically along the bottom to divide the tank in half and to help keep each sediment on the appropriate side of the tank. Mud for the tanks was obtained from Sixty Bass Creek in North Inlet where the sediment was 83.05 % mud and 16.94 % sand. Sand for the tanks was obtained from a sandbar in Debidue Creek in North Inlet where the sediment

was 88.72 % sand and 11.27 % mud. The tank volume was 2810 L with a flow rate of 25 L per minute and a complete turnover in 113 minutes.

Water was pumped directly from North Inlet into the tanks and environmental factors (salinity, temperature, and dissolved oxygen) were monitored each time a ray was introduced to the tank. A fine mesh nylon filter was used to keep additional sediment from flowing into the tanks to maintain the sand and mud compositions on each side of the tank. Activated carbon was used to help remove organic material from the water. When rays were removed from the tanks for release, the sediment was redistributed on each side to cover pits created by each ray.

Rays used in the tank trials were less than 52 cm disk width to be a suitable size for the transport tank. Rays captured in the field were placed in an acclimation pool one at a time. The acclimation pool was a 1.06 m diameter tub submerged in the larger choice tank. The rays were acclimated in the tub for 24 hours and then released freely in the larger choice tank and the acclimation pool was removed from the choice tank. Each ray was observed continuously for 4 hours. The first 15 minutes were not included in statistical analysis to account for any startle response when the ray was released from the holding tank. The total time a ray spent over each sediment was noted and the percent time that the ray spent over sand and over mud was calculated. If the ray was swimming, the tank position could not be recorded; therefore, the time spent over sand and mud may not equal 100%. After each ray was observed in one tank, it was transferred to the holding tank in the other choice tank, acclimated for 24 hours and observed again as stated above to determine whether or not there was a tank effect. Each ray was observed once in each of the two tanks for a total of two trials per ray and the percent time over

each sediment was averaged between the two trials for statistical analysis. After observations were complete, all rays were released into North Inlet. The Wilcoxon signed ranks test was used to determine if there was a significant difference between the two choice tanks and to determine if the rays exhibited a sediment preference between sand and mud in the tanks, which were not independent samples.

<u>RESULTS</u>

Relative Abundance and Sediment Preference

During this study, 118 nets were fished over sand and 69 nets were fished over mud, approximately proportional to the availability of these bottom types in North Inlet estuary. Forty sharks were captured (Table 2), including 31 bonnethead sharks, *Sphyrna tiburo;* 4 blacktip sharks, *Carcharhinus limbatus;* 2 sandbar sharks, *Carcharhinus plumbeus;* 2 bull sharks, *Carcharhinus leucas;* and 1 lemon shark, *Negaprion brevirostris.* All *C. plumbeus, C. leucas, N. brevirostris,* and *C. limbatus* were caught over sand; however, these are insufficient data to conclude whether these species exhibited any sediment preference. The mean CPUE over mud was 0.081 and over sand was 0.208. There was no significant difference between mud and sand mean CPUE (p = 0.180) when compared by the Mann-Whitney U non-parametric test (Figure 3). Tags were attached to 14 of the bonnethead sharks.

Forty-nine rays were captured during this study (Table 3), including 41 bluntnose rays, *Dasyatis sayi;* 4 southern rays, *Dasyatis americana*; 2 smooth butterfly rays, *Gymnura micrura*; 1 Atlantic ray, *Dasyatis sabina*; and 1 spotted eagle ray, *Aetobatus narinari*. The mean CPUE for *D. sayi* over mud was 0.29 and over sand was 0.184. The CPUE for *D. sayi* between sand and mud sites did not differ significantly (p = 0.089; Mann-Whitney U nonparametric test) (Figure 4). The CPUE for male and female rays over mud and sand did not differ significantly (p = 0.104 for females and p = 0.244 for males). Eleven of the 41 *D. sayi* caught were juveniles, and they did not show a preference between sand and mud (p = 0.885). Tags were attached to 37 *D. sayi*.

Sediment samples were divided into classes to further analyze a correlation between CPUE and sediment composition using linear regression. No correlation was found between CPUE and sediment composition for *S. tiburo* over sand (p = 0.502) or mud (p = 0.421) or for *D. sayi* over sand (p = 0.495) or mud (p = 0.355). These categories of sediment were further analyzed by X². Based on the percent fishing effort over each category of sand and mud, the expected number of *S. tiburo* and *D. sayi* were captured in most categories and there was no significant difference between observed and expected catches (Figure 5 for *D. sayi* and Figure 6 for *S. tiburo*).

We also tested for a creek size effect and there was no significant difference between small and large creeks when CPUE within each treatment was compared (p=0.415; Mann-Whitney U nonparametric test) and the number of individuals captured in each creek size is shown in Figure 7. The environmental factors measured over sand and mud were very similar throughout this experiment and were examined by linear regression (Table 5). No linear relationship was found between the number of *D. sayi* captured and dissolved oxygen (p = 0.116), salinity (p = 0.795), temperature (p = 0.174), or turbidity (p = 0.559) (Figures 8-11). No linear relationship was found between the number of *S. tiburo* and dissolved oxygen (p = .063), salinity (p = 0.081), temperature (p = 0.326), or turbidity (p = 0.474) (Figures 12-15).

Distribution

All *S. tiburo* were adult females and all but two of the sharks were captured in small creeks directly connected to Town Creek. Town Creek is one of the main channels in North Inlet and is the most centrally located channel in the inlet. *S. tiburo* was found

throughout Town Creek with 21 sharks found within 2 km of the inlet mouth and 8 sharks found more than 2 km from the inlet mouth. Of the 31 *S. tiburo* captured, 6 died in the net. Total length ranged from 87 to 124 cm (Table 3) (Figure 16). All *S. tiburo* were captured within the following water quality parameters: 3.4 - 6.9 mg/L dissolved oxygen, $26.5 - 31.9^{\circ}$ C, 5-47 NTU and 32.7 - 37.6 psu (Table 6) and no correlation was found between CPUE and any of these parameters (Figure 8).

All *C. plumbeus*, *N. brevirostris*, and 3 of the *C. limbatus* were caught on the Debidue Creek sandbar (site #6) in close proximity to the inlet mouth. Both *C. leucas* were caught at site #4 and one of the *C. limbatus* was caught at site #17. Both of these sites are more than 2 km from the mouth of the inlet but still in the larger, main channels of the inlet. This was the first validated catch of *C. leucas* in North Inlet.

All 49 rays captured were found alive and well in the net, and all rays were caught in or in close proximity to Town Creek. The *D. sayi* disk width size ranged from 26 to 68 cm (Figure 9). All *D. sayi* were captured within the following water quality parameters: 3.92 - 7.7 mg/L dissolved oxygen, $25.9 - 31.9^{\circ}$ C, 30.3 - 37.4 psu, and 5 - 35 NTU turbidity (Table 7), and no correlation was found between CPUE and any of these parameters; however, the number of *D. sayi* captured for classes of each environmental factor measured can be found in Figure 10. No *D. sayi* were captured from July 6, 2011 to August 15, 2011.

Choice Tank Trials

One male *D. sabina*, 10 male *D. sayi*, and 4 female *D. sayi* were tested in the choice tanks (Table 4). The data for *D. sabina* was excluded in all statistical tests to

focus the results on a single species. Using the Wilcoxon signed ranks test, the percentage of time spent over sand for each ray was compared between the two tanks to determine if there was a significant difference between the two tanks. No significant difference was found between the tanks (p = 0.682), therefore, the average time spent over sand and mud was taken from the combined two trials.

The average percentage of time spent over sand and mud was compared for all *D*. *sayi* and also was compared by sex. No significant difference was found between time spent over mud and sand for all rays (p = 0.552) or for male *D*. *sayi* (p = 0.405) (Bonferonni). Since only 4 females were observed, we could not test for a sediment preference of females alone.

DISCUSSION

The purpose of this study was to examine the relative abundance and distribution of elasmobranchs in North Inlet, SC, while determining if sediment was an important factor in the habitat preference of local elasmobranch species. Neither of the elasmobranch species that made up the majority of the catch (*D. sayi* and *S. tiburo*) showed a sediment preference. Additionally, neither *D. sayi* nor *S. tiburo* showed a preference for a specific range of dissolved oxygen, temperature, turbidity, or salinity as habitat preference factors.

When mean CPUE was compared between mud and sand habitats for *D. sayi* using the Mann-Whitney U nonparametric test, there was no significant difference in catch between these two habitats. When mud and sand were divided into narrower categories, and observed versus expected catch were compared with X², no significant difference was found between observed and expected catches, further showing a lack in sediment preference. No sediment preference was shown in the controlled choice tank experiment further supporting that this species does not choose habitat based on sediment composition. The lack of a linear relationship between CPUE and dissolved oxygen, salinity, temperature, or turbidity also supports that this species does not choose habitat based on sediment based on environmental factors.

In field sampling, sharks and rays were being captured during an active period of the day. These animals could only be captured in the tangle nets while swimming from one area to another, and no patterns or habitat preferences were found during this active period. During the choice tank experiment, each individual *D. sayi* was mostly in a

resting period during observation. Each individual ray went back and forth over the sand and mud in the tank, and the lack of a preference in the tank trials shows that this species of ray does not have a sediment preference during resting periods. The combination of these results further supports that the species of sharks and rays studied do not exhibit a habitat preference at any point during the day.

The lack of recaptures during this study further supports the theory that these are motile predators with wide tolerances for environmental factors. In a strong tidal system such as North Inlet estuary, these animals most likely cover many different bottom types during an active period. Since these animals daily cover many different bottom types, this may explain why they do not show a preference for a particular bottom type.

D. sayi are not freshwater tolerant, typically prefer warmer water, and were shown by Snelson *et al.* (1989)to be a migratory species. Supportive of the migratory findings of Snelson *et al.* (1989), *D. sayi* are found in North and South Carolina only during the warm summer months . We had an unexpected cold period in September 2011, which reduced the water temperature much earlier than was expected (from 27°C to less than 25°C). No *D. sayi* were captured after that cold period (sampling continued for 3 weeks), suggesting that they may begin their seasonal migration with the first major temperature drop in the fall season. This was similar to the migration of *D. sayi* into deeper water in Florida during the cooler months (Snelson *et al.* 1989).

D. sayi dominated the catch of rays found in North Inlet, a surprising result in light of a four-year seine and trawl survey of aquatic species in North Inlet that yielded only 3 *D. sayi* (Ogburn *et al.* 1988). Similarly, neither Yednock (2005) nor Maxwell

(2008) caught any *D. sayi* in their hook and line studies in North Inlet on habitat utilization of various shark species and the distribution of *R. terraenovae* respectively, and both studies yielded a heavy bycatch of *D. americana*. North Carolina and South Carolina were previously thought to be at the northern limit for *D. sayi* (Snelson *et al.* 1989). The abundant range of this species may have shifted northward, due to increasing water temperatures along the east coast (Allen *et al.* 2008). A study conducted in Australia found that the range of 45 species of fishes (including some shark and ray species) had extended poleward over the last few decades, which is thought to be due to climate change (Last *et al.* 2010).

The Atlantic *D. sayi* population, as well as other ray populations, seems to be increasing. Pearse Webster (personal communication, March 13, 2012) documented a population increase in *D. sayi*, *D. americana*, and *D. sabina* in an offshore trawl survey from Cape Hatteras, NC to Cape Canaveral, FL between his 2010 and 2011 catches (equal fishing efforts each year). In a single year *D. sayi* increased from 562 to 1003, *D. americana* increased from 77 to 139, and *D. sabina* increased from 94 to 428 (Pearse Webster, personal communication, March 13, 2012). We do not know yet how this putative population increase, if it represents a long-term trend, will affect the North Inlet ecosystem. The structure of the North Inlet ecosystem should continue to be monitored for changes in species distribution.

Although we did not catch many *D. americana* or *D. sabina*, this does not mean that these two species are not abundant in North Inlet. *D. sabina* is known to feed near oyster reefs, and oyster reefs were avoided during this study. *D. americana* may frequent deeper parts of the inlet, which we were unable to sample due to depth restrictions of the nets. A more comprehensive study using multiple capture methods is required to determine the relative abundance of all three *Dasyatis* species found in North Inlet and to determine if *D. sabina* and *D. americana* show the above habitat preferences.

S. tiburo did not show a preference for either mud or sand habitat when the mean CPUE was compared using the Mann-Whitney U nonparametric test. When mud and sand were divided into narrower categories and observed versus expected catch were compared with X², no significant difference was found between observed and expected catches, further showing a lack in sediment preference. There was no linear relationship between CPUE and salinity, temperature, or turbidity; however, there was a significant decrease in CPUE with increasing dissolved oxygen. This preference for lower dissolved oxygen is supported by Belcher and Jennings (2010).

S. tiburo was our second most abundant catch. Prior to this study *S. tiburo* and *R. terraenovae* were known to be an abundant shark species in North Inlet estuary during the summer months (Abel *et al.* 2007), however, the net mesh size in this experiment was too large to catch neonate *R. terraenovae*, therefore limiting our catch to larger individuals.

Female *S. tiburo* on average mature at 94 cm (Lombardi-Carlson *et al.* 2003). Only 3 *S. tiburo* that we caught were below the average length at maturity (87 cm, 91 cm, and 91 cm), but these three sharks still may have been mature. Of the 6 *S. tiburo* (106 to 118 cm TL) that died during this study, all were pregnant. Fetuses were in various stages of development appropriate to the month in which the female was captured. Each female had either 12 or 14 fetuses and the two females that died in August had fully developed

fetuses whose umbilical cords had detached and were very close to birth. This timeline was consistent with the findings of Manire *et al.* (1995), who found in the eastern Gulf of Mexico, *S. tiburo* mate in November, store sperm for fertilization in March or April, and typically give birth in August. The other *S. tiburo* individuals caught in August had enlarged and firm abdomens, suggesting that all of these individuals were pregnant. Based on the individuals that were confirmed pregnant by dissection and the individuals that externally appeared pregnant in our August samples, it is probable that most, if not all, female *S. tiburo* captured during this study were pregnant.

There is no record of a neonate *S. tiburo* from North Inlet, and based on this evidence, North Inlet was not thought to be used as a primary nursery ground by *S. tiburo* (Abel *et al.* 2007). However, two sharks were caught in the present study which were very close to giving birth (umbilical cords had detached), suggesting that parturition occurs in or near North Inlet, and that this inlet may serve as a primary nursery ground for *S. tiburo*. There are several possible reasons why a neonate *S. tiburo* has never been captured in North Inlet. It is possible that the neonates move offshore immediately after birth, the neonates may get flushed out of the inlet by the ebbing tide, the neonates may be consumed by predators soon after birth, or they remain in the inlet for a period of time immediately after birth, but are too small during that time to be caught in a net or that they are uninterested in the kinds of bait used by most fishermen (Cortés *et al.* 1996 and Bethea *et al.* 2007).

Absence of males suggests a segregation of the sexes in Atlantic *S. tiburo* populations. There does not seem to be a segregation of sexes in Gulf of Mexico *S. tiburo* populations as shown by Heupel *et al.* (2006), who found both males and females

in a 1:1 ratio in the same eastern Gulf of Mexico estuary. However, the sex segregation results of the present study were supported by the results of a nursery grounds study conducted in North Inlet by Abel *et al.* (2007) in 2002 and 2003. Thus far there is no explanation for the sex segregation in this population of *S. tiburo*.

At least eight other species of sharks have shown sex segregation including: *Carcharodon carcharias, Carcharhinus plumbeus, Galeus area, Galeorhinus zygopterus, Galeorhinus australis, Negaprion brevirostris, Prionace glauca*, and *Sphyrna lewini* (Klimley 1987). Scalloped hammerheads, *Sphyrna lewini*, are thought to segregate because the females eat different foods than males, allowing for faster growth to reach sexual maturity (Klimley 1987). Most likely this is not the case for this population of *S. tiburo*, since both male and female *S. tiburo* are known to feed primarily on *C. sapidus* (Cortés *et al.* 1996).

Tangle nets were very effective in capturing both sharks and rays while causing little to no injury to the animals, and there was zero net related mortality of rays, which was expected. While we experienced shark mortality, it was at a low rate of 15% of our total shark catch. It was important to use a sampling method without bait since part of this study was sediment preference and we did not want to attract sharks or rays from neighboring habitats. The mesh size was also large enough that bycatch was minimal and in most nets completely absent. Two loggerhead sea turtles, *Caretta caretta*; one green sea turtle, *Chelonia mydas*; one ladyfish, *Elops saurus*; and one red drum, *Sciaenops ocellatus*, were the only large bycatch aside from various species of crabs and jellies caught on the net. All bycatch was returned to the water unharmed. This minimal bycatch is important because sharks may have been attracted to struggling fish in the net.

A few *S. tiburo* were observed to have small recent bite marks upon retrieval from the net, but we cannot state if those bites occurred before or during net capture.

Several species of rays and sharks are known to move with tidal cycles to follow prey, avoid predators, and/or to save energy (Steiner 2007). For example *Dasyatis sabina* (Ackerman *et al.* 2000), *Carcharhinus plumbeus* (Medved and Marshall 1983), and *Rhinoptera bonasus* (Smith and Merriner 1985) have all been observed moving with tidal currents. In this study elasmobranchs were captured during slack tide as they migrated out of the creeks with the outgoing tide. To ensure sediment homogeneity upstream from our nets, each sampled creek was tested for sediment composition in several areas of the creek to ensure that the creek was composed of entirely sand or mud. It is possible that in sand creeks there may have been very narrow areas that we could not access for sampling that may have been classified as muddy areas. In these cases, the narrow, muddy areas were most likely either too narrow or too shallow during slack high tide for the size of elasmobranchs we caught to have been over these areas.

The lack of recaptures in our nets suggests that neither *D. sayi* nor *S. tiburo* had a high site fidelity. Heupel *et al.* (2006) supports the lack of site fidelity found in *S. tiburo*. In the eastern Gulf of Mexico, *S. tiburo* did not show site fidelity, although they did show fidelity to that particular estuary, or an obvious tidal migration (Heupel *et al.* 2006).

D. sayi did not show any statistical abiotic habitat preference factors in field or tank trials and *S. tiburo* did not show any statistical habitat preference factors other than a decreasing CPUE with increasing dissolved oxygen. In North Inlet this lack of habitat

preference may be a result of the well-mixed environment. The lack of habitat preference in this inlet is not necessarily representative of all populations of these species.

Summary

D. sayi and *S. tiburo* did not exhibit a sediment preference, site fidelity, creek size preference, or habitat preferences based on environmental factors in North Inlet, SC. *D. sayi* also did not show a sediment preference in choice tank trials. This lack of sediment or environmental factor preference suggests that both *D. sayi* and *S. tiburo* are able to survive in a wide range of habitats. As climate change continues, the range of these and other elasmobranch species may extend poleward, which could cause many changes in coastal ecosystems.

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Site Name and Number	Avr. % Sand	Avr. % Mud	Max. Depth, m	# Times Fished
1.) Town Creek sandbar	87.4	12.5	1.8	б
2.) Town Creek across from Old	79.8	20.0	1.5	4
Man Creek				
3.) Town Creek S. of Bread and Butter Creek	78.5	21.0	2.1	3
4.) Town Creek N. of Bread and Butter Creek	76.9	15.7	1.8	3
5.) Town Creek N. of Sixty Bass Creek	74.6	19.8	1.8	3
6.) Debidue Creek sandbar	84.6	15.5	1.2	26
7.) Debidue Creek alcove	88.8	11.2	1.8	3
8.) Debidue Creek upper	72.4	14.3	1.8	3
9.) Debidue Creek dune side	82.3	17.5	1.5	7
10.) Sixty Bass Creek upper	16.94	83.0	1.5	7
11.) Sixty Bass Creek mouth	81.5	12.8	2.1	6
12.) Sixty Bass Creek 1 st right	70.6	24.3	1.5	6
13.) Sixty Bass 2 nd right	21.6	76.2	1.2	7
14.) Bread and Butter Creek upper	20.0	76.1	2.1	25
15.) Bread and Butter Creek mouth	68.2	30.0	2.1	4
16.) Jones Creek 1 st left	95.7	3.9	1.2	3
17.) Jones Creek S. of sandbar	93.3	6.7	1.5	3
18.) Mud Creek	74.5	13.0	1.8	3
19.) Bly Creek lake mouth	74.5	12.8	1.8	3
20.) Cutoff Creek upper	23.9	76.0	2.1	7
21.) Shark Spot	69.1	11.3	1.5	24
22.) Across from Shark Spot	90.9	9.0	1.2	5
23.) Old Man Creek across from Bly Creek	83.3	16.7	1.8	3
24.) Clambank Creek mouth	34.9	64.9	1.8	23

Table 1. Name and description of 24 sampling sites.

Table 2. List of all sharks captured during this study. Site numbers correspond with numbers shown on Figure 1.

Site	Species	M/F	Total	Tag #	D. O.,	Temp.,	Salinity,	Turbidity,
#	-		length,	C	mg/L	°C	psu	NTU
			cm		_			
4	C. leucas	Μ	137	No	5.52	31.3	35.4	13
				tag				
4	C. leucas	N/A	137	No	5.52	31.3	35.4	13
				tag				
17	C. limbatus	F	174	78	5.6	30.4	36.7	13
17	C. limbatus	F	174	78	5.46	31.1	35.6	17
6	C. limbatus	Μ	160	No	5.5	29.4	36.1	17
				tag				
6	C. limbatus	F	183	98	6.65	27.4	35.2	14
6	C. plumbeus	F	140	99	6.65	27.4	35.2	14
6	C. plumbeus	F	122	24	6.5	27.7	34.4	9
6	<i>N</i> .	F	124	No	5.5	26.5	35.7	5
	brevirostris			tag				
6	S. tiburo	F	99	21	5.7	27.2	34.4	7
6	S. tiburo	F	98	No	5.7	27.2	34.4	7
				tag				
12	S. tiburo	F	107	12	3.67	28.4	35.4	17
12	S. tiburo	F	118	Died	3.67	28.4	35.4	17
15	S. tiburo	F	106	23	3.36	28.8	35.1	17
21	S. tiburo	F	99	No	3.6	28.2	35.1	17
				tag				
21	S. tiburo	F	108	No	3.6	28.2	35.1	17
				tag				
21	S. tiburo	F	119	No	3.6	28.2	35.1	17
				tag				
2	S. tiburo	F	107	No	4.9	30.7	37.6	N/A
				tag				-
6	S. tiburo	F	115	No	4.8	28.2	35.3	8
				tag				
6	S. tiburo	F	115	No	5.74	29.5	34.5	9
				tag				
22	S. tiburo	F	115	27	6.6	28.5	35.9	15
12	S. tiburo	F	114	Died	4.34	29.5	34.4	15
13	S. tiburo	F	107	Died	4.34	29.5	34.4	13
13	S. tiburo	F	113	85	4	28.9	35	12
16	S. tiburo	F	124	69	6.38	29.5	34.9	10
6	S. tiburo	F	113	0	6.9	28.2	34.1	8
19	S. tiburo	F	87	16	4.79	28	34.7	15
6	S. tiburo	F	119	68	4.45	27.7	34.6	N/A

21	S. tiburo	F	116	72	4.73	28.7	33.9	18
21	S. tiburo	F	107	93	6.2	31.2	32.7	47
11	S. tiburo	F	106	Died	5.8	31.7	32.7	16
5	S. tiburo	F	110	Died	5.5	31.6	32.7	16
24	S. tiburo	F	118	No tag	5.52	31.9	35.5	18
21	S. tiburo	F	91	No tag	5.9	31.4	37.3	19
14	S. tiburo	F	111	No tag	5.7	31.1	37.3	20
3	S. tiburo	F	91	No tag	5.6	31.1	37.4	18
21	S. tiburo	F	118	40	5.8	31.1	37.2	21
11	S. tiburo	F	113	No tag	5.4	30.2	36.6	16
24	S. tiburo	F	121	126	6	30.3	36.6	17
14	S. tiburo	F	107	159	4.9	26.6	34.8	11

Table 3. List of all rays captured during this study. Site numbers correspond with numbers shown on Figure 1.

Site	Species	M/F	Disk	Tag	D. O.,	Temp.,	Salinity,	Turbidity,
#	-		width,	#	mg/L	°Ċ	psu	NTU
			cm		U		•	
6	A. narinari	F	152.4	No	5.4	28.5	34.6	6
				Tag				
1	D. americana	Μ	31	41	6.2	27.8	36.7	14
15	D. americana	Μ	65	79	4.4	27.7	35.3	14
14	D. americana	Μ	63	10	5.9	30.5	35.1	21
1	D. americana	F	107	3	6.49	28.1	34.3	21
6	D. sabina	Μ	28	11	6.3	28.4	36.9	21
15	D. sayi	F	64	47	4.4	27.7	35.3	14
15	D. sayi	F	64	56	4.4	27.7	35.3	14
6	D. sayi	Μ	48	8	6.5	27.7	34.3	9
6	D. sayi	Μ	34	5	6.9	28.2	34.1	8
14	D. sayi	Μ	38	29	7.7	29.4	34.9	25
14	D. sayi	F	34	7	7.7	29.4	34.9	25
6	D. sayi	Μ	43	14	5.4	28.5	34.6	6
6	D. sayi	Μ	43	20	5.4	28.5	34.6	6
6	D. sayi	F	32	25	5.4	28.5	34.6	6
18	D. sayi	Μ	45	13	4.62	28.5	34.7	16
6	D. sayi	F	27	1	6.01	27.5	34.4	6
6	D. sayi	F	26	18	5.5	26.5	35.7	5
20	D. sayi	F	52	15	5.9	30.8	36	14
6	D. sayi	F	41	17	6.9	30	36	8
13	D. sayi	Μ	50	No	3.92	28.4	35.4	16
				Tag				
21	D. sayi	Μ	43	9	5.3	29.9	36.2	20
21	D. sayi	Μ	39	2	5.6	29.9	36.3	18
21	D. sayi	Μ	44	19	6.3	29.1	36.1	16
22	D. sayi	F	62	57	6.6	28.5	35.9	15
9	D. sayi	Μ	37	80	5.5	29.7	30.3	N/A
23	D. sayi	F	65	65	5.02	31.8	31.8	35
21	D. sayi	F	61	No	6.2	30.9	32.7	27
				Tag				
24	D. sayi	F	51	39	5.52	31.9	35.5	18
24	D. sayi	Μ	44	87	5.52	31.9	35.5	18
3	D. sayi	Μ	32	74	5.6	31.1	37.4	18
24	D. sayi	F	45	2	6	30.8	37.1	21
24	D. sayi	F	58	70	6	30.8	37.1	21
9	D. sayi	Μ	45	114	6.24	29.7	35.4	12
9	D. sayi	Μ	38	157	6.24	29.7	35.4	12
24	D. sayi	Μ	41	38	6.3	29.7	36.5	11

24	D. sayi	М	37	48	6.3	29.7	36.5	11
24	D. sayi	Μ	42	6	6.3	29.7	36.5	11
24	D. sayi	F	40	123	5.7	29.8	36.6	16
24	D. sayi	F	68	127	5.7	29.8	36.6	16
24	D. sayi	Μ	43	139	5.7	29.8	36.6	16
24	D. sayi	Μ	43	124	5.8	29.6	36.2	18
24	D. sayi	F	57	128	5.5	29.6	36.3	19
24	D. sayi	F	52	118	4.8	29.6	36.4	21
14	D. sayi	F	62	108	4	25.9	34.8	15
24	D. sayi	Μ	42	152	4.5	26.9	34.9	13
24	D. sayi	Μ	45	113	4.4	27.4	35	15
1	G. micrura	F	52	58	5.1	29.6	38.6	21
1	G. micrura	Μ	36	28	5.1	29.6	38.6	21

Tag #	M/F	Sed. caught	% time over	% time over
		over	sand	mud
9	М	Sand	90.625	9.375
19	М	Sand	41.875	58.125
80	М	Sand	56.25	43.75
87	Μ	Mud	53.125	46.875
74	М	Sand	43.75	53.125
157	М	Sand	43.75	56.25
114	М	Sand	31.25	59.37
124	М	Mud	50	50
152	М	Mud	34.37	46.87
113	Μ	Mud	46.87	53.12
39	F	Mud	43.75	50
2	F	Mud	81.25	18.75
45	F	Sand	93.75	6.25
118	F	Mud	81.25	12.5

Table 4. Percentage of time spent over sand and mud for each tank trial.

	D. O., mg/L		Temperature, °C		Salini	ty, psu	Turbidity, NTU	
	Sand	Mud	Sand	Mud	Sand	Mud	Sand	Mud
Min.	2.7	3.5	24.2	23.7	26.1	32.8	5	6
Mean	5.4	5.5	29.0	28.4	35.2	35.7	14.5	15.7
Max.	7.4	7.7	31.8	31.9	38.6	37.5	47	26

Table 5. Water quality comparison over sand and mud for all nets over all sampling dates.

Table 6. Measured environmental factors throughout the study compared to

	D. O., mg/L		Temper	Temperature, °C		nity, psu	Turbidity, NTU		
	Study	S. tiburo	Study	S. tiburo	Study	S. tiburo	Study	S. tiburo	
Min.	2.7	3.4	23.7	26.5	26.1	32.7	5	5	
Mean	5.5	5.2	28.8	29.2	35.4	35.2	14.9	14.8	
Max.	7.7	6.9	31.9	31.9	38.6	37.6	47	47	

environmental factors at the time sharks were captured.

Table 7. Measured environmental factors throughout the study compared to water quality at the time rays were captured.

	D. O., mg/L		Temperature, °C		Salini	ty, psu	Turbidity, NTU	
	Study	D. sayi	Study	D. sayi	Study	D. sayi	Study	D. sayi
Min.	2.7	3.9	23.7	25.9	26.1	30.3	5	5
Mean	5.5	5.6	28.8	29.2	35.4	35.2	14.9	15
Max.	7.7	7.7	31.9	31.9	38.6	37.4	47	35



Figure 1. Sampling sites in North Inlet estuary from a 1999 aerial image (http://www.northinlet.sc.edu/about/map_jpgs/ADAR1999.jpg.) Numbers correspond with locations listed in Table 1.



Figure 2. A.) A depiction of the tangle net in a small tidal creek showing the buoys and anchors on shore and the net along the bottom all the way across the creek. B.) A depiction of the net in a large inlet channel showing one end of the net on shore and the other end set up-current creating a funnel shape.



Figure 3. Mean CPUE for *S. tiburo* over mud and sand. There was no linear relationship between mud and sand composition and CPUE for this species. The equation for the trend line is found on each graph.



Figure 4. Mean CPUE for *D. sayi* over mud and sand. There was no linear relationship between mud and sand composition and CPUE for this species. The equation for the trend line is found on each graph.



Figure 5. Number of observed vs. expected *D. sayi* captured for each class by percent composition of mud and sand. There was no significant difference between observed and expected catches for any category.



Figure 6. Number of observed vs. expected *S. tiburo* captured for each class by percent composition of mud and sand. There was no significant difference between observed and expected catches for any category.



Figure 7. Number of *D. sayi* and *S. tiburo* captured in small (< 30 m) and large (> 30 m) creeks.



Figure 8. Number of *D. sayi* captured at each dissolved oxygen level. No linear relationship was found between dissolved oxygen and *D. sayi* captures. The regression equation for the trend line is found on this graph.



Figure 9. Number of *D. sayi* captured at each salinity level. No linear relationship was found between salinity and *D. sayi* captures. The regression equation for the trend line is found on this graph.



Figure 10. Number of *D. sayi* captured at each temperature level. No linear relationship was found between temperature and *D. sayi* captures. The regression equation for the trend line is found on this graph.



Figure 11. Number of *D. sayi* captured at each turbidity level. No linear relationship was found between turbidity and *D. sayi* captures. The regression equation for the trend line is found on this graph.



Figure 12. Number of *S. tiburo* captured at each dissolved oxygen level. No linear relationship was found between dissolved oxygen and *S. tiburo* captures. The regression equation for the trend line is found on this graph.



Figure 13. Number of *S. tiburo* captured at each salinity level. No linear relationship was found between salinity and *S. tiburo* captures. The regression equation for the trend line is found on this graph.



Figure 14. Number of *S. tiburo* captured at each temperature level. No linear relationship was found between temperature and *S. tiburo* captures. The regression equation for the trend line is found on this graph.



Figure 15. Number of *S. tiburo* captured at each turbidity level. No linear relationship was found between turbidity and *S. tiburo* captures. The regression equation for the trend line is found on this graph.



Figure 16. Number of female *S. tiburo* captured in each size class. The average size at maturity is 94 cm (Lombardi-Carlson *et al.* 2003).



Figure 17. Number of male and female *D. sayi* captured in each size class. All *D. sayi* < 38 cm were immature, all males > 38 cm were mature, and all females > 50 cm were mature (Snelson *et al.* 1989).