Coastal Carolina University [CCU Digital Commons](https://digitalcommons.coastal.edu/)

[Electronic Theses and Dissertations](https://digitalcommons.coastal.edu/etd) [College of Graduate and Continuing Studies](https://digitalcommons.coastal.edu/graduate)

Spring 5-1-2023

Neonate Atlantic Sharpnose Shark (Rhizoprionodon terraenovae) Relative Abundance and Body Condition in Two South Carolina Estuaries Varying in Urbanization

Rileigh E. Hawk Coastal Carolina University

Follow this and additional works at: [https://digitalcommons.coastal.edu/etd](https://digitalcommons.coastal.edu/etd?utm_source=digitalcommons.coastal.edu%2Fetd%2F171&utm_medium=PDF&utm_campaign=PDFCoverPages) Part of the [Biology Commons,](https://network.bepress.com/hgg/discipline/41?utm_source=digitalcommons.coastal.edu%2Fetd%2F171&utm_medium=PDF&utm_campaign=PDFCoverPages) and the [Ecology and Evolutionary Biology Commons](https://network.bepress.com/hgg/discipline/14?utm_source=digitalcommons.coastal.edu%2Fetd%2F171&utm_medium=PDF&utm_campaign=PDFCoverPages)

Recommended Citation

Hawk, Rileigh E., "Neonate Atlantic Sharpnose Shark (Rhizoprionodon terraenovae) Relative Abundance and Body Condition in Two South Carolina Estuaries Varying in Urbanization" (2023). Electronic Theses and Dissertations. 171.

[https://digitalcommons.coastal.edu/etd/171](https://digitalcommons.coastal.edu/etd/171?utm_source=digitalcommons.coastal.edu%2Fetd%2F171&utm_medium=PDF&utm_campaign=PDFCoverPages)

This Thesis is brought to you for free and open access by the College of Graduate and Continuing Studies at CCU Digital Commons. It has been accepted for inclusion in Electronic Theses and Dissertations by an authorized administrator of CCU Digital Commons. For more information, please contact [commons@coastal.edu.](mailto:commons@coastal.edu)

Neonate Atlantic Sharpnose Shark (*Rhizoprionodon terraenovae***) Relative Abundance and Body Condition in Two South Carolina Estuaries Varying in Urbanization**

By

Rileigh E Hawk

Submitted in Partial Fulfillment of the

Requirements for the Degree of Master of Science in

Coastal Marine and Wetland Studies in the

School of the Coastal Environment

Coastal Carolina University

2023

Dr. Daniel Abel Major Advisor

X

Dr. Robert Young Committee Member

X

Dr. Matthew Kimball Committee Member

X

Dr. Eric Rosch Committee Member

 \sf{X}

Dr. Erin Hackett CMWS Graduate Programs Director X

Dr. Chad Leverette Dean, Gupta College of Science

^{© 2023} by Rileigh E. Hawk (Coastal Carolina University) All rights reserved. No part of this document may be reproduced or transmitted in any form or by any means, electronic, mechanical, photocopying, recording, or otherwise, without prior written permission of Rileigh E. Hawk (Coastal Carolina University).

Acknowledgments

I would like to thank my major advisor, Dr. Daniel Abel, for all of his support throughout every step of this process. I came to Coastal Carolina University with a vision for this project and he provided me with the support and resources to make it a reality. Thank you also to my committee members. Dr. Robert Young was a critical help in developing the methodology for collecting and analyzing the sound data for this study. Thank you to Dr. Matthew Kimball for always being an encouraging face during my time sampling in North Inlet and for your assistance during the writing process. And finally, Dr. Eric Rosch, for jumping in when needed and offering additional support during the writing process.

To all of the volunteers that braved the hot sun and heavy rain to fish with me throughout the summer, I cannot thank you enough. Katie Maddox, Katy Jones, Angela Barreto, Tiffany Bogan, Alexis Davis, Abigail Caviris, Alyssa Antolak, Shelby Pawlikowski, Kristina Flanigan, and Ivy Spratling this project could not have been done without your support. Thank you to the Hodges who helped make my project possible by providing a research vessel for this project. And thank you to the Coastal Carolina University Graduate Incentive Grant for funding my research.

Finally, I would like to extend the largest thank you to my family for encouraging me to follow a dream I have had since childhood. And to my friends for their unwavering support and love during this project.

Abstract

Urbanization near estuaries has been shown to affect the growth and survival of juvenile sharks using the system as a nursery. North Inlet and Murrells Inlet, South Carolina, are similarly-sized, tidally-dominated, bar-built estuaries with extensive *Sporobolus*-lined tidal creeks but differ in degree of human impact. Previously, Murrells Inlet was shown to have a lower abundance and diversity of large sharks than North Inlet and Atlantic Sharpnose Sharks (*Rhizoprionodon terraenovae*) were shown to use North Inlet as a primary nursery. To examine potential differences in neonate shark abundance and growth between a developed estuary, Murrells Inlet, and a protected estuary, North Inlet, fifty-two neonate *R. terraenovae* were captured on hook-and-line gear from May to September 2022. Sharks were measured for length and girth, weighed, sexed, and released. Noise pollution between the two estuaries was investigated using hydrophone recordings. Relative abundance of neonate *R. terraenovae* was much greater for North Inlet ($n = 45$) than for Murrells Inlet ($n = 7$). However, body condition, weight-length relationships, girth-length relationships, and growth rates of the neonate sharks did not differ between the estuaries. Elasmobranch diversity was greater for Murrells Inlet than North Inlet, though bony fish diversity was equal between estuaries. Analysis of sound found no difference in the total loudness of the recordings between estuaries or the sound power of the recordings for shark hearing frequency ranges ($p = 0.57$, $p = 0.45$, respectively). Sampling sites were deeper for North Inlet and there was more boat traffic at Murrells Inlet, however, there was no correlation between the sound recordings and depth or boat traffic for either estuary. Although the difference in urbanization between

estuaries did not affect the growth and body condition of *R. terraenovae*, the drivers behind the difference in abundance of neonates are still unclear.

Table of Contents

List of Tables

List of Figures

Figure 5. Average catch per unit effort (CPUE; with standard error) by month of *R. terraenovae* collected using rod and reel in the Murrells Inlet (green bars) and North Inlet (blue bars) estuaries during May through September 2022. No sharks were caught in Murrells Inlet in July and either estuary in September 2022. ..41

Figure 6. Average catch per unit effort (CPUE; with standard error) by creek of *R. terraenovae* collected using rod and reel in the Murrells Inlet (green bars) and North Inlet (blue bars) estuaries during May through September 2022...42

Figure 7. Percent of total catch using rod and reel in the Murrells Inlet and North Inlet estuaries during May through September 2022. The smaller pie chart represents species that comprised <1% of catch abundance. ...43

Figure 8. Fulton's K values by date for *R. terraenovae* (n = 42) using rod and reel in the Murrells Inlet and North Inlet estuaries during May through September 2022. The red line indicates normal body condition. R²= 0.093. ..44

Figure 9. Regression of girth-length relationship (cm girth to cm FL) for *R. terraenovae* collected in the North Inlet (n = 45; $R^2 = 0.8202$) and Murrells Inlet (n = 7; $R^2 = 0.9753$) estuaries...45

Figure 10. Regression of weight-length relationships (kg weight to cm FL) for *R. terraenovae* collected in the North Inlet ($n = 45$; $R^2 = 0.8846$) and Murrells Inlet ($n = 7$; R ² = 0.9530) estuaries..46

Figure 11. Regression of length (cm FL) by date for *R. terraenovae* collected in the North Inlet (n = 45; $R^2 = 0.4789$) and Murrells Inlet (n = 7; $R^2 = 0.8503$) estuaries........47

Figure 12. Regression of average length by day (cm FL) by date for *R. terraenovae* collected in the North Inlet (n = 45; $R^2 = 0.8385$) and Murrells Inlet (n = 7; $R^2 = 0.8999$) estuaries...48

Figure 13. Average root—mean—square—pressure (RMS) values by estuary (shadowed columns) and creek for the Murrells Inlet (green bars) and North Inlet (blue bars) estuaries during May through September 2022...49

Figure 14. Average Power Density (frequencies within shark hearing, 25 to 1500 Hz) by estuary (shadowed columns) and creek for the Murrells Inlet (green bars) and North Inlet (blue bars) estuaries during May through September 2022. ..50

Introduction

Estuaries are vital transition zones that support some of the most biologically productive systems on Earth (Kennish 2002). These complex coastal ecosystems are characterized by high primary productivity that supports abundant prey populations (Grubbs et al. 2007; Whitfield 2017), as well as shallow habitat that provides prey refuge from larger predators (Ferretti et al. 2010; Heupel & Simpfendorfer 2011; Whitfield 2017), making these systems frequent nursery habitats for a variety of coastal species (Beck et al. 2001; Curtis et al. 2013). Estuaries generally support a high biodiversity and abundance of species. Due to their proximity to rapidly developing areas, estuaries are at risk from urbanization (Kennish 2002; Todd et al. 2019; Freeman et al. 2019).

Estuaries face intense pressure from human development as coastal counties in the United States, which only make up about 10% of the total land mass, are home to 40% of the total population and have approximately five times the population density of the rest of the country (NOAA 2023). Humans receive many ecosystem services from estuaries such as recreation, seafood, cultural experiences, and protection from natural disasters (Freeman et al. 2019; Booi et al. 2022), yet humans also contribute to the destruction of these vital ecosystems. Not only are estuaries affected by localized coastal development, but they are also impacted by anthropogenic activity within the watershed upstream (Freeman et al. 2019). Agricultural runoff and suburban sprawl in upland areas

contribute additional stress on the ecosystem (Kennish 2002; Freeman et al. 2019). Human development adjacent to estuaries increases the degree of impervious surfaces leading to increased runoff, washing pollutants into nearby waterways (Kennish 2002; Freeman et al. 2019). Increases in contaminant, nutrient, and fecal bacteria levels from urbanization adversely affect the turbidity, salinity, and other water quality properties of estuaries (Heupel et al. 2007; Van Dolah et al. 2008; Freeman et al. 2019). Human recreation and activity within estuaries can deteriorate water quality even further by resuspension of sediment from boat use, coastal erosion from beach grooming, and dredging which destroys benthic habitat, deepens the waterways, and introduces additional toxins (Kennish 2002; Vargas-Fonseca et al. 2016; Todd et al. 2019). Coastal urbanization of estuaries has accelerated habitat destruction and resource exploitation, undermining the characteristics that make them critical habitats for juvenile species (Kennish 2002; Werry et al. 2012; Curtis et al. 2013).

Anthropogenic stressors compound to deteriorate essential habitats used by species at all life stages. Reduction in water quality from increased sediment and toxins leads to toxic algal blooms, biodiversity loss, and habitat alteration (Kennish 2002; Vargas-Fonseca et al. 2016; Freeman et al. 2019). Habitat destruction can also modify food webs by reducing fish biomass, increasing the potential for disease, and disrupting trophic interactions (Todd et al. 2019; Rangel et al. 2022). Additionally, pollution from runoff, wastewater, and recreation (boat traffic, fishing activity, sunscreen) affects ecosystem functioning, biodiversity, and behavioral responses (Vargas-Fonseca et al. 2016; Todd et al. 2019; Cartolano et al. 2020). Underwater sound pollution from boat engine noise and other anthropogenic activity is an understudied element of urbanization

that affects communication, hormone levels, development, and predator-prey interactions for a variety of marine organisms, such as fish, mammals, mollusks, arthropods, and reptiles (Kunc et al. 2016; Todd et al. 2019; Cartolano et al. 2020; Chahouri et al. 2022). Unfortunately, many species impacted by these anthropogenic effects use estuaries as nursery habitats during vulnerable stages in their early life history (Vargas-Fonseca et al. 2016).

Estuaries are commonly used as nursery habitats by many coastal shark species (Grubbs et al. 2007; Ulrich et al. 2007; Werry et al. 2012). The heterogeneous structure of estuaries provides refuge for sharks, contributing to lower mortality, while high productivity supports large prey populations that can accelerate the growth rates of young sharks and increase juvenile survival (Heithaus 2007; Heupel et al. 2007; Heupel $\&$ Simpfendorfer 2011; Curtis et al. 2013). The definition of shark nurseries has been debated for the last few decades. Historically they have been defined as productive areas that provide food and shelter where juveniles are abundant and contribute to the adult population (Beck et al. 2001). However, more recent definitions dispute the importance of food availability, instead favoring predator avoidance (Heupel & Simpfendorfer 2011). Updated criteria require that the abundance of juvenile sharks is higher than in other areas, that juveniles remain in the area for distinct time periods, and that the area is used year after year (Heupel et al. 2007). As populations of large coastal sharks continue to decline, the survival of sharks in their youngest stages is crucial to maintain adult populations (Cortes 2002; Ferretti et al. 2010).

Some shark species are philopatric to their natal nursery environments and exhibit strong site fidelity as juveniles making them vulnerable in urbanized systems (Grubbs et al. 2007; Werry et al. 2012). Coastal development, particularly dredging, near estuaries has shown a significant reduction in the survival of juvenile sharks by reducing the amount of shallow, protected habitats (Jennings et al. 2008; Werry et al. 2012). Habitat degradation and a reduction of food availability in urbanized estuaries may reduce metabolic performance and decrease the growth rates of juvenile sharks (Grubbs et al. 2007; Heupel et al. 2007; Whitfield 2017). Furthermore, through the lower nutritional quality of their prey, the health, growth, and development of sharks living in urban areas are decreased when compared to the same shark species in non-urbanized areas (Rangel et al. 2021). The use of estuaries for recreation also puts juvenile sharks at risk through increased fishing activity that can both target sharks and result in incidental catch (Duncan & Holland 2006; Heupel et al. 2009; Ferretti et al. 2010; Wheeler et al. 2020). Sharks are also continually seen at docks and piers, feeding on discarded fish or interacting with fishers via depredation (Mitchell et al. 2018; Martin et al. 2019). Additionally, increased boat traffic may cause habitat avoidance for certain shark species with greater hearing sensitivity in sound ranges that include frequencies emitted by boat engines (Rider et al. 2021; Mickle & Higgs 2022). While nurseries are presumed to increase juvenile survival and growth rates, urbanized estuaries may neutralize these typical nursery benefits.

The northern coast of South Carolina offers an excellent opportunity to study the effects of urbanization on juvenile sharks in estuarine nursery habitats. Murrells Inlet and North Inlet are two similarly sized estuaries that are geographically close with both

located in Georgetown County, South Carolina (Figure 1), that differ greatly in their level of development. Murrells Inlet is surrounded by residential and commercial development and has high seasonal tourism (2012 visitor spending \$497 million, Salvino $\&$ Wachsman, 2013), whereas North Inlet is part of a National Estuarine Research Reserve and is bordered by only a small residential community in an otherwise undeveloped watershed. Total polycyclic aromatic hydrocarbon (PAH) levels, fecal coliform bacterial levels, and nitrate and phosphate levels were significantly higher in Murrells Inlet than in North Inlet (John Vernberg et al. 1992). Sharks in Murrells Inlet were also shown to have a higher concentration of PAH than sharks caught in North Inlet (Prosser 2004). These contaminants can be attributed to increased runoff and dredging in Murrells Inlet, contributing to a decrease in biomass of prey availability and an increase in predation pressure due to habitat loss (John Vernberg et al. 1992). The differences in shark communities between the two estuaries have also been studied, as well as the importance of North Inlet as a possible nursery for juvenile sharks. Murrells Inlet had a significantly lower abundance and diversity of sharks than North Inlet (Prosser 2004; McDonough 2008). In addition, boat traffic was recorded to measure urbanization between the two systems and there was much more boat traffic in Murrells Inlet (McDonough 2008). Neonate Atlantic Sharpnose Sharks (*Rhizoprionodon terraenovae*) in North Inlet exhibited a greater growth rate than their counterparts in a nearshore ocean location (Maxwell 2008). All previous studies indicate that North Inlet is a viable nursery for young sharks, but Murrells Inlet has not been shown to be as such, despite its similar environmental factors.

Ranging in the Northwestern Atlantic from the Bay of Fundy to the Yucatan Peninsula, *R. terraenovae* are the most common small shark species along the southeastern coast of the United States and in the Gulf of Mexico (Loefer & Sedberry 2003). Young-of-year *R. terraenovae* are frequently encountered in the waters of coastal South Carolina during the summer months (Abel et al. 2007). Pups are born offshore from approximately mid-May to early June every year and recruit to coastal bays for the first few months of life, spending the summer between estuaries and other inshore areas, before migrating offshore during the fall (Loefer & Sedberry 2003; Parsons & Hoffmayer 2005; Heupel et al. 2007; Carlson et al. 2008). Neonates are born at approximately 19-24 cm pre-caudal length (PCL) and grow to approximately 45 cm PCL by age one (Loefer $\&$ Sedberry 2003). Young-of-year *R. terraenovae* diet consists mostly of shrimp, but also small teleosts and mollusks (Bethea et al. 2004). They are highly abundant during the pupping season in North Inlet indicating that it may serve as a primary nursery, though more research is required to say for certain (Abel et al. 2007). The high abundance of this species offers the unique opportunity to investigate the impact of urbanization on the quality of shark nurseries and examine how that may impact growth.

Estuaries are used by humans and sharks alike, and the impact that humans have on these vulnerable ecosystems can immensely affect the survival of young sharks. Previous studies of these two estuaries have shown a difference in the abundance and diversity of large shark communities (Prosser 2004; McDonough 2008). North Inlet has been shown to facilitate better growth for neonate *R. terraenovae* when compared to a nearshore area (Maxwell 2008). However, no study has specifically compared the growth rates of neonate *R. terraenovae* between the two estuaries. Although McDonough (2008)

showed increased boat traffic in Murrells Inlet compared to North Inlet, no studies have quantified the noise pollution within the two systems. My study aims to identify possible effects of urbanization on the health and abundance of neonate sharks in South Carolina estuaries. Comparing the diversity and abundance of fish populations and quantifying noise pollution between the two systems will allow further insight into the effects of urbanization on estuarine shark communities. The following questions will be addressed by this study:

- o Do body condition and growth rate of neonate Atlantic Sharpnose Sharks (*Rhizoprionodon terraenovae*) differ between North Inlet (a protected estuary) and Murrells Inlet (a developed estuary)?
- o Does the abundance and diversity of elasmobranchs or fish caught as bycatch differ between North Inlet and Murrells Inlet?
- o Does the loudness of ambient sound differ between North Inlet and Murrells Inlet?

Methods

Survey Site Description

Murrells Inlet (N33° 31.932', W79° 2.127') and North Inlet (N33° 19.711, W79° 10.0143) are two estuaries located along the northern coast of South Carolina with Murrells Inlet being approximately 32 km north of North Inlet (Figure 1). Both are tidally-dominated, bar-built estuaries and are similar in their physical and hydrological characteristics. North Inlet is slightly larger in area, but both are ocean-dominated high salinity estuaries with low freshwater input and are relatively shallow throughout. Both estuaries have *Sporobolus*-lined tidal creeks and a significant *Crassostrea virginica* reef component which dominates the intertidal zone along the edges of creeks. The most important difference between the two is their level of urbanization.

North Inlet is designated as part of the North Inlet- Winyah Bay National Estuarine Research Reserve and is considered relatively undeveloped and pristine. The watershed is undeveloped except for small residential development on the northern boundary of the estuary. There is no commercial development and access is limited because there are no public boat landings in the immediate watershed. In contrast, Murrells Inlet is located at the southern end of the "Grand Strand" region which is highly developed and has heavy seasonal tourism. Murrells Inlet is surrounded by residential and commercial development, except for Huntington Beach State Park at the southern

boundary of the estuary. It is home to three marinas, two public boat landings, and a variety of watersport rental facilities.

Survey Methods

Field sampling was conducted from May $1st$, 2022 to September $15th$, 2022. Four sampling sites per estuary were selected as a representation of the whole estuary for a total of 8 sites. For North Inlet, sampling occurred in Jones Creek, Old Man Creek, Duck Creek, and Crab Haul Creek. For Murrells Inlet, sampling occurred in Main Creek, Oaks Creek, Whale Creek, and Allston Creek (Figure 2 and Figure 3). One creek was visited per sampling day, randomizing the exact location within the creek for each visit. Sampling days alternated between estuaries and creeks, with an average of 3 sampling days per week. Creeks were sampled evenly with 7 visits per site spread throughout the field season.

Past surveys in North Inlet showed that the catch per unit effort (CPUE) of *R. terraenovae* was not dependent on the tide (Maxwell 2008) therefore surveys took place between 9 am and 12 pm to standardize fishing time. Rod and reel fishing was used to target young-of-year *R. terraenovae*. Fishing rods (n=4; 7 ft long) were each spooled with 30lb line and rigged with a nylon-covered steel leader, 4 oz sinker, and 2/0 circle hook. Hooks were baited primarily with frozen squid, though finger mullet was used when squid was not available. Lines were fished in 5-minute intervals or until an animal was caught. If an animal was caught, that line remained out of the water until the 5 minutes were completed. Lines were cast 10 times each day for a total of 40 casts until June $27th$, 2022 when effort was increased to 12 casts per line for a total of 48 casts per day. During

sampling, boat traffic was recorded as the number of boats that passed by while hooks were in the water. This included both motorized and non-motorized watercraft such as kayaks. If the same boat passed later during the sampling period, it was counted again.

For each sampling day, prior to the start of sampling, GPS coordinates and water depth (m) were recorded at the designated site from a GPS-enabled sonar device (STRIKER Cast GPS, Garmin, Olathe, KS). Additionally, secchi depth (cm), surface and bottom water temperature ($\rm{^{\circ}C}$), salinity (ppt), and dissolved oxygen (mg/L) were measured using a refractometer and a handheld multimeter (ProODO D.O. & Temperature meter, YSI, Yellow Springs, OH). Underwater acoustic readings were taken using a hydrophone and audio recorder (Marine Mammal hydrophone, High Tech Inc, Long Beach, MS; H4n Pro, Zoom, Hauppauge, NY) to measure the ambient soundscape of each sampling site. The hydrophone was deployed approximately 1 m under the surface of the water and an audio recording was taken for 10 minutes prior to the first cast. A second 10 minute audio recording was taken after the final fishing lines were retrieved. Recordings were collected using a WAV format at a 44.1 kHz sampling rate using stereo 16-bit samples. All other recorder settings were kept consistent throughout the sampling season for recordings to be accurately compared.

Shark Capture, Handling, and Processing

Sharks caught were identified to species, sexed, and measured for pre-caudal length (PCL), fork length (FL), total length (TL), and girth (all measurements in cm). The girth was measured around the shark between the pectoral and dorsal fins (Figure 4). Sharks were also weighed (g) with a gravity scale either from the hook if they were small

enough or by guiding a pier net behind the shark while it was in the water and then lifting it to weigh. Rays were only identified to species and sexed. After processing, all individuals were released alive back to the waters they were collected from.

Data Analysis

All statistical analyses were conducted using R (v4.2.1; R Core Team 2022) and RStudio (Posit Team 2022). CPUE was calculated daily to assess the catch abundances of *R. terraenovae* between the two estuaries. It was defined as the number of sharks caught per hook minute (5 minutes x # hooks per day). Average CPUE was calculated for each month, estuary, and creek. A Kruskal-Wallis test was used to determine if CPUE differed by month or by creek for each estuary, with a Dunn's post hoc test conducted for any significant results. The difference in CPUE between estuaries was tested with a Mann-Whitney U test.

For all *R. terraenovae* catches with weight and girth measurements, the weightlength and girth-length relationships were investigated with linear regressions and compared between the two estuaries with ANCOVAs. Body condition of neonate *R. terraenovae* was assessed using Fulton's K values. A value of one indicates a normal body condition whereas less than one is poor and greater than one is considered healthy. Fulton's K uses the following equation:

$$
K = 10^3 * \frac{Weight}{PCL^3}
$$

Fulton's K values between both estuaries were compared with a t-test. The growth rate of *R. terraenovae* was analyzed by plotting length (cm FL) versus day. Since multiple sharks were caught on the same day, lengths for all individuals caught on the same day were averaged and those values were also plotted by day to assess the average growth rate over time. Linear regression was used to investigate the relationships between growth rates for the two estuaries and compared with ANCOVAs. Morphometric observations of *R. terraenovae* between estuaries were compared with Mann-Whitney U tests and t-tests.

Multiple linear regression was used to determine if any of the environmental parameters were predictors for the presence or absence of *R. terraenovae*. Mann-Whitney U tests and t-tests were used to determine if environmental parameters were different between the two estuaries. The diversity of elasmobranch (sharks and rays) and fish bycatch were analyzed using Shannon-Wiener diversity indices and compared between the two estuaries using Hutcheson's t-tests.

Ambient sound was evaluated for all creek sampling sites individually. Each acoustic recording was analyzed with Raven Pro 1.6 (K. Lisa Yang Center for Conservation Bioacoustics at the Cornell Lab of Ornithology, 2023) to measure rootmean-square-pressure (RMS) and average power density. The RMS pressure level measures the average loudness of an audio signal based on amplitude for all frequencies. The average power density measures the average sound power over defined frequency ranges. Only the frequencies within elasmobranch hearing range (25 to 1500Hz; Mickle & Higgs 2022) were used to calculate average power density. RMS pressure level and

average power density were calculated for the entire 10 minute recording. The results of the two recordings from each day at the sampling site were averaged to represent the daily noise levels. RMS and average power density were compared between all creeks with a Kruskal-Wallis test and between the two estuaries with a Mann-Whitney U test (RMS) and t-test (average power density). Multiple linear regression was used to determine if any of the environmental factors influenced noise levels. A Mann-Whitney U test and t-test were used to test if RMS and average power density values were different on days when sharks were caught than on days when they were not caught. Spearman Rank correlation tests were used to further investigate the relationship between depth, boat traffic, and noise levels.

Results

A total of 2,496 hooks were deployed from May 1, 2022 to September 15, 2022 resulting in a total of 12,480 minutes of fishing time. Sampling effort was even between Murrells Inlet and North Inlet (1,248 hooks per estuary, 312 hooks per creek). In total, 517 animals were captured during the study, including- 75 elasmobranchs representing 5 species: Smooth Butterfly Ray (*Gymnura micrura*, *n* = 1*)*, Atlantic Stingray (*Hypanus sabinus*, *n* = 14), Bluntnose Stingray (*Hypanus say*, *n* = 7), Atlantic Sharpnose Shark (*Rhizoprionodon terraenovae*, $n = 52$), and Bonnethead (*Sphyrna tiburo*, $n = 1$) [\(Table 1\)](#page-43-0).

Elasmobranch Catch

In North Inlet 45 neonate *R. terraenovae* were caught (23 females, 20 males). Two sharks escaped before being brought on board, however, they were identified as neonate *R. terraenovae*. No other shark species were caught in North Inlet. Two species of ray were caught in North Inlet: *H. sabinus* (1 female, 5 males, 2 unknown) and *H. say* (4 females, 2 males; [Table 1\)](#page-43-0).

Only seven neonate *R. terraenovae* were caught in Murrells Inlet (3 females, 4 males). One immature female (TL < 80cm) *S. tiburo*, the only other shark species caught throughout my study, was caught in Murrells Inlet. Three species of ray were caught in Murrells Inlet: *G. micrura* (1 male), *H. sabinus* (3 females, 3 males), and *H. say* (1 female; [Table 1\)](#page-43-0).

The first *R. terraenovae* was caught in North Inlet on May 16th and in Murrells Inlet on May 21st; the last *R. terraenovae* was caught in North Inlet on August 24th and in Murrells Inlet on August 18th. The CPUE for *R. terraenovae* of North Inlet was significantly greater than Murrells Inlet (means $= 0.0075$ and 0.0014, respectively, $p =$ 0.027). CPUE for North Inlet was significantly greater in June (0.025) than in any other month (all < 0.006) (χ^2 = 11.422, df = 4, p = 0.022; Figure 5). CPUE for Murrells Inlet in June (0.003) was also greater than the other months (all ≤ 0.002), however, this difference was not statistically significant (χ^2 = 5.8235, df = 4, p = 0.213). Old Man Creek in North Inlet had the greatest CPUE (0.016) and was significantly different when compared to all creeks sampled (all < 0.007) (χ^2 = 14.093, df = 7, p = 0.05; Figure 6).

Three species of elasmobranchs were caught in North Inlet, compared to Murrells Inlet's five species [\(Table 1\)](#page-43-0). However, 59 elasmobranchs were caught in North Inlet and only 16 were caught in Murrells Inlet. Elasmobranchs made up 17.9% of the total catch for North Inlet versus 8.4% of the total catch for Murrells Inlet (Figure 7). The diversity index of elasmobranchs between Murrells Inlet and North Inlet was significantly different with a value of 1.40 for Murrells Inlet and 0.71 for North Inlet ($p = 0.004$).

Secchi depth and surface dissolved oxygen had a weak positive effect on CPUE (*p* $= 0.003$ and $p = 0.002$, respectively), while bottom dissolved oxygen had a weak negative effect on CPUE ($p = 0.009$). No other environmental factors affected CPUE. Environmental factors were not significantly different between the two estuaries except for depth and boat traffic (both *p* < 0.001; [Table 2\)](#page-44-0). Additionally, environmental factors were not different between creeks except for depth and boat traffic ($p = 0.005$ and $p <$

0.001, respectively). Boat traffic was greatest in Main Creek of Murrells Inlet with 61.6 boats on average passing during sampling, all other creeks had less than 6 boats on average pass by during sampling [\(Table 3\)](#page-45-0).

Body Condition and Growth

Mean body condition of neonate *R. terraenovae* throughout the sampling season was not different between North Inlet (Fulton's $K = 0.953$, sd = 0.115) and Murrells Inlet (Fulton's K = 0.966, sd = 0.143, $p = 0.82$; Figure 8) and Fulton's K values were positively affected by date $(R^2 = 0.093, p = 0.05;$ Figure 8). Girth-length relationships and weight-length relationships were calculated for all *R. terraenovae* that had the respective measurements ($n = 49, 42$ respectively). The girth length relationships between the two estuaries were not different $(F = 0.0126, p = 0.91$; Figure 9). Weight-length relationships were not different between the two estuaries ($F = 1$, $p = 0.32$; Figure 10). Length (cm FL) was positively affected by date for both estuaries, but the growth relationships were not different between estuaries ($F = 0.3279$, $p = 0.57$; Figure 11). Average length per day (cm FL) was also positively affected by date for both estuaries (Figure 12). The growth rate between estuaries was not different for average fork length per day (F = 0.0474, *p* = 0.83). Morphometric measurements for *R. terraenovae* were also compared between estuaries and all variables were not statistically significant (Table 4).

Bycatch

Fourteen different species of bony fish represented 85.5% of the total catch during the sampling season (Figure 7). In total, 269 bony fish were caught in North Inlet and 173

in Murrells Inlet [\(Table 5\)](#page-47-0). The most abundant species caught as bycatch was Atlantic Croaker (*Micropogonias undulatus*) comprising 58.5% of the total catch (*n* = 111 for Murrells Inlet, *n* = 192 for North Inlet). Pigfish (*Orthopristis chrysoptera*) and Southern Kingfish (*Menticirrhus americanus*) were the only other bony fish species that made up greater than 5% of the total catch. Species richness was equal for each estuary at 11 species. The catch in each estuary was composed of the same eight species and three unique species. Species unique to North Inlet included Bluefish (*Pomatomus saltatrix*), Gafftopsail Sea Catfish (*Bagre marinus*), and Inshore Lizardfish (*Synodus foetens*). Whereas species unique to Murrells Inlet included Crevalle Jack (*Caranx hippos*), Northern Puffer (*Sphoeroides maculatus*), and Oyster Toadfish (*Opsanus tau*; [Table 5\)](#page-47-0). The diversity of fish species was not significantly different between Murrells Inlet and North Inlet (Shannon-Wiener values: 1.29 and 1.13, respectively; $p = 0.193$).

Noise Pollution

RMS and average power density measurements were not significantly different between estuaries (*p*=0.57, *p*=0.45, respectively). Further, sound measurements did not differ between individual creeks (RMS $p = 0.88$, average power density $p = 0.97$, respectively; Figure 13 and Figure 14). None of the measured environmental factors had a significant impact on RMS or average power density ($p = 0.86$, $p = 0.87$ respectively). The effect of depth and boat traffic on the sound measurements were also not significant (depth and RMS $p = 0.68$, depth and power density $p = 0.95$, boat traffic and RMS $p =$ 0.27, boat traffic and power density $p = 0.52$). Finally, RMS and average power density

did not differ on days when sharks were caught versus days when sharks were not caught $(p = 0.25, p = 0.16$ respectively).

Discussion

This study compared populations of neonate *R. terraenovae* between two similar estuaries with differing levels of urbanization to determine the impact of human development on abundance, body condition, and growth. The results from these two estuaries suggest that urbanization may not have as great of an impact on neonate shark growth in the first few months of life as previously thought. Although body condition and growth patterns did not differ between the impacted estuary and the protected estuary, the abundance of *R. terraenovae* was significantly greater in the protected estuary. This study also found that sound levels did not differ between estuaries and did not impact the relative abundance of *R. terraenovae*. These results suggest that much is still not known about how urbanization affects fish and shark communities in estuaries.

Abundance of R. Terraenovae

The abundance of neonate *R. terraenovae* was significantly greater in North Inlet than in Murrells Inlet despite their similar environmental and physical characteristics and close proximity. The urbanization of Murrells Inlet may have contributed to the lower abundance of neonate *R. terraenovae*. Other environmental factors may also play a role in the difference in the abundance of neonate *R. terraenovae*, but none of the environmental factors measured in this study both impacted CPUE and differed between the estuaries. Previous studies have found abiotic factors such as water temperature, salinity, turbidity, or dissolved oxygen can affect the abundance of sharks (Ulrich et al.

2007; Froeschke et al. 2010; Heupel & Simpfendorfer 2014), however, turbidity (secchi depth) and dissolved oxygen had weak effects on CPUE and were not found to be different between estuaries. Additionally, depth is commonly found to impact CPUE (Tickler et al. 2017), but perhaps the variation of depth in this study was not great enough to show a measurable difference. The driver behind the greater abundance in North Inlet could lie in biological factors such as prey abundance and predation pressures or other physical and environmental conditions that were not measured in this study. High primary productivity supports abundant prey populations, but urbanization and habitat alteration decrease prey availability (Todd et al. 2019). An increase in prey density had a positive impact on the abundance of sharks in reef systems and large coastal sharks (Wirsing et al. 2007; Tickler et al. 2017). As the abundance of fish in Murrells Inlet was lower than in North Inlet, this may have contributed to the disparity in the abundance of neonate *R. terraenovae*. Another potential driver in the difference of abundance may lie at the inlet of each estuary. The inlet of Murrells Inlet is reinforced by jetties while the inlet of North Inlet is natural, with no navigation infrastructure. Human development, such as jetties and dredging, was suggested to hinder the recruitment of larval fish communities in another southeastern estuarine system (Korsman et al. 2017), however, this idea has not been explored for juvenile sharks. Predation risk is commonly cited as a determining factor for habitat selection (Heithaus 2007; Carlson et al. 2008), however previous studies have shown that while predatory sharks are found near the inlet of Murrells Inlet, many other species of large sharks are found in North Inlet, which suggests that neonate *R. terraenovae* are likely exposed to predation risk in both systems (Prosser 2004; McDonough 2008).

The catch rates between the two estuaries were vastly different, but the rates between creeks within each estuary did not differ, indicating that abundance is consistent within similar subtidal creeks. Old Man Creek within North Inlet had a significantly greater CPUE than all other creeks which was also found for Maxwell (2008). As there were no differences in environmental and physical characteristics between Old Man Creek and the other creeks in North Inlet, the driver behind the heightened abundance is still unknown. One possible explanation is that juvenile sharks occasionally aggregate, similar to teleost species, to reduce the risk of predation (Yates et al. 2015). On one occasion during sampling, two neonate *R. terraenovae* were caught concurrently on two different rods, and while being reeled in, an additional neonate shark was swimming alongside the caught sharks, which suggests that neonates may school to reduce predation risk. If schooling is a common strategy for this species, a higher CPUE may indicate that sampling was performed in an opportunistic area instead of discovering a unique preferred habitat. Further investigation into the environmental and physical differences, such as creek bottom structure or water flow rate, between Old Man Creek and the other creeks in North Inlet is needed to better understand the factors that contribute to the greater abundance of neonate sharks.

Neonate catches in these two estuaries followed the typical timing of *R. terraenovae* where the first pups are born mid-May and then recruit to coastal bays for the summer before emigrating offshore in the fall (Loefer & Sedberry 2003; Ulrich et al. 2007; Carlson et al. 2008). Neonate abundance was greatest in June for both estuaries, which coincides with the time of parturition. In early July, the number of neonates caught decreased, possibly due to the death of individuals within the system from predation or

starvation, emigration of individuals from the system, decreased immigration or recruitment into the system, or decreased capture success of individuals. Only a few individuals were caught in August with no catch in September in either estuary. This seasonal abundance trend has been previously observed for neonate *R. terraenovae* within North Inlet (Maxwell 2008) indicating that this is their typical residency. Both estuaries demonstrated similar catch trends: the first neonate in North Inlet was caught only five days before the first catch of the season in Murrells Inlet. The last neonate in North Inlet was caught only six days after the final catch in Murrells Inlet. This phenological similarity indicates that neonates were exhibiting similar movements and population trends within the two systems. This suggests that urbanization does not interrupt the timing of migration for neonate *R. terraenovae* into and out of estuaries in the first year of life.

Body Condition and Growth of R. terraenovae

Body condition and growth of *R. terraenovae* did not differ between North Inlet and Murrells Inlet. Body condition can indicate the foraging success of an individual and their likelihood of survival (Logan et al. 2018). Fulton's K values, on average, for the neonate sharks were slightly below one and the same for both estuaries, suggesting that health and foraging success were not impacted by urbanization. However, body condition at birth may be a function of the health of the mother before the neonates begin developing foraging strategies (Weideli et al. 2019). Additionally, weight-length relationships and girth-length relationships did not differ by estuary. These two estuaries attracted neonates of equal body condition, indicating that urbanization was not an

attractor or deterrent to healthy individuals. However, studies in the Northeastern United States and the Mediterranean observed that the body condition and weight of fish were negatively impacted by urbanization and habitat deterioration (Cavraro et al. 2019; Monteiro Pierce et al. 2020). The body condition of Blacktip Reef Sharks (*Carcharhinus melanopterus*) has been shown to decrease in the first few weeks of life as their energy stores from birth were being used while foraging skills were being developed (Weideli et al. 2019). Neonate *R. terraenovae* in Murrells Inlet and North Inlet displayed a positive trend in Fulton's K values throughout the season indicating an overall increase in body condition. This may be due to healthier individuals surviving throughout the season, with less healthy individuals possibly dying rather than improving body condition over the season.

In general, *R. terraenovae* grow rapidly during the first few years of life (Loefer & Sedberry 2003). When comparing Murrells Inlet and North Inlet, urbanization did not seem to hinder growth during the first year of life when compared to the growth of sharks in a pristine habitat. Catch from mid-May to mid-June consisted of mostly newborn sharks in the size range of length at birth $(19-24 \text{ cm } PCL;$ Loefer & Sedberry, 2003). As the season went on, shark size exceeded the newborn length range confirming that the sharks being caught were older and had been growing throughout the season. As it is believed that most neonate *R. terraenovae* are born around the same time in May, the change in length over time shows a rough estimation of growth throughout the summer. However, it should be noted that these observed growth rates may have been a result of new young-of-year sharks entering the system or moving between systems. The use of tag and recapture methods might have presented a more accurate measure of growth rate.

Though reported recapture rates in North Inlet have been low for neonate *R. terraenovae* (< 3%; Maxwell 2008). Given the limited temporal coverage and relatively small number of individuals collected in this study, it is unlikely that the number of individuals recaptured would have been sufficient to accurately measure growth rates. Growth rate did not differ between estuaries suggesting that neither estuary offered an advantage for growth. Unlike earlier work that found that urbanization caused a decrease in length in fish species (Monteiro Pierce et al. 2020), no impacts on length were observed for neonate sharks in these two estuaries. While no impacts of urbanization were observed for the growth of neonate sharks during the summer of 2022, it may be the case that urbanization poses significant long-term effects that are not detectable in the first few months of life. In addition, elevated levels of toxins have been observed in the waters of Murrells Inlet, causing increased levels of PAH levels in sharks (John Vernberg et al. 1992; Prosser 2004). It is currently unknown how exposure to chemical pollutants in the first stages of growth may affect sharks in the long term.

The growth rates of neonate *R. terraenovae* were similar between Murrells Inlet and North Inlet, yet abundances differed. Lower relative abundance of both neonate sharks and prey populations implies that intraspecific competition is similar between estuaries. Higher competition can limit the growth of juveniles from reduced prey availability, but it seems competition was similar due to the lack of a difference in the growth trends of the captured neonate sharks. The similar movement and abundance trends between estuaries suggest that mortality from predation or starvation was also similar between estuaries, though not directly studied. The lack of predators in Murrells Inlet makes it unlikely that predation is driving the unevenness of abundance. As well as
the similarity in body condition and growth rates makes it unlikely that sharks within Murrells Inlet had higher rates of starvation. It is unlikely that neonates recruit into Murrells Inlet and North Inlet at similar rates given that abundance was greater in North Inlet from the start of the season. This suggests that either fewer neonates are selecting Murrells Inlet, due to unmeasured factors, neonates are not staying within the estuary long enough to be captured, or are not evenly distributed throughout the creeks that were sampled within this study.

Diversity

Abundance of elasmobranchs was greater for North Inlet, but in this study, higher elasmobranch diversity was found in Murrells Inlet. These results contradict previous studies that suggest urbanization negatively impacts species diversity (Vargas-Fonseca et al. 2016; Valenti et al. 2017). The calculated diversity may not be representative of elasmobranch diversity for both systems due to small sample sizes and dominance of catch by *R. terraenovae.* In Murrells Inlet, the species richness increased by two-thirds as a result of only two individuals (*G. micrura* and *S. tiburo*). In both estuaries, elasmobranch catch was dominated by neonate *R. terraenovae*. During the sampling season, suspected Bonnethead (*S. tiburo)* and Lemon Shark (*Negaprion brevirostris*) were sighted swimming in the shallows in North Inlet indicating that rod and reel sampling was insufficient to capture the entire diversity of elasmobranchs present. No other species of shark was caught during sampling in North Inlet during summer 2022, although earlier studies have caught *R. terraenovae*, Bonnethead (*S. tiburo*), Blacktip Shark (*Carcharhinus limbatus*), Blacknose Shark (*Carcharhinus acronotus*), Sandbar Shark

(*Carcharhinus plumbeus*), Finetooth Shark (*Carcharhinus isodon*), and Lemon Shark (*N. brevirostris*) in North Inlet (Yednock 2005; Abel et al. 2007). This suggests that rod and reel catches may not be representative of the full breadth of elasmobranch diversity for either estuary and that the impact of urbanization on elasmobranch diversity is still unclear.

The abundance and diversity of teleost species may provide a better picture of the impact of urbanization on species abundance and diversity between the two estuaries as many more individuals were caught resulting in a larger sample size. North Inlet had approximately 1.5 times more catch than Murrells Inlet. Species richness was the same and the diversity was not different between the two estuaries, though teleost species composition differed. This contrasts the widely held idea that urbanization diminishes biodiversity, but perhaps urbanization limits the abundance of prey populations (Lotze et al. 2006; Todd et al. 2019). The most abundant species in both systems, *M. undulatus*, made up over 50% of the catch in each estuary. However, recent surveys of North Inlet found that Spot (*Leiostomus xanthurus*) was the most abundant demersal fish in the estuary (Allen et al. 2014; Kimball et al. 2020). These studies used a wide variety of sampling techniques to capture the full diversity of North Inlet. The abundance and diversity of a system are highly dependent on methodology. The results from this study, although limited by sampling method, contribute to a better understanding of the similarities and differences between these two estuaries.

Hook and line fishing is a cost-effective method of targeting a species while maintaining a high survival rate after release (Gurshin & Szedlmayer 2004). However,

alternative methodologies may better reflect the community assemblage and its response to the effects of urbanization. Hook and line fishing is limited by hook size, bait, sampling location, and individual fisher's experience level. This study used rod-and-reel fishing with 2/0 circle hooks that were specifically targeting neonate *R. terraenovae.* Hook size limits the size of fish that can interact with the gear as larger hook sizes capture larger fish (Campbell et al. 2014). The methodology of this study may have selected for smaller individuals causing the results from this study to be biased toward smaller shark and ray species. Squid, and occasionally mullet, were used as the bait in this study which may explain why certain species are underrepresented. For instance, Bonnethead prey primarily on crab and may not have pursued the bait during sampling. Additionally, smaller pieces of bait on smaller hooks may select for smaller individuals or species (Campbell et al. 2014). Data collection was conducted by fishers with a range of experience during the field season. Though it was not quantified, multiple catches were lost before being successfully brought to the boat which may have impacted CPUE. Additionally, bait loss was common which can greatly impact CPUE (Henderson et al. 2022). Different methodologies, such as longlines, gill nets, or otter trawls would have produced different results for community composition, but rod-and-reel sampling targeted neonate *R. terraenovae* without impacting survivability.

Anthropogenic Factors

Boat traffic was significantly greater for Main Creek in Murrells Inlet with an average of 61.6 boats passing during sampling but this high level of boat traffic was not found to affect the catch of neonate *R. terraenovae*. Main Creek is the main channel from

the marinas in Murrells Inlet out to the ocean and, as such, it is frequently congested with numerous personal watercraft, recreational boats, large yachts $(> 12m)$, and tour vessels, especially during the summer months. The channel is also where jet ski tours frequently pass and, on a few occasions, circled my boat during sampling. Boat traffic for all other creeks was limited to small fishing watercraft (generally $\leq 8m$ in length). For all creeks in North Inlet, average boat traffic was less than 3.5 boats passing per sampling day, whereas only one creek in Murrells Inlet was below 3.5 boats passing per day. Boat traffic was the largest measurable abiotic difference between the two estuaries in this study. Shark behavior in response to boat traffic remains widely understudied, but the hourly presence of Nurse Sharks (*Ginglymostoma cirratum*) has been observed to decrease with increased boat traffic (Rider et al. 2021). Bottlenose dolphins (*Tursiops* sp.) have been found to increase movement speeds, spend greater time traveling, and less time socializing in response to increased boat traffic (Marley et al. 2017; Kassamali-Fox et al. 2020), which may have consequences on energy budgets. Boat traffic as a deterrent for sharks has not been fully explored and may contribute to the lower abundance of neonate *R. terraenovae* and other sharks in Murrells Inlet. Boat traffic can cause turbulence and disturbance to nearshore zones which may impact juvenile shark behavior. Neonate *R. terraenovae* may respond differently to boat traffic than more mature sharks which may become accustomed to the disturbance (Weilgart 2007; Rider et al. 2021). More studies on the effect of boat traffic and noise pollution on sharks of all age classes are needed to better understand the potential short and long-term impacts in estuaries.

Despite the difference in boat traffic between estuaries, average loudness (RMS) was not affected by urbanization. Shark hearing range is most sensitive to low-frequency

sounds that overlap with the frequencies emitted by boat engines (Mickle & Higgs 2022). When isolated to frequencies of shark hearing, the average power density was not different between creeks, was not affected by boat traffic, and was not different for days when sharks were caught. Sharks in aquarium settings have been shown to avoid certain areas, increase swimming activity, and display less foraging behavior when exposed to levels of high anthropogenic sound (Chapuis et al. 2019; de Vincenzi et al. 2021). A decrease in foraging behavior due to boat activity may have caused a lower CPUE in Murrells Inlet than in North Inlet. If future studies further investigate sound pollution in estuaries, slight alterations to methodology may produce better results. Deployment of the hydrophone independently of the fishing vessel would reduce interference with the boat, fixing the hydrophone to a set depth would minimize the effect of biological and environmental interference, and a longer sound recording may allow for a better representation of noise pollution in the system.

North Inlet as a Nursery

Shark nurseries are defined by many qualities, but the current criteria have three requirements established by Heupel et al. (2007); the abundance of juvenile sharks is greater in the proposed area than in other areas, juvenile sharks remain or return to the area for extended periods such as weeks or months, and the area is used year after year whereas other areas are not. North Inlet satisfies these requirements for neonate *R. terraenovae*. Neonate *R. terraenovae* had a greater density in North Inlet than in a similar nearby area (Murrells Inlet). Neonate *R. terraenovae* have been captured in nearshore areas during the early summer which suggests that some individuals may not exclusively

use estuaries during the first few months of life. Neonates in nearshore areas were only captured from mid-May to mid-July, and PCL was significantly greater within North Inlet compared to the nearshore areas so perhaps neonates were captured during recruitment into estuaries (Maxwell 2008). Recaptures of neonates within North Inlet have shown that they display strong site fidelity to the estuary (Maxwell 2008). In this study, neonate *R. terraenovae* occupied North Inlet from mid-May to mid-August. Finally, the presence of neonate *R. terraenovae* in North Inlet has been consistent across years while Murrells Inlet has not had as consistent *R. terraenovae* presence (Prosser 2004; Yednock 2005; Abel et al. 2007; Maxwell 2008; McDonough 2008). Additionally, interviews with local fishers indicated that neonate *R. terraenovae* presence was not consistent within Murrells Inlet (McDonough 2008). As well as meeting the three criteria defining a shark nursery, North Inlet also has a higher abundance of prey species compared to Murrells Inlet (Smith 2012) and, although not explored directly, estuaries are commonly regarded as a refuge for juvenile sharks due to their heterogeneous landscape.

North Inlet has been suggested as a primary nursery for *R. terraenovae* (Abel et al. 2007; Maxwell 2008) and the results of this current study, which directly compared sharks in proximate, analogous estuaries, confirm that North Inlet has a high likelihood of acting as a primary nursery for *R. terraenovae*. To fully understand the drivers behind North Inlet's higher value as a primary nursery, future studies should attempt to quantify the importance of North Inlet compared to other estuaries and coastal areas throughout the *R. terraenovae* range.

Conclusion

This study aimed to compare neonate *R. terraenovae* growth and abundance between North Inlet, a protected estuary, and Murrells Inlet, a developed estuary. Comparison between an urbanized and protected estuary revealed that the abundance of neonate sharks was the greatest difference between the two estuaries. While no body condition or growth differences were found between the two populations, this study provides new insight that urbanization may not impact neonates as harshly as other age classes and that the effects may be delayed. Additionally, the diversity of species, for both elasmobranchs and fishes, does not seem to be affected by urbanization. Boat traffic remains a clear indication of anthropogenic disturbance for urbanized estuaries, but it does not appear to affect the soundscape. Further research is needed to determine if urbanization may affect the body condition or growth rate of other species or later life stages than the ones studied here to evaluate the full impact of urbanization on these valuable estuarine ecosystems.

Tables

Table 1. Elasmobranch catch abundance (total number) collected during rod and reel sampling in the Murrells Inlet and North Inlet estuaries during May through September 2022.

	Murrells Inlet		North Inlet		
Environmental Factor	Mean	SD	Mean	SD	D
Tide Height (m)	0.623	0.43	0.835	0.50	0.122
Depth (m)	2.15	1.35	3.14	0.99	< 0.001 *
Secchi Depth (cm)	75.0	21.98	72.6	20.03	0.682
Surface Temperature (°C)	26.93	2.02	26.86	1.88	0.649
Bottom Temperature (°C)	26.93	2.01	26.86	1.81	0.823
Salinity (ppt)	34	2.18	33.2	2.36	0.185
Surface DO (mg/L)	5.79	1.07	5.389	1.27	0.092
Bottom DO (mg/L)	5.739	1.09	5.180	1.35	0.204
Boat Traffic	18.6	27.82	1.9	2.03	< 0.001 *
RMS	377.14	242.04	334.26	182.97	0.571
Power Density	59.42	5.74	58.28	5.37	0.451

Table 2. Environmental factor data for Murrells Inlet and North Inlet. Means, standard deviations, and p-values. Significance is denoted by a *.

	Murrells Inlet				North Inlet			
Creek	Allston Creek	Main Creek	Oaks Creek	Whale Creek	Crab Haul Creek	Duck Creek	Jones Creek	Old Man Creek
Boat Traffic	4.86 ± 2.04	$61.57 + 24.10$	$5.29 + 2.36$	2.86 ± 4.67	$1.25 + 1.75$	0.29 ± 0.49	3.29 ± 2.56	3.00 ± 1.29
Depth (m)	$.57 \pm 0.52$	2.91 ± 2.32	2.51 ± 0.81	$.59 \pm 0.53$	3.13 ± 0.93	2.80 ± 0.90	$3.43 + 1.14$	3.21 ± 1.10

Table 3. Environmental data for Boat Traffic and Depth between the creeks of Murrells Inlet and North Inlet. Means ± SD.

Table 5. Abundance (total number) of bycatch fish species collected during rod and reel sampling in the Murrells Inlet and North Inlet estuaries during May through September 2022.

Figures

Figure 1. Map of the study areas in Northeastern South Carolina.

Figure 2. Map of North Inlet with sites labeled and individual sampling sites denoted.

Figure 3. Map of Murrells Inlet with sites labeled and individual sampling sites denoted.

Figure 4. Picture of neonate *Rhizoprionodon terraenovae* with morphometric measurements designated.

Figure 5. Average catch per unit effort (CPUE; with standard error) by month of *R. terraenovae* collected using rod and reel in the Murrells Inlet ($n = 7$, green bars) and North Inlet ($n = 45$, blue bars) estuaries during May through September 2022. No sharks were caught in Murrells Inlet in July and either estuary in September 2022.

Figure 6. Average catch per unit effort (CPUE; with standard error) by creek of *R. terraenovae* collected using rod and reel in the Murrells Inlet ($n = 7$, green bars) and North Inlet (*n* = 45, blue bars) estuaries during May through September 2022.

Figure 7. Percent of total catch using rod and reel in the Murrells Inlet and North Inlet estuaries during May through September 2022. The smaller pie chart represents species that comprised <1% of catch abundance.

Figure 8. Fulton's K values by date for *R. terraenovae* (*n* = 42) using rod and reel in the Murrells Inlet and North Inlet estuaries during May through September 2022. The red line indicates normal body condition. $R^2 = 0.093$.

Figure 9. Regression of girth-length relationship (cm girth to cm FL) for *R. terraenovae* collected in the North Inlet ($n = 45$; $R^2 = 0.8202$) and Murrells Inlet ($n = 7$; $R^2 = 0.9753$) estuaries.

Figure 10. Regression of weight-length relationships (kg weight to cm FL) for *R. terraenovae* collected in the North Inlet ($n = 45$; $R^2 = 0.8846$) and Murrells Inlet ($n = 7$; R^2 = 0.9530) estuaries.

Figure 11. Regression of length (cm FL) by date for *R. terraenovae* collected in the North Inlet ($n = 45$; $R^2 = 0.4789$) and Murrells Inlet ($n = 7$; $R^2 = 0.8503$) estuaries.

Figure 12. Regression of average length by day (cm FL) by date for *R. terraenovae* collected in the North Inlet ($n = 45$; $R^2 = 0.8385$) and Murrells Inlet ($n = 7$; $R^2 = 0.8999$) estuaries.

Figure 13. Average root-mean-square-pressure (RMS) levels (with standard error) by estuary (shadowed columns) and creek for the Murrells Inlet $(n = 28$, green bars) and North Inlet (*n* = 28, blue bars) estuaries during May through September 2022.

Figure 14. Average Power Density (frequencies within shark hearing, 25 to 1500 Hz; with standard error) by estuary (shadowed columns) and creek for the Murrells Inlet (*n* = 28, green bars) and North Inlet (*n* = 28, blue bars) estuaries during May through September 2022.

References

- Abel DC, Young RF, Garwood JA, Travaline MJ, Yednock BK. 2007. Survey of the shark fauna in two South Caroline estuaries and the impact of salinity structure. American Fisheries Society Symposium:109–124.
- Allen DM, Allen WB, Feller RF, Plunket JS, Editors. 2014. Site Profile of the North Inlet - Winyah Bay National Estuarine Research Reserve. Georgetown, SC.
- Beck MW et al. 2001. The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. BioScience **51**:633–641.
- Bethea D, Buckel J, Carlson J. 2004. Foraging ecology of the early life stages of four sympatric shark species. Marine Ecology Progress Series **268**:245–264. Available from http://www.int-res.com/abstracts/meps/v268/p245-264/.
- Booi S, Mishi S, Andersen O. 2022. Ecosystem Services: A Systematic Review of Provisioning and Cultural Ecosystem Services in Estuaries. Sustainability (Switzerland) **14**:1–29.
- Campbell MD, Pollack AG, Driggers WB, Hoffmayer ER. 2014. Estimation of Hook Selectivity of Red Snapper and Vermilion Snapper from Fishery-Independent Surveys of Natural Reefs in the Northern Gulf of Mexico. Marine and Coastal Fisheries **6**:260–273.
- Carlson JK, Heupel MR, Bethea DM, Hollensead LD. 2008. Coastal habitat use and residency of juvenile Atlantic sharpnose sharks (*Rhizoprionodon terraenovae*). Estuaries and Coasts **31**:931–940.
- Cartolano MC, Berenshtein I, Heuer RM, Pasparakis C, Rider M, Hammerschlag N, Paris CB, Grosell M, McDonald MD. 2020. Impacts of a local music festival on fish stress hormone levels and the adjacent underwater soundscape. Environmental Pollution **265**:114925. Elsevier Ltd. Available from https://doi.org/10.1016/j.envpol.2020.114925.
- Cavraro F, Bettoso N, Zucchetta M, D'Aietti A, Faresi L, Franzoi P. 2019. Body condition in fish as a tool to detect the effects of anthropogenic pressures in transitional waters. Aquatic Ecology **53**:21–35. Springer Netherlands. Available from https://doi.org/10.1007/s10452-018-09670-4.
- Chahouri A, Elouahmani N, Ouchene H. 2022. Recent progress in marine noise pollution: A thorough review. Chemosphere **291**:132983. Elsevier Ltd. Available from https://doi.org/10.1016/j.chemosphere.2021.132983.
- Chapuis L et al. 2019. The effect of underwater sounds on shark behaviour. Scientific Reports **9**:6924. Available from https://www.nature.com/articles/s41598-019-43078 w.
- Cortes E. 2002. Incorporating Uncertainty into Demographic Modeling: Application to Shark Populations and Their Conservation. Conservation Biology **16**:1048–1062. Available from http://doi.wiley.com/10.1046/j.1523-1739.2002.00423.x.
- Curtis TH, Parkyn DC, Burgess GH. 2013. Use of Human-Altered Habitats by Bull Sharks in a Florida Nursery Area. Marine and Coastal Fisheries **5**:28–38. Available from http://doi.wiley.com/10.1080/19425120.2012.756438.

de Vincenzi G, Micarelli P, Viola S, Buffa G, Sciacca V, Maccarrone V, Corrias V,

Reinero FR, Giacoma C, Filiciotto F. 2021. Biological Sound vs. Anthropogenic Noise: Assessment of Behavioural Changes in *Scyliorhinus canicula* Exposed to Boats Noise. Animals **11**:174. Available from https://www.mdpi.com/2076- 2615/11/1/174.

- Duncan K, Holland K. 2006. Habitat use, growth rates and dispersal patterns of juvenile scalloped hammerhead sharks *Sphyrna lewini* in a nursery habitat. Marine Ecology Progress Series **312**:211–221. Available from http://www.intres.com/abstracts/meps/v312/p211-221/.
- Ferretti F, Worm B, Britten GL, Heithaus MR, Lotze HK. 2010. Patterns and ecosystem consequences of shark declines in the ocean. Ecology Letters **13**:1055–1071. Available from https://onlinelibrary.wiley.com/doi/10.1111/j.1461- 0248.2010.01489.x.
- Freeman LA, Corbett DR, Fitzgerald AM, Lemley DA, Quigg A, Steppe CN. 2019. Impacts of Urbanization and Development on Estuarine Ecosystems and Water Quality. Estuaries and Coasts **42**:1821–1838. Estuaries and Coasts. Available from http://link.springer.com/10.1007/s12237-019-00597-z.
- Froeschke J, Stunz G, Wildhaber M. 2010. Environmental influences on the occurrence of coastal sharks in estuarine waters. Marine Ecology Progress Series **407**:279–292. Available from http://www.int-res.com/abstracts/meps/v407/p279-292/.
- Grubbs RD, Musick JA, Conrath CL, Romine JG. 2007. Delineation of a Summer Nursery for Juvenile Sandbar Sharks in the Chesapeake Bay Region. American Fisheries Society Symposium **50**:87–107.
- Gurshin CWD, Szedlmayer ST. 2004. Short-term survival and movements of Atlantic sharpnose sharks captured by hook-and-line in the north-east Gulf of Mexico. Journal of Fish Biology **65**:973–986. Available from https://onlinelibrary.wiley.com/doi/10.1111/j.0022-1112.2004.00501.x.
- Heithaus M. 2007. Nursery areas as essential shark habitats: a theoretical perspective. American Fisheries Society Symposium **50**:3–13.
- Henderson AC, Smith C, Bruns S. 2022. Fussy eaters, bait loss and escapees: How reliable are baited-hook assessments of shark abundance in shallow, coastal waters? Journal of Experimental Marine Biology and Ecology **556**:151794. Elsevier B.V. Available from https://doi.org/10.1016/j.jembe.2022.151794.
- Heupel M, Carlson J, Simpfendorfer C. 2007. Shark nursery areas: concepts, definition, characterization and assumptions. Marine Ecology Progress Series **337**:287–297. Available from http://www.int-res.com/abstracts/meps/v337/p287-297/.
- Heupel M, Simpfendorfer C. 2011. Estuarine nursery areas provide a low-mortality environment for young bull sharks *Carcharhinus leucas*. Marine Ecology Progress Series **433**:237–244. Available from http://www.intres.com/abstracts/meps/v433/p237-244/.
- Heupel M, Simpfendorfer C. 2014. Importance of environmental and biological drivers in the presence and space use of a reef‑associated shark. Marine Ecology Progress Series **496**:47–57. Available from http://www.int-res.com/abstracts/meps/v496/p47- 57/.

Heupel MR, Williams AJ, Welch DJ, Ballagh A, Mapstone BD, Carlos G, Davies C,

Simpfendorfer CA. 2009. Effects of fishing on tropical reef associated shark populations on the Great Barrier Reef. Fisheries Research **95**:350–361.

- Jennings DE, Gruber SH, Franks BR, Kessel ST, Robertson AL. 2008. Effects of largescale anthropogenic development on juvenile lemon shark (*Negaprion brevirostris*) populations of Bimini, Bahamas. Environmental Biology of Fishes **83**:369–377.
- John Vernberg F, Vernberg WB, Blood E, Fortner A, Fulton M, McKellar H, Michener W, Scott G, Siewicki T, El Figi K. 1992. Impact of urbanization on high-salinity estuaries in the southeastern United States. Netherlands Journal of Sea Research **30**:239–248. Available from

https://linkinghub.elsevier.com/retrieve/pii/007775799290062J.

- Kassamali-Fox A, Christiansen F, May-Collado LJ, Ramos EA, Kaplin BA. 2020. Tour boats affect the activity patterns of bottlenose dolphins (*Tursiops truncatus*) in Bocas del Toro, Panama. PeerJ **8**:e8804. Available from https://peerj.com/articles/8804.
- Kennish MJ. 2002. Environmental threats and environmental future of estuaries. Environmental Conservation **29**:78–107. Available from https://www.cambridge.org/core/product/identifier/S0376892902000061/type/journa l_article.
- Kimball ME, Allen DM, Kenny PD, Ogburn-Matthews V. 2020. Decadal-Scale Changes in Subtidal Nekton Assemblages in a Warm-Temperate Estuary. Estuaries and Coasts **43**:927–939. Estuaries and Coasts. Available from http://link.springer.com/10.1007/s12237-019-00692-1.
- Korsman BM, Kimball ME, Hernandez FJ. 2017. Spatial and temporal variability in ichthyoplankton communities ingressing through two adjacent inlets along the southeastern US Atlantic coast. Hydrobiologia **795**:219–237. Springer International Publishing. Available from http://link.springer.com/10.1007/s10750-017-3131-5.
- Kunc HP, McLaughlin KE, Schmidt R. 2016. Aquatic noise pollution: implications for individuals, populations, and ecosystems. Proceedings of the Royal Society B: Biological Sciences **283**:20160839. Available from https://royalsocietypublishing.org/doi/10.1098/rspb.2016.0839.
- Loefer JK, Sedberry GR. 2003. Life history of the Atlantic sharpnose shark (*Rhizoprionodon terraenovae*) (Richardson, 1836) off the southeastern United States. Fishery Bulletin **101**:75–88.
- Logan RK, White CF, Winkler C, Jorgensen SJ, O'Sullivan JB, Lowe CG, Lyons K. 2018. An evaluation of body condition and morphometric relationships within southern California juvenile white sharks *Carcharodon carcharias*. Journal of Fish Biology **93**:842–849.
- Lotze HK, Lenihan HS, Bourque BJ, Bradbury RH, Cooke RG, Kay MC, Kidwell SM, Kirby MX, Peterson CH, Jackson JBC. 2006. Depletion, Degradation, and Recovery Potential of Estuaries and Coastal Seas. Science **312**:1806–1809. Available from https://www.science.org/doi/10.1126/science.1128035.
- Marley SA, Salgado Kent CP, Erbe C, Parnum IM. 2017. Effects of vessel traffic and underwater noise on the movement, behaviour and vocalisations of bottlenose dolphins in an urbanised estuary. Scientific Reports **7**:13437. Springer US. Available

from https://www.nature.com/articles/s41598-017-13252-z.

- Martin KL, Abel DC, Crane DP, Hammerschlag N, Burge EJ. 2019. Blacktip shark *Carcharhinus limbatus* presence at fishing piers in South Carolina: association and environmental drivers. Journal of Fish Biology **94**:469–480. Available from https://onlinelibrary.wiley.com/doi/10.1111/jfb.13917.
- Maxwell MK. 2008. Distribution of Neonate Atlantic Sharpnose Sharks, Rhizoprionodon terraenovae, in a South Carolina Estuary and Nearby Waters. Coastal Carolina University. Available from https://digitalcommons.coastal.edu/etd/95.
- McDonough MM. 2008. Comparison of Elasmobranch Fauna in Two South Carolina Estuaries Differing in their Degree of Human Impact. Coastal Carolina University. Available from https://digitalcommons.coastal.edu/etd/96.
- Mickle MF, Higgs DM. 2022. Towards a new understanding of elasmobranch hearing. Marine Biology **169**:1–13. Springer Berlin Heidelberg. Available from https://doi.org/10.1007/s00227-021-03996-8.
- Mitchell JD, McLean DL, Collin SP, Langlois TJ. 2018. Shark depredation in commercial and recreational fisheries. Reviews in Fish Biology and Fisheries **28**:715–748. Springer International Publishing. Available from https://doi.org/10.1007/s11160- 018-9528-z.
- Monteiro Pierce R, Limburg KE, Hanacek D, Valiela I. 2020. Effects of urbanization of coastal watersheds on growth and condition of juvenile alewives in New England. Canadian Journal of Fisheries and Aquatic Sciences **77**:594–601. Available from http://www.nrcresearchpress.com/doi/10.1139/cjfas-2018-0434.
- NOAA. 2023. What percentage of the American population lives near the coast? Available from https://oceanservice.noaa.gov/facts/population.html.
- Parsons GR, Hoffmayer ER. 2005. Seasonal Changes in the Distribution and Relative Abundance of the Atlantic Sharpnose Shark *Rhizoprionodon terraenovae* in the North Central Gulf of Mexico. Copeia **4**:914–920.
- Prosser C. 2004. A Preliminary Delineation of Shark Nursery Grounds in Two South Carolina Estuaries. Duke University. Available from http://dukespace.lib.duke.edu/dspace/handle/10161/246.
- Rangel B de S, Hammerschlag N, Moreira RG. 2021. Urban living influences the nutritional quality of a juvenile shark species. Science of The Total Environment **776**:146025. Elsevier B.V. Available from https://doi.org/10.1016/j.scitotenv.2021.146025.
- Rangel BS, Hammerschlag N, Martinelli LA, Moreira RG. 2022. Effects of urbanization on the nutritional ecology of a highly active coastal shark: Preliminary insights from trophic markers and body condition. Science of The Total Environment **826**:154082. Elsevier B.V. Available from

https://linkinghub.elsevier.com/retrieve/pii/S0048969722011743.

Rider MJ, Kirsebom OS, Gallagher AJ, Staaterman E, Ault JS, Sasso CR, Jackson T, Browder JA, Hammerschlag N. 2021. Space use patterns of sharks in relation to boat activity in an urbanized coastal waterway. Marine Environmental Research **172**:105489. Elsevier Ltd. Available from https://doi.org/10.1016/j.marenvres.2021.105489.

- Salvino R, Wachsman Y. 2013. The Economic Impact of the 29576 Zip Code Murrells Inlet/Garden City Beach, SC. Available from http://murrellsinletsc.com/wpcontent/uploads/2016/09/MI2020-Economic-Impact.pdf.
- Smith CA. 2012. The Comparison of Large Nekton between a Pristine and Urbanized Estuary and between two National Estuarine Research Reserve Systems in South Carolina. Coastal Carolina University.
- Tickler DM, Letessier TB, Koldewey HJ, Meeuwig JJ. 2017. Drivers of abundance and spatial distribution of reef-associated sharks in an isolated atoll reef system. PLoS ONE **12**:1–18. Available from http://dx.doi.org/10.1371/journal.pone.0177374.
- Todd PA, Heery EC, Loke LHL, Thurstan RH, Kotze DJ, Swan C. 2019. Towards an urban marine ecology: characterizing the drivers, patterns and processes of marine ecosystems in coastal cities. Oikos **128**:1215–1242. Available from https://onlinelibrary.wiley.com/doi/10.1111/oik.05946.
- Ulrich GF, Jones C, Driggers WB, Drymon JM, Oakley D, Riley C. 2007. Habitat utilization, relative abundance, and seasonality of sharks in the estuarine and nearshore waters of South Carolina. American Fisheries Society Symposium **50**:125–139.
- Valenti JL, Grothues TM, Able KW. 2017. Estuarine Fish Communities along a Spatial Urbanization Gradient. Journal of Coastal Research **78**:254–268. Available from http://www.bioone.org/doi/10.2112/SI78-017.1.
- Van Dolah RF, Riekerk GHM, Bergquist DC, Felber J, Chestnut DE, Holland AF. 2008. Estuarine habitat quality reflects urbanization at large spatial scales in South

Carolina's coastal zone. Science of The Total Environment **390**:142–154. Available from https://linkinghub.elsevier.com/retrieve/pii/S0048969707010492.

- Vargas-Fonseca E, Olds AD, Gilby BL, Connolly RM, Schoeman DS, Huijbers CM, Hyndes GA, Schlacher TA. 2016. Combined effects of urbanization and connectivity on iconic coastal fishes. Diversity and Distributions **22**:1328–1341. Available from https://onlinelibrary.wiley.com/doi/10.1111/ddi.12495.
- Weideli OC, Bouyoucos IA, Papastamatiou YP, Mescam G, Rummer JL, Planes S. 2019. Same species, different prerequisites: investigating body condition and foraging success in young reef sharks between an atoll and an island system. Scientific Reports **9**:13447. Available from https://www.nature.com/articles/s41598-019- 49761-2.
- Weilgart LS. 2007. The impacts of anthropogenic ocean noise on cetaceans and implications for management. Canadian Journal of Zoology **85**:1091–1116. Available from http://www.nrcresearchpress.com/doi/10.1139/Z07-101.
- Werry JM, Lee SY, Lemckert CJ, Otway NM. 2012. Natural or Artificial? Habitat-Use by the Bull Shark, *Carcharhinus leucas*. PLoS ONE **7**:e49796. Available from https://dx.plos.org/10.1371/journal.pone.0049796.
- Wheeler CR, Gervais CR, Johnson MS, Vance S, Rosa R, Mandelman JW, Rummer JL. 2020. Anthropogenic stressors influence reproduction and development in elasmobranch fishes. Reviews in Fish Biology and Fisheries **30**:373–386. Springer International Publishing. Available from https://doi.org/10.1007/s11160-020-09604- 0.
- Whitfield AK. 2017. The role of seagrass meadows, mangrove forests, salt marshes and reed beds as nursery areas and food sources for fishes in estuaries. Reviews in Fish Biology and Fisheries **27**:75–110. Springer International Publishing. Available from http://link.springer.com/10.1007/s11160-016-9454-x.
- Wirsing AJ, Heithaus MR, Dill LM. 2007. Can measures of prey availability improve our ability to predict the abundance of large marine predators? Oecologia **153**:563–568. Available from http://link.springer.com/10.1007/s00442-007-0769-0.
- Yates PM, Heupel MR, Tobin AJ, Simpfendorfer CA. 2015. Ecological Drivers of Shark Distributions along a Tropical Coastline. PLOS ONE **10**:e0121346. Available from https://dx.plos.org/10.1371/journal.pone.0121346.
- Yednock BK. 2005. Habitat Utilization by Multiple Shark Species in a Southeastern Salt Marsh Estuary. Coastal Carolina University. Available from https://digitalcommons.coastal.edu/etd/105.