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Environmental and Individual Factors Shaping the Habitat Use and Trophic Interactions of Juvenile Bull Sharks (Carcharhinus leucas) in a Subtropical Estuary

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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

ENVIRONMENTAL AND INDIVIDUAL FACTORS SHAPING THE HABITAT USE AND TROPHIC INTERACTIONS OF JUVENILE BULL SHARKS (*CARCHARHINUS LEUCAS*) IN A SUBTROPICAL ESTUARY

A dissertation submitted in partial fulfillment of
the requirements for the degree of
DOCTOR OF PHILOSOPHY
in
BIOLOGY
by
Philip Matich

2014

To: Dean Kenneth G. Furton
College of Arts and Sciences

This dissertation, written by Philip Matich, and entitled Environmental and Individual Factors Shaping the Habitat Use and Trophic Interactions of Juvenile Bull Sharks (*Carcharhinus leucas*) in a Subtropical Estuary, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

Craig Layman

Jennifer Rehage

Philip Stoddard

Joel Trexler

Michael Heithaus, Major Professor

Date of Defense: March 19, 2014

The dissertation of Philip Matich is approved.

Dean Kenneth G. Furton
College of Arts and Sciences

Dean Lakshmi N. Reddi
University Graduate School

Florida International University, 2014

DEDICATION

For my mother and father, Anita and Alex Matich, who have dedicated their lives to helping my sister and I succeed and experience the world around us. And for all of my amazing friends, who inspire me and share in my passion for exploration and creative expression.

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And lastly, I would like to thank my parents. Even though you may not understand what I do professionally or personally, or why I live the way that I do, you have provided me with unconditional love and support. I am eternally grateful for everything you have given me. This dissertation is just as much yours as it is mine. You helped cultivate my creativity, work ethic, and adventurous nature, and my drive for success and achievement. I will continue to make you proud.

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ABSTRACT OF THE DISSERTATION

ENVIRONMENTAL AND INDIVIDUAL FACTORS SHAPING THE HABITAT USE AND TROPHIC INTERACTIONS OF JUVENILE BULL SHARKS (*CARCHARHINUS LEUCAS*) IN A SUBTROPICAL ESTUARY

by

Philip Matich

Florida International University, 2014

Miami, Florida

Professor Michael Heithaus, Major Professor

Top predators serve important roles within their respective ecosystem through top-down and bottom-up effects, yet understanding how these roles vary among individuals within predator populations is still in its early stages. Such individuality can have important implications for the functional roles predators play within their respective ecosystems. Therefore, elucidating the factors that drive persistent individual differences within populations is crucial for understanding how individuals, and in turn populations, will respond to environmental changes and anthropogenic stressors, and the implications of these responses for particular ecological functions. In this dissertation I investigated the movements, residency patterns, and trophic interactions of a juvenile bull shark (*Carcharhinus leucas*) population in a coastal estuary that serves as a nursery. I found that bull sharks undergo ontogenetic niche shifts in their diets and habitat use, with a gradual shift from using freshwater and estuarine resources to marine resources as sharks grew. This behavioral shift appeared to be driven by age-based differences in tradeoffs between safety from predators and availability of prey. Nested within population-level trends in behavior, there was considerable, and consistent, individual variation in both movements and trophic interactions suggesting individual specialization and divergent behavioral tactics within the population. Different behavioral types likely play different roles in food web connectivity and ecosystem dynamics, thus understanding the drivers and importance of phenotypic variability among species will be crucial for improving management strategies and predicting the responses of species and ecosystems to impending changes in environmental conditions and human impacts.

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PREFACE

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CHAPTER II

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CHAPTER III

Matich, P., and Heithaus, M.R. (2012). *Effects of an extreme temperature event on the behavior and age structure of an estuarine top predator (*Carcharhinus leucas*)*. Marine Ecology Progress Series, 447:165-178.

CHAPTER IV

Matich, P., Heithaus, M.R., and Layman, C.A. (2010). *Size-based variation in inter-tissue comparisons of stable carbon and nitrogen isotopic signatures of bull sharks (*Carcharhinus leucas*) and tiger sharks (*Galeocerdo cuvier*)*. Canadian Journal of Fisheries and Aquatic Sciences, 67:877-885.

CHAPTER V

Matich, P., Heithaus, M.R., and Layman, C.A. (2011). *Contrasting patterns of individual specialization and trophic coupling in two marine apex predators*. Journal of Animal Ecology, 80:295-304.

The following chapter has been submitted for review and has been formatted for that publication.

CHAPTER VI

Matich, P., and Heithaus, M.R. *Individual and environmental drivers of nested patterns in habitat use and foraging tactics within a large estuarine predator (*Carcharhinus leucas*)*. Animal Behavior: *in review*.

CHAPTER I

GENERAL INTRODUCTION

Top predators can play important roles in their respective ecosystems through both top-down and bottom-up effects (reviewed in Heithaus et al. 2008). Top-down effects on prey populations and ecosystems, which can occur though both consumptive and non-consumptive effects, have been well-studied in a variety of systems (reviewed by Estes et al. 2011). More recently, it has become apparent that some predators also can affect their ecosystems as vectors for the transport of materials within and across habitat boundaries, which may alter bottom-up effects (reviewed in Rosenblatt et al. 2013). Key to both mechanisms of predator impacts on their ecosystems are patterns of habitat use and foraging behavior, and specific trophic interactions.

While most studies of predators' roles in ecosystems focus on the aggregate effects of whole populations, there can be considerable variation among individuals in their behaviors. This variation may result in differential roles in ecosystems and can alter population and community dynamics (Bolnick et al. 2011). Such individual differences are driven by a variety of factors including differences in age, size, sex, morphology, and personalities (Bolnick et al. 2003). Individual variability has been identified in hundreds of animal species across an array of ecosystem types, yet we still lack a functional understanding of what shapes these individual differences in many systems, and its importance in shaping trophic structure, community dynamics, and ecosystem function. Understanding the frequency and persistence of individual differences is especially important in predator populations because of their continued decline in many ecosystems, and the effects these declines can have on ecosystem structure and function (Estes et al. 2011). Gaining such understanding will provide insight into the importance of phentotypic variability in animal's responses to predicted environmental change and anthropogenic disturbance.

In the present dissertation, I investigate the factors shaping the movements, residency patterns, and trophic interactions - and how these may vary among individuals - of juvenile bull sharks (*Carcharhinus leucas*) in an oligotrophic coastal estuary. Bull sharks are top predators in most of their respective coastal ecosystems, and juveniles typically use coastal estuaries as nurseries (Grubbs 2010). Because of their global distribution, bull sharks have been well-studied in many parts of the world, but most of our understanding stems from studies investigating population-level patterns in life-history (e.g. Curtis et al. 2011, Tillett et al. 2012, Daly et al. 2013), and the physical factors that shape shark occurrence (e.g. Wiley and Simpfendorfer 2007, Ortega et al. 2009, Froeschke et al. 2010). Few studies have investigated the effects of biotic factors (e.g. predation risk, food availability, competition) on bull shark behavior (Castro 1993, Steiner 2007), and no studies have investigated individual differences in juvenile bull shark behavior. The goal of this dissertation is to investigate the biotic and abiotic factors influencing the trophic interactions, behavior, and ecological roles of juvenile bull sharks in a dynamic environment, and to elucidate the potential causes and consequences of individual variability in the population.

I begin in Chapter 2 by investigating population-level movement patterns and trophic interactions of juvenile bull sharks in response to seasonal pulses of resources. I quantify variation in the time spent in different microhabitats within the Shark River Estuary relative to the availability of prey from surrounding marshes using acoustic telemetry. I also compare stable isotope values from different tissues of sharks to make predictions about competing models of temporal shifts in diet composition.

In Chapter 3, I describe population- and individual-level movement patterns of juvenile bull sharks in response to an unpredictable, extreme weather event. I quantify the daily movement patterns of sharks and their use of difference microhabitats before, during, and after the extreme cold weather event using passive acoustic telemetry. I also investigate the short-term effects of the "cold snap" on bull shark abundance and population structure using catch rate data from quantitative longline sampling.

In Chapter 4, I investigate ontogenetic shifts in the diets of juvenile bull sharks using stable isotope analysis, and the speed at which bull sharks transition between relying on catabolism and metabolism for energy after birth. I also investigate the usefulness of different body tissues (e.g. fin, muscle, blood) for studying juvenile bull shark trophic interactions in the Shark River Estuary, and verify estimates of relative turnover rates and discrimination differences of fin, muscle, and whole blood $\delta^{13}C$ and $\delta^{15}N$ for juvenile bull sharks.

In Chapter 5, I take advantage of tissue-specific turnover times of stable isotope values to investigate the levels of individual specialization in the trophic interactions of juvenile bull sharks and another large shark species often thought of as a foraging generalist (tiger sharks; *Galeocerdo cuvier*). I also investigate the potential drivers of individual specialization, including food availability and risk, and the importance geographic overlap in disparate food webs in shaping shark trophic interactions.

In Chapter 6, I describe ontogenetic shifts in the habitat use of juvenile bull sharks using passive acoustic telemetry, and the factors that may shape the ontogenetic niche shift. I investigate individual differences in the movement patterns of sharks nested within the context of the niche shift by quantifying variability in risk taking and periodicity in movements. I also explore whether risk-taking by sharks varies with body condition, age, and sex.

I conclude with Chapter 7, where I discuss the implications of my research for studying the behaviors and roles of top predators in their respective ecosystems. I also discuss the factors that may shape individual variability in animal behavior, and its ecological implications, especially in the face of climate change and continued human disturbance.

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CHAPTER II

MULTI-TISSUE STABLE ISOTOPE ANALYSIS AND ACOUSTIC TELEMETERY REVEAL SEASONAL VARIABILITY IN THE TROPHIC INTERACTIONS OF JUVENILE BULL SHARKS IN A COASTAL ESTUARY

Matich, P., and Heithaus, M.R. (2014). Multi-tissue stable isotope analysis and acoustic telemetry reveal seasonal variability in the trophic interactions of juvenile bull sharks in a coastal estuary. Journal of Animal Ecology, 83:199-213.

Summary

1. Understanding how natural and anthropogenic drivers affect extant food webs is critical to predicting the impacts of climate change and habitat alterations on ecosystem dynamics.
2. In the Florida Everglades, seasonal reductions in freshwater flow and precipitation lead to annual migrations of aquatic taxa from marsh habitats to deep-water refugia in estuaries. The timing and intensity of freshwater reductions, however, will be modified by ongoing ecosystem restoration and predicted climate change.
3. Understanding the importance of seasonally pulsed resources to predators is critical to predicting the impacts of management and climate change on their populations. As with many large predators, however, it is difficult to determine to what extent predators like bull sharks (*Carcharhinus leucas*) in the coastal Everglades make use of prey pulses currently.
4. We used passive acoustic telemetry to determine whether shark movements responded to the pulse of marsh prey. To investigate the possibility that sharks fed on marsh prey, we modeled the predicted dynamics of stable isotope values in bull shark blood and plasma under different assumptions of temporal variability in shark diets and physiological dynamics of tissue turnover and isotopic discrimination.
5. Bull sharks increased their use of upstream channels during the late dry season, and although our previous work shows long-term specialization in the diets of sharks, stable isotope values suggested that some individuals adjusted their diets to take advantage of prey entering the system from the marsh, and as such this may be an important resource for the nursery.

6. Restoration efforts are predicted to increase hydroperiods and marsh water levels, likely shifting the timing, duration, and intensity of prey pulses, which could have negative consequences for the bull shark population and/or induce shifts in behavior.
7. Understanding the factors influencing the propensity to specialize or adopt more flexible trophic interactions will be an important step in fully understanding the ecological role of predators and how ecological roles may vary with environmental and anthropogenic changes.

Introduction

Coastal ecosystems experience considerable daily and seasonal variation in environmental conditions (Lewis 2001; Kennish 2002). Also, they have been, and continue to be, heavily influenced by human activities that have contributed to shifts in community composition and have potentially altered the ecological roles of species (e.g. Cloern 2001; Jackson et al. 2001; Parmesan & Yohe 2003). Within coastal ecosystems, predators serve important roles in controlling prey populations, linking disparate food webs, and transporting biomass and nutrients across habitat boundaries (e.g. Bowen 1997; Darimont, Paquet & Reimchen 2009). Thus, understanding how predators are affected by temporally variable and ephemeral food sources is important for understanding the trophic dynamics of a system. However, this can be challenging, because predators are often highly mobile with relatively large home ranges. Consequently, manipulative studies can be difficult to execute and/or lead to biased results. Data quantifying behavioral variability in response to natural variation in food sources can provide valuable insight in the roles predators play. In addition, there is a growing need to understand how both natural and anthropogenic factors influence variability in trophic interactions to predict how they may affect the ecological roles of species and ultimately ecosystem dynamics.

Seasonal changes in temperature, precipitation, and freshwater flow lead to noticeable variation in the distribution, abundance, and behavior of many resident and migratory species in the Florida Coastal Everglades (Chick, Ruetz & Trexler 2004; Ruetz, Trexler & Jordan 2005; Rehage & Trexler 2006). Therefore, trophic interactions are likely to vary in space and time as predators and prey move to stay within suitable environmental conditions or to take advantage of seasonal pulses of prey. These seasonal pulses of prey occur in the coastal Everglades when water levels in freshwater marshes drop and numerous aquatic taxa are forced into deep-water channels (Rehage & Trexler 2006; Rehage & Loftus 2007). The magnitude and timing of these pulses are likely to be affected by ecosystem restoration. Freshwater flow is predicted to increase through freshwater marshes, likely reducing the duration and intensity of marsh dry-down (Sklar et al. 2001; Perry 2004; CERP 2006), and therefore the magnitude and timing of resource pulses into creeks. Thus, understanding the value of this resource pulse in the trophic ecology of estuarine predators will be important for predicting the consequences of restoration efforts within the ecosystem.

Stable isotope analysis has become a popular method used in ecological studies of food webs to investigate trophic interactions (reviewed by Layman et al. 2012). Because the materials eaten by an animal are not immediately incorporated into its tissues, stable isotope values provide dietary data over a previous timeframe based on the isotopic turnover rate of the sampled tissue(s) (Gannes, O'Brien & Martinez del Rio 1997; Post 2002; Martinez del Rio et al. 2009). This lag time can provide a means to investigate the temporal variability in the diet of an organism by serially sampling parts of metabolically inert tissues or comparing the isotopic values of multiple tissues with different turnover rates (Bearhop et al. 2004).

Metabolically inert tissues, like vibrissae in California sea otters (*Enhydra lutris nereis*), provide dietary information about a particular time period or event in the life of an organism, and sequentially sampling inert tissues can provide a dietary record for an organism over its lifetime (Newsome et al. 2009). Unfortunately, many animals do not have easily accessible tissues that can be used for serial sampling. One alternative to sequentially sampling metabolically inert tissues is to sample metabolically active tissue(s) from animals over multiple time periods and quantify the variability in isotope values over time (Bearhop et al. 2004). This can be an effective method when individuals are easily recaptured (e.g. Drago et al. 2010). But many ecosystems are open and animals can move across habitat boundaries, making it difficult to regularly sample the same individuals over time, and limiting the use of this approach.

A third strategy to investigate temporal change in diets is to compare the isotopic values of metabolically active tissues with significantly different turnover rates collected during one sampling event. A tissue with a fast isotopic turnover rate like blood in Japanese quail (*Coturnix japonica*, $\delta^{13}C$ half-life = 11.4 days), provides information on recent trophic interactions, while a tissue with a slower turnover rate like bone collagen ($\delta^{13}C$ half-life = 173.3 days in *C. japonica*), provides a view of the average trophic interactions over an extended time period (Hobson & Clark 1992). If the isotope values of a fast turnover tissue are compared to the isotope values of a slow turnover tissue, the difference in isotope values can provide insight into the temporal variability of an organism's trophic interactions. Comparing the isotope values of multiple metabolically active tissues, however, must be conducted carefully because the values of stable isotopes in the tissues of a consumer are not identical to that of their food due to isotopic discrimination (Gannes, O'Brien & Martinez del Rio 1997), and different tissues from the same organism can have different discrimination factors (e.g. Vander Zanden & Rasmussen 2001; Sweeting et al. 2007; Buchheister & Latour 2010). Thus, understanding the isotopic discrimination values of the tissues being used is important when investigating temporal variability in trophic interactions.

While stable isotope analysis provides useful information on the trophic interactions of animals, data from complimentary approaches strengthen inferences about the trophic ecology of individuals and populations. Acoustic telemetry, for example, provides data on the movements of tagged animals, and when paired with stable isotope analysis, can provide a powerful tool for elucidating individual- and population-level patterns linking habitat use and diet (e.g. Papastamatiou et al. 2010; Rosenblatt & Heithaus 2011; Speed et al. 2012). Here, we used a combination of long-term, passive acoustic tracking, and stable isotopic analysis and modeling using blood plasma (faster turnover tissue) and whole blood (slower turnover tissue) $\delta^{13}C$ values to investigate whether juvenile bull sharks make use of seasonal prey pulses in the coastal Everglades.

Methods

Study species and system

Bull sharks (*Carcharhinus leucas*; Müller & Henle 1839) inhabit coastal and estuarine waters of the tropics and subtropics around the world, and use coastal estuaries as nurseries during early years before moving into coastal ocean habitats (Wiley & Simpfendorfer 2007, Grubbs 2010). Bull sharks can travel between fresh and marine waters with minimal metabolic costs, and young individuals can be found in salinities ranging from 0.2-41.7 parts per thousand (Anderson et al. 2006; Steiner, Michel & O'Donnell 2007; Heupel & Simpfendorfer 2008). As a result, bull sharks can take advantage of a variety of prey types, including teleosts, crustaceans, cephalopods, and other elasmobranchs in marine, brackish, and freshwater habitats (Snelson & Williams 1981; Snelson, Mulligan & Williams 1984; O'Connell et al. 2007).

The Shark River Estuary of Everglades National Park, Florida, USA (Fig. 1) is primarily a braided stream system lined by mangroves that extends more than 30 km upstream from the Gulf of Mexico. The estuary serves as a nursery for juvenile bull sharks year-round, which are found throughout the entire system (Wiley & Simpfendorfer 2007; Heithaus et al. 2009; Matich & Heithaus 2012). Seasonal changes in precipitation and freshwater flow lead to noticeable variation in the distribution, abundance, and behavior of many resident and migratory species in the Florida Coastal Everglades, including the Shark River Estuary (Chick, Ruetz & Trexler 2004; Ruetz, Trexler & Jordan 2005; Rehage & Trexler 2006). Therefore, trophic interactions vary in space and time annually as predators and prey move to stay within acceptable environmental conditions and/or to take advantage of seasonal pulses of prey. Seasonal pulses of freshwater prey into mangrove-lined creeks in the upstream region of the Shark River Estuary occur when water levels in freshwater marshes drop during the dry season (Rehage & Trexler 2006; Rehage & Loftus 2007; Fig. 1), and teleost predators rely on this prey pulse as an important seasonal component of their diets (Boucek & Rehage *in press*). Our previous work revealed that juvenile bull sharks have relatively high levels of individual dietary specialization in the Shark River Estuary (Matich, Heithaus & Layman 2011). Yet, stable isotope analysis revealed that some individuals (*ca.* 13%) exhibit temporal variability in their trophic interactions, possibly driven by use of this seasonal pulse of marsh prey.

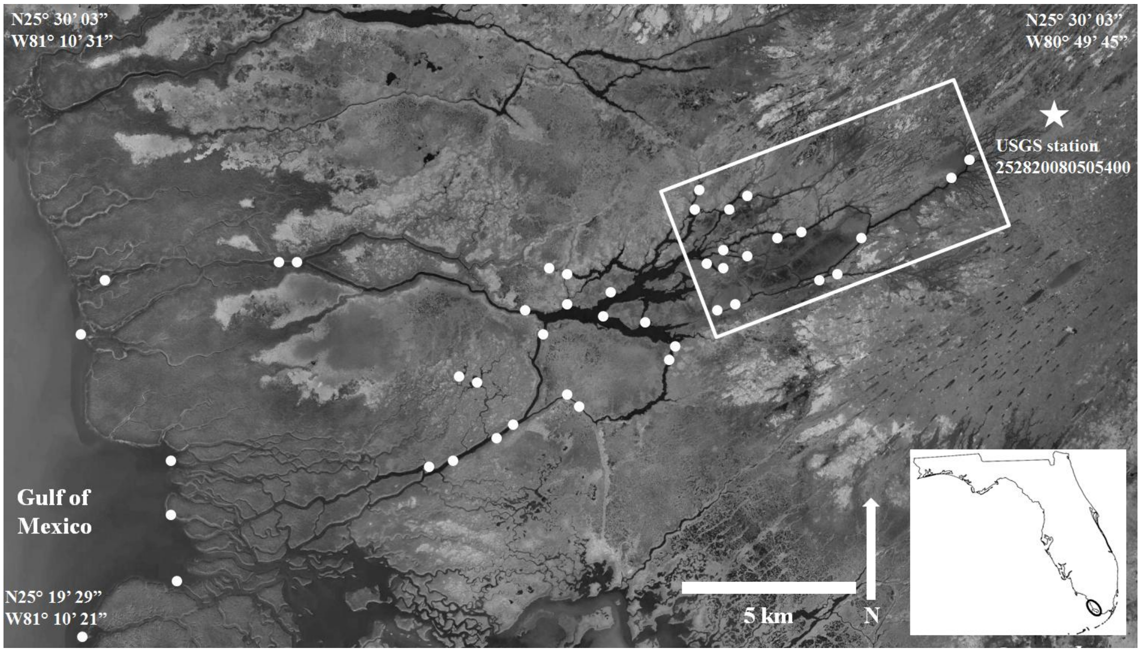

Figure 1: The study occurred in the Shark River Estuary, Florida, USA. The star represents United States Geological Survey water station 252820080505400, which was used to define seasons. Locations of acoustic receivers are indicated by white circles, and the white rectangle encompasses the upstream region where freshwater prey enter the estuary during marsh dry down.

Marsh water levels serve as a seasonal indicator for when taxa migrate from the marsh into deep-water refuges. For the purposes of our analyses, we used water level data from United States Geological Survey water station 252820080505400 Everglades National Park (N25°28'20", W80°50'54"; Fig. 1) adjacent to our study system. When marsh water elevation drops below 10 cm in depth in reference to elevation, the marsh becomes unsuitable for large aquatic taxa (> 8 cm), which are forced to seek out deep-water habitat. As such, the dry season, in reference to water levels, occurs when marsh water elevations are less than 10 cm and the wet season occurs when water levels are greater than 10 cm. These thresholds have been used in studies of movements of Everglades marsh taxa into estuarine creeks (e.g. Chick, Ruetz & Trexler 2004; Rehage & Loftus 2007; Parkos, Ruetz & Trexler 2011). Because the abundance of marsh prey within mangrove-lined creeks changes considerably within seasons (Rehage & Loftus 2007), we further divided each season into sub-seasons (i.e. early and late dry seasons, and early and late wet seasons). During our study, the late wet season of 2008/2009 ended on 29 Feb 2009, and the early dry season was from 1 Mar to 13 Apr 2009. The late dry season was from 14 Apr to 28 May 2009, the early wet season was from 29 May to 16 Oct 2009, and the late wet season began 17 Oct 2009.

Within the confines of the Shark River Estuary, there are two isotopically distinct food webs - freshwater/estuarine ($\delta^{13}C < -25‰$) and marine ($\delta^{13}C > -19‰$; Fry & Smith 2002; Chasar et al. 2005; Williams & Trexler 2006; Fig. 2). Marsh taxa that enter the estuary during the dry season have more depleted $\delta^{13}C$ values (mean ± SE = -30.5 ± 0.5‰) than resident freshwater/estuarine taxa (mean ± SE = -28.0 ± 0.5‰; Matich &

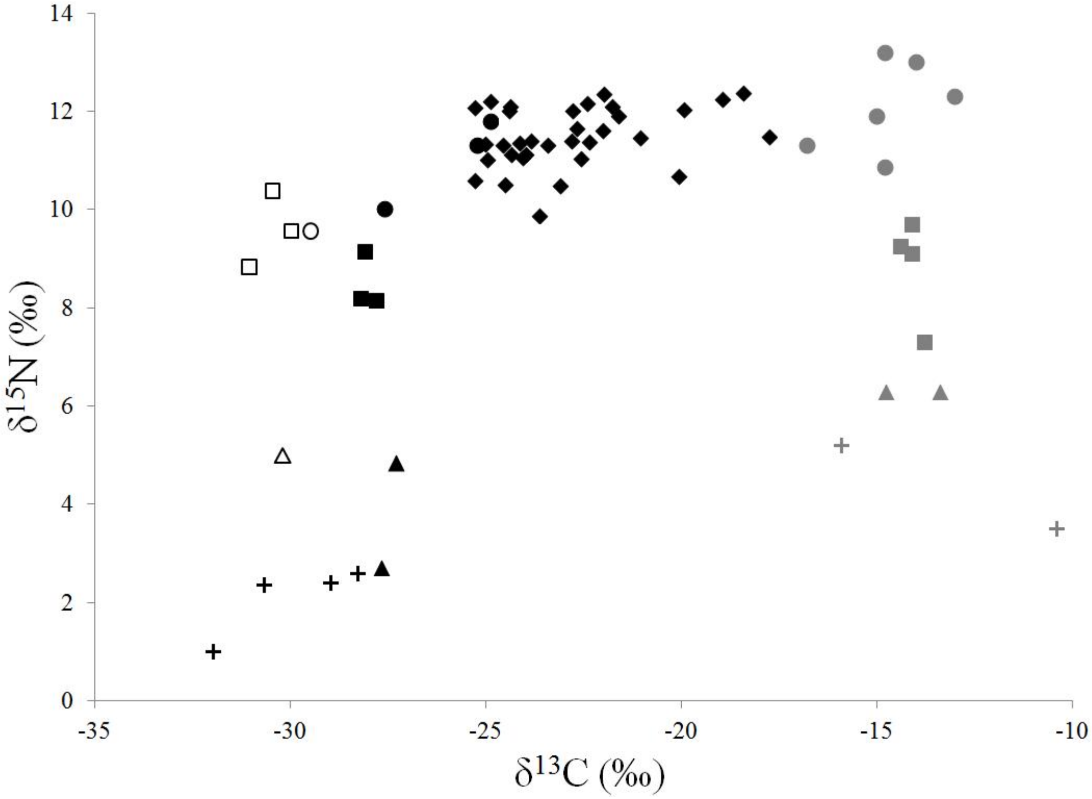

Figure 2: Mean isotope values for producers and consumers in the Shark River Estuary and adjacent marine waters from Fry & Smith (2002), Chasar et al. (2005), Williams & Trexler (2006), and our own sampling. Producers and consumers from the freshwater/estuarine food web are black, those from the marine food web are gray, and migratory marsh taxa are white. Producers are pluses (+), primary consumers are triangles (▲), secondary consumers are squares (■), tertiary consumers are circles (●), and bull sharks (blood isotope values) are diamonds (♦).

Boucek unpublished data). These differences in the δ^{13}C values of potential prey species enabled us to investigate seasonal shifts in bull shark diets between prey with different basal carbon sources, and the potential use of the freshwater prey pulse, by quantifying temporal variability in the δ^{13}C values of bull shark tissues.

Field Sampling

Bull sharks were captured from Oct 2008 to Mar 2012 on ~500 m longlines, fitted with 40-55 14/0 or 15/0 Mustad tuna circle hooks baited with mullet (*Mugil* sp.) and attached to ~2 m of 400 kg monofilament line (see Heithaus et al. 2009 for further details of sampling equipment). Captured sharks were processed alongside the sampling vessel, or within a water-filled, aerated cooler on board. Shark total length was measured to the nearest centimeter. An 18 gauge needle was used to collect 4 mL of blood from the caudal vein. During collection, 3 mL of blood was placed into BD Vacutainer blood collection vials with neither additives nor interior coating, and then immediately separated into its components, including plasma, using a centrifuge spun for one minute at 3000 rpm. The remaining 1 mL of blood was retained in its original composition (whole blood, "blood" hereafter). Based on several lab studies, plasma has an isotopic half-life of ~32 days in elasmobranchs (Kim et al. 2012), and likely serves as a short-term diet indicator for juvenile bull sharks, while blood has an isotopic half-life of ~61 days (MacNeil, Drouillard & Fisk 2006), and likely serves as a longer-term diet indicator for juvenile bull sharks. As such, when more dynamic plasma δ^{13}C values are compared to more stable blood δ^{13}C values they can be used to study short-term changes in the diets of sharks, and provide diet information over the time-frame juvenile bull sharks may respond to the pulse of marsh prey into the Shark River Estuary. Importantly, such inter-tissue comparisons are useful even if tissues do not reach full dietary equilibrium (i.e. four half lives), because they can provide data on the direction (i.e. an increase of decrease in $\delta^{13}C$ depleted prey) and magnitude of dietary change. Tissue samples were put on ice and frozen before laboratory preparations. All samples were dried and homogenized prior to stable isotopic analysis.

Muscle tissue was collected from known estuarine (*Lutjanus griseus*, *Mugil cephalus*) and marsh teleosts (*Lepomis marginatus , L. microlophus, L. punctatus*) that may serve as prey for juvenile bull sharks (based on gape size of sharks, size of teleosts, and stomach contents analysis of juvenile bull sharks in other systems: Snelson & Williams 1981; Snelson, Mulligan & Williams 1984; O'Connell et al. 2007). Samples were collected during ongoing community level surveys using electrofishing, which occurred during the bull shark study (see Rehage & Loftus 2007 for description of sampling method). Samples were frozen until being dried and homogenized in the lab. Stable isotope data from teleosts provided the framework for the difference in $\delta^{13}C$ values of estuarine and freshwater prey for stable isotope diet change models (see below).

Passive acoustic tracking was used to quantify the movement patterns of individual bull sharks to assess their use of upstream areas of the estuary in response to the marsh prey pulse. From Oct 2008 to May 2009, sharks caught in excellent condition (swimming strongly upon capture; n = 23) were surgically fitted with a Vemco V16-4H transmitter (Vemco, Halifax, NS). Transmitters were set to emit a unique series of pulses for each shark at a random interval between 30-90 sec (mean emission interval = 60 sec). Movements of acoustically tagged sharks were tracked within an array of 43 Vemco VR2 and VR2W acoustic receivers (Fig. 1) from Oct 2008 to Nov 2009. In situ measurements revealed mean detection ranges of receivers were ~500 m (see Rosenblatt & Heithaus 2011 for detection ranges of individual receivers). Each receiver was attached to a PVC pipe set in a 10 kg cement anchor. Data from receivers were downloaded every 3-4 months for the duration of the study, and batteries were replaced as needed.

Stable isotope analysis

All shark samples were analyzed at the Florida International University Stable Isotope Facility (29 blood samples and 30 plasma samples) or the Yale Earth System Center for Stable Isotopic Studies (61 blood samples and 60 plasma samples). Lipid extraction was not performed because C:N ratios (mean blood = 2.63 ± 0.25 SD; mean plasma = 2.03 ± 0.26 SD) were below those suggested for extraction or mathematical correction (3.5; Post et al. 2007). To verify analytical consistency, we randomly selected samples to be analyzed at both Florida International University and Yale University, for which the variation between resulting δ^{13}C values and δ^{15}N values were 0.13‰ ± 0.20 SE. The standard deviations of standards run for Yale were 0.14‰ for δ^{13}C and 0.22‰ for δ^{15}N, and 0.29‰ for δ^{13}C and 0.24‰ for δ^{15}N for Florida International. All teleost samples were analyzed at the Florida International University Stable Isotope Facility.

Quantitative Analysis

Acoustic tracking

We quantified the proportion of days each tagged shark was detected in the upstream region based on receiver detections of tagged sharks (Fig. 1). We predicted that if sharks fed from the prey pulse, they would have increased their use of the upstream region where freshwater taxa enter the system early in the dry season, and decreased their use of the upstream region later in the dry season when the abundance of marsh prey decreased (Rehage & Loftus 2007). Sharks were only used for analysis if they were present within the array for > 3 months, and were within the array during the 2009 dry season when the marsh prey pulse was expected to enter the estuary (1 Mar to 28 May). We used a random effects GLMM to test the effect of month on the average proportion of days individual sharks were detected by upstream receivers, with individual as a random effect, and used a Post hoc Tukey's test to test for significant differences across months. We used linear regression to examine the relationship between marsh water level and the proportion of sharks detected per day by upstream receivers during the dry season. Finally, based on the movements of marsh taxa during the dry season, we used t-tests to investigate if there was a significant change in the use of the upstream region by sharks when water elevations were ≤ 0 cm, between 0-5 cm, and between 0-10 cm to develop predictions for our diet change models (see below). Investigating shark habitat use in relation to these water depths allowed us to examine if sharks changed their movement behavior in response to the entry of larger marsh taxa (> 8 cm; enter the estuary from the marsh when water levels drop below 10 cm) or smaller marsh taxa (< 8 cm; enter the estuary from the marsh when water levels drop below 5 cm) (Chick, Ruetz & Trexler 2004; Rehage & Loftus 2007; Trexler & Goss 2009; Parkos, Ruetz & Trexler 2011).

Discrimination difference between blood and plasma

Studies quantifying isotopic discrimination values in sharks are limited. Hussey et al. (2010) investigated discrimination in captive sand tiger (*Carcharias taurus*; n = 3) and lemon sharks (*Negaprion brevirostris*; n = 1), however the only tissue they analyzed that could collected without lethal sampling was muscle tissue, which has a long isotopic half-life (~98 days, MacNeil, Drouillard & Fisk 2006) and was not useful for our study. Both Kim et al. (2012) and Malpica-Cruz et al. (2012) investigated isotopic discrimination in captive leopard sharks (*Triakis semifasciata*; n = 6, n = 16, respectively). Kim et al. (2012) calculated $\Delta^{13}C$ plasma-blood values between 0.5-0.9‰. Despite sampling a variety of tissues, including blood, muscle, and fin tissue, Malpica-Cruz et al. (2012) did not collect plasma, and therefore data from this study was not useful for our analyses.

Tissue-specific incorporation of stable isotopes can be affected by variability in environmental conditions, and can vary between species (reviewed by Vander Zanden & Rasmussen 2001; Crawford, McDonald & Bearhop 2008; Newsome, Clementz & Koch 2010). Therefore, we used linear regression to estimate the inter-tissue discrimination difference between blood and plasma ($\Delta^{13}C$ plasma-blood) in juvenile bull sharks, and compared this to values calculated by Kim et al. (2012). To do so, we plotted paired blood and plasma $\delta^{13}C$ values from 90 juvenile bull sharks against one another, and performed linear regression to quantify the relationship between $\delta^{13}C$ values (see Matich, Heithaus & Layman 2010 for further details). To test whether differences between $\delta^{13}C$ values of blood and plasma varied across the observed range of $\delta^{13}C$ values, we also used a t-test to determine if the slope of the best-fit-line was different from 1:1. We would expect deviation

from a slope of 1:1 if δ^{13}C discrimination varied with δ^{13}C values of shark diets.

Temporal change in diet

In addition to providing an estimate of Δ^{13}C plasma-blood, the regression plot of blood and plasma δ^{13}C values described above also provided data that could be used to investigate dietary variability in sharks. Data points above the best fit line indicate plasma δ^{13}C values more enriched (i.e. less negative) than predicted by the model, and data points below the regression line indicate plasma δ^{13}C values more depleted (i.e. more negative) than predicted by the model (Fig. 3). In elasmobranchs, plasma has a faster isotopic turnover rate (half-life = 32 days, Kim et al. 2012) than blood (half-life = 61 days, MacNeil, Drouillard & Fisk 2006), and therefore dietary changes made by bull sharks should be detected by plasma isotope values considerably faster than blood isotope values. As such, plasma δ^{13}C values more than 0.4‰ enriched above δ^{13}C values of blood (calculated Δ^{13}C plasma-blood - See Results) indicate a recent change to either more marine food web-based diets or a change from feeding on marsh taxa to resident estuarine taxa. Plasma values more depleted than 0.4‰ above blood indicate either a recent change to more estuarine food web-based diets or a change from feeding on resident estuarine taxa to migratory marsh taxa (Fig. 2). Thus, we plotted the residuals from the regression analysis of blood and plasma δ^{13}C against shark capture date [day of year (DOY)] to investigate temporal variability in the diets of bull sharks and to elucidate their potential use of the freshwater prey pulse during the dry season. We then evaluated

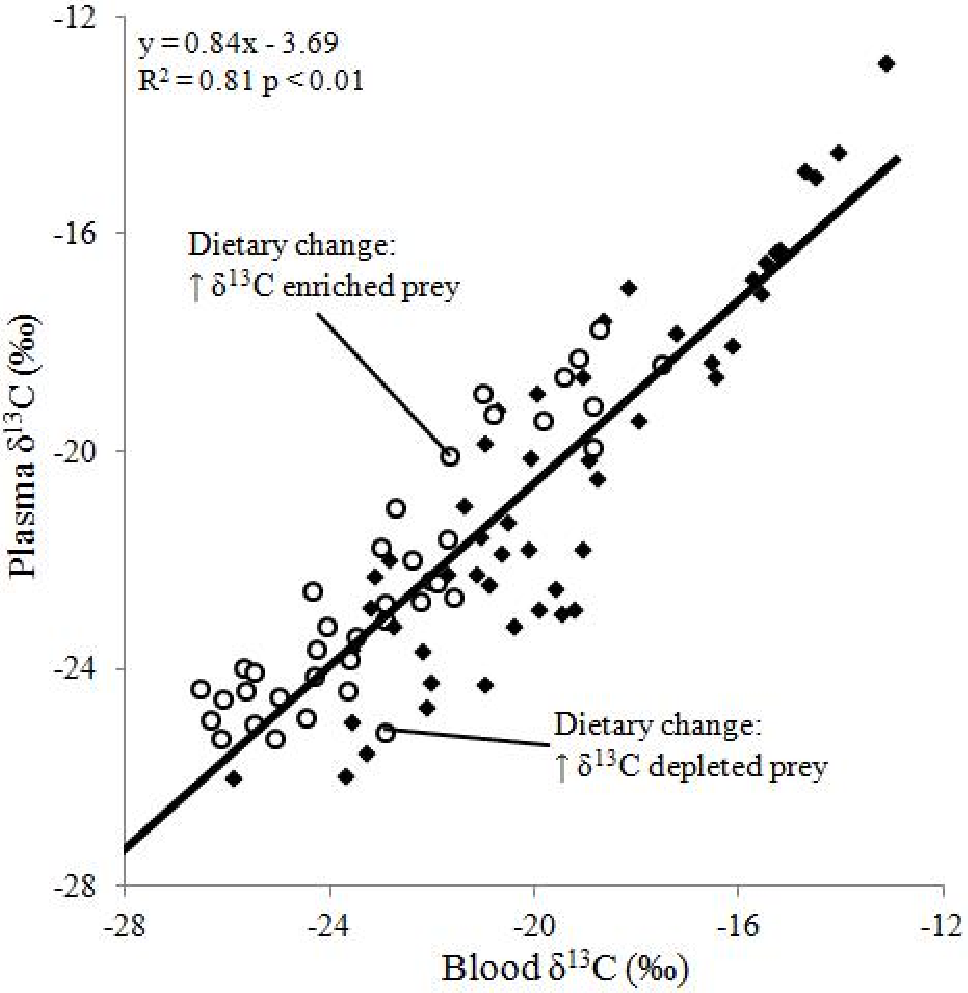

Figure 3: Linear regression of paired blood and plasma $\delta^{13}C$ values. The mean difference between blood and plasma $\delta^{13}C$ values (0.4‰) serves as an approximation for the difference in $\delta^{13}C$ discrimination between blood and plasma in bull sharks. The location of data points relative to the regression line provides insights into whether an individual's diet has become more enriched in $\delta^{13}C$ or more depleted in $\delta^{13}C$ than predicted by differences in discrimination factors of blood and plasma. Open circles are data from sharks caught in 2008-2009, and closed diamonds are data from sharks caught in 2010-2012. Only sharks caught in 2008-2009 were considered for temporal analysis.

the effectiveness of using a piecewise function to describe the data against using the best fit line from linear or non-linear least squares regression (Toms & Lesperance 2003). We selected breakpoints at which $\delta^{13}C$ residuals exhibited a notable change (DOY 128, 150, 163, 175, 213) and quantified the coefficient of determination for each model. We selected the piecewise model with the highest coefficient of determination and compared it to the coefficient of determination for linear and non-linear least squares fits to determine if it was significantly higher in order to choose the best overall model (Fisher 1921). To gain insight into general patterns of bull shark dietary changes in response to the prey pulse, we used ANOVA to test the effect of season on $\delta^{13}C$ residual values. Post hoc Tukey's tests were used to test for significant differences across seasons.

Despite having isotope data from 2008-2012, we only used data from sharks caught from Oct 2008 to Dec 2009 because an extreme weather event in Jan 2010 significantly reduced the number of juvenile bull sharks in the estuary and changed the population structure (Matich & Heithaus 2012). It also possibly affected the community composition, and thereby trophic dynamics, of the ecosystem (Rehage et al. 2010). Additionally, we did not have acoustic tracking data for sharks after 2009 due to this extreme weather event, and therefore could not investigate the correlations between marsh water levels, shark movements, and stable isotope values. Future studies investigating interannual variation in shark trophic interactions and movements, however, would provide additional insights.

Due to the slow isotopic turnover rates of tissues in elasmobranchs (MacNeil, Drouillard & Fisk 2006; Logan & Lutcavage 2010; Kim et al. 2012) and the potential for maternal diets to be reflected in the tissues of newborns sharks (McMeans, Olin & Benz

2009; Matich, Heithaus & Layman 2010; Vaudo, Matich & Heithaus 2010), isotope values of bull sharks may not be indicative of their current diet for individuals less than 90 days old (Belicka et al. 2012). Because bull sharks in the Shark River Estuary are likely born at 60-70 cm TL (based on captures of neonate individuals; Heithaus et al. 2009; Matich & Heithaus 2012) between May and August (based on the presence of umbilical scars; Curtis, Adams & Burgess 2011), and grow 10-20 cm/year (based on recaptured individuals; Neer, Thompson & Carlson 2005), we only included tissues from individuals that were greater than 84 cm total length (at least one year of age) and individuals less than 85 cm TL that were caught between December and April with closed umbilical scars (at least 90 days old).\

To determine if bull sharks changed their diets during the freshwater pulse, we developed a series of theoretical models to predict the differences in plasma and blood $\delta^{13}C$ values to determine if plasma had recently become more enriched or depleted in $\delta^{13}C$ in response to a dietary change (Fig. 4). We modeled six plausible dietary shifts. These included 1) estuarine prey → marsh prey (E→F); 2) marine + estuarine prey → estuarine + marsh prey (M+E→E+F); 3) marine + estuarine prey → marsh prey (M+E→F); 4) marine prey → estuarine prey (M→E); 5) marine prey → estuarine + marsh prey (M→ E+F); and 6) marine prey → marsh prey (M→F) (Fig. 4b; Table 1). We used turnover data from MacNeil, Drouillard & Fisk (2006) (blood half-life = 61 days) and Kim et al. (2012) (plasma half-life = 32 days) to predict the rate of change in blood and plasma isotopes based on the differences in $\delta^{13}C$ values of prey items from different food webs (mean $\delta^{13}C \pm SE$; marine = -14.1 ± 0.2‰, estuarine = -28.0 ± 0.5‰, and marsh = -30.5 ± 0.5‰). The models assumed that sharks would change their diets in

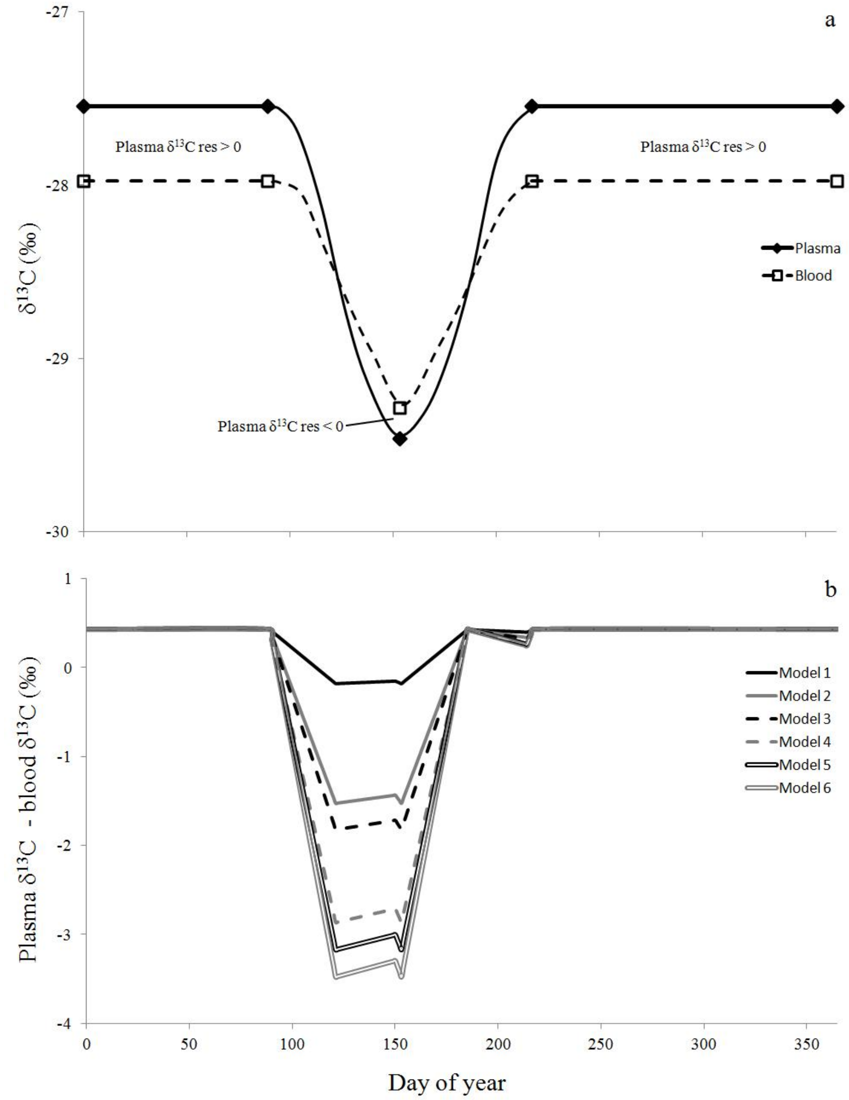

Figure 4: Model predictions for changes in a) $\delta^{13}C$ values of plasma and blood and b) $\delta^{13}C$ residuals if bull sharks switched to using freshwater prey during the dry season. If bull shark diets consist of resident estuarine taxa and are at equilibrium during the wet season, mean blood $\delta^{13}C$ values should be -28.0‰ and mean plasma $\delta^{13}C$ values should be -27.6‰. When marsh taxa enter the estuary during the dry season, if bull sharks switch to feeding on marsh taxa (Model 1), plasma $\delta^{13}C$ values will become more depleted faster than blood $\delta^{13}C$ values because plasma $\delta^{13}C$ turnover (~32 day half-life) is faster in elasmobranchs than blood $\delta^{13}C$ turnover (~ 61 day half-life). In this scenario, differences between plasma and blood $\delta^{13}C$ values are predicted to switch from being positive to negative on DOY 112 and then revert to being positive on DOY 162 after marsh taxa have become depleted and bull sharks return to feeding on estuarine taxa. Note that in b) the inconsistencies at the ends of the diet switch periods (near DOY 148 and 200) are attributed to the different rates of change in plasma and blood $\delta^{13}C$ (plasma approaches it asymptote much sooner than blood).

Model	$\delta^{13}C$ at equilibrium	$\delta^{13}C$ at prey pulse
1 (E→F)	-28.0	-30.5
2 (M+E→E+F)	-21.0	-29.3
3 (M+E→F)	-21.0	-30.5
4 (M→E)	-14.1	-28.0
5 (M→E+F)	-14.1	-29.3
6 (M→F)	-14.1	-30.5

Table 1: Predicted $\delta^{13}C$ values (in ‰) of prey in bull shark diets during periods of dietary equilibrium (wet season) and during dietary change (attributed to the freshwater prey pulse) used to predict $\delta^{13}C$ residuals for the theoretical models. M = marine prey, E = estuarine prey, F = marsh prey entering channels during marsh dry down.

response to marsh prey entering the system, with the timing of the modeled change based on the movements of bull sharks (see Results). Thus, the model assumed that shark diets changed when marsh water levels were ≤ 0 cm, which corresponds to the time when sharks significantly increased their use of the upstream region [31 Mar 2009 (DOY 90)]. A second diet switch, to a diet similar to that before the prey pulse, was modeled to occur when water levels rose above 0 cm [3 Jun 2009 (DOY 154)]. During predicted periods of dietary equilibrium (wet season), we assumed that blood and plasma values would differ by our calculated $\Delta^{13}C$ plasma-blood (0.4‰; see Results).

Differences in $\delta^{15}N$ values of marine, estuarine, and marsh taxa bull sharks may have fed upon (mean $\delta^{13}C$ ± SE; marine = 8.8 ± 0.5‰, estuarine = 9.0 ± 0.5‰, and marsh = 9.1 ± 0.3‰) did not provide the same resolution as differences in $\delta^{13}C$ values for taxa from each food web. Therefore, we only modeled changes in $\delta^{13}C$, rather than both $\delta^{13}C$ and $\delta^{15}N$. Stable isotope mixing models have become a popular analytical tool to investigate the trophic interactions of animals (reviewed by Layman et al. 2012), but mixing models do not provide adequate output to investigate temporal variability in the diets of individuals without repeated sampling, which is often difficult for highly mobile species, such as sharks. Our modeling approach, however, enabled us to quantify variability in the diets of each sampled shark in response to the freshwater prey pulse, and therefore we chose not to employ a mixing model.

Despite recent lab studies quantifying the turnover rates and discrimination values of blood and plasma stable isotopes in elasmobranchs (MacNeil, Drouillard & Fisk 2006; Logan & Lutcavage 2010; Kim et al. 2012; Malpica-Cruz et al. 2012), these processes can vary among similar species (reviewed by Vander Zanden & Rasmussen 2001;

Crawford, McDonald & Bearhop 2008; Newsome, Clementz & Koch 2010). To investigate whether our estimates of discrimination and turnover rates might affect the performance of our models, we tested additional models in which we varied blood and plasma isotopic half-lives and $\Delta^{13}C$ plasma-blood. We created models with the half-lives of blood and plasma decreased to half of published values (31 and 16 days, respectively) and increased to twice published values (122 days and 64 days, respectively; MacNeil, Drouillard & Fisk 2006; Kim et al. 2012) (Fig. 5a). We also created models with $\Delta^{13}C$ plasma-blood of 0.9‰, 0.7‰, and 0.2‰, representing a range of $\Delta^{13}C$ plasma-blood values across the calculated values of Kim et al. (2012) (Fig. 5b). As such, we created six different models (each diet change scenario; see above) for 12 different treatments of isotopic half-life and $\Delta^{13}C$ plasma-blood.

Because a piecewise function best described $\delta^{13}C$ residuals across time (see Results), we used piecewise linear regression with the same breakpoint as the true $\delta^{13}C$ residuals and DOY model (DOY = 169) to investigate the relationship between the predicted difference in $\delta^{13}C$ values (from theoretical models) and DOY. Because regression plots of predicted and true differences in $\delta^{13}C$ values produced best fit lines with the same correlation coefficients and f-values for each diet change model across each isotopic half-life and $\Delta^{13}C$ plasma-blood, we could not use traditional model selection. We therefore compared the best fit lines of the theoretical models to that of the model for true $\delta^{13}C$ residuals and DOY. This approach allowed us to qualitatively select the best model(s) describing if and how bull sharks changed their trophic interactions in response to the prey pulse, and how isotopic half-life and $\Delta^{13}C$ plasma-blood affected model selection. Criteria for qualitatively selecting the best theoretical models included

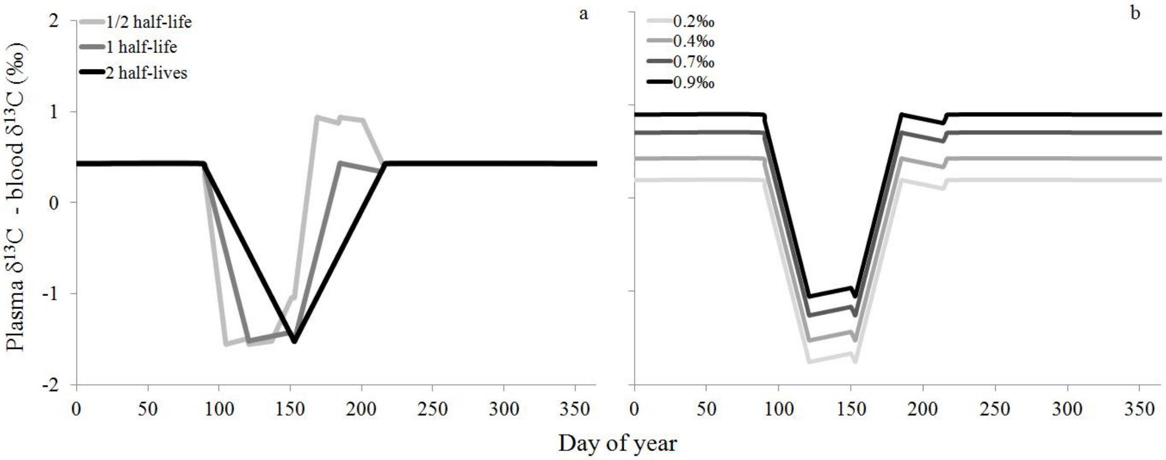

Figure 5: Effects of changing a) isotopic half-life at Δ^{13}C plasma-blood = 0.4‰ and b) Δ^{13}C plasma-blood at one half-life on predictions of diet-change model 2.

1) slopes of the piecewise functions with the same direction (positive or negative) as the model for true $\delta^{13}C$ residuals and DOY; 2) slopes not significantly different from that of true $\delta^{13}C$ residuals and DOY (t-test); and 3) piecewise

functions with the closest mean distance to the true $\delta^{13}C$ residuals and DOY regression lines. ANOVA was used to test the effects of model, isotopic half-life, and $\Delta^{13}C$ plasma-blood on mean distance from the true $\delta^{13}C$ residuals and DOY piecewise function for theoretical models that passed the first two criteria. Post-hoc Tukey's tests were used to test for significant differences across these factors. All statistical analyses were conducted in JMP 6.0.0.

Results

From 2008 to 2012, we captured 90 juvenile bull sharks. Twenty-three individuals between 71-142 cm total length (mean TL ± SD = 102 ± 22 cm) had acoustic transmitters surgically implanted in them, and were tracked from 10 Oct 2008 to 30 Nov 2009 for a total of 5343 tracking days. Three individuals were not present for > 3 months within the system, and therefore were not included in movement analyses. Shark detections by upstream receivers varied by month, and were highest in April and May (DOY 91-151; $R^2 = 0.59$, $p < 0.01$; Fig. 6). During the dry season, there was a negative correlation between shark use of the upstream region and marsh water levels (DOY 60-148; $R^2 = 0.52$, $p < 0.01$; Fig 6), and the proportion of sharks detected in the upstream region was significantly higher when water levels were ≤ 0 cm (mean ± SD = 0.38 ± 0.14) compared to > 0-5 (0.12 ± 0.07) and > 0-10 cm (0.10 ± 0.06) ($t = 6.09$, $p < 0.01$; $t = 8.54$, $p < 0.01$, respectively; Fig. 6).

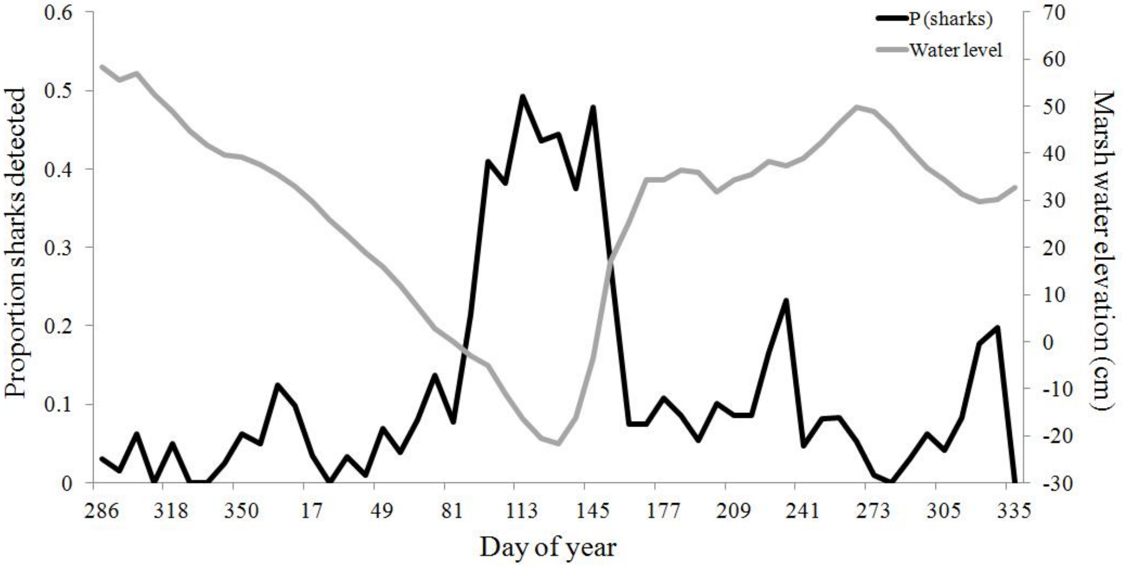

Figure 6: Relationship between marsh water elevation (gray line) at United States Geological Survey water station 252820080505400 and the proportion of sharks detected by upstream receivers per day (black line) from 10 Oct 2008 (DOY 284) to 31 Nov 2009 (DOY 335).

Thirty-nine of the sampled sharks (n = 17 males, 22 females) captured from Oct 2008 to Dec 2009 and ranging in size from 75-182 cm TL (mean TL ± SD = 116.5 ± 28.3 cm) were used to investigate seasonal shifts in shark diets relative to the 2009 pulse of prey from the marsh. Sharks had blood and plasma $\delta^{13}C$ values that ranged from -17.5‰ to -26.5‰ (mean $\delta^{13}C \pm SD = -22.9 \pm 2.4‰$) and -17.8‰ to -25.3‰ (mean $\delta^{13}C \pm SD = -22.4 \pm 2.3‰$), respectively, and blood and plasma $\delta^{15}N$ values that ranged from 10.5‰ to 12.8‰ (mean $\delta^{15}N \pm SD = 11.6 \pm 0.5‰$) and 9.9‰ to 12.4‰ (mean $\delta^{15}N \pm SD = 11.5 \pm 0.6‰$), respectively (Fig. 2).

There was a significant, positive relationship between blood and plasma $\delta^{13}C$ values with a high coefficient of determination (Fig. 3; $R^2 = 0.81$, $p < 0.01$), and the slope of the best fit line was not different from one (slope = 0.84, $t_{(90)} = 0.18$). This suggests the mean difference between blood and plasma $\delta^{13}C$ values (plasma was 0.4‰ greater than blood) was consistent across the $\delta^{13}C$ range of the sampled sharks.

The $\delta^{13}C$ residuals of bull sharks caught in 2008-2009 varied significantly with DOY and capture season. A piecewise function with a breakpoint at DOY 169 was significantly better than a polynomial fit or linear fit for the relationship between DOY and $\delta^{13}C$ residuals (z-score = 5.48, $p < 0.01$; Fig. 7), with the magnitude of the slope for the first section (DOY 0-169) more than three times greater than the magnitude of the second section (DOY 169-365). Mean $\delta^{13}C$ residuals decreased significantly between the early dry season and the late dry season, and then increased from the late dry season to the early wet season (Fig. 8).

Changing the parameters of the models (isotopic half-life and $\Delta^{13}C$ plasma-blood) changed their predictions of $\delta^{13}C$ residuals. As the duration of isotopic half-life increased

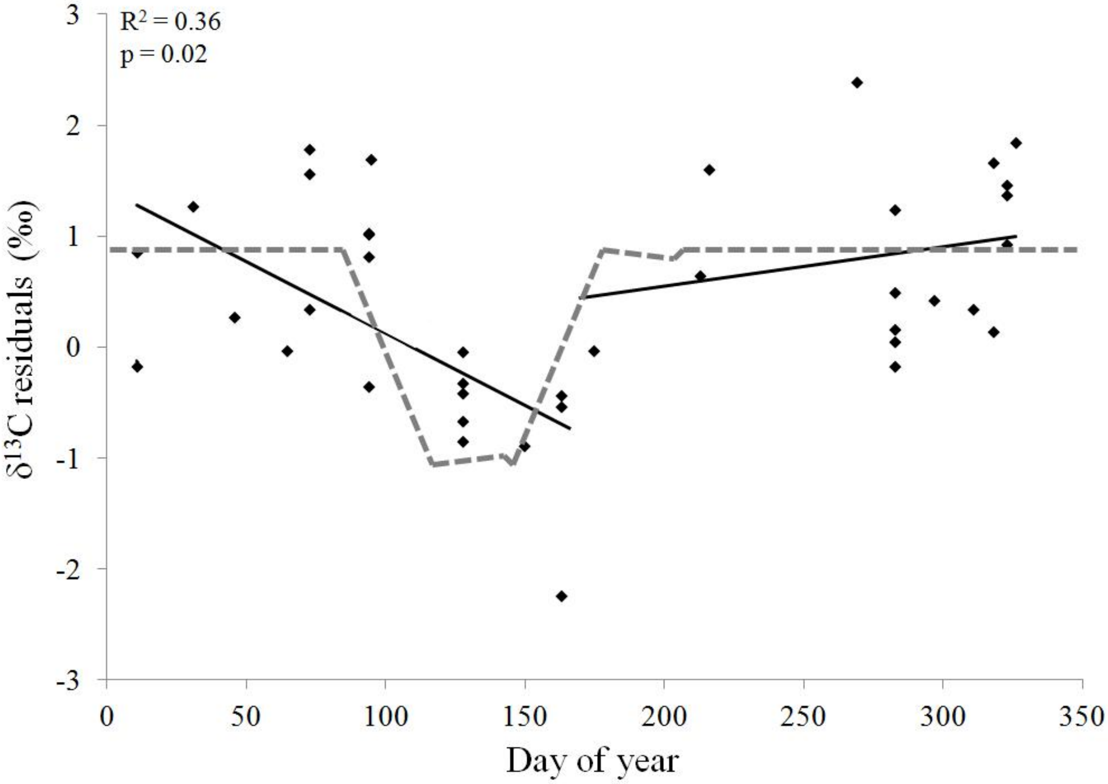

Figure 7: Temporal variation in δ^{13}C residuals. The black lines are predicted residuals based on a piecewise function and the gray dashed line is the predicted change in the difference between plasma and blood δ^{13}C values for model 2 (marine + estuarine prey → estuarine + freshwater prey attributed) at one isotopic half-life and Δ^{13}C plasma-blood = 0.9‰, which was the best model for predicting changes in δ^{13}C residuals. Model selection was not influenced by assumptions about isotopic half life or Δ^{13}C.

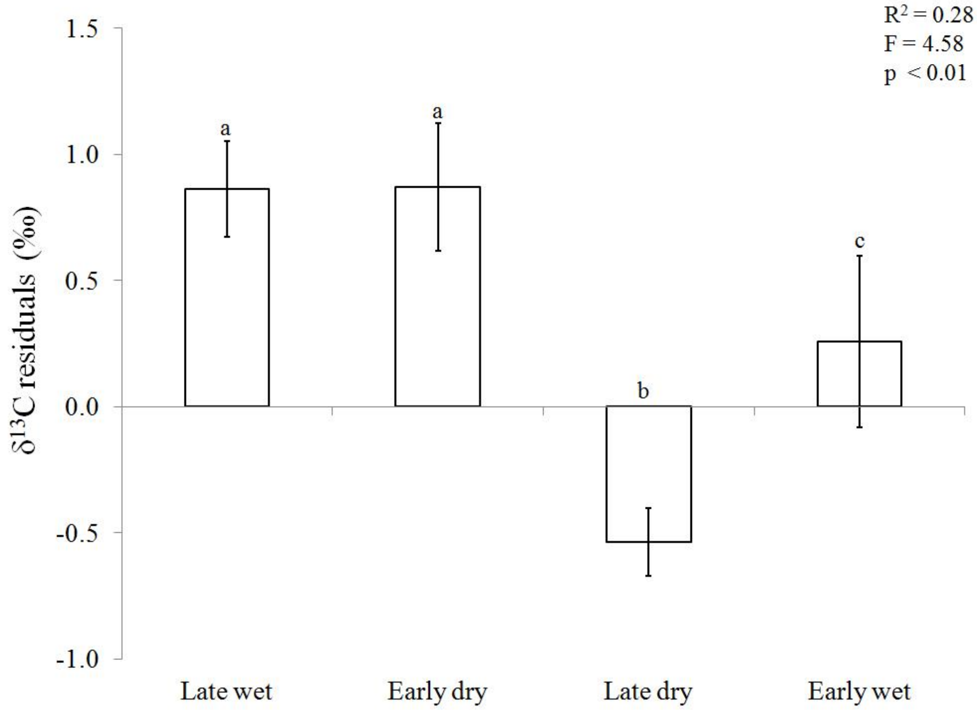

Figure 8: Seasonal variation in mean δ^{13}C residuals. Error bars are ± SE, and bars with different letters are significantly different based on post hoc Tukey's tests.

(i.e. from 0.5 half-lives to 2 half-lives), models predicted an increase in the duration of time $\delta^{13}C$ residuals were in a state of change in response to diet shifts, and as $\Delta^{13}C$ plasma-blood increased (i.e from 0.2-0.9‰), models predicted greater positive $\delta^{13}C$ residuals during non-pulse periods and smaller negative $\delta^{13}C$ residuals during the prey pulse (Fig. 5). ANOVA revealed that model ($F = 10.26$, $p < 0.01$) and $\Delta^{13}C$ plasma-blood ($F = 14.08$, $p < 0.01$) were significant factors explaining variability in mean distance between piecewise functions of theoretical models and the model of true $\delta^{13}C$ residuals and DOY (Appendix 1). Models with $\Delta^{13}C$ plasma-blood = 0.7‰ and 0.9‰ had significantly lower mean distances from the true $\delta^{13}C$ residuals and DOY piecewise function than discrimination differences of 0.2‰ and 0.4‰ (Fig. 9a), and models with $\Delta^{13}C$ plasma-blood = 0.9‰ were the best fit (mean distance ± SE = 0.5 ± 0.1‰, 0.4 ± 0.1‰ for $\Delta^{13}C$ plasma-blood = 0.7‰ and 0.9‰, respectively). Models 2 and 3 (marine + estuarine prey → estuarine + freshwater prey, and marine + estuarine prey → freshwater prey, respectively) had the lowest mean distances from the $\delta^{13}C$ residuals and DOY piecewise function (Fig. 9b), with model 2 having the best fit (distance ± SE = 0.4 ± 0.1‰, 0.5 ± 0.1‰ for model 2 and model 3, respectively). This suggests that model 2 at $\Delta^{13}C$ plasma-blood = 0.9‰ was the best model for predicting changes in $\delta^{13}C$ values of blood and plasma, (Table 2, Appendix 1). Parameter estimates for isotopic half-life did not affect model performance ($F = 0.90$, $p = 0.42$).

Discussion

Seasonal resource pulses are important components of annual energy budgets for many species (reviewed by Otsfeld & Keesing 2000, Yang et al. 2008). For example,

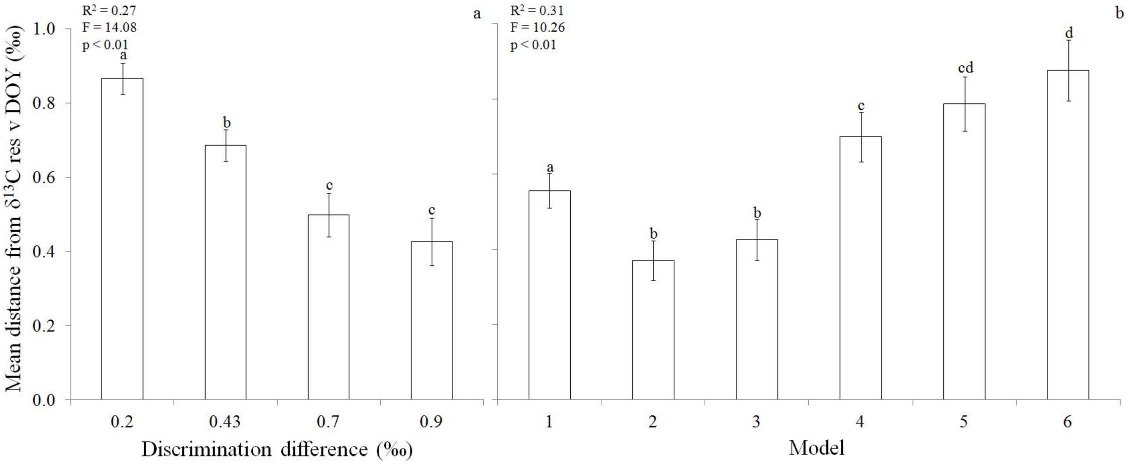

Figure 9: Mean differences between actual $\delta^{13}C$ residuals and those predicted by a) all models at each $\delta^{13}C$ discrimination difference between tissues and b) across each model for all discrimination differences and half-lives**.** Data are means and error bars are ± SE, and bars with different letters are significantly different based on post hoc Tukey's tests.

	δ^{13}C Discrimination difference			
Model	0.2‰	0.4‰	0.7‰	0.9‰
1 (E→F)	0.7 ± <0.1	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
2 (M+E→E+F)	0.7 ± <0.1	0.5 ± <0.1	0.2 ± <0.1	0.1 ± <0.1
3 (M+E→F)	0.8 ± <0.1	0.5 ± <0.1	0.3 ± <0.1	0.2 ± <0.1
4 (M→E)	1.0 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.5 ± 0.2
5 (M→E+F)	1.0 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.6 ± 0.2
6 (M→F)	1.1 ± 0.1	0.9 ± 0.1	0.8 ± 0.2	0.7 ± 0.2

Table 2: Mean distances ± SE (in ‰) between actual δ^{13}C residuals of bull shark blood and plasma isotope values, and those predicted by theoretical models for each tissue-specific discrimination difference between plasma and blood (Δ^{13}C plasma-blood with plasma more enriched for each scenario) to determine the best fit model(s) for sharks' diet change during the freshwater prey pulse. Smaller distances indicate a better fit. M = marine prey, E = estuarine prey, F = marsh prey entering channels during marsh dry down.

brown bears (*Ursus arctos*) in North American Pacific riparian ecosystems rely on predictable annual pulses of spawning salmon to sustain their biomass levels for overwinter hibernation (Naimen et al. 2002, Helfield & Naimen 2006). Along the South African coastline, sardines (*Sardinops sagax*) make annual migrations into nearshore areas and serve as an important pulse of food for marine mammals, birds, bony fishes, and elasmobranchs (Dudley & Cliff 2010; O'Donoghue, Drapeau & Peddemors 2010). Within the Shark River Estuary, the influx of marsh taxa into upstream channels comprises a considerable proportion of the annual energy budget of teleost predators in the ecotone region (e.g. *Amia calva*, Centropomus undecimalis, *Micropterus salmoides*; Rehage & Loftus 2007; Boucek & Rehage *in press*), suggesting this resource pulse is likely a seasonally important component of estuarine food webs within the ecosystem. Our study shows that numerous juvenile bull sharks move upstream to take advantage of this influx of marsh prey, despite relatively high levels of individual specialization within the population found in our previous work (Matich, Heithaus & Layman 2011).

Previously, we found that juvenile bull sharks in the Shark River Estuary show considerable inter-individual variation in trophic interactions, and many individuals (*ca.* 57%) showed relatively high degrees of specialization on one type of resource pool (i.e. marine food webs vs freshwater/estuarine food webs; Matich, Heithaus & Layman 2011). Our results from this study suggest the trophic interactions of some sharks in the estuary (i.e. those identified previously as specialists) are flexible, at least during the dry season when marsh taxa enter the system and provide an additional food source. Blood and plasma δ^{13}C values (mean ± SD = -22.9 ± 2.4‰ and -22.4 ± 2.3‰, respectively) suggest that many bull sharks fed on marine and freshwater/estuarine prey throughout the year, and during the wet and early dry seasons, sharks had $\delta^{13}C$ residuals (plasma-blood) similar to our predictions attributed to $\Delta^{13}C$ plasma-blood (*ca.* 0.3-0.9‰), suggesting they had relatively stable diets. Yet, sharks had significantly lower $\delta^{13}C$ residuals during the late dry season (mean ± SE = -0.5 ± 0.4‰), and model selection predicted diet switches from marine and estuarine prey to estuarine and marsh prey during the marsh prey pulse with a relatively rapid return to the previous diet at the terminus of the prey pulse when marsh prey were depleted, suggesting bull sharks fed on this seasonal resource pulse from freshwater marshes despite many individuals specializing on other resources outside this time period (Matich, Heithaus & Layman 2011).

Individual specialization has been hypothesized as a means to reduce intraspecific competition, especially in ecosystems with limited resources (reviewed by Bolnick et al. 2003). The Shark River Estuary is an oligotrophic system, and limited food abundance may be a driver of individual specialization in juvenile bull sharks (Matich, Heithaus & Layman 2011), however the additional suite of resources from the marsh during the prey pulse may relax intraspecific competition for food. Apparently similar to bull sharks in the Shark River Estuary, specialist bluegill sunfishes (Lepomis macrochirus) were more successful foragers than generalists, but individual specialists exhibited flexibility in their habitat use and switched foraging behaviors when preferred resources became depleted (Werner, Mittlebach & Hall 1981). When preferred prey were scarce, silver perch (*Bidyanus bidyanus*) in aquaculture ponds switched from specializing on *Daphnia* to specializing on calanoids and insects, suggesting individual specialization may be a flexible trait in some populations (Warburton, Retif & Hume 1998). If bull shark trophic specialization is driven by density dependent processes, then the influx of marsh taxa into the system may considerably increase the availability of food, and reduce the need for sharks to have specialized diets when the prey pulse enters the estuary (Svanback & Persson 2004).

Drivers of bull shark behavior

Previous studies in the Everglades suggest that large marsh taxa (> 8 cm TL) vacate the marsh in search of deep water refugia early in the dry season when water levels drop below 10 cm in reference to elevation, and small marsh taxa (< 8 cm TL) enter the estuary later when water levels drop below 5 cm (Rehage & Loftus 2007; Trexler & Goss 2009). Thus, we predicted bull sharks would use the upstream region of the estuary early in the dry season to take advantage of all marsh taxa entering the system. Yet, bull sharks began using upstream areas heavily later than we predicted, which may be due to several reasons. Because many sharks in the estuary are less than three years old (Heithaus et al. 2009; Matich & Heithaus 2012), a lack of foraging experience may hinder their ability to detect when marsh prey are available (e.g. Werner and Giliam 1984). Interannual variation in timing and magnitude of the prey pulse due to variation in precipitation and freshwater flow (Boucek & Rehage *personal communication*), may further reduce the ability of bull sharks to detect the start of this event. Unfortunately, our data set will not currently allow us to test these hypotheses.

Prey preference may also play a role in the timing of the bull sharks' responses to the prey pulse (Lanszki & Sallai 2006; Hawlena & Perez-Mellado 2009). If bull sharks preferred to eat large mesopredators like bass or bowfin, we would have expected them to use the upstream region earlier than observed, and their diets and isotope values would have changed accordingly. Instead, bull sharks did not significantly increase their use of the upstream region of the estuary until marsh water levels dropped below 0 cm, when all aquatic taxa have vacated the marsh. Thus, bull sharks may wait until the overall abundance of marsh taxa of all sizes in the system is relatively high, or they may be targeting smaller prey that arrive in the estuary later. Shark $\delta^{15}N$ values suggest that bull sharks likely targeted smaller prey from the marsh. Plasma and blood $\delta^{15}N$ values of bull sharks caught during the dry season (mean ± SE = 11.8 ± 0.1‰ and 11.9 ± 0.2‰, respectively) were comparable to $\delta^{15}N$ values of muscle tissue of other large aquatic predators like snook (mean ± SE = 11.3 ± 0.3‰) and bass (mean ± SE =10.93 ± 0.14‰) that are known to feed on small marsh taxa. Therefore, bull sharks likely compete for with these large mesopredators for small prey that decline in abundance as the dry season progresses rather than consuming them (Boucek & Rehage *in press*). Comparison of $\delta^{15}N$ values must be made cautiously, however. For example, muscle tissue in elasmobranchs has a slow turnover rate (half-life = 98 days, MacNeil, Drouillard & Fisk 2006), and thus we may not expect to detect large seasonal changes in $\delta^{15}N$ values. Additionally, $\delta^{15}N$ turnover and discrimination rates may vary with diet quality, trophic pathway, metabolic activity, and body size (reviewed by Vander Zanden & Rasmussen 2001; Martinez del Rio et al. 2009; Hussey et al. 2012). Future studies incorporating stomach content analysis and fatty acid analysis should help further elucidate the importance of resource pulses to bull shark diets as well as intraspecific variation in the use of these resources.

Alternative explanations

Alternative explanations are unlikely to account for observed temporal variation in habitat use and $\delta^{13}C$ values of sharks within the estuary. For example, shifts in habitat use by sharks could be driven by upstream movements of preferred prey (e.g. Ford et al. 1998; Rolstad, Loken & Rolstad 2000). Yet, if sharks were feeding on the same prey year round, $\delta^{13}C$ residuals would be expected to remain similar during the year or exhibit longer lag-times if the prey of sharks had moved upstream to feed on the marsh prey pulse (i.e. the time for preferred prey to integrate marsh prey into tissues which would then be integrated into shark tissues).

Increased use of the upstream area by bull sharks when marsh water levels were ≤ 0 cm may have been driven by changes in environmental conditions. Juvenile bull sharks in other estuaries modify their space use in accordance with changes in salinity (e.g. Heupel & Simpfendorfer 2008; Froeschke, Stunz & Wildhaber 2010). Thus, bull sharks may have increased their use of the upstream region of the estuary during the dry season when salinities in areas further downstream increased and became higher than sharks preferred. However, salinity remains relatively low in the upstream region year-round (Heithaus et al. 2009, Rosenblatt & Heithaus 2011) and bull sharks are found in all areas of the estuary in all seasons (Matich & Heithaus 2012), suggesting physical factors are unlikely to be driving the significant increase in the use of the upstream area when marsh water levels are ≤ 0 cm.

Alterations in metabolic processes in response to environmental change can cause variability in stable isotope values (Kelly 2000; McCutchan et al. 2003; Vanderklift & Ponsard 2003). Although bull sharks experience seasonal changes in salinity that may lead to changes in stable isotope values of tissues (Heithaus et al. 2009; Rosenblatt & Heithaus 2011), daily and weekly changes in salinity within the estuary would be expected to buffer a detectable change in isotope values attributed to osmoregulatory processes. Additionally, changing the isotopic half-lives and discrimination differences of our theoretical models did not affect the performance of our models or model selection (models 2 and 3 were the best models for all permutations), suggesting changes in metabolic processes attributed to environmental variability are unlikely to have produced the trends in $\delta^{13}C$ observed during our study. As such, our results do indeed suggest sharks changed their diets during the dry season in response to the prey pulse, which may be a seasonally important source of nutrients and energy as observed in other predators within the system (Boucek & Rehage *in press*).

Conclusions

Stable isotope analysis is an attractive tool for ecologists because it can provide a time-integrated view of trophic interactions (Bearhop et al. 2004). While stable isotopes often provide only course information with regard to prey identity (reviewed by Gannes, O'Brien & Martinez del Rio 1997; Post 2002), employing this tool with complimentary approaches can be used to elucidate patterns and drivers of variability in trophic interactions and make predictions about how future conditions may lead to changes in food webs. Here we used a combination of stable isotope analysis, acoustic telemetry, and predictive modeling to elucidate changes in bull shark behavior in response to a resource pulse of taxa from adjacent marshland. Isotope data suggest sharks increased the proportion marsh prey in their diets during the dry season, and movement data show that bull sharks increased their use of areas adjacent to freshwater marshes during this time. Annual variability in hydrology and planned changes in Everglades water management, however, may affect the importance of marsh taxa in the diets of bull sharks during the dry season.

Restoration efforts are planned to increase freshwater flow through the Everglades (CERP 2006), which will likely affect marsh water elevations (Obeysekera et al. 2011) and, in turn, the onset and duration of this resource pulse and the total biomass entering the Shark River Estuary. Increased freshwater flow and higher water levels in the marsh may lead to increased productivity, but may diminish the magnitude of the resource pulses into estuarine creeks, which could have negative consequences for the bull shark population and/or affect their ecological role within the ecosystem. If marsh taxa are not available within the estuary during the dry season, prey availability within the upper areas of the estuary may decrease and force bull sharks to increase their use of marine resources. This potential dietary shift may lead to decreased survival of young sharks, because downstream areas where marine taxa are most abundant are the riskiest habitats for small sharks to forage in due to high predation risk from large sharks (Heithaus et al. 2009; Matich, Heithaus & Layman 2011). However, this shift in behavior may lead to upstream nutrient transport if juvenile sharks forage in areas where marine taxa are prominent, but rest upstream where large sharks are rarely found (Rosenblatt & Heithaus 2011). Additionally, if marsh taxa are not available to bull sharks, levels of individual specialization may further increase in the face of increased intraspecific competition (Matich, Heithaus & Layman 2011). Understanding how environmental variability currently affects the magnitude and timing of this pulse and the effects it has on aquatic communities is important for making predictions about how changes in freshwater flow may alter slough communities in the Everglades. Using modeling approaches such as the one developed for this study can advance our understanding of temporal variation in trophic interactions, and provide predictions about how changes in the environment should affect food webs. Further research investigating the importance of resource pulses and disturbance regimes on the trophic dynamics of systems should increase our understanding of how predicted environmental changes due to natural and anthropogenic drivers may cause important ecological changes, and affect the role of predators within their respective ecosystems.

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Isotopic half-life	Discrimination difference		Slope part 1	Slope part 2	Distance part 1	Distance part 2
		Residuals v DOY	-0.013	0.004	0	0
0.5	0.2	Model 1	-0.003	-0.001	0.763	NA
		Model 2	-0.009	-0.002	0.694	NA
		Model 3	-0.011	-0.002	0.788	NA
		Model 4	-0.016	-0.003	1.109	NA
		Model 5	-0.017	-0.003	1.203	NA
		Model 6	-0.019	-0.004	1.297	NA
	0.43	Model 1	-0.003	-0.001	0.763	NA
		Model 2	-0.009	-0.002	0.464	NA
		Model 3	-0.011	-0.002	0.558	NA
		Model 4	-0.016	-0.003	0.879	NA
		Model 5	-0.017	-0.003	0.973	NA
		Model 6	-0.019	-0.004	1.067	NA
	0.7	Model 1	-0.003	-0.001	0.763	NA
		Model 2	-0.009	-0.002	0.272	NA
		Model 3	-0.011	-0.002	0.288	NA
		Model 4	-0.016	-0.003	0.609	NA
		Model 5	-0.017	-0.003	0.703	NA
		Model 6	-0.019	-0.004	0.797	NA
	0.9	Model 1	-0.003	-0.001	0.763	NA
		Model 2	-0.009	-0.002	0.272	NA
		Model 3	-0.011	-0.002	0.161	NA
		Model 4	-0.016	-0.003	0.409	NA
		Model 5	-0.017	-0.003	0.503	NA
		Model 6	-0.019	-0.004	0.597	NA
1	0.2	Model 1	-0.005	0.001	0.639	0.570
		Model 2	-0.015	0.002	0.725	0.654
		Model 3	-0.017	0.003	0.824	0.674
		Model 4	-0.025	0.004	1.162	0.739
		Model 5	-0.027	0.004	1.260	0.758
		Model 6	-0.029	0.005	1.359	0.777
	0.43	Model 1	-0.005	0.001	0.639	0.566
		Model 2	-0.015	0.002	0.495	0.424
		Model 3	-0.017	0.003	0.594	0.444
		Model 4	-0.025	0.004	0.932	0.509
		Model 5	-0.027	0.004	1.062	0.528
		Model 6	-0.029	0.005	1.235	0.547
	0.7	Model 1	-0.005	0.001	0.639	0.214
		Model 2	-0.015	0.002	0.225	0.154

		Model 3	-0.017	0.003	0.324	0.174
		Model 4	-0.025	0.004	0.890	0.239
		Model 5	-0.027	0.004	1.062	0.258
		Model 6	-0.029	0.005	1.235	0.277
	0.9	Model 1	-0.005	0.001	0.639	0.214
		Model 2	-0.015	0.002	0.125	0.090
		Model 3	-0.017	0.003	0.298	0.062
		Model 4	-0.025	0.004	0.890	0.039
		Model 5	-0.027	0.004	1.062	0.063
		Model 6	-0.029	0.005	1.235	0.091
2	0.2	Model 1	-0.004	0.001	0.666	0.608
		Model 2	-0.013	0.005	0.574	0.777
		Model 3	-0.016	0.006	0.649	0.815
		Model 4	-0.023	0.008	0.906	0.945
		Model 5	-0.025	0.009	0.981	0.983
		Model 6	-0.027	0.010	1.059	1.021
	0.43	Model 1	-0.004	0.001	0.666	0.378
		Model 2	-0.013	0.005	0.344	0.547
		Model 3	-0.016	0.006	0.419	0.585
		Model 4	-0.023	0.008	0.741	0.715
		Model 5	-0.025	0.009	0.900	0.753
		Model 6	-0.027	0.010	1.059	0.791
	0.7	Model 1	-0.004	0.001	0.666	0.157
		Model 2	-0.013	0.005	0.074	0.277
		Model 3	-0.016	0.006	0.196	0.315
		Model 4	-0.023	0.008	0.741	0.445
		Model 5	-0.025	0.009	0.900	0.483
		Model 6	-0.027	0.010	1.059	0.521
	0.9	Model 1	-0.004	0.001	0.666	0.157
		Model 2	-0.013	0.005	0.126	0.092
		Model 3	-0.016	0.006	0.196	0.149
		Model 4	-0.023	0.008	0.741	0.342
		Model 5	-0.025	0.009	0.900	0.398
		Model 6	-0.027	0.010	1.059	0.455

Appendix 1: Slopes of piecewise models for all theoretical models and distances from the piecewise model for δ^{13}C residuals and DOY. The distance from δ^{13}C residuals and DOY for all revised models at a half-life of 0.5 for the second part of the piecewise function are not included because the slopes of the lines were the opposite sign of δ^{13}C residuals and DOY.

CHAPTER III

EFFECTS OF AN EXTREME TEMPERATURE EVENT ON THE BEHAVIOR AND AGE STRUCTURE OF AN ESTUARINE TOP PREDATOR (*CARCHARHINUS LEUCAS*)

Matich, P., and Heithaus, M.R. (2012). Effects of an extreme temperature event on the behavior and age structure of an estuarine top predator (*Carcharhinus leucas*). Marine Ecology Progress Series, 447:165-178.

Abstract

The frequency of extreme environmental events is predicted to increase in the future. Understanding the short- and long-term impacts of these extreme events on large-bodied predators will provide insight into the spatial and temporal scales at which acute environmental disturbances in top-down processes may persist within and across ecosystems. Here, we use long-term studies of movements and age structure of an estuarine top predator - juvenile bull sharks - to identify the effects of an extreme 'cold snap' from 2-13 Jan 2010 over short (weeks) to intermediate (months) time scales. Juvenile bull sharks are typically year-round residents of the Shark River Estuary until they reach 3-5 years of age. However, acoustic telemetry revealed that almost all sharks either permanently left the system or died during the cold snap. For 116 days after the cold snap, no sharks were detected in the system with telemetry, or were captured during longline sampling. Once sharks returned, both the size structure and abundance of the individuals present in the nursery had changed considerably. During 2010, individual longlines were 70% less likely to capture any sharks, and catch rates on successful longlines were 40% lower than during 2006-2009. Also, all sharks caught after the cold snap were young-of-the-year or neonates, suggesting that the majority of sharks in the estuary were new recruits and several cohorts had been largely lost from the nursery. The longer-term impacts of this change in bull shark abundance to the trophic dynamics of the estuary, and the importance of episodic disturbances to bull shark population dynamics will require continued monitoring, but are of considerable interest due to the ecological roles of bull sharks within coastal estuaries and oceans.

Introduction

Many ecosystems experience predictable disturbances in their physical environment, and these shifts in conditions can be important in structuring and/or restructuring communities (e.g. Doan 2004, Tabacchi et al. 2009, Tyler 2010). Less attention has been given to the impacts of unpredictable extreme environmental events on ecosystem dynamics (Turner 2010). However, these acute events may also be important in shaping communities, and their effects can be widespread and long-lasting (e.g. Mulholland et al. 2009, Byrnes et al. 2011, Foster et al. 2011). Gaining an understanding of extreme weather events is important because their frequency is expected to increase in the future (Easterling et al. 2000, Meehl et al. 2000, IPCC 2007).

Acute changes in environmental conditions generally require a rapid behavioral response from animals, and in the case of extreme events, individuals may not have previously encountered such conditions and populations may not have adapted to cope with them physiologically. Thus, rapid and extreme changes can lead to both short- and long-term alterations in the size and structure of populations (e.g. Gabbert et al. 1999, Chan et al. 2005, Daufresne et al. 2007). These shifts in population density and structure can lead to considerable shifts in the habitat use, trophic and social interactions, and resource use of both individuals and populations after extreme events (e.g. Frederick & Loftus 1993, Frederiksen et al. 2008, Lea et al. 2009). In turn, these changes in populations and behaviors can be transmitted through communities and ultimately affect ecosystem stability (e.g. Bennets et al. 2002, Thibault & Brown 2008, Mantzouni & MacKenzie 2010).

Bull sharks (*Carcharhinus leucas*; Müller & Henle 1839) are a widely distributed, coastal predator found in tropical, subtropical, and temperate ecosystems worldwide (Compagno 1984). Because bull sharks are highly efficient osmoregulators, they can travel between fresh and marine waters, and respond to sudden changes in salinity with minimal metabolic costs (Anderson et al. 2006). Subadult and mature individuals typically reside in coastal waters, while juveniles use coastal estuaries as nurseries during early years (Heithaus et al. 2007, Wiley & Simpfendorfer 2007, Castro 2011). Within estuaries, juvenile bull sharks experience environmental variability, including acute and seasonal shifts in local salinities and temperatures (e.g. Simpfendorfer et al. 2005, Steiner et al. 2007, Wiley & Simpfendorfer 2007). This variability in the physical environment can lead to seasonal and intermittent patterns in shark occurrence within nurseries (e.g. Heupel & Simpfendorfer 2008, Yeiser et al. 2008, Heupel et al. 2010). However, seasonal variability in temperature and/or salinity does not cause all populations to leave the confines of their respective nurseries (e.g. Heithaus et al. 2009), and whether acute changes in water temperature may cause large changes in behavior or survivorship are unknown. Understanding the impacts of acute events on bull sharks in nurseries is important, however, because of their possible roles in linking coastal and estuarine food webs (Matich et al. 2011), and their position as an upper trophic level predator in these habitats.

South Florida, USA experiences predictable seasonal changes in air temperature that contribute to annual shifts in the community composition of aquatic and terrestrial ecosystems (e.g. McIvor et al. 1994, Ruetz et al. 2005, Rehage & Loftus 2007). These changes are typically moderate and gradual (Duever et al. 1994), but from 2-13 Jan 2010, South Florida experienced a dramatic and extended drop in air temperature (mean low air temperature = 6.1°C ± 0.7 SD; NOAA 2010) that led to an extreme mortality event of both terrestrial and aquatic species on a scale not recorded in Everglades National Park for more than 50 years (Rehage et al. 2010). Here, we take advantage of an ongoing long-term study conducted before, during, and after this event, to investigate the effects of this extreme cold event on the behavior and age structure of bull sharks that typically exhibit year-round residency within a South Florida coastal estuary.

Methods

Study location

The Shark River Estuary of Everglades National Park, Florida, USA (Fig. 1) is primarily a braided stream system lined by mangroves that extends from the Gulf of Mexico to freshwater vegetated marshes ~30 km upstream (Childers 2006). Juvenile bull sharks use the estuary as a nursery year-round, and reside in the ecosystem for their first 3-5 years of life (Wiley & Simpfendorfer 2007, Heithaus et al. 2009). For the purpose of this study, the area was divided into four different sampling regions based on spatial variability in salinity documented during long-term sampling. The Downriver (DR) region includes the coastal waters of Ponce de Leon Bay and relatively deep (3-5 m) and wide (50-400 m) channels extending up to 5 km upstream, with an annual salinity range of 16-39 parts per thousand (ppt) (mean = 29 ppt ± 4.9 SD). The Shark River (SR) region includes relatively deep (3-7 m) channels 6-14 km upstream, and salinity varies seasonally from 1-34 ppt (mean = 14 ppt ± 8.9 SD). Tarpon Bay (TB) is a relatively shallow bay (1-3 m deep) with several smaller bays 15-19 km upstream, and salinity

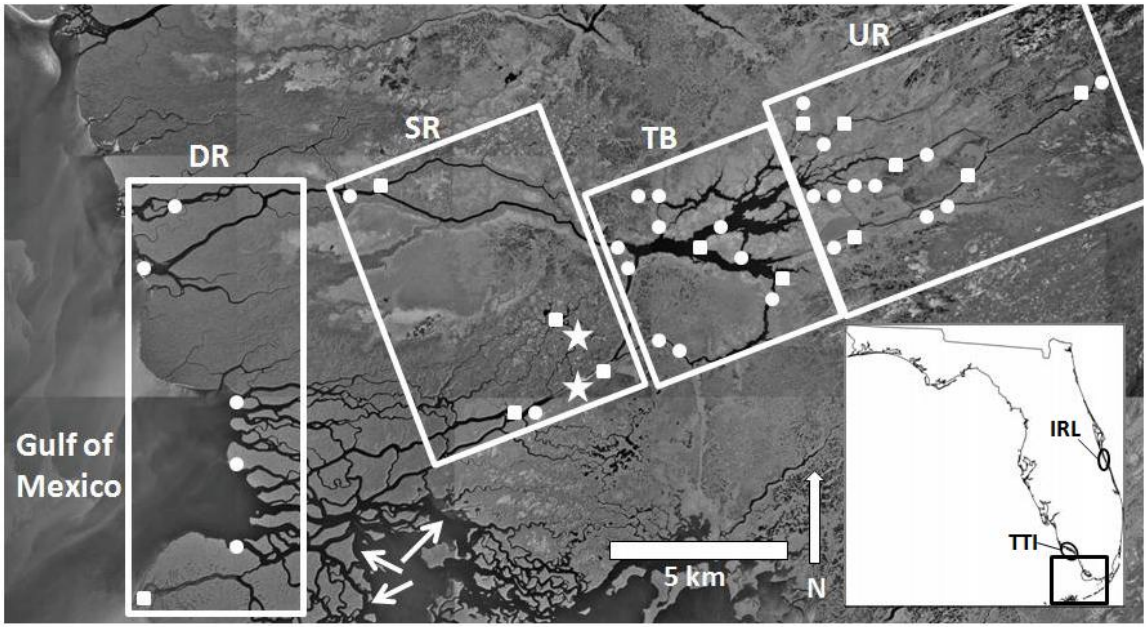

Figure 1: Longline and acoustic telemetry sampling regions (DR: Downriver, SR: Shark River, TB: Tarpon Bay, and UR: Upriver) within the Shark River Estuary of Florida, USA. Locations of acoustic receivers are indicated by white circles, squares, and stars. Acoustic receivers with Hobo temperature loggers are white squares. White stars are the locations of receivers that last detected sharks the six sharks lost within the system during the cold snap (i.e. last detected within the SR region). Note that those locations are in relatively close proximity to receivers both upstream and downstream and exiting the system without a detection on another receiver would have been unlikely. Although there appear to be unmonitored exits from the estuary (general area indicated by white arrows), sharks moving into this portion of the system cannot exit into the Gulf of Mexico without passing by one of the monitored exits (i.e. all exits to the Gulf of Mexico are monitored by acoustic receivers). Locations of the Indian River Lagoon (IRL) and Ten Thousand Islands (TTI) are indicated on the inset map.

ranges from 0.3-25 ppt annually (mean = 5 ppt ± 6.0 SD). And finally, the Upriver (UR) region includes relatively narrow channels 2-4 m deep, which are 20-27 km upstream, that temporally vary in salinity from 0.2-21 parts per thousand (ppt) (mean = 3 ppt ± 4.6 SD) (Fig. 1).

Field sampling

Spatial and temporal variability in water temperature were measured using Hobo Pro v2 data loggers (Onset, Cape Cod, MA) deployed at 13 locations throughout the system (Fig. 1) from Jul 2007 - Jan 2011. Water temperature was measured by loggers every 10-15 minutes throughout the study, and data were downloaded every 3-4 months. Throughout the study, water temperatures also were measured during all sampling events using a YSI 85 handheld water quality meter (YSI Incorporated, Yellow Springs, OH). Because of the superior spatial and temporal resolution of data from Hobo data loggers, we used only these data in analyses from Jul 2007 - Jan 2011.

Spatial and temporal variability in bull shark abundance was quantified from 2006-2010 using ~500 m longlines fitted with 40-55 14/0 or 15/0 Mustad tuna circle hooks. Hooks were baited with mullet (*Mugil* sp.) and attached to ~2 m of 400 kg monofilament line (see Heithaus et al. 2009 for details of sampling equipment). Longline sampling took place in all four regions (DR, SR, TB, and UR) quarterly for the duration of the study (Table 1, Appendix 1). In 2008, however, sampling only took place during Jan and Oct-Dec. We therefore excluded data from 2008 in our analyses of bull shark relative abundance. Captured sharks (n = 121 from 2006-2007 and 2009-2010) were tagged, measured, and sexed alongside the sampling vessel, or within a water-filled,

	Longlines (n)	Sharks (n)	Temperature (°C)
Jan-Mar			
2006	19	16	23.3 ± 3.5
2007	7	8	24.5 ± 0.8
2009	39	12	21.0 ± 3.1
2010	31	0	17.2 ± 3.9
Apr-Jun			
2006	18	11	28.2 ± 1.7
2007	30	5	24.3 ± 1.1
2009	56	18	28.0 ± 2.2
2010	33	5	27.6 ± 2.3
Jul-Sep			
2006	8	4	29.6 ± 1.1
2007	21	6	30.8 ± 1.4
2009	39	12	30.7 ± 1.2
2010	25	2	30.1 ± 1.0
Oct-Dec			
2006	38	14	25.7 ± 1.8
2007	4	3	19.8 ± 1.4
2009	43	3	25.1 ± 2.0
2010	30	2	23.1 ± 4.9

Table 1: Number of longline sets, number of juvenile bull sharks caught on longlines, and average water temperatures with standard deviations for each sampling period.

aerated cooler on board. Shark stretched total length was measured over the top of the body to the nearest centimeter, the presence or absence of an umbilical scar on the ventral side of the body was recorded, and sharks were externally tagged using a plastic roto tag affixed through the first dorsal fin prior to being released.

Passive acoustic tracking was used to quantify the movement patterns of individual bull sharks. From Dec 2007 - Dec 2009 sharks caught in excellent condition (swimming strongly upon capture) ranging from 67-149 cm total length (n = 40 individuals with active transmitters at the time of full acoustic array establishment; see below; Appendix 2) were surgically fitted with a Vemco V16-4H transmitter (Vemco, Halifax, NS). Transmitters were set to emit a unique series of pulses for each shark at a random interval between 30-90 sec (mean emission interval = 60 sec; mean battery life = 2 yr). Movements of acoustically tagged sharks were tracked within an array of 43 Vemco VR2 and VR2W acoustic receivers (Fig. 1), that was fully established by October 2008. In most areas, acoustic receivers were deployed in pairs, such that the location and direction of movement for each acoustically tagged shark could be monitored continuously throughout most of the study system. Due to the complexity of the channels at the mouth of the estuary this could not be achieved in the DR region. However, based on the detection ranges of the acoustic receivers (in situ measurements revealed mean detection ranges were ~500 m; see Rosenblatt & Heithaus 2011 for detection ranges of individual receivers), and their locations at the estuary mouth, sharks entering the Gulf of Mexico would have been detected by at least one of the receivers as they exited the Shark River Estuary. Between the DR and SR regions, there are several exit points from the estuary that lead into Whitewater Bay, but there are no connecting bodies of water that allow for sharks to travel between the Gulf of Mexico and Whitewater Bay (i.e. the only exit points from the system are at the mouths of the Shark and Harney Rivers, where acoustic receivers were in place; Fig. 1). Each receiver was attached to a PVC pipe set in a 10 kg cement anchor. Data from receivers were downloaded every 3-4 months for the duration of the study, and batteries were replaced as needed.

Data analysis

Passive acoustic telemetry was used to assess the effects of the cold snap on bull shark behavior and survival. Data downloaded from acoustic receivers were converted to times of entry into and exit from the sampling regions (DR, SR, TB, and UR; Fig. 1) using a custom computer program (GATOR; Andrew Fritz, FritzTech, Houston, TX). Logistic regression was used to test the effects of sampling month, year, region and their interactions on 1) the probability of detecting all sharks with active transmitters within the system, and 2) the probability of detecting at least one shark with an active transmitter within the system. After analyses of full models with all factors and interactions, interactions with $P > 0.10$ were sequentially removed from models. All main factors (month, year, and region) were included in final models regardless of p-values. Logistic regression was used to test the probability that each shark had left the system (i.e. emigrated) or was 'lost' in the system (i.e. last detected by an acoustic receiver within the array that was not adjacent to an exit point of the estuary) each month from Nov 2008 - Jan 2010.

Longline catch data were analyzed to assess changes in bull shark abundance, distribution and size/age structure relative to the cold snap. Due to the large number of zeros in the data, we used a conditional approach (e.g. Fletcher et al. 2005, Serafy et al. 2007) to quantify the change in shark abundance and distribution in relation to the cold snap. First, we used logistic regression to test the effects of sampling month, year, region, and their interactions on the probability of catching at least one juvenile bull shark on a particular longline set ("occurrence"). Next, we used a general linear model to determine how these factors and possible interactions influenced the number of sharks caught on longlines when they were present ("concentration"). We pooled months into four sampling periods: Jan-Mar, Apr-Jun, Jul-Sep, and Oct-Dec for each year. Concentration data were transformed using Box-Cox transformations. All interactions with $P > 0.10$ were sequentially removed from models, but main factors were included in final models regardless of significance level. Post hoc Tukey's test was used to test for significant differences across treatments.

To determine the effects of the cold snap on the size structure of the bull shark nursery, we used a Kruskal-Wallis one-way analysis of variance to investigate whether the sizes of sharks caught from May-Dec varied across sampling years. Sharks caught from Jan-Apr for all years were not included in body size analyses because no sharks were caught from Jan-Apr in 2010 (sharks were captured during these months in other years; Table 1, Appendix 1), and including sharks from these months in other years could have confounded our ability to investigate changes in size structure between previous years and that present in 2010 after the cold snap. In addition, logistic regression was used to examine the effects of capture year on the probability of capturing sharks with umbilical scars (i.e. neonates <2 months old; Compagno 1984) and of the probability of capturing sharks <90 cm total length (i.e. young of the year; Branstetter & Stiles 1987,Neer et al. 2005). All statistical analyses were conducted in JMP 6.0.0.

Results

Environmental Conditions

Prior to the cold snap, water temperatures in the estuary ranged from 14.2 °C (6 Feb 2009) to 33.1 °C (15 Jul 2009), with the coldest temperatures occurring from Jan-Mar (mean = 22.0 °C ± 3.0 SD), and the warmest temperatures occurring from Jul-Sep (mean = 30.6 °C ± 1.2 SD) (Fig. 2). Water temperatures in the Shark River Estuary during the cold snap were considerably lower (mean = 12.9 °C ± 2.8 SD, 4-15 Jan 2010) than any other time period during the study (Figs. 2 & 3), and mean daily water temperatures dropped as low as 9.1 °C at the peak of the event (12 Jan 2010 at DR). Mean daily air temperature lows in the Florida Everglades were below 10°C from 1-14 Jan 2010 (Flamingo Ranger Station NOAA).

Effects on Bull Sharks

From 2006-2009, we captured 112 juvenile bull sharks (66-200 cm TL; 57 females and 55 males; Table 1). After 20 Dec 2009, no sharks were caught until 22 May 2010, and only nine sharks were caught from 22 May 2010 to 16 Dec 2010 despite sampling effort similar to previous years (68-86 cm TL; 2 females, 8 males, one individual escaped before its sex was determined; Table 1, Appendix 1). During sampling in Jan 2010, two bull sharks (~100 cm TL) were found dead within the confines of the estuary, presumably from

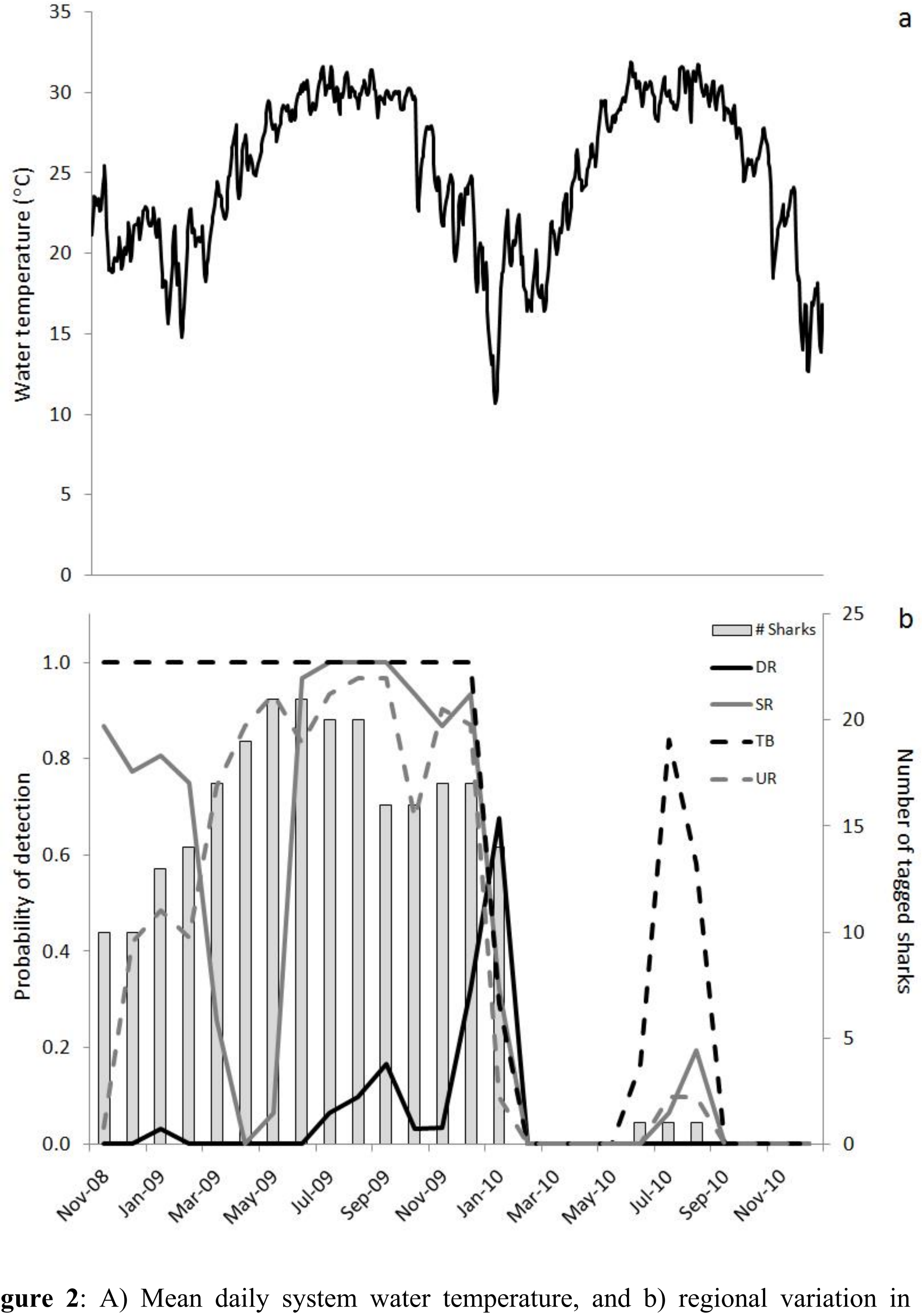

Figure 2: A) Mean daily system water temperature, and b) regional variation in the probability of detecting at least one acoustically tagged bull shark. Bars indicate the number of sharks with transmitters active within the study area.

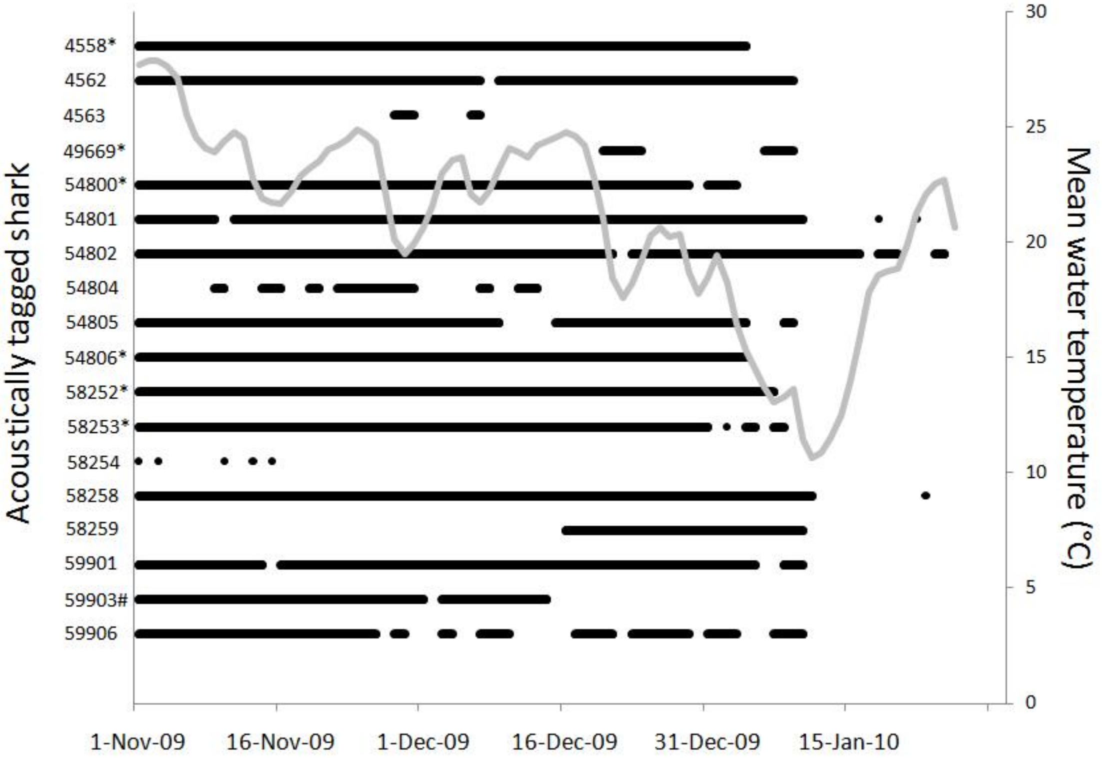

Figure 3: Acoustic receiver detections of tagged sharks from 1 Nov 2009 until departure from the system (black line or dot represents detection in system; * indicates shark last detected within Shark River region (i.e. was not detected on any of the most downstream monitors before disappearing permanently); # indicates the shark that was detected in the system after 23 Jun 2010). Gaps in detections include days in which sharks were in areas within the system but outside the detection range of acoustic receivers. Mean system water temperature is displayed in gray.

temperature-induced mortality - these were the only sharks found dead during the study (2006-2011).

From Oct 2008 - Dec 2009, 40 bull sharks (67-149 cm TL; 21 females and 19 males, Appendix 2) with surgically implanted acoustic transmitters were active in the tracking array. Of these, 14 individuals were present during the cold snap (2-25 Jan 2010) and had transmitters that were implanted at least 18 days before the event. Six of the 14 individuals present during the cold snap (43%) were 'lost' within the confines of the system during the cold snap (see Fig. 1 for the last detection locations of these individuals), suggesting they probably died in the system. The other eight individuals left the system (i.e. were last detected in the DR region) during the cold snap. The proportion of acoustically tagged sharks that were lost (43%) and that left the system (57%) were considerably greater than any other month during the study ($F_{46,211} = 3.56$, $p < 0.01$; $F_{46,211} = 2.72$, $p < 0.01$, respectively; Fig. 4). The 26 acoustically tagged individuals not present during the cold snap either 1) left prior to the cold snap - permanently emigrating to other estuaries or coastal waters (n = 17), 2) had acoustic transmitter malfunctions (e.g. battery failure) immediately after release (n = 5), 3) likely died due to stress incurred during surgery (n = 2), or 4) disappeared inside the array because of natural or anthropogenic mortality (e.g. fishing, boat traffic, other research projects; n = 2; Appendix 2). The acoustically tagged sharks lost during the cold snap (n = 6) were last detected by the receivers within the southeast part of the Shark River region (Fig. 1) where it is highly unlikely that they could have left the system or entered Whitewater Bay without being detected by at least one of the two receivers farther downstream in the SR region. The region where acoustically tagged sharks were last detected during the cold snap

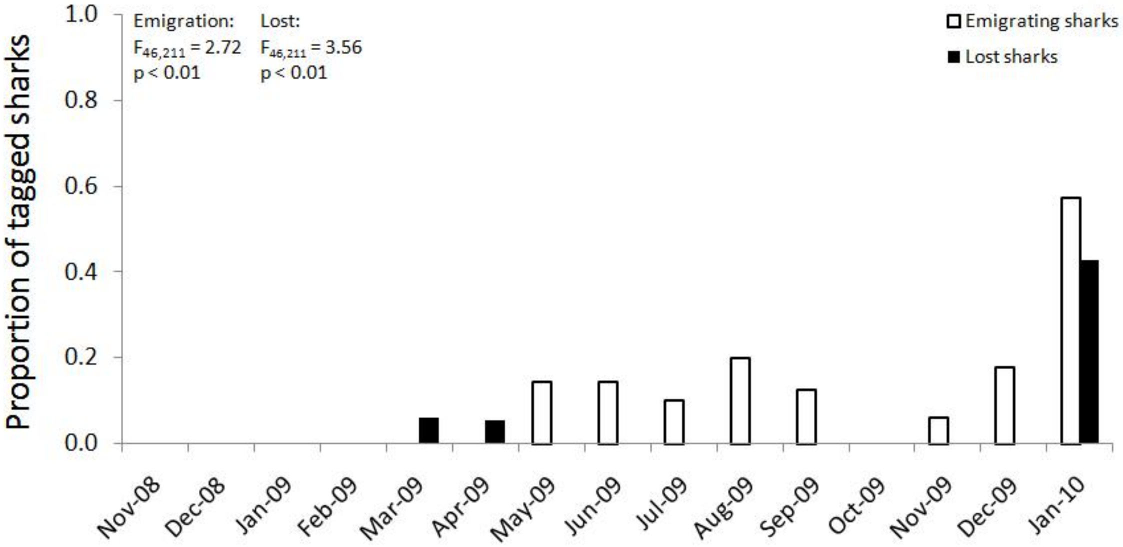

Figure 4: Proportion of acoustically tagged sharks that left (i.e. emigrated) from the estuary and the proportion of sharks that were 'lost' (i.e. last detected by an acoustic receiver within the array that was not adjacent to an exit point of the estuary) from Nov 2008 - Jan 2010.

(i.e. DR or SR) was not influenced by shark total length (t = 1.13, p = 0.28, df = 12). No acoustically tagged sharks were detected on acoustic receivers after the cold snap until 24 Jun 2010.

The probability of detecting at least one shark and all sharks on acoustic receivers within the Shark River Estuary varied with all main factors (region, month, and year) and the interaction between sampling region and year (Table 2; Fig. 2). From Nov 2008 - Dec 2009, more sharks were detected in Tarpon Bay (6.18 sharks/day ± 0.18 SE) than any other region, and the fewest number of sharks were detected in the Downriver region (0.13 sharks/day ± 0.03 SE). The Shark River (2.06 sharks/day ± 0.10 SE) and Upriver (1.39 sharks/day ± 0.10 SE) regions had intermediate numbers of sharks detected (Fig. 2). In Jan 2010, the cold snap caused a considerable shift in detections at all sites. Detections decreased sharply in TB (1.92 sharks/day ± 0.68 SE) and UR (0.24 sharks/day ± 0.14 SE), but increased in DR (1.88 sharks/day ± 0.36 SE) before all sharks exited the system or were no longer detected within the system by 26 Jan 2010 (Figs. 2 & 3). Most acoustically tagged sharks present during the cold snap were no longer detected after 11 Jan 2010, however three individuals (54801, 54802, 58258), which moved into DR during the cold snap, remained in the vicinity throughout the cold snap and were detected intermittently on DR monitors before disappearing permanently by 26 Jan 2010 (Fig. 3). All acoustically tagged individuals that were detected immediately before and during the cold snap had transmitters that should have been active at the time of the last acoustic monitor download on 22 Jan 2011. Only one shark (59903) reappeared in the system after the cold snap on 24 Jun 2010, and remained in the system until it was last detected heading into the DR region (based on detection sequence in SR) on 29 Aug 2010 (Fig. 2).

	Region	Month	Year	Region*Month	Region*Year	Month*Year	N	Adj. R^2
Longlines								
Occurrence	**6.83, 3 (<0.01)**	2.53, 3 (0.06)	**11.5, 3 (<0.01)**	0.69, 9 (0.71)	0.60, 9 (0.79)	**3.65, 9 (<0.01)**	105	0.40
Concentration	0.52, 3 (0.67)	0.57, 3 (0.64)	**5.86, 3 (<0.01)**	**2.38, 9 (0.04)**	0.47, 6 (0.82)	1.27, 8 (0.31)	48	0.40
Acoustic tracking								
P (1 shark)	**30.4, 3 (<0.01)**	**2.51, 11 (0.01)**	**56.6, 2 (<0.01)**	0.69, 33 (0.84)	**11.7, 6 (<0.01)**	0.67, 8 (0.72)	88	0.81
P (all sharks)	**34.5, 3 (<0.01)**	**2.55, 11 (<0.01)**	**7.73, 2 (<0.01)**	0.72, 33 (0.81)	**3.50, 6 (<0.01)**	0.89, 8 (0.53)	88	0.71

Table 2: Results from logistic regression investigating the factors influencing bull shark occurrence and concentration (longline sampling) and the probability of detecting at least one shark [P(1 shark)] and all sharks [P(all sharks)] on acoustic receivers. Significant factors are in bold. Non-significant interactions (P>0.10) were excluded from final models.

Nine juvenile bull sharks were caught on longlines from 22 May 2010 to 16 Dec 2010 (Table 1). Occurrence and concentration of bull sharks varied across sampling years, and occurrence varied across regions (Table 2; Fig. 5). The probability of catching at least one shark on a longline set (i.e. occurrence) was highest in 2006 and lowest in 2010, and was highest in Tarpon Bay and lowest Upriver (Fig. 5a). The number of sharks caught on longlines when present (i.e. concentration) was highest in 2007 and lowest in 2010, and exhibited minimal variability across regions (Fig. 5c). Thus, sharks were encountered less often after the cold snap, and when they were encountered in 2010, they were in smaller numbers than when encountered in previous years. Both occurrence and concentration were least variable across years and regions from Apr-Sep, and exhibited considerable variability between years and regions from Oct-Mar (Fig. 5d).

Mortality and abandonment of the system during the cold snap resulted in changes in the size structure of bull sharks directly following the event. Bull sharks caught after the cold snap from May-Dec 2010 were significantly smaller (mean total length = 77 cm ± 1.7 SE) than all previous sampling years (mean TL = 106 cm ± 4.7 SE) during these months ($\chi^2 = 17.33$; $p < 0.01$; Fig. 6a). The probability of catching a shark less than 90 cm total length, and the probability of catching a shark with an umbilical scar (neonate) varied significantly across years ($F_{3,38} = 8.28$, $p < 0.01$; $F_{3,38} = 6.37$, $p < 0.01$, respectively). All of the bull sharks caught in 2010 were young-of-the-year and 67% were neonates, which was higher than other years (of the sharks caught from 2006-2009, 41% were young-of-the-year, and only 11% were neonates, respectively; Fig. 6).

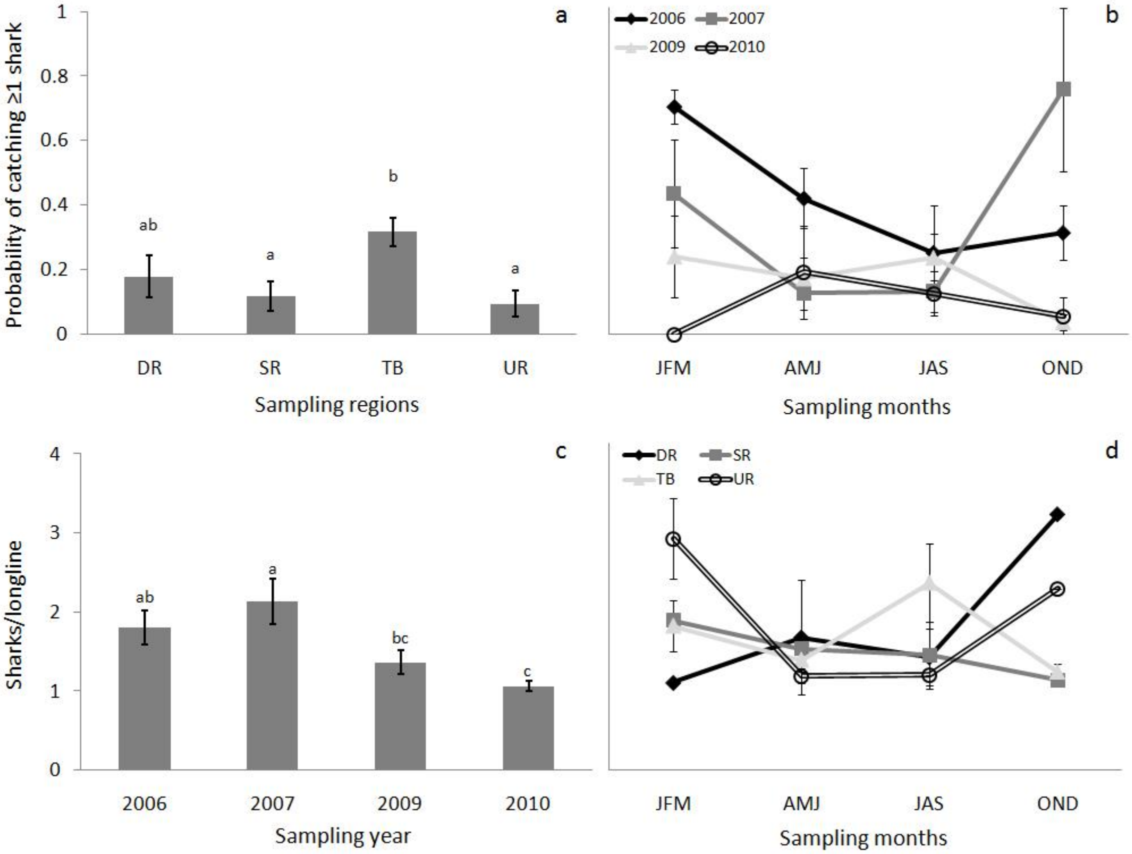

Figure 5: Bull shark occurrence varied across regions (a) and with an interaction of season and year (b). The number of sharks captured on longlines with sharks (concentration) varied across years (c) and with an interaction of months and region (d). Bars are SE and bars with different letters are significantly different based on post hoc Tukey's test.

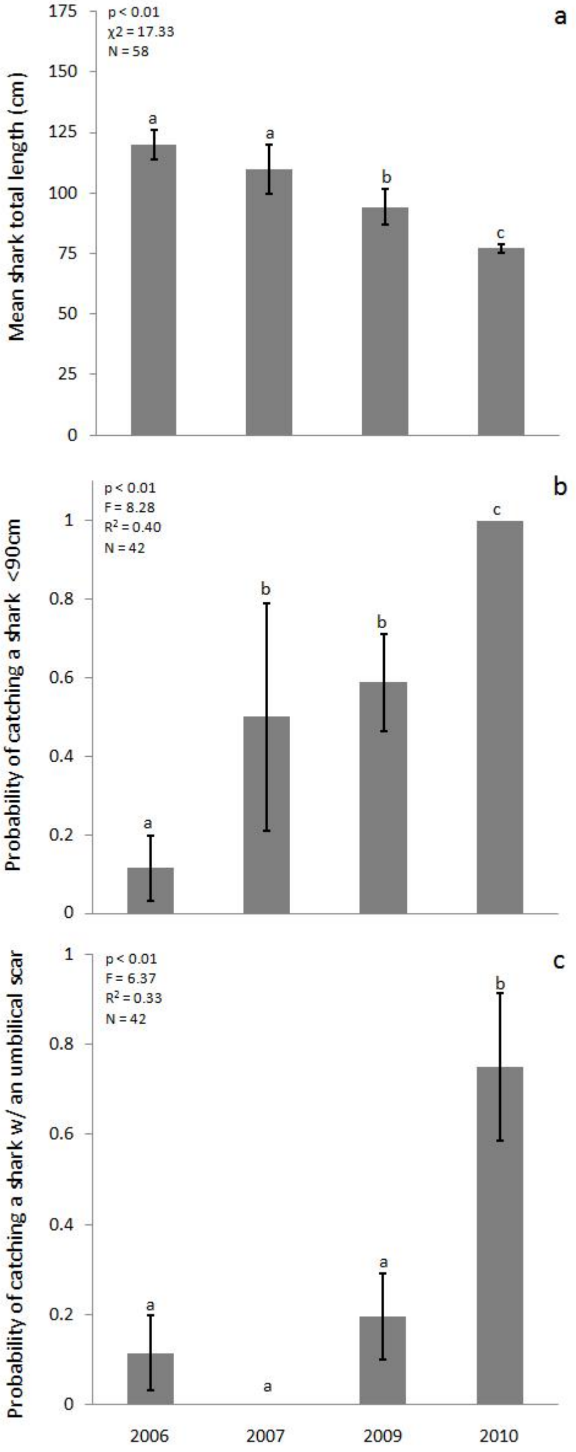

Figure 6: Annual differences in a) mean bull shark total length in cm, b) mean probability of a caught bull sharks being less than 90 cm TL, and c) mean probability of a caught bull shark having an umbilical scar, for sharks caught from May 22 - December 16. Bars are SE and bars with different letters are significantly different based on post hoc Tukey's test.

Discussion

Population-level Effects

Populations often experience daily and seasonal shifts in environmental conditions, and individuals adjust to these predictable changes by making local or long-distance migrations, changing their behavior, and/or making metabolic adjustments (e.g. Heupel & Hueter 2001, Klimley et al. 2002, Swenson et al. 2007, Holdo et al. 2009, Speed et al. 2010). However, unpredictable and rapid fluctuations in environmental conditions may occur too quickly for individuals to appropriately adjust their behavior or respond physiologically in order to meet metabolic needs and survive (e.g. Aebischer 1986, Schoener et al. 2001). An inability to adapt to such events may have important consequences for the structure and function of populations and ecosystems (e.g. Easterling et al. 2000, Daufresne et al. 2007, Thibault & Brown 2008), and is a concern for conservation because the frequency of extreme environmental events is predicted to increase in the future (IPCC 2007).

Extreme cold events have led to fish kills in Florida about every ten years in the last 100 years (Gilmore et al. 1978, Snelson & Bradley 1978 and references within), suggesting the cold snap in 2010 was not unique. However, in comparison to previous cold events, the magnitude of individuals killed as a result of cold temperatures in Jan 2010 was considerably greater. During the cold snap of 1976-77 in the Indian River Lagoon, central Florida, USA - the last published account of an extensive fish kill in Florida attributed to an extended drop in temperature - mean water temperatures were 10.8 °C, which is comparable to water temperatures in the Shark River Estuary in Jan 2010, and resulted in dead individuals from 56 species, including bull sharks (n = 2;

Gilmore et al. 1978, Snelson & Bradley 1978). Yet, the number of fish reported dead in 1977 was several orders of magnitude lower (tens to hundreds), compared to the effects of the cold snap in Jan 2010 (thousands to tens of thousands of fishes killed; Rehage et al. 2010, personal observation), suggesting the impacts on survivorship were much greater in general in the Shark River Estuary during the 2010 event, and the recovery period may be longer.

Before the cold snap, bull shark use of the Shark River Estuary was characterized by individuals <3 years old being year-round residents (Heithaus et al. 2009, P Matich & MR Heithaus *unpublished data*), which may be facilitated by the relatively warm winter water temperatures (e.g. Garla et al. 2006, Chapman et al. 2009, Cortes et al. 2011). The absolute temperatures in Jan 2010, and the duration of the extreme cold event, appear to have exceeded the thermal tolerance of bull sharks using the Shark River Estuary, and resulted in profound impacts on abundance and subsequent size/age structure in the nursery.

Acoustically tagged bull sharks displayed uncharacteristic movement patterns during the cold snap, with mass movements out of Tarpon Bay and into the Downriver region (where, even in past winters, there had been low detection frequencies), before disappearing into the Gulf of Mexico. Mass movements out of estuaries in response to atypical environmental conditions has been observed in juvenile blacktip sharks (*Carcharhinus limbatus*) in Terra Ceia Bay, central Florida, which left the bay in response to the drop in barometric pressure prior to the arrival of a tropical storm (Heupel et al. 2003). All individual blacktip sharks returned to Terra Ceia Bay within two weeks of their departure. Like blacktips, sea snakes (*Laticauda* spp.) in Lanyu, Taiwan vacated their normal coastal habitat in response to changes in barometric pressure prior to a typhoon, and returned less than two weeks later after its passage (Liu et al. 2010). In addition to the bull sharks that left during and only days after the cold snap (n = 14), three tagged sharks (75-107 cm TL) left the system a few weeks prior to the event in Dec 2009. One of these early-departing individuals was the only acoustically tagged shark to return to the estuary after the cold snap (in June 2010), and was one of the smallest individuals (75 cm TL) acoustically tagged at the time of the cold snap. The departure of sharks just before and during the cold snap was unusual, because unlike juvenile bull sharks within coastal estuaries in more northern portions of Florida (e.g. Heupel and Simpfendorfer 2008, Yeiser et al. 2008, Heupel et al. 2010), bull sharks in this nursery are typically year-round residents and do not seasonally or intermittently travel into or out of the estuary (Heithaus et al. 2009, P Matich & MR Heithaus *unpublished data*).

Despite water temperatures returning to normal (>18 °C) within three weeks of the cold snap, no acoustically tagged bull sharks returned to the estuary at this time, and only one individual returned during the study. Previous tag-recapture studies in Everglades National Park and along the Florida coast of the Gulf of Mexico revealed that some bull sharks will relocate to estuaries more than 100 km from initial capture locations (Wiley & Simpfendorfer 2007). Yet, the number of sharks making these long migrations (n = 3 of 302; 1%) was small, and tracking data from the Shark River Estuary suggest such movements are uncommon under normal conditions. Therefore, some individuals that left the estuary may have permanently emigrated, while others may have died.

The behavior resulting from the sudden drop in temperature caused reductions in the occurrence and concentration of bull sharks in the system by 70% and 40% respectively (i.e. approximately a 73% reduction in overall catch rates). This decline in shark abundance may have been due to temperature stress, increased predation, and/or permanent relocation. During the cold snap, two bull sharks (~100 cm total length) were found dead within the confines of the estuary, almost certainly from temperature-induced mortality. Finding even two dead sharks is notable, however, because sharks are negatively buoyant and sink upon death (Helfman et al. 1997), and the Shark River Estuary is turbid. Indeed, to our knowledge dead sharks have not been found in the system previously, despite considerable research effort in the study area. In addition, six (43%) of the acoustically tagged bull sharks were last detected by receivers in the southeastern part of the Shark River sampling region, suggesting they died within the estuary, but outside of the detection range of any individual receiver. Prior to the cold snap, only two of 23 (9%) acoustically tagged individuals (82 and 83 cm TL at capture in Jan 2009 and Nov 2008, respectively) may have died of natural causes (e.g. stress, starvation) in Mar and Apr 2009 in Tarpon Bay, suggesting the survival rate of juvenile bull sharks is relatively high in the Shark River Estuary (Heupel & Simpfendorfer 2011). There are virtually no predators of bull sharks within the estuary (MR Heithaus & P Matich *unpublished data*), and because all of the sharks that died during the cold snap died within days of each other, and movements during detection did not reveal abnormal movement patterns attributed to predation (i.e. faster rate of movement of a large predator that had consumed a smaller shark; Heupel & Simpfendorfer 2002), all of these individuals likely succumbed to the low temperatures. Temperature-related mortality may also be responsible for the low rate of return of individuals that left the system - in more northern estuaries in Florida, bull sharks (Indian River Lagoon) and smalltooth sawfish (*Pristis pectinata*; Ten Thousand Islands) also died due to thermal stress attributed to the 2010 cold snap (J Imhoff personal communication; D Bethea personal communication, respectively; see Fig. 1), suggesting the effects of the cold snap extended beyond the Shark River Estuary, and sharks that emigrated towards or into other estuaries or coastal areas during this time may not have been able to locate thermal refugia. However, three sharks did remain in the proximity of the DR region until Jan 22, 24, and 25 (54801, 58258, and 54802, respectively). By the dates of their final detection, water temperatures were comparable to previous years (mean = 20.3 °C from 22-25 Jan 2010 at DR), suggesting that some sharks that did not succumb to temperature stress.

Juvenile bull sharks that left the estuary may also have experienced increased mortality from predation. Small sharks in Florida's coastal waters are at considerable risk of predation from large predatory sharks (e.g. *C. leucas, Negaprion brevirostris*; Compagno 1984, Snelson et al. 1984, Castro 2011, P Matich & MR Heithaus *unpublished data*). During typical years, juvenile bull sharks almost exclusively remained in areas at least 10-15 km upstream from the DR region, probably to avoid larger sharks that live at the mouth of the estuary (Heithaus et al. 2009, P Matich *unpublished data*). However, in escaping their rapidly chilling estuarine habitat during the cold snap, juvenile bull sharks entered high-risk coastal habitats where predation may have reduced the number of sharks that returned to the estuary afterwards. It is also possible that despite temperatures returning to normal relatively quickly, departing bull sharks may have remained within coastal waters or traveled to other estuaries where they took up residence (Wiley & Simpfendorfer 2007, Yeiser et al. 2008, Heupel et al. 2010).

Regardless of whether departing sharks died from temperature stress, were eaten by predators, or relocated to another estuary, the abundance and size range of juvenile bull sharks was altered within the Shark River Estuary. Prior to the event, the size range of bull sharks in the system was relatively wide (66-200 cm TL). But for 12 months after the event, all sharks caught (n = 9) were less than 90 cm TL (68-86 cm TL), and most (n = 6; 67%) had umbilical scars indicating they were only weeks old. The variability in the size of captured sharks was very small, further suggesting they were from the same cohort, and that virtually all individuals of several age classes were lost from the nursery. Although nine individuals is a relatively small sample, the sampling effort in 2010 was comparable to previous years, and these nine individuals are reflective of the abundance and sizes of bull sharks in the estuary. Unless there is immigration, it will likely take several years for bull shark densities in the Shark River Estuary to recover and resemble the size structure present before the cold snap. Indeed, if the largest individuals in 2010 were 80-90 cm TL (the largest individual caught in 2010 was 86 cm TL), and exhibited fast growth rates for bull sharks (e.g. 20 cm TL per year; Neer et al. 2005), then these sharks will attain total lengths similar to the third quartile of sharks found in the estuary before the cold snap (130 cm TL) in at least 2-3 years.

Community- and Ecosystem-level Effects

Within Florida, acute cold events of at least eight straight days occur about every five years in south Florida; there were 12 such events from 1950-2009 (Flamingo Ranger

Station). However, the last recorded occurrence of a cold snap with a duration of 12 days or longer prior to 2010 was in 1940 (Flamingo Ranger Station, Rehage et al. 2010), and there have been no published reports of massive fish kills in south Florida since the winter of 1976-77 (Gilmore et al. 1978, Snelson & Bradley 1978), and even this event was not as extreme as that in 2010. Considering the rare nature of these extended extreme events (occur every 30-40 years) with the low proportion of acoustically tagged bull sharks returning to the Shark River Estuary (n = 1; 6% of tagged individuals), and the probable ages of all bull sharks caught in 2010 (age-class 0), it suggests there has not been strong selection for the ability to withstand such events within this nursery.

The resulting change in bull shark density and sizes could have important consequences. Prior to the cold snap, bull sharks in the Shark River Estuary showed a relatively high degree of individual specialization in trophic interactions, with some large and small juveniles exclusively feeding from marine food webs and others from food webs based in the estuary or upstream marshes, in spite of being captured in the same location in the estuary (Matich et al. 2011). This specialization appeared to be driven by high levels of intraspecific competition (Matich et al. 2011), which combined with the risk of cannibalism and predation might have driven spatial size structuring of the sharks in the estuary (Simpfendorfer et al. 2005, Heithaus et al. 2009). As a result of the cold snap, and subsequent changes in shark abundance and size structure, intraspecific competition and the risk of cannibalism likely decreased considerably. Based on theory and studies of other taxa (e.g. Estes et al. 2003, Svanback & Persson 2004, Keren-Rotem et al. 2006, Bolnick et al. 2010), this would be predicted to result in an expansion of bull shark activity areas for small size classes and more generalized diets until the nursery recovers. Lower competition also could permit more juvenile bull sharks to feed in low-risk (upstream) areas, and thus avoid the high-food, high-risk areas that include marine-based food webs at the mouth of the estuary. Since bull sharks are the only sharks that regularly use estuaries and freshwater areas in Florida, this shift in habitat use could at least temporarily interrupt the role bull sharks play in linking marine and freshwater food webs (Matich et al. 2011). If structural changes like those that occurred in the Shark River Estuary occurred in other shark populations throughout South Florida, it could alter the dynamics of coastal ecosystems across a large spatial area for several years (e.g. Finstad et al. 2009, Holt & Barfield 2009), unless changes in immigration and/or density-dependent recruitment and survival increase the rate of recovery. Based on the relatively low rate of departures of sharks from the Shark River Estuary prior to the cold snap, studies in other bull shark nurseries (e.g. Steiner et al. 2007, Heupel & Simpfendorfer 2008,), and the presence of almost exclusively new cohorts since the cold snap, it appears that juvenile bull sharks tend to remain within their natal nurseries, and the rate of immigration into the Shark River from other nurseries is low and is unlikely to speed the recovery of densities and age structure.

Our study suggests that rare, but extreme environmental fluctuations can lead to marked localized changes in population size and structure, even in relatively large-bodied, highly mobile species. However, the importance of extreme events to long-term population and ecosystem dynamics remains unclear. To understand the long-term effects of these events, we must better understand how individual shark nurseries contribute to adult populations, the importance of density-dependence within shark nurseries, and how shark populations affect these estuarine ecosystems.

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DR	Jan-Mar			Apr-Jun			Jul-Sep			Oct-Dec		
	Longlines	Sharks	Temp.	Longlines	Sharks	Temp.	Longlines	Sharks	Temp.	Longlines	Sharks	Temp.
2006	0	0	NA	0	0	NA	4	1	30.1 ± 0.1	2	0	24.4 ± 0.4
2007	0	0	NA	9	2	24.5 ± 1.2	3	0	31.6 ± 0.4	1	2	20.8
2009	9	2	22.6 ± 2.0	7	1	26.0 ± 1.9	8	2	30.5 ± 1.1	4	0	23.6 ± 2.8
2010	11	0	18.0 ± 2.5	3	0	27.3 ± 3.5	5	0	30.9 ± 1.1	7	0	25.8 ± 2.6

SR	Jan-Mar			Apr-Jun			Jul-Sep			Oct-Dec		
	Longlines	Sharks	Temp.	Longlines	Sharks	Temp.	Longlines	Sharks	Temp.	Longlines	Sharks	Temp.
2006	6	5	18.9 ± 1.8	7	5	26.6 ± 1.3	2	0	30.1 ± 0.6	8	2	25.8 ± 1.8
2007	0	0	NA	6	0	24.7 ± 0.5	5	2	31.6 ± 0.6	1	0	21.2
2009	5	0	22.7 ± 0.7	6	0	27.9 ± 1.7	6	1	30.5 ± 0.4	7	0	24.5 ± 1.9
2010	8	0	15.3 ± 3.2	6	0	27.8 ± 2.6	9	0	29.7 ± 1.0	5	0	25.1 ± 3.0

TB	Jan-Mar			Apr-Jun			Jul-Sep			Oct-Dec		
	Longlines	Sharks	Temp.	Longlines	Sharks	Temp.	Longlines	Sharks	Temp.	Longlines	Sharks	Temp.
2006	8	6	25.2 ± 2.1	7	5	29.2 ± 0.5	2	3	28.0 ± 0.0	18	10	25.5 ± 2.2
2007	4	5	24.6 ± 0.8	8	2	23.7 ± 1.2	5	3	31.3 ± 0.9	2	1	18.5 ± 0.6
2009	19	10	20.7 ± 3.3	29	13	28.6 ± 1.9	19	9	30.8 ± 1.2	22	3	25.0 ± 3.0
2010	9	0	20.0 ± 3.9	15	5	27.6 ± 2.2	7	2	30.5 ± 1.1	10	2	22.5 ± 5.5

UR	Jan-Mar			Apr-Jun			Jul-Sep			Oct-Dec		
	Longlines	Sharks	Temp.	Longlines	Sharks	Temp.	Longlines	Sharks	Temp.	Longlines	Sharks	Temp.
2006	5	5	25.7 ± 0.8	4	1	29.1 ± 0.9	0	0	NA	10	2	25.9 ± 1.1
2007	3	3	24.4 ± 0.8	7	1	24.4 ± 1.0	8	1	29.8 ± 1.7	0	0	NA
2009	6	0	18.7 ± 2.2	14	4	27.9 ± 1.7	6	0	31.1 ± 0.5	10	0	26.5 ± 2.9
2010	3	0	10.6 ± 0.2	9	0	27.7 ± 2.2	4	0	29.3 ± 0.4	8	0	19.8 ± 4.9

Appendix 1: Table 1: Number of longline sets, number of juvenile bull sharks caught on longlines, and average water temperatures with standard deviations for each sampling region for each sampling period. Note that sample effort was consistently high throughout the study in the region with the highest catch rates.

ID	Capture date	Last detection	Tracking outcome	Sex	Total length (cm)
2064	6 Mar 09	23 Mar 09	Malfunction	M	142
4558	18 Dec 07	4 Jan 10	Lost	M	90
4562	7 Nov 08	9 Jan 10	Emigrated	F	105
4563	31 Jan 08	7 Dec 09	Emigrated	F	77
4564	8 Jan 08	13 Jul 09	Emigrated	F	107
49663	10 Oct 08	4 May 09	Emigrated	M	105
49664	10 Oct 08	5 May 09	Emigrated	M	124
49665	10 Oct 08	4 Jun 09	Emigrated	F	71
49667	10 Oct 08	2 Sep 09	Emigrated	M	110
49668	10 Oct 08	9 Aug 09	Emigrated	F	123
49669	10 Oct 08	9 Jan 10	Lost	F	131
49670	7 Nov 08	14 Apr 09	Lost	F	83
49671	31 Jan 09	29 Jul 09	Emigrated	F	116
49672	11 Jan 09	26 Aug 09	Emigrated	M	93
49673	11 Jan 09	9 Mar 09	Lost	M	82
54799	14 Mar 09	8 Aug 09	Emigrated	F	75
54800	4 Apr 09	3 Jan 10	Lost	M	110
54801	15 Feb 09	22 Jan 10	Emigrated	M	75
54802	4 Apr 09	25 Jan 10	Emigrated	M	112
54803	14 Mar 09	21 Aug 09	Emigrated	M	75
54804	14 Mar 09	13 Dec 09	Emigrated	F	105
54805	8 May 09	9 Jan 10	Emigrated	F	129
54806	5 Apr 09	4 Jan 10	Lost	F	125
54807	4 Apr 09	7 May 09	Malfunction	F	82
54808	8 May 09	Not detected	Not detected	M	149
58250	8 May 09	14 Jun 09	Emigrated	F	86
58251	30 May 09	21 Jun 09	Emigrated	M	132
58252	8 May 09	7 Jan 10	Lost	M	81
58253	12 Jun 09	8 Jan 10	Lost	F	125
58254	12 Jun 09	15 Nov 09	Emigrated	M	75
58255	25 Jul 09	1 Aug 09	Died	F	77
58256	24 Jun 09	18 Dec 09	Died	M	77
58257	24 Jun 09	17 Oct 09	Malfunction	M	69
58258	4 Aug 09	24 Jan 10	Emigrated	M	115
58259	16 Dec 09	10 Jan 10	Emigrated	F	75
59901	25 Jul 09	10 Jan 10	Emigrated	M	79
59902	30 Jul 09	Not detected	Not detected	F	73
59903	31 Oct 09	29 Aug 10	Emigrated	F	75

59906	24 Oct 09	10 Jan 10	Emigrated	F	136
59907	17 Sep 09	20 Sep 09	Emigrated	F	67

Appendix 2: Acoustically tagged sharks with dates of capture and last date detected in the array of acoustic receivers, cause of tracking termination, sex, and total length in cm. Individuals with identification numbers in bold were present in the Shark River Estuary during the cold snap.

CHAPTER IV

SIZE-BASED VARIATION IN INTER-TISSUE COMPARISONS OF STABLE CARBON AND NITROGEN ISOTOPE SIGNATURES OF BULL SHARKS AND TIGER SHARKS

Matich, P., Heithaus, M.R., and Layman, C.A. (2010). Size-based variation in inter-tissue comparisons of stable carbon and nitrogen isotopic signatures of bull sharks (*Carcharhinusn leucas*) and tiger sharks (*Galeocerdo cuvier*). Canadian Journal of Fisheries and Aquatic Sciences, 67:877-885.

Abstract

Stable isotopes are an important tool for understanding the trophic roles of elasmobranchs. However, whether different tissues provide consistent stable isotope values within an individual are largely unknown. To address this, the relationships among carbon and nitrogen isotope values were quantified for blood, muscle, and fin from juvenile bull sharks (*Carcharhinus leucas*), and blood and fin from large tiger sharks (*Galeocerdo cuvier*) collected in two different ecosystems. We also investigated the relationship between shark size and the magnitude of differences in isotopic values between tissues. Isotope values were significantly positively correlated for all paired tissue comparisons, but R^2 values were much higher for $\delta^{13}C$ than $\delta^{15}N$. Paired differences between isotopic values of tissues were relatively small, but varied significantly with shark total length, suggesting shark size can be an important factor influencing the magnitude of differences in isotope values of different tissues. For studies of juvenile sharks, care should be taken in using slow turnover tissues like muscle and fin, because they may retain a maternal signature for an extended time. While correlations were relatively strong, results suggest correction factors should be generated for the desired study species, and may only allow course-scale comparisons between studies using different tissue types.

Introduction

Elasmobranchs (sharks, skates, and rays) play crucial roles in marine ecosystems (Heithaus et al. 2008), but gaps in our knowledge of their trophic interactions hinder understanding of marine community dynamics and ecosystem function. Current studies of trophic interactions of elasmobranchs, especially sharks, are particularly important because populations of many species are declining rapidly worldwide (e.g. Dulvy et al. 2008). These declines already may be causing drastic shifts in food web structure and function (Heithuas et al. 2008).

Most studies of elasmobranch trophic interactions have employed stomach content analysis (see Weatherbee and Cortes 2004 for a review). Although stomach content analysis allows identification of specific prey taxa, it has drawbacks, including the need for large sample sizes and often destructive sampling. Sharks also often have empty stomachs (Weatherbee and Cortes 2004), further limiting information that can be gleaned from this approach. Stable isotope analysis provides an alternative, or complementary, method for gaining insights into the trophic interactions of sharks (e.g. Fisk et al. 2002, Domi et al. 2005, MacNeil et al. 2005), especially because samples can be collected without sacrificing individuals. This method is based on the principle that a consumer's tissues isotopically resemble those of its food (Post 2002), and thus present an extended dietary record (Bearhop et al. 2004). However, stable isotopes are incorporated into different body tissues at different rates, which can affect interpretation of data (Martinez del Rio et al. 2009).

Our understanding of the dynamics of stable isotope values in elasmobranchs lags behind that of other taxa. For example, isotopic turnover rates in tissues of elasmobranchs have only been reported for two species (δ^{15}N in captive *Potamotrygon motoro*; MacNeil et al. 2006; δ^{15}N and δ^{13}C in captive *Carcharhinus plumbeus*; Logan and Lutcavage 2010), compared to numerous studies investigating isotopic turnover rates in mammals (e.g. MacAvoy et al. 2006, Miller et al. 2008), birds (e.g. Hobson and Clark 1992, Haramis et al. 2007), and bony fishes (e.g. Jardine et al. 2004, Perga and Gerdeaux 2005, McIntyre and Flecker 2006). In addition to understanding turnover rates, it is important to understand the variability of isotopic values for various tissue types within an individual in order to make full use of stable isotopic data and compare information among studies (e.g. Pinnegar and Polunin 1999, Vander Zanden and Rasmussen 2001, Sweeting et al. 2005).

The purpose of this study was to (1) compare the δ^{13}C and δ^{15}N values of muscle, blood, and dorsal fin tissues from juvenile bull sharks (*Carcharhinus leucas*) and blood and dorsal fin tissues of large (juvenile and adult) tiger sharks (*Galeocerdo cuvier*) to determine if resulting intra-specific values from one tissue are comparable to those of other tissues for each species, and (2) gain insights into how differences among tissues within individuals may vary with shark size. Understanding if stable isotope analysis provides relatively consistent dietary data across tissue types, and if this consistency is similar across size-classes, may allow for less invasive sampling of tissues, and provide insight into ecological drivers of dietary variation.

Methods

Muscle, whole blood ("blood" hereafter), and dorsal fin ("fin") tissues were collected from 81 juvenile bull sharks (70-162 cm total length) captured on 500m longlines within the Shark River estuary of Everglades National Park, Florida, USA (see Heithaus et al. 2009 for specific details of the study area and capture methods). We used a biopsy punch to collect a 0.5 cm^3 muscle tissue biopsy *ca.* 5 cm lateral to the first dorsal fin, scissors to collect a 0.5 cm^3 tissue clip from the dorsal fin, and an 18 gauge needle to collect 2 ml of blood from the caudal vein. Tissues were placed on ice and frozen upon return to the laboratory. Skin was removed from muscle samples before laboratory preparations. All samples were dried and homogenized. Blood and fin clips were collected from 46 tiger sharks (159-396 cm TL) captured on drumlines during long-term studies in the hypersaline seagrass ecosystem of Shark Bay, Western Australia (see Wirsing et al. 2006 for study site and sampling details). Sample collection, storage, and processing protocols were identical to those for bull sharks.

All samples were analyzed at the Florida International University Stable Isotope Facility (43 *C. leucas* blood samples, 50 *C. leucas* muscle samples, and 26 *C. leucas* fin samples) or the Yale Earth System Center for Stable Isotopic Studies (34 *C. leucas* blood samples, 27 *C. leucas* muscle samples, 19 *C. leucas* fin samples, 46 *G. cuvier* blood samples, and 46 *G. cuvier* fin samples). Lipids were not extracted from any tissues, and C:N ratios indicated that corrections for lipid content were not necessary (Post et al. 2007). To verify analytical consistency, we randomly selected samples to be analyzed at both Florida International University and Yale University, for which the variation between resulting $\delta^{13}C$ $\delta^{15}N$ values were 0.13‰ ± 0.20SE.

We used least squares regression analysis to determine (1) the relationships between $\delta^{13}C$ and $\delta^{15}N$ values for all paired tissues of bull sharks (i.e. blood-muscle, blood-fin, muscle-fin) and tiger sharks (i.e. blood-fin), and (2) the relationship between shark length and paired differences between tissues. Each paired difference was calculated by taking the absolute difference between the $\delta^{13}C$ or $\delta^{15}N$ values of two tissue types for each shark (e.g. if muscle = -13.1‰ and blood = -13.8‰, then the paired difference = 0.7‰). Cook's test was used to identify outliers, each tissue comparison regression model slope was tested to determine if it deviated significantly from a slope of one, and paired difference models were tested as linear and polynomial models to identify the best fitting model. Because isotope assimilation into body tissues experiences a lag time based on the turnover rate of the specific tissue type (reviewed by Martinez del Rio et al. 2009), and sharks can experience ontogenetic shifts in diet (reviewed by Weatherbee and Cortes 2004), in some cases polynomial models may produce the best fit for determining the relationship between isotope values and shark size.

Results

Comparisons of $\delta^{13}C$ and $\delta^{15}N$ values revealed highly significant positive correlations for all tissue pairs in bull sharks. The slopes of all three bull shark $\delta^{13}C$ comparisons did not differ from 1:1 and all R^2 values were >0.71 (Fig.1a, c, e). Blood was on average 0.57‰ ± 0.055 SE more depleted (i.e. more negative) than muscle and on average 2.8‰ ± 0.10 SE more depleted than fin, and muscle was on average 2.1‰ ± 0.092 SE more depleted than fin (Fig. 1a, c, e). Relationships between $\delta^{15}N$ values were significant, but weaker than those of $\delta^{13}C$, with R^2 values between 0.15-0.43 (Fig. 1b, d, f). Only the relationship between muscle and fin deviated from a slope of one (slope = 0.6, $t_{41} = -7.8$, $p = <0.001$). Mean differences for bull shark blood and muscle $\delta^{15}N$ was 0.80‰ ± 0.064 SE, blood and fin was 0.65‰ ± 0.16 SE, and muscle and fin was 0.20‰

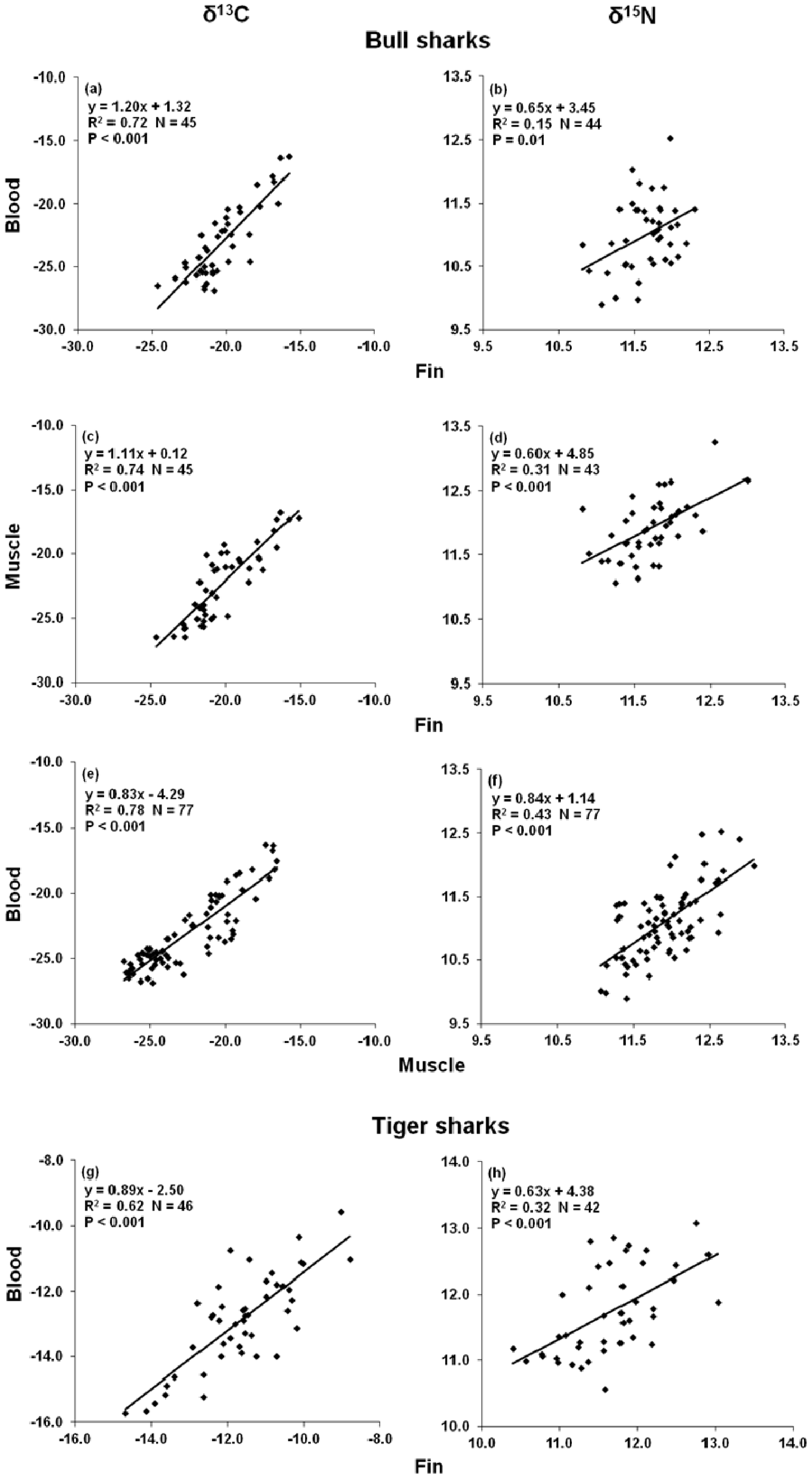

Figure 1: Comparisons of δ^{13}C for blood and fin (a), muscle and fin (c), and blood and muscle (e), and comparisons of δ^{15}N for blood and fin (b), muscle and fin (d), and blood and muscle (f) for *Carcharhinus leucas*, and δ^{13}C for blood and fin (g), and δ^{15}N for blood and fin (h) for *Galeocerdo cuvier*.

± 0.15 SE (Fig. 1b, d, f). The ranges of $\delta^{13}C$ values were relatively wide for all bull shark tissue types, while the ranges of $\delta^{15}N$ values were relatively narrow (Table 1).

Relationships between tissue types were similar in tiger sharks. Correlations for $\delta^{13}C$ and $\delta^{15}N$ of blood and fin were positive and significant, but the relationship was tighter for $\delta^{13}C$ ($R^2 = 0.62$) than for $\delta^{15}N$ ($R^2 = 0.32$) (Fig. 1g, h). The slope for $\delta^{13}C$ was not significantly different from one, but the slope for $\delta^{15}N$ was (slope = 0.63, $t_{40} = -10.0$, $p = <0.001$). For tiger sharks, the $\delta^{13}C$ of blood was on average 1.2‰ ± 0.26 SE more depleted than fin while the mean difference in $\delta^{15}N$ was only 0.09‰ ± 0.21 SE (Fig. 1g, h). Similar to the bull sharks, the ranges of $\delta^{13}C$ values were relatively wider than those of $\delta^{15}N$ values (Table 1).

Based on the tight relationships in isotopic values of tissues, it is not surprising most tissue types showed similar relationships between $\delta^{13}C$ and $\delta^{15}N$ and shark total length. For both $\delta^{13}C$ and $\delta^{15}N$ in bull sharks, all tissues declined until 110-130 cm TL, and then increased (Fig. 2a-f). All relationships between isotope values and shark total length were significant ($p < 0.05$) for bull sharks. For tiger sharks, $\delta^{13}C$ of fin and blood slightly increased with size until 250-300 cm TL, and then declined (Fig. 2g and i), while $\delta^{15}N$ declined with size until 250-300 cm TL, and then increased (Fig. 2h and j). Only the relationship between blood $\delta^{13}C$ values and tiger shark total length was significant.

The difference in $\delta^{13}C$ values between tissue types for bull sharks was influenced by shark total length for all pairings. In all cases for bull sharks, paired differences in $\delta^{13}C$ values were highest for the smallest individuals and decreased with size. This relationship was strongest for fin and blood ($R^2 = 0.64$), and weakest for fin and muscle ($R^2 = 0.21$; Fig. 3a, c, e). The paired difference between muscle and blood dropped

		Min $\delta^{13}C$	Max $\delta^{13}C$	Min $\delta^{15}N$	Max $\delta^{15}N$
Bull Sharks	Blood	-26.86	-16.27	9.91	12.53
	Muscle	-26.79	-16.51	11.07	13.26
	Fin	-24.62	-15.13	10.81	13.00
Tiger Sharks	Blood	-15.72	-9.56	10.57	13.09
	Fin	-14.69	-8.77	10.41	13.03

Table 1: Minimum and maximum values for $\delta^{13}C$ and $\delta^{15}N$ values for blood, muscle, and fin for *Carcharhinus leucas* and blood and fin for *Galeocerdo cuvier* in ‰.

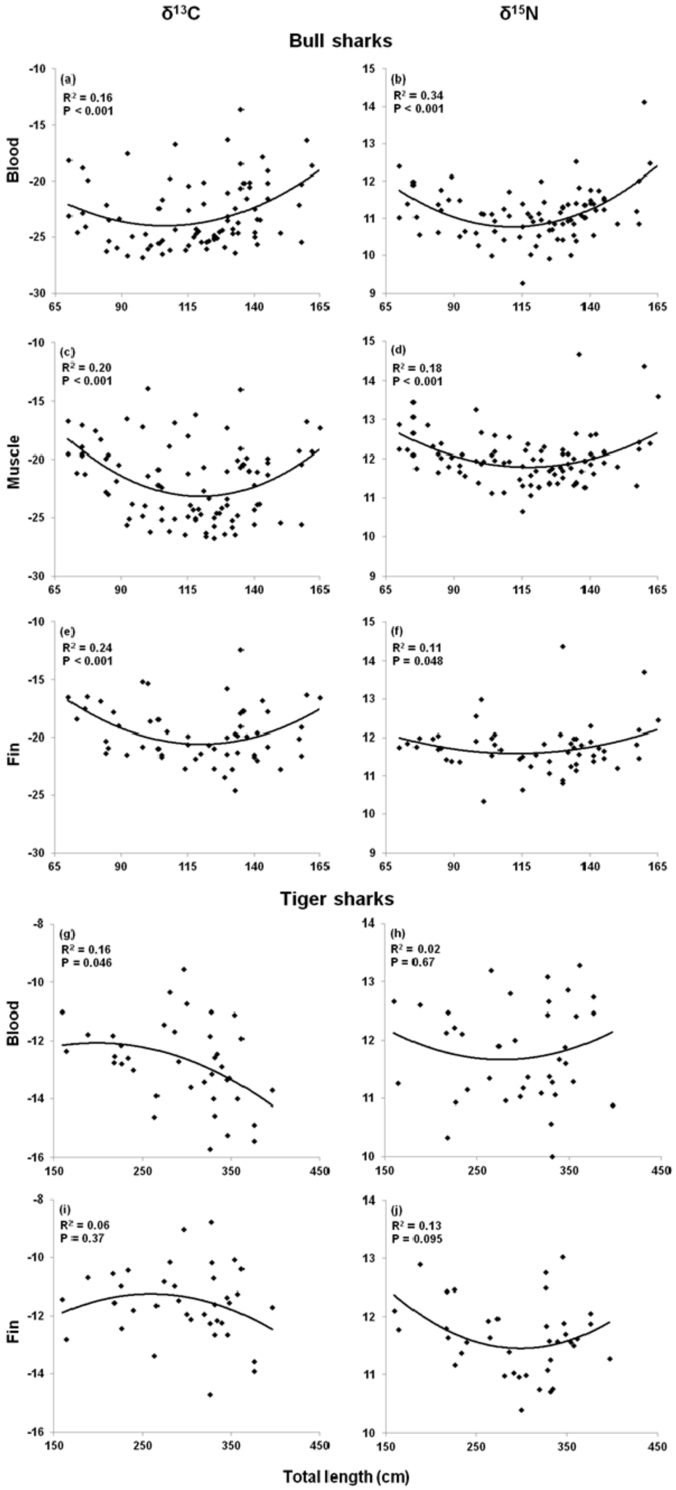

Figure 2: Comparisons of δ^{13}C and shark total length for fin (a), blood (c), and muscle (e), and comparisons of δ^{15}N and shark total length for fin (b), blood (d), and muscle (f) for *Carcharhinus leucas*, and δ^{13}C and shark total length for fin (g) and blood (i), and δ^{15}N and shark total length for fin (h) and blood (j) for *Galeocerdo cuvier.*

rapidly until ~110cm TL, when the direction of the difference became less predictable. The difference between fin and blood dropped linearly and approached zero at approximately 165cm TL, and the difference between fin and muscle showed a relatively weak relationship with shark length. Paired differences for $\delta^{15}N$ of bull sharks showed a different pattern. There was no significant relationship between shark size and tissue difference in $\delta^{15}N$ of fin and muscle, while somewhat weak, but significant, nonlinear relationships were found for comparisons between blood and muscle ($R^2 = 0.18$), and blood and fin ($R^2 = 0.39$; Fig. 3b, d, f). The difference in $\delta^{15}N$ for these comparisons was relatively low at small total lengths, increased slightly with size, and then declined in the largest individuals.

For tiger sharks, there was a significant but relatively weak ($R^2 = 0.27$), positive effect of shark size on differences in $\delta^{13}C$ of fin and blood, and shark size explained no variation in differences between $\delta^{15}N$ of fin and blood (Fig. 3g, h).

Discussion

Our study of two shark species at different life history stages, and from two different environments, has important implications for using stable isotope data in studies of elasmobranchs. Variability in stable isotope values within and among individuals can be driven by many ecological factors, including environmental conditions, metabolic processes, food quality, or changes in behavior, among many other factors (reviewed by Martinez del Rio et al. 2009). Yet, patterns of variability in stable isotope values among individuals can provide important insights into the trophic ecology of individuals within a population, as well as into differences among population and species.

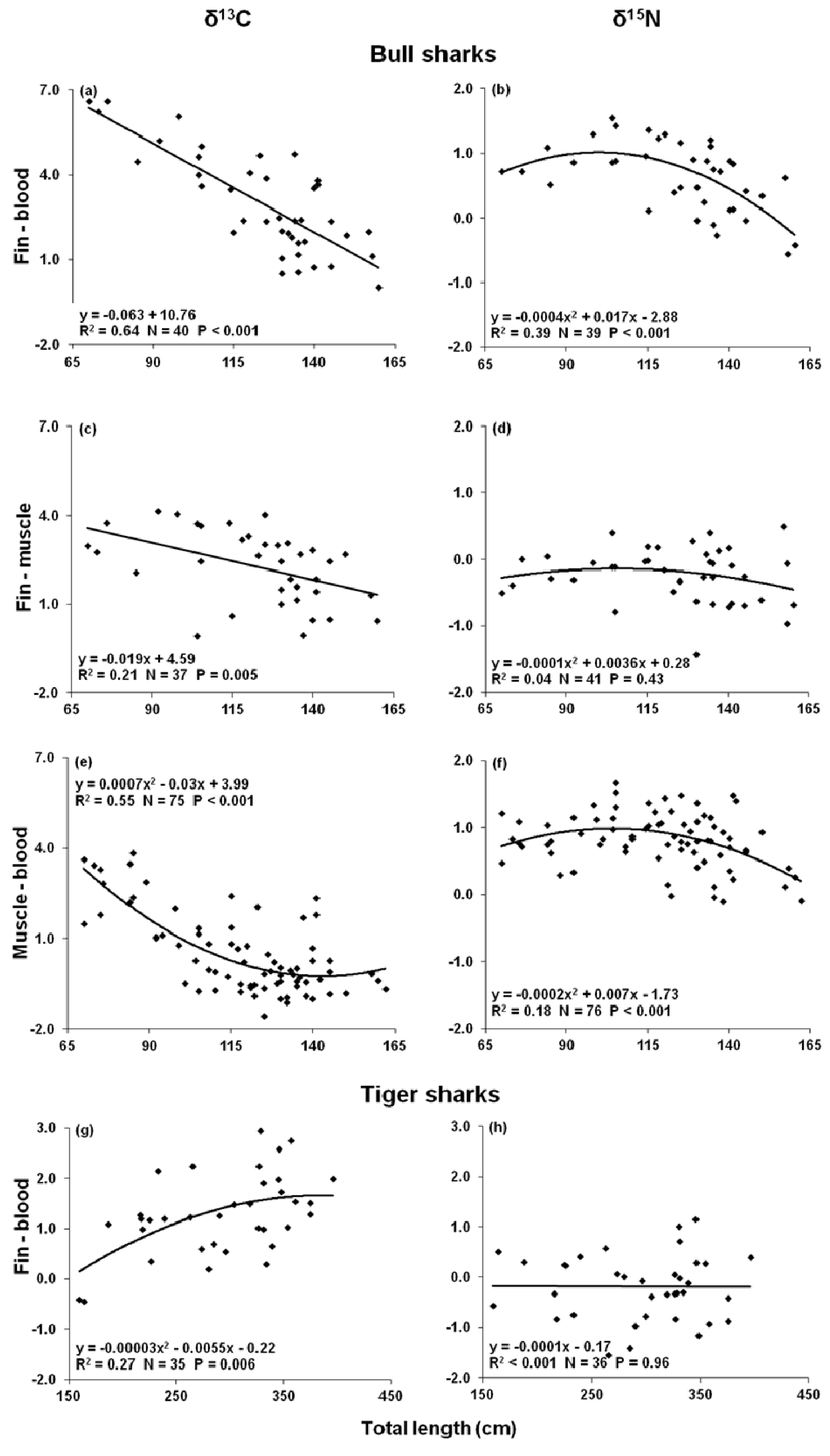

Figure 3: Paired differences of δ^{13}C for blood and fin (a), muscle and fin (c), and blood and muscle (e), and of δ^{15}N for blood and fin (b), muscle and fin (d), and blood and muscle (f) for *Carcharhinus leucas*, and δ^{13}C for blood and fin (g), and δ^{15}N for blood and fin (h) for *Galeocerdo cuvier.*

Body size appears to be one factor that explained the regression slopes for some of the inter-tissue paired differences for our sample populations (Fig. 3). The paired differences in $\delta^{13}C$ of bull shark tissues were greatest in smaller individuals and decreased with size, indicating that isotopic values of different tissues were more similar for larger individuals. Prior to birth, bull sharks are directly connected to their mothers by an umbilical cord, which serves as a pathway through which nutrients and energy are transferred between mother and fetus. Based on the presence of open umbilical scars, bull sharks in the coastal Everglades are born between 65-75 cm TL. Because of their connection to their mothers, pups should have $\delta^{13}C$ values similar to their mothers (coastal predators; $\delta^{13}C$ ~-15‰ in our study area; Chasar et al. 2005), as seen in cetaceans (e.g. bottlenose dolphins, *Tursiops truncatus,* Knoff et al. 2008; sea lions, *Zalophus californianus,* Porras-Peters et al. 2008). After birth, juvenile sharks spend several years in low-salinity estuaries and nearshore waters (e.g. Wiley and Simpfendorfer 2007, Heithaus et al. 2009), and therefore $\delta^{13}C$ values should begin to diverge from their mothers as they adopt a more $\delta^{13}C$-depleted estuarine diet (consumer taxa $\delta^{13}C$ is typically < -25‰ in the Shark River; Williams and Trexler 2006, M. Heithaus *unpublished data;* see also Fig 2). The change in $\delta^{13}C$ values should occur earlier in tissues that turnover more rapidly. For example, differences between blood and both fin and muscle in the smallest bull sharks suggests that fin tissue largely maintains the maternal signature, likely due to a slower turnover rate. In contrast, blood reflects the young sharks' diet within two years of birth, likely due to a faster turnover rate in this tissue type (MacNeil et al. 2006).

The regression model for the paired difference of $\delta^{13}C$ for muscle and blood appears to reach equilibrium around 110 cm TL and two years of age (based on growth rates in Branstetter and Stiles 1987 and estimated sizes at birth; Heithaus et al. 2009). This may indicate the time period for which muscle $\delta^{13}C$ values are no longer influenced by the maternal diet for juveniles, and accurately portray that individual's diet over its lifetime. Deviations in isotope values of larger individuals may reflect other underlying ecological patterns, for example seasonal shifts in diet, which may be displayed more rapidly in blood values than in muscle or fin (P. Matich et al. *unpublished data*). In contrast to bull sharks, differences in $\delta^{13}C$ among blood and fin clips increased with size in tiger sharks. This likely reflects a difference in the feeding ecology of the two species, and the increasing difference in $\delta^{13}C$ of blood and fin may reflect a shift in the diets of tiger sharks as they grow (e.g. Lowe et al. 1996, Simpfendorfer et al. 2001).

Size-based differences among tissues in stable isotope values are important to consider when investigating the ecological drivers of dietary variation within populations. $\delta^{13}C$ values (Fig. 2a, c, e) support the hypothesis that the maternal influence on isotopic values of juvenile bull sharks is evident for several years, but individual variability in isotopic values makes it difficult to draw conclusions about the precise timing of tissue values equilibrating. Especially for $\delta^{13}C$ of both species, the range of isotope values was relatively wide, even for sharks of a given size, suggesting that other factors, like habitat use (e.g. Darimont et al. 2009, Quevedo et al. 2009), body condition (e.g. Tinker et al. 2008, Tucker et al. 2009), and/or seasonal shifts (e.g. Inger et al. 2006, Cherel et al. 2007) may affect the diet patterns for individuals of these two populations.

The strong positive correlations between tissues in $\delta^{13}C$ for both bull sharks and tiger sharks (Fig. 1) suggest that for a species, multiple tissues may be compared after applying a correction factor. A strict 1:1 substitution of values among tissues is not recommended, and we suggest correction factors should be generated for individual populations because ecological differences may lead to variability in isotopic assimilation across individuals of the same taxa (Post 2002). Using correction factors generated for a species in one ecosystem may differ from those generated for the same species collected from a different ecosystem, and therefore it is currently most appropriate to generate correction factors on a per-population basis.

Tissue comparisons may allow for gaps within data sets to be filled and to increase the number of individuals that can be directly compared. Individuals for which isotope values of a particular tissue are not available may have correction factors applied to estimate isotopic value(s) of the uncollected tissue. Yet, it is important to consider potential factors that limit the use of correction factors. Species that experience ontogenetic shifts in diet may experience variability in inter-tissue relationships between isotope values (e.g. Quillfeldt et al. 2008, Tierney et al. 2008, Young et al. 2010), and therefore correction factors may be more accurate for certain age/size-classes of animals. For example, the difference between tissues for bull sharks (paired differences; Fig. 3) were largest (7‰ fin-blood) for the smallest individuals sampled, and tended to decrease and approach equilibrium (1:1 relationship) as bull shark total length increased. This suggests that correction factors may be more useful for larger individuals, which generally had smaller differences in isotope values for different tissues. Therefore, care must be taken when using correction factors and variability in factors that affect trophic role (such as body size) must be taken into consideration prior to using estimated isotope values produced by correction factors for diet analysis.

Relationships among tissues in $\delta^{15}N$ were relatively weak, raising doubts as to whether tissues can be compared reliably. The relatively small range in $\delta^{15}N$ for both species (3.3‰ and 3.4‰ for tiger sharks and bull sharks, respectively), however, could be responsible for these patterns, and the question of interest may determine the magnitude of potential error when substituting $\delta^{15}N$ values for different tissue types when using correction factors. The paired differences in $\delta^{15}N$ for bull sharks ($R^2 = 0.04$ to 0.39) and tiger sharks ($R^2 < 0.01$) were relatively weak, suggesting that combining data sets with multiple tissue types may be problematic for $\delta^{15}N$. Because we found the $\delta^{15}N$ relationships to be relatively weak, we suggest that further ecological and physiological studies are needed to elucidate the factor(s) affecting inter-tissue differences in $\delta^{15}N$.

Published turnover rates for elasmobranch tissues (MacNeil et al. 2006), combined with the long duration before convergence of $\delta^{13}C$ values of blood and muscle of bull sharks in our study, suggest that using stable isotopes from these tissues are most appropriate for elucidating long-term dietary patterns. Such long-term information may be useful for investigating questions such as the degree of specialization within populations, how changes in environmental factors may influence consumer diets, and what ecological factors influence inter-population variation in feeding behaviors. Other taxa exhibit considerably faster turnover rates for blood (e.g. ~52 days ($\delta^{13}C$) and ~46 days ($\delta^{15}N$) for mice (*Mus musculus*) MacAvoy et al. 2006), muscle (e.g. 4-5 months ($\delta^{15}N$) for whitefish (*Coregonus lavaretus*) Perga and Gerdeaux 2005), and fin (e.g. ~37 days ($\delta^{15}N$) for armored catfish (*Ancistrus triradiatus*) McIntyre and Flecker 2006) tissues, allowing for more fine-scale diet studies. Therefore, stomach content analysis remains an important complimentary method for studying elasmobranch trophic ecology, especially when investigating short-term variability in diets.

Our understanding and application of stable isotopes in elasmobranchs is still in its infancy. Sharks and rays are important top and mesopredators in multiple ecosystems (Heithaus et al. 2010). With many populations jeopardized worldwide, stable isotope analysis provides an important tool for studying their trophic ecology non-lethally. Yet, further studies in the field and laboratory, and across a variety of taxa, environments, and life history stages, are needed to better understand how stable isotopes can be best applied and interpreted for studies of their trophic ecology.

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CHAPTER V

CONTRASTING PATTERNS OF INDIVIDUAL SPECIALIZATION AND TROPHIC COUPLING IN TWO MARINE APEX PREDATORS

Matich, P., Heithaus, M.R., and Layman, C.A. (2011). Contrasting patterns of individual specialization and trophic coupling in two marine apex predators. Journal of Animal Ecology, 80:295-304.

Summary

1. Apex predators are often assumed to be dietary generalists and, by feeding on prey from multiple basal nutrient sources, serve to couple discrete food webs. But there is increasing evidence that individual level dietary specialization may be common in many species, and this has not been investigated for many marine apex predators.
2. Because of their position at or near the top of many marine food webs, and the possibility that they can affect populations of their prey and induce trophic cascades, it is important to understand patterns of dietary specialization in shark populations.
3. Stable isotope values from body tissues with different turnover rates were used to quantify patterns of individual specialization in two species of "generalist" sharks (bull sharks, *Carcharhinus leucas*, and tiger sharks, *Galeocerdo cuvier*).
4. Despite wide population-level isotopic niche breadths in both species, isotopic values of individual tiger sharks varied across tissues with different turnover rates. The population niche breadth was explained mostly by variation within individuals suggesting tiger sharks are true generalists. In contrast, isotope values of individual bull sharks were stable through time and their wide population level niche breadth was explained by variation among specialist individuals.
5. Relative resource abundance and spatial variation in food-predation risk tradeoffs may explain the differences in patterns of specialization between shark species.
6. The differences in individual dietary specialization between tiger sharks and bull sharks results in different functional roles in coupling or compartmentalizing distinct food webs.

7. Individual specialization may be an important feature of trophic dynamics of highly mobile marine top predators and should be explicitly considered in studies of marine food webs and the ecological role of top predators.

Introduction

Populations of large marine predators are declining rapidly worldwide (e.g. Myers & Worm 2003; Estes *et al*. 2007; Ferretti *et al*. 2010), which may lead to marked changes in community structure and ecosystem function (Heithaus *et al.* 2008). While numerous studies have shown that removal of top predators can have significant consequences for marine communities, the scope, magnitude, and context-dependence of these effects are only starting to be realized (Heithaus *et al.* 2008). In many cases, our understanding of the ecological role of large marine predators, and potential consequences of their declines, is hindered by a lack of data on their trophic ecology.

In addition to top-down impacts on prey species, an important ecological function of predators is the coupling of energy pathways from distinct food webs (Rooney *et al.* 2006). This occurs when lower trophic level consumers derive their energy from a single source (i.e. primary producer base), but at increasing trophic levels consumers tend to incorporate energy from a wider range of prey serving to couple multiple energetic pathways (Rooney *et al.* 2006; Rooney, McCann & Moore 2008). Such coupling is often evaluated at a population level, ignoring the behaviors and habits of individuals. Populations of "generalist" predators may in fact be a collection of individual-level trophic specialists that vary considerably in their resource use (e.g. Urton & Hobson 2005; Woo *et al.* 2008). At a population level, predator species may incorporate prey taxa from multiple food webs into their diets, but individual-level dietary specialization may serve to keep energy pathways from discrete food webs separate. For example, Eurasian perch (*Perca fluviatilis*), which have a wide niche width at the population level, segregate into littoral and pelagic specialists, and consequently individuals do not couple these two components of freshwater food webs (Quevedo, Svanback & Eklov 2009).

Individual specialization within populations may be more likely under conditions of 1) resource scarcity, 2) interhabitat differences in resource availability, 3) fitness trade-offs that result in individual-specific behavior, 4) cultural transmission of foraging traditions, and/or 5) cognitive constraints that limit the use of diverse sets of resources (e.g. Rendell & Whitehead 2001; Estes *et al.* 2003; Svanback & Persson 2004; Araujo & Gonzaga 2007; Darimont, Paquet & Reimchen 2009). Recent studies have investigated individual dietary specialization in birds (e.g. Inger *et al.* 2006; Martinez del Rio *et al.* 2009a), mammals (e.g. Urton & Hobson 2005; Newsome *et al.* 2009), and bony fishes (e.g. Beaudoin *et al.* 1999; Quevedo *et al*. 2009), but few studies have considered individual specialization in large, non-mammalian, marine predators that use multiple ecosystems.

Here we investigate whether two species of sharks, in two distinct ecosystems, exhibit individual trophic specialization. Specifically, we used stable isotope analysis of multiple tissues with different turnover rates, to reveal patterns of variation in diets within and among individual bull sharks (*Carcharhinus leucas* Müller & Henle, 1839) inhabiting an oligotrophic coastal estuary, and among individual tiger sharks (*Galeocerdo cuvier* Peron & LeSueur, 1822) in a relatively pristine seagrass community. Our study investigates if predator populations can be treated as homogeneous units, or if an individual level approach is essential to understand the full range of trophic roles that these populations fill (Estes *et al.* 2003; Svanback & Persson 2004; Ravigne, Dieckmann & Olivieri 2009).

Methods

Coastal Everglades, Florida

The Shark River Estuary of Everglades National Park, Florida, USA (Fig. 1), is the main drainage basin for the Everglades (Childers 2006). The ecosystem is primarily a braided stream lined by mangroves that extend more than 30km upstream from the Gulf of Mexico, before giving way to freshwater vegetated marshes. It is considered a relatively oligotrophic, phosphorus-limited system (Childers *et al.* 2006). The Shark River Estuary is a nursery for juvenile bull sharks, which may be found from the mouth of the river to more than 27 km upstream (Wiley & Simpfendorfer 2007; Heithaus *et al.* 2009). Bull sharks are one of the largest-bodied predators in the ecosystem. Bull sharks in coastal ecosystems have a relatively wide dietary niche at the population level, preying on teleosts, mollusks, crustaceans, cephalopods, and other elasmobranchs (Snelson & Williams 1981; Snelson, Mulligan & Williams 1984; O'Connell *et al.* 2007).

Bull sharks were captured from 2005-2009 on ~500m longlines fitted with 40-55 14/0 or 15/0 Mustad tuna circle hooks baited with mullet (*Mugil* sp.) and attached to ~2m of 400kg monofilament line (see Heithaus *et al.* 2009 for details). Captured sharks were processed alongside the sampling vessel, or within a water-filled, aerated cooler on board. We used a biopsy punch to collect a 0.5 cm^3 muscle tissue biopsy *ca.* 5 cm lateral to the first dorsal fin, and an 18 gauge needle to collect 4ml of blood from the caudal vein. From the blood, 3ml was placed into BD Vacutainer blood collection vials with neither additives nor interior coating, and separated into its components, including plasma, using a centrifuge spun for one minute at 3000rpm. The remaining 1ml of blood was retained in its original composition (whole blood, "blood" hereafter). Tissues were

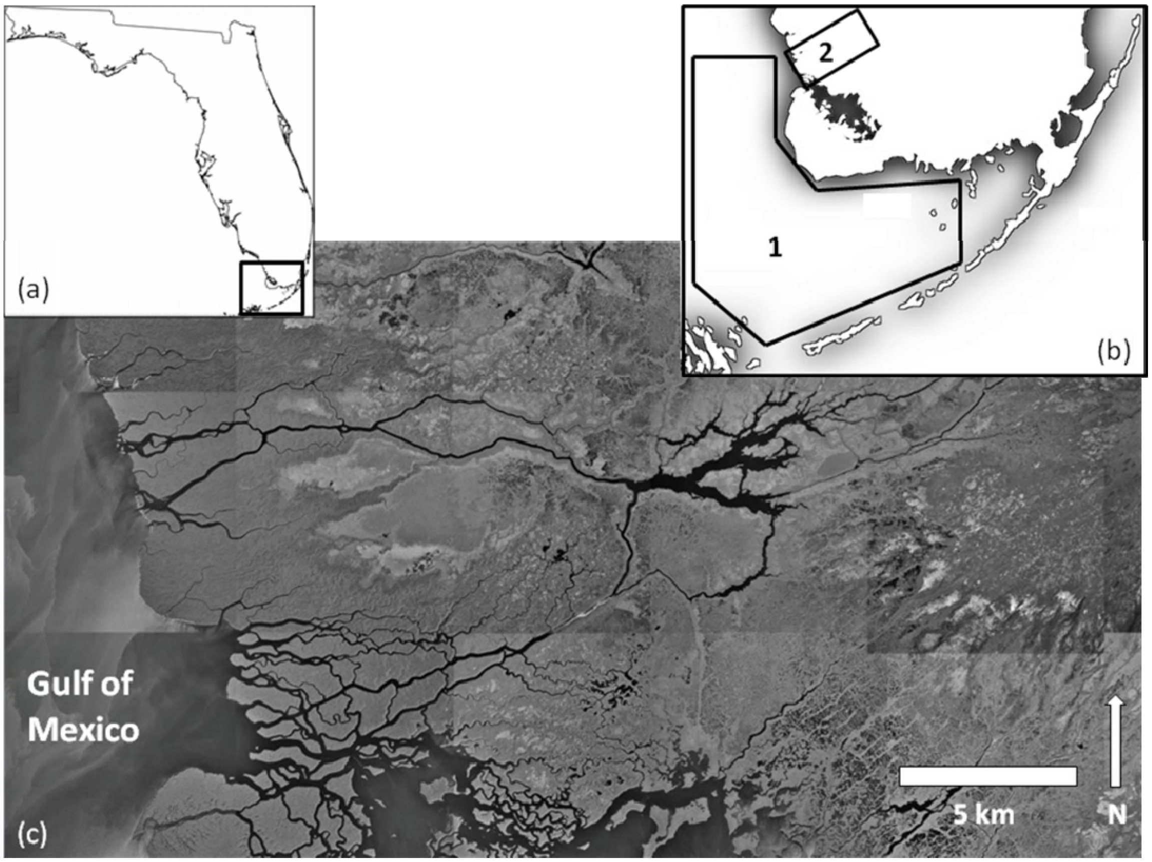

Figure 1. Coastal habitats of south Florida (a) can be divided into marine (1) and freshwater/estuarine (2) food webs (b). Juvenile bull sharks were sampled in the Shark River Estuary (c), which is within the freshwater/estuarine food web.

placed on ice and frozen upon return to the laboratory. Skin was removed from muscle samples before laboratory preparations. Because muscle tissue of sharks may incorporate isotopes from their diet over a temporal scale of many months (e.g. MacNeil, Drouillard & Fisk 2006; Logan & Lutcavage 2010; Matich, Heithaus & Layman 2010; S. Kim personal communication), only bull sharks over 99cm in total length (approximately 1-2 years old and older) were included in analyses to eliminate any potential maternal isotopic influence.

To determine the community context of trophic interactions in the Shark River Estuary, we defined ranges of δ^{13}C that were representative of two "endpoint" habitats: (1) mangrove creeks and estuarine marshes within the Shark River Slough (i.e. from the mouth of the estuary and upstream, termed the "freshwater/estuarine food web") (Fry & Smith 2002; Williams & Trexler 2006), and (2) fully marine habitats (e.g., seagrass beds) in Florida Bay ("marine food web") (Chasar *et al.* 2005) (Fig. 1). From the existing literature and our own analyses, we compiled both primary producers and "resident" consumers, i.e., taxa that are largely restricted in their distribution to one of the two habitat "endpoints" and would be unlikely to move between them. Sampling of bull sharks in this study was entirely within the boundaries of the "freshwater/estuarine food web".

Shark Bay, Australia

Shark Bay is a large, seagrass-dominated, subtropical bay located along the central Western Australian coast. The study took place in the Eastern Gulf, offshore of Monkey Mia Dolphin Resort. The study site is made up of a series of shallow (<4m depth) seagrass-covered banks and deep channels (see Wirsing, Heithaus & Dill 2006 for detailed description). Tiger sharks are the top predator in the ecosystem, and more than 95% of catches of sharks >2m are tiger sharks (Heithaus 2001; Wirsing *et al.* 2006). Tiger sharks are widely considered to be one of the most generalized of sharks in terms of diet, which may include mollusks, cephalopods, elasmobranchs, teleosts, reptiles (sea snakes, sea turtles), and marine mammals (Compagno 1984; Lowe *et al.* 1996; Simpfendorfer, Goodreid & McAuley 2001).

Tiger sharks were captured from 2007-2009 on drumlines equipped with a single Mustad shark hook (12/0-14/0) (see Wirsing *et al.* 2006 for details). Captured sharks were processed alongside the sampling vessel. Blood and plasma were collected in the same manner as with bull sharks, and scissors were used to collect a 0.5 cm^2 tissue clip from the dorsal fin (fin tissue was collected and used for analyses rather than muscle tissue because of the difficulty in collecting muscle from large tiger sharks). Samples were processed in the same manner as those for bull sharks.

Similar to the Shark River Estuary, we defined ranges of $\delta^{13}C$ that were representative of two discrete food webs to establish the ecosystem context of trophic interactions in Shark Bay: (1) "benthic food web" (likely based on seagrass and associated microphytobenthos) and (2) "pelagic food web", which would be expected to be based on autochthonous seston production.

Stable isotope analysis

All shark samples were analyzed at the Florida International University Stable Isotope Facility (43 *C. leucas* blood samples and 50 *C. leucas* muscle samples) or the

Yale Earth System Center for Stable Isotopic Studies (15 *C. leucas* plasma samples, 28 *C. leucas* blood samples, 21 *C. leucas* muscle samples, 21 *G. cuvier* plasma samples 46 *G. cuvier* blood samples, and 46 *G. cuvier* fin samples). Lipid extraction was not performed because C:N ratios (bull shark mean muscle = 3.1 ± 0.3 SD , mean blood = 2.7 ± 0.2 SD, mean plasma = 2.0 ± 0.2 SD; tiger shark mean fin = 2.7 ± 0.1 SD, mean blood = 2.4 ± 0.0 SD, mean plasma = 2.1 ± 0.1 SD) were generally below those suggested for extraction or mathematical correction (3.5; Post *et al.* 2007), and lipid extraction appears have minimal effects (<0.6‰) on δ^{13}C values of shark muscle (Hussey *et al.* 2010). Samples from producers and invertebrates with carbonate shells were acidified for δ^{13}C values (δ^{15}N run separately). Producer and community consumer samples were analyzed at Yale Earth System Center for Stable Isotopic Studies.

To verify analytical consistency, we randomly selected samples to be analyzed at both Florida International University and Yale University, for which the variation between resulting δ^{13}C values and δ^{15}N values were 0.13‰ ± 0.20SE. The standard deviations of standards run for Yale were 0.14‰ for δ^{13}C and 0.22‰ for δ^{15}N, and 0.29‰ for δ^{13}C and 0.24‰ for δ^{15}N for Florida International.

Quantitative Analysis

Trophic specialization can be assessed by measuring the variation in the diets of individuals, and is accomplished by calculating the dietary variation within individuals (WIC: within individual component of variation) and between individuals (BIC: between individual component of variation) of a population (Roughgarden 1972, Bolnick *et al.* 2002). The WIC of a population measures how variable an individual's diet is over a given time frame. This is typically expressed as a mean value for an entire population, but also can be calculated for individuals (see 'individual variance' calculations below). The BIC of a population measures how different each individual's diet is from the other members of the population (Bolnick *et al.* 2002). For individuals and populations that are more specialized, WIC should be relatively small because individual diets show little variation and should be consistent over time. Generalist individuals should have a relatively higher WIC because these individuals have a broader dietary niche width (Bolnick *et al.* 2003). The variation between individuals (BIC) varies based on total niche width (TNW), but in general, the degree of individual specialization should increase as the BIC:WIC specialization ratio increases for a given TNW (Newsome *et al.* 2009).

Stable isotope analysis has become a popular method for addressing questions about trophic ecology and dietary specialization because 1) stable $\delta^{13}C$ isotopes can be used to assess the flow of basal nutrients through food webs and gain insights into trophic coupling (e.g. France 1997; Vander Zanden & Vadeboncoeur 2002), and 2) body tissues of individuals incorporate isotope values of their diets at various rates (e.g. Hobson & Clark 1992, Bearhop *et al.* 2004). Comparing isotopic values of multiple tissues that vary in turnover rate within an individual, therefore, provides insight into the relative temporal stability of an individual's diet, and can be used to investigate questions about individual trophic specialization (Bearhop *et al.* 2004).

Isotopic turnover rates of elasmobranchs studied to date suggest that muscle and fin have relatively long turnover rates (complete isotope turnover in 390-540 days and 576 days) and whole blood has an intermediate turnover rate (complete isotope turnover in 240-300 days; MacNeil *et al*. 2006; Logan & Lutcavage 2010, Matich *et al*. 2010) (Fig. 2). Blood plasma turns over at an even faster rate than whole blood (S. Kim, personal communication; complete isotopic turnover in plasma occurs in 72-102 days; Fig. 2), so we used plasma to provide insight into diets at shorter temporal scales. Although most of these isotope turnover rates were calculated for relatively small elasmobranchs in captive trials (*Potamotrygon motoro*: mean mass = 0.1kg; *Carcharhinus plumbeus*: mean mass = 6.4kg), and isotope turnover rates can vary with body size (e.g. Carleton & Martinez del Rio 2005, Martinez del Rio *et al*. 2009b), field studies of size-based variation among fin, muscle, and blood of bull sharks suggest that these lab-based estimates are likely similar to those found in natural settings (Matich *et al.* 2010; see discussion for further consideration of turnover rates). Furthermore, even if there is variation in absolute turnover rates based on body size, the relative turnover times of tissues (muscle/fin > whole blood > plasma) is expected to be the same.

Delta values (δ) are often used to express stable isotope data, but in order to make comparisons in specialization between tiger sharks and bull sharks, it was necessary to account for difference in their potential isotope niche width (i.e. differences in the range of $\delta^{13}C$ values). Therefore, to normalize isotope data for bull sharks and tiger sharks, we converted $\delta^{13}C$ values for tissues to proportional values (p-values; Newsome *et al.* 2007). Each system has two discrete basal resource pools with distinct $\delta^{13}C$ values: the Shark River has a "freshwater/estuarine food web" (mean $\delta^{13}C$ = -29.7‰ ± 0.7SE) and a "marine food web" (-14.5‰ ± 0.3SE), and Shark Bay has a "benthic food web" (-8.5‰ ± 0.3SE) and a "pelagic food web" (-16.1‰ ± 0.8SE). Therefore, "p-values" were calculated based on mean $\delta^{13}C$ values of available food sources for each system using a

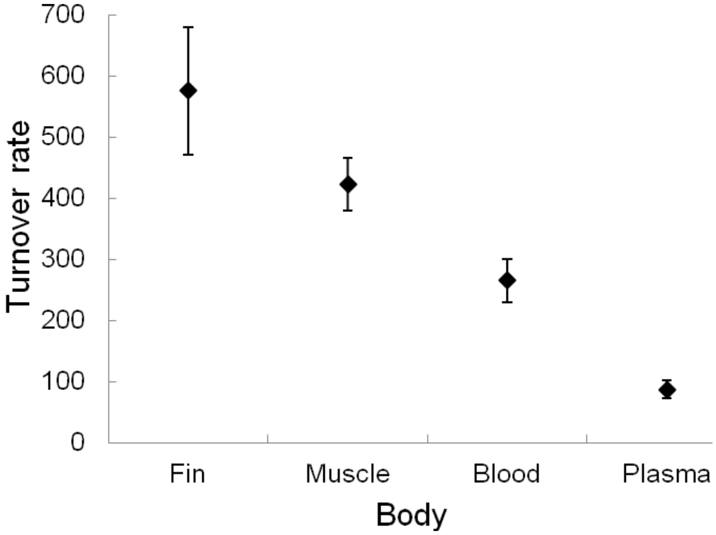

Figure 2: Estimated turnover rates (± SE) of body tissues from freshwater stingray *(Potamotrygon motoro*: MacNeil *et al*.2006 – fin, muscle, and blood) and leopard shark (*Triakis semifasciata*: Sora Kim personal communication – plasma). These turnover rates are from controlled studies using relatively small individuals, which are comparable in size to the bull sharks in this study. Tiger sharks in Shark Bay are considerably larger, and therefore turnover rates may be slower (see Discussion).

two-source mixing model (Phillips & Gregg 2001). These p-values provide a measure of the relative position of δ^{13}C values between endpoints of potential energy pathways.

To quantify dietary specialization in bull sharks and tiger sharks, we employed four different models (see below for details of each): 1) General Linear Model (GLM) using isotope p-values of two body tissues (bull sharks: muscle and whole blood; tiger sharks: fin and whole blood) with individual included as a random effect, 2) GLM using isotope p-values of three body tissues (bull sharks: muscle, whole blood, and plasma; tiger sharks: fin, whole blood, and plasma) with individual included as a random effect, 3) calculation of variance for each individual using isotope p-values of two body tissues (bull sharks: muscle and blood; tiger sharks: fin and blood), and 4) calculation of variance for each individual using isotope p-values of three body tissues (bull sharks: muscle, blood, and plasma; tiger sharks: fin, blood, and plasma). In addition, we calculated individual specialization metrics using IndSpec (Bolnick *et al*. 2002) to supplement our novel analytical framework.

Two-tissue GLM

The mean sum of squares of the two-tissue model ($E(SS_B)$), which is defined as

$$E(SS_B) = \frac{n\sum_{i=1}^{m}(\bar{Y}_i - \bar{Y})^2}{n(m-1)} \qquad (1)$$

measures the variability between individuals (a proxy for the between individual component of variation – BIC), where *m* is the total number of individuals, *i* is any individual, and *n* is the total number of tissues. The mean sum of squares of the error ($E(SS_W)$), where

$$E(SS_w) = \frac{\left[\sum_{i=1}^{m}\sum_{j=1}^{n}(Y_{ij}-Y_i)^2\right]}{m(n-1)} \qquad (2)$$

measures the variability within individuals (a proxy for the within individual component of variation – WIC), where *j* is any tissue. The resulting F-ratio ($E(SS_B)$:$E(SS_W)$) is a proxy for individual specialization within the population (a proxy of BIC:WIC). As the variation between individuals increases (i.e. BIC increases), and/or the variation within individuals decreases (i.e. WIC decreases), the ratio, and therefore relative degree of individual specialization, increases (Bolnick *et al.* 2003).

Three-tissue GLM

Employing plasma with muscle and blood (bull sharks), and fin and blood (tiger sharks), is a more rigorous test of specialization because of the rapidity with which plasma turns over. If the relatively short term isotope values of plasma are consistent with those of the slower turnover tissues, this provides additional evidence for specialization within a population. GLMs were conducted as described above, but with three tissue types employed.

Two-tissue individual variance

A GLM produces values that can be used to assess the relative specialization of a population, and these can be compared between populations. Yet, the two- and three-tissue GLMs do not provide a way to make multiple pair-wise comparisons amongindividuals within a population, and assess the frequency of individuals that are more or less specialized. To this end, variance of p-values for bull sharks (muscle and blood) and for tiger sharks (fin and blood) was calculated for each individual separately, using

$$\mathrm{Var}(X) = E[(X - \mu)^2] \qquad (3)$$

resulting in a WIC value for each individual in the two populations. The population BIC (estimated from the two-tissue random effects models) was then divided by each individual WIC, yielding a relative specialization value for each individual shark. Higher values of this index suggest a greater degree of dietary specialization, i.e., the different tissue types had more similar isotope p-values. Analysis of variance (ANOVA) was run to determine if specialization index values were related to body size (total length in cm), body condition (based on residual of length-mass relationship; only bull sharks), sex, capture season, or capture year.

Three-tissue individual variance

Similar to the three-tissue GLM, plasma was included in the individual variance analyses as a more rigorous test of individual specialization. Calculations were carried out in the same fashion as the two-tissue individual variance analysis. ANOVA was run to test the significance of the same factors (body size, condition, sex, season, and year) on specialization.

IndSpec

IndSpec is a program developed to calculate the specialization parameters described by Bolnick *et al*. (2002) from diet data. The program calculates the variability between each isotope value and relates this to individuals within the population using

$$TNW = Var(x_{ij}) \qquad (4)$$

$$WIC = E\left[Var\left(x_j \middle| i\right)\right] \qquad (5)$$

$$BIC = Var\left[E\left(x_j \middle| i\right)\right] \qquad (6)$$

where x is the diet parameter ($\delta^{13}C$ value for our study), *j* is tissue type, and *i* is individual.

Results

Characterization of food webs

The $\delta^{13}C$ values of primary producers and consumers of the Shark River Estuary (freshwater/estuarine food web) differed substantially from those found in the marine food web (Fig. 3a). Resident consumers' mean $\delta^{13}C$ values from the freshwater/estuarine food web were always lower than -25‰, and usually lower than -28‰. In contrast, consumers feeding in marine habitats had $\delta^{13}C$ values between -11‰ and -19‰. Although consumers with intermediate $\delta^{13}C$ values (-19‰ to -25‰) are found in the Shark River Estuary (e.g. snook, *Centropomus undecimalis*, $\delta^{13}C$ range = -18.9‰ to -27.3‰, M. Heithaus *unpublished data*), a review of the literature and our own sampling suggests that these consumers are relatively uncommon, and they tend to have $\delta^{13}C$ values relatively close to freshwater/estuarine consumers (e.g. snook mean $\delta^{13}C$ = -25.0‰ ± 0.6SE, M. Heithaus *unpublished data*). Several species are found in both the freshwater/estuarine and marine habitats, and often have a $\delta^{13}C$ value of the habitat where they were captured (e.g. blue crabs, *Callinectes sapidus*, Florida Bay $\delta^{13}C$ = -14.3‰; Shark River mid-estuary $\delta^{13}C$ = -27.8‰ ± 0.3SE; gray snapper, *Lutjanus griseus*, Florida

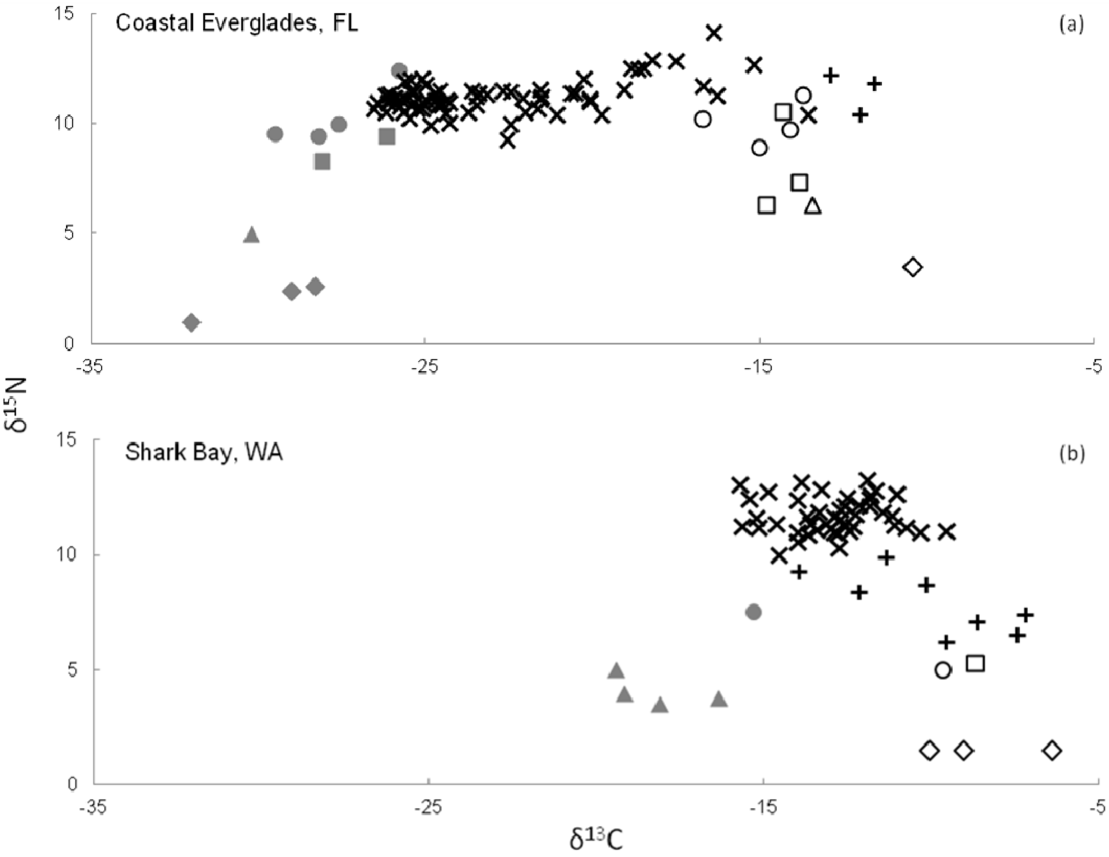

Figure 3: (A) Mean isotope values for producers and consumers in the Shark River Estuary (freshwater/estuarine food web) and surrounding marine waters. Producers and consumers from the freshwater/estuarine food web are gray and those from the marine food web are white. Producers are diamonds (♦), molluscs are triangles (▲), crustaceans are squares (■), teleosts are circles (●), other elasmobranchs are crosses (+), and bull sharks (whole blood) are X's. (B) Mean isotope values for producers and consumers in Shark Bay. Producers and consumers from the pelagic food web are gray and those from the benthic food web are white. Producers are diamonds (♦), molluscs are triangles (▲), other invertebrates are squares (■), megagrazers (i.e. dugongs and turtles) are circles (●), other elasmobranchs are crosses (+), and tiger sharks (whole blood) are X's. Standard deviations around mean values are omitted for simplicity.

Bay $\delta^{13}C$ = -13.4‰ ± 1.2SE; Shark River $\delta^{13}C$ = -28.4‰ ± 0.4SE; Chaser *et al.* 2005; C. McIvor *et al.* personal communication).

Consumers of Shark Bay, Australia were not as separated in $\delta^{13}C$ values as consumers in the Shark River, but there were still distinctions between taxa of the benthic and pelagic food webs (Fig. 3b). Primary consumers from the pelagic food web had $\delta^{13}C$ values lower than -16‰, while those from the benthic food web had $\delta^{13}C$ values higher than -10‰. Unlike the Shark River ecosystem, consumers with intermediate values were common in Shark Bay. As the trophic position (inferred by $\delta^{15}N$ value) of taxa increased, taxa mean $\delta^{13}C$ values converged toward intermediate values.

General isotope trends in sharks

Overall, we sampled 71 bull sharks (100-187cm TL) in the Shark River Estuary and 46 tiger sharks (160-396 cm TL) in Shark Bay, Australia. The mean $\delta^{13}C$ of bull sharks were: -22.8‰ ± 0.4SE (muscle), -22.9‰ ± 0.4SE (whole blood), and -21.5‰ ± 0.7SE (plasma). Mean $\delta^{13}C$ values, however, masked considerable variability, i.e. a $\delta^{13}C$ range of 12.7‰, which was 60% of the $\delta^{13}C$ range of producers and consumers in the Shark River Estuary and surrounding coastal waters of Florida Bay (~22‰). Nineteen bull sharks fell within the range of isotope values for those taxa identified in the freshwater/estuarine food web and eight fell within values of the marine food web; the rest (N= 44) had $\delta^{13}C$ values that fell between these two food webs (Fig. 3a). Tiger sharks had a narrower $\delta^{13}C$ range (5.9‰) that was 42% of the entire $\delta^{13}C$ range of producers and consumers in Shark Bay (14‰), and all individuals, except for one, had

δ^{13}C values that were intermediate between mean values of species in the benthic and pelagic food webs (Fig. 3b).

Patterns of individual specialization

Two-tissue GLMs based on muscle and blood (bull sharks), and fin and blood (tiger sharks) revealed that bull sharks tended to be more specialized and tiger sharks tended to be more generalized in their respective diets (Fig. 4a). Within-individual variation (WIC) of bull sharks (0.003) was considerably lower than that of tiger sharks (0.021), while between individual variation (BIC) was greater in bull sharks (0.08) than tiger sharks (0.06). The specialization index for tiger sharks was relatively low (2.84), suggesting that individuals were more generalized in their diet. In contrast, the specialization index was much greater for bull sharks (23.7; Fig. 4a), indicating individuals were more specialized in their diet.

Three-tissue GLM based on muscle, blood, and plasma (bull sharks), and fin, blood, and plasma (tiger sharks) strengthened trends found in the two-tissue random effects models (Fig. 4a). For individuals with more specialized diets, the value of the three-tissue specialization index should be comparable, or increase, relative to that based on two tissues, because short-term and long-term dietary variation should be similar when diets are temporally stable. In contrast, for generalists, variation within individuals (WIC) should be greater on average when including fast turnover tissues, and therefore should result in lower specialization values for analyses based on three tissues than those based on two tissues. Consistent with these predictions, when plasma was included with fin, muscle, and blood, the specialization index was considerably greater than that of the

Figure 4: a) Specialization indices of bull sharks and tiger sharks based on isotope p-values derived from muscle (M), blood (B), and plasma (P) (bull sharks) and fin (F), blood (B), and plasma (P) (tiger sharks); b) specialization comparison between bull sharks and tiger sharks using isotope p-values from muscle (M), blood (B), and plasma (P) (bull sharks) and fin (F), blood (B), and plasma (P) (tiger sharks), derived from IndSpec.

two-tissue analysis for bull sharks (42.5). And, while the specialization index increased for tiger sharks (4.37), suggestive of a more specialized diet, it was still considerably smaller than that of the bull sharks quantified using the two and three tissue models, and tiger shark WIC was greater for the three-tissue analysis (0.06), indicating tiger sharks are more generalized.

IndSpec revealed very similar patterns in the dietary specialization of bull sharks and tiger sharks (Fig. 4b) when compared to the GLMs (Fig. 4a). WIC was considerably lower for bull sharks (0.002 for both the two- and three-tissue analyses) than for tiger sharks (0.01 and 0.02 for the two- and three-tissue analyses, respectively), and BIC was comparable for the two species (0.04 for both bull shark analyses, and 0.03 and 0.04 for the tiger shark two- and three-tissue data sets, respectively). The specialization index values for bull sharks (23.4 and 19.8 for the two- and three-tissue data sets, respectively) were considerably higher than those for tiger sharks (2.8 and 2.1 for the two- and three-tissue data sets, respectively).

The two-tissue individual variance analysis revealed similar trends. A large proportion of bull shark individuals had relatively high specialization indexes (92% had specialization index vales greater than ten), while most tiger shark individuals had relatively low specialization index values (74% had a specialization index less than ten; Fig. 5a). The distribution of sharks falling into each range of specialization values was significantly different for tiger sharks and bull sharks (Kolmogorov-Smirnov test: $T_{samples} = 0.96$; $p < 0.01$). Similarly, the three-tissue individual variance analysis showed that tiger sharks were less specialized than bull sharks (Kolmogorov-Smirnov test: $T_{samples} = 1$; $p < 0.01$; Fig. 5b). In this analysis, more than 71% of tiger sharks had specialization

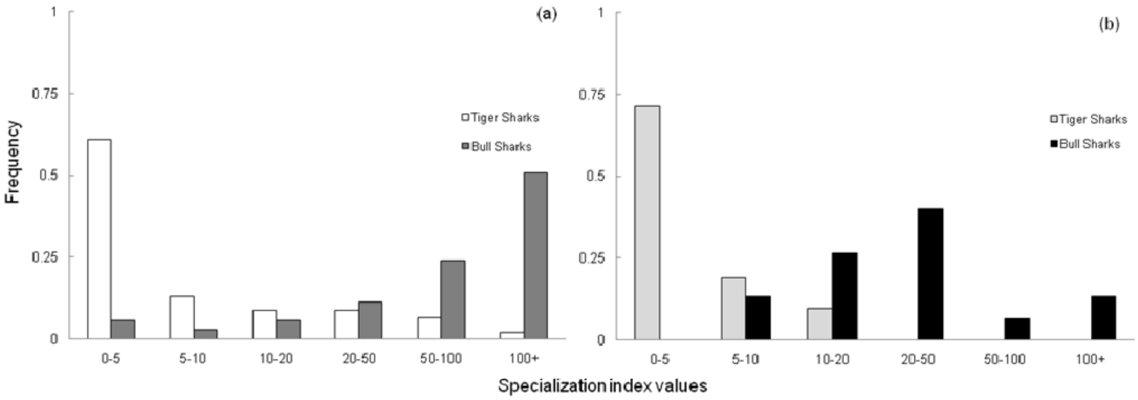

Figure 5: a) Frequency of tiger sharks and bull sharks within each range of specialization values calculated from the a) two-tissue and b) three-tissue individual variance analyses. Higher specialization index values indicates greater dietary specialization.

values below ten, while all bull sharks had specialization values above ten. Specialization index values were not directly related to shark body size, body condition, sex, season, or year (Table 1; Fig. 6).

Discussion

Variation in trophic specialization

Individual-level specialization is relatively widespread, and can be an important factor driving population-level trophic dynamics (Bolnick *et al.* 2003). Yet, with the exception of foraging behavior in marine mammals (e.g. *Orcinus orca*: Williams *et al.* 2004; *Arctocephalus gazella* and *Arctocephalus tropicalis*: Cherel *et al.* 2007; *Tursiops aduncus*: Mann *et al.* 2008; *Enhydra lutris nereis*: Newsome *et al.* 2009) and marine birds (e.g. *Phalacrocorax albiventer*: Kato *et al.* 2000; five penguin species: Cherel *et al*. 2007; *Uria lomvia*: Woo *et al*. 2008), individual specialization has been largely overlooked in marine systems, and the implications of specialization on food web dynamics has not been adequately investigated. Because of the important role sharks can play in ecosystems (see Heithaus *et al.* 2008; 2010), it is especially important to elucidate patterns of individual specialization in this group of elasmobranch fishes, and the implications this may have for food web structure and ecosystem function.

The two species of sharks studied here are widely considered to be generalist top predators in their respective ecosystems (e.g. Simpfendorfer *et al*. 2001; Weatherbee & Cortes 2004), but both the GLMs and IndSpec revealed considerable differences in the patterns of trophic specialization between them. Tiger sharks apparently were relatively generalized in their diets. Values of $\delta^{13}C$ over multiple time scales revealed that there

		Two-tissue ANOVA			Three-tissue ANOVA		
		N	F	p	N	F	p
Bull Sharks	Total length	71	0.36	0.55	15	0.16	0.69
	Sex	71	0.68	0.41	15	<0.01	0.99
	Capture season	71	2.84	0.10	15	NA	NA
	Capture year	71	0.21	0.89	15	NA	NA
	Body condition	13	0.82	0.39	13	0.07	0.79
Tiger Sharks	Total length	46	1.12	0.30	21	0.83	0.38
	Sex	46	0.34	0.57	15	NA	NA
	Capture season	46	1.28	0.28	21	0.83	0.57
	Capture year	46	1.55	0.22	21	NA	NA

Table 1. ANOVA results for effects of size, sex, season, year, and condition on dietary specialization in bull sharks and tiger sharks. NA: sample sizes not adequate for tests.

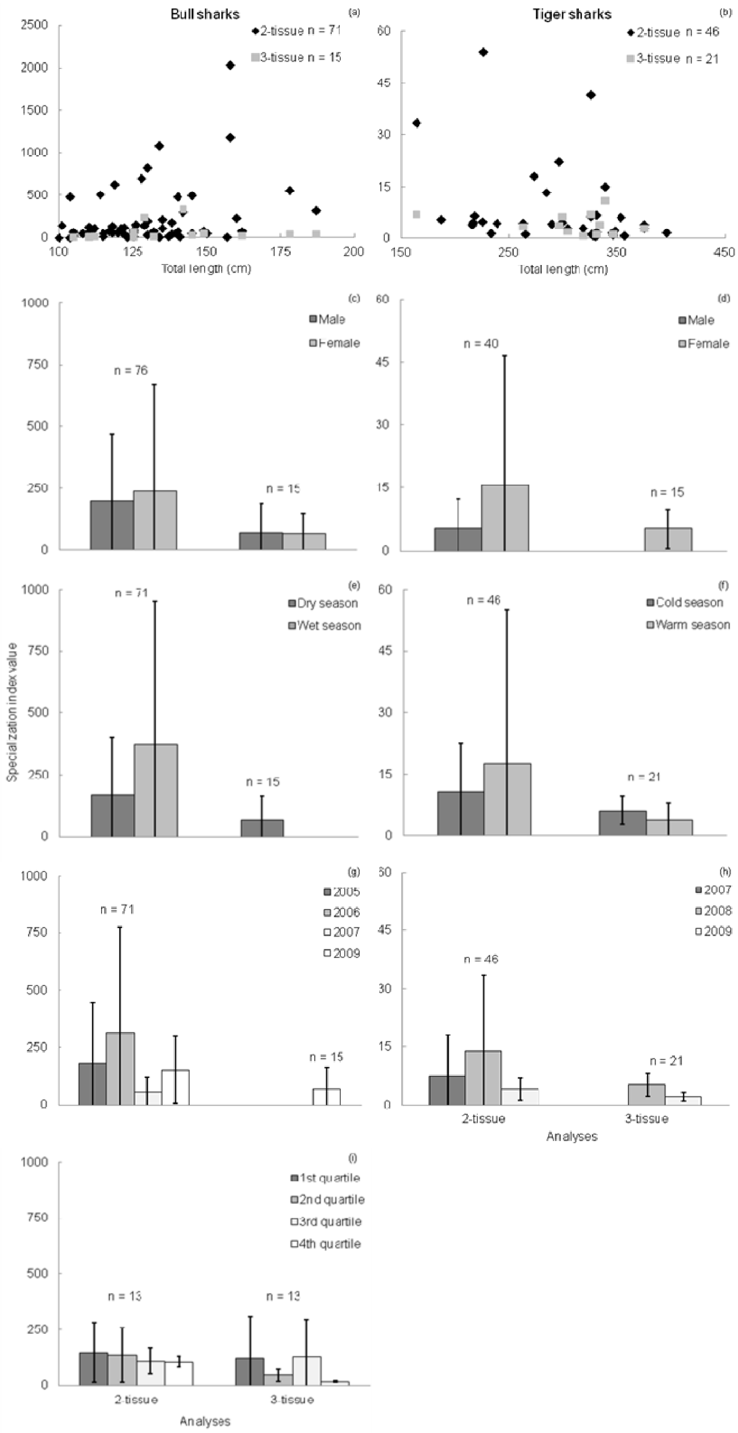

Figure 6: Individual specialization index values based on two- (♦) and three-tissue (■) GLMs of a) bull sharks and b) tiger sharks in relation to shark total length (cm). Mean individual specialization index values (± S.D.) based in two- and three-tissue GLMs of bull sharks separated by c) sex, e) capture season, g) capture year, and i) body condition, and mean individual specialization index values of tiger sharks separated by d) sex, f) capture season, and h) capture year.

was considerable variation in the diet of tiger sharks over time, indicating that individuals have relatively unspecialized diets. In contrast, bull sharks showed temporal stability in their diets, and most individuals were relatively specialized despite having a broad isotopic niche width at the population level. Therefore, although both species are "generalists" at the population level, they differ considerably at the individual level.

Often, individual specialization can be documented by observing the behavior of particular individuals over time. But for sharks and many other upper trophic level marine predators, this is not possible. Analyzing stable isotopic signatures of multiple tissues with differing rates of turnover is a powerful tool for assessing individual specialization when an individual can only be sampled once (e.g. Bearhop *et al*. 2004; Quevedo *et al*. 2009; Jaeger *et al*. 2010). Because analytical techniques for determining specialization patterns using isotope data from tissues with different turnover rates are still being developed, we used two methods to assess specialization: GLMs and the computer program IndSpec. Despite differences in output, both analytical frameworks produced the same trends in individual dietary specialization, or lack thereof, for bull sharks and tiger sharks – bull sharks are relatively more specialized than tiger sharks.

Body condition (reviewed by Vanderklift & Ponsard 2003) and the presence of lipids (Post *et al*. 2007) can be important factors to consider when interpreting isotopic values. Neither of these factors though, appeared to likely confound the results in our study. First, body condition tends to affect $\delta^{15}N$ more than $\delta^{13}C$ (e.g. Hobson, Alisauskas & Clark 1993; Kurle and Worthy 2001; Polischuk, Hobson & Ramsay 2001), and there was no affect of body condition on $\delta^{13}C$ of bull sharks (body condition data were not available for tiger sharks). Lipid content of tissues also is likely to have little effect on our results. Although sharks store lipids in muscle and liver tissues (Bone 1999; Remme *et al*. 2006), the mean C:N ratio of bull shark muscle tissue was low with little variation (3.1 ± 0.3 SD), suggesting lipid content resulted in minimal variation in muscle $\delta^{13}C$ between individuals (i.e. little effect on BIC). Mean C:N ratios of fin (2.7 ± 0.1 SD; tiger sharks), whole blood (2.7 ± 0.2 SD, 2.4 ± 0.0 SD), and plasma (2.0 ± 0.2 SD, 2.1 ± 0.1 SD) were also low for bull sharks and tiger sharks, respectively.

Shifts in metabolic activity in response to variation in abitoic conditions (e.g. temperature) can modify isotope discrimination and routing, and lead to variability in $\delta^{13}C$ values (reviewed by Kelly 2000). However, this likely did not affect the interpretation of the results from this study. Seasonal variation in water temperature occurs in the Shark River Estuary and Shark Bay, Australia, but they occur over a similar range (Shark River: 15-33°C; Heithaus *et al*. 2009; Shark Bay: 13-28°C; Wirsing, Heithaus & Dill 2007). Therefore, it seems unlikely that the differing patterns of specialization we observed can be attributed to differential effects of temperature on isotopic routing and discrimination.

Interpretation of isotope values can also be affected by whether tissues are in dietary equilibrium (reviewed by Martinez del Rio *et al*. 2009b), which may be influenced by seasonal changes in diets or prey switching within the timescale of a tissue's turnover (e.g. Matich *et al*. 2010). It is quite possible that tissues – especially those with longer turnover times – are not in equilibrium (at least for larger tiger sharks). The possibility of non-equilibrium of tissues in tiger sharks and some bull sharks, however, does not confound our basic findings of interspecific differences in individual specialization, because we are explicitly making use of differential rates of change in signatures of various tissues and do not need to assume that they are in equilibrium.

Finally, knowing the timeframe over which isotopic values are incorporated into tissues is important for determining the timescale over which specialization is measured using our methods. Isotopic turnover rates generally decrease with increasing body size (Martinez del Rio *et al*. 2009b), at a rate of $x^{-0.25}$ (Carelton & Martinez del Rio 2005). For slow-growing species like elasmobranchs, this relationship however, may overestimate differences in turnover rates. For example, freshwater stingrays (*Potamotrygon motoro*) and sandbar sharks (*Carcharhinus plumbeus*) had comparable turnover rates (muscle 422 days and 390-540 days, respectively; blood 265 days and 240-300 days, respectively) despite an order of magnitude difference in body mass (0.1kg and 6.4kg; MacNeil *et al*. 2006; Logan & Lutcavage 2010). Previous studies of bull sharks (Matich *et al*. 2010) suggest that turnover rates of muscle and whole blood of bull sharks in the Shark River are similar to laboratory estimates for freshwater stingrays, leopard sharks, and sandbar sharks, and body size differences may not result in major changes in isotopic turnover rates in this group of fishes. Nonetheless, if the standard scaling relationships apply, then tiger sharks should exhibit complete turnover times on the order of ~230 days for blood plasma, ~720 days for whole blood, and ~1500 days for fin (back-calculations based on turnover times of sandbar sharks; Logan & Lutcavage 2010; and regression model from Carleton & Martinex del Rio 2005). It is important to note, that if tiger sharks exhibit these loger turnover times, it would be expected to result in patterns of specialization that are *opposite* to those we found. Because the faster turnover tissues (i.e. plasma, whole blood) would incorporate diets over longer time frames, short-term variation in diets would not be reflected to the same degree and WIC should be smaller than in species with faster turnover rates.

Drivers of specialization and implications

Our results suggest that individual dietary specialization in elasmobranchs, and resulting community trophodynamics, is context dependent. Differences in resource availability and distribution as well as intraspecific competition, between the Shark River Estuary and Shark Bay ecosystems suggest that density-dependence may be an important factor affecting individual trophic specialization in sharks (see Estes *et al.* 2003; Svanback & Persson 2004; Tinker, Bentall & Estes 2008 for non-shark examples). Density-dependence generally occurs in early life-stages of sharks, including in nursery habitats like the Shark River Estuary, where population sizes are relatively large with respect to resource availability (see Heithaus 2007; Heupel, Carlson & Simpfendorfer 2007 for reviews). Conditions of resource scarcity can lead to specialization in trophic niches, because individuals exploiting a narrow range of resources can be more efficient than those exploiting more diverse resources (Bolnick *et al.* 2003). For example, sea otters (*Enhydra lutris nereis*) foraging in resource-limited habitats of the central Californian coastline were relatively specialized in their diets (Tinker *et al*. 2008) compared to more generalized sea otters along the Washington coastline where diverse food sources were readily available (Laidre & Jameson 2006). Resources for tiger sharks are relatively abundant in Shark Bay (Heithaus *et al.* 2002), likely leading to lower levels of competition, which may result in individual tiger sharks being relatively unspecialized in their diets. In comparison, the oligotrophic nature of the Shark River Estuary leads to low aquatic productivity and limited resource availability in much of the system (Childers 2006). Such resource limitation is likely a driver of the individual trophic specialization found in the bull shark population.

In ecosystems with multiple potential energetic pathways, the spatial arrangement of discrete food webs may also be an important factor determining levels of individual specialization. Ecosystems with discrete food webs that have a high degree of geographical overlap are more likely to support generalist individuals, because individuals can readily exploit resources from both food webs without significant movements between resource patches (e.g. Miller, Karnovsky & Trivelpiece 2009; Montevecchi *et al.* 2009). When food webs are spatially distinct with little or no geographic overlap, however, individual dietary specialization may be relatively widespread across a population (e.g. Darimont *et al.* 2009; Quevedo *et al*. 2009). In Shark Bay, both pelagic and benthic food webs overlap spatially, providing tiger sharks with access to each food web within the same habitat. In contrast, within the Shark River Estuary the marine and freshwater/estuarine food webs are spatially distinct. Specialization would be expected if sharks segregated into individuals that strictly resided in marine waters and those that stayed within the estuary. However, the bull sharks used for this study were all captured within the estuary, suggesting they move between habitats that encompass each food web.

Mean population $\delta^{13}C$ values suggest bull shark individuals derive carbon from multiple food webs, but the $\delta^{13}C$ values and specialization index values indicated that many individuals specialized in feeding from the marine food web despite being captured within the estuary. Indeed, taxa representing the marine food web are found more than

30 km from the capture locations of some sharks that had enriched $\delta^{13}C$, suggesting that they had moved long distances to feed. Movement data from bull sharks in the Shark River suggest that some individuals do move from the estuary into marine waters and back (P. Matich *unpublished data*) and further work will be needed to link individual behavior with patterns of specialization. Why wouldn't all sharks move into, or remain in, the potentially more resource-rich marine food web? Although the juvenile bull sharks in our study are among the largest-bodied predators in the Shark River Estuary, larger sharks that can prey upon these juvenile sharks inhabit the furthest areas downstream in the marine food web where risk is greatest for juvenile sharks (P. Matich *unpublished data*). Therefore, in addition to the trade-off between opportunistic feeding and foraging efficiency that favors specialization in resource-poor environments (Bolnick 2004), specialization in the bull sharks of the Shark River Estuary may also occur because of a trade-off between foraging opportunities and the risk of predation. These data support the view that behavioral and dietary specialization may be closely linked (Bolnick *et al.* 2007).

Individual specialization affects trophic dynamics, and previous studies suggest it may prevent resources of spatially distinct food webs from being coupled by individual predators (e.g. Quevedo *et al*. 2009). However, highly mobile predators, like sharks, have the potential to forage at a great distance from sites where they spend considerable amounts of time, and may serve to couple ecosystems through this trophic role. Isotope values suggest that some bull sharks move into and out of the system, which may enable them to feed on taxa from both the marine and freshwater/estuarine food webs, and would likely result in low specialization index values based on a generalized diet. Yet,

$\delta^{13}C$ values indicated that these individuals were dietary specialists and fed primarily in the marine food web located outside of the nursery in which they were captured. These specialized individuals may serve as important links in the connectivity of multiple food webs through a bottom-up mechanism of nutrient transport to the oligotrophic upper reaches of the Everglades (Polis, Anderson & Holt 1997), while tiger sharks of Shark Bay may serve a more traditional role of a generalist top predator that couples discrete food webs (Rooney *et al.* 2006). Food web structure and dynamics may be more complex in ecosystems with specialist top predators, and a "species-level" approach to conservation and management may be over simplistic in such situations. Therefore, studies of foraging ecology of highly mobile marine predators should explicitly consider the possibility of individual specialization. The use of stable isotopes sampled from multiple tissues would allow such studies to be conducted non-lethally and/or alongside traditional diet studies employing stomach contents analysis.

In summary, our study suggests that individual specialization can occur in non-mammalian marine top predators, but is not ubiquitous. Factors including resource availability, competition, food-predation risk trade-offs, and spatial overlap of food webs may contribute to the observed levels of specialization. Future studies should explore the level of individual dietary specialization that occurs within other shark populations, as well as other highly mobile apex predators, and the potential effects this may have on ecosystem processes. Studies that investigate the mechanisms by which among individual specialization is manifest in highly mobile predators, the heritability or drivers of this variation in trophic niches, and the effects specialization has on the trophic dynamics within and across ecosystems will be particularly important for future conservation efforts, especially in light of widespread top predator declines in marine ecosystems.

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CHAPTER VI

INDIVIDUAL AND ENVIRONMENTAL DRIVERS OF NESTED PATTERNS IN HABITAT USE AND FORAGING TACTICS WITHIN A LARGE ESTUARINE PREDATOR (*CARCHARHINUS LEUCAS*)

Matich, P., and Heithaus, M.R. Individual and environmental drivers of nested patterns in habitat use and foraging tactics within a large estuarine predator (*Carcharhinus leucas*). Animal Behavior: *in review*.

Abstract

Ontogenetic niche shifts are common among animals, and can lead to size- and/or age-based differences in habitat use and trophic interactions. However, individual differences nested within behavioral shifts can lead to divergent behaviors within size-/age-classes, and cause variability in the ecological roles individuals play in their respective ecosystems. Using acoustic telemetry, we tracked the movements of juvenile bull sharks in the Shark River Estuary of Everglades National Park, FL, USA, and found that sharks increased their use of marine microhabitats with age, likely to take advantage of more abundant resources, but continued to use freshwater and estuarine microhabitats, likely as refuge from marine predators. Nested within this ontogenetic niche shift, however, divergent movement patterns were exhibited by sharks at various temporal scales, likely in response to both external and internal factors, including spatial variability in productivity, intraspecific competition, and individual responses to food-risk trade-offs and body condition. Such nested behavior suggests individual specializations and behavioral syndromes, which can strongly influence population-level dynamics, may develop early in the life-histories of animals. With continued changes in environmental conditions affecting the distribution and abundance of species, understanding the factors that shape animal behavior and lead to intraspecific variability is becoming progressively more important, especially as we increasingly recognize the importance of genotypic and phenotypic diversity in natural systems.

Introduction

Changes in energetic requirements and risk of predation through ontogeny often lead to changes in home range sizes and activity areas as individual needs, and the nature of trade-offs change (Werner & Gilliam 1984). When energetic needs are the sole driver of foraging behavior, animals should select habitats that lead to the highest energetic intake/growth rates (reviewed by Pyke 1984). However, for most animals, especially juveniles, the risk of predation is often higher in energetically profitable habitats creating food-risk trade-offs (Gilliam & Fraser 1987; reviewed by Lima & Dill 1990; Lima 1998; Brown & Kotler 2004). As animals grow, vulnerability to predation generally decreases because of greater size, speed, and escape ability, often leading to increased use of more profitable areas that have become less risky for larger individuals (Werner & Gilliam 1984). As such, ontogenetic shifts in habitat use are common among vertebrates, and size-related differences in food-safety trade-offs can lead to size segregation within populations (Wilbur 1980; Werner & Gilliam 1984; Klemetsen et al., 2003; Barton 2010), as well as size-related differences in the ecological roles of individuals across age-classes.

Nested within size-/age-defined differences, intraspecific variability in behavior (e.g. individual specialization, animal personalities, behavioral syndromes) can be important in shaping the structure and functional role of populations by altering niche widths, resource use efficiencies, spatiotemporal dynamics of trophic interactions and dissimilar roles among population members (reviewed by Bolnick et al., 2003; Sih et al., 2004a). It now appears that such differences in behaviors within populations are widespread (Sih et al., 2004b; Bolnick et al., 2011), and, therefore, ontogenetic niche shifts are unlikely to be uniform across individuals within age-classes. Such within age-class divergence in behavior can lead to substantially different trajectories later in life. In the northeastern Pacific, for example, anadromous male coho salmon (*Oncorhynchus kisutch*) diverge into two mating tactics - fighters and sneakers - early in their life history as a result of larger body size of sneakers than fighters at the fry (juvenile) stage coupled with genetic differences (Gross 1991; Gross & Repka 1998; Paez et al., 2010). Fighters (i.e. hooknoses) reside and grow in marine waters for 18 months before spawning in freshwater systems where they engage in physical bouts for access to spawning females. In comparison, sneakers (i.e. jacks) only reside in marine waters for six months before returning to spawn, and their smaller body size at maturity compared to fighters enables them to use stealth tactics to gain access to spawning females. A similar divergence in early behavior has been documented in bluegill sunfish (*Lepomis macrochirus*; Gross 1985), with late-maturing males exhibiting parental care and nest guarding, and early-maturing males exhibiting cuckholdry (Gross & Charnov 1980).

Inter-individual variation is not always attributed to consistent differences in behavior types or specialization though. Indeed, individual state (e.g. residual reproductive value, gut fullness, body condition) can lead also to divergence in behavior (e.g. Houston et al., 1988; Clark 1994). For example, individuals closer to starvation (i.e. low body condition) often will accept higher predation risk in order to obtain greater energy intake rates (e.g. wildebeest, *Connochaetes taurinus*, Sinclair & Arcese 1995; green turtles, *Chelonia mydas*, Heithaus et al., 2007a). Despite a growing number of studies that have identified individual behavioral differences within populations, for most species it is unclear how intraspecific variability in behavior develops, and if it persists over the lifetime of individuals. Further investigation is needed to understand the prevalence of individual differences among juveniles, and the drivers of behavioral strategies that develop in early life-history stages.

Bull sharks (*Carcharhinus leucas*; Müller & Henle 1839) undergo ontogenetic shifts in habitat use, moving from estuaries to marine habitats (Simpfendorfer et al., 2005; Heupel et al., 2007; Grubbs 2010). During early years, estuaries provide low risk of predation combined with adequate food for growth (Heupel et al., 2007; Heithaus 2007). As bull sharks transition to new habitats, they often encounter different suites of prey, resulting in ontogenetic shifts in feeding habits (Matich et al., 2010). However, juvenile bull sharks are not uniform in their diets, and at least older juveniles residing in estuaries can exhibit considerable differences in their trophic interactions, ranging from freshwater specialists and marine specialists to trophic generalists (Matich et al., 2011). Whether such individual differences in behavior may develop early in the life of bull sharks, however, remains unclear. Similarly, no studies have investigated whether sharks modify their behavior in response to changes in body condition, and such studies are generally few for large-bodied taxa (but see Sinclair & Arcese 1995; Gaillard et al., 2000; Heithaus et al., 2007 for examples). Investigating condition-dependent habitat use, however, is important since it can mediate impacts of top-down and bottom up disruptions to food webs (Heithaus et al., 2008).

Here, we used passive acoustic telemetry to quantify ontogenetic shifts in the habitat use of juvenile bull sharks, and to investigate individual differences in movement behavior through ontogeny. We also used drumline shark fishing to quantify spatial variability in predation risk, and published literature on nutrient availability and productivity to investigate how food-risk trade-offs may influence individual differences in juvenile bull shark habitat use.

Methods

Study location

The Shark River Estuary within the Florida Coastal Everglades (Fig. 1) serves as a nursery year-round for juvenile bull sharks (Heithaus et al., 2009). The estuary is oligotrophic and limited by phosphorous inputs from marine waters, leading to greater productivity at the mouth of the estuary than in areas upstream (Childers 2006). As such, prey availability for juvenile bull sharks is likely greatest in the lower portions of the estuary during most of the year (Matich & Heithaus 2014) as a result of high levels of nutrients and productivity compared to upstream areas (Simard et al., 2006). However, predation risk for juvenile bull sharks is also likely greatest in downstream areas where large predatory sharks reside (Wiley & Simpfendorfer 2007). Thus, areas that are safe may provide a more limited suite of resources with the exception of a brief pulse of prey during the dry season (Boucek & Rehage 2013; Matich & Heithaus 2014). To understand the general patterns in habitat use and movements of juvenile bull sharks, and how predation risk varies spatially, we divided the estuary into four regions on the basis of variation in abiotic conditions (e.g. salinity) documented during long-term sampling and used in previous studies (see Matich & Heithaus 2012 for details of sampling regions): 1) Downriver (DR), 2) Shark River (SR), 3) Tarpon Bay (TB), and 4) Rookery Branch (RB) (Fig 1).

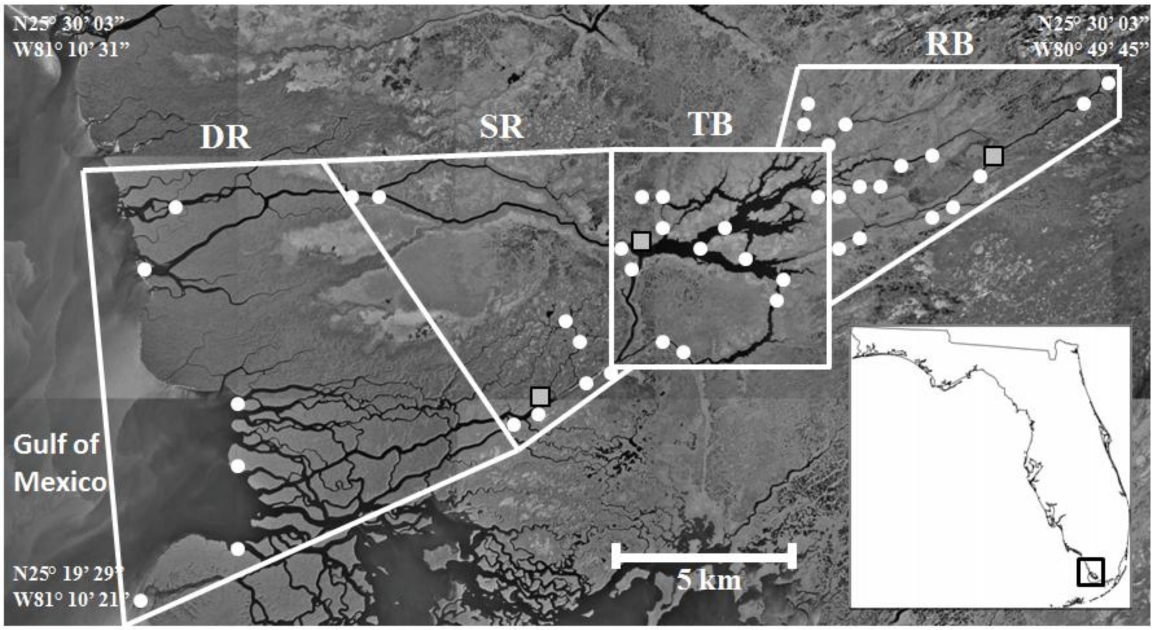

Figure 1: Study zones (DR, SR, TB, and RB) within Shark River Estuary, FL. Locations of acoustic receivers are denoted by white circles, and locations of water quality loggers are denoted by gray squares.

Field sampling

To quantify predation risk, we used bottom-set drumlines deployed in three sampling regions (DR, SR, and TB; Fig. 1) from 2009-2011. The fishing gear targets large sharks (Heithaus et al., 2007b) including species like bull sharks, lemon sharks (*Negaprion brevirostris*) and blacktip sharks (*Carcharhinus limbatus*) that prey upon smaller elasmobranchs (Castro 2011; Ebert et al., 2013). Drumlines consisted of a 25 kg cement weight (used to anchor the line), with 20-30 m of 400 kg monofilament terminating at a 16/0 circle-hook baited with bonito (*Sarda sarda*). A line with two surface buoys was also attached to the cement weight to mark the line (see Heithaus et al., 2007b for details of sampling equipment). Each fishing day, ten individual lines were spaced 300-500 meters apart from one another and allowed to soak for *ca.* 2 hours before being checked (mean = 2 hr 16 min ± 38 min). Once caught, sharks were brought alongside the sampling vessel, identified to species, total length was measured to the nearest centimeter, and a numbered roto tag was put in the first dorsal fin for identification. Drumlines were rebaited and replaced after each check until sunset or until weather conditions deteriorated.

To quantify shark movements, juvenile bull sharks were caught from 2008-2009 using ~500 m longlines fitted with 40-55 14/0 or 15/0 Mustad tuna circle hooks. Hooks were baited with mullet (*Mugil* sp.) and attached to ~2 m of 400 kg monofilament line (see Heithaus et al., 2009 for details of sampling equipment). Sharks were processed alongside the sampling vessel, or within a water-filled, aerated cooler on board. Shark total length was measured to the nearest centimeter, shark body mass was measured to the nearest 0.5 kg using a Macro-Line spring scale (Pesola, Switzerland), sex was determined by the presence or absence of claspers, and sharks were externally tagged using a numbered roto tag affixed through the first dorsal fin. Sharks swimming strongly upon capture (n = 40) were surgically fitted with a Vemco V16-4H transmitter (Vemco, Halifax, NS) to track their movements. Transmitters were set to emit a unique series of pulses for each shark at a random interval between 30-90 sec (mean emission interval = 60 sec; mean battery life = 2 yr). Movements of acoustically tagged sharks were tracked within an array of 43 Vemco VR2 and VR2W acoustic receivers strategically placed throughout the estuary to detect the location and direction of movement of tagged sharks across the estuary (Fig. 1). Each receiver had a detection range of ~500 m (see Rosenblatt & Heithaus 2011 for further details of the sampling array) making it highly unlikely tha a shark was not detected moving past a receiver. Data from receivers were downloaded every 3-4 months and batteries were replaced as needed.

To quantify spatial and temporal variability in environmental conditions, three YSI 6920 Sonde water quality loggers (YSI Incorporated, Yellow Springs, OH) were deployed in SR, TB, RB (Fig. 1) from Feb 2010 - Jan 2011. Water quality loggers measured and recorded salinity, water temperature, and dissolved oxygen saturation every 30 minutes. Data from loggers were downloaded every four months and batteries were changed as needed.

Data analysis

We used catch per unit effort (CPUE) of large predatory sharks that posed a threat to juvenile bull sharks (bull sharks, lemon sharks, and blacktip sharks) as an estimate of predation risk (Fig. 2). Catch per unit effort serves as a proxy for predator encounter

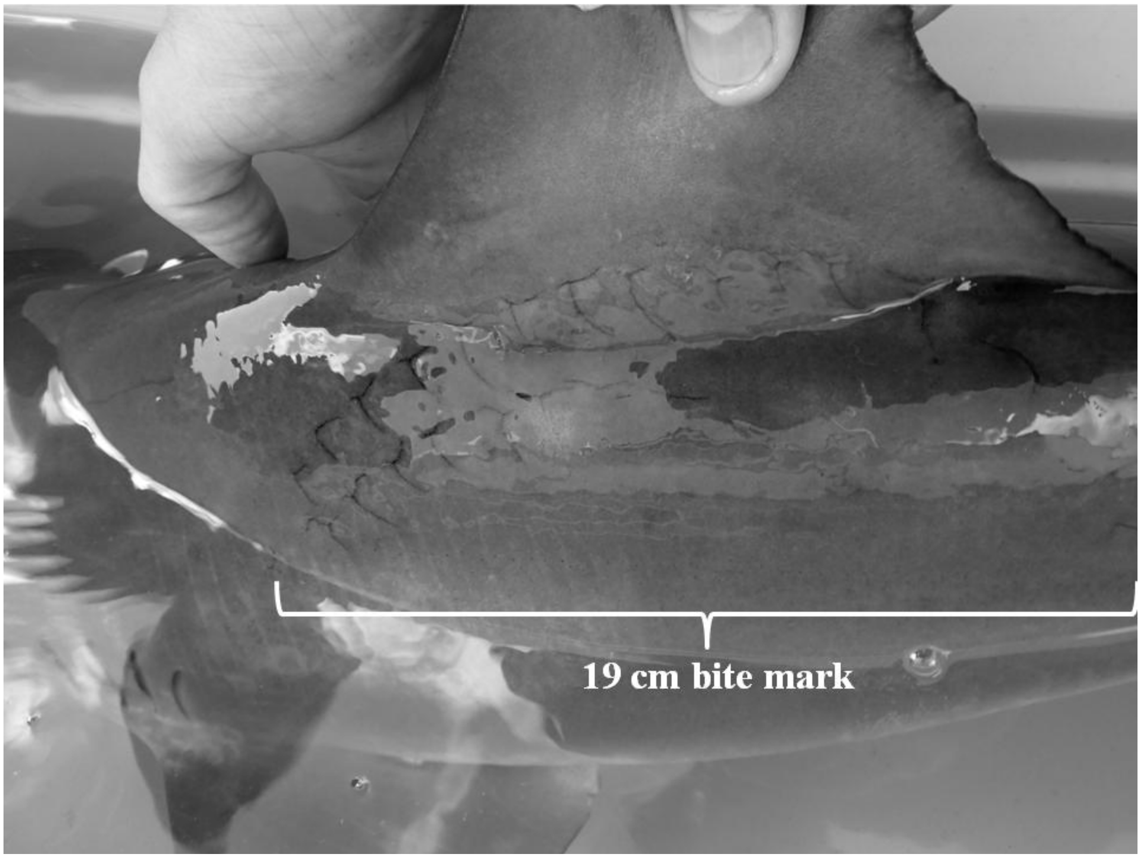

Figure 2: Recent bite mark on a 100 cm TL bull shark caught 18 km from the mouth of the estuary. Based on mouth width-total length relationships (Lowry et al. 2009), the attacker is estimated to be a 162 cm TL lemon shark.

rates, and likely is an adequate estimate of relative predation risk at the scale of the present study because 1) we do not suspect that escape probabilities in an encounter situation vary spatially or are lower in low-CPUE habitats and 2) CPUE variability at the scale of this study (see results) was high enough to make it unlikely that spatial variation in the probabilities of other steps in the predator-prey interaction are of greater importance in driving variation in probabilities of prey death.

Catch per unit effort was calculated as the number of predatory sharks greater than 160 cm total length (TL) caught per hour drumline hooks soaked. We selected 160 cm as a minimum size based on the calculated size of a shark - based on bite width-total length relationships - that had attacked a juvenile bull shark in the SRE (Lowry et al., 2009; Fig 2). Using larger minimum sizes, however, did not affect the general pattern. For empty hooks retrieved without bait, we reduced the soak time by half to account for spatial variation in bait loss rates (Wirsing et al., 2006; Heithaus et al., 2007b). We used ANOVA to assess spatial and temporal variation in predation risk throughout the estuary. Analysis revealed annual differences in CPUE of predatory sharks, however year and the interaction of year and sampling region were not significant factors in ANOVA (F = 0.87, p = 0.43, F = 1.09, p = 0.38, respectively), and therefore we pooled data from 2009-2011. A post hoc Tukey's test was used to test for significant differences across sampling sites.

To investigate variation in the movement patterns and habitat use of juvenile bull sharks, we quantified the monthly 1) minimum linear distance each shark travelled within the estuary per month, 2) minimum linear distance of the river each shark used within the estuary per month, and 3) proportion of time spent in each sampling region (DR, SR, TB, and RB). We quantified residence times of each shark using predicted age at departure from the estuary (when sharks were last detected at SR or DR receivers and no longer detected during the study). We also conducted fast Fourier transforms (FFTs) to identify periodic movements between the four sampling regions and to quantify the timescale over which periodic movements were observed (Papastamatiou et al., 2009). Among sharks that exhibited periodic movements, we quantified the time of day when recurrent movements were made between sampling regions and the duration of time spent in each sampling region. Data were only analyzed for sharks that were tracked within the estuary for at least four months.

To investigate ontogenetic shifts in habitat use, we grouped sharks into age-classes determined by total length at capture and capture date. Bull sharks in the Shark River Estuary are likely born at 60-70 cm TL (based on captures of neonate individuals) between May and August (based on the presence of open umbilical scars; see also Curtis et al., 2011), and grow 10-20 cm/year (based on recaptured individuals; see also Neer et al., 2005). Therefore, we used the classifications in Table 1 to indentify age-classes for each shark. Using these criteria, we predicted the age of each shark after capture and release for the duration of the study. Sharks that were born before 2009 and tracked after July 2009 were reassigned into the next oldest age-class on 1 July 2009. We used general linear models to test the effects of age-class on monthly distance travelled, monthly distance used within the estuary, and the proportion of time spent in each sampling region. We used ANOVA to elucidate the effects of age on shark movement patterns, and post hoc Tukey’s tests were used to detect significant differences across age-classes. We used a chi-squared test to assess the effects of age-class on movement periodicity, and used logistic regression to test the effects of periodicity (sharks either exhibited

Maximum total lengths Capture month	age-class 0	age-class 1	age-class 2	age-class 3
Jul-Sep	78 cm	98 cm	118 cm	138 cm
Oct-Dec	83 cm	103 cm	123 cm	143 cm
Jan-Mar	88 cm	108 cm	128 cm	148 cm
Apr-Jun	93 cm	113 cm	133 cm	153 cm

Table 1: Maximum total lengths used to define shark age classes.

periodic movements or did not exhibit periodic movements) on distance travelled, distance used within the estuary, and time spent in sampling regions. Because intraspecific variability in behavior can lead to, or be driven by differences in the body condition of individuals (Gross & Charnov 1980; Sinclair & Arcese 1995; Clark & Mangel 2000), we used linear regression to test the effects of body condition (calculated using residuals of body length v body mass) on the proportion of time spent in each sampling region, distance travelled, and distance used within the estuary. We also used ANOVA to quantify differences in movements and habitat use attributed to shark sex. Finally, we used ANOVA to quantify temporal and spatial variability in salinity, temperature, and dissolved oxygen and used FFTs to identify periodicity in salinity, temperature, and dissolved oxygen both within regions and between adjacent regions.

Our previous work revealed that during the late dry season (Mar-May), bull sharks exhibit significant changes in their movement patterns and trophic interactions in response to an annual resource pulse that enters the estuary from adjacent freshwater marshes (Matich & Heithaus 2014). When included in analyses, sampling month was a significant factor in all tests used to investigate shark movements, likely because of this response to the resource pulse. To investigate the movements of bull sharks within the Shark River Estuary outside of this three month period, we removed movement data from Mar-May 2009. JMP 10 was used for all statistical analyses besides FFTs, which were conducted in STATISTICA 10.

Results

Environmental conditions

Salinity and dissolved oxygen (concentration and saturation) were significantly different across sampling regions, with mean dissolved oxygen saturation higher in TB than RB and SR at all hours (Fig. 3c & d), and salinity predictably decreasing as the distance from the Gulf of Mexico increased (Fig. 3a). Environmental factors did not significantly vary with time of day (F = 0.07, p = 0.99; F = 0.58, p = 0.94; F = 0.30, p = 0.99; F = 0.54, p = 0.96 for temperature, salinity, dissolved oxygen concentration and saturation, respectively) and the interaction of time of day and sampling region was not significant for any environmental factor (F = 0.01, p = 0.99; F = 0.30, p = 0.99; F = 0.25, p = 0.99; F = 0.10, p = 0.99 for temperature, salinity, dissolved oxygen concentration and saturation, respectively; Fig. 3). Environmental parameters exhibited no periodicity in RB or SR, but all environmental parameters in TB exhibited periodicity, with salinity and dissolved oxygen saturation having the strongest frequency of occurrence. Cross-region analyses also revealed periodicity in the differences in environmental parameters between RB and TB, and SR and TB likely because of periodic trends in environmental conditions within TB. Frequency of periodicity for dissolved oxygen saturation was 1-3 orders of magnitude higher than all other parameters, however differences across regions were relatively small (Fig. 3d).

Predation risk

From May 2009 - Oct 2011, we caught 53 predatory sharks >160 cm TL during more than 2700 hook hours. Large shark catch rates varied among sampling sites, with

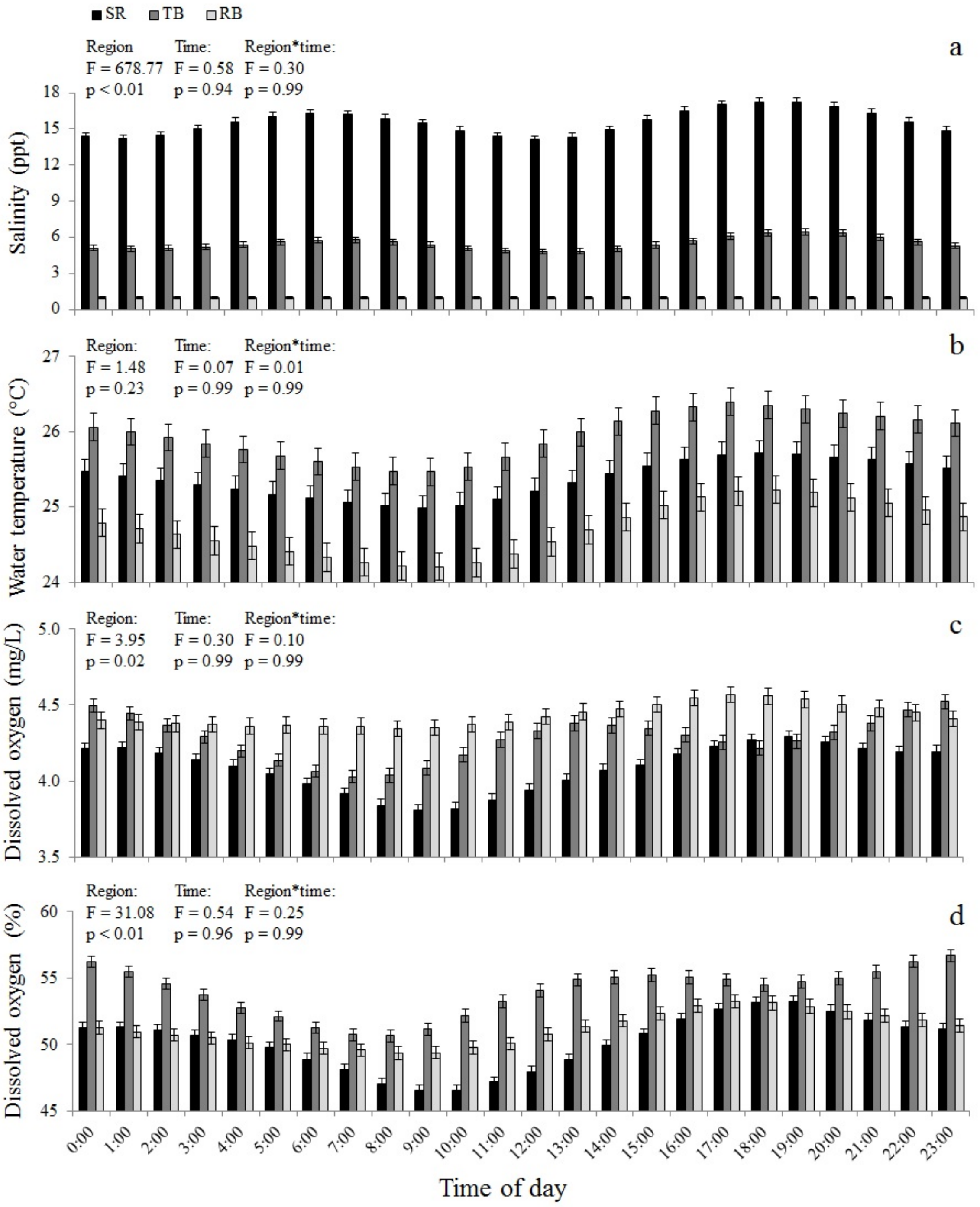

Figure 3: Mean hourly a) salinity, b) water temperature, c) dissolved oxygen concentration, and d) dissolved oxygen saturation for sampling regions SR, TB, and RB. Error bars are standard errors.

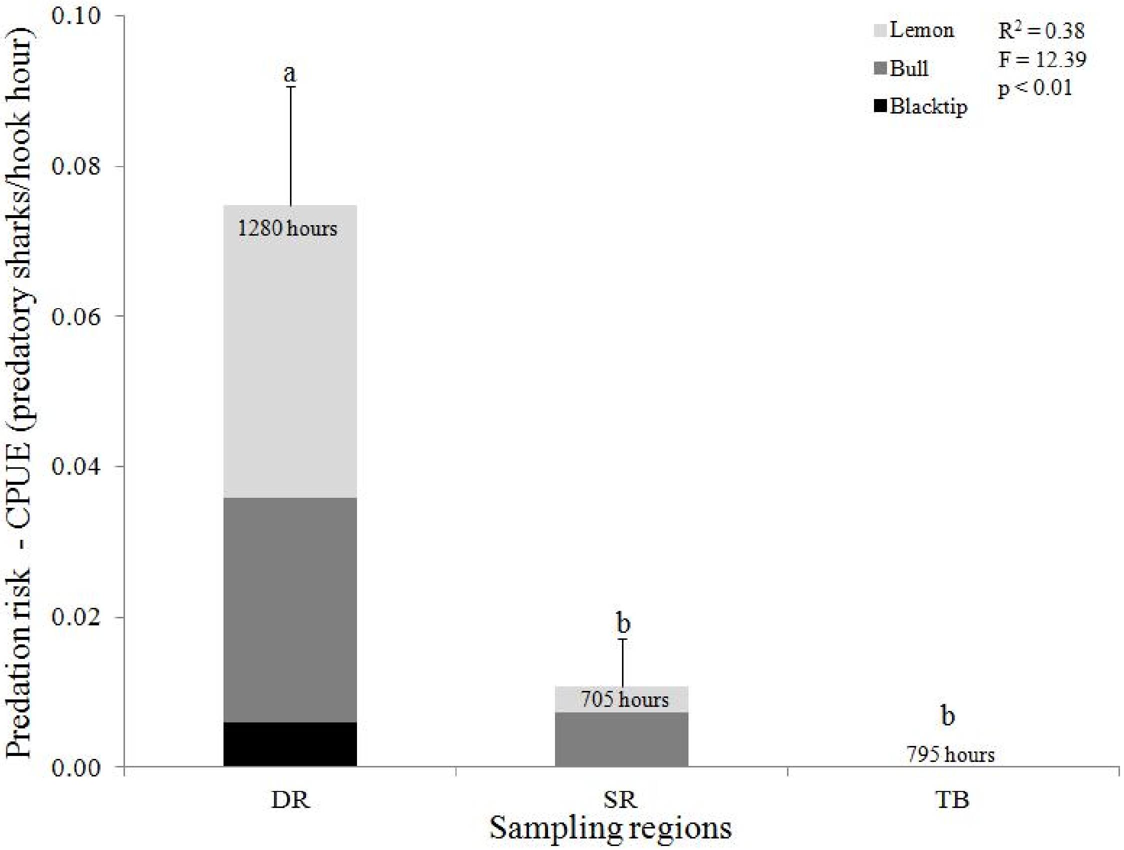

Figure 4: Catch rates of large sharks in the Downriver (DR), Shark River (SR), and Tarpon Bay (TB) regions of the estuary. Data include three predatory shark species - *N. brevirostris* (light gray), *C. luecas* (dark gray), and *C. limbatus* (black). Error bars are ± SE. Bars with different letters are significantly different at $p < 0.05$ based on post hoc Tukey's tests.

the highest catches at DR (Fig. 4). Only three sharks were caught at SR (two bull sharks, 182 and 187 cm TL, one lemon shark 215 cm TL), and no sharks were caught at TB (Fig. 4). At DR, blacktip sharks >160 cm TL (n = 4) ranged from 162-181 cm TL (mean ± SD = 169 ± 8 cm TL), bull sharks >160 cm TL (n = 20) ranged from 160-220 cm TL (mean ± SD = 177 ± 18 cm TL), and lemon sharks >160 cm TL (n = 26) ranged from 160-230 cm TL (mean ± SD = 207 ± 17 cm TL).

Juvenile bull sharks movements

During Jan 2010, an extreme cold weather event lead to the mortality of many terrestrial and aquatic taxa throughout south Florida, including bull sharks in the Shark River Estuary (Matich & Heithaus 2012). During or immediately after (days) the event, all acoustically tagged bull sharks either left the estuary for deep water refugia provided by the ocean, or died in the estuary (Matich & Heithaus 2012). As a result, our analyses are restricted to 27 juvenile bull sharks (71-131 cm TL) tracked for at least four months before this event. These 27 sharks were relatively evenly distributed across age-classes 0-2 upon capture (n = 7, 8, and 10 for age-classes 0, 1, and 2, respectively) and only two individuals were classified in age-class 3 upon capture. During the study, 14 sharks from age-classes 0, 1, and 2 (n = 4, 5, and 5, respectively) were tracked beyond July, and thus were reclassified into the next age-class after 1 July 2009. Of the 27 sharks used for analyses, 14 individuals were present during the 2010 cold snap, and therefore residence times could not be determined for these sharks, because the event disrupted movement behavior likely to occur outside of this extreme event. The 13 sharks that emigrated from the estuary prior to this event had a mean residency time of 2.69 ± 0.95 years (SD; based on predicted age-classes at departure date), with most individuals (10 of 13; 77%) emigrating between May and September. Two individuals (15%) left during their fourth year, and one shark (8%) left during its first year.

The linear distance sharks traveled within the estuary significantly increased with age from *ca.* 75 km to more than 250 km, and the and linear distance sharks used increased from *ca.* 12 km to 26 km (Fig. 5). The proportion of time spent in the DR and SR sampling regions increased with shark age from *ca*. 26% to 45%, while the proportion of time spent in TB decreased from *ca*. 56% to 34% (Fig. 6). Periodic movements between sampling zones were exhibited by 42% of tracked sharks (n = 11; Fig. 7, Table 2), and occurred between TB and either RB or SR. Among periodic commuters, only one of nine age-class 0 sharks (14%) exhibited periodic movements between TB and SR. For age-class 1, three sharks (38%) exhibited periodic movements between TB and RB and one shark (13%) exhibited periodic movements between TB and SR. Five of ten age-class 2 sharks (50%) exhibited periodic movements between TB and SR, as did one of two age-class 3 sharks (50%; Fig. 8a). Ten of these periodic commuters spent the greatest proportion of their time in TB (mean ± SD = 65% ± 27%; Fig. 6b), and left for SR or RB just before or after sunset (62% of these movements occurred between 18:00-21:00 EST) and returned to TB before sunrise (77% of these movements occurred between 22:00-5:00 EST; Figs. 9 & 10, Table 2). Among periodic commuters, the duration of time spent in transit zones (sampling region where the least amount of time was spent between the two regions where periodic movements were observed; i.e. SR or RB) significantly increased with age from *ca*. 1 hr/day to 10 hrs/day, and the proportion

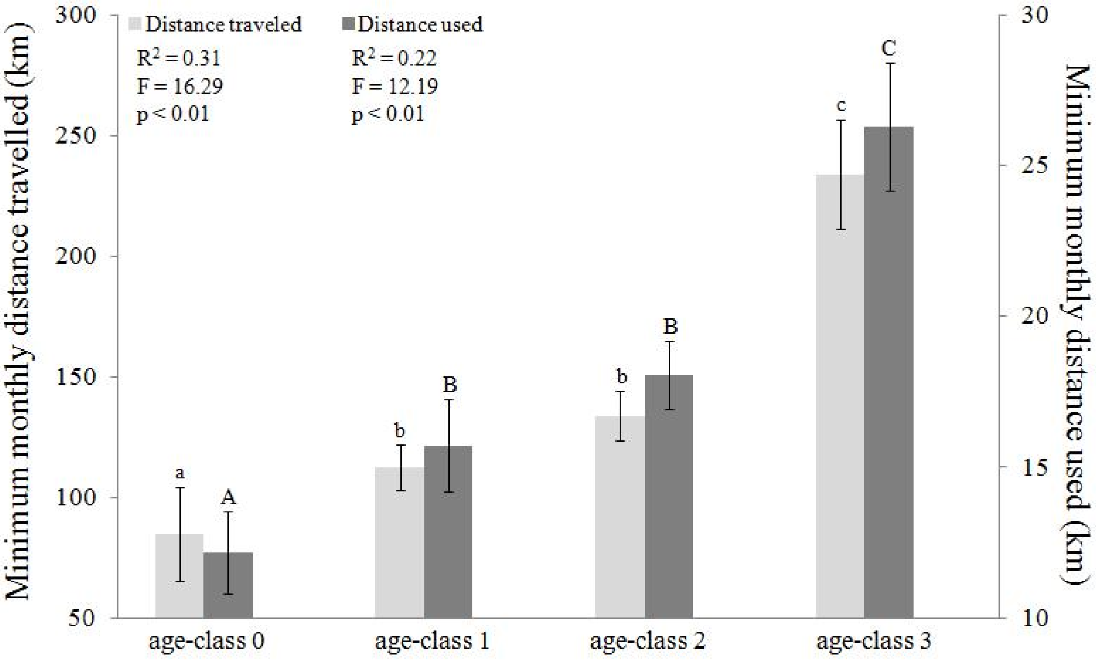

Figure 5: Population means of minimum monthly distances travelled (primary y-axis) and minimum monthly distance used within the estuary (secondary y-axis) for sharks age-class 0-3. Error bars are ± SE, and bars with different letters are significantly different at $p < 0.05$ based on post hoc Tukey's tests.

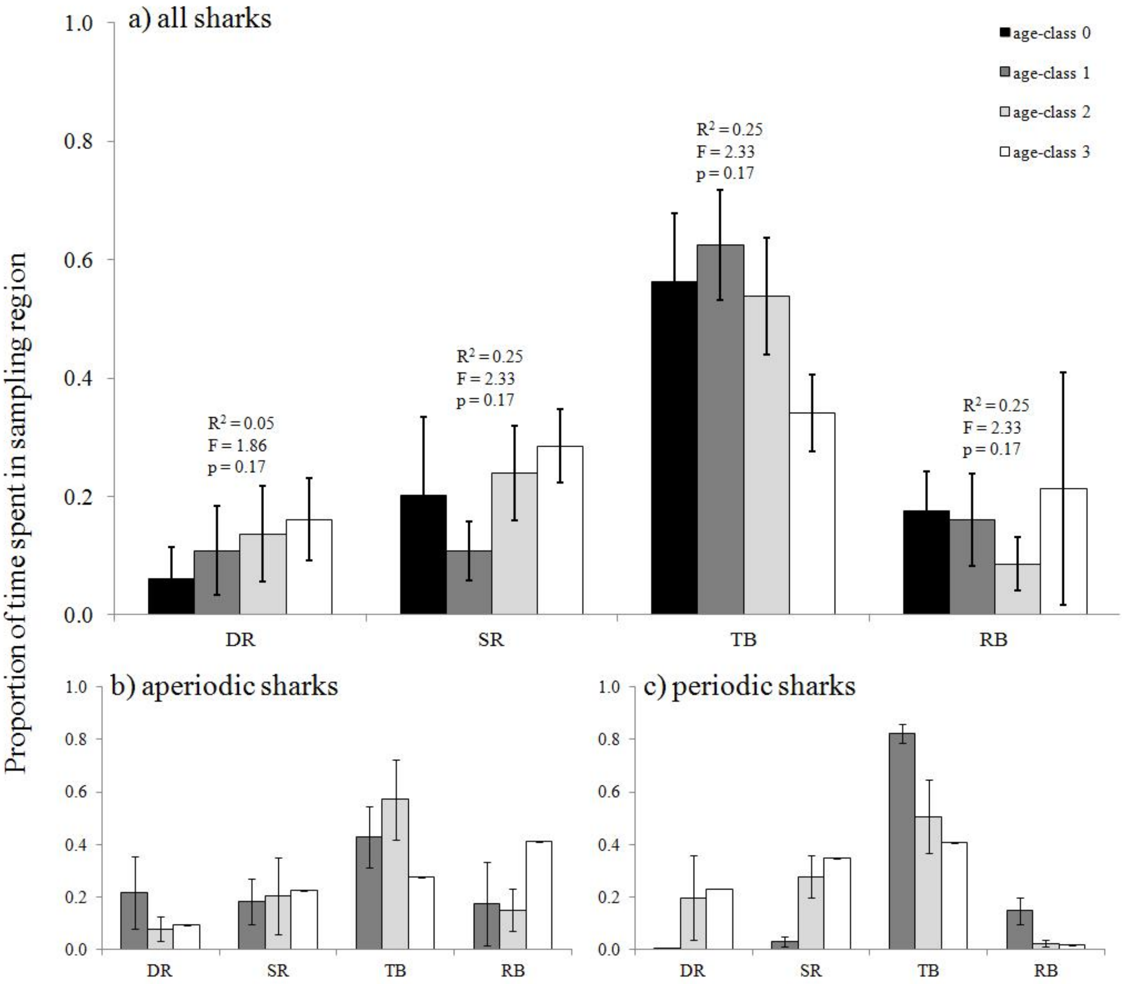

Figure 6: Proportion of time spent in each sampling region for a) sharks age-class 0-3, b) aperiodic sharks in age-classes 1-3, and c) periodic sharks in age-classes 1-3. Error bars are ± SE.

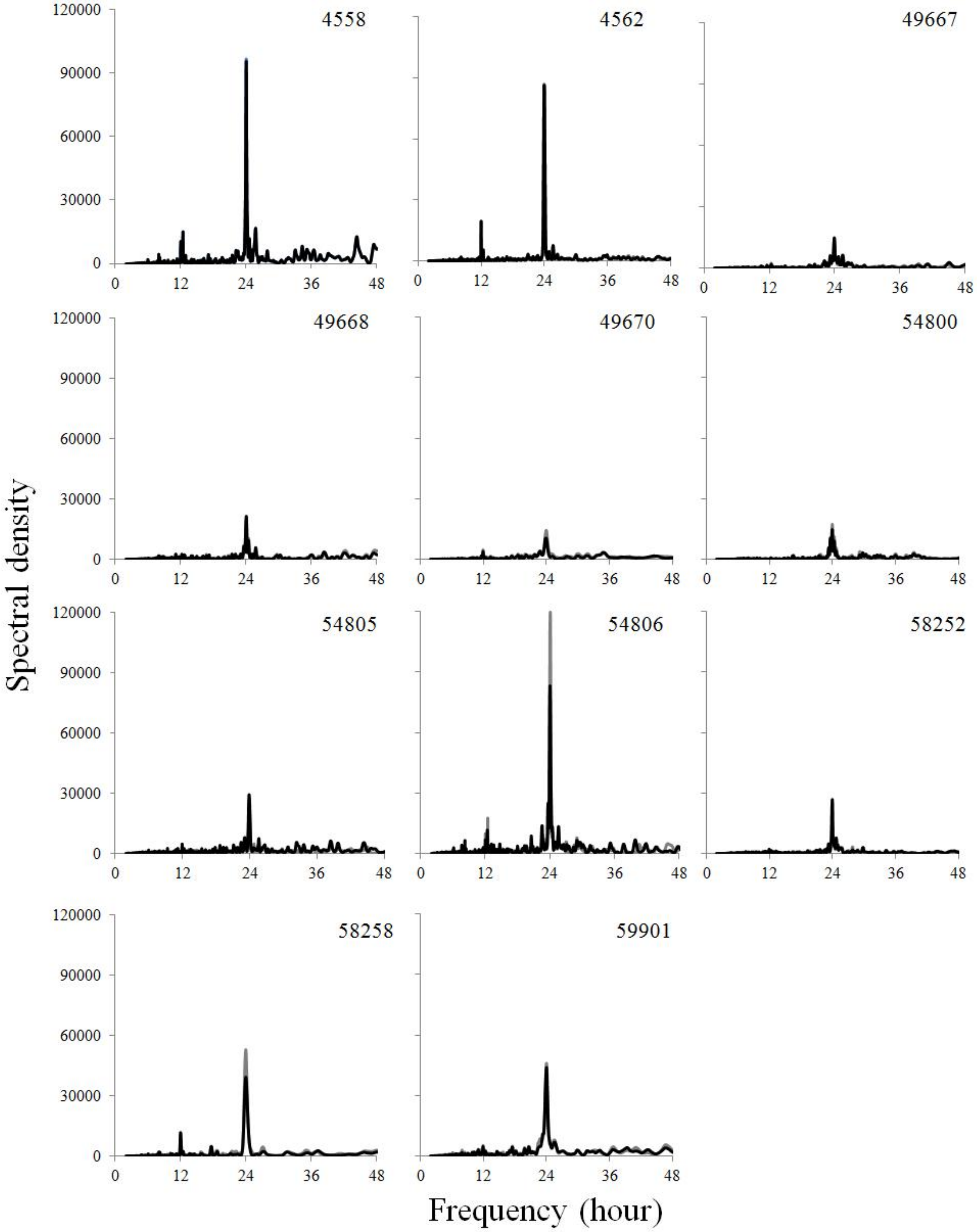

Figure 7: Periodogram of fast Fourier transformations for sharks that exhibited periodic movements between adjacent sampling regions (TB-SR and TB-RB).

Shark	Age at capture	Body condition at capture	Periodic movement	Departure time	Return time	Proportion of days tracked
4558	2		TB to SR	20:46 ± 2:13 hr	01:54 ± 2:41 hr	0.52
4562	1		TB to SR	20:11 ± 2:28 hr	01:13 ± 2:14 hr	0.42
4563	1		None			
49663	1		None			
49664	2		None			
49665	0		None			
49667	2		SR to TB*	08:00 ± 5:11 hr	11:42 ± 5:35 hr	0.38
49668	2		TB to SR	19:55 ± 2:47 hr	02:05 ± 3:10 hr	0.40
49669	2		None			
49670	0		TB to SR	23:02 ± 2:16 hr	00:10 ± 2:13 hr	0.24
49671	2	7.63	None			
49672	2	3.15	None			
49673	0	-0.21	None			
54799	0	1.47	None			
54800	1	2.07	TB to RB	23:12 ± 3:22 hr	02:16 ± 3:53 hr	0.34
54801	0	4.46	None			
54802	2	1.59	None			
54803	0	-1.50	None			
54804	1	-2.73	None			
54805	3	1.51	TB to SR	19:45 ± 2:41 hr	05:44 ± 4:29 hr	0.33
54806	2	-0.03	TB to SR	19:46 ± 1:46 hr	04:46 ± 2:25 hr	0.46
58250	1	-1.17	None			
58252	1	0.03	TB to RB	18:18 ± 3:20 hr	22:09 ± 3:25 hr	0.48
58253	3	0.47	None			
58254	0	2.66	None			
58258	2	-0.97	TB to SR	20:58 ± 1:20 hr	01:56 ± 1:54 hr	0.42
59901	1	1.40	TB to RB	18:53 ± 2:34 hr	23:53 ± 3:25 hr	0.56

Table 2: Periodic movements of sharks detected using FFTs. Departure time is the average time of day when sharks traveled from the sampling region they spent most of their time in to transition zones (with SD) and return time is the average time of day when sharks returned to the sampling region they spent most of their time in from transition zones (with SD). Note that all but one shark exhibiting periodic movements spent most of their time in TB and made periodic movements between TB and either RB or SR - shark 49667* spent most of its time in SR and moved between SR and TB. Proportion of days tracked is the proportion of days periodic movements were detected while tracking each shark.

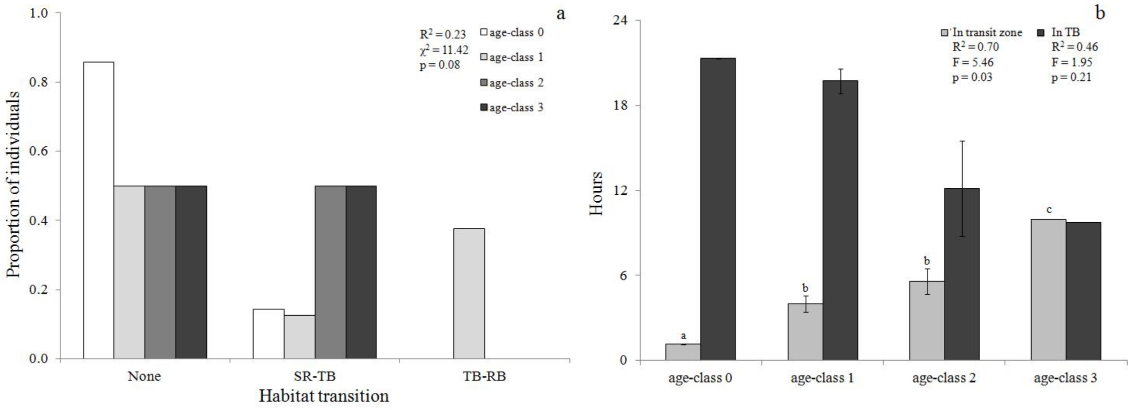

Figure 8: a) Proportion of individuals exhibiting periodic movements between SR and TB, between TB and RB, and no periodic movements. b) Mean duration (h) spent in transit zones by periodic sharks and mean time in TB for each age-class. Error bars are ± SE, and bars with different are letters significantly different at $p < 0.05$ based on post hoc Tukey's tests.

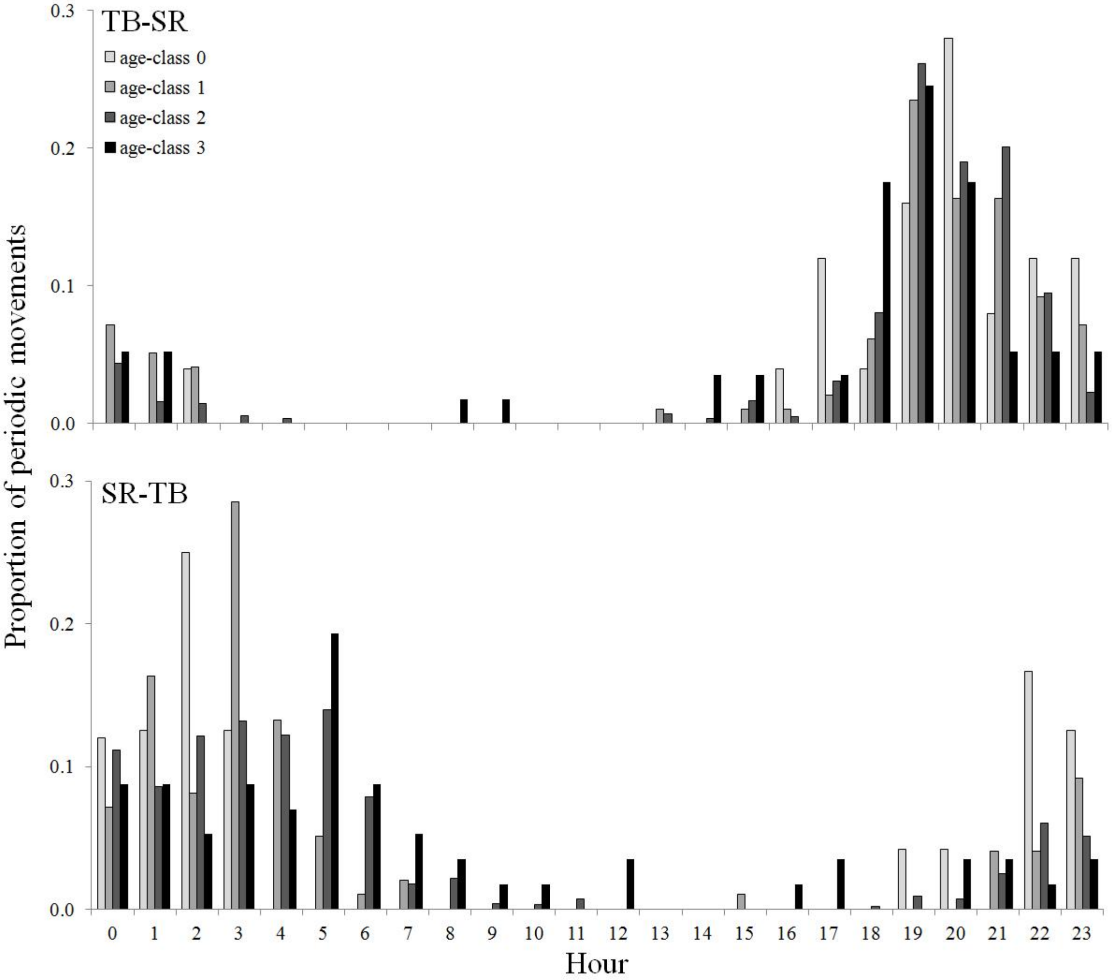

Figure 9: The hourly average proportions of periodic movements from TB to SR (top) and from SR to TB (bottom) for sharks of each age-class.

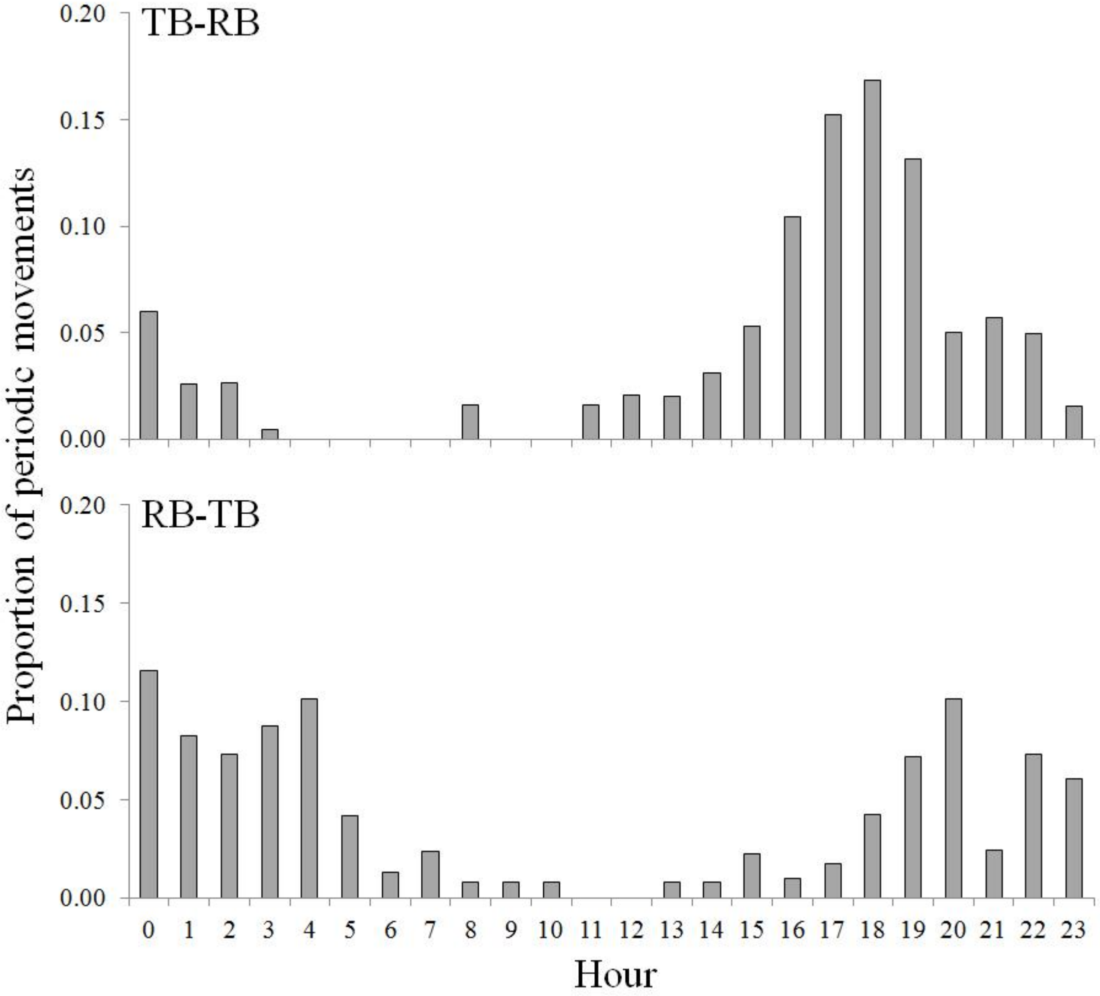

Figure 10: The hourly average proportions of periodic movements from TB to RB (top) and from RB to TB (bottom) for sharks of age-class 1 (only sharks age-class 1 exhibited periodic movements between TB and RB).

of time spent in TB decreased with age from *ca*. 82% to 41% (Fig. 8b). Sampling month was not a significant factor for any test used to investigate shark movements.

Differences in movement periodicity or body condition lead to intraspecific variability in shark movements (Fig. 11). Within age-classes 1-3, periodic commuters (n=10) had 60% greater monthly travel distances and were detected, on average, 3 km farther upstream (mean ± SE = 168 km/month ± 17 km, 9.8 ± 1.4 km from the mouth, respectively) than aperiodic sharks (n = 10; mean ± SE = 106 km/month ± 17 km, F = 8.06, p <0.01; 6.8 ± 1.1 km, F = 4.25, p = 0.03, respectively). Periodic commuters increased their use of downstream areas (SR and DR) from *ca*. 3% of their time to 58% of their time, and decreased in their use of upstream areas (TB and RB) from *ca*. 97% of their tie to 42% of their time with age. Aperiodic sharks decreased in their use of DR from *ca*. 22% of their time to 9% of their time, and increased in their use of RB from *ca*. 17% of their time to 41% of their time with age (Fig. 6).

As a result of changes in sampling protocols, body mass was only measured for 17 tracked sharks (63%), with no more than five individuals from each age-class. As such, we could only investigate the effects of body condition at the population level. Differences in body condition revealed that more emaciated individuals (i.e. those in poor condition) spent more time in the DR region than healthy individuals (F = 26.65, p < 0.01; Fig. 12), however there were no differences in the use of the other three sampling regions (SR, TB, and RB) with body condition (F = 2.14, p = 0.15; F = 1.75, p = 0.19; and F = 3.18, p = 0.08; respectively). Also, there were no clear trends in how body condition affected distance traveled (F = 1.05, p = 0.31) or used (F = 0.13, p = 0.72), or movement periodicity ($\chi^2 = 0.42$, p = 0.52). There were also no significant differences in

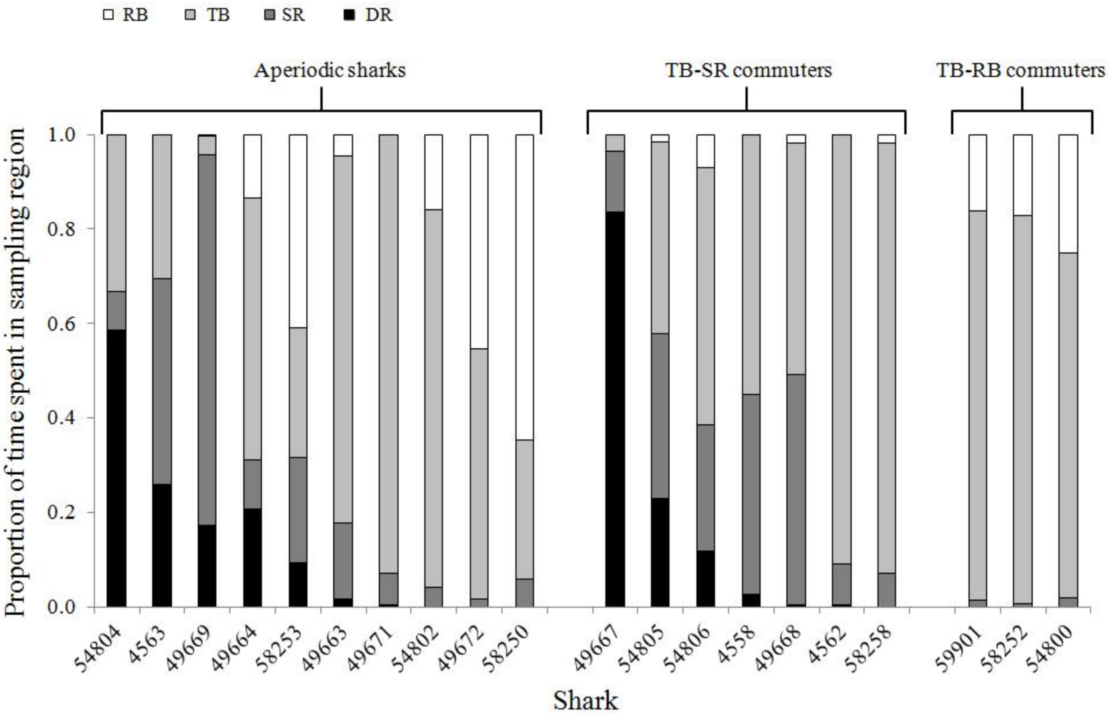

Figure 11: Proportion of time spent in each sampling region for aperiodic sharks (left), TB-SR commuters (middle), and TB-RB commuters (right) of sharks in age-classes 1-3.

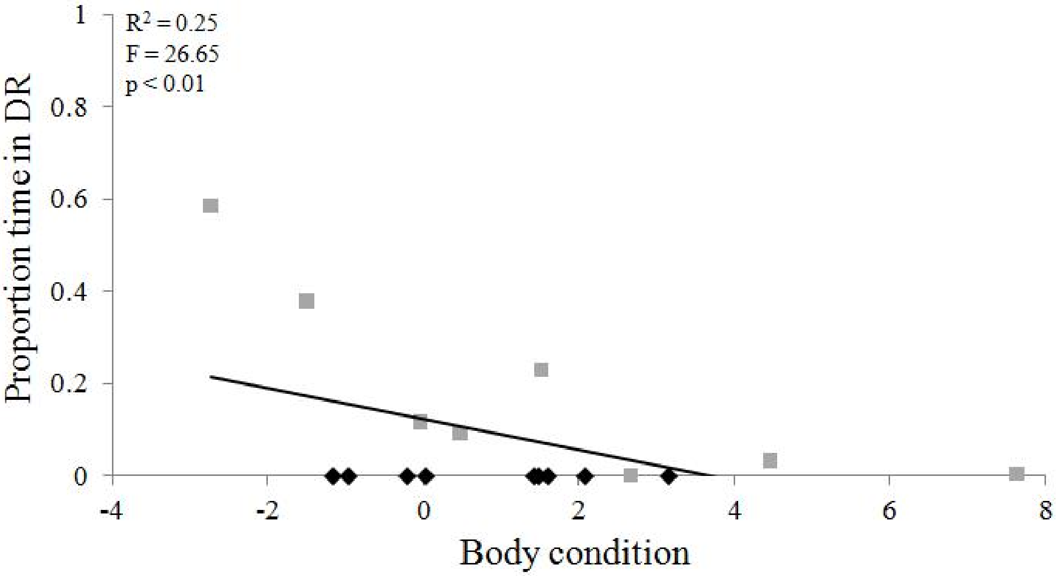

Figure 12: Effects of body condition on the proportion of time spent in DR. Black diamonds are data from individuals that were never detected in DR and gray squares are data from individuals that were detected in DR.

shark movements or distributions between to sexes (P(DR): F = 0.14, p = 0.71; P(SR): F = 3.69, p = 0.07; P(TB): F = 2.80, p = 0.11; P(RB): F = 0.07, p = 0.79; distance traveled : F = 1.33 , p = 0.27; distance used: F = 1.04, p = 0.32).

Discussion

As animals grow, changes in energetic needs and risk of predation often lead to shifts in habitat use and foraging behavior, with fitness gains associated with increased access to food in dangerous habitats increasing as overall risk declines with prey body size (Werner & Gilliam 1984). Increased body size in Nassau grouper (*Epinephelus striatus*) and bluegill sunfish, for example, leads to a decrease in predation risk and an increase in the use of more exposed, but more energetically profitable habitats, creating dietary differences across size-classes (Werner & Hall 1988; Eggleston et al., 1998; Dahlgren & Eggleston 2000). Similarly, juvenile anolis lizards (*Anolis aeneus*) avoid risky areas, which limits foraging opportunities and exposes individuals to more adverse physical conditions, whereas adults use microhabitats with more preferred conditions and dietary options (Stamps 1983). Such size- and age-related changes in behavior are common, including in sharks, but not ubiquitous among vertebrates (Wilbur 1980, Werner & Gilliam 1984; Grubbs 2010). Therefore, quantifying these ontogenetic shifts and the factors that drive them is important for understanding when and how behavior may change with size and age as well as how ontogenetic habitat shifts and ecological roles of juveniles might vary with increasing anthropogenic impacts to ecosystems (Barton 2010; Yang & Rudolf 2010).

Juvenile bull sharks in the Shark River Estuary undergo an ontogenetic niche shift with an increase in the use of more saline habitats (this study) and an increase in the proportion of marine taxa in their diets as they grow (Matich et al., 2010). Within the estuary, limited availability of marine-derived phosphorous leads to food-risk trade-offs for bull sharks, with an increase in productivity (Childers et al., 2006; Simard et al., 2006) - and likely prey availability - as well as predation risk closer to the Gulf of Mexico. As expected, the average use of more productive downstream areas increased with bull shark age from *ca*. 6% to 16% of their time, with larger body size and increased swimming speed of older individuals likely facilitating the use of riskier areas within the estuary. However, bull sharks in older age-classes continued to use upstream areas of the estuary. In the Bahamas, juvenile lemon sharks undergo ontogenetic shifts in habitat use and increase their use of risky areas as they grow, but older juveniles continue to use shallow refuge areas, likely to avoid larger cannibalistic conspecifics and other large sharks in deeper waters (Guttridge et al., 2012). Evidence of predation attempts (see Fig. 2) suggest that juvenile bull sharks within the Shark River Estuary are at risk of attack from large sharks, at least at the mouth of the estuary, for the majority, if not the duration, of their residency. Thus, larger juvenile bull sharks likely forage in more productive downstream regions, but continue to use upstream areas of the estuary as a refuge from larger sharks in between foraging bouts. These findings suggest food-risk trade-offs are important in shaping the habitat use and trophic interactions of bull sharks in the Shark River Estuary, and such trade-offs likely are widespread for sharks using coastal nurseries (Heithaus 2007; Heupel et al., 2007).

Not all individuals, however, display the same pattern of ontogenetic niche shifts. Some bull sharks appear to take more risks by frequently using downstream habitats while others rarely enter these areas until they emigrate from the nursery, while still other sharks actually increase their use of upstream areas with age. Isotopic data, which integrates diets over long time frames in sharks (months-years; e.g. MacNeil et al., 2006), suggest that many of these movement differences likely persist over periods of many months to more than a year (Matich et al., 2011). Among tracked sharks in age-classes 1-3, 50% of individuals made diel periodic movements between adjacent sampling regions (periodic sharks) and 50% of individuals made irregular and sporadic movements within and between sampling regions (aperiodic sharks). The divergence in movement tactics appears to occur in the first 6-18 months of residency in the Shark River Estuary, and consistent differences in movement tactics across age-classes (each age-class, besides newborn sharks, was comprised of 50% periodic sharks and 50% aperiodic sharks) suggest these tactics persists throughout the duration of sharks' residencies within the estuary.

Such individual differences in behavioral tactics have been documented in a variety of taxa including fishes, birds, mammals, amphibians, reptiles, and arthropods (reviewed by Gross 1996; Smith & Skulason 1996), and similar to many species, the observed divergence between periodic and aperiodic sharks in the Shark River Estuary is likely shaped by, or results in, variability in trophic interactions (e.g. Edwards et al., 2011; Henaux et al., 2011; Rosenblatt & Heithaus 2011). Populations of sharks from multiple families [e.g. Carcharinidae (e.g. *Carcharhinus plumbeus*), Triakidae (e.g. *Mustelus californicus*), Sphyrnidae (e.g. *Sphyrna lewini*)], appear to make diel movements in response to food-risk trade-offs (Holland et al., 1993; Conrath & Musick 2010; Espinoza et al., 2011). In these taxa, juvenile sharks generally use safe microhabitats during daylight hours, but at night - when sharks gain a sensory (e.g. chemosensory, electrochemical) advantage over many of their prey - are thought forage in risky microhabitats that are hypothesized to be more profitable than microhabitats used during daylight. Within the Shark River Estuary, periodic commuters of all ages spent most of their time in the safety of TB where no predatory sharks were detected, and regularly made nightly trips upstream to RB or downstream to SR and returned to TB before sunrise, likely for foraging purposes. The differences in periodic movements between individuals (either between TB-RB or TB-SR) may be attributed to size-based differences in food-risk trade-offs (for TB-SR commuters) or foraging considerations (for TB-RB commuters). At night, younger periodic sharks predominantly travelled to RB, where predation risk was negligible but food abundance is likely similar to TB, while older periodic sharks traveled to SR where productivity and predation risk is higher than TB. These age-specific differences in periodic movements result in an increase in the use of downstream habitats and a decrease in the use of upstream habitats with age, which helped drive population-level trends in habitat use.

In contrast, aperiodic sharks did not make consistent diel movements between adjacent sampling regions, suggesting individual differences in movement tactics are nested within the observed ontogenetic niche shift of bull sharks. Anadromous coho salmon undergo ontogenetic niche shifts, with transitions between freshwater and marine habitats, and nested within this ontogenetic niche shift, males diverge into sneakers and fighters that employ different behavioral strategies for reproductive purposes (Gross

1991; Gross & Repka 1998). Similarly, male Iberian wall lizards (*Podarcis hispanica*) are more frequently exposed to predators than females, which enables males to habituate to novel situations faster than females, and nested within sex-specific differences, less social individuals spend less time in refuge and habituate to novel conditions faster than more social individuals (Rodriguez-Prieto et al., 2011). However, Iberian wall lizards that habituate faster may suffer greater mortality because of their increased exposure to predators. Interestingly, among bull sharks few measureable differences were detected between periodic and aperiodic individuals during the study - periodic and aperiodic sharks did not exhibit differences in body condition, age-based differences in size, detectable differences in residency time, or overall use of risky areas, however the relatively short duration of the study may mask the long-term benefits of choosing one movement strategy over another.

While individual differences in movement periodicity and trophic interactions (Matich et al., 2011) suggest intraspecific variability is consistent through time, state-dependent variation in the use of DR by commuting sharks (both periodic and aperiodic) suggests that other individual differences in movements are more transitory and shaped by both internal and external factors. In general, individuals are expected to take greater risks to gain energy when they are close to starvation. For example, green sea turtles (*Chelonia mydas*) in poor body condition used profitable, but risky microhabitats more often than turtles in good condition (Heithaus et al., 2007a), and similar condition-dependent risk taking is seen in taxa as diverse as copepods (*Metridia pacifica;* Hays et al., 2001) and wildebeest (*Connochaetes taurinus*; Sinclair & Arcese 1995). Bull sharks fit this pattern with an increase in the use of the more productive but dangerous DR as body condition declined. This result only pertained to individuals that used DR at least occasionally, however. Indeed, 53% of individuals in which body condition was measured (n = 9) were never detected in DR, even for individuals in poor condition (n = 4). Therefore, some individuals appear risk-averse, even when faced with energetic challenges, which may be attributed to individual differences in their personalities (Sih et al., 2004b).

Investigating the drivers and consequences of individual differences and nested patterns of behavior is important for elucidating the scale(s) at which intraspecific variability occurs in order to improve our ability to preserve variability (genotypic and phenotypic) within populations. Among juvenile bull sharks, limited productivity within the Shark River Estuary apparently leads to heightened intraspecific competition within the nursery that could drive the observed considerable degree of individual differences in trophic interactions (Matich et al., 2011), as has been observed in other systems (e.g. Smith & Skukason 1996; Day & Young 2004; Pfennig et al., 2007). As such, limited productivity in the Shark River Estuary may drive the individual differences in movement tactics and foraging decisions observed during this study and heterogeneity in trajectories through ontogenetic shifts. This includes both long-term specialization in feeding within a single food web and consistent movement tactics, as well as more flexible movements and trophic interactions (Matich et al., 2011). The nesting of movement patterns highlights the interplay of long-term and short-term variation in behavioral tactics that can shape life history events. Past studies of such individual variability and nesting has predominantly focused on behavioral differences nested within sexual polymorphisms (reviewed by Svensson et al., 2009; Galeotti et al., 2013), with much less attention focusing on other nested traits (but see Gross 1984; Smith & Skulason 1996 for reviews). However, research on divergent strategies suggests that nested behavioral differences can result in greater population densities by reducing intraspecific competition (e.g. Maynard Smith 1976; Gross 1984; Moran 1992). Our study adds to a limited body of work that shows behavioral divergence in large-bodied, highly-mobile species can occur early in the life-history of individuals and persist through their lifetimes, and shows how intrapopulation variation in behavior may contain both long- and short-term components. With a growing need to understand the development and persistence of behavioral differences within populations, further research investigating the long-term effects of individual differences and modeling how changes in external factors affect individual fitness will aid in the development of strategies to preserve genotypic and phenotypic diversity in the face of ecosystem-wide environmental changes.

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CHAPTER VII

GENERAL CONCLUSIONS

Predators affect food web structure through their trophic interactions, which in turn can impact ecosystem function. Such top-down effects have been relatively well studied (Estes et al. 2011), but recent work suggests predators can play less appreciated, but perhaps no less important, roles in ecosystems including stimulating primary productivity through material translocation (e.g. Schmitz et al. 2010, Rosenblatt et al. 2013). Many predator populations, in the oceans and freshwater as well as on land, are still in decline, necessitating an understanding of the factors that shape their ecological roles and importance in order to predict and ameliorate environmental changes that may result (Heithaus et al. 2008, Ripple et al. 2014). Increasingly, it is becoming obvious that one particular area of inquiry where we still lack a strong functional understanding of predators' roles is how behavior might vary consistently within and among individuals and, in turn, how these behavioral polymorphisms might affect individuals' roles in ecosystems. Such studies are important for understanding the ecological implications of individual differences in the responses of animals to environmental drivers and human disturbance.

To help fill this gap in knowledge on the drivers of predator behavior and the persistence of individual variability within predator populations, I investigated the factors that shape bull shark movements, residency patterns, and trophic interactions in the Shark River Estuary, Florida, USA. I also investigated how bull sharks responses to such drivers vary among individuals, and in turn how intraspecific variability affects the niche widths and particular roles sharks play within the ecosystem. The results of my research have helped elucidate the importance of food-risk trade-offs (Matich and Heithaus *in review;* Chapter 6), resource limitations (Matich et al. 2011, Matich and Heithaus 2014, *in review*; Chapters 2,5, and 6), and environmental variability (Matich and Heithaus 2012; Chapter 3) in shaping juvenile shark behavior and life-history, and the importance of each in driving the divergence of behavioral tactics among juvenile sharks within the Shark River Estuary (Matich et al. 2011, Matich and Heithaus *in review*; Chapters 5 and 6). My research provides insight into the diversity of factors that shape bull shark behavior, and how changes in these factors attributed to restoration and climate change may affect the roles sharks play in the ecosystem. My research also provides a framework in which to investigate behavioral variability at multiple organizational levels to better understand what shapes phenotypic variability within wild populations, and its consequences within populations and communities.

In Chapter 2, I used passive acoustic tracking to investigate temporal variability in the movement patterns and trophic interactions of bull sharks in the Shark River Estuary, and to gain insight into the importance of resource subsidies for juvenile sharks within the estuary. My data suggest that when water levels of marshland adjacent to the Shark River Estuary decrease beyond a certain threshold (0 cm in reference to elevation), sharks significantly increase their use of upstream channels where migrating marsh taxa enter the estuary due to marsh drying (Boucek and Rehage 2013). In turn, stable isotope analysis suggests bull shark trophic interactions also change in response to this environmental change, with a significant increase in the consumption of freshwater taxa, presumably from the marsh. Studies of other predators in the system (e.g. *Centropomus undecimalis*) also show significant changes in diets during late spring, when gut fullness of predators increases, and taxa predators target as prey change in response to the resource subsidy provided by migrating marsh taxa (Boucek and Rehage 2013). As such, my research supports the contention that allocthonous resources may be critical for consumers in oligotrophic systems, like the Shark River Estuary (e.g. Polis and Hurd 1996, Post et al. 1998, Helfield and Naiman 2001). Chapter 2 also highlights the flexibility in trophic interactions within individual sharks, and provides an analytical framework for using stable isotope values of dynamic tissues to investigate such flexibility within populations.

In Chapter 3, I used passive acoustic telemetry to investigate the response of juvenile bull sharks to a pulsed extreme environmental event. In January 2010, an extended bout of cold temperatures not experienced for more than 50 years swept through south Florida (NOAA report), and water temperatures in the Shark River Estuary dropped to 9°C in some areas. The extended drop in temperatures led to behavioral changes among many species, and death rates significantly increased for many animals in the region (Rehage et al. 2010). During and immediately after the event, juvenile bull sharks in the Shark River Estuary adopted two different behavioral responses to the cold temperatures - 43% of tracked sharks remained in the system and died (representing a 700% increase in the death rate compared to before the event), and 57% left the estuary, presumably to seek out deep water refuges in marine waters (representing a 400% increase in emigration rate compared to before the event). Only one shark (13% of sharks that emigrated) returned to the estuary following the event. Similar reactions to extreme weather events (e.g. tropical storms) have been observed in other shark species (*Carcharhinus amboinensis*, *C. limbatus, C. sorrah*, and *C. tilstoni*), however in such cases, sharks were observed to leave their respective ecosystems prior to extreme events and return weeks later (Heupel et al. 2003, Udyawer et al. 2013). Because bull sharks did not depart the estuary prior to the event, and no sharks returned immediately afterward, the cold snap in January 2010 may shape the demographics for years to come. The importance of such episodic mortality/emigration events on the dynamics of shark nurseries and the potential consequences for adult populations remain unknown. Chapter 3 adds to a growing literature on species' responses to environmental change, especially extreme episodic events, which is of concern since such events are predicted to occur with increasing frequency with climate change (Christensen and Kanikicharla 2013).

In Chapter 4, I used stable isotope analysis to investigate size-based shifts in the trophic interactions of juvenile bull sharks in the Shark River Estuary to gain insight into individual differences in shark behavior. Previous studies suggest bull sharks undergo an ontogenetic shift in habitat use, from predominantly using freshwater and estuarine habitats to using marine habitats (e.g. Wiley and Simpfendorfer 2007, Heupel and Simpfendorfer 2008, Curtis et al. 2011). However, ontogenetic shifts in bull shark diets were previously undocumented beyond anecdotal accounts. My data suggest that shark trophic interactions in the Shark River Estuary follow - at the population level - a similar pattern to habitat use in other systems. Sharks gradually shift from predominantly feeding upon freshwater and estuarine taxa to feeding on marine taxa. This ontogenetic shift in shark diets is likely attributed to a combination of shifts in habitat use patterns in response to size-based changes in energetic needs and vulnerability to predation risk, and prey capture abilities in response to age-/size-based changes in prey recognition, swimming speed, and gape width (reviewed in Wilbur 1980, Werner and Gilliam 1984, Grubbs 2010). Interestingly, I found that size-isotope relationships were not uniform across all individuals, however, with some sharks appearing to change diets sooner/later than others. Also, although newborn juvenile bull sharks switch from catabolism for energy by breaking down energy stores in their livers to self-provisioning fairly rapidly (McMeans et al. 2009), the transition between catabolism and metabolism appears to vary among individuals. Thus individual differences in trophic interactions may develop during the early life-history of juvenile bull sharks in the Shark River Estuary, and persist through time.

In Chapter 5, I used stable isotope analysis to delve deeper into the possibility for persistent interindividual differences in juvenile bull shark trophic interactions within the Shark River Estuary. Using a novel analytical framework for stable isotope data, I suggest that bull sharks exhibit relatively high levels of temporal stability in their diets compared to tiger sharks (*Galeocerdo cuvier*) - another species thought of as a trophic generalist at the population level (e.g. Simpfendorfer et al. 2001, Weatherbee and Cortes 2004). Many bull sharks specialized on feeding on prey from one food web or a consistent mix of prey (16% of sharks specialized on marine taxa and 41% of sharks specialized on freshwater/estuarine taxa), and resource limitation within the Shark River Estuary appears to be an important driver of individual differences in trophic interactions. Interestingly, individual specializations among bull sharks were not geographically dependent. Sharks caught throughout the estuary specialized on either marine or estuarine food webs, while many other individuals (43%) exhibited mixed or generalized diets. This lack of spatial pattern in foraging specializations (i.e. marine specialists can be found over 20 km from the ocean) suggests that some sharks may facilitate the movement of nutrients across microhabitat boundaries within the Shark River Estuary, which could be important in this oligotrophic system (Childers 2006, Heithaus et al. 2009, Rosenblatt and Heithaus 2011).

In Chapter 6, I used passive acoustic telemetry to investigate how shark movement patterns vary with age. I observed a gradual shift from the youngest sharks predominantly using freshwater and estuarine areas, to older individuals using marine areas more frequently in the Shark River Estuary. Age/size-based shifts in shark habitat use are likely attributed to size-based differences in foraging decisions and vulnerability to predation risk, with larger sharks using more productive but risky downstream microhabitats to meet higher energetic needs (reviewed in Heithaus 2007, Grubbs 2010). However, similar to patterns in trophic interactions (Chapters 4 and 5), juvenile bull sharks were not uniform in their movement patterns, and there was considerable variability in habitat use and risk taking among individuals of the same age-classes. Among sharks in age-classes 1-3, 50% of individuals exhibited periodic, diel movement patterns between adjacent regions of the Shark River Estuary, likely for foraging purposes, and the other 50% of sharks did not exhibit periodic movements. Similarly, 47% of tracked sharks exhibited condition-dependent risk taking, with an increase in the use of risky downstream microhabitats as body condition decreased, while 53% of sharks avoided risky downstream areas regardless of body condition. These individual differences in movement patterns suggest that juvenile bull sharks in the Shark River Estuary diverge into various movement tactics at an early age. Nested patterns of behavior are not well documented among predator populations outside of sexual dimorphism, and Chapter 6 shows that individual variation within the Shark River

Estuary shark population may contain both long- (periodic movements) and short-term (condition-dependent movements) components.

Overall, my results suggest that within the Shark River Estuary, juvenile bull shark behavior is shaped by a complex array of external and internal factors (Fig. 1), which appear to interact in some contexts - e.g. ontogenetic niche shifts are likely due to size-related changes in energetic needs and spatial variability in predation risk and food availability (Chapters 4 and 6); temporal variability in shark foraging behavior is likely attributed to spatial variability in food resources and flexible foraging decisions of sharks (Chapter 2), which can vary among individuals (Chapter 6). Food availability and predation risk, however, appear to be the most important drivers of juvenile bull shark behavior in the Shark River Estuary, with unpredictable changes in environmental conditions also affecting nursery dynamics, and relatively minimal impacts from variation in other abiotic parameters (e.g. salinity, dissolved oxygen) within normal bounds.

Despite population-level trends, bull sharks responses to limited resources, allocthonous food inputs, and food-risk trade-offs were not uniform. It is likely that individual variation among sharks is driven largely by ecosystem oligotrophy, which causes individuals to specialize in feeding within distinct food webs or on stable mixes of resources from multiple food webs. Trophic specializations and niche width divergence likely reduce intraspecific competition within the nursery, and may increase resource use efficiency among sharks (e.g. Estes et al. 2003, Pfennig 2007, Martin and Pfennig 2009). Some sharks, however, remain more flexible in their behavior and display similarly variable trophic interactions and movement patterns, as would be expected by an

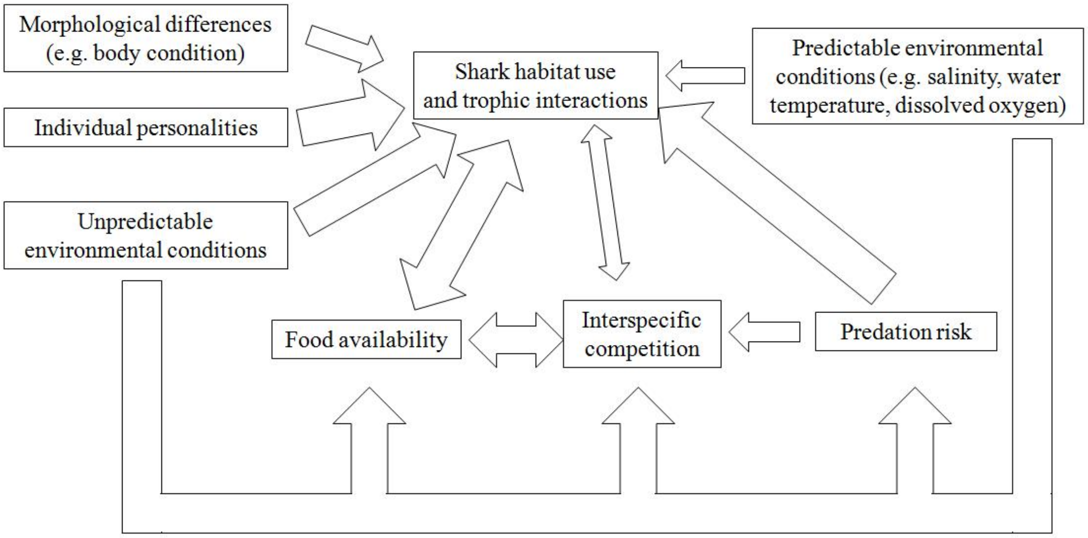

Figure 1: Conceptual model of the factors affecting the habitat use and trophic interactions of juvenile bull sharks inhabiting the Shark River Estuary. The size of arrows suggests the hypothesized importance of each factor in shaping bull shark behavior.

"opportunistic generalist species" (e.g. Pandit et al. 2009, Ravigne et al. 2009, Poisot et al. 2011). Even within individuals that appear to be specialized, there is behavioral flexibility (e.g. condition-dependent habitat use), which likely is critical to survival in resource-limited conditions (e.g. Clark 1994). Indeed, some individuals that appear to adopt more risk-prone, but high-reward, behavior by using productive but dangerous downstream areas vary in their amount of risk-taking in relation to their body condition. Upstream, many individuals take advantage of allochthonous resources from freshwater marshes. The recurring frequency of divergent strategies among all sharks (besides newborns) suggests these individual differences likely develop early in the life-history of bull sharks, and persist at least throughout their residency within the Shark River Estuary. Limited food resources appears to be the main driver leading to intraspecific variability among juvenile bull sharks, but individual differences are likely not exclusively driven by oligotrophy, and innate differences (e.g. personalities; Sih et al. 2004) in responsiveness to predation risk also likely play a role in driving the observed patterns (Fig. 2). Less information, however, is available on how such factors lead to divergent strategies, and thus testing the strength of multiple factors in shaping individual variability is a critical need for future research, especially with predicted changes in community structure in response to climate change (e.g. Parmesan and Yohe 2003, Berg et al. 2010, Hof et al. 2011).

The interplay of external factors, along with individual differences in boldness and/or exploratory nature may also play a role in the nesting of divergent strategies within bull sharks in the Shark River Estuary (e.g. Smith and Skulason 1996, Sih et al. 2004, Rodriguez-Prieto et al. 2011). Individual differences in foraging behavior and

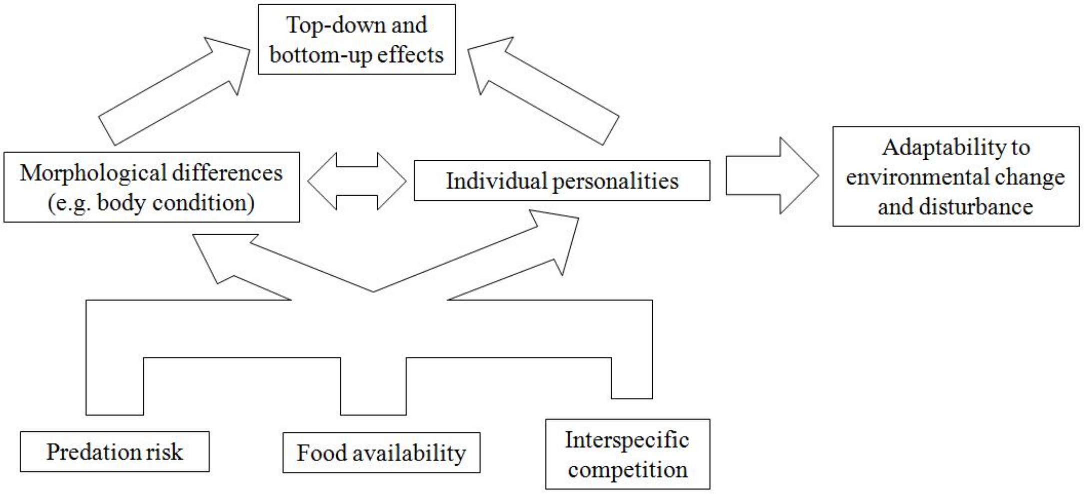

Figure 2: Conceptual model of the factors affecting individual differences in the behavior of juvenile bull sharks inhabiting the Shark River Estuary, and in turn their roles in the ecosystem, and potentially their ability to adjust to environmental change.

movement patterns were prevalent within population-level changes in habitat use and trophic interactions, suggesting intraspecific variability occurs over multiple organizational levels in juvenile bull sharks - e.g. in general sharks increase their use of more productive downstream areas to account for increased energetic needs, but some individuals appear risk-averse and avoid risky microhabitats regardless of energetic needs attributed to size or body conditions, while others specialize on feeding from food webs in risky marine habitats. Studies on behavioral nesting have received relatively little attention outside of reproductive capacities (e.g. Gross 1984, 1996, Gross and Repka 1998), though, suggesting such patterns may be rare, or simply are overlooked. Thus, more attention should be committed to understanding the drivers of individual differences at multiple levels of organization, which may reveal the importance of behavioral layering in shaping the ecological roles of species, as well as improve our ability to preserve variability (genotypic and phenotypic) within populations, and increase our understanding of its function for species in the face of ecosystem-wide environmental changes.

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