Multi-Tissue Stable Isotope Analysis and Acoustic Telemetry Reveal Seasonal Variability in the Trophic Interactions of Juvenile Bull Sharks in a Coastal Estuary

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Recommended Citation


This material is based upon work supported by the National Science Foundation through the Florida Coastal Everglades Long-Term Ecological Research program under Cooperative Agreements #DBI-0620409 and #DEB-9910514. Any opinions, findings, conclusions, or recommendations expressed in the material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

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Multi-tissue stable isotope analysis and acoustic telemetry reveal seasonal variability in the trophic interactions of juvenile bull sharks in a coastal estuary

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Running head: seasonal changes in bull shark diets

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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.

**Key words:** $\delta^{13}$C, food webs, habitat use, movements, shark nursery, trophic ecology
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.

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

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 prey pulse 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 & Boucek unpublished data).
These differences in the $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ values are compared to more stable blood $\delta^{13}C$ 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 δ¹³C 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 δ¹³C values and δ¹⁵N values were 0.13‰ ± 0.20 SE. The standard deviations of standards run for
Yale were 0.14‰ for $\delta^{13}C$ and 0.22‰ for $\delta^{15}N$, and 0.29‰ for $\delta^{13}C$ and 0.24‰ for $\delta^{15}N$ 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 ∆¹³C 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 (∆¹³C 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 δ¹³C values from 90 juvenile bull sharks against one another, and performed linear regression to
quantify the relationship between δ¹³C values (see Matich, Heithaus & Layman 2010 for further details). To test whether differences between δ¹³C values of blood and plasma varied across the observed range of δ¹³C 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 δ¹³C discrimination varied with δ¹³C values of shark diets.

Temporal change in diet

In addition to providing an estimate of Δ¹³C plasma-blood, the regression plot of blood and plasma δ¹³C 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 δ¹³C values more enriched (i.e. less negative) than predicted by the model, and data points below the regression line indicate plasma δ¹³C 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 δ¹³C values more than 0.4‰ enriched above δ¹³C values of blood (calculated Δ¹³C 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 δ¹³C 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 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 $\rightarrow$ marsh prey ($E\rightarrow F$); 2) marine + estuarine prey $\rightarrow$ estuarine + marsh prey ($M+E\rightarrow E+F$); 3) marine + estuarine prey $\rightarrow$ marsh prey ($M+E\rightarrow F$); 4) marine prey $\rightarrow$ estuarine prey ($M\rightarrow E$); 5) marine prey $\rightarrow$ estuarine + marsh prey ($M\rightarrow E+F$); and 6) marine prey $\rightarrow$ marsh prey ($M\rightarrow F$) (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 ± 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 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 $\leq 0$ 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 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).

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 δ^{13}C values that ranged from -17.5‰ to -26.5‰ (mean δ^{13}C ± SD = -22.9 ± 2.4‰) and -17.8‰ to -25.3‰ (mean δ^{13}C ± SD = -22.4 ± 2.3‰), respectively, and blood and plasma δ^{15}N values that ranged from 10.5‰ to 12.8‰ (mean δ^{15}N ± SD = 11.6 ± 0.5‰) and 9.9‰ to 12.4‰ (mean δ^{15}N ± SD = 11.5 ± 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 (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
\[ \Delta^{13}C \text{ plasma-blood} = 0.9\% \] were the best fit (mean distance ± SE = 0.5 ± 0.1\%, 0.4 ± 0.1\% for
\[ \Delta^{13}C \text{ plasma-blood} = 0.7\% \text{ and } 0.9\%, \text{ respectively} \). Models 2 and 3 (marine + estuarine prey
\rightarrow \text{ estuarine + freshwater prey, and marine + estuarine prey } \rightarrow \text{ 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 \( \Delta^{13}C \text{ plasma-blood} = 0.9\% \) 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, brown bears
(\textit{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.
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. \textit{Amia calva}, \textit{Centropomus undecimalis}, \textit{Micropterus salmoides}; Rehage
& Loftus 2007; Boucek & Rehage \textit{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 $\delta^{13}$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.
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 $\leq 0$ 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 $\leq 0$ 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.

**Acknowledgments**

This work was made possible by the Florida Coastal Everglades LTER, and funding for this project was provided by the National Science Foundation (DBI0620409, DEB9910514, DRL0959026) and Florida International University’s Marine Sciences Program. We thank the many volunteers who assisted with shark fishing, especially Cynthia Aceves, Greg Mineau, and Cate Pritchard. Special thanks to Adam Rosenblatt for establishing the array of acoustic receivers, and helping with downloading data from receivers and loggers. Thanks to Andrew Fritz for help with managing acoustic data. Thanks to Dr Joel Trexler for providing logistical and analytical support for this project, and Dr Craig Layman and Dr Jennifer Rehage for comments on the manuscript. Thanks also to the United States Geological Survey for providing data in which to classify capture dates into seasons, and thanks to Dr Jennifer Rehage and Ross Boucek for providing tissue samples of teleosts for stable isotope analysis. Research was conducted under Everglades National Park permits EVER-2009-SCI-0024, EVER-2007-SCI-0025, and EVER-2005-SCI-0030 and with Florida International University IACUC approval.
Capture data and isotopic data are available upon request through the FCE LTER website:

http://fce.lternet.edu/.

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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.

<table>
<thead>
<tr>
<th>Model</th>
<th>$\delta^{13}$C at equilibrium</th>
<th>$\delta^{13}$C at prey pulse</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (E→F)</td>
<td>-28.0</td>
<td>-30.5</td>
</tr>
<tr>
<td>2 (M+E→E+F)</td>
<td>-21.0</td>
<td>-29.3</td>
</tr>
<tr>
<td>3 (M+E→F)</td>
<td>-21.0</td>
<td>-30.5</td>
</tr>
<tr>
<td>4 (M→E)</td>
<td>-14.1</td>
<td>-28.0</td>
</tr>
<tr>
<td>5 (M→E+F)</td>
<td>-14.1</td>
<td>-29.3</td>
</tr>
<tr>
<td>6 (M→F)</td>
<td>-14.1</td>
<td>-30.5</td>
</tr>
</tbody>
</table>
Table 2: Mean distances ± SE (in ‰) between actual δ¹³C 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 (Δ¹³C 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.

<table>
<thead>
<tr>
<th>Model</th>
<th>0.2‰</th>
<th>0.4‰</th>
<th>0.7‰</th>
<th>0.9‰</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (E→F)</td>
<td>0.7 ± &lt;0.1</td>
<td>0.6 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>2 (M+E→F+E+F)</td>
<td>0.7 ± &lt;0.1</td>
<td>0.5 ± &lt;0.1</td>
<td>0.2 ± &lt;0.1</td>
<td>0.1 ± &lt;0.1</td>
</tr>
<tr>
<td>3 (M+E→F)</td>
<td>0.8 ± &lt;0.1</td>
<td>0.5 ± &lt;0.1</td>
<td>0.3 ± &lt;0.1</td>
<td>0.2 ± &lt;0.1</td>
</tr>
<tr>
<td>4 (M→E)</td>
<td>1.0 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>5 (M→E+F)</td>
<td>1.0 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>6 (M→F)</td>
<td>1.1 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>0.8 ± 0.2</td>
<td>0.7 ± 0.2</td>
</tr>
</tbody>
</table>
Figure captions:

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.

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 (♦).

Figure 3: Linear regression of paired blood and plasma δ^{13}C values. The mean difference between blood and plasma δ^{13}C values (0.4‰) serves as an approximation for the difference in δ^{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 δ^{13}C or more depleted in δ^{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.

Figure 4: Model predictions for changes in a) δ^{13}C values of plasma and blood and b) δ^{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 δ^{13}C values should be -28.0‰ and mean plasma δ^{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 δ^{13}C values will become more depleted faster than blood δ^{13}C values because plasma δ^{13}C turnover (~32 day half-life) is faster in elasmobranchs than blood δ^{13}C turnover (~61 day half-life). In this scenario, differences between plasma and blood δ^{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 δ^{13}C (plasma approaches it asymptote much sooner than blood).

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.

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).
Figure 7: Temporal variation in $\delta^{13}$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 $\delta^{13}$C values for model 2 (marine + estuarine prey $\rightarrow$ estuarine + freshwater prey attributed) at one isotopic half-life and $\Delta^{13}$C plasma-blood = 0.9‰, which was the best model for predicting changes in $\delta^{13}$C residuals. Model selection was not influenced by assumptions about isotopic half life or $\Delta^{13}$C.

Figure 8: Seasonal variation in mean $\delta^{13}$C residuals. Error bars are $\pm$ SE, and bars with different letters are significantly different based on post hoc Tukey’s tests.

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 $\pm$ SE, and bars with different letters are significantly different based on post hoc Tukey’s tests.
Figure 2:
Figure 4:
Figure 5:
Figure 6:
Figure 7:
Figure 8: