1-2014

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

Philip Matich
Marine Sciences Program, Florida International University

Michael R. Heithaus
Marine Sciences Program, Florida International University, heithaus@fiu.edu

Recommended Citation

Matich, P., M.R. Heithaus. 2013. 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 DOI: 10.1111/1365-2656.12106

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.

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

Philip Matich* and Michael R. Heithaus

Marine Sciences Program

Florida International University

3000 NE 151st

North Miami, FL 33181, USA

Running head: seasonal changes in bull shark diets

*To whom correspondence should be addressed, pmati001@fiu.edu, (305) 919-5602 voice,

(305) 919-4030 fax

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

91 effective method when individuals are easily recaptured (e.g. Drago et al. 2010). But many
92 ecosystems are open and animals can move across habitat boundaries, making it difficult to
93 regularly sample the same individuals over time, and limiting the use of this approach.
94 A third strategy to investigate temporal change in diets is to compare the isotopic values
95 of metabolically active tissues with significantly different turnover rates collected during one
96 sampling event. A tissue with a fast isotopic turnover rate like blood in Japanese quail (*Coturnix*
97 *japonica*, $\delta^{13}C$ half-life = 11.4 days), provides information on recent trophic interactions, while a
98 tissue with a slower turnover rate like bone collagen ($\delta^{13}C$ half-life = 173.3 days in *C. japonica*),
99 provides a view of the average trophic interactions over an extended time period (Hobson &
100 Clark 1992). If the isotope values of a fast turnover tissue are compared to the isotope values of
101 a slow turnover tissue, the difference in isotope values can provide insight into the temporal
102 variability of an organism's trophic interactions. Comparing the isotope values of multiple
103 metabolically active tissues, however, must be conducted carefully because the values of stable
104 isotopes in the tissues of a consumer are not identical to that of their food due to isotopic
105 discrimination (Gannes, O'Brien & Martinez del Rio 1997), and different tissues from the same
106 organism can have different discrimination factors (e.g. Vander Zanden & Rasmussen 2001;
107 Sweeting et al. 2007; Buchheister & Latour 2010). Thus, understanding the isotopic
108 discrimination values of the tissues being used is important when investigating temporal
109 variability in trophic interactions.
110 While stable isotope analysis provides useful information on the trophic interactions of
111 animals, data from complimentary approaches strengthen inferences about the trophic ecology of
112 individuals and populations. Acoustic telemetry, for example, provides data on the movements
113 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

137 variation in the distribution, abundance, and behavior of many resident and migratory species in
138 the Florida Coastal Everglades, including the Shark River Estuary (Chick, Ruetz & Trexler 2004;
139 Ruetz, Trexler & Jordan 2005; Rehage & Trexler 2006). Therefore, trophic interactions vary in
140 space and time annually as predators and prey move to stay within acceptable environmental
141 conditions and/or to take advantage of seasonal pulses of prey. Seasonal pulses of freshwater
142 prey into mangrove-lined creeks in the upstream region of the Shark River Estuary occur when
143 water levels in freshwater marshes drop during the dry season (Rehage & Trexler 2006; Rehage
144 & Loftus 2007; Fig. 1), and teleost predators rely on this prey pulse as an important seasonal
145 component of their diets (Boucek & Rehage *in press*). Our previous work revealed that juvenile
146 bull sharks have relatively high levels of individual dietary specialization in the Shark River
147 Estuary (Matich, Heithaus & Layman 2011). Yet, stable isotope analysis revealed that some
148 individuals (*ca.* 13%) exhibit temporal variability in their trophic interactions, possibly driven by
149 use of this seasonal pulse of marsh prey.
150 Marsh water levels serve as a seasonal indicator for when taxa migrate from the marsh
151 into deep-water refuges. For the purposes of our analyses, we used water level data from United
152 States Geological Survey water station 252820080505400 Everglades National Park
153 (N25°28'20", W80°50'54"; Fig. 1) adjacent to our study system. When marsh water elevation
154 drops below 10 cm in depth in reference to elevation, the marsh becomes unsuitable for large
155 aquatic taxa (> 8 cm), which are forced to seek out deep-water habitat. As such, the dry season,
156 in reference to water levels, occurs when marsh water elevations are less than 10 cm and the wet
157 season occurs when water levels are greater than 10 cm. These thresholds have been used in
158 studies of movements of Everglades marsh taxa into estuarine creeks (e.g. Chick, Ruetz &
159 Trexler 2004; Rehage & Loftus 2007; Parkos, Ruetz & Trexler 2011). Because the abundance of

160 marsh prey within mangrove-lined creeks changes considerably within seasons (Rehage &
161 Loftus 2007), we further divided each season into sub-seasons (i.e. early and late dry seasons,
162 and early and late wet seasons). During our study, the late wet season of 2008/2009 ended on 29
163 Feb 2009, and the early dry season was from 1 Mar to 13 Apr 2009. The late dry season was
164 from 14 Apr to 28 May 2009, the early wet season was from 29 May to 16 Oct 2009, and the late
165 wet season began 17 Oct 2009.

166 Within the confines of the Shark River Estuary, there are two isotopically distinct food
167 webs - freshwater/estuarine ($\delta^{13}C < -25‰$) and marine ($\delta^{13}C > -19‰$; Fry & Smith 2002; Chasar
168 et al. 2005; Williams & Trexler 2006; Fig. 2). Marsh taxa that enter the estuary during the dry
169 season prey pulse have more depleted $\delta^{13}C$ values (mean ± SE = -30.5 ± 0.5‰) than resident
170 freshwater/estuarine taxa (mean ± SE = -28.0 ± 0.5‰; Matich & Boucek unpublished data).
171 These differences in the $\delta^{13}C$ values of potential prey species enabled us to investigate seasonal
172 shifts in bull shark diets between prey with different basal carbon sources, and the potential use
173 of the freshwater prey pulse, by quantifying temporal variability in the $\delta^{13}C$ values of bull shark
174 tissues.

175

176 *Field Sampling*

177 Bull sharks were captured from Oct 2008 to Mar 2012 on ~500 m longlines, fitted with
178 40-55 14/0 or 15/0 Mustad tuna circle hooks baited with mullet (*Mugil* sp.) and attached to ~2 m
179 of 400 kg monofilament line (see Heithaus et al. 2009 for further details of sampling equipment).
180 Captured sharks were processed alongside the sampling vessel, or within a water-filled, aerated
181 cooler on board. Shark total length was measured to the nearest centimeter. An 18 gauge needle
182 was used to collect 4 mL of blood from the caudal vein. During collection, 3 mL of blood was

183 placed into BD Vacutainer blood collection vials with neither additives nor interior coating, and
184 then immediately separated into its components, including plasma, using a centrifuge spun for
185 one minute at 3000 rpm. The remaining 1 mL of blood was retained in its original composition
186 (whole blood, "blood" hereafter). Based on several lab studies, plasma has an isotopic half-life
187 of ~32 days in elasmobranchs (Kim et al. 2012), and likely serves as a short-term diet indicator
188 for juvenile bull sharks, while blood has an isotopic half-life of ~61 days (MacNeil, Drouillard &
189 Fisk 2006), and likely serves as a longer-term diet indicator for juvenile bull sharks. As such,
190 when more dynamic plasma $\delta^{13}C$ values are compared to more stable blood $\delta^{13}C$ values they can
191 be used to study short-term changes in the diets of sharks, and provide diet information over the
192 time-frame juvenile bull sharks may respond to the pulse of marsh prey into the Shark River
193 Estuary. Importantly, such inter-tissue comparisons are useful even if tissues do not reach full
194 dietary equilibrium (i.e. four half lives), because they can provide data on the direction (i.e. an
195 increase of decrease in $\delta^{13}C$ depleted prey) and magnitude of dietary change. Tissue samples
196 were put on ice and frozen before laboratory preparations. All samples were dried and
197 homogenized prior to stable isotopic analysis.

198 Muscle tissue was collected from known estuarine (*Lutjanus griseus*, *Mugil cephalus*)
199 and marsh teleosts (*Lepomis marginatus , L. microlophus, L. punctatus*) that may serve as prey
200 for juvenile bull sharks (based on gape size of sharks, size of teleosts, and stomach contents
201 analysis of juvenile bull sharks in other systems: Snelson & Williams 1981; Snelson, Mulligan &
202 Williams 1984; O'Connell et al. 2007). Samples were collected during ongoing community
203 level surveys using electrofishing, which occurred during the bull shark study (see Rehage &
204 Loftus 2007 for description of sampling method). Samples were frozen until being dried and
205 homogenized in the lab. Stable isotope data from teleosts provided the framework for the

206 difference in $\delta^{13}C$ values of estuarine and freshwater prey for stable isotope diet change models
207 (see below).
208 Passive acoustic tracking was used to quantify the movement patterns of individual bull
209 sharks to assess their use of upstream areas of the estuary in response to the marsh prey pulse.
210 From Oct 2008 to May 2009, sharks caught in excellent condition (swimming strongly upon
211 capture; n = 23) were surgically fitted with a Vemco V16-4H transmitter (Vemco, Halifax, NS).
212 Transmitters were set to emit a unique series of pulses for each shark at a random interval
213 between 30-90 sec (mean emission interval = 60 sec). Movements of acoustically tagged sharks
214 were tracked within an array of 43 Vemco VR2 and VR2W acoustic receivers (Fig. 1) from Oct
215 2008 to Nov 2009. In situ measurements revealed mean detection ranges of receivers were ~500
216 m (see Rosenblatt & Heithaus 2011 for detection ranges of individual receivers). Each receiver
217 was attached to a PVC pipe set in a 10 kg cement anchor. Data from receivers were downloaded
218 every 3-4 months for the duration of the study, and batteries were replaced as needed.
219
220 *Stable isotope analysis*
221 All shark samples were analyzed at the Florida International University Stable Isotope
222 Facility (29 blood samples and 30 plasma samples) or the Yale Earth System Center for Stable
223 Isotopic Studies (61 blood samples and 60 plasma samples). Lipid extraction was not performed
224 because C:N ratios (mean blood = 2.63 ± 0.25 SD; mean plasma = 2.03 ± 0.26 SD) were below
225 those suggested for extraction or mathematical correction (3.5; Post et al. 2007). To verify
226 analytical consistency, we randomly selected samples to be analyzed at both Florida
227 International University and Yale University, for which the variation between resulting $\delta^{13}C$
228 values and $\delta^{15}N$ values were 0.13‰ ± 0.20 SE. The standard deviations of standards run for

229 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
230 Florida International. All teleost samples were analyzed at the Florida International University
231 Stable Isotope Facility.
232
233 *Quantitative Analysis*
234 Acoustic tracking
235 We quantified the proportion of days each tagged shark was detected in the upstream
236 region based on receiver detections of tagged sharks (Fig. 1). We predicted that if sharks fed
237 from the prey pulse, they would have increased their use of the upstream region where
238 freshwater taxa enter the system early in the dry season, and decreased their use of the upstream
239 region later in the dry season when the abundance of marsh prey decreased (Rehage & Loftus
240 2007). Sharks were only used for analysis if they were present within the array for > 3 months,
241 and were within the array during the 2009 dry season when the marsh prey pulse was expected to
242 enter the estuary (1 Mar to 28 May). We used a random effects GLMM to test the effect of
243 month on the average proportion of days individual sharks were detected by upstream receivers,
244 with individual as a random effect, and used a Post hoc Tukey's test to test for significant
245 differences across months. We used linear regression to examine the relationship between marsh
246 water level and the proportion of sharks detected per day by upstream receivers during the dry
247 season. Finally, based on the movements of marsh taxa during the dry season, we used t-tests to
248 investigate if there was a significant change in the use of the upstream region by sharks when
249 water elevations were ≤ 0 cm, between 0-5 cm, and between 0-10 cm to develop predictions for
250 our diet change models (see below). Investigating shark habitat use in relation to these water
251 depths allowed us to examine if sharks changed their movement behavior in response to the entry

252 of larger marsh taxa (> 8 cm; enter the estuary from the marsh when water levels drop below 10
253 cm) or smaller marsh taxa (< 8 cm; enter the estuary from the marsh when water levels drop
254 below 5 cm) (Chick, Ruetz & Trexler 2004; Rehage & Loftus 2007; Trexler & Goss 2009;
255 Parkos, Ruetz & Trexler 2011).
256
257 Discrimination difference between blood and plasma
258 Studies quantifying isotopic discrimination values in sharks are limited. Hussey et al.
259 (2010) investigated discrimination in captive sand tiger (*Carcharias taurus*; n = 3) and lemon
260 sharks (*Negaprion brevirostris*; n = 1), however the only tissue they analyzed that could
261 collected without lethal sampling was muscle tissue, which has a long isotopic half-life (~98
262 days, MacNeil, Drouillard & Fisk 2006) and was not useful for our study. Both Kim et al.
263 (2012) and Malpica-Cruz et al. (2012) investigated isotopic discrimination in captive leopard
264 sharks (*Triakis semifasciata*; n = 6, n = 16, respectively). Kim et al. (2012) calculated $\Delta^{13}C$
265 plasma-blood values between 0.5-0.9‰. Despite sampling a variety of tissues, including blood,
266 muscle, and fin tissue, Malpica-Cruz et al. (2012) did not collect plasma, and therefore data from
267 this study was not useful for our analyses.
268 Tissue-specific incorporation of stable isotopes can be affected by variability in
269 environmental conditions, and can vary between species (reviewed by Vander Zanden &
270 Rasmussen 2001; Crawford, McDonald & Bearhop 2008; Newsome, Clementz & Koch 2010).
271 Therefore, we used linear regression to estimate the inter-tissue discrimination difference
272 between blood and plasma ($\Delta^{13}C$ plasma-blood) in juvenile bull sharks, and compared this to
273 values calculated by Kim et al. (2012). To do so, we plotted paired blood and plasma $\delta^{13}C$
274 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 $\delta^{13}C$ discrimination varied with $\delta^{13}C$ values of shark diets.

Temporal change in diet

In addition to providing an estimate of $\Delta^{13}C$ plasma-blood, the regression plot of blood and plasma $\delta^{13}C$ 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 $\delta^{13}C$ values more enriched (i.e. less negative) than predicted by the model, and data points below the regression line indicate plasma $\delta^{13}C$ 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 $\delta^{13}C$ values more than 0.4‰ enriched above $\delta^{13}C$ values of blood (calculated $\Delta^{13}C$ 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 $\delta^{13}C$ 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

298 pulse during the dry season. We then evaluated the effectiveness of using a piecewise function
299 to describe the data against using the best fit line from linear or non-linear least squares
300 regression (Toms & Lesperance 2003). We selected breakpoints at which $\delta^{13}C$ residuals
301 exhibited a notable change (DOY 128, 150, 163, 175, 213) and quantified the coefficient of
302 determination for each model. We selected the piecewise model with the highest coefficient of
303 determination and compared it to the coefficient of determination for linear and non-linear least
304 squares fits to determine if it was significantly higher in order to choose the best overall model
305 (Fisher 1921). To gain insight into general patterns of bull shark dietary changes in response to
306 the prey pulse, we used ANOVA to test the effect of season on $\delta^{13}C$ residual values. Post hoc
307 Tukey's tests were used to test for significant differences across seasons.
308 Despite having isotope data from 2008-2012, we only used data from sharks caught from
309 Oct 2008 to Dec 2009 because an extreme weather event in Jan 2010 significantly reduced the
310 number of juvenile bull sharks in the estuary and changed the population structure (Matich &
311 Heithaus 2012). It also possibly affected the community composition, and thereby trophic
312 dynamics, of the ecosystem (Rehage et al. 2010). Additionally, we did not have acoustic
313 tracking data for sharks after 2009 due to this extreme weather event, and therefore could not
314 investigate the correlations between marsh water levels, shark movements, and stable isotope
315 values. Future studies investigating interannual variation in shark trophic interactions and
316 movements, however, would provide additional insights.
317 Due to the slow isotopic turnover rates of tissues in elasmobranchs (MacNeil, Drouillard
318 & Fisk 2006; Logan & Lutcavage 2010; Kim et al. 2012) and the potential for maternal diets to
319 be reflected in the tissues of newborns sharks (McMeans, Olin & Benz 2009; Matich, Heithaus
320 & Layman 2010; Vaudo, Matich & Heithaus 2010), isotope values of bull sharks may not be

321 indicative of their current diet for individuals less than 90 days old (Belicka et al. 2012).
322 Because bull sharks in the Shark River Estuary are likely born at 60-70 cm TL (based on
323 captures of neonate individuals; Heithaus et al. 2009; Matich & Heithaus 2012) between May
324 and August (based on the presence of umbilical scars; Curtis, Adams & Burgess 2011), and grow
325 10-20 cm/year (based on recaptured individuals; Neer, Thompson & Carlson 2005), we only
326 included tissues from individuals that were greater than 84 cm total length (at least one year of
327 age) and individuals less than 85 cm TL that were caught between December and April with
328 closed umbilical scars (at least 90 days old).
329 To determine if bull sharks changed their diets during the freshwater pulse, we developed
330 a series of theoretical models to predict the differences in plasma and blood $\delta^{13}C$ values to
331 determine if plasma had recently become more enriched or depleted in $\delta^{13}C$ in response to a
332 dietary change (Fig. 4). We modeled six plausible dietary shifts. These included 1) estuarine
333 prey → marsh prey (E→F); 2) marine + estuarine prey → estuarine + marsh prey (M+E→E+F);
334 3) marine + estuarine prey → marsh prey (M+E→F); 4) marine prey → estuarine prey (M→E);
335 5) marine prey → estuarine + marsh prey (M→ E+F); and 6) marine prey → marsh prey (M→F)
336 (Fig. 4b; Table 1). We used turnover data from MacNeil, Drouillard & Fisk (2006) (blood half-
337 life = 61 days) and Kim et al. (2012) (plasma half-life = 32 days) to predict the rate of change in
338 blood and plasma isotopes based on the differences in $\delta^{13}C$ values of prey items from different
339 food webs (mean $\delta^{13}C \pm$ SE; marine = -14.1 ± 0.2‰, estuarine = -28.0 ± 0.5‰, and marsh = -
340 30.5 ± 0.5‰). The models assumed that sharks would change their diets in response to marsh
341 prey entering the system, with the timing of the modeled change based on the movements of bull
342 sharks (see Results). Thus, the model assumed that shark diets changed when marsh water levels
343 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 $\delta^{13}C \pm SE$; 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

367 days, respectively; MacNeil, Drouillard & Fisk 2006; Kim et al. 2012) (Fig. 5a). We also
368 created models with $\Delta^{13}C$ plasma-blood of 0.9‰, 0.7‰, and 0.2‰, representing a range of $\Delta^{13}C$
369 plasma-blood values across the calculated values of Kim et al. (2012) (Fig. 5b). As such, we
370 created six different models (each diet change scenario; see above) for 12 different treatments of
371 isotopic half-life and $\Delta^{13}C$ plasma-blood.

372 Because a piecewise function best described $\delta^{13}C$ residuals across time (see Results), we
373 used piecewise linear regression with the same breakpoint as the true $\delta^{13}C$ residuals and DOY
374 model (DOY = 169) to investigate the relationship between the predicted difference in $\delta^{13}C$
375 values (from theoretical models) and DOY. Because regression plots of predicted and true
376 differences in $\delta^{13}C$ values produced best fit lines with the same correlation coefficients and f-
377 values for each diet change model across each isotopic half-life and $\Delta^{13}C$ plasma-blood, we
378 could not use traditional model selection. We therefore compared the best fit lines of the
379 theoretical models to that of the model for true $\delta^{13}C$ residuals and DOY. This approach allowed
380 us to qualitatively select the best model(s) describing if and how bull sharks changed their
381 trophic interactions in response to the prey pulse, and how isotopic half-life and $\Delta^{13}C$ plasma-
382 blood affected model selection. Criteria for qualitatively selecting the best theoretical models
383 included 1) slopes of the piecewise functions with the same direction (positive or negative) as the
384 model for true $\delta^{13}C$ residuals and DOY; 2) slopes not significantly different from that of true
385 $\delta^{13}C$ residuals and DOY (t-test); and 3) piecewise functions with the closest mean distance to the
386 true $\delta^{13}C$ residuals and DOY regression lines. ANOVA was used to test the effects of model,
387 isotopic half-life, and $\Delta^{13}C$ plasma-blood on mean distance from the true $\delta^{13}C$ residuals and
388 DOY piecewise function for theoretical models that passed the first two criteria. Post-hoc

389 Tukey's tests were used to test for significant differences across these factors. All statistical
390 analyses were conducted in JMP 6.0.0.
391
392 **Results**
393 From 2008 to 2012, we captured 90 juvenile bull sharks. Twenty-three individuals
394 between 71-142 cm total length (mean TL ± SD = 102 ± 22 cm) had acoustic transmitters
395 surgically implanted in them, and were tracked from 10 Oct 2008 to 30 Nov 2009 for a total of
396 5343 tracking days. Three individuals were not present for > 3 months within the system, and
397 therefore were not included in movement analyses. Shark detections by upstream receivers
398 varied by month, and were highest in April and May (DOY 91-151; $R^2 = 0.59$, $p < 0.01$; Fig. 6).
399 During the dry season, there was a negative correlation between shark use of the upstream region
400 and marsh water levels (DOY 60-148; $R^2 = 0.52$, $p < 0.01$; Fig 6), and the proportion of sharks
401 detected in the upstream region was significantly higher when water levels were ≤ 0 cm (mean ±
402 SD = 0.38 ± 0.14) compared to > 0-5 (0.12 ± 0.07) and > 0-10 cm (0.10 ± 0.06) ($t = 6.09$, $p <$
403 0.01; $t = 8.54$, $p < 0.01$, respectively; Fig. 6).
404 Thirty-nine of the sampled sharks (n = 17 males, 22 females) captured from Oct 2008 to
405 Dec 2009 and ranging in size from 75-182 cm TL (mean TL ± SD = 116.5 ± 28.3 cm) were used
406 to investigate seasonal shifts in shark diets relative to the 2009 pulse of prey from the marsh.
407 Sharks had blood and plasma $\delta^{13}C$ values that ranged from -17.5‰ to -26.5‰ (mean $\delta^{13}C$ ± SD
408 = -22.9 ± 2.4‰) and -17.8‰ to -25.3‰ (mean $\delta^{13}C$ ± SD = -22.4 ± 2.3‰), respectively, and
409 blood and plasma $\delta^{15}N$ values that ranged from 10.5‰ to 12.8‰ (mean $\delta^{15}N$ ± SD = 11.6 ±
410 0.5‰) and 9.9‰ to 12.4‰ (mean $\delta^{15}N$ ± SD = 11.5 ± 0.6‰), respectively (Fig. 2).

411 There was a significant, positive relationship between blood and plasma $\delta^{13}C$ values with
412 a high coefficient of determination (Fig. 3; $R^2 = 0.81$, $p < 0.01$), and the slope of the best fit line
413 was not different from one (slope = 0.84, $t_{(90)} = 0.18$). This suggests the mean difference
414 between blood and plasma $\delta^{13}C$ values (plasma was 0.4‰ greater than blood) was consistent
415 across the $\delta^{13}C$ range of the sampled sharks.
416 The $\delta^{13}C$ residuals of bull sharks caught in 2008-2009 varied significantly with DOY and
417 capture season. A piecewise function with a breakpoint at DOY 169 was significantly better
418 than a polynomial fit or linear fit for the relationship between DOY and $\delta^{13}C$ residuals (z-score =
419 5.48, $p < 0.01$; Fig. 7), with the magnitude of the slope for the first section (DOY 0-169) more
420 than three times greater than the magnitude of the second section (DOY 169-365). Mean $\delta^{13}C$
421 residuals decreased significantly between the early dry season and the late dry season, and then
422 increased from the late dry season to the early wet season (Fig. 8).
423 Changing the parameters of the models (isotopic half-life and $\Delta^{13}C$ plasma-blood)
424 changed their predictions of $\delta^{13}C$ residuals. As the duration of isotopic half-life increased (i.e.
425 from 0.5 half-lives to 2 half-lives), models predicted an increase in the duration of time $\delta^{13}C$
426 residuals were in a state of change in response to diet shifts, and as $\Delta^{13}C$ plasma-blood increased
427 (i.e from 0.2-0.9‰), models predicted greater positive $\delta^{13}C$ residuals during non-pulse periods
428 and smaller negative $\delta^{13}C$ residuals during the prey pulse (Fig. 5). ANOVA revealed that model
429 ($F = 10.26$, $p < 0.01$) and $\Delta^{13}C$ plasma-blood ($F = 14.08$, $p < 0.01$) were significant factors
430 explaining variability in mean distance between piecewise functions of theoretical models and
431 the model of true $\delta^{13}C$ residuals and DOY (Appendix 1). Models with $\Delta^{13}C$ plasma-blood =
432 0.7‰ and 0.9‰ had significantly lower mean distances from the true $\delta^{13}C$ residuals and DOY
433 piecewise function than discrimination differences of 0.2‰ and 0.4‰ (Fig. 9a), and models with

434 $\Delta^{13}C$ plasma-blood = 0.9‰ were the best fit (mean distance ± SE = 0.5 ± 0.1‰, 0.4 ± 0.1‰ for
435 $\Delta^{13}C$ plasma-blood = 0.7‰ and 0.9‰, respectively). Models 2 and 3 (marine + estuarine prey
436 → estuarine + freshwater prey, and marine + estuarine prey → freshwater prey, respectively) had
437 the lowest mean distances from the $\delta^{13}C$ residuals and DOY piecewise function (Fig. 9b), with
438 model 2 having the best fit (distance ± SE = 0.4 ± 0.1‰, 0.5 ± 0.1‰ for model 2 and model 3,
439 respectively). This suggests that model 2 at $\Delta^{13}C$ plasma-blood = 0.9‰ was the best model for
440 predicting changes in $\delta^{13}C$ values of blood and plasma, (Table 2, Appendix 1). Parameter
441 estimates for isotopic half-life did not affect model performance ($F = 0.90$, $p = 0.42$).
442
443 **Discussion**
444 Seasonal resource pulses are important components of annual energy budgets for many
445 species (reviewed by Otsfeld & Keesing 2000, Yang et al. 2008). For example, brown bears
446 (*Ursus arctos*) in North American Pacific riparian ecosystems rely on predictable annual pulses
447 of spawning salmon to sustain their biomass levels for overwinter hibernation (Naimen et al.
448 2002, Helfield & Naimen 2006). Along the South African coastline, sardines (*Sardinops sagax*)
449 make annual migrations into nearshore areas and serve as an important pulse of food for marine
450 mammals, birds, bony fishes, and elasmobranchs (Dudley & Cliff 2010; O'Donoghue, Drapeau
451 & Peddemors 2010). Within the Shark River Estuary, the influx of marsh taxa into upstream
452 channels comprises a considerable proportion of the annual energy budget of teleost predators in
453 the ecotone region (e.g. *Amia calva*, *Centropomus undecimalis*, *Micropterus salmoides*; Rehage
454 & Loftus 2007; Boucek & Rehage *in press*), suggesting this resource pulse is likely a seasonally
455 important component of estuarine food webs within the ecosystem. Our study shows that
456 numerous juvenile bull sharks move upstream to take advantage of this influx of marsh prey,

457 despite relatively high levels of individual specialization within the population found in our
458 previous work (Matich, Heithaus & Layman 2011).

459 Previously, we found that juvenile bull sharks in the Shark River Estuary show
460 considerable inter-individual variation in trophic interactions, and many individuals (*ca.* 57%)
461 showed relatively high degrees of specialization on one type of resource pool (i.e. marine food
462 webs vs freshwater/estuarine food webs; Matich, Heithaus & Layman 2011). Our results from
463 this study suggest the trophic interactions of some sharks in the estuary (i.e. those identified
464 previously as specialists) are flexible, at least during the dry season when marsh taxa enter the
465 system and provide an additional food source. Blood and plasma $\delta^{13}C$ values (mean ± SD = -
466 22.9 ± 2.4‰ and -22.4 ± 2.3‰, respectively) suggest that many bull sharks fed on marine and
467 freshwater/estuarine prey throughout the year, and during the wet and early dry seasons, sharks
468 had $\delta^{13}C$ residuals (plasma-blood) similar to our predictions attributed to $\Delta^{13}C$ plasma-blood (*ca.*
469 0.3-0.9‰), suggesting they had relatively stable diets. Yet, sharks had significantly lower $\delta^{13}C$
470 residuals during the late dry season (mean ± SE = -0.5 ± 0.4‰), and model selection predicted
471 diet switches from marine and estuarine prey to estuarine and marsh prey during the marsh prey
472 pulse with a relatively rapid return to the previous diet at the terminus of the prey pulse when
473 marsh prey were depleted, suggesting bull sharks fed on this seasonal resource pulse from
474 freshwater marshes despite many individuals specializing on other resources outside this time
475 period (Matich, Heithaus & Layman 2011).

476 Individual specialization has been hypothesized as a means to reduce intraspecific
477 competition, especially in ecosystems with limited resources (reviewed by Bolnick et al. 2003).
478 The Shark River Estuary is an oligotrophic system, and limited food abundance may be a driver
479 of individual specialization in juvenile bull sharks (Matich, Heithaus & Layman 2011), however

480 the additional suite of resources from the marsh during the prey pulse may relax intraspecific
481 competition for food. Apparently similar to bull sharks in the Shark River Estuary, specialist
482 bluegill sunfishes (*Lepomis macrochirus*) were more successful foragers than generalists, but
483 individual specialists exhibited flexibility in their habitat use and switched foraging behaviors
484 when preferred resources became depleted (Werner, Mittlebach & Hall 1981). When preferred
485 prey were scarce, silver perch (*Bidyanus bidyanus*) in aquaculture ponds switched from
486 specializing on *Daphnia* to specializing on calanoids and insects, suggesting individual
487 specialization may be a flexible trait in some populations (Warburton, Retif & Hume 1998). If
488 bull shark trophic specialization is driven by density dependent processes, then the influx of
489 marsh taxa into the system may considerably increase the availability of food, and reduce the
490 need for sharks to have specialized diets when the prey pulse enters the estuary (Svanback &
491 Persson 2004).
492
493 *Drivers of bull shark behavior*
494 Previous studies in the Everglades suggest that large marsh taxa (> 8 cm TL) vacate the
495 marsh in search of deep water refugia early in the dry season when water levels drop below 10
496 cm in reference to elevation, and small marsh taxa (< 8 cm TL) enter the estuary later when
497 water levels drop below 5 cm (Rehage & Loftus 2007; Trexler & Goss 2009). Thus, we
498 predicted bull sharks would use the upstream region of the estuary early in the dry season to take
499 advantage of all marsh taxa entering the system. Yet, bull sharks began using upstream areas
500 heavily later than we predicted, which may be due to several reasons. Because many sharks in
501 the estuary are less than three years old (Heithaus et al. 2009; Matich & Heithaus 2012), a lack of
502 foraging experience may hinder their ability to detect when marsh prey are available (e.g.

503 Werner and Giliam 1984). Interannual variation in timing and magnitude of the prey pulse due
504 to variation in precipitation and freshwater flow (Boucek & Rehage *personal communication*),
505 may further reduce the ability of bull sharks to detect the start of this event. Unfortunately, our
506 data set will not currently allow us to test these hypotheses.

507 Prey preference may also play a role in the timing of the bull sharks' responses to the
508 prey pulse (Lanszki & Sallai 2006; Hawlena & Perez-Mellado 2009). If bull sharks preferred to
509 eat large mesopredators like bass or bowfin, we would have expected them to use the upstream
510 region earlier than observed, and their diets and isotope values would have changed accordingly.
511 Instead, bull sharks did not significantly increase their use of the upstream region of the estuary
512 until marsh water levels dropped below 0 cm, when all aquatic taxa have vacated the marsh.
513 Thus, bull sharks may wait until the overall abundance of marsh taxa of all sizes in the system is
514 relatively high, or they may be targeting smaller prey that arrive in the estuary later. Shark $\delta^{15}N$
515 values suggest that bull sharks likely targeted smaller prey from the marsh. Plasma and blood
516 $\delta^{15}N$ values of bull sharks caught during the dry season (mean ± SE = 11.8 ± 0.1‰ and 11.9 ±
517 0.2‰, respectively) were comparable to $\delta^{15}N$ values of muscle tissue of other large aquatic
518 predators like snook (mean ± SE = 11.3 ± 0.3‰) and bass (mean ± SE =10.93 ± 0.14‰) that are
519 known to feed on small marsh taxa. Therefore, bull sharks likely compete for with these large
520 mesopredators for small prey that decline in abundance as the dry season progresses rather than
521 consuming them (Boucek & Rehage *in press*). Comparison of $\delta^{15}N$ values must be made
522 cautiously, however. For example, muscle tissue in elasmobranchs has a slow turnover rate
523 (half-life = 98 days, MacNeil, Drouillard & Fisk 2006), and thus we may not expect to detect
524 large seasonal changes in $\delta^{15}N$ values. Additionally, $\delta^{15}N$ turnover and discrimination rates may
525 vary with diet quality, trophic pathway, metabolic activity, and body size (reviewed by Vander

526 Zanden & Rasmussen 2001; Martinez del Rio et al. 2009; Hussey et al. 2012). Future studies
527 incorporating stomach content analysis and fatty acid analysis should help further elucidate the
528 importance of resource pulses to bull shark diets as well as intraspecific variation in the use of
529 these resources.
530
531 *Alternative explanations*
532 Alternative explanations are unlikely to account for observed temporal variation in
533 habitat use and $\delta^{13}C$ values of sharks within the estuary. For example, shifts in habitat use by
534 sharks could be driven by upstream movements of preferred prey (e.g. Ford et al. 1998; Rolstad,
535 Loken & Rolstad 2000). Yet, if sharks were feeding on the same prey year round, $\delta^{13}C$ residuals
536 would be expected to remain similar during the year or exhibit longer lag-times if the prey of
537 sharks had moved upstream to feed on the marsh prey pulse (i.e. the time for preferred prey to
538 integrate marsh prey into tissues which would then be integrated into shark tissues).
539 Increased use of the upstream area by bull sharks when marsh water levels were ≤ 0 cm
540 may have been driven by changes in environmental conditions. Juvenile bull sharks in other
541 estuaries modify their space use in accordance with changes in salinity (e.g. Heupel &
542 Simpfendorfer 2008; Froeschke, Stunz & Wildhaber 2010). Thus, bull sharks may have
543 increased their use of the upstream region of the estuary during the dry season when salinities in
544 areas further downstream increased and became higher than sharks preferred. However, salinity
545 remains relatively low in the upstream region year-round (Heithaus et al. 2009, Rosenblatt &
546 Heithaus 2011) and bull sharks are found in all areas of the estuary in all seasons (Matich &
547 Heithaus 2012), suggesting physical factors are unlikely to be driving the significant increase in
548 the use of the upstream area when marsh water levels are ≤ 0 cm.

549 Alterations in metabolic processes in response to environmental change can cause
550 variability in stable isotope values (Kelly 2000; McCutchan et al. 2003; Vanderklift & Ponsard
551 2003). Although bull sharks experience seasonal changes in salinity that may lead to changes in
552 stable isotope values of tissues (Heithaus et al. 2009; Rosenblatt & Heithaus 2011), daily and
553 weekly changes in salinity within the estuary would be expected to buffer a detectable change in
554 isotope values attributed to osmoregulatory processes. Additionally, changing the isotopic half-
555 lives and discrimination differences of our theoretical models did not affect the performance of
556 our models or model selection (models 2 and 3 were the best models for all permutations),
557 suggesting changes in metabolic processes attributed to environmental variability are unlikely to
558 have produced the trends in $\delta^{13}C$ observed during our study. As such, our results do indeed
559 suggest sharks changed their diets during the dry season in response to the prey pulse, which
560 may be a seasonally important source of nutrients and energy as observed in other predators
561 within the system (Boucek & Rehage *in press*).
562
563 *Conclusions*
564 Stable isotope analysis is an attractive tool for ecologists because it can provide a time-
565 integrated view of trophic interactions (Bearhop et al. 2004). While stable isotopes often provide
566 only course information with regard to prey identity (reviewed by Gannes, O'Brien & Martinez
567 del Rio 1997; Post 2002), employing this tool with complimentary approaches can be used to
568 elucidate patterns and drivers of variability in trophic interactions and make predictions about
569 how future conditions may lead to changes in food webs. Here we used a combination of stable
570 isotope analysis, acoustic telemetry, and predictive modeling to elucidate changes in bull shark
571 behavior in response to a resource pulse of taxa from adjacent marshland. Isotope data suggest

572 sharks increased the proportion marsh prey in their diets during the dry season, and movement
573 data show that bull sharks increased their use of areas adjacent to freshwater marshes during this
574 time. Annual variability in hydrology and planned changes in Everglades water management,
575 however, may affect the importance of marsh taxa in the diets of bull sharks during the dry
576 season.

577 Restoration efforts are planned to increase freshwater flow through the Everglades
578 (CERP 2006), which will likely affect marsh water elevations (Obeysekera et al. 2011) and, in
579 turn, the onset and duration of this resource pulse and the total biomass entering the Shark River
580 Estuary. Increased freshwater flow and higher water levels in the marsh may lead to increased
581 productivity, but may diminish the magnitude of the resource pulses into estuarine creeks, which
582 could have negative consequences for the bull shark population and/or affect their ecological role
583 within the ecosystem. If marsh taxa are not available within the estuary during the dry season,
584 prey availability within the upper areas of the estuary may decrease and force bull sharks to
585 increase their use of marine resources. This potential dietary shift may lead to decreased survival
586 of young sharks, because downstream areas where marine taxa are most abundant are the riskiest
587 habitats for small sharks to forage in due to high predation risk from large sharks (Heithaus et al.
588 2009; Matich, Heithaus & Layman 2011). However, this shift in behavior may lead to upstream
589 nutrient transport if juvenile sharks forage in areas where marine taxa are prominent, but rest
590 upstream where large sharks are rarely found (Rosenblatt & Heithaus 2011). Additionally, if
591 marsh taxa are not available to bull sharks, levels of individual specialization may further
592 increase in the face of increased intraspecific competition (Matich, Heithaus & Layman 2011).
593 Understanding how environmental variability currently affects the magnitude and timing of this
594 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.

618 Capture data and isotopic data are available upon request through the FCE LTER website:
619 http://fce.lternet.edu/.
620
621 **References**

622 Anderson, G.W., Pillans, R.D., Hyodo, S., Tsukada, T., Good, J.P., Takei, Y., Franklin, C.E. &
623 Hazon, N. (2006) The effects of freshwater to seawater transfer on circulating levels of
624 angiotensin II C-type natriuretic peptide and arginine vasotocin in the euryhaline
625 elasmobranch, *Carcharhinus leucas*. *General and Comparative Endocrinology*, **147**, 39-
626 46.
627 Bearhop, S., Adams, C.E., Waldron, S., Fuller, R.A. & MacLeod, H. (2004) Determining trophic
628 niche width: a novel approach using stable isotope analysis. *Journal of Animal Ecology*,
629 **73**, 1007-1012.
630 Belicka, L.L., Matich, P., Jaffé, R. & Heithaus, M.R. (2012) Fatty acid and stable isotopic
631 composition as indicators of the effect of maternal resource dependency on early-life
632 feeding ecology of the bull shark, *Carcharhinus leucas*. *Marine Ecology Progress Series*,
633 **455**, 245-256.
634 Bolnick, D.I., Svanback, R., Fordyce, J.A., Yang, L.H., Davis, J.M., Hulsey, C.D. & Forister,
635 M.L. (2003) The ecology of individuals: incidence and implications of individual
636 specialization. *American Naturalist*, **161**, 1-28.
637 Boucek, R.E. & Rehage, J.S.R. No free lunch, displaced marsh consumers a regulate prey
638 subsidy to an estuarine consumer. *Okios*, **in press**.
639 Bowen, W.D. (1997) Role of marine mammals in aquatic ecosystems. *Marine Ecology Progress*
640 *Series*, **158**, 267-274.

641 Buchheister, A. & Latour, R.J. (2010) Turnover and fractionation of carbon and nitrogen stable
642 isotopes in tissues of a migratory coastal predator, summer flounder (*Paralichthys*
643 *denatatus*). *Canadian Journal of Fisheries and Aquatic Sciences*, **67**, 445-461.
644 CERP (Comprehensive Everglades Restoration Plan). (2006) The journey to restore America's
645 Everglades. U.S. Army Corps of Engineers and South Florida Water Management
646 District.
647 Chasar, L.C., Chanton, J.P., Koenig, C.C. & Coleman, F.C. (2005) Evaluating the effect of
648 environmental disturbance on the trophic structure of Florida Bay, U.S.A.: Multiple
649 stable isotope analyses of contemporary and historical specimens. *Limnology and*
650 *Oceanography*, **50**, 1059-1072.
651 Chick, J.H., Ruetz III, C.R. & Trexler, J.C. (2004) Spatial scale and abundance patterns of large
652 fish communities in freshwater marshes of the Florida Everglades. *Wetlands*, **24**, 652-
653 664.
654 Cloern, J.E. (2001) Our evolving conceptual model of the coastal eutrophication problem.
655 *Marine Ecology Progress Series*, **210**, 223-253.
656 Crawford, K., McDonald, R.A. & Bearhop, S. (2008) Applications of stable isotope techniques
657 to the ecology of mammals. *Mammal Review*, **38**, 87-107.
658 Curtis, T.H., Adams, D.H. & Burgess, G.H. (2011) Seasonal distribution and habitat associations
659 of bull sharks in the Indian River Lagoon, Florida: a 30-year synthesis. *Transactions of*
660 *the American Fisheries Society*, **140**, 1213-1226.
661 Darimont, C.T., Paquet, P.C. & Reimchen, T.E. (2009) Landscape heterogeneity and marine
662 subsidy generate extensive intrapopulation niche diversity in a large terrestrial vertebrate.
663 *Journal of Animal Ecology*, **78**, 126-133.

664 Drago, M., Cardona, L., Aguilar, A., Crespo, E.A., Ameghino, S. & Garcia, N. (2010) Diet of
665 lactating South American sea lions, as inferred from stable isotopes, influences pup
666 growth. *Marine Mammal Science*, **26**, 309-323.
667 Dudley, S.F.J., & Cliff, G. (2010) Influence of the annual sardine run on catches of large sharks
668 in the protective gillnets off KwaZulu-Natal, South Africa, and the occurrence of sardine
669 in shark diet. *African Journal of Marine Science*, **32**, 383-397.
670 Fisher, R.A. (1921) On the "probable error" of a coefficient of correlation deduced from a small
671 sample. *Metron*, **1**, 3-32.
672 Ford, J.K.B., Ellis, G.M., Barrett-Lennard, L.G., Morton, A.B., Palm, R.S. & Balcomb, K.C.
673 (1998) Dietary specialization in two sympatric populations of killer whales (*Orcinus*
674 *orca*) in coastal British Columbia and adjacent waters. *Canadian Journal of Zoology*, **76**,
675 1456-1471.
676 Froeschke, J., Stunz, G.W. & Wildhaber, M.L. (2010) Environmental influences on the
677 occurrence of coastal sharks in estuarine waters. *Marine Ecology Progress Series*, **407**,
678 279-292.
679 Fry, B., and T.J. Smith III. 2002. Stable isotope studies of red mangroves and filter feeders from
680 the Shark River estuary, Florida. *Bulletin of Marine Science*, **70**, 871-890.
681 Gannes, L.Z., O'Brien, D.M. & Martinez del Rio, C. (1997) Stable isotopes in animal ecology:
682 assumptions, caveats, and a call for more laboratory experiments. *Ecology*, **78**, 1271-
683 1276.
684 Goss-Custard, J.D., & Sutherland, W.J. (1997) Individual behavior, populations and
685 conservation. *Behavioral Ecology Fourth Edition* (eds J.R. Krebs & N.B. Davies), pp.
686 373-395. Blackwell Science, Oxford.

687 Grubbs, R.D. (2010) Ontogenetic shifts in movements and habitat use. *Sharks and their Relatives*
688 *II: Biodiversity, Adaptive Physiology, and Conservation* (eds J.C. Carrier, J.A. Musick &
689 M.R. Heithaus), pp. 319-350. CRC Press, Boca Raton, FL.
690 Hawlena, D., & Perez-Mellado, V. (2009) Change your diet or die: predator-induced shifts in
691 insectivorous lizard feeding ecology. *Oecologia*, **161**, 411-419.
692 Heithaus, M.R., Delius, B.K., Wirsing, A.J. & Dunphy-Daly, M.M. (2009) Physical factors
693 influencing the distribution of a top predator in a subtropical oligotrophic estuary.
694 *Limnology and Oceanography*, **54**, 472-482.
695 Helfield, J.M. & Naiman, R.J. (2006) Keystone interactions: salmon and bear in riparian forests
696 of Alaska. *Ecosystems*, **9**, 167-180
697 Heupel, M.R. & Simpfendorfer, C.A. (2008) Movement and distribution of young bull sharks
698 *Carcharhinus leucas* in a variable estuarine environment. *Aquatic Biology*, **1**, 277-289.
699 Hobson, K.A. & Clark, R.G. (1992) Assessing avian diets using stable isotopes I: turnover of
700 13C in tissues. *Condor*, **94**, 181-188.
701 Hussey, N.E., Brush, J., McCarthy, I.D. & Fisk, A.T. (2010) $\delta^{15}N$ and $\delta^{13}C$ diet-tissue
702 discrimination factors for large sharks under semi-controlled conditions. *Comparative*
703 *Biochemistry and Physiology A*, **155**, 445-453.
704 Hussey, N.E., MacNeil, M.A., Olin, J.A., McMeans, B.C., Kinney, M.J., Chapman, D.D. & Fisk,
705 A.T. (2012) Stable isotopes and elasmobranchs: tissue types, methods, applications and
706 assumptions. *Journal of Fish Biology*, **80**, 1449-1484.
707 Jackson, J.B.C., Kirby, M.X., Berger, W.H., Bjorndal, K.A., Botsford, L.W., Bourque, B.J.,
708 Bradbury, R.H., Cooke, R., Erlandson, J., Estes, J.A., Hughes, T.P., Kidwell, S., Lange,
709 C.B., Lenihan, H.S., Pandolfi, J.K., Peterson, C.H., Steneck, R.S., Tegner, M.J. &

Warner, R.R. (2001) Historical overfishing and the recent collapse of coastal ecosystems. *Science*, **293**, 629-638.

Kelly, J.F. (2000) Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Canadian Journal of Zoology*, **78**, 1-27.

Kennish, M.J. (2002) Environmental threats and environmental future of estuaries. *Environmental Conservation*, **29**, 78-107.

Kim, S.L., Martinez del Rio, C., Casper, D. & Koch, P.L. (2012) Isotopic incorporation rates for shark tissues from a long-term captive feeding study. *Journal of Experimental Biology*, **215**, 2495-2500.

Lanszki, J. & Sallai, Z. (2006) Comparison of the feeding habits of Eurasian otters on a fast flowing river and its backwater habitats. *Mammalian Biology*, **71**, 336-346.

Layman, C.A., Araujo, M.S., Boucek, R., Harrison, E., Jud, Z.R., Matich, P., Hammerschlag-Peyer, C.M., Rosenblatt, A.R., Vaudo, J.J., Yeager, L.A., Post, D. & Bearhop, S. (2012) Applying Stable Isotopes to Examine Food Web Structure: An Overview of Analytical Tools. *Biological Reviews*, **87**, 542-562.

Lewis Jr, W.M. (2001) Wetlands Explained. Oxford University Press, Oxford. Pp. 147.

Logan, J.M. & Lutcavage, M.E. (2010) Stable isotope dynamics in elasmobranch fishes. *Hydrobiologia*, **644**, 231-244.

MacNeil, M.A., Drouillard, K.G., & Fisk, A.T. (2006) Variable uptake and elimination of stable nitrogen isotopes between tissues in fish. *Canadian Journal of Fisheries and Aquatic Sciences*, **63**, 345-353.

731 Malpica-Cruz, L., Herzka, S.Z., Sosa-Nishizaki, O. & Lazo, J.P. (2012) Tissue-specific isotope
732 trophic discrimination factors and turnover rates in a marine elasmobranch: empirical and
733 modeling results. *Canadian Journal of Fisheries and Aquatic Sciences*, **69**, 551-564.
734 Martinez del Rio, C., Wolf, N., Carleton, S.A., & Gannes, L.Z. (2009) Isotopic ecology ten years
735 after a call for more laboratory experiments. *Biological Reviews*, **84**, 91-111.
736 Matich, P., Heithaus, M.R. & Layman, C.A. (2010) Size-based variation in intertissue
737 comparisons of stable carbon and nitrogen isotopic signatures of bull sharks
738 (*Carcharhinus leucas*) and tiger sharks (*Galeocerdo cuvier*). *Canadian Journal of*
739 *Fisheries and Aquatic Sciences*, **67**, 877-885.
740 Matich, P., Heithaus, M.R. & Layman, C.A. (2011) Contrasting patterns of individual
741 specialization and trophic coupling in two marine apex predators. *Journal of Animal*
742 *Ecology*, **80**, 295-304.
743 Matich, P. & Heithaus, M.R. (2012) Effects of an extreme temperature event on the behavior and
744 age structure of an estuarine top predator (*Carcharhinus leucas*). *Marine Ecology*
745 *Progress Series*, **447**, 165-178.
746 McCutchan Jr, J.H., Lewis Jr, W.M., Kendall, C. & McGrath, C.C. (2003) Variation in trophic
747 shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos*, **102**, 378-390.
748 McMeans, B.C., Olin, J.A. & Benz, G.W. (2009) Stable-isotope comparisons between embryos
749 and mothers of a placentatrophic shark species. *Journal of Fish Biology*, **75**, 2464-2474.
750 Naiman, R.J., Bilby, R.E., Schindler, D.E. & Helfield, J.M. (2002) Pacific salmon, nutrients, and
751 the dynamics of freshwater and riparian ecosystems. *Ecosystems*, **5**, 399-417.

752 Neer, J.A., Thompson, B.A. & Carlson, J.K. (2005) Age and growth of Carcharhinus leucas in
753 the northern Gulf of Mexico: incorporating variability in size at birth. *Journal of Fish*
754 *Biology*, **67**, 370-383.
755 Newsome, S.D., Tinker, M.T., Monson, D.H., Oftedal, O.T., Ralls, K., Staedler, M.M., Fogel,
756 M.L. & Estes, J.A. (2009) Using stable isotopes to investigate individual diet
757 specialization in California sea otters (*Enhydra lutris nereis*). *Ecology*, **90**, 961-974.
758 Newsome, S.D., Clementz, M. & Koch, P.L. (2010) Using stable isotope biogeochemistry to
759 study marine mammal ecology. *Marine Mammal Science*, **26**, 509-572.
760 Obeysekera, J., Kuebler, L., Ahmed, S., Chang, M.L., Engel, V., Langevin, C., Swain, E. &
761 Wan, Y.S. (2011) Use of hydrologic and hydrodynamic modeling for ecosystem
762 restoration. *Critical Reviews in Environmental Science and Technology*, **41**, 447-488.
763 O'Connell, M.T., Shepherd, T.D., O'Connell, A.M.U. & Myers, R.A. (2007) Long-term declines
764 in two apex predators, bull sharks (*Carcharhinus leucas*) and alligator gar (*Atractosteus*
765 *spatula*), in Lake Pontchartrain, an oligohaline estuary in southeastern Louisiana.
766 *Estuaries and Coasts*, **30**, 567-574.
767 O'Donoghue, S.H., Drapeau, L. & Peddemors, V.M. (2010) Broad-scale distribution patterns of
768 sardine and their predators in relation to remotely sensed environmental conditions
769 during the KwaZulu-Natal sardine run. *African Journal of Marine Science*, **32**, 279-291.
770 Ostfeld, R.S. & Keesing, F. (2000) Pulsed resources and community dynamics of consumers in
771 terrestrial ecosystems. *Trends in Ecology and Evolution*, **15**, 232-237.
772 Papastamatiou, Y.P., Friedlander, A.M., Caselle, J.E. & Lowe, C.G. (2010) Long-term
773 movement patterns and trophic ecology of blacktip reef sharks (*Carcharhinus*

774 *melanopterus*) at Palmyra Atoll. *Journal of Experimental Marine Biology and Ecology*,
775 **386**, 94-102.
776 Parkos III, J.J., Ruetz III, C.R. & Trexler, J.C. (2011) Disturbance regime and limits on benefits
777 of refuge use for fishes in a fluctuating hydroscape. *Oikos*, **120**, 1519-1530.
778 Parmesan, C. & Yohe, G. (2003) A globally coherent fingerprint of climate change impacts
779 across natural systems. *Nature*, **421**, 37-42.
780 Perry, W. (2004) Elements of South Florida's Comprehensive Everglades Restoration Plan.
781 *Ecotoxicology*, **13**, 185-193.
782 Post, D.M. (2002) Using stable isotopes to estimate trophic position: models, methods, and
783 assumptions. *Ecology*, **83**, 703-718.
784 Post, D.M., Layman, C.A., Arrington, D.A., Takimoto, G. , Montaña, C.G. & Quattrochi, J.
785 (2007) Getting to the fat of the matter: models, methods and assumptions for dealing with
786 lipids in stable isotope analyses. *Oecologia*, **152**, 179-189.
787 Rehage, J.S. & Trexler, J.C. (2006) Assessing the net effect of anthropogenic disturbance on
788 aquatic communities in wetlands: community structure relative to distance from canals.
789 *Hydrobiologia*, **596**, 359-373.
790 Rehage, J.S. & Loftus, W.F. (2007) Seasonal fish community variation in headwater mangrove
791 creeks in the southwestern Everglades: an examination of their role as dry-down refuges.
792 *Bulletin of Marine Science*, **80**, 625-645.
793 Rehage, J., Gaiser, E., Heithaus, M., Ross, M. & Ruiz, P. (2010) Effects of a rare cold snap on
794 Everglades biota: what are the long-term consequences for the ecosystem? LTER
795 Network News Spring 2010. http://news.lternet.edu/Article348.html.

796 Rolstad, J., Loken, B., & Rolstad, E. (2000) Habitat selection as a hierarchical spatial process:
797 the green woodpecker at the northern edge of its distribution range. *Oecologia*, **124**, 116-
798 129.
799 Rosenblatt, A.E. & Heithaus, M.R. (2011) Does variation in movement tactics and trophic
800 interactions among American alligators create habitat linkage? *Journal of Animal*
801 *Ecology*, **80**, 786-798.
802 Ruetz III, C.R., Trexler, J.C., & Jordan, F. (2005) Population dynamics of wetland fishes: spatio-
803 temporal patterns synchronized by hydrological disturbance? *Journal of Animal Ecology*,
804 **74**, 322-332.
805 Sklar F.H., Fitz, H.C., Wu, Y., Van Zee, R. & McVoy, C. (2001) The design of ecological
806 landscape models for Everglades restoration. *Ecological Economics*, **37**, 379-401.
807 Snelson Jr, F.F. & Williams, S.E. (1981) Notes on the occurrence, distribution, and biology of
808 elasmobranch fishes in the Indian River Lagoon System, Florida. *Estuaries*, **4**, 110-120.
809 Snelson Jr, F.F., Mulligan, T.J. & Williams, S.E. (1984) Food habits, occurrence, and population
810 structure of the bull shark, *Carcharhinus leucas*, in Florida coastal lagoons. *Bulletin of*
811 *Marine Science*, **34**, 71-80.
812 Speed, C.W., Meekan, M.G., Field, I.C., McMahon, C.R., Abrantes, K. & Bradshaw, C.J.A.
813 (2012) Trophic ecology of reef sharks determined using stable isotopes and telemetry.
814 *Coral Reefs*, **31**, 357-367.
815 Steiner, P.A., Michel, M. & O'Donnell, P.M. (2007) Notes on the occurrence and distribution of
816 elasmobranches in the Ten Thousand Islands estuary, Florida. *American Fisheries Society*
817 *Symposium*, **50**, 237-250.

818 Svanback, R. & Persson, L. (2004) Individual diet specialization, niche width, and population
819 dynamics: implications for trophic polymorphisms. *Journal of Animal Ecology*, **73**, 973-
820 982.
821 Sweeting, C.J., Barry, J.T., Polunin, N.V.C. & Jennings, S. (2007) Effects of body size and
822 environment on diet-tissue $\delta^{13}C$ fraction in fishes. *Journal of Experimental Marine*
823 *Biology and Ecology*, **352**, 165-176.
824 Toms, J.D. & Lesperance, M.L. (2003) Piecewise regression: a tool for identifying ecological
825 thresholds. *Ecology*, **84**, 2034-2041.
826 Trexler, J.C. & Goss, C.W. (2009) Aquatic fauan as indicators for Everglades restoration:
827 applying dynamic targets in assessments. *Ecological Indicators*, **9S**, S108-S119.
828 Vander Zanden, M.J. & J.B. Rasmussen. (2001) Variation in $\delta^{15}N$ and $\delta^{13}C$ trophic fractionation:
829 implications for aquatic food web studies. *Limnology and Oceanography*, **46**, 2061-2066.
830 Vanderklift, M.A. & Ponsard, S. (2003) Source of variation in consumer-diet $\delta^{15}N$ enrichment: a
831 meta-analysis. *Oecologia*, **136**, 169-182.
832 Vaudo, J.J., Matich, P. & Heithaus, M.R. (2010) Mother-offspring fractionation in two species of
833 placentatrophic sharks. *Journal of Fish Biology*, **77**, 1724-1727.
834 Warburton, K., Retif, S. & Hume, D. (1998) Generalists as sequential specialists: diets and prey
835 switching in juvenile silver perch. *Environmental Biology of Fishes*, **51**, 445-454.
836 Werner, E.E., Mittlebach, G.G. & Hall, D.J. (1981) The role of foraging profitability and
837 experience in habitat use by the bluegill sunfish. *Ecology*, **62**, 116-125.
838 Werner, E.E. & Gilliam, J.F. (1984) The ontogenetic niche and species interactions in size-
839 structured populations. *Annual Review of Ecology and Systematics*, **15**, 393-425.

840 Wiley, T.R. & Simpfendorfer, C.A. (2007) The ecology of elasmobranches occurring in the
841 Everglades National Park, Florida: implications for conservation and management.
842 *Bulletin of Marine Science*, **80**, 171-189.
843 Williams, A.J. & Trexler, J.C. (2006) A preliminary analysis of the correlation of food-web
844 characteristics with hydrology and nutrient gradients in the southern Everglades.
845 *Hydrobiologia,* **569**, 493-504.
846 Yang, L.H., Bastow, J.L., Spence, K.O. & Wright, A.N. (2008) What can we learn from resource
847 pulses? *Ecology*, **89**, 621-634

Tables

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.

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

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

	$\delta^{13}C$ 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

859

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 $\delta^{13}C$ 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.

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).

Figure 5: Effects of changing a) isotopic half-life at $\Delta^{13}C$ plasma-blood = 0.4‰ and b) $\Delta^{13}C$ 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).

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

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

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

Figure 1:

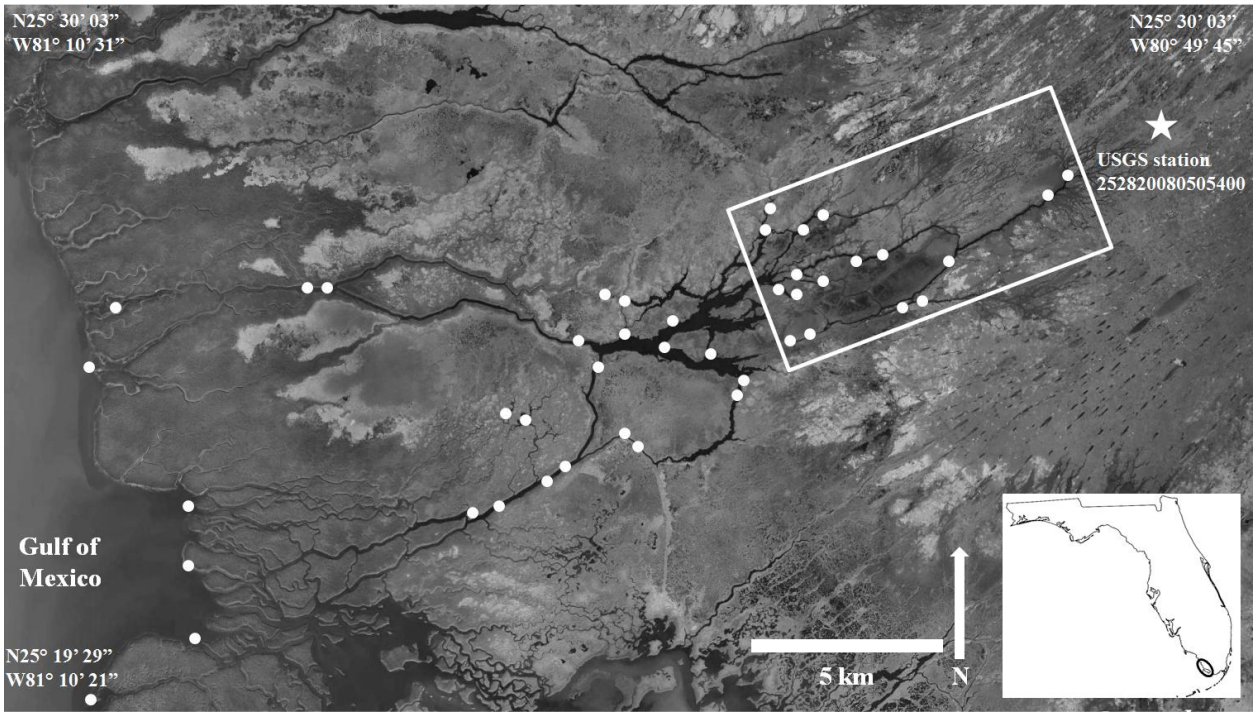

N25° 30' 03"
W81° 10' 31"
N25° 30' 03"
W80° 49' 45"
USGS station
252820080505400
Gulf of
Mexico
N25° 19' 29"
W81° 10' 21"
5 km
N

Figure 2:

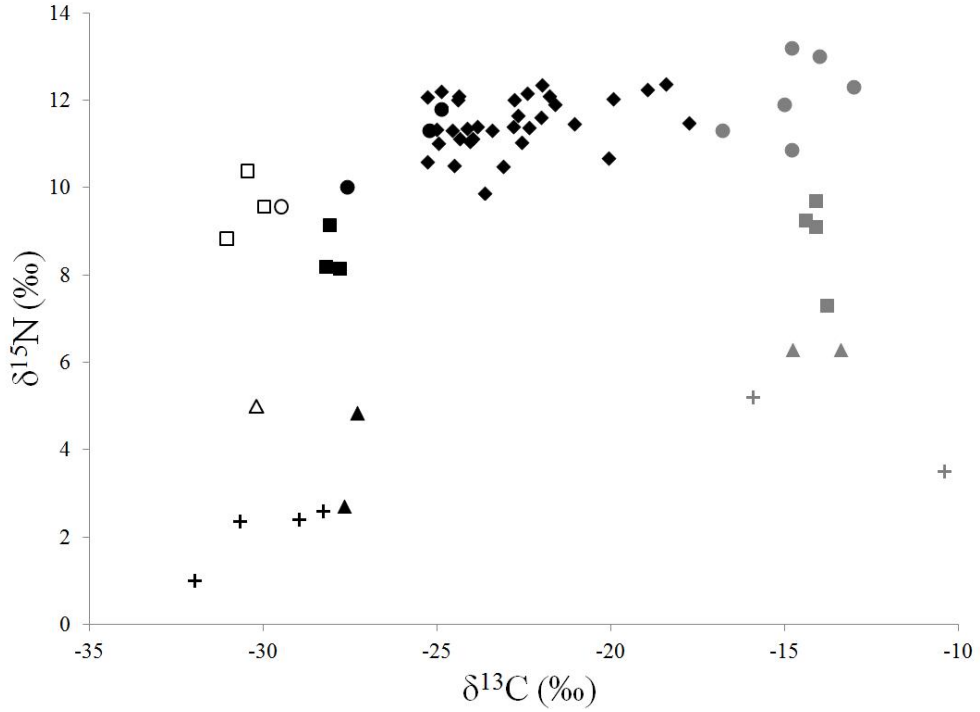


Figure 3:

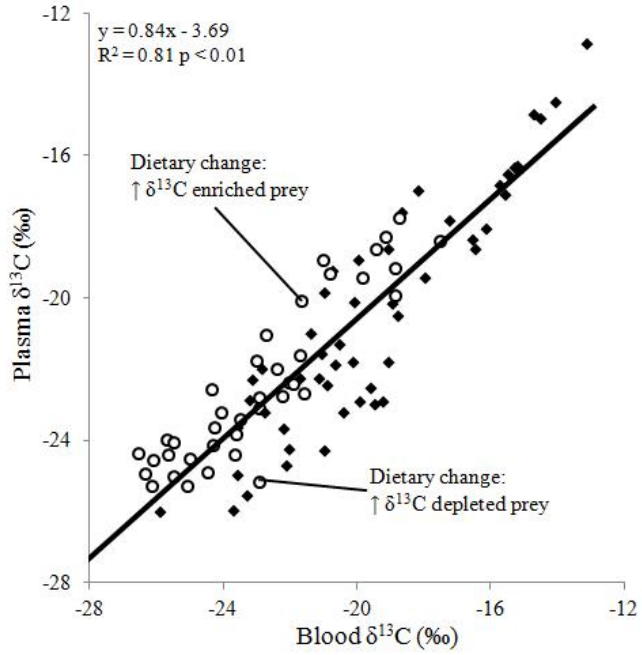

y = 0.84x - 3.69
R² = 0.81 p < 0.01
Dietary change:
↑ δ13C enriched prey
Dietary change:
↑ δ13C depleted prey
Plasma δ13C (‰)
Blood δ13C (‰)
-12
-16
-20
-24
-28
-28
-24
-20
-16
-12

Figure 4:

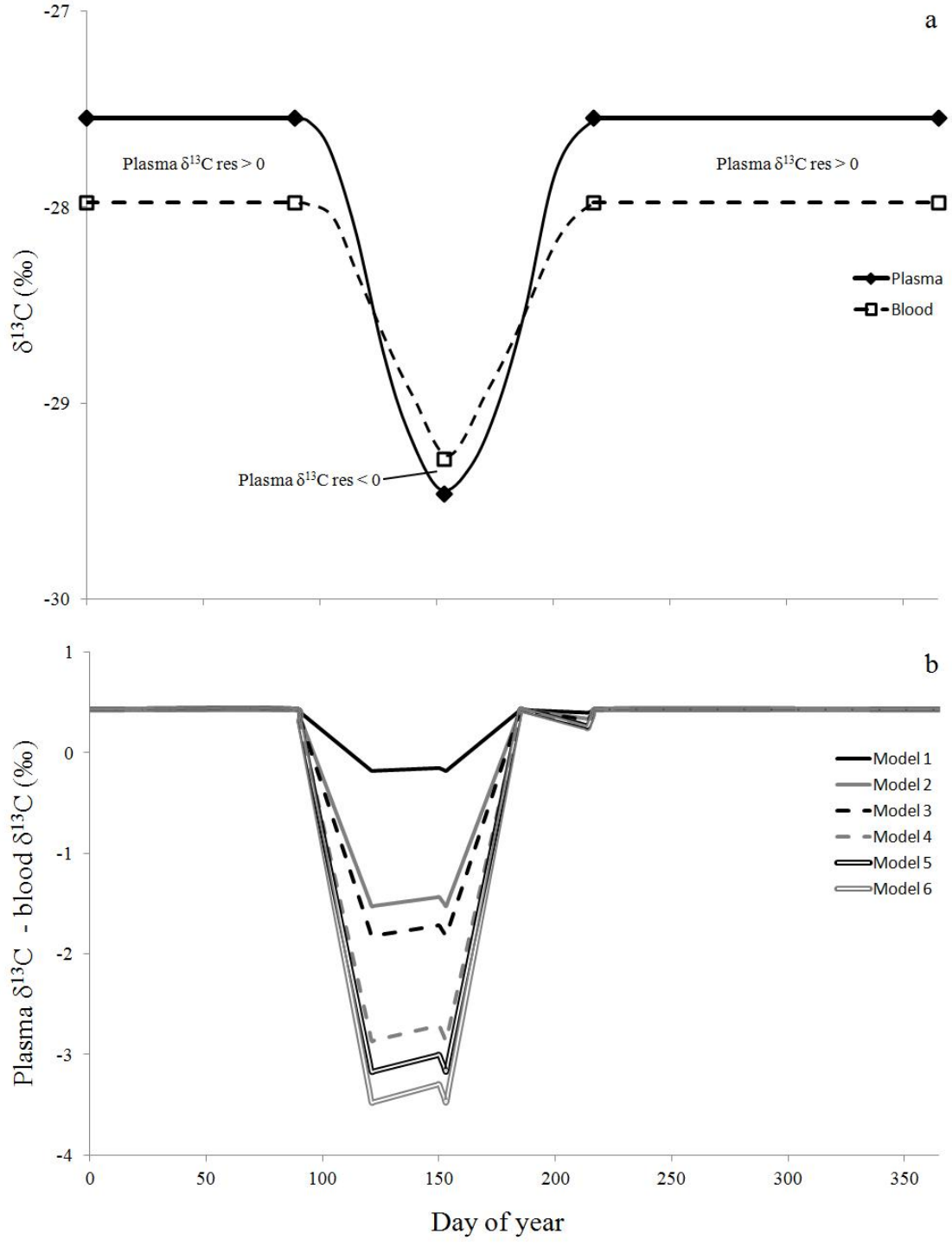

a
-27
-28
-29
-30
δ13C (‰)
Plasma δ13C res > 0
Plasma δ13C res > 0
Plasma δ13C res < 0
Plasma
Blood
b
1
0
-1
-2
-3
-4
Plasma δ13C - blood δ13C (‰)
Model 1
Model 2
Model 3
Model 4
Model 5
Model 6
0
50
100
150
200
250
300
350
Day of year

Figure 5:

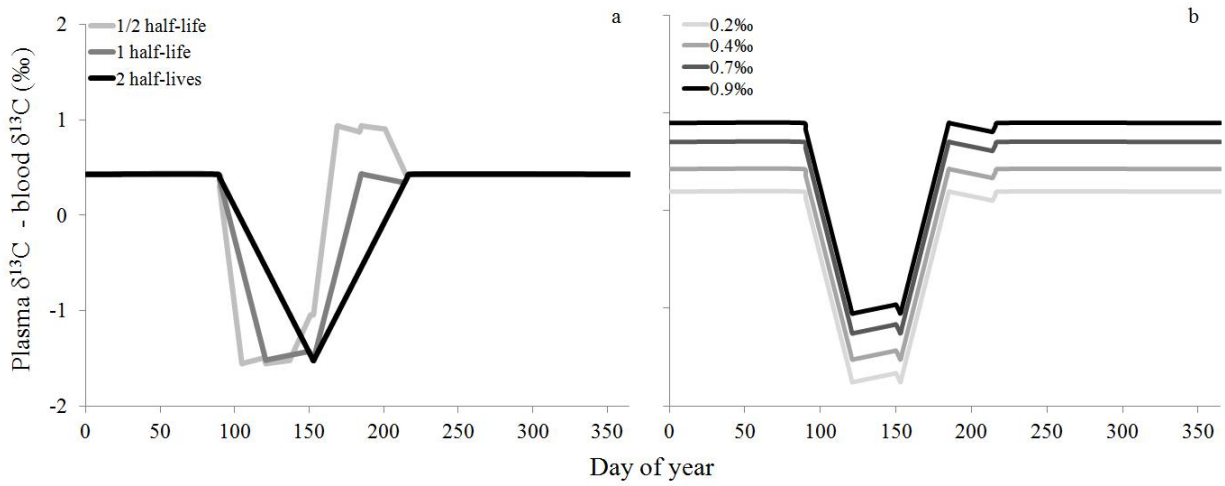

a
b
1/2 half-life
1 half-life
2 half-lives
0.2‰
0.4‰
0.7‰
0.9‰
Plasma δ13C - blood δ13C (‰)
Day of year
2
1
0
-1
-2
0
50
100
150
200
250
300
350

Figure 6:

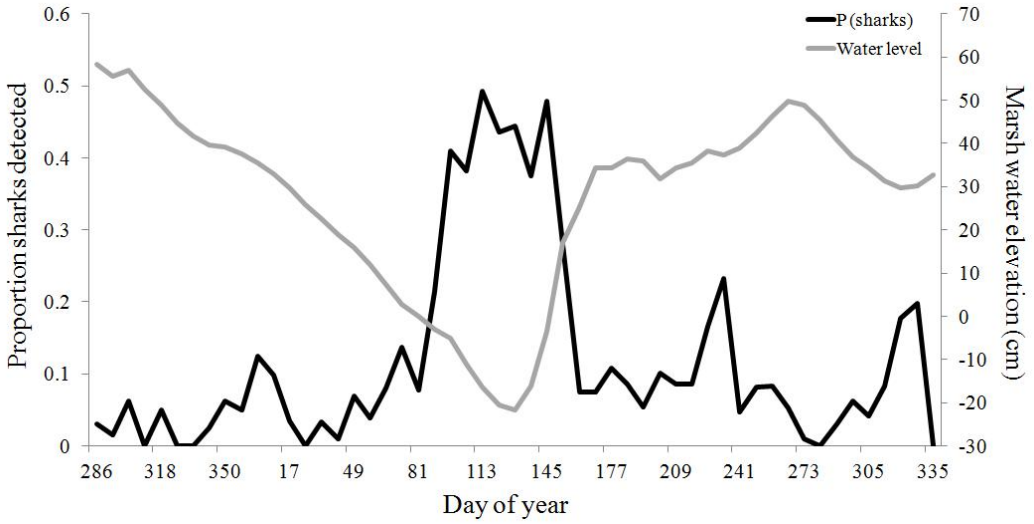

P (sharks)
Water level
Proportion sharks detected
Marsh water elevation (cm)
Day of year
0.6
0.5
0.4
0.3
0.2
0.1
0
70
60
50
40
30
20
10
0
-10
-20
-30
286
318
350
17
49
81
113
145
177
209
241
273
305
335

Figure 7:

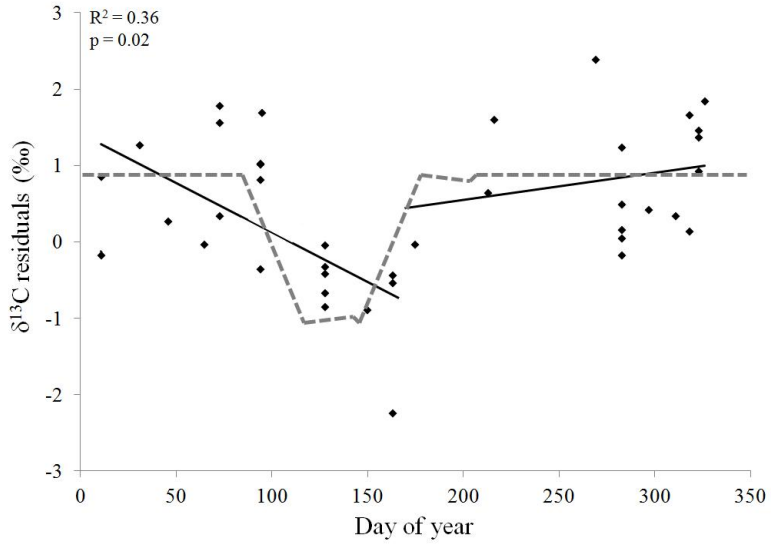

R² = 0.36
p = 0.02
δ13C residuals (‰)
Day of year
3
2
1
0
-1
-2
-3
0
50
100
150
200
250
300
350

Figure 8:

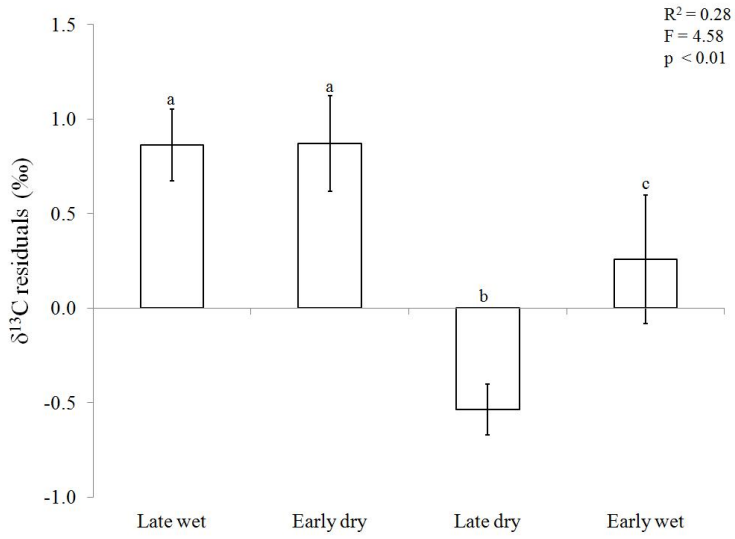
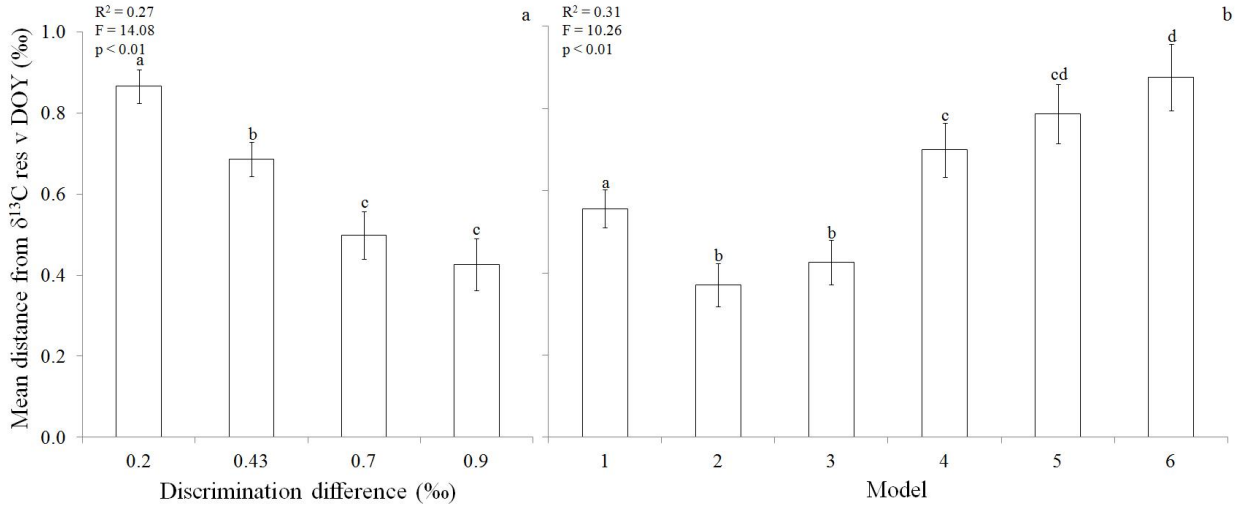

R² = 0.28
F = 4.58
p < 0.01
δ13C residuals (‰)
1.5
1.0
0.5
0.0
-0.5
-1.0
a
a
b
c
Late wet
Early dry
Late dry
Early wet

Figure 9:


R² = 0.27
F = 14.08
p < 0.01
a
Mean distance from δ13C res v DOY (‰)
1.0
0.8
0.6
0.4
0.2
0.0
a
b
c
c
0.2
0.43
0.7
0.9
Discrimination difference (‰)
R² = 0.31
F = 10.26
p < 0.01
b
a
b
b
c
cd
d
1
2
3
4
5
6
Model