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USING STABLE ISOTOPES TO INVESTIGATE TROPHIC INTERACTIONS OF
BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS*) IN THE FLORIDA
COASTAL EVERGLADES

An Undergraduate Honors Thesis submitted in partial fulfillment of
the requirements for the degree of Bachelors of Science

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To: Dr. Timothy Collins, Chairperson
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This Undergraduate Honors Thesis in Biological Sciences, written by *Valeria Paz* entitled "Using stable isotopes to investigate trophic interactions of bottlenose dolphins (*Tursiops truncatus*) in the Florida Coastal Everglades", is submitted to you in partial fulfillment of the requirements for Undergraduate Honors in Biological Sciences. The Biological Sciences Undergraduate Honors Committee and the candidate's research supervisor(s) have read this thesis. We recommend that it be approved.



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DEDICATION

In the loving memory of Jaime Pajares

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ABSTRACT

Top predators are known for their ability to 1) affect their communities through predation and 2) induce behavioral modifications. Recent research suggests that they may also play “bottom-up” roles in ecosystems, including transporting materials within and across habitat boundaries. The Florida Coastal Everglades (FCE) is an “upside-down” oligotrophic estuary where productivity decreases from the mouth of the estuary to freshwater marshes. Therefore, movements of predators may be important in ecosystem dynamics. While other estuarine predators in the FCE have been shown to potentially move nutrients among ecosystems, the potential for bottlenose dolphins (*Tursiops truncatus*) to play a similar role in the systems has not been investigated. Stable isotope analysis of biopsy samples were used to investigate spatial variation in trophic interactions of dolphins to see if they might transport nutrients. Values of $\delta^{15}\text{N}$ suggest dolphins feed at a trophic level similar to other top predators in the ecosystem while $\delta^{13}\text{C}$ suggest that dolphins forage largely within food webs where they were sampled rather than transporting nutrients across ecosystem boundaries. The exception may be dolphins foraging in rivers, which may transport nutrients downstream; a pattern opposite to that of bull sharks and alligators in these habitats. Further research is necessary to predict how future changes occurring due to restoration and climate will affect the ecological roles of dolphins.

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INTRODUCTION

Top predators are best known for their ability to affect their communities through consumption of prey and by inducing behavioral modifications such as risk avoidance (Heithaus et al. 2008). However, recent research suggests that they may have additional roles, including the transport of materials within and across habitat boundaries and thus may play a part in bottom-up processes (e.g. Schmitz et al. 2010; Vanni 2012). For example, hippos (*Hippopotamus amphibius*) foraging in terrestrial habitats return to freshwater habitats where they defecate. In some cases, these terrestrial nutrient inputs can be critical for riverine productivity (Subalusky et al. 2014). In Pacific North-West, salmon return marine-derived nutrients to the streams they return to for spawning (Naiman et al. 2002) and bears can further transport these nutrients into forests after consuming salmon (Helfield and Naiman 2006). Understanding such dynamics in ecosystems requires an understanding of the trophic interactions of top predators relative to their movements.

Estuaries are an essential habitat for species of recreational, commercial, and ecological value because they are characterized by high primary and secondary productivity and serve as nurseries for many invertebrates and fish species (Beck et al. 2001). Unlike most estuaries, The Florida Coastal Everglades (FCE) does not have productivity peak where the marine and freshwater meet (Childers 2006). Thus, the FCE is considered an “upside-down” oligotrophic estuary where productivity decreases from the mouth of the estuary to freshwater marshes. In such oligotrophic systems, consumers could be important vectors for the movement of nutrients upstream. Although a number of studies have examined the dynamics of estuaries,

and their connections to neighboring terrestrial and aquatic ecosystems (i.e., Hickey and Banas 2003, Peterson et al. 1994), the role of top predators as potential vectors for nutrient transport into estuaries has been largely overlooked until recently. For example, in the FCE some individual bull sharks (*Carcharhinus leucas*) and American alligators (*Alligator mississippiensis*) found in upstream and freshwater habitats have diets derived from marine food webs and may actively transport marine nutrients into nutrient-poor regions of the estuary.

An interesting aspect of potential nutrient transport by alligators and juvenile bull sharks is that individuals differ in their ecological roles and appear to specialize in particular foraging tactics (“individual specialization;” Bolnick et al. 2003). The drivers of individual specialization within these populations differ. In bull sharks, individuals appear to differ in their willingness to risk predation to access productive downstream waters. Some individuals repeatedly move between safer and less productive upstream waters to dangerous downstream waters in order to feed while others remain for years in safer habitats (Matich et al. 2011, Matich and Heithaus 2015). Unlike some crocodylians, alligators lack a functioning salt-secreting gland, which sets a physiological limitation to accessing high salinity waters (Taplin 1998). For alligators, individuals appear to differ in their willingness to trade-off salinity stress and food availability. Some individuals accept salt stress to forage downstream, returning to low salinity waters between foraging trips, while others remain in low-salinity waters continuously (Rosenblatt and Heithaus 2011, Rosenblatt et al. 2015). Therefore, both bull sharks and alligators appear to either recycle nutrients within

freshwater/estuarine habitats (resident individuals) or move marine nutrients upstream (“commuting individuals”).

Common Bottlenose dolphins (*Tursiops truncatus*) are widely distributed in coastal waters of the Gulf of Mexico and the US Atlantic coast (Shane et al. 1986). Resident populations occur in many bays and estuaries (Barros et al. 2010, Mazzoil et al. 2005, Simoes-Lopes and Fabian 1999) in the SE United States. Because of their high metabolic rates and high local abundance, bottlenose dolphins have the potential to consume a large portion of primary productivity within habitats (Young and Phillips 2002). Therefore, studies of their trophic interactions are important for understanding community and ecosystem dynamics where they occur.

Bottlenose dolphins also might be expected to show considerable variation in ecological roles within populations. Indeed, bottlenose dolphins are well-known for the wide array of foraging behaviors they display, many of which are specific to restricted groups of individuals or particular locations (e.g., Nowacek 2002, Sargeant et al. 2005). For example, mud ring feeding has only been documented in Florida Bay. During mud ring feeding, one dolphin creates a mud plume in the shape of a ring around a school of mullet. Once the ring has closed, the individual that made the ring and any other dolphins present (up six) wait at the surface for fish to jump out of the water from inside the ring, and then catch the fish in the air (Torres & Read 2009). Such specialized behaviors can lead to ecological niche partitioning. For example, in Shark Bay, dolphins in the same habitats display different foraging behaviors that allow them to access different prey taxa (Mann et al. 2008). Dolphins have also been

known to display high site fidelity of resident groups in bays and estuaries (Barros et al. 2010, Mazzoil et al. 2005, Simões-Lopes and Fabian 1999).

Bottlenose dolphins are a large and relatively abundant predator in the coastal and estuarine waters of the Everglades (Sarabia 2012) and could play important roles in the ecosystem through both top-down and bottom-up pathways. To date, however, little is known about trophic interactions of bottlenose dolphins in the FCE and how they might vary within and among habitats.

Stable isotope analysis has become an important method for assessing trophic interactions (Layman et al. 2012), especially for highly mobile species such as marine mammals where obtaining stomach contents is difficult or impossible. Naturally occurring isotopic signatures, such as nitrogen and carbon, are passed from prey to consumers and are reflected in the tissue of the consumer. Carbon and Nitrogen isotopes of a predator can express foraging habitat and trophic level, but they do not elucidate the exact diet of the individual although it may be documented by direct observations when no biological material is available (Kiszka et al. 2011). Briefly, the ratio of ^{12}C to ^{13}C relative to a standard ($\delta^{13}\text{C}$) provides insights into the food webs in which a consumer is feeding while the ratio of ^{14}N to ^{15}N relative to a standard ($\delta^{15}\text{N}$) provides insights into relative trophic level (Layman et al. 2012). Of particular value is its ability to resolve foraging locations (e.g. freshwater vs. marine) in ways that traditional stomach contents analysis does not (Matich et al. 2011).

Stable isotopic investigations within the FCE are facilitated by the presence of isotopically distinct food webs: freshwater/estuarine, which is characterized by $\delta^{13}\text{C}$ less than -25‰, and a marine food web characterized by $\delta^{13}\text{C}$ greater than -19‰

(Matich and Heithaus 2014). Values of $\delta^{13}\text{C}$, therefore, can be used to determine the food webs (or mixes of them) in which consumers are feeding relative to the locations in which they are sampled.

The objective of this study was to determine the trophic interactions of bottlenose dolphins in the FCE using stable isotope analysis. Specifically, we set out to determine if 1) bottlenose dolphins foraged primarily within food webs where they were sampled or if they might move nutrients across habitat boundaries and 2) if trophic interactions of individual dolphins sampled within the same habitat were similar or, like other top predators, diverged among individuals.

MATERIALS AND METHODS

Data collection

The Florida Coastal Everglades is composed of freshwater marshes that are linked to mangrove-lined channels into estuarine coastal waters, where productivity decreases from the mouths of the river to upstream marshes (Childers et al. 2006). During dry season, salinities greater than 20‰ could occur up to 17 km from the mouth of the estuary (Rosenblatt and Heithaus 2011). While the salinities where the channels meet the ocean can drop below 15‰ during the wet season (Childers et al. 2006). Due to seasonal dry down of marshes, prey enters the channels creating a prey pulse that is available to estuarine predators (Rehage and Loftus 2007).

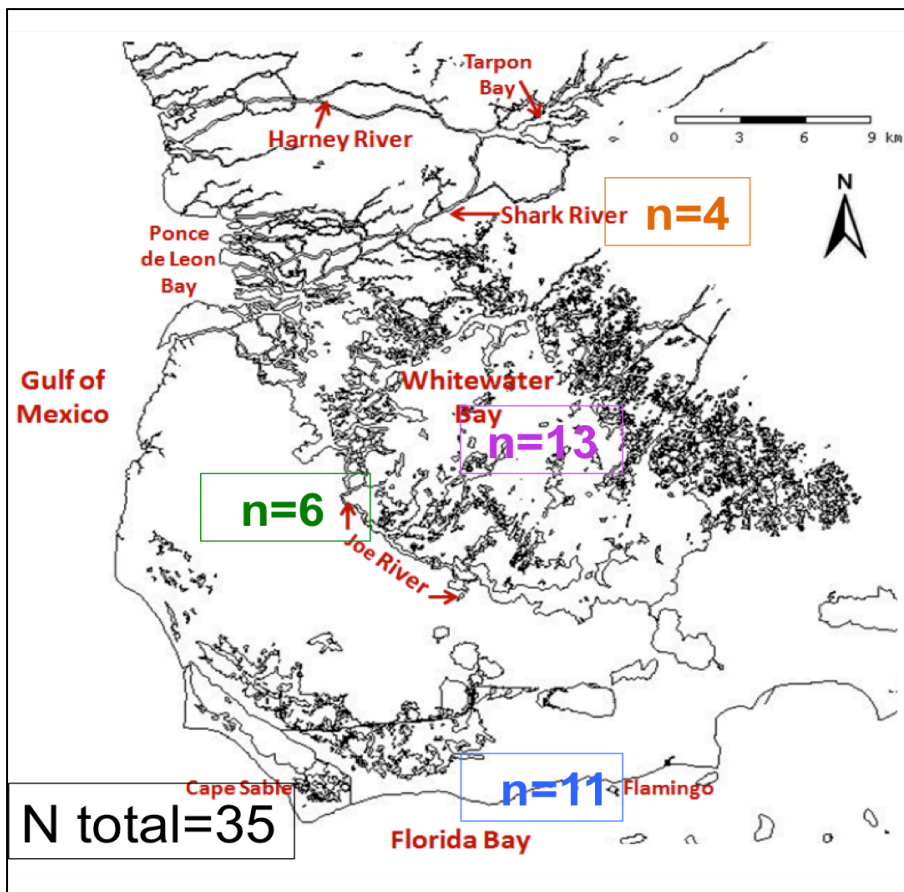


Figure 1. Florida Coastal Everglades Map after Sarabia (2012), with respective sample size per area (n).

Field sampling occurred between February 2013-May 2014 from 6.4m Mako vessel and 4.8m Carolina Skiff. Between 0700 and 1800, the sampling vessel haphazardly searched the study area (Figure 1) for dolphins. When dolphins were encountered, the boat was maneuvered slowly towards the group and we recorded group size (maximum, minimum, best estimate), approximate age of all individuals (adult, juvenile, neonate), GPS position, predominate group behavior, and environmental data. During surveys of the group, photographs of the dorsal fins of every individual in the group were taken with a Nikon D80 and a 70-300mm lens. These photos were later used to identify all individuals possible on the basis of variation in dorsal fin size and shape, as well as nicks and scars (see Wursig and Wursig 1977).

Small-boat-based surveys were undertaken in the Florida Coastal Everglades during daylight hours between 7:00 and 18:00h. The survey vessel did not follow pre-determined transects, but every attempt was made to sample during the whole daylight period. Cetacean sighting and behavioral data, as well as biopsy samples were collected opportunistically. When dolphins were encountered, standard sighting data were recorded: group size (maximum, minimum, best estimate), approximate age (adult, juvenile, neonate), geographic position, and behavioral activity.

We collected biopsy samples using a BARNETT (Veloci-Speed Class, 68-kg draw weight) crossbow with Finn Larsen CetaDart, bolts and tips (dart 25-mm long, 5-mm-diameter). The conical plastic stopper on the dart caused the bolt to rebound after impact with the dolphin and then float on the surface, allowing retrieval (Kiszka et al. 2010). Darting only occurred in Beaufort wind conditions 2 or less and when

dolphins were engaged in resting or traveling behavior and no calves were present. We recorded the behavior of dolphins before and after darting and recorded acute reactions of the targeted individual and focal group. Dolphins were typically hit below the dorsal fin when they were *ca.* 3-10 m from the research vessel (Figure 2). Once the biopsy bolt was retrieved, samples were removed from the dart tip with tweezers and wrapped individually in aluminum foil and Ziploc bags then stored on ice until being transferred to a freezer.



Figure 2. Dolphin being sampled with a modified crossbow dart

Stable Isotopes

Stable isotope analysis of carbon-13 and nitrogen-15 using skin and blubber samples was used to assess foraging habitats and relative trophic position of bottlenose dolphins. Samples were obtained from Florida Bay, Joe River, Whitewater Bay, and Shark River (see Figure 1). Samples were first separated into skin and blubber using a sterile, surgical scalpel blade. Each sample was labeled and placed in a drying oven for a minimum of 24 hours at 60°C, then homogenized using ceramic mortar and pestle.

To extract the lipids, powdered samples were immersed in 2:1 chloroform:methanol solution 3-5 times greater than the volume of the samples. Samples were then agitated and placed in a centrifuge (3000 rpm, 10 min), and then the solvent was removed using a micropipette. Lipid extraction was successively performed three times, and then samples were rinsed with deionized water and re-dried for at least 24 hours at 60°C. Sample weighing 0.4-0.7 mg were placed into 4x6 mm tin capsules and analyzed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ using standard elemental analyzer isotope ratio mass spectrometer (EA-IRMS) at the Southeast Environmental Research Center Stable Isotope Lab at Florida International University. The EA was used to combust organic material, producing N_2 and CO_2 , which were then measured on a Thermo Electron Delta C IRMS in a continuous flow mode. Isotopic ratios (R) were reported in the standard delta (d) notation relative to the international standards of Vienna Pee Dee belemnite (for $\delta^{13}\text{C}$) and atmospheric nitrogen ($\delta^{15}\text{N}$):

$$\delta(\text{‰}) = \left[\left(R_{\text{Sample}} / R_{\text{Standard}} \right) - 1 \right] \times 1000.$$

Analytical reproducibility was based on replicates of internal standards (e.g. bovine liver, glycine).

Data Analysis

All statistical analyses were conducted using IBM SPSS Statistics data editor 21 All assumptions for Kruskal Wallis one-way ANOVA and Related Samples Wilcoxon Signed Ranks Test were met.

RESULTS

I collected a total of 35 biopsy samples of skin and blubber from the FCE (figure 1). δC^{13} values ranged from -26.89‰ to -9.66‰ for skin samples and from -22.06‰ to -7.79 ‰ for blubber samples. The values of δN^{15} from the skin samples and blubber samples ranged from 9.96‰ to 12.87‰ and 11.4 ‰ to 14.70‰, respectively. Means, standard deviation (SD), and sample size (N) for each sample site are summarized in table 1.

Both blubber (N=34) and skin (N = 35) δC^{13} values varied among the sites (Kruskal-Wallis tests; blubber: $H= 23.68$, $df=3$, $P<0.001$, skin: $H= 23.68$, $df=3$, $P<0.001$). No significant variation was detected between skin and blubber δC^{13} values from Whitewater Bay and Joe River (Table 2). When comparing δC^{13} values from Joe River to that of Shark River's and Shark River's to that of Whitewater Bay's no significant variation was detected among the blubber samples but there were significantly different skin samples (Table 2).

Values of δN^{15} in blubber varied significantly different among the sites (Kruskal-Wallis test; blubber: $H= 7.941$, $df=3$, $P=0.047$), with a significant difference among Florida Bay and Whitewater Bay (Kruskal-Wallis test; blubber: $H= 5.515$ $df=1$, $P<0.05$), and a significant difference between Whitewater bay and Shark River samples (Kruskal-Wallis test; blubber: $H= 4.628$ $df=1$, $P<0.05$). Skin tissue, however, did not show any significant variation among sites for δN^{15} (Kruskal-Wallis test; $H= 4.88$, $df=3$, $P=0.181$)

In Figure 3, a bi-variable plot δC^{13} & δN^{15} was used to illustrate the potential isotopic niche of the dolphins sampled. Dolphin δC^{13} ratios are shown along with the mean isotopic ratios for producers and consumers used in Matich and Heithaus (2014). Two isotopically distinct food webs –freshwater/estuarine ($\delta C^{13} < -25\text{‰}$) and adjacent marine ($\delta C^{13} > -19\text{‰}$) found in the FCE are used for qualitative comparison.

There are significant isotopic differences between δN^{15} values of blubber and skin, and δC^{13} values of blubber and skin (Related Samples Wilcoxon Signed Ranks Test: δN^{15} N=34, $Z=-5.086$, $P<0.001$; C^{13} N=34, $Z=-5.035$, $P<0.001$). In figure 4, δN^{15} and δC^{13} data for skin and blubber are summarized: δC^{13} differ among locations and between tissues samples and δN^{15} ratios seem to be similar among sample locations but differ overall among skin and blubber samples.

Table 1. Summary data for stable isotope values of skin and blubber from Florida Bay (FBay), Whitewater Bay (WWB), Joe River, and Shark River. .

Sample Location		$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
		Blubber	Skin	Blubber	Skin
FBay	Mean	-9.92	-12.64	13.48	11.05
	Std. Deviation	1.30	1.46	0.72	0.85
	N	11	12	11	12
Joe	Mean	-18.21	-19.93	13.31	11.26
	Std. Deviation	1.31	1.58	0.66	0.79
	N	6	6	6	6
Shark	Mean	-20.17	-24.10	13.70	11.52
	Std. Deviation	1.51	2.62	0.39	0.38
	N	4	4	4	4
WWB	Mean	-18.45	-20.11	12.71	11.70
	Std. Deviation	1.27	1.37	0.76	0.75
	N	13	13	13	13

Table 2. Summary data comparing skin and blubber δC^{13} values among sites.

Sites comparison using independent-samples Kruskal-Wallis test					
Sites	$\delta^{13}\text{C}$	n	Test Statistic	DF	P-value
Fbay, Joe	Blubber	17	11.013	1	<0.05
	Skin	18	11.38	1	<0.05
Shark, WWB	Blubber	17	3.71	1	0.054
	Skin	17	6.79	1	<0.05
Fbay, WWB	Blubber	24	17.175	1	<0.05
	Skin	25	18.014	1	<0.05
Joe, WWB	Blubber	19	0.277	1	0.559
	Skin	19	0.008	1	0.93
Fbay, Shark	Blubber	15	8.265	1	<0.05
	Skin	16	8.483	1	<0.05
Shark, Joe	Blubber	10	2.909	1	0.088
	Skin	10	4.545	1	<0.05

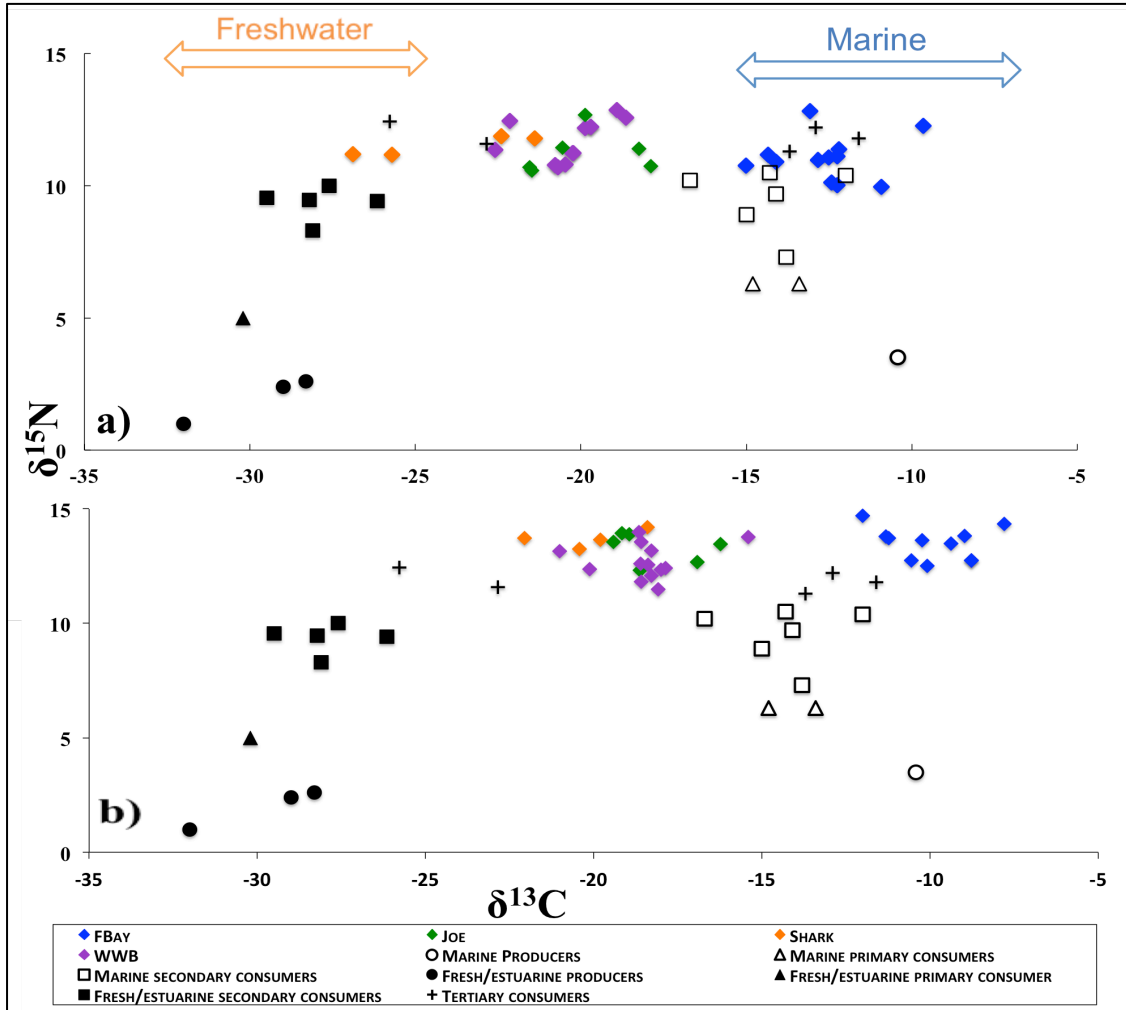


Figure 3. Isotopic biplot of $\delta^{13}\text{C}$ vs $\delta^{15}\text{N}$ of dolphins (colored symbols) and representative producers and consumers from freshwater/estuarine food webs of the Shark River Estuary and marshes as well as from adjacent marine waters (data based on Fry & Smith 2002, Chasar et al. 2005, William and Trexler 2006, Matich & Heithaus 2014.). Dolphin data in (a) are from skin samples while (b) is based on blubber samples. Producers and consumers from the freshwater/estuarine food web are solid black; those from the marine food web are white. Producers are circles (●), primary consumers are triangles (▲), secondary consumers are squares (■), tertiary consumers are (+) and dolphins isotopes are diamonds (◆)

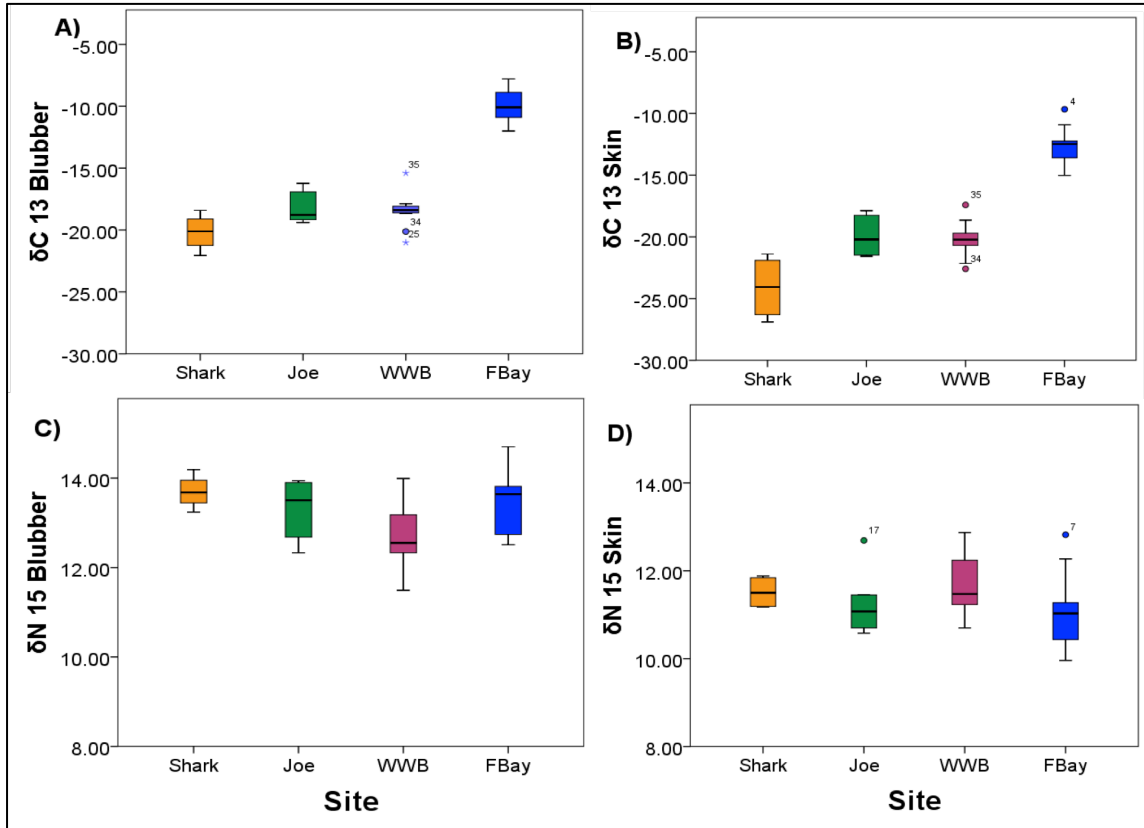


Figure 4. Box plots of isotopic values of blubber (left) and skin (right). The midpoint of each box represents the median, the box edges are 25% quartiles, and whiskers represent maximum and minimum values that are not outliers.

DISCUSSION

Dolphins have the potential of being an essential driver in FCE, yet not much is known about them in this system. Stable isotope analysis has become a popular method for addressing questions about trophic ecology and dietary specialization of high mobile predators (e.g. Kiszka et al 2011, Matich and Heithaus 2014, Rosenblatt and Heithaus 2011). δC^{13} isotopic values display very little change with trophic transfers and its used depict the basal carbon source of an individual, while $\delta^{15} N$ isotopic values are used to determine trophic level since it exhibits stepwise enrichment with trophic transfers. Consumer stable isotope ratios will slightly differ from its prey; this variance is referred as diet-tissue discrimination (Browning et al. 2014a). In order to interpret determine δC^{13} & δN^{15} isotope values, proper information in diet discrimination such as metabolic rates, age, dietary quality, tissue sampled must be determined (i.e Minagawa and Wada 1984; Layman et al 2012; Browning et al. 2014b).

When comparing dolphin skin δN^{15} values among sites there was relatively small variation (Figure 4). Although there was a difference in δN^{15} values from the blubber samples detected among sites that could have been due to a small sample size or due to the use of untransformed $\delta^{15} N$ values. The untransformed δN^{15} isotope data collected from FCE provide a rough estimation of the trophic level from which these individuals are feeding. Based on $\delta^{15} N$ isotopic values, dolphins appeared to be of a tertiary consumer (top predator), at a similar level to bull sharks and alligators in Florida Coastal Everglades (Figure 3).

When comparing δC^{13} of dolphins and other taxa in the FCE (Figure 3), dolphins seem feed from a wide range of food webs, which is not surprising because they inhabit all of them. Shark River's δC^{13} ratio is characterized with that of freshwater/estuarine food web and Florida Bay's δC^{13} ratio is depicted with that of a marine food web. While the isotopic values of Whitewater bay and Joe River might have an intermediate δC^{13} ratio. Dolphins δC^{13} ratios fall within the overall isotopic variance found in the FCE but most importantly it shows a spatial distinction between individuals sampled in the rivers (Shark, Joe, WWB) and in the marine waters (FB) (Figure 3, 4).

Unlike bull sharks and alligators where you have individuals in the same area apparently feeding from different food webs (Rosenblatt and Heithaus 2011, Rosenblatt et al. 2015, Matich et al. 2011, Matich and Heithaus 2015), dolphins seems to feed consistently within the food web where they were sampled. This is similar to what has been seen in Indian River dolphins where they were able to differentiate between dolphins from adjacent lagoons based on isotope signatures (Browning et al. 2014). δC^{13} isotopic values of dolphins sampled in Shark River and Florida Bay seem to suggest that dolphins are foraging within single ecosystems (e.g estuarine vs. marine). It still remains unclear if dolphins sampled in Whitewater Bay and Joe River are feeding in both marine and estuarine food webs or if prey in this area have intermediate isotopic values. If different potential resource pools have overlapping δ values, as it might be the case of in Whitewater Bay, stable isotopes alone may not be able to indicate the particular source pool being utilized (Layman et al., 2012).

Body tissues of individuals incorporate isotope values of their diets at different rates, this known as the turnover rate (Matich et al. 2011). Isotope turnover in organisms is largely controlled by their metabolic rate as it involves metabolic exchange of elements, growth, and protein turnover (Hussey et al. 2012). The use of two tissues (e.g. skin and blubber) with different turnover rates can provide an insight of temporal variability of the individual. (e.g. Matich and Heithaus 2014). Therefore further data analysis to correct the isotope values of blubber and skin is needed before being able to make temporal assumptions. Unlike the alligators and bull sharks, bottlenose dolphins have a high metabolic rate so therein lies the opportunity to investigate a more fine-scale temporal difference by using a predator that assimilates isotopes at a faster rate.

Dolphins seem to be recycling nutrients within their locations and do not take part in transporting nutrients upstream like alligators and bull sharks. Dolphins residing in the rivers might be exception to this assumption since they are thought to be moving out of the rivers in between foraging bouts according to Sarabia (2012). The supporting isotope and behavioral data suggests that dolphins residing in the rivers could potentially be vectors of nutrients downstream.

The FCE is subject to impacts by the Comprehensive Everglades Restoration Plan (CERP), that was created to restore and preserve the natural water function and resources (CERP 2015), and climate change. In order to predict the potential impact of the eminent threats, it is critical to understand of spatiotemporal variation in biotic and abiotic factors, their interactions, and how they affect the abundance and behavior of key species. As a predator with a high metabolic rate, long

distance movement capabilities, and capability to vertically learn foraging techniques (Pilot et al. 2012), bottlenose dolphins have the potential to affect the structure and function of ecosystems (Bowen 2007). Dolphins in the FCE might be impacted differently depending on their location. Therefore it is necessary to use a combination of multiple methods such as investigating the targeted prey taxa, estimating trophic niche throughout seasons, using a larger sample size, focal follows and photo-identification information to further elucidate the spatial, temporal, and trophic role of the bottlenose dolphins.

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