

Isotopic Discrimination Factors ($\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$) between Tissues and Diet of the Broad-Snouted Caiman (*Caiman latirostris*)

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21 ABSTRACT.—Natural variation in stable isotope ratios is a useful tool in diet studies. However, the correct interpretation of isotopic data is
22 reliant on proper estimates of discrimination factors. This study aimed to describe the magnitude of the discrimination factors of carbon and
nitrogen isotopes between diet and tissues ($\Delta^{13}\text{C}_{\text{tissue-diet}}$ and $\Delta^{15}\text{N}_{\text{tissue-diet}}$) of the Broad-snouted Caiman (*Caiman latirostris*) and to verify
potential differences between age classes. The isotopic ratios of carbon and nitrogen ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) were determined in samples of two tissue
types (claw and scute) collected from 18 captive animals and in 15 samples of their diet. The $\Delta^{13}\text{C}_{\text{tissue-diet}}$ was $1.2 \pm 0.1\text{‰}$ for claw and $0.9 \pm$
 0.2‰ for scutes; the $\Delta^{15}\text{N}_{\text{tissue-diet}}$ was $1.1 \pm 0.1\text{‰}$ for claw and $0.8 \pm 0.2\text{‰}$ for scutes. These values were much lower than the values assumed
commonly in ecological studies (3–5‰), and similar to a previous study with crocodylians. Our results emphasize the need to determine
discrimination factors specific to taxa instead of assuming average values derived from the literature.

The application of stable isotope analysis (SIA) in animal ecology studies has increased in recent decades as a result of technological development in this area and the information that can be obtained by this methodology (Gannes et al., 1997, 1998; Martinez Del Rio et al., 2009; Ramos and González, 2012). SIA has been used to trace the migration routes of wild animals (Hobson, 1999; Hobson and Wassenaar, 2008), to reconstruct dietary sources and to determine the trophic level of organisms (Kelly, 2000; Kupfer et al., 2006; Casey and Post, 2011).

The basis of these analyses is that animal tissues reflect the isotopic ratios of the food consumed (DeNiro and Epstein, 1978, 1981; Crawford et al., 2008). The stable isotope ratios ($\delta^{13}\text{C}$: $^{13}\text{C}/^{12}\text{C}$; $\delta^{15}\text{N}$: $^{15}\text{N}/^{14}\text{N}$) should be defined in delta notation (δ) and be reported in parts per mil (‰) relative to international standards (see Fry, 2006). Therefore, it is possible to identify the various dietary sources contributing to the tissue formation (Fry, 2006), for example, the contribution of C_3 and C_4 plants to the diet (e.g., Magnusson et al., 1999; Cerling et al., 2006). These photosynthetic cycles correspond to markedly different $\delta^{13}\text{C}$ values (C_3 : -35 to -22‰ , average 27‰ ; C_4 : -16 to -9‰ , average -12.5‰ ; Farquhar et al., 1989). Moreover, it is possible to determine the trophic levels of consumers due to the progressive increase of $\delta^{15}\text{N}$ from the bottom to the top of the food chain (Minagawa and Wada, 1984).

The successful use of this methodology depends on the estimates of discrimination factors ($\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$) between animal tissues and diet (Caut et al., 2009). The discrimination factor can be defined as the change in the isotope ratio due to the contrasting behavior of different isotopes that occurs in natural processes such as evaporation, burning, or any metabolic pathway (Farquhar et al., 1989). Many researchers use mean values determined by literature reviews (McCutchan et al., 2003; Vanderklift and Ponsard, 2003) because of the impossibility of conducting experiments with their species of interest under controlled conditions (Martinez del Rio et al., 2009). However, mistakes can result from the use of values in the literature (Caut et al., 2008). Erroneous estimates of the discrimination factor may result in errors in determining the contribution of food sources in the diet or in the establishment

of their trophic level (Caut et al., 2009). Mixture models that use isotopic values require accurate estimates of the discrimination factor to provide reliable data (Phillips and Gregg, 2001).

Taxonomic identity appears to play a major role in the discrimination factors of organisms (Caut et al., 2009). Therefore, in the absence of information on discrimination factors for the organism of interest, researchers should, whenever possible, use values estimated from closely related species. This approach requires the documentation of the discrimination factors of as many taxonomic groups as possible.

Despite a few recent studies focusing on discrimination factors in reptiles (Seminoff et al., 2007; Fisk et al., 2009; Murray and Wolf, 2012), this group has been neglected in the literature. For crocodylians, there is little information on discrimination factors (Caut, 2013; Rosenblatt and Heithaus, 2013). Therefore, we investigated the discrimination factors of the Broad-snouted Caiman (*Caiman latirostris*) experimentally. The Broad-snouted Caiman is a crocodylian distributed over a wide latitudinal range ($5\text{--}34^\circ\text{S}$) within South America, including Argentina, Bolivia, Paraguay, Uruguay, and Brazil (Verdade and Piña, 2006). This species can be found in a wide variety of habitats, including wetlands, floodplains, rivers, streams, and ponds (Medem, 1983; Borteiro et al., 2006). *Caiman latirostris* is also one of the few alligatorids that inhabit estuarine habitats (Grigg et al., 1998) and in the future stable isotope analysis could be very important in determining contributions from marine versus freshwater/terrestrial prey and water resources in their populations (e.g., Rosenblatt and Heithaus, 2011; Wheatley et al., 2012). In Brazil, this species is commercially farmed in intensive systems (Verdade, 2004; Verdade et al., 2006). These characteristics (broad geographical distribution and multiple habitat use and captive breeding) make this caiman an interesting model to study discrimination factors in neotropical crocodylians. The aim of this study was to describe the magnitude of the discrimination factor for carbon and nitrogen isotopes between the diet and tissues of the Broad-snouted Caiman and to verify possible differences between age groups. We hope that this information will help to provide an empirical basis for interpreting future studies that apply isotopic analysis to reptiles in general and crocodylians in particular.

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MATERIALS AND METHODS

Eighteen female Broad-snouted Caimans from a commercial breeding facility were maintained on the same controlled diet (ground chicken necks, adapted from Sarkis-Gonçalves et al. 2002) since hatching. Chicken necks came from a single facility, where the chickens were fed with commercial feed made up of corn, soy, and wheat throughout their lives. The caimans were housed in greenhouse enclosures (length: 7 m, width: 5 being 1.5 m dried, deep pool: 1 m, adapted from Verdade et al., 2006) and fed daily. During the sampling period, they belonged to two distinct age classes, 1–2 yr ($n = 9$) and 3–4 yr ($n = 9$). The mean total length for age class 1–2 yr was 91 ± 12 cm (range: 71–104 cm) and the mean total length for age class 3–4 yr was 107 ± 6 cm (range: 97–115 cm). The 3–4-yr-old animals were significantly larger than 1–2-yr-old animals (t -test: $t_{17} = -3.5$, $P = 0.005$).

The animals were snared or captured manually and physically restrained without the use of chemical immobilization (Verdade, 2000, 2001). Total length of each individual was measured with a tape (1-mm precision). Claw and scute tissues were chosen for the experiment because they could be collected easily without sacrificing animals. Claw samples (terminal 0.5 cm of the claw) were collected on the same finger in all animals, whereas scute samples (2 cm^2) were collected from the tail of each caiman with the use of surgical scissors. The samples were stored in individual containers without the use of chemicals and frozen prior to sample processing. Diet samples were collected 3 mo before ($n = 7$) and during the experiment ($n = 8$).

Lipid contents among different organisms or tissues may introduce considerable bias in SIA (Post et al., 2007). We analyzed the samples in two ways: with the lipids extracted and without the lipids extracted. Lipids were extracted by a solution of chloroform and methanol that physically removed lipids from samples. After extraction, lipids were oven dried at 50°C until they reached a constant mass.

The claw and scute samples were fragmented to the smallest possible size; the diet samples were macerated finely using mortar and pestle. Mass was determined for the resulting material (0.8 to 1.2 mg) and placed in small tin capsules.

The isotopic compositions of carbon and nitrogen were determined by combustion “online” from the sample with the use of continuous flow–isotope ratio mass spectrometers (CF-IRMS) on a Carlo Erba elemental analyzer (CHN-1110) coupled to a Delta Plus mass spectrometer in the Laboratório de Ecologia Isotópica de Centro de Energia Nuclear na Agricultura (CENA) at the Universidade de São Paulo (USP). The isotopic composition of carbon and nitrogen was calculated with the following equation:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = \left(\frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \right) * 1,000,$$

where R is the molar ratio $\text{C}^{13}/\text{C}^{12}$ or $\text{N}^{15}/\text{N}^{14}$ in the sample

and standard, expressed as delta (δ) per mil (‰) (see Fry, 2006). The standard deviations of an internal standard (sugarcane leaves) were 0.07‰ for $\delta^{15}\text{N}$ and 0.14‰ for $\delta^{13}\text{C}$ based on 15 within-run samples during four runs. The standards used for nitrogen and carbon were atmospheric nitrogen and Vienna-Pee-Dee Belemnite (calcium carbonate comes from a Cretaceous belemnite from the Pee Dee formation in South Carolina, USA), respectively.

We tested the data for normality with the use of the Anderson Darling test and for homoscedasticity with the use of Levene’s test prior to statistical analyses. The t -test (Zar, 1999) was used to investigate possible differences in isotope composition between treatments with and without lipids extraction, and to check differences in diet isotope composition between the two collection periods.

The discrimination factor was determined by the equation $\Delta(p/s) = \delta(p) - \delta(s)$, where $\Delta(p/s)$ = discrimination factor per mil, $\delta(p)$ = isotope ratio per mil of the animal tissues and $\delta(s)$ = isotope ratio per mil of the diet. We performed a two-way ANOVA (Zar, 1999) to detect possible differences involving $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in tissues (claw and scute) and age classes (1–2 and 3–4 yr) and the possible interaction between these factors. All statistical analyses were performed with the use of Minitab 16. The measurements are expressed as mean \pm standard deviation.

RESULTS

The isotope compositions of the treatments with and without lipids extraction are statistically similar ($\delta^{13}\text{C}$ scutes: $t_{35} = -1.0$, $P = 0.302$; $\delta^{15}\text{N}$ scutes: $t_{35} = -1.2$, $P = 0.217$; $\delta^{13}\text{C}$ claw: $t_{35} = -0.2$, $P = 0.794$; $\delta^{15}\text{N}$ claw: $t_{35} = 0.2$, $P = 0.8$). For this reason, we used the results from the treatment with lipid extraction to determine the discriminations factors. The mean C:N ratio for samples with and without lipids extraction were 2.75 ± 0.04 and 2.70 ± 0.04 for scutes, and 2.6 ± 0.01 and 2.68 ± 0.01 for claw, respectively.

The $\delta^{13}\text{C}$ was $-17.8 \pm 0.1\text{‰}$ (range: -18.1 to -17.4‰) for claw tissue and $-18.1 \pm 0.2\text{‰}$ (range: -18.4 to -17.6‰) for scute tissue; the $\delta^{15}\text{N}$ was $4.7 \pm 0.1\text{‰}$ (range: 4.5 – 5.2‰) for claw tissue and $4.4 \pm 0.2\text{‰}$ (range: 4.1 – 5‰) for scute tissue (Table 1; Fig. 1). The mean values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ diet were $-19 \pm 0.6\text{‰}$ (range: -20.1 to -18‰) and $3.6 \pm 0.1\text{‰}$ (range: 3.3 – 4‰), respectively. There was no difference in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ diet between the two different diet sampling periods ($\delta^{13}\text{C}$: $t_{14} = 1.4$, $P = 0.17$; $\delta^{15}\text{N}$: $t_{14} = -1.7$, $P = 0.09$).

The discrimination factors between the tissues and diet $\Delta^{13}\text{C}$ values were $1.2 \pm 0.1\text{‰}$ (range: 0.9 – 1.5‰) for claw and $0.9 \pm 0.2\text{‰}$ (range: 0.6 – 1.4‰) for scute. The corresponding discrimination factors $\Delta^{15}\text{N}$ were $1.1 \pm 0.1\text{‰}$ (range: 0.9 – 1.5‰) for claw and $0.9 \pm 0.2\text{‰}$ (range: 0.5 – 1.4‰) for scute (Fig. 2).

There were minor differences in the $\Delta^{13}\text{C}$ values between tissues ($df = 1$, $F = 29.4$, $P < 0.001$) and age classes ($F_{1,35} = 5.9$, P

TABLE 1. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ mean of *C. latirostris*, showing different tissue types (scute and claw) and age classes (1–2 and 3–4 yr).

	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
	Mean \pm SD	Range	Mean \pm SD	Range
Claw (1–2 yr)	-17.9 ± 0.1	-18.1 to -17.7	4.8 ± 0.1	4.5 – 5.2
Claw (3–4 yr)	-17.6 ± 0.1	-17.8 to -17.4	4.7 ± 0.1	4.5 – 5
Scute (1–2 yr)	-18.1 ± 0.1	-18.3 to -17.9	4.4 ± 0.1	4.2 – 4.7
Scute (3–4 yr)	-18 ± 0.2	-18.4 to -17.6	4.4 ± 0.2	4.1 – 5



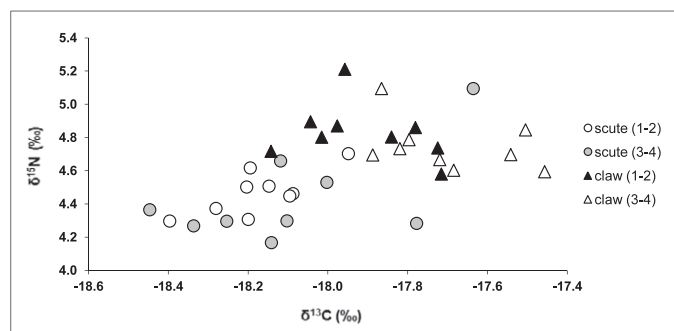


FIG. 1. Biplot of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of *Caiman latirostris*, showing different tissue types (scute and claw) and age classes (1–2 and 3–4 yr).

= 0.02), but no interaction between these factors was found ($F_{1,35} = 1.18$, $P = 0.28$). In addition, there were minor differences in the $\Delta^{15}\text{N}$ values between tissues ($F_{1,35} = 29.4$, $P < 0.001$) but not between age classes ($F_{1,35} = 0.7$, $P = 0.39$), nor was there interaction between factors ($F_{1,35} = 0.1$, $P = 0.67$).

DISCUSSION

Literature on the discrimination factors for different animal tissues in experimental and field conditions are scarce, especially in the case of reptiles (Warne et al., 2010). The discrimination factors we report, ranging from 0.9 to 1.2‰ for carbon and from 0.9 to 1.1‰ for nitrogen, differ from those values assumed commonly in ecological studies.

The $\delta^{15}\text{N}$ discrimination factors for *C. latirostris* are lower than those used in the literature (3–5‰) (DeNiro and Epstein, 1981; Minagawa and Wada, 1984; Peterson and Fry, 1987). However, no clear pattern of differentiation between species has yet been described for reptiles (Table 2). For example, Seminoff et al. (2009) found values ranging from 1.49 to 2.86‰ for various tissues (red blood cells, blood plasma, whole blood, and skin) of the marine turtle *Dermochelys coriacea*, and Seminoff et al. (2007) found values of –0.80 to 3.8‰ for the Common slider, *Trachemys scripta*, by examining many type of tissues (see Table 2). In the case of crocodylians, previous studies have also detected lower levels relative to $\Delta^{15}\text{N}$. Rosenblatt and Heithaus (2013) found values ranging from –1.75 to 1.22‰ for red blood cells, blood plasma, and whole scute tissues of *Alligator mississippiensis*, and Caut (2013) found values of –2.5 to 0.39‰ for red blood cells, blood plasma, and muscle of *C. latirostris* hatchlings. Caut (2013), in a recent study on discrimination factors in Broad-snouted Caiman hatchlings, examined different tissues than those used in this study (claws and scutes). This variation among species can be due to species-specific growth patterns, isotopic routing pathways patterns of protein synthesis, and different strategies for elimination of nitrogenous excreta (Rosenblatt and Heithaus, 2013).

The $\delta^{13}\text{C}$ discrimination factors for *C. latirostris* are within the range described in literature (0–1‰: DeNiro and Epstein, 1978; Peterson and Fry, 1987) and for other reptiles (Table 2). Alligators (–0.65 to 0.61‰; Rosenblatt and Heithaus, 2013) and *C. latirostris* hatchlings (–0.52 to 1.06‰; Caut, 2013) presented $\Delta^{13}\text{C}$ values similar to this study. The mechanisms that control these differences among reptiles are not yet completely understood (Rosenblatt and Heithaus, 2013).

The use of stable isotope models to quantify the contribution of different food resources to the diet of wild animals has increased lately (Caut et al., 2009). Stable isotope methodology

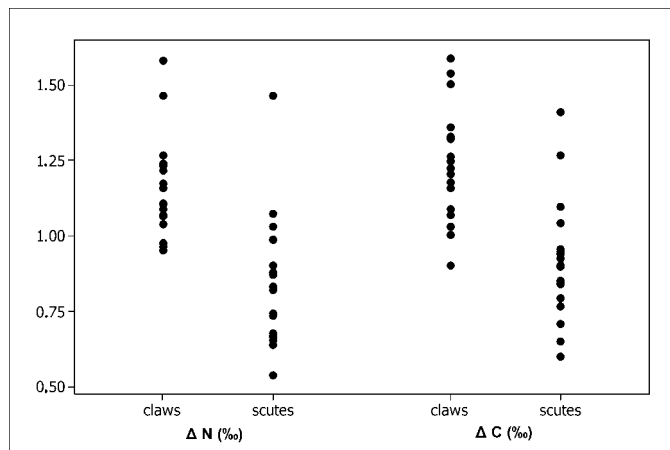


FIG. 2. $\Delta^{13}\text{C}_{\text{tissue-diet}}$ and $\Delta^{15}\text{N}_{\text{tissue-diet}}$ of claw and scute tissues of *Caiman latirostris*.

can be useful when it is difficult to determine the diet by conventional techniques (Seminoff et al., 2006). In addition, this methodology permits diet analyses on different time scales depending on the tissues used (Hobson and Clark, 1992; Dalerum and Angerbjörn, 2005).

The discrimination factor is one of the most important parameters in these models, directly influencing their results (Ben-David and Schell, 2001). However, the wide variation in the values for different taxa should be considered. In this context, the discrimination factors we report for the Broad-snouted Caiman serve to highlight the importance of determining such values at the species level.

Discrimination factors show several sources of variation, like food type, physiological stress, lipid extraction from the sample, diet quality, nutritional status, taxa, tissues and age (Hobson et al., 1993; Pinnegar and Polunin, 1999; Roth and Hobson, 2000; Pearson et al., 2003). However, the mechanism of thermoregulation in the Broad-snouted Caiman may be a primary factor determining the discrimination values found in this study. Most literature data are generally based on experiments with endothermic animals (mammals and birds) and on a few ectotherms (fish) (Caut et al., 2009). As a consequence, these studies should be used with caution in possible comparisons with ectotherms and heterotherms as they have a distinct physiological system (Pough et al., 2003; Zug et al., 2008) that may influence carbon and nitrogen incorporation dynamics.

The physiological strategies used by different animals to eliminate waste nitrogen may also influence the results of this study (see Seminoff et al., 2009). Animals can be divided into three groups according to the principal form of waste nitrogen excreted: ammonotelic (ammonia), uricotelic (uric acid), and ureotelic (urea) (Pough et al., 2003). Crocodylians, which live in aquatic environments, have the advantage of being able to excrete ammonia (an extremely toxic compound) directly, investing less energy and fewer biochemical steps in forming excreta. Ureotelic and uricotelic organisms are significantly more enriched in ^{15}N than ammonotelics (Vanderklift and Ponsard, 2003). Crocodylians in osmotic stress can also excrete uric acid (Leslie and Spotila, 2000). Future studies should focus on these physiological strategies in order to understand their consequences in nitrogen incorporation.

The diet type and quality can also directly influence the discrimination factors in this study. This effect is registered for a wide range of taxa and must be considered when interpreting

TABLE 2. Discrimination factors for carbon and nitrogen isotopes ($\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$) in several tissues of ectotherms (RBC = red blood cells).

Species	Tissue	$\Delta^{13}\text{C}$ (‰)	$\Delta^{15}\text{N}$ (‰)	Reference
<i>Alligator mississippiensis</i> (American alligator)	RBC	-0.49-0.03	-0.78-0.95	Rosenblatt and Heithaus (2013)
	Blood plasma	-0.65--0.04	-1.75-0.35	
	Whole scutes	0.28-0.61	-1.39-1.22	
<i>Caiman latirostris</i> (Broad-snouted caiman)	RBC	-0.52-0.66	-0.93-0.39	Caut (2013)
	Blood plasma	-0.11--0.08	-2.24-0.08	
	Muscle	-0.04-1.06	-2.5 to -1.59	
<i>Trachemys scripta</i> (Common slider)	Claw	0.23	2.3	Aresco (2005)
<i>Trachemys scripta</i> (Common slider)	RBC	-	1.90	Seminoff et al. (2007)
	Blood plasma	-	2.50-3.80	
	Whole blood	-	-0.80-2.20	
	Liver	-	0.40-3.00	
	Brain	-	2.90	
	Pectoralis major muscle	-	2.7	
	Pubioshiofemoralis internus muscle	-	3.4	
<i>Gopherus agassizii</i> (Desert tortoise)	RBC	0.20-0.80	-	Murray and Wolf (2012)
	Blood plasma	1.00-1.60	-	
	Scutes	0.60-0.80	-	
<i>Caretta caretta</i> (Loggerhead sea turtle)	RBC	-0.64-1.53	-0.25-0.16	Reich et al. (2008)
	Blood plasma	-0.38-0.29	-0.32-1.50	
	Whole blood	1.11-0.92	0.14-0.19	
	Skin	1.11-2.62	1.60	
	Scutes	-0.86-1.77	-0.64-0.61	
<i>Chelonia mydas</i> (Green turtle)	RBC	-1.11	0.22	Seminoff et al. (2006)
	Blood plasma	-0.12	2.92	
	Whole blood	-0.92	0.57	
<i>Chelonia mydas</i> (adults; Green turtle)	Epidermis	0.17	2.80	Vander-Zaden et al. (2012)
	RBC	0.30	2.48	
	Blood plasma	0.24	4.17	
	Dermis	2.58	4.93	
<i>Chelonia mydas</i> (juveniles; Green turtle)	Epidermis	1.62	4.04	Vander-Zaden et al. (2012)
	RBC	0.51	2.36	
	Blood plasma	1.16	4.06	
	Dermis	2.18	4.15	
<i>Dermochelys coriacea</i> (Leatherback sea turtle)	Epidermis	1.87	3.77	Seminoff et al. (2009)
	RBC	0.46	1.49	
	Blood plasma	-0.58	2.86	
	Whole blood	0.35	1.98	
	Skin	2.26	1.85	
<i>Elaphe guttata guttata</i> (Corn snake)	Whole blood	2.25	-	Fisk et al. (2009)
	Liver	1.73	-	
	Muscle	2.29	-	
<i>Sceloporus undulatus consobrinus</i> (Prairie lizard)	RBC	-1.10	-	Warne et al. (2010)
	Blood plasma	-0.50	-	
	Skin	-0.80	-	
	Liver	-1.00	-	
<i>Crotaphytus collaris</i> (Collared lizard)	Muscle	-1.90	-	Warne et al. (2010)
	RCB	-1.00	-	
	Blood plasma	0.20	-	

isotopic data (Mirón et al., 2006; Caut et al., 2010; Florin et al., 2011; Lecomte et al., 2011). Caut et al. (2009) found a significant negative relationship between both $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$, with their corresponding diet isotopic ratios. A recent laboratory experiment with *Alligator mississippiensis* had similar results for $\Delta^{15}\text{N}$ in relation to this study (Rosenblatt and Heithaus, 2013).

The diet used in this study is the most common diet used in Broad-snouted Caiman farms in Brazil (Sarkis-Gonçalves et al., 2002). Although it is expected to be considerably different from the diet of wild animals, it is useful to show the discrimination factor between diet and tissues for *C. latirostris*. In addition, a large intraspecific variation in diet is expected for this species in the wild because of its dramatic growth from hatchling (30 g) to adult (60 kg) and its wide latitudinal (5–34°S) and altitudinal (0–800 m) range. Future studies on discrimination factors should consider these sources of variation.

The tissues from animals maintained on controlled diets in captivity may have different isotopic ratios in relation to wild animals because of variations in the proportion of proteins, carbohydrates, and lipids (Seminoff et al., 2006) and/or due to a different fractionation of isotopes (Tieszen et al., 1983). The distinct metabolic pathway through which the dietary elements reach the tissues may contribute to variation in the discrimination factor (Podlesak and McWilliams, 2006).

Age may influence the discrimination factor, depending on the taxa of interest. This effect has been described in the loggerhead turtle (*Caretta caretta*) (Reich et al., 2008), the green turtle (*Chelonia mydas*) (Vander-Zaden et al., 2012), and the red fox (*Vulpes vulpes*) (Roth and Hobson, 2000). However, no clear relationship has been found for cattle (Sutoh et al., 1987), mussels (Minagawa and Wada, 1984) or birds (Hobson and Clark, 1992).

Broad-snouted Caiman showed age-related differences in discrimination factor only for carbon, whereas no effect was found for nitrogen. However, these results should be considered with caution, because the age classes overlapped in body size, as all animals used were juveniles. Future studies involving older specimens and, consequently, a wider range of size classes of *C. latirostris* should be prioritized to better evaluate such effect.

The discrimination factors quantified in this study are relatively different from those described previously for other taxa, which highlights the need for studies at the species level. However, many important questions about discrimination factors remain unanswered. Future studies should prioritize the possible effects of sex, body size, growth rate, type/quality of the diet, and the type of tissue on discrimination factors. As crocodylians are considerably plastic in relation to growth rates (Abercrombie and Verdade, 2002), ecophysiological processes can have a consistent intraspecific (i.e., interpopulation) variation. The elucidation of the dynamics of stable isotopes in a wide range of species will allow a better understanding of the applications and limitations of the analysis of stable isotopes.

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