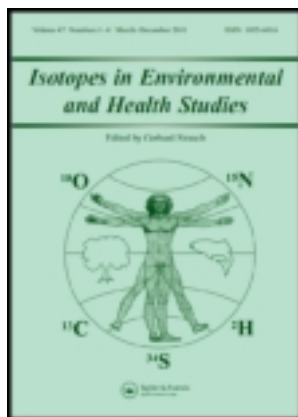


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## Isotopes in Environmental and Health Studies

Publication details, including instructions for authors and subscription information:

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To cite this article: Thiago S. Marques, Neliton R.F. Lara, Luis A.B. Bassetti, Carlos I. Piña, Plínio B. Camargo & Luciano M. Verdade (2013) Intraspecific isotopic niche variation in broad-snouted caiman (*Caiman latirostris*), *Isotopes in Environmental and Health Studies*, 49:3, 325-335

To link to this article: <http://dx.doi.org/10.1080/10256016.2013.835309>

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## Intraspecific isotopic niche variation in broad-snouted caiman (*Caiman latirostris*)

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(Received 22 December 2012; accepted 11 August 2013)

Strategies to minimise intraspecific competition are common in wild animals. For example, individuals may exploit food resources at different levels of the food chain. Analyses of stable isotopes are particularly useful for confirming variations in an intraspecific niche because the chemical composition of animals tends to reflect both the food consumed and the habitats occupied by the species. However, studies using this methodology to investigate neotropical crocodylians are scarce. This study aimed to verify the existence of ontogenetic and sexual niche variation in broad-snouted caiman in a silvicultural landscape in Brazil through the use of carbon and nitrogen stable isotopes. The isotopic ratios of carbon and nitrogen were determined in claw samples collected from 24 juveniles, 8 adults, and 16 hatchlings of *C. latirostris*. We identified a discrete ontogenetic variation in the isotopic niche and sexual difference only for juveniles. These results may indicate differences in the exploitation resources and a consequent reduction in competition between age classes.

**Keywords:** carbon-13; crocodylians; food resources; isotope ecology; niche variation; nitrogen-15

### 1. Introduction

The ecological niche is traditionally defined as the multidimensional space comprising the resources used by an organism [1]. However, conceptual advances and technological developments in isotope ecology in recent decades have caused an increase in the application of this methodology to investigate niche relationships between organisms, and these advances and developments have caused the concept of the niche to be adapted. The isotopic niche can be defined as the area in space of  $\delta$  values ( $n$ -dimensional), with the isotopic values ( $\delta$  values) as the coordinates [2].

The analysis of stable isotopes is particularly useful within this context because the chemical composition of animals (isotope ratio) tends to reflect both the food consumed and the habitats occupied by the species [3–5], providing an integrated representation in space and time. Therefore, the isotopic niche is not the same as the dietary niche because isotopic axes can provide information on the biogenic and scenopoetic aspects traditionally used to define niche hypervolumes [6].

The carbon isotope ratio ( $^{13}\text{C}/^{12}\text{C}$ ) is widely used to differentiate carbon sources in diets [7,8]. Similarly, the nitrogen isotope ratio ( $^{15}\text{N}/^{14}\text{N}$ ) is used to determine the trophic level of an organism

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in view of the progressive increase in  $^{15}\text{N}$  from the base to the top of the food chain [9]. This isotope methodology can also serve to identify differences in resource use at different time scales [10], favouring the ecological assessment of long-term trends in management and conservation projects [2].

Strategies to minimise intraspecific competition are common in wild animals because they can maximise the exploitation of available resources (e.g. habitat or food) and increase the fitness of competitors [11,12]. Intraspecific variations in resource use have important evolutionary implications for the ecological attributes of species [13] and may occur during the ontogenetic development of individuals [14,15] and/or between the sexes [16].

Body size is an important factor in the biology of animals because it directly influences energy requirements [15]. Ontogenetic niche variation can involve changes in habitat use and in the exploitation of food resources [11], and ontogenetic variation in resource exploitation has been extensively studied in a wide range of taxa, including invertebrates [17], amphibians [18], reptiles [19,20], birds [21,22], and mammals [23,24]. Niche variation between sexes is also common [25,26].

Crocodylian species show a dramatic increase in body mass from hatchling to adult, which results in changes in the exploitation of food resources [27]. An understanding of changes in diet is of key importance for species conservation and is directly related to changes in ecological niches [28]. Although occasionally vague [29], the concept of niche is of fundamental importance for the understanding of life history strategies and ecological interactions [30]. However, few studies using this approach have been conducted in neotropical crocodylians, particularly in anthropic environments, where resource availability may be altered.

This study aimed to test the possible occurrence of ontogenetic and sexual niche variation in broad-snouted caiman in a silvicultural landscape of Southeastern Brazil through the use of stable isotopes of carbon and nitrogen. The null hypothesis in this study is that no intraspecific niche variation (ontogenetic or sexual) occurs in this species under such circumstances.

## 2. Materials and methods

### 2.1. Study area

This study was conducted at two silvicultural farms (Três Lagoas:  $23^{\circ}22'0'' - 23^{\circ}20'41''\text{S}/48^{\circ}28'0'' - 48^{\circ}27'57''\text{W}$  and Arca:  $23^{\circ}20'0'' - 23^{\circ}18'51''\text{S}/48^{\circ}27'30'' - 48^{\circ}28'20''\text{W}$ ) located in the municipality of Angatuba, state of São Paulo, Brazil. The climate is considered subtropical, with slight variation occurring along the Alto Paranapanema watershed [31].

The study area has experienced cycles of deforestation and the regeneration of native vegetation [32]. The Três Lagoas and Arca farms present total areas (TAs) of 3242 and 1122 ha, respectively. The landscape on the two farms is a mosaic formed by a matrix of eucalyptus plantations, fragments of native vegetation (transition between Cerrado and Atlantic Forest) [33], and water bodies (lakes, ponds, and streams).

### 2.2. Methods of capture and immobilisation of animals

Captures were performed from February 2010 to April 2011 during the night with the aid of steel cable snares and sealed-beam headlights to locate the animals. The approach was performed by boat, as described by Hutton et al., [34] or by foot at the edge of the water body (if small) while imitating hatchling vocalisation. We also used baited traps (three iron hoops, one metre in diameter, with 6 cm mesh size) placed within the water bodies near the edge. The animals were physically restrained and blindfolded with a cloth, and the mouth was closed with adhesive tape.

The individuals were marked by cutting single and double tail scutes [35,36], with a previously established combination of numbers, in addition to microchips (transponders) (Destron, TX1414B). The total length (TTL) and snout–vent length (SVL) were measured with a measuring tape of 1 mm precision [37]. The animals were sexed by visual examination of the genitalia morphology, using a speculum of appropriate size [36,38]. Claw fragments (the terminal 5 mm of the claw in adults and juveniles and the entire claw in hatchlings) were collected from all of the captured animals to analyse stable carbon and nitrogen isotopes. Claws are considered to be metabolically inert tissues, thus recording the isotopic composition of food resources close to the time of formation [39].

### 2.3. Sample preparation

The claw samples were washed with a solution of chloroform and methanol to extract the lipids, dried at 50°C to a constant mass, and homogenised and powdered using a grinder. The resulting material was weighed (0.8–1.0 mg) and placed in small tin capsules.

The isotopic composition of carbon and nitrogen was determined by the combustion of an ‘online’ sample with CF-IRMS on a Carlo Erba elemental analyser (CHN-1110) coupled to a Delta Plus mass spectrometer. The analysis was conducted in the Laboratório de Ecologia Isotópica of the Centro de Energia Nuclear na Agricultura of the Universidade de São Paulo, in Piracicaba, São Paulo, Brazil. The calculation of the isotopic composition of carbon and nitrogen was based on the following equation:

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

where  $R$  denotes the  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$  molar ratio in the sample and standard [40]. The results are presented in delta ( $\delta$ ) per mil (‰). The international standards used for nitrogen and carbon were atmospheric  $\text{N}_2$  (Air) and Vienna Peedee Belemnite, respectively. The standard deviations of secondary isotopic reference materials (sugarcane leaves) were 0.08 ‰ for  $\delta^{15}\text{N}$  and 0.14 ‰ for  $\delta^{13}\text{C}$  based on seven within-run samples during two runs.

### 2.4. Data analysis

The captured animals were separated into the following age classes adapted from Larriera et al. [41]: hatchlings (class I) <50 cm, juveniles (class II) 50–129.9 cm, and adults (class III and IV) >130 cm TTL. These age classes were converted into measures based on SVL, according to the regression equations available in Verdade [42] for *C. latirostris* (TTL–SVL relationship:  $r^2 = .97$ ,  $p < .001$ ) because crocodylians usually lose part of their tail in agonistic interactions [43]. Therefore, the age classes can be represented as follows: hatchlings (class I) < 25 cm, juveniles (class II) 25–67.9 cm, and adults (class III and IV) > 68 cm SVL. The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of the captured hatchlings were not incorporated in the analyses because the individuals were captured from the same nest, possibly resulting in sampling bias due to pseudoreplication. However, the means and standard deviations are presented.

The ontogenetic niche was evaluated according to the hypothesis-testing framework proposed by Hammerschlag-Peyer et al. [44] for stable isotope ratios. Incorporating important characteristics of food resource use, such as niche widths (Bayesian standard ellipse), niche position (test of central tendency), and niche overlap, this framework identifies three possible types of situations: 1 – no niche shift, 2 – niche expansion/reduction, and 3 – discrete niche shift between size classes [15].

Quantitative metrics based on the position of individuals within the  $\delta^{15}\text{N}$ – $\delta^{13}\text{C}$  data were used to reveal aspects of the trophic structure of wild animals at the population and community levels [45–47]. The TA metric (the convex hull area formed by extreme individuals within  $\delta^{15}\text{N}$ – $\delta^{13}\text{C}$ ) indicates the niche widths of the population or community studied [45]; however, the area of the polygon is strongly influenced by the sample size, making it impractical to compare groups having different sample sizes [48]. Therefore, we used the Bayesian standard ellipse metric ( $\text{SEA}_B$ ) to measure the niche widths to ensure robustness with regard to the variation in sample size [48]. This metric was calculated from the variance and covariance of  $x$  and  $y$  data (bivariate data-set), containing approximately 40% of the data and determining the core niche. Probability values for differences between the groups were obtained by calculating the proportion of the total number of simulations (10,000), where one group had a larger  $\text{SEA}_B$  than the other. Details of the calculations can be found in Jackson et al. [48]. The metrics were calculated using Stable Isotope Analysis in the R package [49] for R [50].

The niche position was evaluated by a test of central tendency that was based on the Euclidean distance between the groups' centroids [51]. Nested linear models and residual permutation procedures were generated, and the measurements (Euclidean distance) were compared between the groups. The tests were conducted using R software [50] following the script provided by Turner et al. [51].

The question of whether isotopic compositions ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) vary between age classes (juvenile and adults) and sexes was addressed using MANOVA. In case of significance, ANOVA was performed for each isotope composition to determine which of these contributed to any of the significant effects found in the MANOVA results considering a Bonferroni correction. An analysis of mean (ANOM) was used as a *post hoc* test. Linear regression was applied to the juvenile males and juvenile females to verify the possible relationship between the isotopic compositions and body length (i.e. SVL). In addition, an analysis of covariance (ANCOVA) was applied to verify the interaction between sex and body length (SVL) in juveniles. The normality and homoscedasticity of the data were analysed prior to the application of the statistical analyses, which were performed with Minitab 16. The significance level was set at  $\alpha = 0.05$  for the statistical analyses and the measurements are expressed as the mean  $\pm$  standard deviation.

### 3. Results

In total, 48 broad-snouted caiman were captured (16 hatchlings, 24 juveniles, and 8 adults). The average TTLs were  $22.8 \pm 0.6$  cm (range: 21.5–23.5 cm) for hatchlings,  $80.0 \pm 13.4$  cm (range: 56.5–108.5 cm) for juveniles, and  $164.6 \pm 19.0$  cm (range: 136.0–198.0 cm) for adults. Their respective SVLs were  $10.9 \pm 0.3$  cm (range: 10.2–11.4 cm),  $37.8 \pm 6.1$  cm (range: 27.0–49.0 cm), and  $82.2 \pm 9.1$  cm (range: 72.0–98.0 cm).

Discrete niche variation between the age classes was found using the framework of hypothesis-testing proposed by Hammerschlag-Peyer et al. [44]. The niche widths calculated for each age class by  $\text{SEA}_B$  using Bayesian inference were statistically similar (juveniles – mean:  $2.89 \text{‰}^2$ , median:  $2.81 \text{‰}^2$ , 95% Cr.I. :  $1.85\text{--}4.11 \text{‰}^2$ ; adults – mean:  $2.63 \text{‰}^2$ , median:  $2.43 \text{‰}^2$ , 95% Cr.I.:  $1.22\text{--}4.73 \text{‰}^2$ ;  $p = .631$ ; Figure 1). However, the positions of the isotopic niches were significantly different (Euclidean distance = 5.75;  $p = .001$ ), with no niche overlap between the two age classes (Figure 2).

The stable isotope compositions were significantly different among the age classes (MANOVA:  $F_{1,31} = 66.0$ ;  $p < .001$ ), and separate ANOVA indicated that both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  were different among the age classes ( $\delta^{15}\text{N}$ :  $F_{1,31} = 85.2$ ;  $p < .001$ ;  $\delta^{13}\text{C}$ :  $F_{1,31} = 115.3$ ;  $p < .001$ ). A similar pattern was found for sex because the isotope compositions were different between the sexes

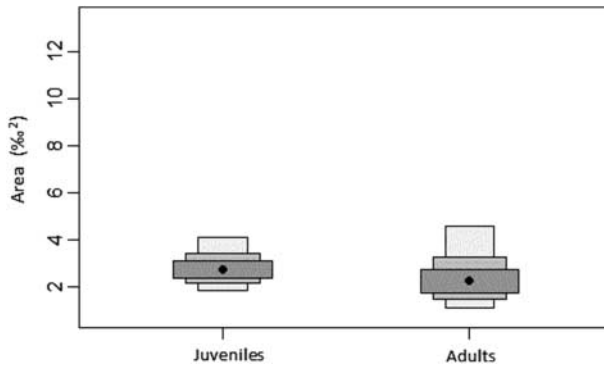


Figure 1. Similarity between estimates of Bayesian standard ellipse areas ( $SEA_B$ ) in juveniles and adults of broad-snouted caiman (*C. latirostris*) inhabiting silvicultural areas in Southeastern Brazil. The black points correspond to the mean  $SEA_B$  for each group, while shaded boxes representing the 50%, 75% and 95% credible intervals from dark to light grey.

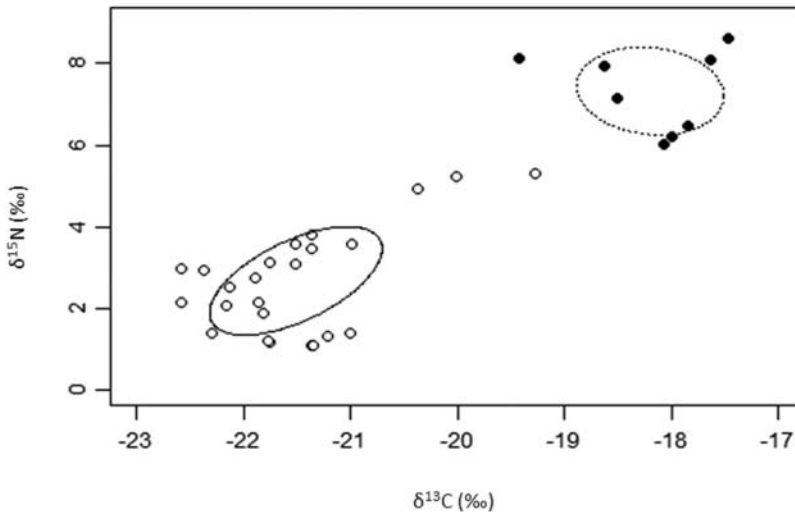


Figure 2. Spatial divergence of standard ellipse between juveniles (white balls) and adults (black balls) of broad-snouted caiman (*C. latirostris*) in  $\delta^{15}N$ - $\delta^{13}C$  space.

(MANOVA:  $F_{1,31} = 25.5$ ;  $p < .001$ ), and ANOVA indicated that both  $\delta^{15}N$  and  $\delta^{13}C$  were different between the sexes ( $\delta^{15}N$ :  $F_{1,31} = 72.2$ ;  $p < .001$ ;  $\delta^{13}C$ :  $F_{1,31} = 58.7$ ;  $p < .001$ ). There was no difference between the adult males and females, but there was a significant difference between the sexes in juveniles (Table 1).

There was a significant relationship between  $\delta^{15}N$  and SVL for the juvenile males ( $F_{1,16} = 40.4$ ;  $p < .001$ ;  $r^2 = .73$ ) but not for the females ( $F_{1,6} = 0.2$ ;  $p = .650$ ;  $r^2 = .04$ ; Figure 3). In addition, there was no significant relationship between  $\delta^{13}C$  and SVL for the juveniles (males:  $F_{1,16} = 2.60$ ;  $p = .124$ ;  $r^2 = .09$ ; females:  $F_{1,6} = 1.60$ ;  $p = .251$ ;  $r^2 = .01$ ; Figure 4). SVL in juveniles was found to be a significant covariant of sex to  $\delta^{15}N$  (ANCOVA:  $F_{1,23} = 19.90$ ;  $p < .001$ ), but not to  $\delta^{13}C$  (ANCOVA:  $F_{1,23} = 0.20$ ;  $p = .649$ ).

The mean  $\delta^{15}N$  and  $\delta^{13}C$  values for the hatchlings were  $5.6 \pm 0.1$  ‰ (5.4–5.9 ‰) and  $-18.6 \pm 0.1$  ‰ ( $-18.8$  to  $-18.4$  ‰), respectively. However, the hatchlings were not considered for the above analysis, as the individuals captured in the same nest are likely siblings, potentially resulting in sampling bias due to pseudoreplication [52].

Table 1. Stable isotope compositions ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) differ between age classes and sexes of broad-snouted caiman (*Caiman latirostris*) inhabiting silvicultural areas in Southeastern Brazil as revealed by ANOVA and ANOM tests (superscript letters indicate similarities or differences).

	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
Juveniles	$-21.5 \pm 0.7^{\text{A}}$ (-22.5 to -19.2)	$2.6 \pm 1.3^{\text{A}}$ (1.0 to 5.3)
Adults	$-18.1 \pm 0.6^{\text{B}}$ (-19.4 to -17.4)	$7.3 \pm 0.9^{\text{B}}$ (6.0 to 8.5)
Juveniles: males	$-21.8 \pm 0.4^{\text{A}}$ (-22.5 to -21.0)	$2.0 \pm 0.8^{\text{A}}$ (1.0 to 3.4)
Juveniles: females	$-20.7 \pm 0.9^{\text{B}}$ (-21.7 to -19.2)	$4.2 \pm 0.9^{\text{B}}$ (3.1 to 5.3)
Adults: males	$-18.0 \pm 0.9^{\text{C}}$ (-19.4 to -17.4)	$7.8 \pm 0.9^{\text{C}}$ (6.4 to 8.5)
Adults: females	$-18.3 \pm 0.3^{\text{C}}$ (-18.6 to -17.9)	$6.8 \pm 0.8^{\text{C}}$ (6.0 to 7.9)

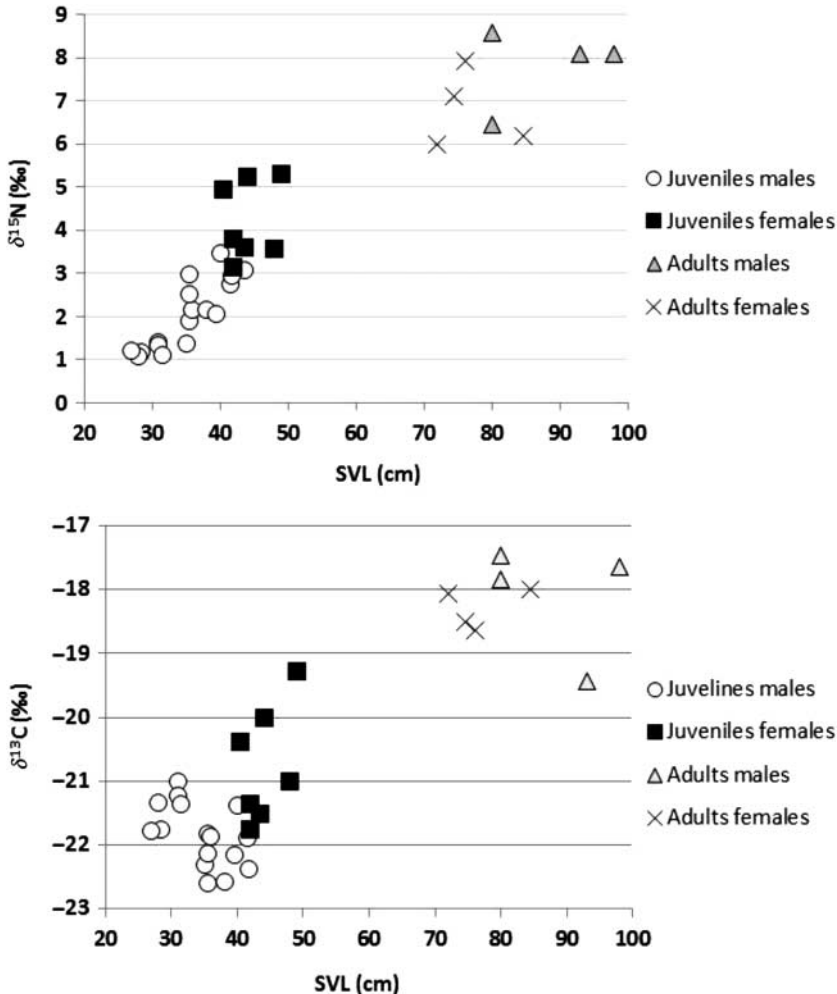


Figure 3. Progressive increase of stable isotope compositions ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) in relationships of SVL in broad-snouted caiman (*C. latirostris*) in silvicultural areas in Southeastern Brazil.

#### 4. Discussion

Crocodylians are top predators in the food chain in most freshwater ecosystems [53]. These animals can exploit a wide range of food resources and occupy a variety of habitats, such as wetlands,



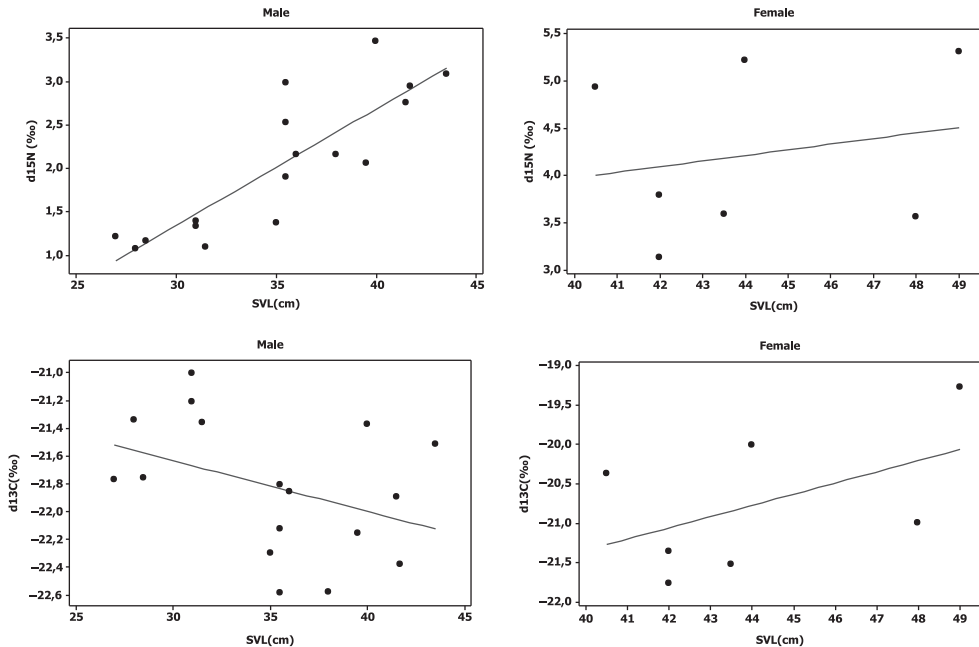


Figure 4. Different relationships between stable isotope compositions ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) and (SVL) of males and females juveniles of broad-snouted caiman (*C. latirostris*) in silvicultural areas in Southeastern Brazil.

floodplains, lakes, rivers, streams, and ponds [53–57]. *C. latirostris* has a broad latitudinal range within its geographical distribution [55]. Therefore, this species exploits a wide variety of prey, including invertebrates (e.g. arthropods and crustaceans) and vertebrates (from fish to mammals) [58,59].

Distinct isotopic niches between juveniles and adults may be due to ontogenetic differences in diet. This is consistent with the data obtained using traditional diet analyses in this species [58,59] and other crocodylians [60–63] in which a diet shift from invertebrate to vertebrate items occurs with ontogenetic development. Considering that there is a dramatic change ( $1000\times$ ) in body size for this species from hatchling (50 g) to adult (50 kg), we can expect a dramatic ontogenetic change in prey size. As a matter of fact, crocodylian (from hatchling to adult) feeding behaviour is typically characterised by inertial ingestion of any possible food item they can grab and manage to swallow [64], and this ontogenetic shift in diet may result in a decrease in intraspecific niche competition [65]. However, there is generally a consistent intraspecific variation in crocodylian growth rates, with a possible influence on this pattern.

Traditional techniques of diet analyses based on stomach-flushing are considerably invasive. Radloff et al. [28] proposed a new approach to crocodylian studies based on stable isotope composition ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) and, as a result, found an ontogenetic shift in the isotopic niche of Nile crocodiles (*Crocodylus niloticus*), a finding that is corroborated by the present study. Age/size classes can be identified by marked changes in isotopic composition in comparison to body size.

The difference in isotopic niche found between *C. latirostris* juveniles and adults can be either due to differential habitat use by age classes or to a distinct resource use on a microhabitat scale. The stable isotope composition of a food web can vary spatially based on a variety of biogeochemical processes [5]. In addition, recent studies with estuarine crocodylians, *Crocodylus acutus* and *Alligator mississippiensis*, showed the importance of a marine environment compared to an inland/freshwater environment to this species [66,67]. *C. latirostris* can also be found in

an estuarine habitat [68], and these two environments (marine and freshwater) can be exploited by the species in the search for resources necessary for their survival. However, the animals were captured at the same site in this study, with most likely the same baseline isotopic values. Under such a circumstance, the use of distinct food resources on a microhabitat scale would be most likely.

The social behaviour of crocodylians can also influence the results of this study. Aggressive interactions are usually related to the use of space in *C. latirostris* [43] and other crocodylians [69]. Indeed, social hierarchy may limit the access of individuals to certain microhabitats and may consequently produce a limitation of food resources [27].

*C. latirostris* individuals show consistent sexual dimorphism in the shape and size of the skull [70,71], and this morphological variation between the sexes could result in intraspecific niche variation [72]. However, the differences between sexes found in the juveniles in the present study are likely due to the clutch effect [37]. The clutch effect – not sex – could also be responsible for the difference in body size between the juvenile males and females in this study in which we likely sampled a clutch of males and another clutch of females. Although there are molecular markers that are sufficiently effective to identify siblings in this species [73–75], these analyses were not performed in this study due to logistical reasons.

There appears to be a complex relationship between nesting habitat [76], temperature-dependent sex determination [77], and growth rate [78] in this species and most likely in other crocodylians [79]. These processes would, by hypothesis, affect dispersal patterns [80] and habitat use [81]. Consequently, the isotopic niche would be affected by the sex of the individual and by the genetic and phenotypic compounds shared by siblings, usually called the clutch effect. Future studies should focus on this issue.

The values of the isotopic ratios of nitrogen and carbon for hatchlings (mean  $\delta^{15}\text{N}$ : 5.6 ‰; mean  $\delta^{13}\text{C}$ : –18.6 ‰) were closer to the values of the adult females than to those of the adult males. The hatchling group captured in this study was apparently moving from the nest site to the nearest pond and most likely had not started feeding yet, as they survive for some weeks or months on the reserves of their yolk sac. For this reason, the hatchlings possibly still reflect their maternal isotopic pattern [82]. Indeed, a maternal influence on the isotopic ratios of hatchlings has been reported for snakes [83] and sea turtles [84], highlighting the need for future studies that focus on understanding this relationship in crocodylian species. In addition, the possibility of testing whether the isotopic ratios of the hatchlings could be used to estimate their maternal ratios should be addressed; as such a measure can be useful in large species that are more difficult to capture [84].

Woodborne et al. [85] investigated *C. niloticus* mass mortality events using claw tissues, establishing the aquatic food web structure in the system in relation to the presence/absence of pansteatitis (dietary disease). Being relatively inert, claws integrate the isotopic composition of the time they are formed, as opposed to metabolically active tissues that alter their isotopic composition during the replacement of dead cells [38]. The use of claws for isotopic studies as a novel approach for crocodylians has the great advantage of indicating the relatively long-term dietary isotopic composition without requiring the sacrifice of animals or the use of invasive procedures (such as bone biopsy). However, claws can also record dietary temporal variations because this tissue grows continuously [86]: the proximal region reflects the most recent diet/habitat, whereas the distal region reflects the oldest. Therefore, the interpretation of the isotopic composition of claw tissue should be made with caution, as its turnover rate is likely to be slow. Rosenblatt and Heithaus [87] found the isotope turnover rates of American alligators (*A. mississippiensis*) to be considerably slower than those of most other taxa studied (blood plasma, red blood cells, and scutes: 252, 566, and 590 d for  $\delta^{13}\text{C}$  and 249.6, 1109.2, and 414 d for  $\delta^{15}\text{N}$ , respectively). Caut [88] found higher isotope turnover rates for broad-snouted caiman hatchlings (blood plasma, red blood cells, and muscle: 83, 256–306, and 134 d for  $\delta^{13}\text{C}$  and 48–91, 160–283, and 188 d for

$\delta^{15}\text{N}$ , respectively) than for alligators, possibly because the study was conducted with hatchlings, which have a faster growth rate than juveniles and adults. Future studies should prioritise the turnover rate in crocodylian claws by considering their distal gradient and the possible differences between anterior and posterior claws parts. In addition, as claws are inert tissues, they can also be useful to study differences among age classes.

## Acknowledgement

The animals used in the study were captured under IBAMA licence [N° 19221-6].

## Funding

This study was funded by the Biota Program/FAPESP [Proc. N° 2006/60954-4]. Luciano M. Verdade and Plínio B. de Camargo hold CNPq Productivity Scholarships. Thiago S. Marques had scholarships from FAPESP [Procs. No. 2008/07076], CNPq [Procs. No. 142682/2011-0] and the Student Research Assistance Scheme at the Crocodile Specialist Group SSC/IUCN.

## References

- [1] Hutchinson GE. Concluding remarks. *Cold Spring Harbor Symp Quant Biol.* 1957;22:415–427.
- [2] Newsome SD, Martinez Del Rio M, Bearhop S, Phillips DL. A niche for isotopic ecology. *Front Ecol Environ.* 2007;5:429–436.
- [3] DeNiro MJ, Epstein S. Influence of diet on the distribution of carbon isotopes in animals. *Geochim Cosmochim Acta.* 1978;42:495–506.
- [4] DeNiro MJ, Epstein S. Influence of diet on the distribution of nitrogen isotopes in animals. *Geochim Cosmochim Acta.* 1981;45:341–351.
- [5] Hobson KA. Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia.* 1999;120:314–326.
- [6] Hutchinson GE. *An introduction to population ecology.* New Haven (CT): Yale University Press; 1978.
- [7] Peterson BJ, Fry B. Stable isotopes in ecosystem studies. *Annu Rev Ecol Syst.* 1987;18:293–320.
- [8] Fry B. *Stable isotope ecology.* New York: Springer Science & Business Media; 2006.
- [9] Minagawa M, Wada E. Step-wise enrichment of  $^{15}\text{N}$  along food chains: further evidence and the relation between  $\Delta^{15}\text{N}$  and animal age. *Geochim Cosmochim Acta.* 1984;48:1135–1140.
- [10] Dalerum F, Angerbjörn A. Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. *Oecologia.* 2005;144:647–658.
- [11] Morin P. Competition: mechanisms, models, and niches. In: Morin P, editor. *Community ecology.* Malden (MA): Wiley-Blackwell; 2011. p. 25–55.
- [12] Ricklefs RE. *The economy of nature.* New York: W.H. Freeman; 2008.
- [13] Bolnick DI, Amarasekare P, Araújo MS, Bürger R, Levine JM, Novak M, Rudolf VHW, Schreiber SJ, Urban MC, Vasseur D. Why intraspecific trait variation matters in community ecology. *Trends Ecol Evol.* 2011;26:183–192.
- [14] Polis GA. Age structure component of niche width and intraspecific resource partitioning: can age-groups function as ecological species? *Am Nat.* 1984;123:541–564.
- [15] Werner EE, Gilliam JF. The ontogenetic niche and species interactions in size-structured populations. *Annu Rev Ecol Syst.* 1984;15:393–425.
- [16] Shine R. Ecological causes for the evolution of sexual dimorphism: a review of the evidence. *Q Rev Biol.* 1989;64:419–461.
- [17] Hentschel TB. Intraspecific variation in  $\delta^{13}\text{C}$  indicates ontogenetic diet changes in deposit-feeding polychaetes. *Ecology.* 1998;79:1357–1370.
- [18] Rosa I, Canavero A, Maneyro R, Camargo A. Trophic niche variation and individual specialization in *Hypsiboas pulchellus* (Duméril and Bibron, 1841) (Anura, Hylidae) from Uruguay. *S Am J Herp.* 2011;6:98–106.
- [19] Hatase H, Takai N, Matsuzawa Y, Sakamoto W, Omuto K, Goto K, Arai N, Fujiwara T. Size-related differences in feeding habitat use of adult female loggerhead turtles *Caretta caretta* around Japan determined by stable isotope analyses and satellite telemetry. *Mar Ecol Prog Ser.* 2002;233:273–281.
- [20] Willson JD, Winne CT, Pilgrim MA, Romanek CS, Gibbons JW. Seasonal variation in terrestrial resource subsidies influences trophic niche width and overlap in two aquatic snake species: a stable isotope approach. *Oikos.* 2010;119:1161–1171.
- [21] Forero MG, Hobson KA, Bortolotti GR, Donazar JA, Bertellotti M, Blanco G. Food resource utilization by the megalanic penguin evaluated through stable-isotope analysis: segregation by sex and age and influence on offspring quality. *Mar Ecol Prog Ser.* 2002;234:289–299.
- [22] Forero MG, Gonzalez-Solis J, Hobson KA, Donazar JA, Bertellotti M, Blanco G, Bortolotti GR. Stable isotopes reveal trophic segregation by sex and age in the southern giant petrel in two different food webs. *Mar Ecol Prog Ser.* 2005;296:107–113.

- [23] Knoff A, Hohn A, Macko SA. Ontogenetic diet changes in bottlenose dolphins (*Tursiops truncatus*) reflected through stable isotopes. *Mar Mammal Sci.* 2008;24:128–137.
- [24] Newsome SD, Etnier MA, Monson DH, Fogel ML. Retrospective characterization of ontogenetic shifts in killer whale diets via  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  analysis of teeth. *Mar Ecol Prog Ser.* 2009;374:229–242.
- [25] Houston D, Shine R. Sexual dimorphism and niche divergence: feeding habitats of the Arafura filesnake. *J Anim Ecol.* 1993;62:737–748.
- [26] Bulté G, Gravel MA, Blouin-Demers G. Intersexual niche divergence in northern map turtles (*Graptemys geographica*): the roles of diet and habitat. *Can J Zool.* 2008;86:1235–1243.
- [27] Huchzermeyer FW. Crocodiles: biology, husbandry and diseases. Wallingford: CABI Publishing; 2003.
- [28] Radloff FGT, Hobson KA, Leslie AJ. Characterising ontogenetic niche shifts in Nile crocodile using stable isotope ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) analyses of scute keratin. *Isotopes Environ Health Stud.* 2012;48:439–456.
- [29] Peters RH. A critique for ecology. Cambridge: Cambridge University Press; 1991.
- [30] Kawanishi K, Liang SHN, Darimont C, Reimchen TE, Sunquist ME. Isotopic niche differentiation among mammals from a rainforest in peninsular Malaysia. *Raffles Bull Zool.* 2012;60:233–239.
- [31] CBHAP: Comitê da bacia hidrográfica do Alto Paranapanema – Diagnóstico da situação dos recursos hídricos da bacia hidrográfica do Alto Paranapanema – Relatório Zero UGRHI-14 [Internet] [cited 2010 Mar 17]. Available from: <http://www.sigrh.sp.gov.br/sigrh/ARQS/RELATORIO/CRH/CBH-ALPA/629/index.html>
- [32] Lisboa MAM. A política dos coronéis e a difusão do ensino primário em Angatuba/SP (1870–1930) [thesis]. Campinas: Universidade Estadual de Campinas; 2008.
- [33] Athayde EA. Importância da heterogeneidade natural na conservação da diversidade de árvores em paisagem agrossilvicultural [thesis]. Rio Claro: Universidade Estadual Paulista ‘Júlio de Mesquita Filho’; 2012.
- [34] Hutton JM, Loveridge JP, Blake DK. Capture methods for the Nile crocodile in Zimbabwe. In: Webb GJW, Manolis SC, Whitehead PJ, editors. *Wildlife management: crocodiles and alligators*. Chipping Norton, Australia: Surrey Beatty; 1987. p. 211–216.
- [35] Bolton M. La explotación del cocodrilo en cautividad. Roma: Guía FAO Conservación; 1994.
- [36] Chabreck R. Methods of capturing, marking and sexing alligators. *Proc Ann Conf Southeast Assoc Game Fish Comm.* 1963;17:45–50.
- [37] Verdade LM. Morphometric analysis of the broad-snouted caiman (*Caiman latirostris*): an assessment of individuals’ clutch, body size, sex, age, and area of origin [thesis]. Gainesville: University of Florida; 1997.
- [38] Allstead J, Lang JW. Sexual dimorphism in the genital morphology of young American alligators, *Alligator mississippiensis*. *Herpetologica.* 1995;51:314–325.
- [39] Ethier DM, Kyle CJ, Kyser TK, Nocera JJ. Variability in the growth patterns of the cornified claw sheath among vertebrates: implications for using biogeochemistry to study animal movement. *Can J Zool.* 2010;88:1043–1051.
- [40] Bond AL, Hobson KA. Reporting stable-isotope ratios in ecology: recommended terminology, guidelines and best practices. *Waterbirds.* 2012;35:324–331.
- [41] Larriera A, Siroski P, Piña CI, Imhof A. Sexual maturity of farm-released *Caiman latirostris* (Crocodylia: Alligatoridae) in the wild. *Herpetol Rev.* 2006;37:26–28.
- [42] Verdade LM. Regression equations between body and head measurements in the broad-snouted caiman (*Caiman latirostris*). *Rev Brasil Biol.* 2000;60:469–482.
- [43] Verdade LM. Agonistic social behavior of broad-nosed caiman (*Caiman latirostris*) in captivity: implications to reproductive management. In: Working meeting of The Crocodile Specialist Group. Switzerland: IUCN – The World Conservation Union; 1992. p. 200–217.
- [44] Hammerschlag-Peyer CM, Yeager LA, Araújo MS, Layman CA. A hypothesis-testing framework for studies investigating ontogenetic niche shifts using stable isotope ratios. *PloS One.* 2011;6:e27104:1–7.
- [45] Layman CA, Arrington DA, Montaña CG, Post DM. Can stable isotope ratios provide for community-wide measures of trophic structure? *Ecology.* 2007;88:42–48.
- [46] Layman CA, Quattrochi JP, Peyer CM, Allgeier JE. Niche width collapse in a resilient top predator following ecosystem fragmentation. *Ecol Lett.* 2007;10:937–944.
- [47] Quevedo M, Svanback R, Eklov P. Intrapopulation niche partitioning in a generalist predator limits food web connectivity. *Ecology.* 2009;90:2263–2274.
- [48] Jackson AL, Inger RI, Parnell AC, Bearshop S. Comparing isotopic niche widths among and within communities: SIBER – Stable Isotope Bayesian Ellipses in R. *J Anim Ecol.* 2011;80:595–602.
- [49] Parnell AC, Inger R, Bearshop S, Jackson AL. Source partitioning using stable isotopes: coping with too much variation. *PloS One.* 2010;5:e9672:1–5.
- [50] R Core Team. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria [Internet] [cited 2013 Feb 10]. Available from: <http://www.R-project.org/>
- [51] Turner TF, Collyer ML, Krabbenhoft TJ. A general hypothesis-testing framework for stable isotope ratios in ecological studies. *Ecology.* 2010;91:2227–2233.
- [52] Hurlbert SH. Pseudoreplication and the design of ecological field experiments. *Ecol Monogr.* 1981;54:187–211.
- [53] Thorbjarnarson JB, Ross JP, Espinosa E. Crocodiles: an action plan for their conservation. Switzerland: IUCN/SSC Crocodile Specialist Group; 1998.
- [54] Pooley AC. Dieta y hábitos alimentarios. In: Ross CA, Garnett S, editors. *Cocodrilos y caimanes*. Barcelona: Encuentro Editorial; 1992. p. 76–91.
- [55] Verdade LM, Piña CI. *Caiman latirostris*. *Cat Am Soc Amph Rep.* 2006;833:1–21.
- [56] Medem F. Los crocodylia de Sur America. Bogotá: Editora Carrera; 1983.

- [57] Borteiro C, Prigioni C, Garcia JE, Tedros M, Gutiérrez F, Kolenc F. Geographic distribution and conservation status of *Caiman latirostris* (Crocodylia, Alligatoridae) in Uruguay. *Phyllomedusa*. 2006;5:97–108.
- [58] Melo MTQ. Dieta do *Caiman latirostris* no sul do Brasil. In: Verdade LM, Larriera A, editors. *Conservação e manejo jacaré e crocodilos da América Latina*. Piracicaba: C.N. Editoria; 2002. p. 116–125.
- [59] Borteiro C, Gutiérrez F, Tedrosa M, Kolenc F. Food habits of the broad-snouted Caiman (*Caiman latirostris*: Crocodylia, Alligatoridae) in northwestern Uruguay. *Stud Neotrop Fauna Environ*. 2009;44:31–36.
- [60] Magnusson WE, De Silva EV, Lima AP. Diets of amazonian crocodilians. *J Herpetol*. 1987;21:85–95.
- [61] Thorbjarnarson JB. Diet of the spectacled caiman (*Caiman crocodilus*) in the central Venezuelan Llanos. *Herpetologica*. 1993;49:108–117.
- [62] Tucker AD, Limpus CJ, McCallum HI, McDonald KR. Ontogenetic dietary partitioning by *Crocodylus johnstoni* during the dry season. *Copeia*. 1996(4):978–988.
- [63] Delany MF, Linda SB, Moore CT. Diet and condition of American alligators in 4 Florida lakes. In: *Proceedings of the 53rd Annual Conference of the Southeast Association of Fish and Wildlife Agencies*; 1999 Oct 28. Baton Rouge, USA. p. 220–222.
- [64] Gans C. Comments on inertial feeding. *Copeia*. 1969(4):855–857.
- [65] Bolnick DI, Svanback R, Fordyce JA, Yang LH, Davis JM, Hulsey CD, Forister ML. The ecology of individuals: incidence and implications of individual specialization. *Am Nat*. 2003;161:1–28.
- [66] Rosenblatt AE, Heithaus MR. Does variation in movement tactics and trophic interactions among American alligators create habitat linkages? *J Anim Ecol*. 2011;80:786–798.
- [67] Wheatley PV, Peckham H, Newsome SD, Koch PL. Estimating marine resource use by the American crocodile *Crocodylus acutus* in southern Florida, USA. *Mar Ecol Prog Ser*. 2012;447:211–229.
- [68] Grigg GC, Beard LA, Moulton T, Mello MTQ, Taplin LE. Osmoregulation by the broad-snouted Caiman, *Caiman latirostris*, in estuarine habitat in southern Brazil. *J Comp Physiol B*. 1998;168:445–452.
- [69] Lang J. Crocodilian behavior: implications for management. In: Webb GJW, Manolis SC, Whitehead, PJ, editors. *Wildlife management: crocodiles and alligators*. Chipping Norton, Australia: Surrey Beatty & Sons; 1987. p. 273–294.
- [70] Verdade LM. Cranial sexual dimorphism in captive adult broad-snouted caiman (*Caiman latirostris*). *Amphibia-Reptilia*. 2003;24:92–99.
- [71] Piña CI, Larriera A, Siroski P, Verdade LM. Cranial sexual discrimination in hatchling broad-snouted caiman (*Caiman latirostris*). *Iheringia*. 2007;97:17–20.
- [72] Hedrick AV, Temeles EJ. The evolution of sexual dimorphism in animals: hypotheses and tests. *Trends Ecol Evol*. 1989;4:136–138.
- [73] Zucoloto RB, Verdade LM, Coutinho LL. DNA microsatellite library for the broad-snouted caiman (*Caiman latirostris*). *J Exp Zool*. 2002;294:346–351.
- [74] Zucoloto RB, Villela PMS, Verdade LM, Coutinho LL. Interspecific transference of microsatellite markers in South American Caimans (*Caiman* spp and *Paleosuchus palpebrosus*). *Genet Mol Biol*. 2006;29:75–78.
- [75] Zucoloto RB, Verdade LM, Villela PMS, Regitano L.C.A, Coutinho L.L. Parentage test in broad-snouted caimans (*Caiman latirostris*, Alligatoridae) using microsatellite DNA. *Genet Mol Biol*. 2009;32:874–881.
- [76] Montini JP, Piña CI, Larriera A, Siroski P, Verdade LM. The relationship between nesting habitat and hatching success in Caiman latirostris (Crocodylia, Alligatoridae). *Phyllomedusa*. 2006;5:91–96.
- [77] Piña CI, Siroski P, Larriera A, Lance VA, Verdade LM. The temperature-sensitive period (TSP) during incubation of broad-snouted caiman (*Caiman latirostris*) eggs. *Amphibia-Reptilia*. 2007;28:123–128.
- [78] Sarkis-Gonçalves F, Castro AMV, Verdade LM. Uso de descartes de origem animal e ganho de peso de jacaré-de-papo-amarelo (*Caiman latirostris*) em cativeiro. *Sci Agric*. 2002;59:243–250.
- [79] Abercrombie C, Verdade LM. A análise de crescimento em crocodilianos. In: Verdade LM, Larriera A, editors. *La Conservación y Manejo de los Crocodylia de America Latina*. Piracicaba: CN Editoria; 2002. p. 1–20.
- [80] Magnusson WE. Dispersal of hatchling crocodiles (*Crocodylus porosus*) (Reptilia: Crocodylidae). *J Herpetol*. 1979;13:227–231.
- [81] Ferraz SFB, Vilaça AM, Vettorazzi CA, Gerhard P, Ferraz KMPMB, Verdade LM. Uso de imagens CBERS2 (sensor CCD) para identificação de habitats aquáticos de crocodilianos no reservatório da usina Luiz Eduardo Magalhães, Tocantins. In: *Anais do XII Simpósio Brasileiro de Sensoriamento Remoto*; 2005 Ab. 16–21; Goiania, Brasil. p. 937–944.
- [82] Jenkins SG, Partridge ST, Stephenson TR, Farley SD, Robbins CT. Nitrogen and carbon isotope fractionation between mothers, neonates, and nursing offspring. *Oecologia*. 2001;129:336–341.
- [83] Pilgrim MA. Expression of maternal isotopes in offspring: implications for interpreting ontogenetic shifts in isotopic composition of consumers. *Isotopes Environ Health Stud*. 2007;43:155–166.
- [84] Frankel NS, Vander Zanden HB, Reich KJ, Williams KL, Bjorndal KA. Mother-offspring stable isotope discrimination in loggerhead sea turtles *Caretta caretta*. *Endang Spec Res*. 2012;17:133–138.
- [85] Woodborne S, Huchzermeyer KDA, Govender D, Pienaar DJ, Hall LG, Myburgh JG, Deacon AR, Venter J, Lubcker N. Ecosystem change and the Olifants River crocodile mass mortality events. *Ecosphere*. 2012;3:1–17.
- [86] Hénaux V, Powell LA, Hobson KA, Nielsen CK, LaRue MA. Tracking large carnivore dispersal using isotopic clues in claws: an application to cougars across the Great Plains. *Meth Ecol Evol*. 2011;2:489–499.
- [87] Rosenblatt AE, Heithaus MR. Slow isotope turnover rates and low discrimination values in the American alligator: implications for interpretation of ectotherm stable isotope data. *Physiol Biochem Zool*. 2013;86:137–148.
- [88] Caut S. Isotope incorporation in broad-snouted caimans (crocodilians). *Biology Open*. 2013;2:629–634.