CROCODILE SPECIALIST GROUP – CSG/IUCN/SSC
Student Research Assistance Scheme (SRAS)

Final Report

Population structure, space use and trophic ecology of broad-snouted caiman (*Caiman latirostris*) in silvicultural landscape

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1. REPORT PRESENTATION

This report presents the main results from the study "Population structure, space use and trophic ecology of broad-snouted caiman (*Caiman latirostris*) in silvicultural landscape" that received Student Research Assistance Scheme (SARS) from the Crocodile Specialist Group (CSG/IUCN /SSC). Further discussion in relation to these data can be found in the doctoral thesis of Thiago S. Marques or in the following scientific papers:

a) Thesis

Marques TS. 2013. Population structure, space use and trophic ecology of broad-snouted caiman (*Caiman latirostris*) in silvicultural landscape. Doctoral thesis, Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, Brazil.

b) Articles

Marques TS, Lara NRF, Bassetti LAB, Piña CI, Camargo PB, Verdade LM. 2013. Intraspecific isotopic niche variation in broad-snouted caiman (*Caiman latirostris*). Isotopes in Environmental and Health Studies 49: 325-335.

Marques TS, Bassetti LAB, Lara NRF, Araujo MS, Piña CI, Camargo PB, Verdade LM. In press. Isotopic discrimination factors ($\Delta^{13}C$ and $\Delta^{15}N$) between tissues and diet of the Broad-snouted Caiman (*Caiman latirostris*). Journal of Herpetology.

c) Manuscripts

Marques TS, Bassetti LAB, Lara NRF, Millan CH, Piña CI, Verdade LM. Population structure of the broad-snouted caiman (*Caiman latirostris*) in a silvicultural landscape. Zoological Studies (SUBMITTED).

2. INTRODUCTION

The broad-snouted caiman (*Caiman latirostris*) is a medium-sized crocodilian, with rare individuals longer than 2 meters in nature (VERDADE, 1998). This species exhibits a broad latitudinal distribution (5-34 °S) in South America, including Argentina, Bolivia, Brazil, Paraguay and Uruguay (VERDADE; PIÑA, 2006; VERDADE et al. 2010).

The broad geographical distribution of the species may be a result of the ability to use various habitat types, such as rivers, lakes, wetlands and floodplains (MEDEM, 1983; VERDADE; PIÑA, 2007). However, the species faced complex conservation issues during last century (VERDADE et al. 2010). Water bodies originally occupied by the species have suffered extreme pressure as a result of anthropogenic pollution from urban sewage, industrial waste and agrochemicals (SCOTT et al. 1990; MECHE et al. 2009; PIÑA et al. 2009). For this reason, the species was considered endangered in the past (GROOMBRIDGE 1987; BERNARDES et al. 1990). However, currently the broad-snouted caiman is no longer considered as threatened (MMA, 2008; IUCN, 2014) due to its high adaptive capacity to colonize man-made habits such as decanting pounds and artificial reservoirs for agriculture or livestock (VERDADE; LAVORENTI, 1990; BORTEIRO et al. 2001, 2005, 2008). Apparently, these anthropic environments provide a stable water supply throughout the year, decreasing the effect of a lack of precipitation on reproduction (SIMONCINI et al. 2011).

Recently, the action plan for the broad-snouted caiman of Crocodile Specialist Group (IUCN - International Union for Conservation of Nature) has identified priority conservation actions to be developed with the species (VERDADE et al. 2010). The recommendations highlight the need for studies about distribution and status of populations throughout its geographical range, as well as researches focusing their ability to colonize man-made environments in response to the destruction of their natural habitats.

In this context, the main goals of this study were to determine the population structure, space use, and niche variation of broad-snouted caiman in silvicultural landscape in the state of São Paulo. In addition, we also determined the isotopic discrimination factors for the species in controlled environment.

2. MATERIALS AND METHODS

2.1 Study area

This study was conducted at two silvicultural farms, Três Lagoas (23°22'0'' - 23°20'41'' S/48°28'0'' - 48°27'57'' W) and Arca (23°20'0'' - 23°18'51'' S/48°27'30'' - 48°28'20'' W), located in the municipality of Angatuba, south-central São Paulo state, Brazil. These farms include ponds, streams and several small artificial reservoirs originally built to supply water for the cattle, but currently maintained as part of the fire control program of the *Eucalyptus* production. Crocodilians were captured in five water bodies: Lagoão, Lagoa Suja, Três Lagoas, Açude Vermelho and Açude do Casemiro (Figure 1).

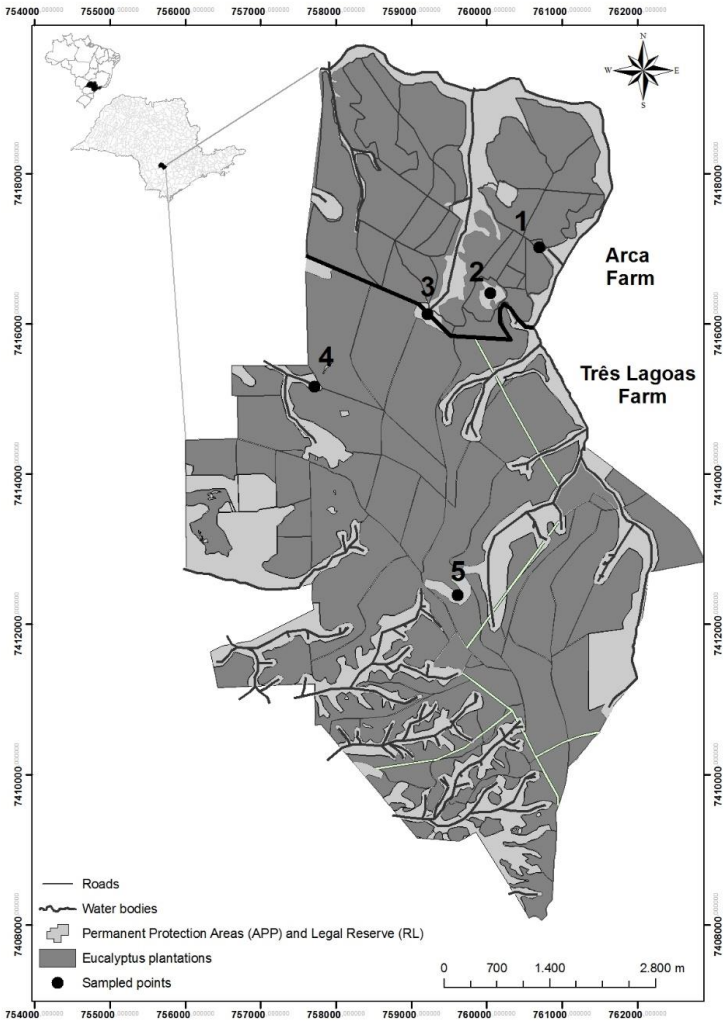


Figure 1. Study Area (Três Lagoas and Arca farms) localized in Angatuba, São Paulo state, Brazil, showing water bodies where individuals of *Caiman latirostris* were captured (1 - Açude Vermelho; 2 – Lagoão; 3 - Lagoa Suja; 4 - Açude do Casemiro; 5 - Três Lagoas).

2.2 Population structure

Caimans were captured during three days/nights each month from February 2010 to June 2010 and from October 2010 to April 2011 by boat with the aid of steel cable snares following detection with sealed-beam headlights (HUTTON et al. 1987). If the water body was small, the animals were attracted by an observer imitating hatchlings' vocalization and were captured by hand near the shore (WALSH, 1987). In addition, baited traps (three iron hoops, 1 meter in diameter with a 6 cm mesh size) were placed in the water bodies near the shore. The animals were snared or captured manually and physically restrained without the use of chemical immobilization. Total length of each individual was measured with a tape (1-mm precision).

The captured animals were individually marked with microchips (Destron, TX1414B) and by notching mark (by cut single and double external tail scutes using previously established numerical combinations). Sex determination was based on the visual examination of genital morphology with the aid of a speculum. Biometric measurements (total length and snout-vent length) were taken with a measuring tape, and body mass was recorded with a dynamometer.

Size classes proposed by LARRIERA et al. (2006) were adapted to snout-vent length measures, according to regression equations established for the species by Verdade (2000), as follows: class I (hatchlings) < 25 cm, class II (juveniles) 25 - 67.9 cm, class III (adults) 68 - 101 cm and class IV (large males) > 102 cm.

2.3 Space use

Telemetry Solutions transmitters (Q4000 Enhanced EXT memory_LS26500 SIRFIII, 85 mm, 50 mm, 54 mm, 257 g) were fixed at tail base of the eight adult animals (4 males and 4 females) with subcutaneous passage of flexible steel wire and with acrylic resin. Crocodilians are ectothermic vertebrates that depend on the external temperature to get body heat. Due to the influence of external temperature on animal movements two sampling periods were considered: spring/summer (September to April) and autumn/winter (May-August). The transmitters have been programmed to record the

crocodiles locations three times a day in spring/summer and one point per day in autumn/winter. The total monitoring period was from February 2010 to October 2011.

Home range was estimated by minimum convex polygon (MCP; convex polygon with the union of the outermost external points of the locations distribution; MOHR, 1947; FULLER et al., 2006) and kernel 95% method (K95; grids contoured probabilities based kernels; WORTON, 1987, 1989). The size and number of activity centers (AC) (HAYNE, 1949) were determined by the method kernel 50%. Spatial analyzes of the data were performed using *Animal Movement* and *Hawth's Analysis Tools* extensions of the ArcView 3.2 and ArcGIS 9.

Analyses of normality and variance homogeneity were performed previously in order to guide the choice of parametric or nonparametric tests. Home range analyses were done in two ways: considering the estimates of all individuals and removing an outlier (148.180 individual). Possible sex differences in the home range (MPC and K95) were verified by the Mann -Whitney test considering all individuals and by t-test removing the outlier.

2.3 Niche variation

Claw fragments (the terminal 0.5 cm) were collected from all of the captured animals to analyze stable carbon and nitrogen isotopes. The claw samples dried at 50 °C to a constant mass, and homogenized 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 analyzer (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* (CENA), of the *Universidade de São Paulo* (USP), in Piracicaba, São Paulo, Brazil. The calculation of the isotopic composition of carbon and nitrogen was based on the following equation:

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

where R denotes the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ molar ratio in the sample and standard. The results are presented in delta (δ) per mil (‰). The international standards used for nitrogen and carbon were atmospheric N_2 (Air) and Vienna Peedee Belemnite (VPDB), respectively.

The ontogenetic niche was evaluated according to the hypothesis-testing framework proposed by HAMMERSCHLAG-PEYER et al. (2011) 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.

We used the Bayesian standard ellipse metric (SEA_B) to measure the niche widths to ensure robustness with regard to the variation in sample size (JACKSON et al. 2011). 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 SEA than the other. Details of the calculations can be found in Jackson et al. (2011). The metrics were calculated using Stable Isotope Analysis in the R package (SIAR – PARNELL et al. 2010) for R (R Core Team).

The niche position was evaluated by a test of central tendency that was based on the Euclidean distance between the groups' centroids (TURNER et al. 2010). Nested linear models and residual permutation procedures were generated, and the measurements (Euclidean distance) were compared between the groups (TURNER et al. 2010). The tests were conducted using R software (R Core Team) following the script provided by TURNER et al. (2010).

The question of whether isotopic compositions ($\delta^{15}N$ and $\delta^{13}C$) 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 (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 analyzed prior to the application of the statistical analyses, which were performed with Minitab 16.

2.4 Discrimination factor

Eighteen female broad-snouted caimans from a commercial breeding facility were maintained on the same controlled diet (ground chicken necks) 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 m (3.5 m water surface and 1.5 m dried), deep pool: 1 m] 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 (71–104 cm) and the mean total length for age class 3–4 yr was 107 ± 6 cm (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).

Claw and scute's 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's samples (2 cm^2) were collected from the tail of each caiman with the use of surgical scissors.

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 as described in the previous section.

The discrimination factor was determined by the equation D(p/s) = d(p) - d(s), where D (p/s) = discrimination factor per mil, d(p) = isotope ratio per mil of the animal tissues and d(s) = isotope ratio per mil of the diet. We performed a two-way ANOVA to detect possible differences involving $\delta^{13}C$ and $\delta^{15}N$ 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.

3. RESULTS

3.1 Population structure

We captured 52 individual broad-snouted caiman in the study area, 16 of class I (30.8%), 28 of class II (53.8%; ♀: 7; ♂: 21) and 8 of class III (15.4%; ♀: 4; ♂: 4). No individuals of class IV were captured. The number of individuals captured varied markedly among water bodies: 27 individuals (51.9%) in Lagoa Suja, 22 (42.4%) in Lagoão, 1 (1.9%) in Três Lagoas, 1 (1.9%) in Açude Vermelho and 1 (1.9%) in Açude do Cazemiro (Figure 2).

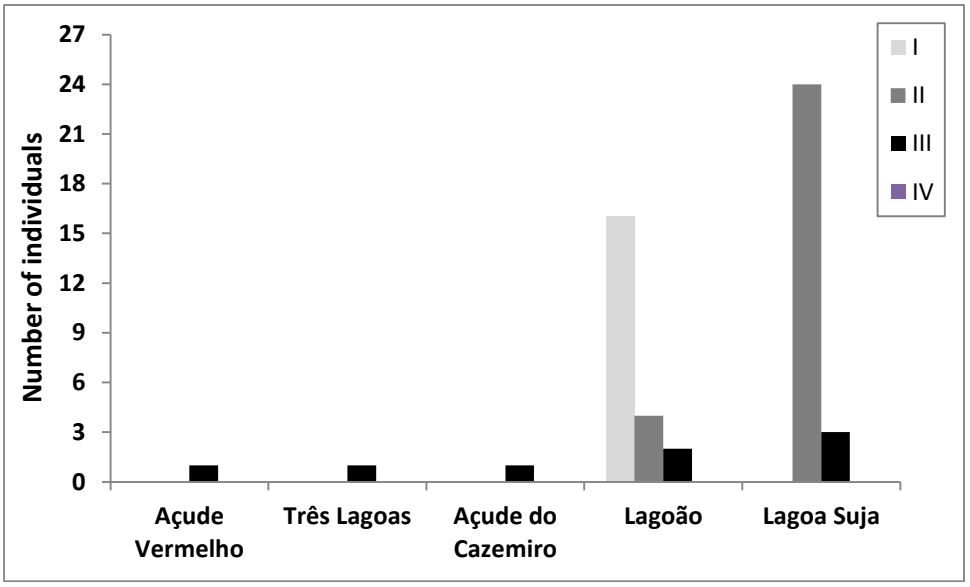


Figure 2. Number of broad-snouted caiman captured by size-class and water body (I hatchlings: < 25.0 cm, II juveniles: 25.0 - 67.9 cm, III adults: 68.0 - 101.0 cm and IV large males: > 102.0 cm snout-vent length) in the present study.

3.2 Space use

Home range mean according MPC and K95 estimated for species was 96.6 ± 183.9 ha (7.2 - 548.5 ha) and 43.2 ± 78.6 ha (2.8 - 236.2 ha), respectively (Table 1, Figure 3 and 4). The home range of females and males was similar for both MPC [$w_7 = 20$, $p = 0.665$; ♀: 32.4 ± 17.5 ha (7.2 - 43.5 ha); ♂: 160.8 ± 260.0 ha (10.8 - 548.5 ha)] and K95 [$w_7 = 19$, $p = 0.885$; ♀: 17.9 ± 13.7 ha (2.8 - 35.8 ha); ♂: 68.5 ± 112.0 ha (6.2 - 236.2 ha)].

Home range average removing the outlier (individual 148.180) according to MPC and K95 were 32.0 ± 23.8 ha (7.1 - 72.2 ha) and 15.6 ± 11.0 ha (2.8 - 35.7 ha), respectively. The home range of females and males removing the outlier were also similar for both MPC [$t_6 = 0.04$, $p = 0.973$; ♀: 32.4 ± 17.6 ha (7.1 - 46.4 ha), ♂: 31.6 ± 35.2 ha (10.8 - 72.2 ha)] and K95 [$t_6 = 0.64$, $p = 0.554$; ♀: 17.8 ± 13.6 ha (2.8 - 35.7 ha); ♂: 12.5 ± 7.8 ha (6.1 - 21.3 ha)].

Table 1 - Frequency VHF (Hz), sex (F: female, M: male), snout-vent length (SVL, cm), body mass (kg), number of points, home range calculated by the minimum convex polygon method (MPC), Kernel 95%, number of activity centers (AC), area of activity centers (ha) and monitoring period of each individual *Caiman latirostris* in landscape silvicultural

Freq. VHF (Hz)	Sex	SVL (cm)	Mass (kg)	N° points	MCP (ha)	Kernel 95% (ha)	N° AC	Area AC (ha)	Period
148.240	F	72	8.75	187	46.5	35.8	2	9.1	02/2010 – 08/2010 01/2011 – 07/2011
148.200	F	76	14.75	79	7.2	2.8	1	0.4	03/2010 – 06/2010
148.320	F	74.5	11.00	195	41.9	14.0	2	2.8	03/2010 – 12/2010
148.300	F	84.5	15.50	214	34.2	18.9	1	2.8	04/2010 -01/2011
148.280	M	98	27.00	134	11.8	6.2	2	0.5	04/2010 – 10/2010
148.260	M	80	15.00	124	72.2	21.4	1	4.0	11/2010- 02/2011
148.220	M	80	14.00	148	10.8	10.2	2	0.8	12/2010 - 06/2011
148.180	M	93	26.00	295	548.5	236.2	3	82.5	12/2010 – 09/2011

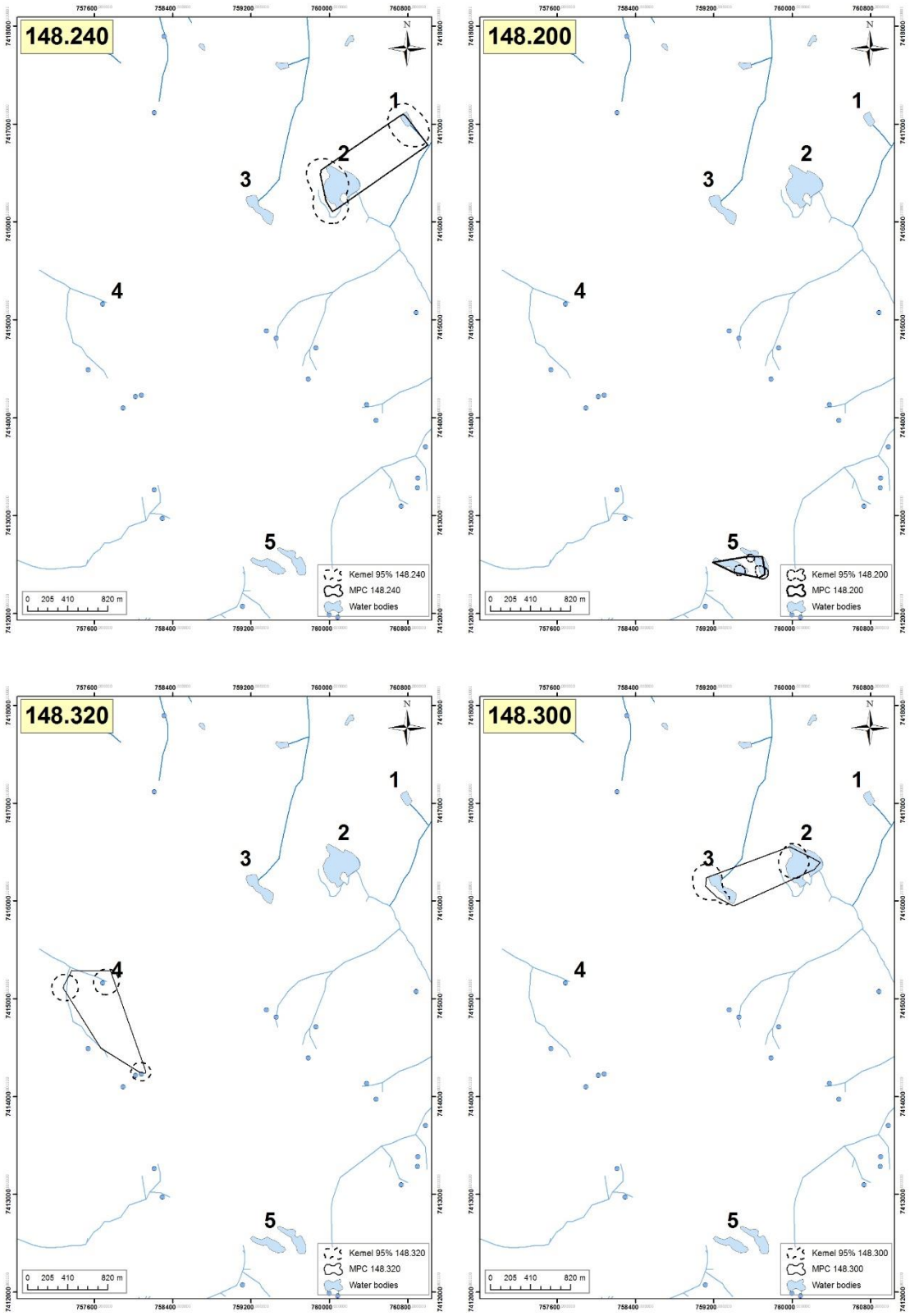


Figure 3. Home range calculated by minimum convex polygon method (MCP) and Kernel 95% (K95) for females *Caiman latirostris* in silvicultural landscape (Datum: SAD 69 - UTM). The numbers indicate the capture locations of individuals: 1- Açude Vermelho, 2- Lagoão, 3- Lagoa Suja, 4- Açude do Casemiro e 5- Três Lagoas

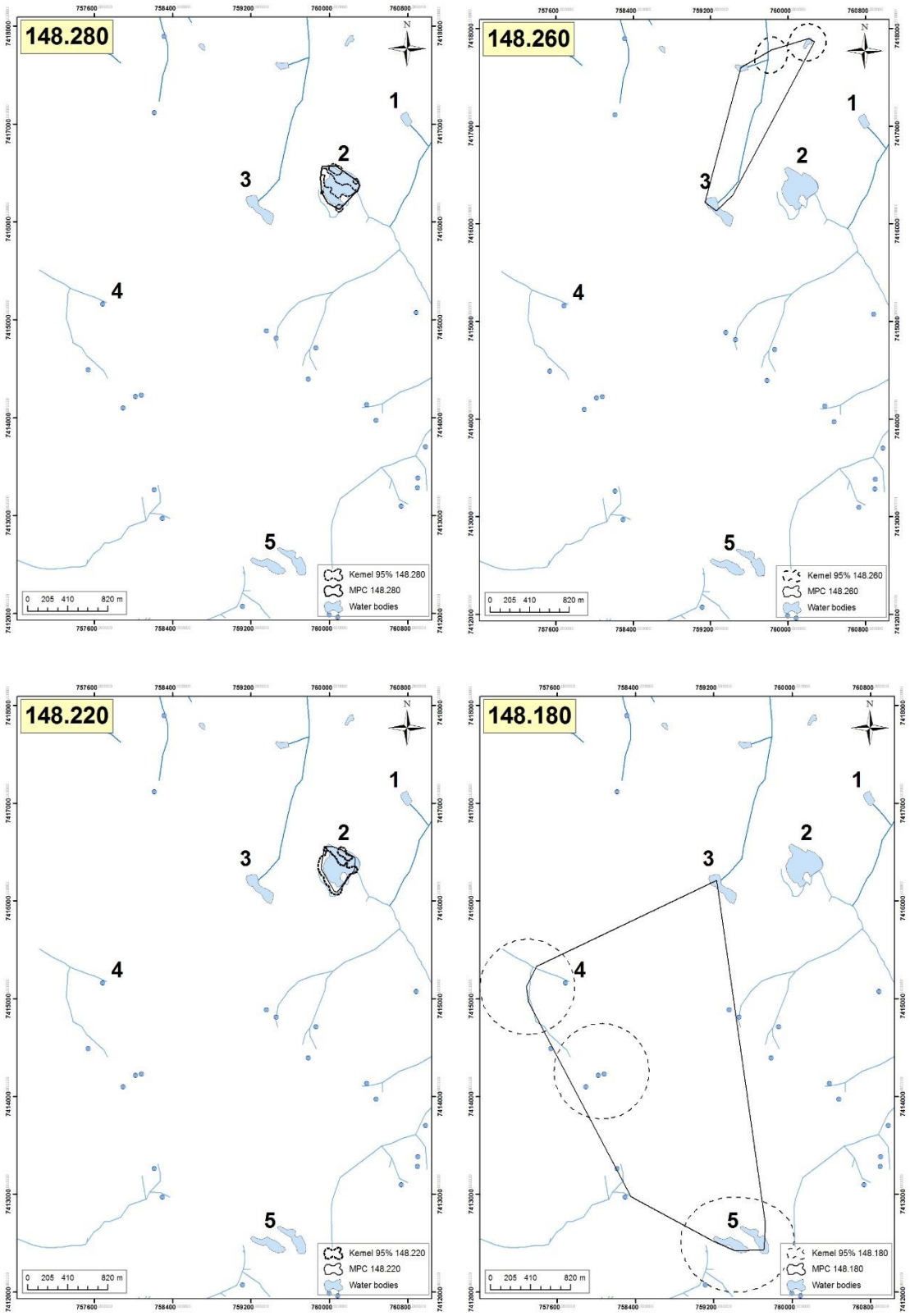


Figure 4. Home range calculated by minimum convex polygon method (MCP) and Kernel 95% (K95) for males *Caiman latirostris* in silvicultural landscape (Datum: SAD 69 - UTM). The numbers indicate the capture locations of individuals: 1- Açude Vermelho, 2- Lagoão, 3- Lagoa Suja, 4- Açude do Casemiro e 5- Três Lagoas

3.3 Niche variation

Discrete niche variation between age classes was found using the framework of hypothesis testing proposed by HAMMERSCHLAG-PEYER et al. (2011). The niche widths calculated for each age class by SEA_B using Bayesian inference were statistically similar (juveniles - mean: 2.89 ‰^{2}, median: 2.81 ‰^{2}, 95% Cr.I.: 1.85 to 4.11 ‰^{2}; adults - mean: 2.63 ‰^{2}, median: 2.43 ‰^{2}, 95% Cr.I.: 1.22 to 4.73 ‰^{2}; p = 0.631; Figure 5). However, the positions of the isotopic niches were significantly different (Euclidean distance = 5.75; p = 0.001), with no niche overlap between the two age classes (Figure 6).

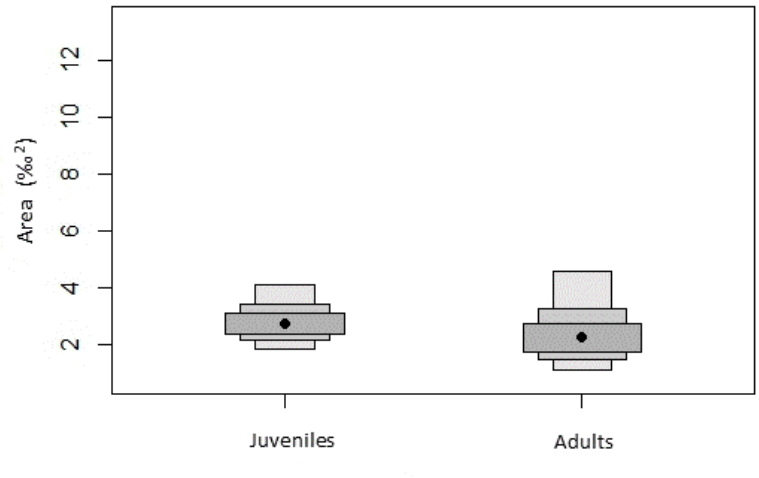


Figure 5. Similarity between estimates of Bayesian standard ellipses (SEA_B) area in juveniles and adults of broad-snouted caiman (*Caiman latirostris*) inhabiting silvicultural areas in southeastern Brazil. The black dots correspond to the mean SEA_B area for each group, while shaded boxes representing the 50%, 75% and 95% credible intervals from dark to lightgrey.

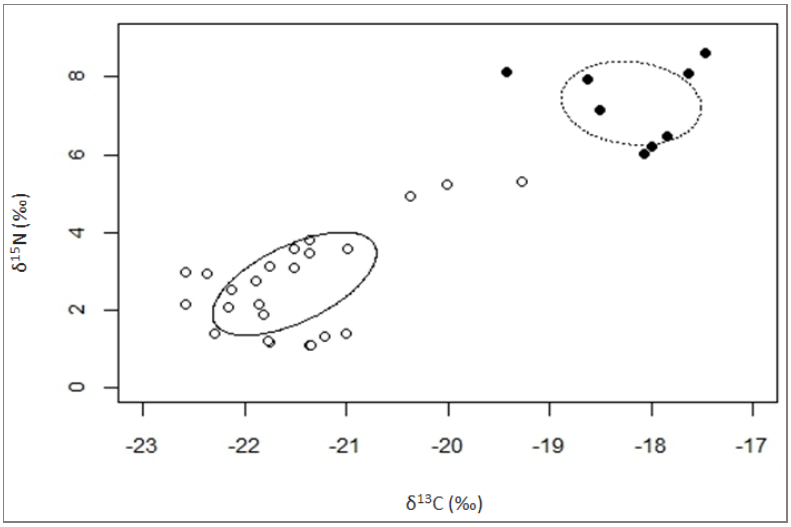


Figure 6. Spatial divergence of standard ellipse between juveniles (white balls) and adults (black balls) of broad-snouted caiman (*Caiman latirostris*) in $\delta^{15}N$ - $\delta^{13}C$ space.

The stable isotope compositions were significantly different among the age classes (MANOVA: $F_{1,31}$ = 66.0; $p < 0.001$), and separate ANOVA with Bonferroni correction indicated that both $\delta^{15}N$ and $\delta^{13}C$ were different among the age classes ($\delta^{15}N$: $F_{1,31}$ = 85.2; $p < 0.001$; $\delta^{13}C$: $F_{1,31}$ = 115.3; $p < 0.001$). A similar pattern was found for sex because the isotope compositions were different between sexes (MANOVA: $F_{1,31}$ = 25.5; $p < 0.001$), and ANOVA indicated that both $\delta^{15}N$ and $\delta^{13}C$ were different between sexes ($\delta^{15}N$: $F_{1,31}$ = 72.2; $p < 0.001$; $\delta^{13}C$: $F_{1,31}$ = 58.7; $p < 0.001$). There was no difference between the adult males and females, but there was a significant difference between sexes in juveniles (Table 2).

Table 2. Stable isotope compositions ($\delta^{15}N$ and $\delta^{13}C$) differ between age classes and sexes of broad-snouted caiman (*Caiman latirostris*) inhabiting silvicultural areas in southeastern Brazil as reveled by ANOVA and ANOM's tests (superscript letters indicate similarities or differences).

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

There was a significant relationship between $\delta^{15}N$ and SVL for juvenile males ($F_{1,16}$ = 40.4; $p < 0.001$; r^2 = 0.73) but not for females ($F_{1,6}$ = 0.2; p = 0.650; r^2 = 0.04; Figure 8). In addition, there was no significant relationship between $\delta^{13}C$ and SVL for juveniles (males: $F_{1,16}$ = 2.60; p = 0.124; r^2 = 0.09; females: $F_{1,6}$ = 1.60; p = 0.251; r^2 = 0.01; Figure 7). SVL in juveniles was found to be a significant covariant of sex to $\delta^{15}N$ (ANCOVA: $F_{1,23}$ = 19.90; $p < 0.001$) but not to $\delta^{13}C$ (ANCOVA: $F_{1,23}$ = 0.20; p = 0.649).

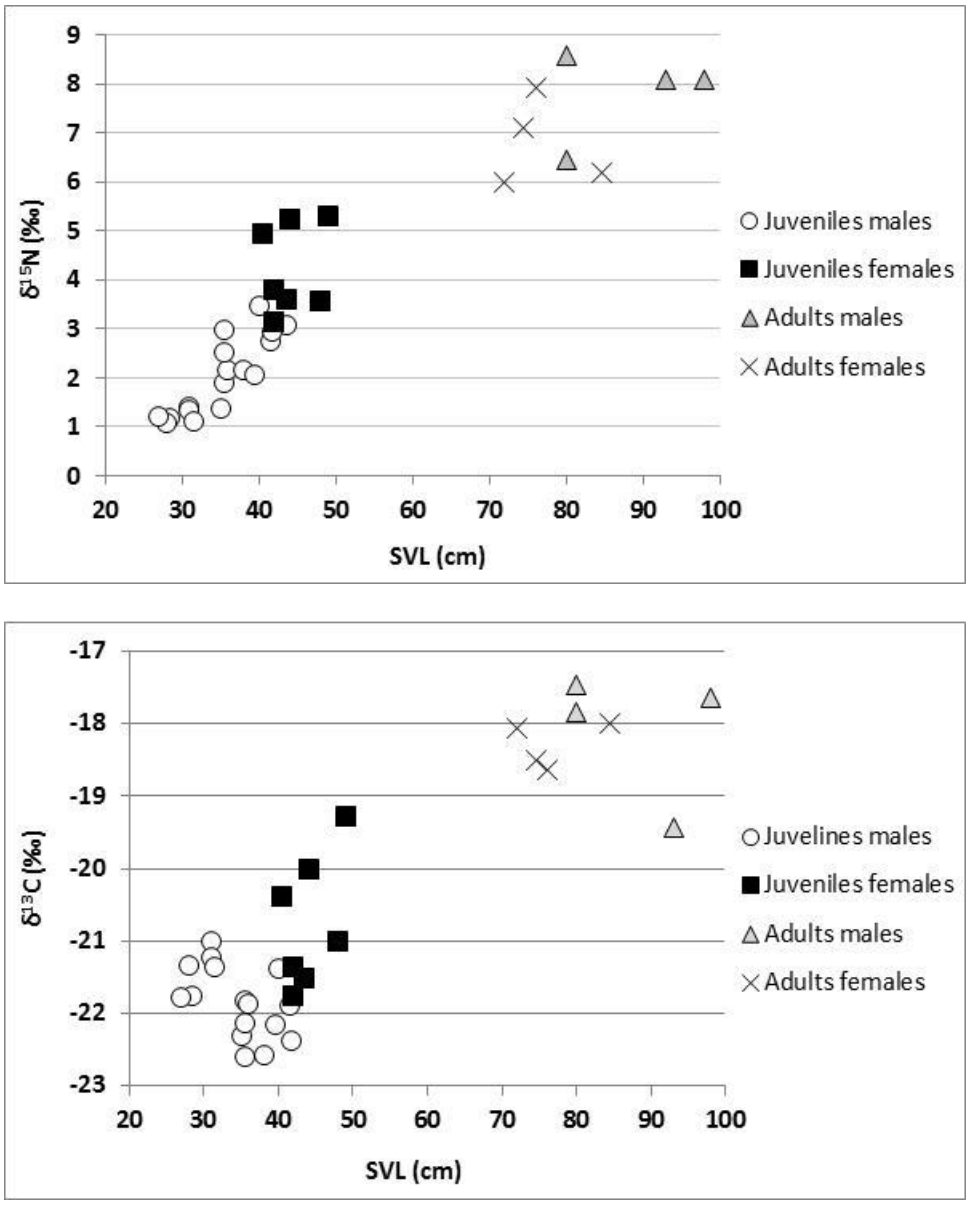


Figure 7. Progressive increase of stable isotope compositions ($\delta^{15}N$ and $\delta^{13}C$) in relationships of snout-vent length (SVL) in broad-snouted caiman (*Caiman latirostris*) in silvicultural areas in southeastern Brazil.

The mean $\delta^{15}N$ and $\delta^{13}C$ values for the hatchlings were 5.6 ± 0.1 ‰ (5.4 – 5.9 ‰) and -18.6 ± 0.1 ‰ (-18.8 – -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.

3.4 Discrimination factor

The discrimination factors between the tissues and diet $\Delta^{13}C$ values were 1.2 ± 0.1 ‰ (0.9 – 1.5‰) for claw and 0.9 ± 0.2 ‰ (0.6 – 1.4‰) for scute. The corresponding discrimination factors $\Delta^{15}N$ were 1.1 ± 0.1 ‰ (0.9 – 1.5‰) for claw and 0.9 ± 0.2 ‰ (0.5 – 1.4‰) for scute (Figure 8). There were minor differences in the $\Delta^{13}C$ values between tissues ($F_{1.35} = 29.4$, $p < 0.001$) and age classes ($F_{1.35} = 5.9$, $p = 0.020$), but no interaction between these factors was found ($F_{1.35} = 1.18$, $p = 0.28$). In addition, there were minor differences in the $\Delta^{15}N$ values between tissues ($F_{1.35} = 29.4$, $p < 0.001$) but not between age classes ($F_{1.35} = 0.7$, $p = 0.390$), nor was there interaction between factors ($F_{1.35} = 0.1$, $p = 0.670$).

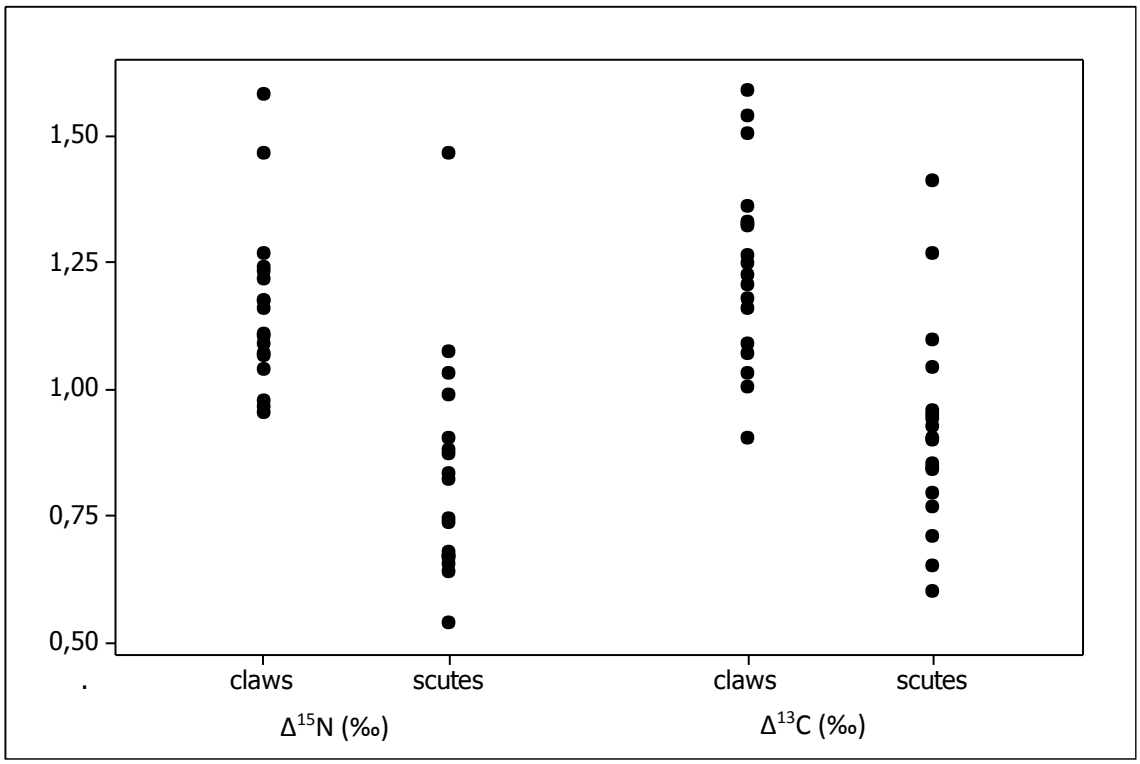


Figure 8. $\Delta^{13}C_{tissue\text{-}diet}$ and $\Delta^{15}N_{tissue\text{-}diet}$ of claw and scute tissues of *Caiman latirostris*.

4. USE OF RESOUCES

The funds of the Student Research Assistance Scheme at the Crocodile Specialist Group were fully used in the expenditures of field activities. The transmitters for monitoring

of crocodilians were acquired by a grant coordinated by Dr. Luciano M. Verdade (Biota Program/FAPESP Proc. No 2006/60954-4). Thiago S. Marques had scholarships from FAPESP (São Paulo Research Foundation; grant 2008/07076) and CNPq (National Council for Scientific and Technological Development; grant 142682/2011-0).

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