Summary Report "Dieta, condição corporal e bioquímica do sangue das fêmeas nidificantes de jacaré-tinga na Amazônia Central**"**

Introduction

Although nesting ecology is well studied in several crocodilian species, there is still little information regarding how aspects related to reproduction (e.g. nest location and attendance) may influence diet, physiology and body condition of nesting females during nesting season (Mazzotti et al., 2012). The spectacled caiman (*Caiman crocodilus*) is a mid-size crocodilian that builds its nests either along the margins of lakes and canals or inside the forest, sometimes at distances of hundreds of meters from a permanent water body (Da Silveira *et al*., 2010; Villamarín *et al*., 2011). Nesting female *C. crocodilus* (Figure 1) usually hide near the nest in an almost comatose state, frequently under leaf litter, or in the debris of fallen trees (Marioni *et al*., 2007). This study addressed if nest attendance influences dietary composition, body condition and physiological state of nesting females of *C*. *crocodilus* in Central Amazonia.

Methods

We carried out the study between November and December of 2012 in the várzea forests of the Piagaçu-Purus Sustainable Development Reserve (PP-SDR)PP-SDR, which is located between the Purus and Madeira Rivers (04º05'S, 61º44'W and 5º20'S, 63º20'W), approximately 350 km southwest of Manaus, Amazonas State, Brazil. This period coincides with the annual dry season, when water levels are at their lowest and when caimans are nesting (Marioni *et al*., 2007).

Nesting females were captured and physically restrained using a pole-snare (Ketch-All Animal Restraining Pole), ropes and tapes (Da Silveira *et al*., 1999). Mass was determined and captured females were measured (snout-vent length - SVL) with a metric scale (± 0.1 mm) and had their stomach content examined by stomach flushing (Da Silveira *et al*., 1999). Blood samples were collected immediately after physical immobilization, within a maximum period of 15 minutes (Guillette et al., 1997), just before the measuring and stomach flushing procedures described above (Figure 1). Approximately 5 mL of blood was

withdrawn from the supravertebral sinus (Sykes and Klaphake, 2008) into a sterile syringe using a 22G x 1" needle (0.7 x 25 mm).

During the same period, nocturnal surveys were conducted from an aluminium boat with a 15 HP motor in deep-water canals with permanent water flow, locally known as Paranãs, in order to find non-nesting females that were of similar sizes to those at nests (Thorbjarnarson, 1994). Non-nesting females were captured using a pole with a break-away nose (Figure 2A), physically restrained using ropes and tapes (Da Silveira & Magnusson, 1999), measured (SVL), weighed, and had their stomach contents examined and blood withdrawn (Figure 2B) as described above for nesting females. Adult males of similar size ($60 \leq SVL \leq 90$) were also captured to add an additional control group in the comparison between nesting and non-nesting females, but did not have their stomach contents examined. All caimans (including nesting females) were released within a maximum period of 30 minutes after capture.

Caiman body condition was estimated with Fulton's K body condition factor, according to the following equation: $K = (\text{body mass}/SVL^{b}) \times 10^{n}$, where $b = 3$ and $n = 5$ (Zweig et al., 2014). All blood samples were processed immediately upon our arrival to the field research facility (Figure 2C). Plasmatic glucose, triglycerides and lactate concentrations were determined in Cobas Mira Plus® automated system using specific commercial kits of colorimetric reaction (Glucose liquiform, Triglycerides liquiform, Lactate liquiform and Uric Acid liquiform). Non-metric multidimensional scaling ordination (NMDS) was used to test for differences in dietary composition between nesting and non-nesting females (Horna *et al*. 2001).

Results

Morphological measurements were obtained for 49 nesting females, 16 non-nesting females and 27 adult males. Fulton's "K" body condition was significant different between nesting females, non-nesting females and adult males ($F = 18.63$; $P < 0.001$), but not between non-nesting individuals ($P = 0.15$; Figure 1A). Glucose ($F = 34.99$; $P > 0.001$), triglycerides (Krustal-Wallis Test Statistic = 24.42; $P > 0.001$), lactate (Krustal-Wallis Test Statistic = 30.82; $P < 0.001$) and uric acid ($F = 14.84$; $P < 0.001$) were significantly different among C. *crocodilus* nesting and non-nesting individuals (Figures 3B, 1C, 1D). However, no

significant difference was found in all four blood parameters between non-nesting females and males (glucose: P = 0.72; triglycerides: P = 0.97; lactate: P = 0.84).

We obtained stomach contents of 33 nesting and 16 non-nesting females. About 24% of stomachs of nesting females were empty, while stomachs of all non-nesting females had at least one food item (U = 234.0; $p < 0.05$). Recently-ingested food items were found in only 39% of stomach contents of nesting females, but all non-nesting females had recently-ingested prey items in their stomachs (U = 84; $p < 0.05$). Diet composition of individual females was affected by nesting status (nesting or non-nesting; Figure 4) but not by female size (ANCOVA: $F_{1,1} = 22.449$; $p < 0.001$; $F_{1,40} = 0.001$; $p = 0.972$). Occurrence (O), percent occurrence (%O), frequency (N) and relative frequency (%N) of prey items found in stomach contents are shown in Table 1.

Discussion

This study represents the first eco-physiologically approach on nesting strategies of *Caiman crocodilus* and provides first evidence that nest attendance may impose a metabolic cost to nesting females. Fulton's "K" body condition factor enabled the quantification and comparison of energetic reserves and was able to show significant differences between caimans attending and not attending nests. The results from body condition analysis were supported by plasmatic concentrations of glucose, triglycerides, lactate, which were significantly lower in nesting females when compared to non-nesting females and adult males. These differences could be related to variation in diet between nesting and non-nesting caimans (Taylor, 1979; Santos et al., 1994) because correlation between high values of body condition scores and a diet dominated by fish has been found in *Alligator mississippiensis* (Delany *et al*., 1999). In RDS-PP, while fish represents the most important prey item for non-nesting females of *C*. *crocodilus*, the occurrence of this prey item in the stomach contents of nesting females was very low. Our results also demonstrate that nest even though nesting females may endure periods of food deprivation, associated with nest attendance, they may occasionally leave the nest unattended in order to feed. However, without data on movements of nesting females it is difficult to determine if nesting females undertake regular

journeys between nest and water in order to feed or to rehydrate (Coppo *et al*., 2006). Future studies should attempt to combine more detailed information on nesting female movements (e.g. radio-telemetry) with information on diet and body condition.

References

Coppo, J. A., Mussart, N.B., Barboza, N.M., Fioranelli, S.A., Koza, G.A. & Prado, W.S. (2006). Physiological variations of serum electrolytes (Na, K, Ca, P, Mg, Cu) in farm-housed *Caiman latirostris* and *Caiman yacare* (Crocodylia: Alligatoridae). *Analecta Veterinaria*, 26, 9-15.

Da Silveira, R. & Magnusson, W. E. (1999). Diet of spectacled caiman and black caiman in the Anavilhanas archipelago, Central Amazonia, Brazil. *Journal of Herpetology*, 33, 181-192.

Da Silveira, R., Ramalho, E., E. Thorbjarnarson, J. B. & Magnusson, W. E. (2010). Depredation by Jaguars on Caimans and importance of reptiles in the diet of Jaguar. *Journal of Herpetology*, 44, 418-424.

Delany, M. F. & Abercrombie, C. L. (1986). American Alligator food habits in north central Florida. *Journal of Wildlife Management*, 50, 348-353.

Guillette, L. J., Woodward, A. R., Crain, D. A., Masson, G. R., Palmer, B. D., Cox, M. C., You-Xiang, Q. & Orlando, E. F. (1997). The Reproductive cycle of the female American alligator (*Alligator mississippiensis*). *General and Comparative Endocrinology*, 108, 87-101.

Horna, J. V., Cintra, R. & Ruesta, P. V. (2001). Feeding ecology of black caiman *Melanosuchus niger* in a western Amazonian Forest: The effects of ontogeny and seasonality on diet composition. *Ecotropica*, 7, 1-11.

Marioni, B., Von Muhlen, E. M. & Da Silveira, R. (2007). Nesting of *Melanosuchus niger* and *Caiman crocodilus* in the Piagaçu-Purus Sustainable Development Reserve, Central Amazonia, Brazil. *Crocodile Specialist Group Newsletter*, 26, 8-9.

Mazzotti, F. J., Cherkiss, M. S., Brandt, L. A., Fujisaki, I., Hart, K., Jeffery, B., McTurry, S. T., Platt, S. G., Rainwater, T. R. & Vinci, J. (2012). Body condition of Morelet's crocodile (*Crocodylus moreletti*) from Northern Belize. *Journal of Herpetology,* 46, 356-362.

Santos, S. A., Nogueira, M. S., Pinheiro, M. S., Campos, Z., Magnusson, W. E. & Mourão, G. M. (1996). Diets of *Caiman crocodilus yacare* from different habitats in the brazilian Pantanal. *Herpetological Journal*, 6, 111-117.

Sykes IV, J. M. & Klaphake, E. (2008). Reptile Hematology. *Veterinary Clinics of North America,* 11, 481-500*.*

Thorbjarnarson, J. B. (1994). Reproductive ecology of the spectacled caiman (*Caiman crocodilus*) in the Venezuelan Llanos. *Copeia*, 1994, 907-919.

Villamarín, F., Marioni, B., Botero-Arias, R., Thorbjarnarson, J. B. & Magnusson, W. E. (2011). Conservation and management implications of nest-site selection of the sympatric crocodilians *Melanosuchus niger* and *Caiman crocodilus* in Central Amazonia, Brazil. *Biological Conservation*, 144, 913-919.

Zweig, C. L. Rice, K. G. Percival, F. & Mazzotti, F. J. (2014). Body condition factor analysis for the American alligator (*Alligator mississippiensis*)*. Herpetological Review*, 45:216-219.

Table 1. Occurrence (O), percent occurrence (%O), frequency (N) and relative frequency (%N) of prey items found in stomach contents of nesting and non-nesting females of *Caiman crocodilus* in PP-SDR. Prey items were identified to the lowest taxonomic level possible (see text for more information on lower classifications) and grouped into five prey categories: Terrestrial Invertebrates (TI), Aquatic Invertebrates (AI), Molluscs, Fish or Other Vertebrates (OV). Prey items were considered fresh if they had been recently ingested or had little evidence of active digestion (detailed information on the criteria can be found in the Material and Methods section).

			Nesting					Non-nesting				
			Occurrence		Frequency		Fresh?	Occurrence		Frequency		Fresh?
Category	Order	Family	O	%O	N	%N	---	O	%O	N	%N	---
AI	Decapoda	Trichodactylidae	2	0,06	2	0,02	Yes	1	0,06	1	0,01	Yes
AI	Decapoda	Palaemonidae	0	0	0	0	---	1	0,06	1	0,01	Yes
AI	Hemiptera	Belostomatidae	2	0,06	2	0,02	No	4	0,25	4	0,03	Yes
TI	Blattaria	Blaberidae	1	0,03	1	0,01	Yes	0	0	0	0	---
TI	Coleoptera	Carabidae	6	0,18	9	0,11	No	2	0,13	2	0,02	No
TI	Diptera	---	2	0,06	2	0,02	Yes	5	0,31	7	0,06	Yes
TI	Hemiptera	Cicadidae	1	0,03	3	0,04	Yes	4	0,25	20	0,16	Yes
TI	Hemiptera	Cicadellidae	0	0	0	0	---	1	0,06	1	0,01	Yes
TI	Hymenoptera	Formicidae	16	0,48	36	0,44	Yes	7	0,44	51	0,4	Yes
TI	Hymenoptera	Vespidae	2	0,06	2	0,02	Yes	2	0,13	2	0,02	Yes
TI	Isoptera	---	1	0,04	1	0,01	No	0	0	0	0	---
TI	Orthoptera	---	1	0,04	1	0,01	Yes	2	0,13	2	0,02	Yes
TI	Scolopendromorpha	---	1	0,03	1	0,01	Yes	0	0	0	0	---
Mollusc	Gastropoda	Ampullariidae	8	0,24	16	0,20	No	0	0	0	0	---
Fish	Beloniformes	Belonidae	0	0	0	0	---	1	0,06	1	0,01	Yes
Fish	Characiformes	Anostomidae	0	0	0	0	---	2	0,13	3	0,02	Yes
Fish	Characiformes	Erythrinidae	0	0	0	0	---	1	0,06	1	0,01	Yes
Fish	Characiformes	Characidae	0	0	0	0	---	1	0,06	1	0,01	Yes
Fish	Characiformes	Serrasalmidae	0	0	0	0	---	1	0,06	1	0,01	Yes
Fish	Characiformes	Unidentified	0	0	0	0	---	1	0,06	1	0,01	Yes
Fish	Osteoglossiformes	Osteoglossidae	0	0	0	0	---	1	0,06	1	0,01	No
Fish	Perciformes	Cichlidae	0	0	0	0	---	3	0,19	3	0,02	Yes
Fish	Siluriformes	Auchenipteridae	0	0	0	0	---	1	0,06	1	0,01	Yes
Fish	Siluriformes	Callichthyidae	0	0	0	0	---	6	0,38	6	0,05	Yes
Fish	Siluriformes	Loricaridae	0	0	0	0	---	2	0,13	2	0,02	Yes
Fish	Siluriformes	Pimelodidae	0	0	0	0	---	1	0,06	2	0,02	Yes
Fish	Synbranchiformes	Synbranchidae	0	0	0	0	---	1	0,06	3	0,02	Yes
Fish	Unidentified	---	1	0,04	1	0,01	No	1	0,06	2	0,02	No
OV	Squamata	Amphisbaenidae	0	0	0	0	---	1	0,06	1	0,01	Yes
OV	Unidentified reptile	---	1	0,04	1	0,01	No	1	0,06	1	0,01	No
OV	Primates	Atelidae/Cebidae	1	0,04	1	0,01	No	0	0	0	0	---
Empty	---	---	8	0,24	---	---		0	0	---	---	
Total	---	---	33	1	81	1		16	1	125	1	



Figure 1. Nesting female (A) and the blood sampling (B), stomach flushing (C) and measurement (D) procedures performed on *Caiman crocodilus* in PP-SDR.



Figure 2. Capturing (A) and blood sampling (B) a non-nesting female of *Caiman crocodilus* and processing the blood in the improvised laboratory in PP-SDR.

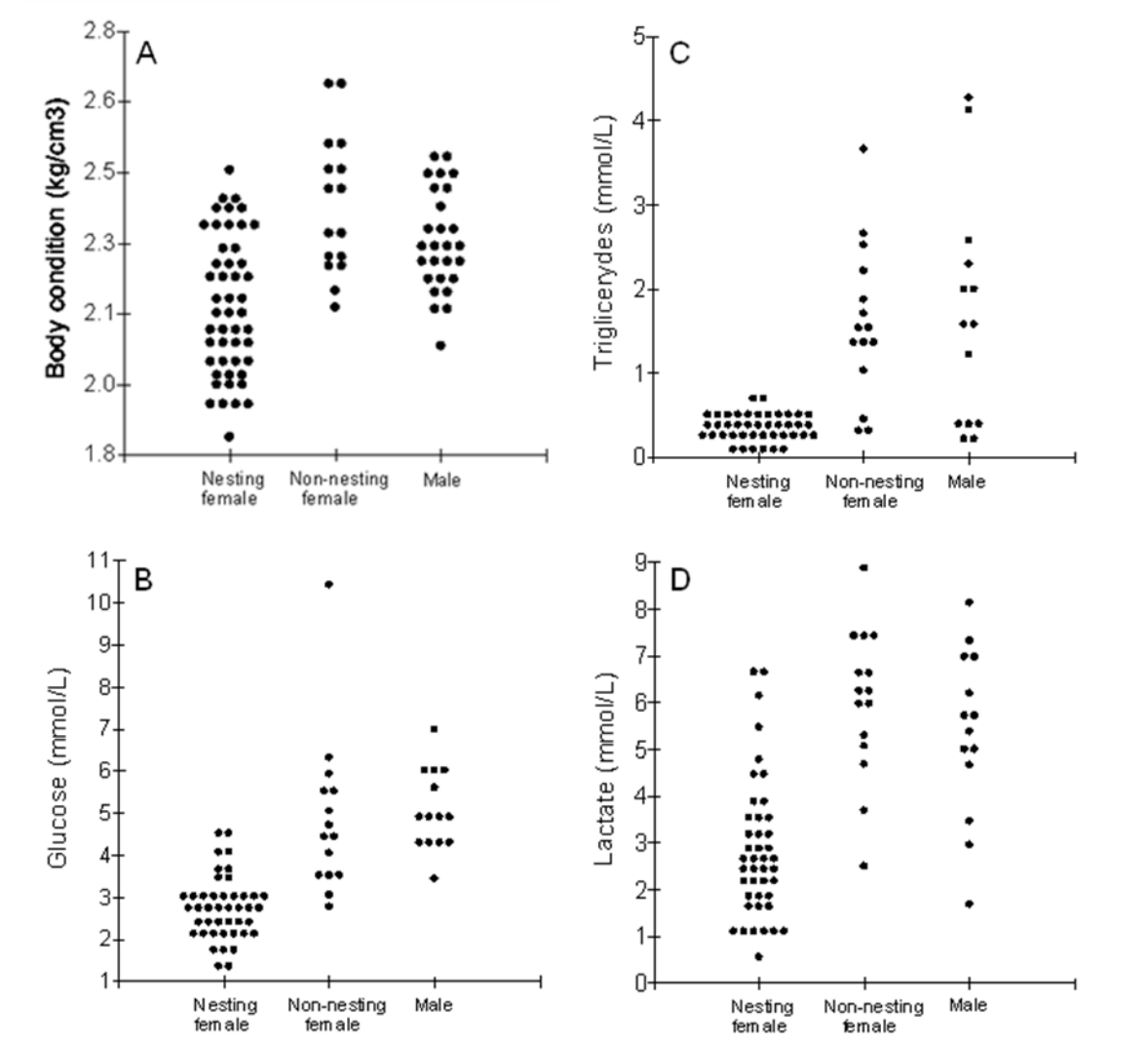


Figure 3. Body condition (A) and plasmatic concentrations of glucose (B), triglycerides (C) and lactate (D) of females captured near the nest (nesting females), females captured away from nests (non-nesting females) and males of *Caiman crocodilus* in PP-SDR. Each point represents a caiman.

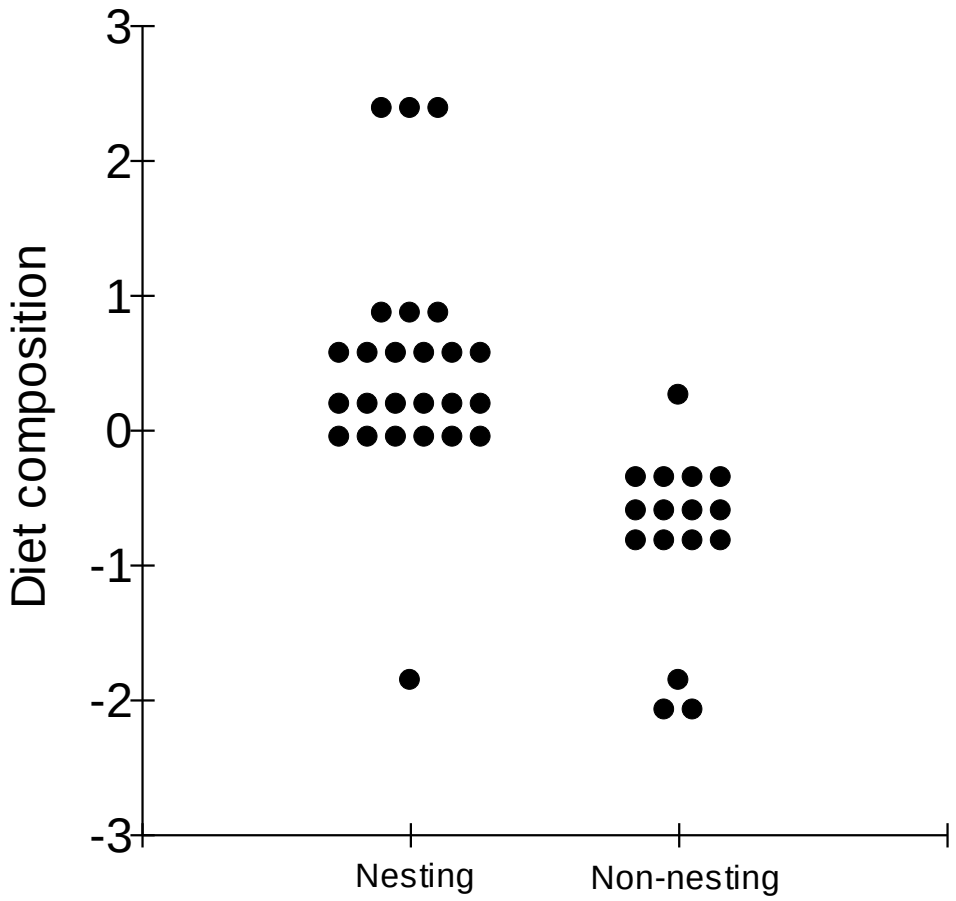


Figure 4. Diet composition (represented by score values derived from multidimensional scaling analysis) between nesting and non-nesting females of *Caiman crocodilus* in PP-SDR. Each point represents a female.