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Hormone Levels and Ultrasound Evaluation of *Caiman latirostris* (Crocodylia, Alligatoridae) Ovulation

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Abstract. Although there is much information available about reproduction in *Caiman latirostris,* knowledge related to steroid hormone levels and follicle development for wild adult females is still lacking. In this study we monitored and assessed the development of follicles and eggs and correlated these results with plasma steroid hormone levels in 32 adult females captured in Santa Fe, Argentina. Fieldwork was carried out over two reproductive seasons (October–January) between 2010 and 2012. Using an ultrasound device to take images of the reproductive structures of adult females, we observed individuals with vitellogenic follicles ($n = 5$), eggs ($n = 4$), atretic follicles ($n = 11$), and inactive reproductive structures ($n = 12$). We found no reproductive females smaller than 65 cm snout–vent length. High levels of estradiol were found during the ovulation period (November) only in reproductive females. Reproductive females showed no differences in progesterone levels during the study period (November–January) compared to non-reproductive females; however, reproductive females showed higher progesterone levels during nesting (December). We found no differences in progesterone levels between reproductive females and females with atretic follicles at the end of the nesting period (January). Ultrasound imaging was found to be an efficient technique to study reproductive structures at the beginning of reproductive cycle of the broad-snouted caiman. Isolated analyses of hormonal levels are not sufficient to determine the reproductive condition of *C. latirostris* females.

Keywords. Crocodilians; Radioimmunoassay (RIA); Reproduction; Reproductive hormones; Ultrasonography.

Resumo. Apesar do grande número de informações disponíveis sobre a biologia reprodutiva do *Caiman latirostris,* pouco se sabe sobre os níveis hormonais e o desenvolvimento de folículos e ovos para fêmeas adultas na natureza. No presente estudo, nós monitoramos e determinamos o desenvolvimento folicular e correlacionamos esses resultados com os valores dos níveis dos hormônios esteroides de 32 fêmeas adultas capturadas na província de Santa Fe, Argentina. O trabalho de campo foi realizado durante duas estações reprodutivas (outubro–janeiro) entre os anos de 2010 e 2012. Um ultrassom portátil foi utilizado para avaliar e obter imagens das estruturas reprodutivas das fêmeas, sendo possível observar folículos vitelogênicos ($n = 5$), ovos ($n = 4$), folículos atrésicos ($n = 11$) e ausência de estrutura reprodutiva ($n = 12$). Não foram encontradas fêmeas menores que 65 cm de comprimento rostro-cloacal com características que pudessem indicar sinais de reprodução. Os maiores valores de estradiol foram encontrados durante a ovulação (novembro) para as fêmeas reprodutivas. Não foram encontradas diferenças nos níveis de progesterona para as fêmeas reprodutivas durante o período estudado (novembro–janeiro), apesar de terem apresentado maiores valores durante a nidificação (dezembro) do que fêmeas não reprodutivas. Não encontramos diferenças nos níveis de progesterona entre fêmeas reprodutivas e fêmeas com folículos atrésicos no final do período de nidificação (janeiro). A ultrassonografia demonstrou ser uma técnica eficiente para o estudo das estruturas reprodutivas no começo do ciclo reprodutivo do jacaré-de-papo-amarelo. Somente análises dos níveis hormonais, de forma isolada, não são suficientes para determinar a condição reprodutiva das fêmeas de *C. latirostris.*

INTRODUCTION

In recent years some non-invasive methods, such as palpation, radiography, and ultrasound, have been used to evaluate aspects of the reproductive dynamics of female reptiles (Rostal et al., 1990; Martínez-Torres et al., 2006; Gilman and Wolf, 2007), including ovarian follicular development (Uribe et al., 1996). The use of ultrasound to study follicular development allows reproductive structures to be observed, revealing the female caiman reproductive condition without invasive procedures (Hildebrandt et al., 2000). Some studies have highlighted the efficiency of ultrasound to identify, measure, and follow the development of vitellogenic follicles and eggs in wild and captive crocodilians (Tucker and Limpus, 1997; Palacios and Beltrán, 2005; Lance et al., 2009), including Neotropical crocodilians (Vac et al., 1992; Coutinho, 2000; Palacios and Beltrán, 2005) such as the broad-snouted caiman, *Caiman latirostris* (Daudin, 1802).

Although there is much information available on *Caiman latirostris* reproduction in the wild and in captivity (Verdade and Piña, 2006), knowledge about egg development, hormonal fluctuation, and percentage of actively reproductive females per breeding season is still lacking.

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The only information about follicular development for this species was reported by Vac et al. (1992), who used ultrasound to study captive females and observed follicles with diameters of 1.0–1.5 cm at the beginning of the reproductive season. Determining the percentage of adult females that are reproductively active in a given season can help in estimating population parameters, such as population size, based on number of nests observed (Wilkinson, 1983; Taylor et al., 1991).

Lance et al. (2009) reported the relationship between follicle development (using ultrasound imaging) and hormone levels in female *Alligator mississippiensis* (Daudin, 1802). They observed high estradiol levels in individuals with large vitellogenic follicles and increasing levels of progesterone and testosterone levels during follicle growth. Likewise, for marine turtles and desert tortoises Rostal et al. (1996) and Lance and Rostal (2002) showed a strong relationship between follicle growth and hormonal levels (estradiol and testosterone) and observed that progesterone levels increase during ovulation. Therefore, besides cytology, studies using ultrasound techniques and hormonal data would be accurate tools to identify and recognize the reproductive status of reptiles.

Although the broad-snouted caiman has a wide geographic distribution and economic importance in Argentina (Larriera, 1993; Larriera and Imhof, 2006), there is no information in the literature concerning ultrasound or hormonal data for wild individuals that could be used to understand its reproductive cycle and nesting frequency. However, it is known that mating begins in October and females build their nests and oviposit an average of 36 eggs (range: 16–45) from December through January (Larriera, 1994; Piña et al., 2002; Simoncini et al., 2009); females exhibit nest care (Verdade, 1995), clutch size increase with latitude (Simoncini et al., 2009), and females can reach sexual maturity at the age of 5 years in captivity (Verdade et al., 2003) or wild conditions (Larriera et al., 2006).

This study aimed at: 1) identifying the reproductive structures (follicles and eggs), 2) describing follicle and egg development, and 3) correlating follicle and egg development with levels of estradiol, progesterone, and testosterone in *Caiman latirostris*. This information can be useful for the sustainable use of the species.

MATERIALS AND METHODS

Study area and capture methods

Fieldwork was carried out in the northern region of Santa Fe Province, Argentina, during two reproductive seasons from November 2010–January 2011 and November 2011–January 2012 at four study sites: Caminos (30°02´46˝S, 59°58´30˝W), Espín (29°58´04˝S, 60°04´55˝W), Fisco (30°11´40˝S, 61°00´50˝W), and Lucero (29°54´25˝S, 60°50´23˝W) (Fig. 1).

The climate is temperate with a mean minimum annual temperature of 14.9°C and maximum of 25.6°C and an annual rainfall of 1,380 mm (National Meteorological Service web site: www.smn.gov.br). The main habitats and nesting environments of *Caiman latirostris* in Argentina are characterized as forest, savanna, and floating vegetation (Montini et al., 2006).

Females were caught with a steel cable noose and immobilized manually without the use of anesthetics and then sexed by cloacal palpation (Yanosky, 1990; Verdade, 1997). Blood samples were collected within 10 min of capture and then ultrasound procedures were performed. The following measurements were taken from all captured individuals: total length (TL) and snout–vent length (SVL) were measured with a tape measure (± 1 mm) and body mass (BM) was measured with a scale (± 0.1 kg). Females were marked individually by notching tail scutes (Bolton, 1994). Individuals were released at the capture site after data collection.

Blood samples and ultrasonography

Blood samples were collected in the field from the dorsal spinal vein (Olson et al., 1975) and placed in 15 mL heparinized tubes. Time between capture and blood extraction never exceeded 10 min to avoid stress-induced suppression of estradiol (Elsey et al., 1991; Lance et al., 2009). Each tube was centrifuged for 10 min at 4,000 revolutions per min. Separated plasma was stored in Eppendorf tubes (1.5 mL) refrigerated with ice until arrival at the laboratory, where they were frozen (-20°C) for subsequent hormone analyses.

To determine reproductive condition and visualize follicles and eggs we used a portable ultrasound device (Welld®, model WED-3000V) with a 5.0 MHz convex linear transducer. Females of different sizes (including juveniles, n = 10; TL: 87.5–125 cm, $\overline{X}$ 107 ± 12 cm,) were examined, but we were only able to visualize reproductive structures in individuals with SVL > 65 cm, consistent with previous results for *Caiman latirostris* (Larriera et al., 2006). Individuals were scanned on both sides of the ventrolateral region of the abdomen to determine reproductive stage (Tucker and Limpus, 1997; Lance et al., 2009). An ultrasound coupling gel was applied to facilitate visualization during scanning. Static images were captured on-screen, measuring follicles and eggs with an electronic caliper (± 1 mm). Ultrasound resolution allowed the identification of only well-developed vitellogenic follicles, eggs, and atretic follicles. Females with vitellogenic follicles and eggs were considered reproductive and females without visible reproductive structures were considered non-reproductive for that season. Females with atretic

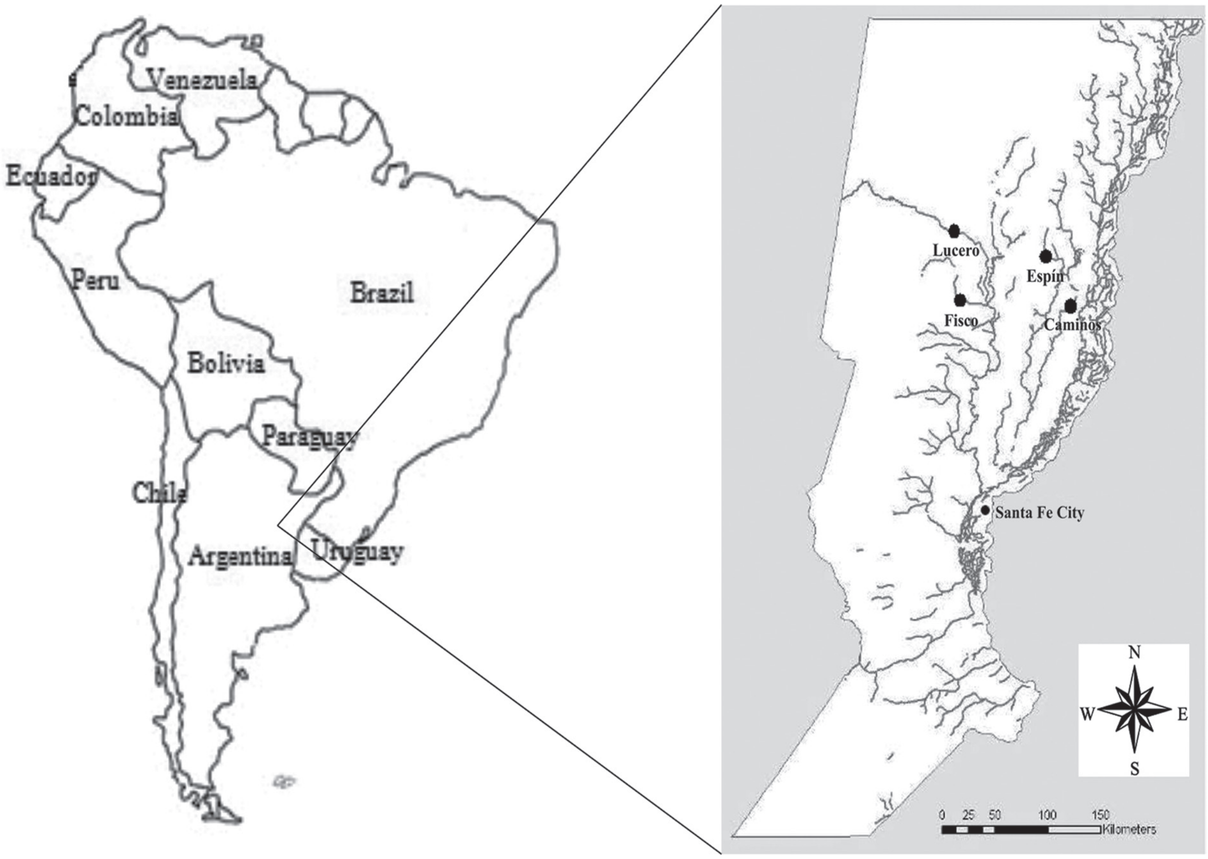

Figure 1. Study sites (Caminos, Espín, Fisco, and Lucero) in Santa Fe Province, Argentina. Adapted from Simoncini et al. (2011).

follicles captured on their nests were considered to be reproductive for the hormone analysis but were not used to calculate the percentage of actively reproductive females (see Data analysis, below). The remaining females with atretic follicles were not included in the hormone analysis because it was not possible to identify the reproductive season to which the follicles corresponded (Lance et al., 2009). Individuals were classified according to the identified structures following Palacios and Beltrán (2005) and Lance et al. (2009), as:

a) Non-reproductive (NR): no active reproductive structures were found
b) Vitellogenic follicles (VF): presence of multiple black spherical corpuscles > 1.5 cm diameter
c) Eggs (EG): structures perfectly delineated with or without calcified shells, generally > 4.0 cm diameter
d) Atretic follicles (AT): presence of multiple follicles of variable shape and size, generally < 1.5 cm in diameter

Hormonal analysis

Frozen plasma samples were transported, processed, and analyzed by radioimmunoassay (RIA) in the laboratory. Prior to RIA procedures, we evaluated the need for extraction of steroids (performed as described below for estradiol determinations) to eliminate the sex hormone binding proteins that could interfere with the assay, according to the standards established by Boretto et al. (2010) and Lance et al. (2015). We found that for accurate determination of 17β-estradiol the plasma samples had to be extracted. However, progesterone and testosterone were measured directly in plasma because extraction did not modify the results, showing that plasma proteins did not interfere significantly with RIA for these hormones.

Progesterone and testosterone levels in plasma were measured using radioimmunoassay commercial kits for total hormones (PITKPG-11 and PITKTT-9, Coat-A-Count solid phase respectively; Siemens Healthcare Diagnostics Inc. Los Angeles, CA, USA). For measurements of 17β-estradiol, 250 µL of plasma were extracted twice adding 1 mL of diethyl ether, the tubes were vortexed briefly, and frozen at -80°C during approximately 5 minutes. Supernatant was carefully pipetted to glass culture tubes and all extracts were evaporated to dryness at room temperature and under a stream of nitrogen. Extracts were reconstituted in 0.5 mL PBS EDTA 1 mM, gelatin 0.1%, incubated at 37°C for 2 hours and aliquots of 60 µL were used to measure [^{125}I]- 17β-estradiol using a commercial kit for

Table 1. Cross-reactivity of estradiol, progesterone, and testosterone kits used for measurements in *Caiman latirostris* with other hormones/metabolites. Kits are from Siemens Healthcare Diagnostics Inc. Los Angeles, CA, USA. ND = not detectable; NI = not informed.

Compound	**% Cross-reactivity**		
	17β-estradiol (PIKE2D-11)	**Progesterone (PITKPG-11)**	**Testosterone (PITKTT-9)**
Aldosterone	ND	ND	0
Corticosterone	ND	0.9	0.002
Danazol	ND	0.006	0.09
DHEA-SO_4	ND	0.002	0.006
Estradiol	100	ND	0.02
Estriol	0.235	NI	NI
Estrone	12.5	NI	0.01
Progesterone	ND	100	0
Testosterone	ND	0.1	100

total hormone (PIKE2D-11 double antibody radioimmunoassay from Siemens Healthcare Diagnostics Inc. Los Angeles, CA, USA). To validate the extraction procedure, commercial serum standards of known hormone concentration were extracted and recovery estimated to be between 80–90%. The results were not corrected for recovery since all the samples were processed and measured at the same time. For further validation of the three assays, a pool of caiman plasma was run as an additional control and used for parallelism estimation. Different dilutions of the pool were run in the assay, and we found that the curve was parallel to the respective standard curves. Additional information about cross-reactivity of the kits used for hormonal measurements can be found in Table 1.

Data analysis

Because data were not normally distributed, we used a Kruskal-Wallis test followed by post-hoc multiple comparisons to compare females hormone levels and the reproductive condition between months. The proportion of actively reproductive females per year was calculated by adding the number of females with follicles and eggs and dividing by the total number of adult females captured. Females captured on their nests were not considered for this calculation, because their reproductive condition was previously known since they had already laid eggs and they do not overlap reproductive periods. Females with atretic follicles were also not considered for

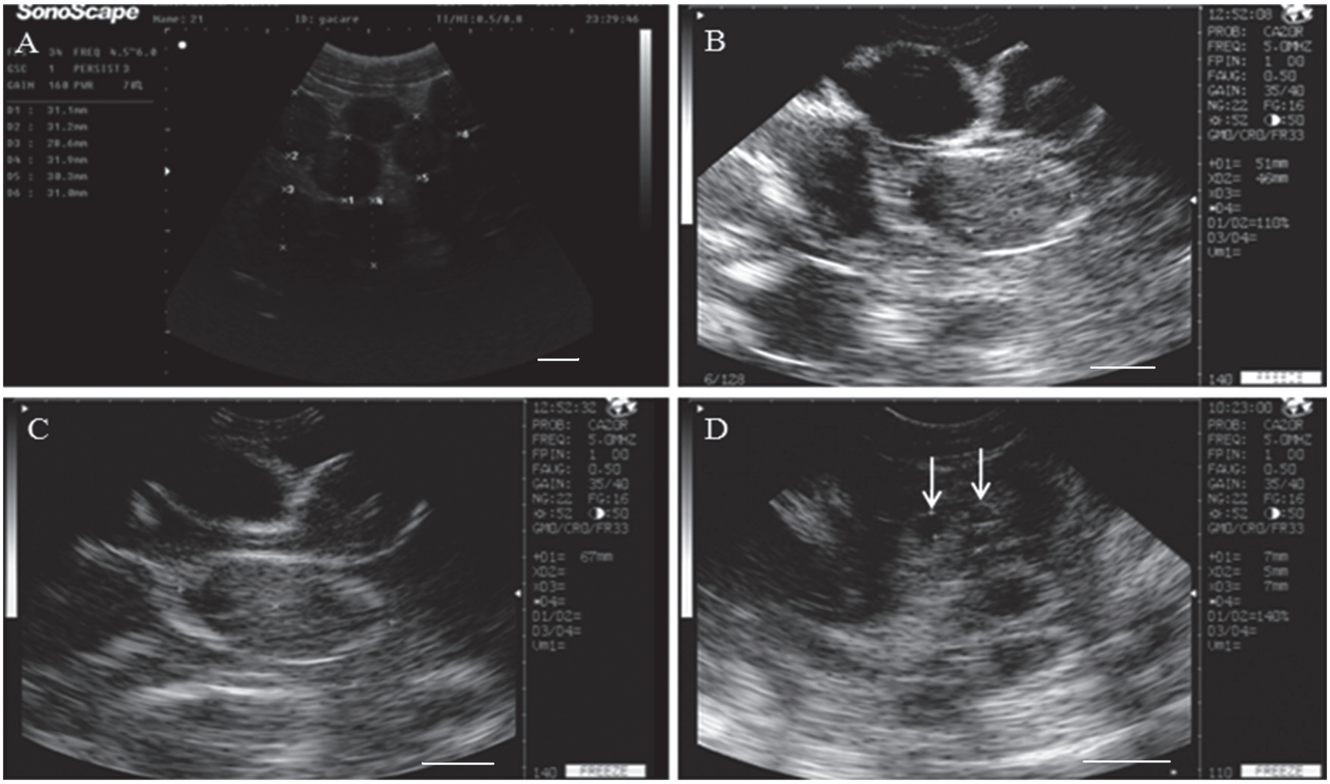

Figure 2. Ultrasound images of reproductive structures of *Caiman latirostris.* A = vitellogenic follicles; B and C = eggs; D = atretic follicles (arrows). Scale bar = 20 mm.

this calculation, because the low resolution of the ultrasound made it impossible to identify whether reproductive structures corresponded to the current or previous breeding seasons.

To evaluate if the reproductive activity was related to female body condition we calculated a body condition index (BCI). This index was considered as the residuals of the linear regression between SVL and body BM (Litzgus et al., 2008). All data were analyzed in the software InfoStat (Di Renzo et al., 2008). The values are expressed as means ± standard error and range, and were considered to be statistically different when $P < 0.05$.

RESULTS

We examined the ultrasound images of 32 adult females (SVL > 65 cm) captured during the reproductive season (October–January; Table 2). Their reproductive status was vitellogenic follicles ($n = 5$), eggs ($n = 4$), atretic follicles ($n = 11$), and non-reproductive ($n = 12$) (Fig. 2). Fifty percent of the females were reproductively active in 2010/2011 ($n = 5/10$) and 36% were active in 2011/2012 ($n = 4/11$). Reproductive activity was not related to female body condition between reproductive and non-reproductive females (BCI; $H = 0.05$, df = 1, $P = 0.831$); however, we observed that 80% of reproductive females were > 77 cm SVL and 75% of non-reproductive females were < 77 cm SVL (Fig. 3).

Hormonal levels and reproductive structures

Despite the wide variation in hormone levels between groups (vitellogenic follicles, eggs, atretic follicles and non-reproductive), we observed higher estradiol levels in females with vitellogenic follicles and eggs than females with atretic follicles or non-reproductive (Table 3, Fig. 4A). Progesterone levels were low in females with follicles but increased during ovulation in females with eggs (Table 3, Fig. 4B). Generally, mean values for testosterone were low (Table 3, Fig. 4C) and similar among the different groups ($P > 0.05$).

Hormonal levels per month

Reproductive females showed higher estradiol levels during follicular development (November; $H = 8.42$, df = 2, $P = 0.014$; Fig. 5A) compared with the other reproductive stages. During nesting (December–January) estradiol levels were lower (107.2 ± 45.8 pg/mL and 71.8 ± 17 pg/mL, respectively) than prior to nesting (November, 1603.9 ± 605.4 pg/mL; $H = 8.42$, df = 2, $P = 0.014$), and we found no differences between reproductive and

Table 2. *Caiman latirostris* females examined by ultrasound. Capture date = day/month/year; TL = total length; SVL = snout–vent length; BM = body mass (kg); Follicles = reproductive structures; VF = vitellogenic follicles; EG = eggs; NR = no reproductive structures observed; AT = atretic follicles; * = Females caught at nests.

Capture date	TL (cm)	SVL (cm)	BM (kg)	Follicles (average mm)
11/11/2010	152.0	78.0	18.2	VF (32)
29/11/2010	157.0	79.0	20.6	EG (51)
29/11/2010	146.0	77.0	17.8	EG (50)
01/12/2010	125.0	72.0	10.4	NR
01/12/2010	164.0	84.0	19.4	EG (53)
01/12/2010	144.0	79.0	14.1	NR
08/12/2010	134.0	68.0	11.6	NR
08/12/2010	138.5	68.0	12.1	NR
08/12/2010	152.0	79.0	18.2	EG (67)
08/12/2010	149.5	74.0	13.5	NR
28/12/2010	162.0	80.0	17.6	AT (14)*
29/12/2010	155.6	79.0	15.4	AT (18)*
06/01/2011	157.0	76.0	16.6	AT (8)*
06/01/2011	175.0	85.0	22.0	AT (7)*
12/01/2011	158.0	79.0	16.5	AT (4)*
13/01/2011	142.0	77.0	15.5	AT (11)*
01/11/2011	157.0	79.0	16.9	VF (18)
01/11/2011	158.0	80.0	18.4	VF (29)
01/12/2011	138.0	69.0	9.5	VF (17)
01/12/2011	141.0	71.0	11.5	NR
01/12/2011	140.0	70.0	10.4	NR
01/12/2011	141.0	67.5	11.2	NR
21/12/2011	169.0	84.5	21.3	NR
21/12/2011	141.0	70.0	11.8	NR
28/12/2011	134.0	69.0	10.0	VF (15)
04/01/2012	144.0	73.0	12.6	AT (7)
26/01/2012	163.0	85.5	20.3	AT (9)
26/01/2012	161.0	83.0	20.2	NR
26/01/2012	139.0	70.5	12.5	AT (8)
27/01/2012	159.5	87.0	19.1	AT (10)
27/01/2012	140.0	70.5	12.0	NR
27/01/2012	156.0	76.5	13.8	AT (8)

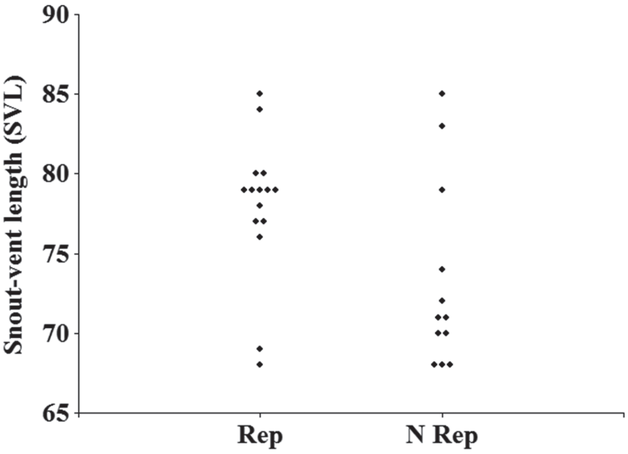

Figure 3. Body length (snout–vent length) and reproductive condition for adult female *Caiman latirostris* in Argentina. Rep = reproductive; NRep = non-reproductive.

Table 3. Mean estradiol, progesterone, and testosterone levels for *Caiman latirostris* adult females in different reproductive conditions. The values were analyzed by Kruskal-Wallis test followed by post-hoc multiple comparisons and are expressed as mean ± standard error. Groups identified with the same superscript letter have similar level of hormone (P > 0.05). VF = vitellogenic follicles; EG = eggs; AT = atretic follicles; NR = no reproductive structures observed.

Hormone	**Reproductive condition**				**Kruskal-Wallis**	
	VF	**EG**	**AT**	**NR**	***H***	***P***
17β-estradiol (pg/mL)	1517.9 ± 700.7^{A}	360 ± 263.6^{A}	52.9 ± 14.2^{B}	76 ± 13.6^{B}	10.76	0.013
Progesterone (ng/mL)	0.3 ± 0.1^{A}	2.1 ± 0.6^{B}	1.0 ± 0.5^{A}	0.3 ± 0.1^{A}	8.20	0.035
Testosterone (ng/mL)	0.3 ± 0.2^{A}	1.5 ± 1.3^{A}	0.01 ± 0.01^{A}	0.05 ± 0.03^{A}	1.70	0.544

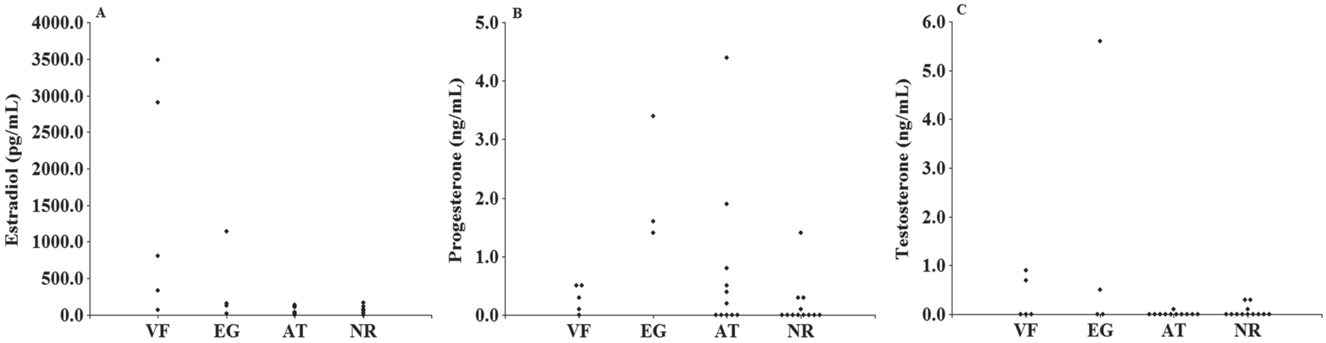

Figure 4. Estradiol **(A)**, progesterone **(B)**, and testosterone **(C)** levels and reproductive condition for adult female *Caiman latirostris*. VF = vitellogenic follicles; EG = eggs; AT = atretic follicles; NR = no reproductive structures observed.

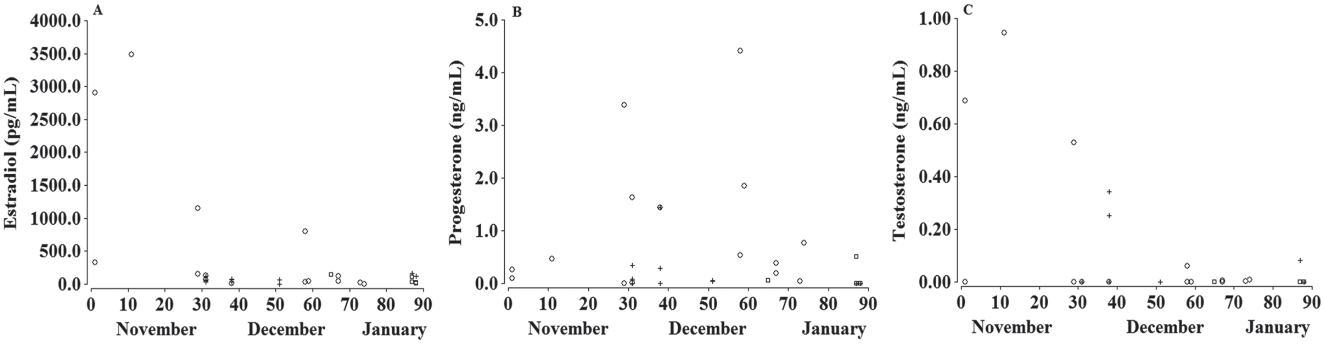

Figure 5. Estradiol **(A)**, progesterone **(B)**, and testosterone **(C)** levels during reproductive seasons (2010–2012) for *Caiman latirostris*. Circle = reproductive females; Square = atretic follicles; Cross = non-reproductive females.

non-reproductive females (*H* = 0.35, df = 1, *P* = 0.553). At the end of the nesting period (January) estradiol levels were similar to those of non-reproductive females, and we found no differences between the studied groups (reproductive, non-reproductive, and atretic follicles; *H* = 0.95, df = 2, *P* = 0.267). Females that were captured at their nests after oviposition had lower estradiol values than females with follicles or eggs (*H* = 7.34, df = 2, *P* = 0.025).

Reproductive females had higher progesterone values during nesting (December) than non-reproductive females (*H* = 5.56, df = 1, *P* = 0.035; Fig. 5B). We found no differences between testosterone levels for non-reproductive females during the nesting period (December–January; *H* = 0.18, df = 1, *P* = 0.999) or between reproductive females, non-reproductive females, and females with atretic follicles (*H* = 2.32, df = 2, *P* = 0.120) at the end of the nesting period (January; Fig. 5C).

DISCUSSION

Generally, the reproductive cycle of crocodilians begins in early spring as temperatures rise (Lance, 1989; Larriera and Imhoff, 2006). Based on this, the reproductive cycle of *Caiman latirostris* appears well defined, with vitellogenic follicles and eggs found in November/December (late spring and early summer) and atretic follicles ocurring in December/January. A well-defined reproductive cycle has also been observed for other crocodilians, such as *Alligator mississippiensis* (Joanen and McNease, 1979), *Caiman yacare* (Daudin, 1802) (Coutinho, 2000), and *Crocodylus johnsoni* Krefft, 1873 (Tucker and Limpus, 1997). However, it should be emphasized that the eight females captured in their nests had atretic follicles (Table 2) and that these can be found throughout the year in mature crocodilians. However, atretic follicles are not found in immature females and are rarely found in very old, barren females (Lance et al., 2009).

This is the first study reporting data about ultrasonography and hormone levels for wild individuals of *Caiman latirostris*. The only published report about the follicular dynamics of *C. latirostris* was presented by Vac et al. (1992) for captive individuals in Brazil. Those authors reported the presence of 3.0–3.8 cm diameter follicles in December. In our study, females presented follicles of 1.5–3.2 cm diameter in November and December, similar to the pattern above. Possibly, due to the ultrasound limitations we could not identify previtellogenic follicles and corpora lutea by ultrasound images. We were not able either to accurately count follicles or eggs with ultrasonography because of the overlapping of reproductive structures and the presence of intestinal fecal material, as previously reported by Tucker and Limpus (1997) and Lance et al. (2009).

For the broad-snouted caiman in Argentina we observed that follicles and eggs can be detected by ultrasonography during November–December and atretic follicles can be found in postovulatory females from late December–early January. No females were caught in other months. The reproductive cycle of *Caiman latirostris* showed a similar pattern to that described for other crocodilians (Lance, 1989; Thorbjarnarson, 1996). Because we started our study in November, future studies should perform ultrasound scans from early September (beginning of the breeding season) to identify the possible presence of developing follicles at this time and enable a better understanding of the reproductive cycle of this species.

Estradiol values in non-reproductive females and females with atretic follicles were very low compared to those that had developing follicles and eggs (Fig. 4); this can be explained because estradiol is a strong stimulant of vitellogenesis in reptiles (Ho et al., 1985; Cree et al., 1992), with low levels after nesting or for non-reproductive females. Additionally, estradiol is mainly produced by preovulatory follicles and should decrease after oviposition. This was observed in the present study, with estradiol levels being lower in females that had laid eggs than in those with developing follicles or eggs. Higher progesterone levels were found in females with eggs, indicating that this hormone increases during ovulation until the moment of egg deposition, and decreases after nesting. The main function of progesterone in oviparous species is to stimulate follicular maturation and prepare the oviduct for reproduction (Wibbels et al., 1992). The same pattern has been reported for other crocodilians (Lance et al., 2009) and turtles (Rostal et al., 1998; Rostal et al., 2001; Schramm et al., 1999).

The percentage of *Caiman latitrostris* females actively reproducing (50% in 2010, 36% in 2011) was somewhat higher than that reported for this species in captivity (27–33%, Vac et al., 1992) and similar for other wild alligatorid populations (29–50% in *Alligator mississippiensis,* Lance et al., 2009; 33% in *Paleosuchus trigonatus* (Schneider, 1801), Magnusson and Lima, 1991), but was lower than that described for *Crocodylus* (90% in *Crocodylus johnsoni,* Webb et al., 1983; 63.8% in *Crocodylus acutus* (Cuvier, 1807), Thorbjarnarson, 1988). Rainfall is one of the conditions that might affect reproductive frequency in crocodilians (Kushlan and Jacobsen, 1990; Simoncini et al., 2011). In years with more rainfall more females would reproduce due to greater availability of food, better body condition, and lower stress levels, which should favor reproduction (Joanen and McNease, 1989).

In this study, we did not observe a relationship between body condition index and reproductive activity; however, we observed that females would be physiologically able to reproduce > 69 cm SVL. Nevertheless, female sexual maturity should not be based on the breeding success of a few individuals, as they might not represent the entire population (Joanen and McNease, 1987; Coutinho, 2000). In this study, 80% of reproductive females were > 77 cm SVL, which can be considered a threshold for field studies; however, it should be noted that the 75% of non-reproductive females that were < 77 cm SVL (Fig. 3) could be immature, quiescent (mature, but did not nest that year) or barren (old and possibly no longer able to reproduce) females. Knowing the variables that can affect reproduction is important for sustainable use programs, since this information could help in estimating nest production and spatial distribution (Simoncini et al., 2011). However, this study encompassed only two consecutive years. A long-term monitoring program on the reproductive biology of the species is needed to support its ranching program in Argentina.

Information about hormone levels alone is not enough to diagnose the reproductive condition or the stage of the follicular cycle for *Caiman latirostris* females. Therefore, it is recommended that studies on hormone levels should be accompanied by a technique that allows a view of reproductive structures, such as ultrasound. With a simple nest counting it is possible to determine the number of reproductive actively females in a season (Wilkinson, 1983; Taylor et al., 1991). Knowing the reproductive frequency would allow the total adult female population to be estimated.

Most of the information related to the dynamics of ovarian follicle development for crocodilians has been obtained from the sacrifice of adult individuals (Lance, 1989; Palacios and Beltrán, 2005) or the use of animals found dead on roads (Thorbjarnarson, 1996). The use of ultrasound allows reproductive data to be obtained from living animals (Robeck et al., 1990; Bertona and Chiaraviglio, 2003; Valdez et al., 2011; Sacchi et al., 2012), which is particularly important for endangered species (e.g., *Alligator sinensis* Fauvel, 1879). With technological advances in equipment and its portability, the use of ultrasound in wild populations might become viable. However, only the integrative use of cytology, hormone profiles, and ultrasound can lead to precise estimates of reproductive status of female crocodilians, as already done for turtles (Rostal et al., 1998) and alligators (Lance et al., 2009).

Future studies should assess the hormonal cycle of *Caiman latirostris* from the beginning of courtship until mating (presumably August–September) to identify possible variations in estradiol, progesterone, and testosterone levels and the relationship between hormone levels and reproductive condition in females. Such information might be crucial to the estimate of reproductive activity for the population, which is particularly relevant for both endangered and economic species.

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