REPTILES & AMPHIBIANS

Intraspecific Variation in Black Caiman (*Melanosuchus niger*) Embryonic Development

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Abstract.—Steps in the embryonic development of crocodilians are usually species-specific, highlighting the plasticity of the process. Herein we describe intraspecific variation in the growth of embryos in three Black Caiman (*Melanosuchus niger*) nests monitored *in situ* in the Central Brazilian Amazon. Nests differed in which embryonic stages the first increases in total length and mass occurred. Differences from a previous study of the same species included the timing of morphological characteristics such as the closure of the abdominal wall. Therefore, our data are indicative of the plasticity of embryonic development of Black Caimans in natural conditions.

The Black Caiman, *Melanosuchus niger* (Spix 1825) (Alligatoridae), occurs throughout Amazonia, where the highest population densities are associated with flooded habitats. Nesting females lay their eggs on floodplains isolated from the floods of main rivers that theoretically provide hydrological stability during incubation and reduce the risk of nest flooding and embryonic mortality (Thorbjarnarson and Silveira 2000). Nesting begins at the end of the dry season, which is characterized by high temperatures and low water levels (Schú et al. 2015). Embryonic development in crocodilians is generally divided into three phases, the first two of which comprise formation and sexual determination, and the third is associated primarily with growth (Ferguson 1985).

The first studies on crocodilian embryonic development, which considered body measurements and age as references to describe patterns for embryonic development, were followed by descriptions of embryonic developmental stages because differences in incubation period and metabolic rates within and among species usually led to biased results (Donayo et al. 2002). More recent studies establishing embryonic developmental stages for crocodilians account for the continuous appearance of morphological structures during specific stages of development, resulting in more reliable criteria for evaluation (Ferguson, 1985, 1987; Iungman et al. 2008).

Vieira et al. (2011) divided the embryonic development of Black Caimans into 28 stages, defined according to morphological changes from pharyngeal arch formation to hatchling eclosion, but did not consider embryonic growth. To address this, we herein propose to: (1) describe growth in total length and mass in relation to embryonic stages based on observations of three nests in natural conditions, and (2) evaluate our results within a phylogenetic framework, comparing growth curves with other crocodilian species.

Materials and Methods

Study area.—We conducted this study at the Lago Cuniã Extractivist Reserve (Resex), located along the lower Rio Madeira in the western Brazilian Amazon in the State of Rondônia (**Fig. 1**). The reserve comprises 75,876.67 ha of Amazon forest, of which 18,000 ha are principal crocodilian habitats, characterized by floodplains, locally designated as varzeas, and several lakes, the water levels of which are closely associated with the Rio Madeira flood season. The rainy season extends from October to March, the dry season from April to September.

Data sampling.—Data were obtained during the 2019 nesting period from three intact nests at Lago Cuniã Extrativist Reserve. We monitored the nests (designated 10, 11 and 12) throughout the incubation period (23 September–19 November 2019). All were located along the bank of the Igarape Grande, a flooding canal near the reserve's main lake, at: nest 10 = -44.5065, -1.151503; nest 11= -29.6364, -8.06994 W; and nest 12: -29.607, -8.0619.

We measured the dimensions and mass of eggs before opening them to assess embryonic total length and mass using a digital calliper (to 20 cm) and, when total lengths exceeded 20 cm, a 30-cm ruler. Masses were obtained using a digital balance (SF-400) with accuracy to 1 g. Embryos were stored in 70% alcohol. During each sampling period, we also measured nest temperatures.

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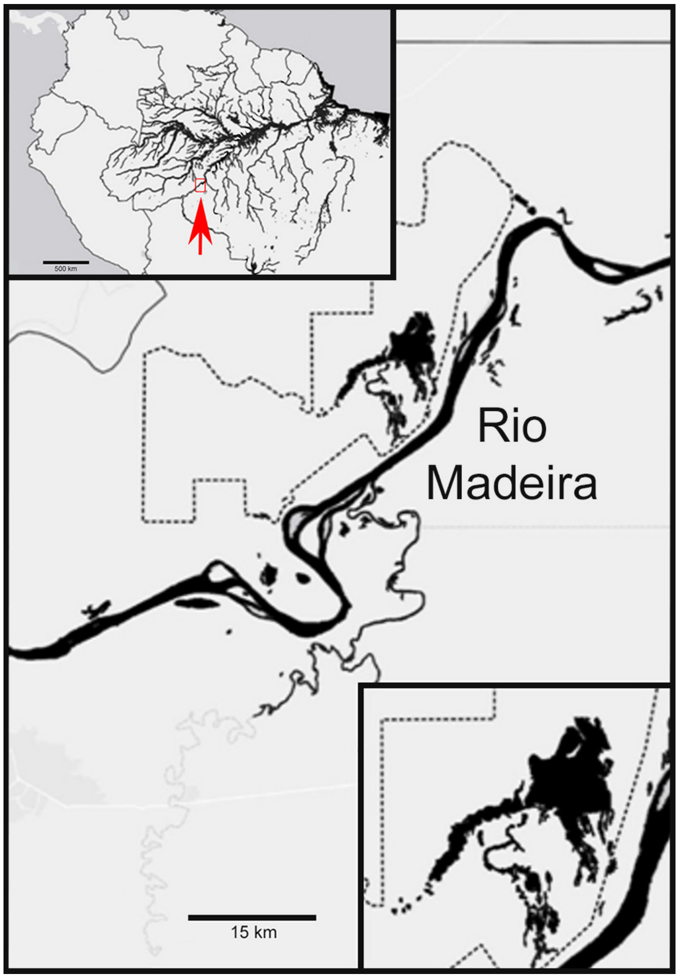

Figure 1. Map showing the location of the Lago do Cuniã Extractivist Reserve, Amazon, Brazil.

Embryonic stage identification and growth curves.—Embryonic stages and estimated ages of embryos were determined according to criteria of Vieira et al. (2011) using a magnifier (Stemi 351, Zeiss, Jena, German) at the Universidade Federal de Minas Gerais (UFMG). Growth curves were obtained using the RStudio graphical package "plot" by graphing each embryo's total length and mass according to embryonic stage. In accord with the licence issued by the Brazilian System of Biodiversity Information (SISBIO), all embryos were accessioned in the Herpetological Collection of the Universidade Federal de Minas Gerais (UFMG) (nos. 3386–3418. The invoice authorizing the use of the embryos was issued on 31 January 2022 (no. 01/2022) by the coordinator of the herpetological collection.

Results

By the end of the incubation period, we had collected a total of 34 eggs: 12 from nests 10 and 11, and ten from nest 12. Eggs were collected at intervals of five days, except on four occasions, when sampling was postponed one day due to bad weather or difficulties accessing the nests, resulting in a following interval of four days.

Nest temperatures were 29.0–33.9 ºC (n = 33, mean = 31.7 °C). In the first sample, the earliest embryonic stage was stage 10 in nest 10, in which the total length of the embryo

Table 1. Total length, mass, and developmental stage of Black Caiman (*Melanosuchus niger*) embryos from three nests in Brazilian Amazonia during the 2019 nesting season. Embryonic stages are according to Vieira (2011). The m-dashes (—) indicate the lack of data from an infertile egg collected from nest 11, for which the embryonic stage was impossible to determine.

	Total		Embryonic	
Nest	Date	Length	Mass	Stage
10	23 SEP	5.2	<1	10
10	28 SEP	5.1	<1	11
10	03 OCT	7.5	3	12
10	09 OCT	9.2	4	14
10	13 OCT	10.7	8	15
10	19 OCT	13.3	12	16
10	23 OCT	15.3	18	16
10	28 OCT	17	19	18
10	02 NOV	23.7	35	21
10	07 NOV	24.5	40	22
10	13 NOV	27.8	49	25
10	19 NOV	28.8	82	26
11	23 SEP	7	2.3	13
11	28 SEP	8.5	4	14
11	03 OCT	10.5	7	15
11	09 OCT	13.1	12	16
11	13 OCT	14.8	18	18
11	19 OCT	—	—	—
11	23 OCT	21.5	39	21
11	28 OCT	25.5	47	22
11	02 NOV	30	65	22
11	07 NOV	29.8	67	23
11	13 NOV	31	93	26
11	19 NOV	30	93	27
12	23 SEP	10	7	14
12	28 SEP	12.7	7	16
12	03 OCT	15.5	17	18
12	09 OCT	18.2	25	21
12	13 OCT	22	32	22
12	19 OCT	26	45	23
12	23 OCT	26	41	25
12	28 OCT	30	90	26
12	02 NOV	28.5	90	27
12	07 NOV	33	91	28

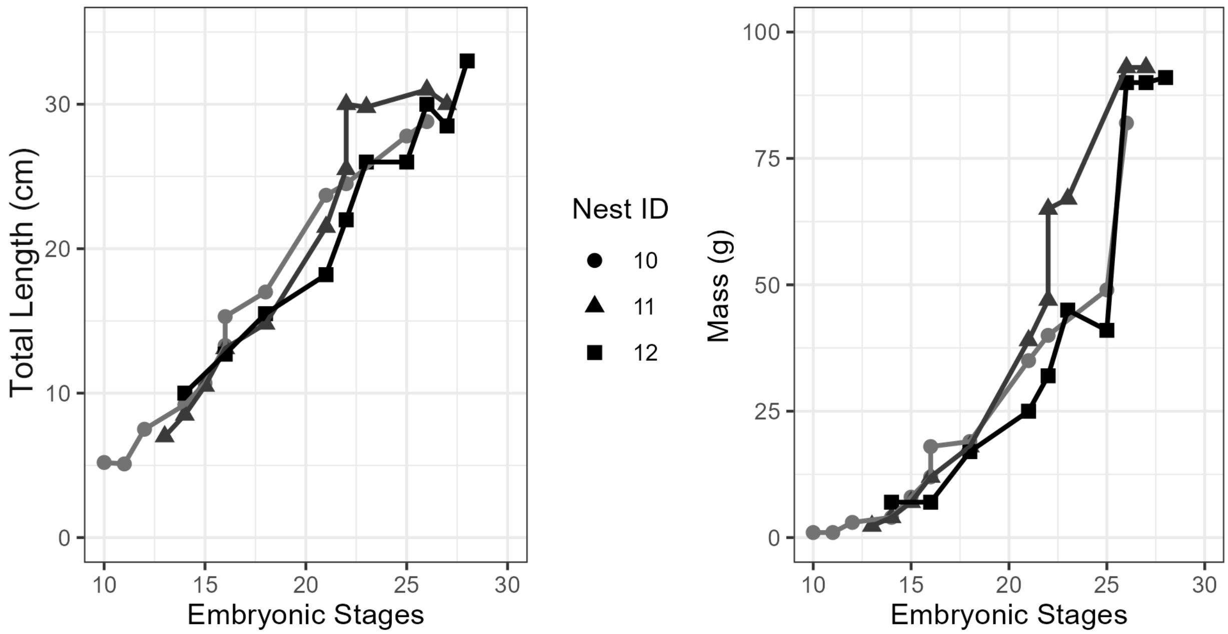

Figure 2. Embryonic Black Caiman (*Melanosuchus niger*) total length (cm) and mass (g) during embryonic development in three monitored nests in the Amazon rainforest in 2019.

was 5.2 cm and weight was <1 g; the latest was stage 14 in nest 12, with an embryo of 10 cm in length and a weight of 7 g. These data suggested that eggs in nest 10 were laid in early September with an incubation period of 82 days, whereas eggs in nests 11 and 12 were likely laid in mid- to late August, with incubation periods of 75 and 83 days, respectively. Sampling dates, embryonic stages, and biometric data for embryos in all three nests are listed in Table 1.

During fieldwork, one embryo from nest 12, at the final stage of embryonic development, hatched on 7 November 2019. Embryos from nests 10 and 11 hatched shortly after, when females were seen at the nests. Embryonic lengths and masses at various stages of embryonic development are illustrated in Fig. 2.

Relationships between growth and developmental stages in the three nests followed essentially the same pattern to stage 15; thereafter, however, each nest followed a different pattern. In terms of mass, considerable differences were evident after stage 17.

Discussion

Our findings are suggestive of plasticity in Black Caiman reproduction, an adaptation to varying environmental conditions which likely affect both incubation period and embryonic development. Populations living in different hydrological regimes adjust nesting period to avoid flooding, whereas embryonic mass and total length could be influenced by factors such as nest temperature.

The estimated age of the monitored nests suggests that the nesting season at Lake Cunia Extrativist Reserve extends from mid-August to mid-November, an incubation period of 75–83 days. Those results differ from data reported by Heron et al. (1990), Vieira et al. (2011), and Villamarín et al. (2011), who indicated that *M. niger* nested from September to December/January, with an incubation period of 90 or even 96 days under a semi-controlled temperature environment.

Local water regimes may explain the differences we observed in the incubation period. In the Amazon River Basin, the wet season begins in February and the dry season ends in October (Nölscher et al. 2016); while in the Rio Madeira Basin, the wet season begins in November/December and the dry season ends in August (Ronchail and Gallaire 2006). Villamarín et al. (2011) studied nests in the Amazon River Basin, where lakes remained isolated for more than 81 days. In contrast, Lago Cuniã's lakes and channels are directly influenced by the rising water level of the Rio Madeira, limiting the end of the nesting season to mid-November.

Analysis of embryonic development revealed a key difference in the three nests. Embryos lacked complete intestinal retraction and abdominal wall closure, which Vieira (2011) described as occurring in stage 17, after the appearance of the egg tooth in stage 18. Despite this difference, the embryos exhibited typical development in other aspects, including egg tooth emergence at stage 18, increased skin pigmentation from stage 19 onward, cloacal orifice closure at stage 21, tail scute formation at stage 22, and emergence of protuberances indicating the first teeth at stage 23.

We observed the first growth spurt (total length and mass) at different stages; in nest 10 during stages 18–21 (45 days) and in nest 12 during stages 22–23 (55 days). An infertile egg in nest 11 made it impossible to determine when the first size change occurred in this nest. A second growth spurt (mass only) attributable to yolk absorption occurred at stage 26 (72 days) in all nests. At that time, embryos reached maximum mass without substantive changes in length. Vieira (2011) reported the first size differences at stage 12 (26 days), which is suggestive of intraspecific variations in the timing of development for Black Caimans, which aligns with findings of interspecific variations in other non-squamate reptiles (Andrews 2004). Factors that can influence development include egg size (Piña et al. 2003) and incubation temperature (Piña et al. 2007). Nest factors like material and sun exposure (Campos 2003) and nest size (Piña et al. 2007; Do Val et al., in press) affect chamber temperature, which can lead to variation between clutches. Our recorded temperature range (29.0–33.9 °C, mean = 31.7 °C) was slightly warmer than the 28.2–31.5 °C measured by Vieira et al. (2011). Although Campos (2003) suggested that higher temperatures accelerated development, our results might indicate slower growth attributable to more stable temperatures with fewer fluctuations. However, our limited data prevents definitive conclusions. Further studies are needed to fully explain this discrepancy.

Although most studies of embryonic development in crocodilians have focused on the emergence of specific morphological features, few studies presented the timing of size and/or mass increases. Among crocodilids, size in *Crocodylus porosus* embryos increased substantially at 24 and again at 90 days of incubation (Brien et al. 2014); mass of *Crocodylus niloticus* embryos varied little until 20 days of age, then increased moderately at 35 days, followed by a progressive increase during the final days of incubation (Peterka et al. 2010); and for *Crocodylus johnstoni*, Deeming and Ferguson (1990) showed a fast initial increase in mass during stage 20 and in body size during stage 10, but did not provide the ages of the embryos at those stages. As for alligatorids, increases in body sizes of embryonic *Alligator mississippiensis* peaked at approximately 40–50 days and declined gradually thereafter (Bardsely et al. 1995), and embryonic *Paleosuchus trigonatus* increased simultaneously in mass and size between the fifth and the sixth weeks of incubation (about 36–43 days) (Vasquez 1983). Our data, although based on a small dataset, were essentially similar to those of other alligatorids, especially *P. trigonatus*, another Amazonian caiman.

However, other than Viera et al. (2011), this study is the only one to examine embryonic development of Black Caimans under natural conditions. Nevertheless, despite the observed intraspecific variations, all three nests hatched during the flooding season, suggesting that neither the delay in closing the abdominal wall nor the increases in length and mass in any way impaired the progress of embryonic development and thus the reproductive fitness of the females at Lago Cuniã Reserve.

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