NESTING THERMAL ECOLOGY AND EMBRYONIC DEVELOPMENT IN BLACK CAIMAN'S (*MELANOSUCHUS NIGER*, SPIX 1758)

ABSTRACT

The black caiman (*Melanosuchus niger*, Spix 1825) is a crocodilian species endemic to the Amazon biome, that nests within a defined nesting season bridging the dry and wet seasons. Eggs are deposited in a mound of fresh and old vegetation, which buffers the eggs against ambient weather extremes. Relatively little is known about the thermal relations within *M. niger* nests. We present data on both nest temperatures and embryonic development rates in wild nests (N = 7) from the southwestern Brazilian Amazon, gathered in 2019 and 2024. The mean incubation period from laying to hatching was estimated as 83 (± SD 4.88) days. Nest temperatures had a mean of 31.6 ± 1.65°C, with extremes between 27.2ºC and 35.9ºC, with daily cycling and baseline shifts over time. High nest temperatures seems to have caused an indirect negative effect on embryos during development. Embryo development rate for six wild nests are provided and the interactions between nest temperatures, embryo development rate and survival rate for the black caiman are discussed.

INTRODUCTION

The black caiman (*Melanocushus niger* Spix, 1758) is the largest member of the crocodilian Family Alligatoridae, endemic to the Amazon Biome in Colombia, Guyanas, Ecuador, Peru, Bolivia and Brazil (Thorbjarnarson, 2010). Nesting occurs in a pulse, lasting around one month, beginning in late August early September, during the drier season, and ending in November, when wet season rains have begun (Do Val et al. 2024). Like other Alligatorid species, *M. niger* is a mound-nester, building nests above water level, from branches, leaves and litter, often along seasonally flooded channels (Villamarín-Jurado and Suárez, 2007; Banon et al. 2019).

All crocodilian embryos are reasonably well-developed at laying (10-20 somites) relative to birds and turtles (Magnusson and Taylor, 1980; Ferguson, 1987; Iungman et al. 2008), and within a day of laying, embryos attach to the inside, upper surface of the egg-shell membrane causing an opaque spot to form on the shell (Webb et al. 1987). Early embryological development stages, based on morphology, are similar across species although variation occurs in the later growth stages preceding hatching. Those stages have been described for *M. niger* by Vieira et al. (2011). The rate at which embryological development proceeds is determined largely by temperature, if the gas and moisture environments are not compromised (Webb et al. 1987). But within both crocodilian nest mounds (Gurra 2013) and hole nests (Sharma et al. 2025), variation in egg temperature is dampened relative to outside ambient temperature (Gurra, 2013).

The incubation temperatures that occur in wild *M. niger* nests, and the degree to which they affect embryological development rates in wild nests, are largely unknown. The *M. niger* embryological stages reported by Vieira et al. (2011) were drawn from a series of wild nests and were not defined in terms of the nest environment. No other studies appear to report variation in *M. niger* embryo development rates, nor total development times from laying to hatching, in wild or controlled incubation conditions.

This study was carried out in the southwest Brazilian Amazon, in the same area Vieira et al. (2011) collected their embryo series from wild nests. We report on the incubation temperatures within 7 wild *M. niger* nests over time, and examine its relationship to ambient weather conditions. We quantify embryological development rates by sampling embryos in the nests, define times to hatching, and examine the influence of nest temperature on both. Results are discussed in relation to existing knowledge on embryo development.

MATERIALS AND METHODS

Study site

This study was conducted at the Lago Cuniã Extractivist Reserve (Resex) in Rondônia, western Brazilian Amazon, along the lower reaches of the extensive Madeira River (Fig. 1). The reserve encompasses 759 km^2 of Amazon forest, with 180 km^2 considered prime black caiman habitat characterized by floodplains (várzeas and igarapés) with numerous lakes, joined in the wet season but isolated during the dry season. Water levels within the Reserve are dictated by those in the Madeira River. The transition from dry season (June to September) to wet season (October to May) involves precipitation changing from 165 mm to 1253 mm, which causes flooding. It also involves an increase in minimum ambient temperatures (19.3ºC to 23.4ºC), but a decrease in maximum ambient temperatures (33.2ºC to 32.2ºC) (INMET, 2025a; 2025b).

Nests

Data were collected from seven *M. niger* nests, two from 2019 (11 and 12) and five from 2024 (1-5). Nest 1was located by the margin of Lago Cuniã and the other six along Igarapé Grande a tributary of Madeira River (Fig. 1). Nests 1 and 12 were partially exposed to the sun, at some period of the day (sunset/ sunrise), while the other five were completely shaded by trees. The nest mounds were constructed largely of dead vegetation.

Temperature monitoring

In 2019 data, preliminary data on temperature cycling were recorded with Incoterm 7665.02.0.00 devices (accuracy ±1.0°C), that record maximum and minimum temperatures each hourly, At each nest, one thermistor with two probes, one positioned approximately 1 meter above the nest (e.g attached to a branch), in shade, and the other within the nest chamber among the eggs. In 2024, Onset MX2201 HOBO Pendant® MX Bluetooth-enabled water temperature data loggers (accuracy ±0.5°C) were used to record chamber and ambient temperatures in the same way, but every two hours. In both years, nests were visited each 5-7 days after deployment to check the integrity of nests, download the data, and in 2019 to reset the Incoterm recorders. Mean nest data for both years were calculated either by the average between minimum and maximum temperatures (2019) or from average of the entire dataset for each nest (2024).

Embryo Samples

In 2019 two embryos from each nest were sampled each 5 days, and in 2024 two embryos were sampled early, in the initial visit, and again after 30 days from nest 2-5. Sample 2 for nest one occurred 48 days after the first sample.

The embryo series described by Vieira et al. (2011) were drawn from six wild nests, in the same study area, and thus as an interim measure, a relative age for our embryos was assigned based on the age-stage relationship reported by Vieira et al. (2011). In our 2019 and 2024 samples, the overall mean nest temperature we report here, in both years, was 31.6°C. Hence the relative age probably reflects mean embryo development rate at or around a mean of 31.6°C.

From the embryos drawn from each nest over time, we used real and relative times between two embryo stages, to calculate the degree to which embryo development rate in each of our nests was higher or lower than the mean rate reported by Vieira et al. (2011). This allowed us to estimate the likely day of laying, the predicted time when development was advanced to the stage of yolk internalization (Stage 27; 76 to 77-days old), and the range of days over which hatching could be expected (Stage 28; 78-91 days).

Hatching Date

Hatching occurs when adult females excavate the nest mound and release the hatchlings. In 2019 we were able to determine hatch day confidently, for both nests, by daily visits during the final days of incubation. In 2024, visits to determine whether the nests had hatched were carried out from November 28th twice a week, on Monday and Friday. The total incubation time was calculated by the estimated age at the first visit until a mid point between Monday and Friday, when the hatching may have happened.

RESULTS

Nests and Eggs

The seven nest mounds had a mean height of 48.42 ± 8.69 cm (SD) and a basal diameter of 140 ± 10.51 cm. Clutch size was 28 ± 7.2 eggs (N=7; range 15 to 37), representing a total clutch mass within the nest mound of 3.66 ± 1.15 kg of eggs (M=7; range 1.6 to 5 kg).

Variation in nest temperature

In the two nests examined in 2019 mean ambient temperature, across each five days, was 27±5.94°C (N = 17; range 18.8°C to 35.1°C), which was significantly lower than the mean clutch temperature of 31.6 ± 1.65°C (N = 17; range 28.5°C to 34.2°C, for the same period. In the 2024 data, mean ambient temperature was 27.2 ±3.6°C (N= 2590; range 21.3ºC to 40ºC) which is not significantly different from 2019, but significantly lower than the mean nest temperature 31.6±1.57°C (N = 2590; range 27.2ºC to 35.9ºC) , which was identical to the 2019 mean nest temperature. The mean difference between ambient and nest temperatures in 2019 (5.2°C) was similar to that in 2024 (4.4°C). The results are consistent with the nest mound maintaining a more stable temperature, with a higher mean than ambient temperature, through a combination of insulation (from both the lowest and highest ambient temperatures) and heating via direct exposure to the sun and/or potential endogenous heat sources (decomposition, metabolic heat)

Embryonic development

The embryos recovered from the 2019 and 2024 nests ranged from stage 5 (12 days) to stage 14 (31 days) of Vieira et al. (2011), assumed to be at a similar overall mean nest temperature of 31.5 ± 1.17 ºC (N = 7; range 30.4ºC to 33.6ºC) found in this study.

Based on the embryonic age at the first sampling (Table 1) the time of laying was mid-to-late August 2019 and mid-August to late September in 2024. In 2019, embryos were 29-31 days old (Stages 13 and 14) when found. In 2024, one nest was 55 days old (Stage 22) and the others 11-29days old (Stages 5-13) when found. The second embryo recovered in 2024 was after a 30-day interval for nests 2 to 5 and 48 days for nest 1.

Nest 1 appeared to be anomalous, with very high temperatures initially (35-36ºC) which declined (32-33 ºC) but remained high relative to other nests (Fig. 2). Based on the estimated age of Embryo 1 (55 days), embryos could have been expected to start internalizing yolk (stage 26; 75-76 days) in about 20-21 days or less (given the higher temperature), and hatch in 78-91, 23-36 days after Embryo 1 was collected. The clutch had not hatched by the 36 days, and so a single egg was removed. It contained a reasonably fresh dead embryo, maybe 5 days dead, that was structurally abnormal. It had swollen feet, pale coloration on the head and body, and was a small hatchling that had internalized a very large yolk volume, with a little yolk still external, and a wide, stretched umbilical area. This nest was left in the field, but subsequently flooded and no further information on the embryos was gained. We could not reject the possibility that all embryos within the nest were compromised or dead when last visited, before the nest was inundated by flood waters.

The embryo development rates we quantified for individual nests (N = 6; Nest 1 excluded) between Embryo 1 and 2 did not deviate significantly from the mean rates reported by Vieira et al (2011). The mean Development Rate Coefficient (hereafter DRC) linking the two was identical (1.005±0.29; SD; N = 6; range 0.96 to 1.04). Nor was variation in the DRC explained by mean nest temperatures recorded ($r^2 = 0.007$;p = 0.87). The general trend was for total incubation time from laying to hatching to increase as the DRC increased, as expected, but it did not reach statistical significance within this small sample ($r^2 = 0.24$;p = 0.325. Total incubation time was not significantly related to the nest temperature ($r^2 = 0.08$;p = 0.57), with the non-significant trend being an increase rather than decrease (slope = 3.3 days per 1 ^{o}C). Whilst accepting the mean ages from Vieria et al (2011) as a form of reference series, the duration of stages, and variation around mean ages is not consistent. The early stages tend to last 1-2 days, but for example Stage 28 (hatching) can range from 1 to 14 days.

The time relations between an advanced embryo internalizing its yolk, and the process of hatching, are not documented in detail here. Vieria e al (2011) considered the yolk enclosing process started in Stage 25 (67-71 days) but was not complete until Stage 27 (76-77 days). So they estimate hatching (78-91 days) occurs 1-14 days after yolk enclosure. In our study the mean predicted total incubation time (based on DRC) was 84.9 ± 2.51. (SD; N = 6; range 81.1 to 87.9), and the real incubation time was 88.7 ± 11.0 (range 69 to 99 days). What actually triggers hatching is not known, and delays could play a role in synchronising the final stages of development, before hatching, if temperature gradients across the clutch were large. On Table 1 we have included some calculations on the potential extent of hatching delay [11.8 ± 10.0 days; range -6.0 to 21.7 days) if yolk internalization was complete at 76.5 days, to provide some guidance as to what may be occurring.

During the monitoring, nest 5 was partially predated by a jaguar, exposing the data logger for two days. At the next visit after the depredation, we reconstructed the nest to continue monitoring the remaining eggs. All the other nests remained intact until the end of the incubation period. After November 28th, nests continued to be monitored by locals until they hatched, which occurred within 4 to 10 days. Only nest 1 did not hatch, as it was flooded by the rising waters.

In addition to variations in incubation time, we observed distinct nest temperature profiles among nests throughout embryonic development (Fig. 2). While concurrent temperature reductions were noted during rainy days from October 28th onward, temperature increases, decreases, and peak temperatures varied substantially across nests. For example, nest 1 maintained consistently high temperatures (>33°C) throughout most of the sampling period, whereas nests 2-5 exhibited lower, more stable temperatures (<33°C). Given the age difference among nests, the potential influence of metabolic heat (MH) production on nest temperature also differed. The late stage for the first sampled Embryo in nest 1 indicated it had already reached the higher embryo growth phase (Vieira, 2011), in which higher MH could be expected (Fig. 2). All nests showed an increase in temperature in the final days of incubation (until the end of the monitoring period) assumed to be due to MH. This increase is more evident in nests 2 and 3, which are their overall maximum temperatures at this time.

DISCUSSION

This study provides new insights into the temperature relations of seven black caiman wild nests, the rates of embryological development that take place within those nests, and the variation in total development time between egg-laying and hatching.

Variation in nest temperature

The 2024 data confirmed that ambient and nest temperatures not only have different means and variances, but they cycle on different time scales. The maximum ambient and nest temperatures occurred at 2pm and 10pm respectively, and minimum temperatures at 3am and 7pm. There is thus an offset of daily temperature cycling of 8 hours for maximum temperatures, and 16 hours for minimum temperatures. A similar process was previously observed in birds (Mersten-Katz et al. 2012) in which the delay between ambient and nest tempertature cycling was attributed to the insulation of the nest provided, increasing its capacity to retain heat within the chamber.

Embryonic development and nest temperature

Temperature is a pivotal environmental variable during the incubation period in reptiles (Gatto and Reina, 2022). It governs the biochemical and biophysical functioning of embryos, and determines embryo developmental rate (Booth, 2017). It determines sex in many reptile species including all crocodilians, shapes phenotype in various ways, and changes growth physiology and developmental plasticity (Singh et al. 2020). Significant changes in phenotype and post-hatching survival probabilities can result from chronic or acute thermal stress (Carlo et at. 2018; Sanger et al. 2018). Embryos have no mechanism for adjusting the temperatures to which they are exposed, and are vulnerable to adverse effects of high or low incubation temperatures (Webb and Cooper-Preston 1989; Hall and Warner, 2021). Embryo developmental rate is highly predictable from temperature if the gas and moisture environments are not limiting (Webb et al. 1987). The changes in general biochemical activity due to temperature (Booth 2017) may speed reaction rates to the point of protein modification and/or and gene expression (Singh et al. 2020).

In our study with *M. niger*, nest 1 had a mean temperature of 33.6ºC, but this was higher (35-36ºC) in the earlier incubation period. None of the clutch hatched, and an advanced dead embryo had a small body and very large yolk. This syndrome in some hatchlings has been linked to metabolic stress, when the embryo enters the growth phase, but gas exchange is limited (oxidative stress), exacerbated by high temperatures: they appear to be trying to enhance development to exit the egg (Hall and Warner 2021; Sanger et al. 2021). Such high temperatures are known to cause high rates of abnormality in *Crocodylus porosus* (Webb and Cooper-Preston 1989), and chronic thermal stress causes morphological abnormalities in other reptiles (Carlo et at. 2018; Sanger et al. 2018). Similar results were also observed by López-Luna et al. (2015) regarding low hatching success in eggs from nests of *Crocodylus moreletii* with extremely variable temperatures, which fluctuated more than 10ºC and rose above 34ºC.

No significant relationship between nest temperatures and total incubation time was apparent in the 6 nests for which we had data, but four nests with mean temperatures 30.4º C to 31.2ºC hatched after 73-76 days, whereas the remaining two, at nest temperatures higher than 32 ºC hatched after 83 and 90 days (Table 1). For *Paleosuchus palpebrosus* (Campos 2022), *Alligator mississippiense* (Rhodes and Lang 1996), *Crocodylys johnstoni* (Webb et at 1983; Webb et al. 1987), *Crocodylus porosus* (Webb et al. 1987), *Caiman Latirostris* (Pina et al. 2003), *Crocodylus acutus* (Simoncini et al. 2019; Cherkiss et al. 2020) and perhaps all crocodilians, incubation time is negatively correlated with incubation temperature. However, *M. niger* may have the option of extending the period between yolk internalisation and hatching, which is likely independent of temperature. The trigger for hatching is unknown, but our nests were uncovered and hatched during the night, presumably by the female, and hence the trigger may be somewhat independent of the hatchlings. That metabolic heat may be responsible for the increasing temperatures observed towards the end of incubation (Fig. 2) is consistent with what has been found in *C. johnstoni* (Webb et al 1987), *C. porosus* (Webb et al. 1987, Webb and

Cooper-Preston 1989) and *Alligator mississippiensis* (Ewert and Nelson, 2003; Rhodes and Lang 1996).

Crocodylia thermal sensitivity

Incubation nest temperatures allowing normal development to hatching, and the higher temperature limits that cause abnormalities and increased probabilities of mortality, have been described for some Crocodylian species but for others, including *M. niger*. Magnusson et al (1985) assembled the historical information available for 14 species, and our *M. niger* results, with mean nest temperatures of 31.6ºC, are typical of most other species. The upper and lower critical thermal limits for normal development are not well understood for most species, and are likely to depend on the embryo stage at which exposure occurs, the period of exposure, whether it represents a baseline shift in temperature change or of a short-term exposure during extreme daily heating or cooling cycle, and ultimately, how it was measured or determined.

For *M. niger*, a mean nest temperature of 33.6 ± 1.39ºC, with short-term peaks reaching 35.9ºC (Nest 1) appears to have exceeded lethal limits. Yet a mean of 32.7ºC ± 1.20ºC, with peaks reaching 34.2ºC (Nest 12) did not. Hence the upper critical limit is likely to be mean nest temperature around 33ºC with peaks not exceeding 34.5ºC. The lowest mean nest temperatures we recorded (30.4ºC ± 0.80ºC; 30.4ºC ± 0.80ºC), nor the minimal spot temperature (27.3ºC) appeared to have adversely affected development. However, Magnusson et al (1985) reported no survivors from two species with nest temperatures around 26ºC, and at 27-28ºC, total development time could be expected to be doubled relative to incubation time at 31.6ºC incubation time (Webb et al. 1987), hence if development abnormalities did not occur, for *M. niger* at least the probability of flood mortality would increase greatly. Table 2 summarizes data on thermal sensitivities for other crocodilian species indicating *M. niger* is broadly consistent with other species.

CONCLUSION

The present study establishes the temperature regime eggs are exposed to in wild *M. niger* nests, and the embryo development rates that occur in those wild nests. Mean nest temperatures between 30.4 and 32.7 ºC resulted in normal development, but a nest with a mean temperature of 33.7 ºC appeared to exceed lethal limits. The cycling of ambient and nest temperatures is out of phase with each other, with a lag time of around 8 hours for maximum temperatures and 16 hours for minimum temperatures. The hatching delay for *M. niger,* between embryos internalizing their yolk and actually hatching, may be more flexible and on occasion unusually extended, but few data are available for comparison.

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FIGURE LEGENDS

Figure 1. Map of the Lago do Cuniã Reserve in the Brazilian Amazon. The blue spot in the map (top left) indicates the location of the reserve on a National scale, as shown in the bottom left figure. The graph (top right) shows historical maximum, minimum and mean water levels at the Madeira River's station near the reserve (1970-2024). Green (2019) and red and yellow (2024) dots in the lower right figure show the location of nests studied.

Figure 2. Mean daily nest temperature in the 5 nests examined in 2024. Red lines reflect when embryos are from stage 22, when growth begins to be significant compared to previous stages

(Vieira, 2011) and the embryos metabolism should be increasing (Whitehead and Seymour, 1990).

TABLE LEGENDS

Table 1. Embryo development rate calculations (DRC). "V. Age" is the mean age in days assigned to development stages by Vieira et al (2011). The "Real Age" between Embryos 1 and 2 ranged from 23 to 40 days. N = no Embryo 2 obtained. DRC = "Real Age Diff."/"V. Age Diff". The "V. Age" time for total development (84.5 days), yolk internalization complete (76.5 days) and laying date (Day 1) were all corrected according to the DRC to calculate predicted real times.

Table 2. Summarized information on thermal tolerance range among Crocodylia families and their respective species

Figure 1

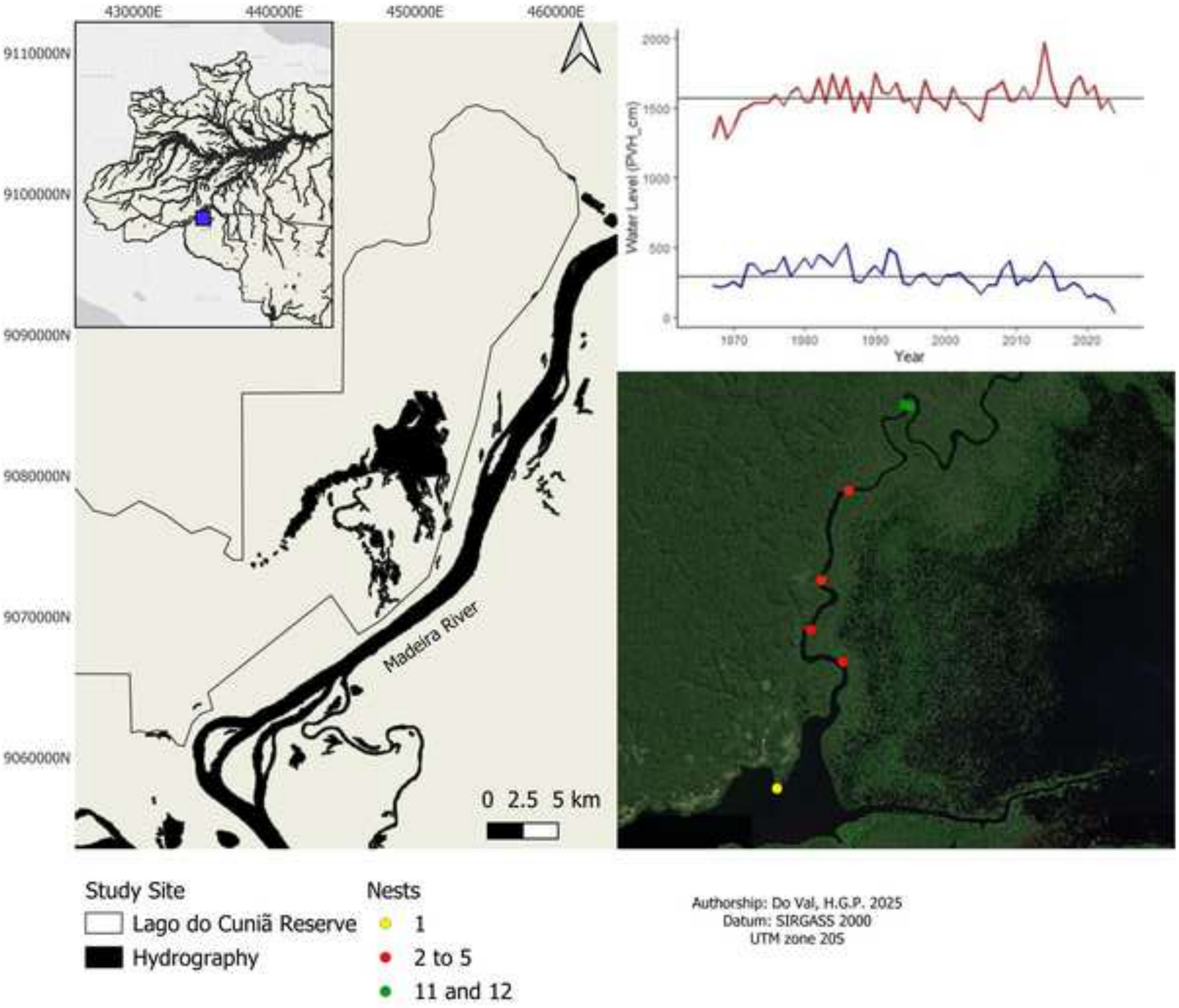
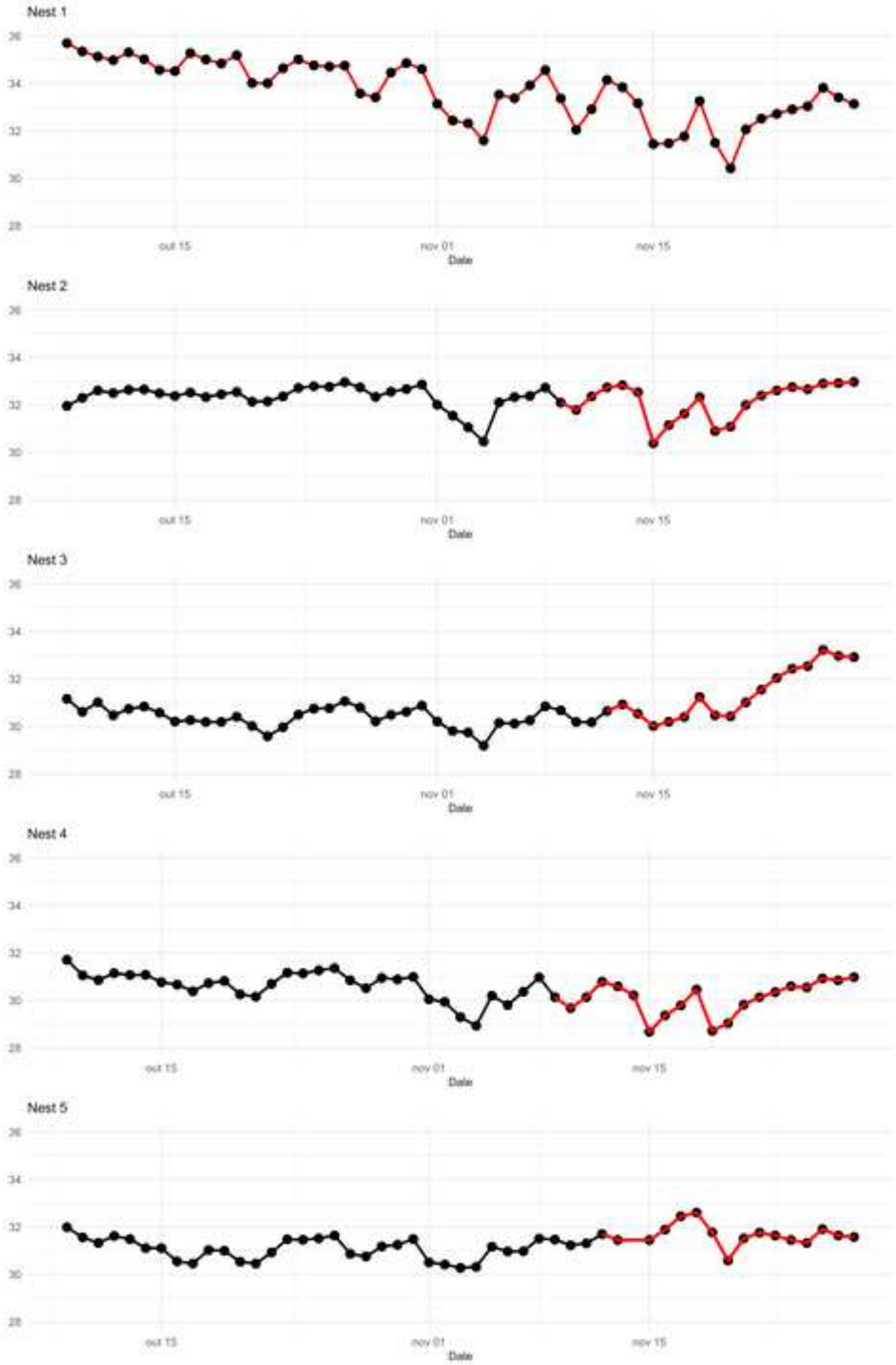

430000E
440000E
450000E
460000E
9110000N
9100000N
9090000N
9080000N
9070000N
9060000N
Madeira River
0 2.5 5 km
2000
1500
1000
500
0
Water Level (PVH_cm)
1970
1980
1990
2000
2010
2020
Year
Study Site
Lago do Cuniã Reserve
Hydrography
Nests
1
2 to 5
11 and 12
Authorship: Do Val, H.G.P. 2025
Datum: SIRGASS 2000
UTM zone 20S

Figure 2


Nest 1
36
34
32
30
28
out 15
nov 01
nov 15
Date
Nest 2
36
34
32
30
28
out 15
nov 01
nov 15
Date
Nest 3
36
34
32
30
28
out 15
nov 01
nov 15
Date
Nest 4
36
34
32
30
28
out 15
nov 01
nov 15
Date
Nest 5
36
34
32
30
28
out 15
nov 01
nov 15
Date

Nest No	Nest Temp. (Mean SD) (ºC)	V. Age Embryo 1	V. Age Embryo 2	V. Age Diff	Real Age Diff	DRC	Pred. Inc time	Real Inc time	Pred Yolk intern	Hatch delay	Est. Inc. time
1	33.6±1.39	54	N	N	N	N	N	N	N	N	N
2	32.2±0.74	28	56	28	27	0.96	81.1	88	73.4	14.6	90
3	30.6±0.85	11.5	49	37.5	38	1.01	85.3	99	77.3	21.7	73
4	30.4±0.80	21.5	54	32.5	33	1.02	86.2	86	78	8	75
5	31.2±0.63	13.5	49	35.5	36	1.02	86.2	98	78	20	76
11	30.4±1.13	28	51.5	32.5	23	0.98	82.8	69	75	-6	75
12	32.7±1.20	30.5	69	38.5	40	1.04	87.9	92	79.7	12.3	83

A Family/ Species	Thermal tolerance range	Bibliography
Alligatoridae		
Caiman latirostris	29 ºC to 33 ºC	Piña et al. 2003
Caiman yacare	29 ºC to 33 ºC	Gomes et al. 2024
Melanosuchus niger	27 ºC to 34 ºC	present study
Alligator mississippiensis	26 ºC < and < 36 ºC	Ferguson and Joanen, 1982
Crocodylidae		
Crocodylus porosus	29 ºC < and < 33 ºC	Webb and Cooper-Preston, 1989; Liu et al. 2023
Crocodylus siamensis	30 °C to 33 °C	Liu et al. 2023
Crocodylus johnstoni	< 34 ºC	Webb et al. 1983b; Webb and Cooper-Preston, 1989
Crocodylus niloticus	29 °C to 33 °C	Liu et al. 2023
Crocodylus moreletti	Up to 33 ºC	López-Luna et al. 2015
Osteolaemus tetraspis	28 ºC < and <32 ºC	Pollard et al. 2017