

Nesting of *Phrynops geoffroanus* (Testudines: Chelidae) on sandy beaches along the Upper Xingu River, Brazil

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ABSTRACT. This work presents the first data on incubation temperature of *Phrynops geoffroanus* (Schweigger, 1812) in a natural environment, and provides information on nest predation, hatching success and size of offspring born in the nests on sandy beaches along the Upper Xingu River. Thirty-one *P. geoffroanus* nests were found, of which eleven were completely predated, mainly by *Cerdocyon thous* (Linnaeus, 1766). Incubation was completed in nine out of the 17 nests protected by netting. The nests presented an average of 13.1 eggs and were distributed over the various geomorphological sectors of the nine sampled beaches. The size and weight of the hatchlings varied significantly between nests, and the incubation period in protected nests lasted for 76.5 days, less than reported for controlled incubation in the laboratory. Daily variation in incubation temperature in the three nests monitored for temperature was lower in those situated in fine sand sediments. Incubation temperature varied from 22 to 39 °C and may have affected hatching success, which reached 60.8% in protected nests. Nest distribution in different geomorphological sectors indicated the plasticity of *P. geoffroanus* in terms of variation in nesting environment, which partly explains the species' broad geographical distribution.

KEY WORDS. Geoffroy's side-necked turtle; hatching success; incubation; nest predation; Xingu Indigenous Park.

Geoffroy's side-necked turtle, *Phrynops geoffroanus* (Schweigger, 1812), is the freshwater chelid turtle with the broadest geographical distribution in South America; it is found in all the main river basins (PRITCHARD & TREBBAU 1984, BALDO *et al.* 2007, VOGT 2008). Despite this broad distribution and common occurrence in the most varied aquatic environments (SOUZA 2005, RUEDA-ALMONACID *et al.* 2007), and although it is one of the most commonly found neotropical urban animals (SOUZA & ABE 2001, MARTINS *et al.* 2010) there are still taxonomic doubts about the occurrence of sub-species or cryptic species within the group. Some authors call the various occurrences of this freshwater turtle the “*geoffroanus* complex” (RHODIN & MITTERMEIER 1983, MCCORD *et al.* 2001, SOUZA 2005, VOGT 2008).

Egg-laying often takes place in shady areas and clay soils along the banks of rivers and lakes (MEDEM 1960). However, the females may migrate hundreds of meters from the river to lay eggs in open sites and among creeping vegetation (VOGT 2008). Although *P. geoffroanus* does not show gregarious behavior, its nests may be placed near one another (MEDEM 1960, VOGT 2008). Incubation is relatively prolonged, as with the other

chelid turtles. There are no data available about incubation temperature in a natural environment, but in the laboratory embryonic development is viable at least between 25 and 32 °C (KARDON 1981, EWERT *et al.* 2004, LISBOA *et al.* 2004). It is worth noting that, unlike some turtle species, the sex of *P. geoffroanus* is genetically determined rather than being affected by environmental temperature during incubation (EWERT *et al.* 2004). Females lay eggs at least four times in any one reproductive season (VOGT 2008), and the nests may contain up to 30 spherical eggs, about 3 cm in diameter, with a hard shell (SOUZA *et al.* 2006, SOUZA & ABE 2001). The nests are generally shallow, not exceeding 20 cm in depth (MEDEM 1960).

Reproductive parameters, such as litter size, predation rate and hatching success, are important components in the species' life history and essential to management and conservation projects (SOUZA & VOGT 1994, CONGDON *et al.* 2000, FERREIRA JÚNIOR & CASTRO 2010). For neotropical freshwater turtles these parameters are little known (SOUZA *et al.* 2006), and for *P. geoffroanus* data on reproduction are mainly taken from captive individuals. This work presents the first data on incuba-

tion temperature in the natural environment and provides information on predation, hatching success and size of *P. geoffroanus* hatchling born in nests on sandy beaches along the Upper Xingu River, in the Parque Indígena do Xingu (Xingu Indigenous Park), state of Mato Grosso, Brazil.

MATERIAL AND METHODS

The Parque Indígena do Xingu, the first indigenous territory recognized by the Brazilian government in 1961, covers about 30,000 km² and is a good example of well preserved forest. In recent years deforestation has increased in the area surrounding the Park, motivated by the expanding frontiers of agriculture and livestock grazing. This has made the Park a particularly important area in which to study *P. geoffroanus* in a natural environment. The region is situated in a transition zone between the Cerrado and the Amazon biome, so the monitored laying sites are often covered by sparse bushy herbaceous vegetation that provides little shade. These areas may be classified within the common Cerrado types as grassland, wet campo, savannah woodland, and gallery forest.

During the period of low water, from June to October, sand-bars known as beaches appear along the principal rivers of the Amazon basin. These are used as a nesting area by numerous species of turtle, especially those of the genus *Podocnemis*. In the Xingu Indigenous Park the beaches are formed in point bars made up of sandy sediments. In some beaches the stretches downstream and near the internal bank of the channel made by this point bar may have a centimetric to decimetric clay-silt layer that covers the sandy sediments.

The area monitored in this project includes nine consecutive beaches that lie along 13 km of river (from 11°55'49.34"S, 53°32'56.16"W to 11°59'08.19"S; 53°28'12.62"W). The edges and banks of the rivers and lakes were not monitored, and the nests were only searched for on beaches (Fig. 1).

Three nests located on beaches 4 (11°56'03.67"S, 53°31'04.83"W), 5 (11°56'58.03"S, 53°30'21.58"W) and 6 (11°56'38.12"S, 53°29'32.19"W) were monitored with dataloggers (Ibuttons Maxim DSG1921G), which recorded the temperature every 60 min during incubation. The dataloggers were inserted among the eggs at the center of the nest. The nests were protected with wire netting to prevent predation, which is intense in the area. The net was made of metal wire, 2 cm mesh, with 40 cm diameter, covering the nest completely. The possible influence of the netting on the nest temperature was not evaluated. After hatching, the hatchlings were measured (margin of error ± 0.01 mm) at the height of the shell, at the straight carapace length and width; they were weighed (± 0.1 g) and released into an internal lagoon on beach 5. On hatching, 100 g of sediments were collected from each nest to evaluate the texture characteristics in the egg-laying areas. The sediments were sieved and the granulometric fractions were classified into pebble, granule, very coarse sand, coarse sand,

medium sand, fine sand, very fine sand and mud (Folk 1974). To calculate the height of the nest in relation to the river level a CST Berger (± 1 cm) was used and the daily variations in river level were monitored with a limnetic ruler. October 3rd was adopted as the reference date for comparison of height of nests where eggs had been laid on different days and beaches. On this day the Xingu River reached its lowest level during the 2010 reproductive season.

Variation in size and weight of the hatchling and in the incubation temperature of the monitored nests was analyzed using a Kruskal-Wallis One Way Analysis of Variance on Ranks with an *a posteriori* Dunn test.

RESULTS

In October, when the wet season begins, the lowest parts of the beaches are flooded, often submerging the nests of *Podocnemis unifilis* (Troschel, 1848), which are common in these parts. In the study area within the Xingu National Park, *P. unifilis* nests in almost every parts of the beach. *Phrynops geoffroanus* shares the nesting areas with *P. unifilis*, with overlap in the sites. The large size of the beaches and the low nest density, however, prevents females from destroying other nests while they are laying eggs.

Thirty-one *P. geoffroanus* nests were found on nine beaches along the Xingu River during the 2010 reproductive season. Eleven nests (35.5%) were completely predated; of these, one was predated by ants, five were predated before being protected by netting, five were completely predated by the crab-eating fox, *Cerdocyon thous* (Linnaeus, 1766), after being covered by netting. This fox's tracks were sighted relatively frequently and one individual of the species was seen circling the nests of *P. geoffroanus* and *P. unifilis*. Predation of the nests by *C. thous* was identified based on footprints and claw marks along the edges of the nests situated in clay silt substrate. The eggs in one nest did not present any sign of embryo development. One nest that was found after partial predation was then protected and incubation was completed. Eight nests were identified after hatching, when hatchlings and their tracks were found. Two protected nests were flooded and all the embryos died. Out of the 17 protected nests, 10 completed incubation.

The mean hatchling weight (9.1 ± 1.4 g; range = 6 to 11.5), length of the carapace (38.3 ± 2 mm; range = 31.5 to 41.3), width of carapace (30.1 ± 1.83 mm; range = 24.7 to 33.6), and height of shell (13.9 ± 1.06 mm; range = 11.3 to 16.1) were calculated for the 83 individuals from the 10 protected nests in the Xingu Indigenous Park. The nests presented a mean of 13.1 ± 2.38 eggs ($n = 10$; range = 9 to 17 eggs). The size (length of the carapace: Kruskal-Wallis, $H_{5,56} = 22.8$; $p < 0.001$; width of carapace: $H_{5,56} = 33.8$; $p < 0.001$; height of shell: $H_{5,56} = 13.7$; $p < 0.001$) and the weight of hatchlings (Kruskal-Wallis, $H_{5,56} = 43.1$; $p < 0.001$) varied significantly between nests.

Hatching success was considered to be the relationship between the number of hatchlings and the total number of

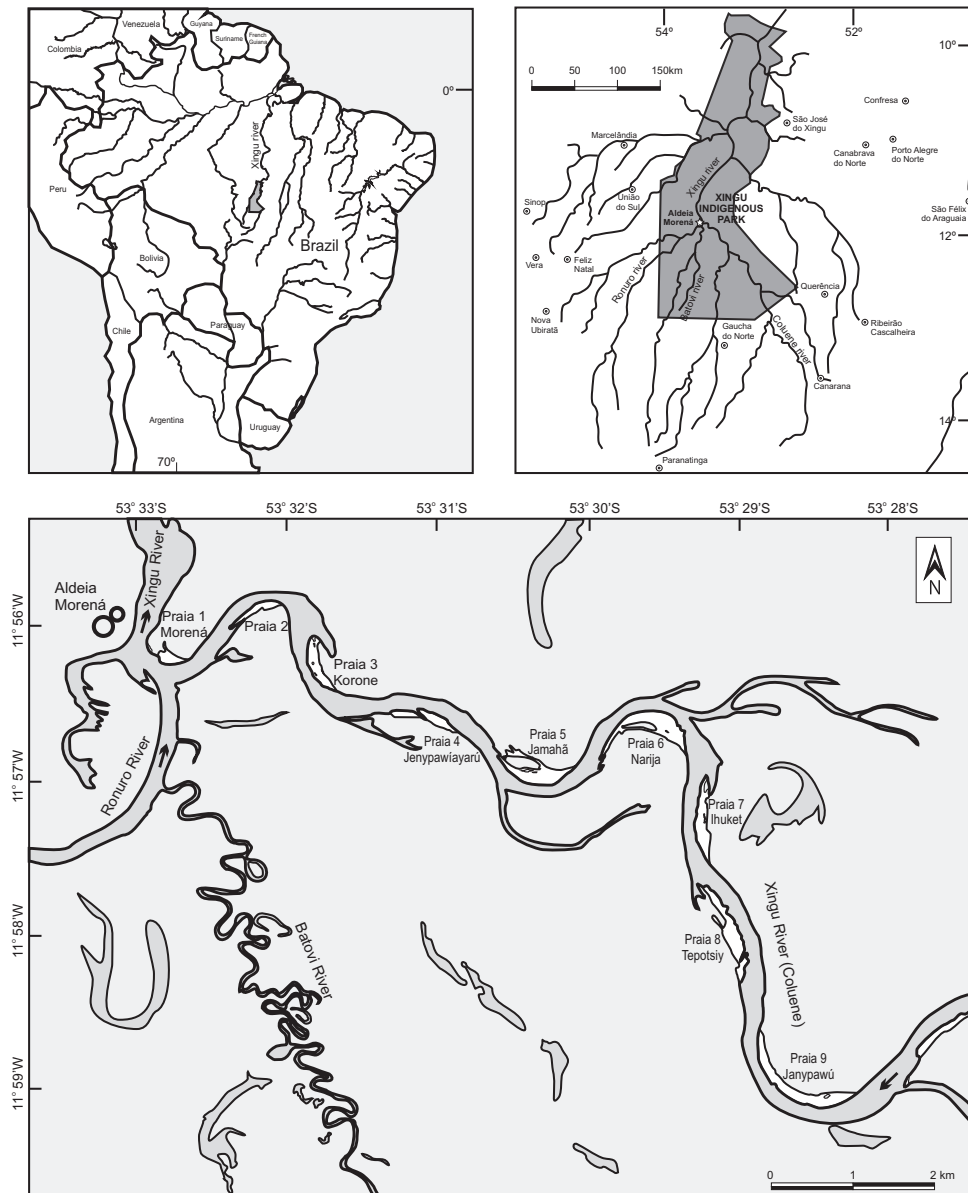


Figure 1. Localization map of the studied area.

eggs containing an embryo; in the 10 protected nests it was $60.8 \pm 37.5\%$ (range of 0 to 100%). The duration of incubation, considered as the period, in days, which passed between laying and first hatching, was 76.5 ± 9.65 days for six protected nests (range between 65 and 94 days). The daily variation in incubation temperature in three monitored nests (Tab. I) was lowest in the nest situated in sediments classified as well selected fine sand ($D_{50} = 164 \mu\text{m}$) and differed significantly from the nests found in well selected medium sand ($D_{50} = 305 \mu\text{m}$ and $D_{50} = 289 \mu\text{m}$) (Kruskal-Wallis, $H = 118.6$; $p < 0.001$).

The mean maximum height of the nests, in relation to the Xingu River level, was 211 ± 60.1 cm ($n = 10$; range 144 to 219 cm). The nests were situated mainly in the upper parts of the beaches, within open areas near the transition to vegetation. In this transition belt, the soil frequently has a sandy-silt layer, several centimeters deep and with a crisp consistency, over which creeping vegetation grows. The nests in this belt were normally found only after predation, because it is difficult to preserve and notice tracks and other signs of excavation in the sandy silt.

Table I. Variation in incubation temperature for *P. geoffroanus* on beaches along the Xingu River. The temperatures were measured every hour. Mean \pm SD (range).

Nest	Beach	Period	Average temperature (°C)	Daily variation (°C)	Substrate
1	6	4/8 – 18/10	28.6 \pm 2.01 (23.5 – 33.5)	4.7 \pm 1.03 (2.5 – 8.0)	Well selected fine sand
2	4	21/8 – 12/11	28.9 \pm 2.79 (22.0 – 37.0)	7.1 \pm 1.62 (2.0 – 11.5)	Well selected medium sand
3	5	28/8 – 12/10	29.7 \pm 3.87 (19.0 – 39.0)	10.7 \pm 2.38 (3.0 – 18.5)	Well selected medium sand

DISCUSSION

The open sandy beaches of the Xingu River presented a low density of nests, which suggests that *P. geoffroanus* nests in these places may occur in a secondary manner, subsidiary to other nesting sites, such as along the edges of rivers, creeks and lakes (MEDEM 1960, SOUZA & ABE 2001). The nests are found distributed throughout the various geomorphological sectors of the beaches, as occurs with *P. unifilis*, which lays eggs on the same beaches. The nests were found on the open beach, at the river's edge and in the belt between the beach and the adjacent vegetation (e.g. gallery forest and typical savannah woodland).

Phrynops geoffroanus lays its eggs in synchrony with the variation in level of the Xingu River, as do other species of Amazonian turtles, such as *Podocnemis expansa* (Schweigger, 1812) (ALHO & PÁDUA 1982, FERREIRA JÚNIOR & CASTRO 2006, 2010), *Podocnemis sextuberculata* (Cornalia, 1849) (PEZZUTI & VOGT 1999) and *P. unifilis* (FERREIRA JÚNIOR & CASTRO 2010). When nesting occurs on the beaches, the duration of incubation should be compatible with the low water period, and hatching should take place before the beaches flood. The length of time that *P. geoffroanus* incubates on the Xingu River is less than durations recorded in the laboratory, which were between 115 and 186 days (LISBOA *et al.* 2004); between 156 and 173 days (KARDON 1981); between 206 and 319 days (GUIX *et al.* 1989) and between 149 and 331 days (Flavio de B. Molina, unpubl. data). These durations would be incompatible with incubation on the Xingu River, since at the beginning of October the river level started to rise, flooding the beaches. VOGT (2008) suggested that the incubation period of *P. geoffroanus* varies according to temperature and humidity, which control embryo diapause and aestivation (DOODY *et al.* 2001). In natural surroundings, under a fluctuating daily temperature regime, embryo diapause would not take place. If it did, it would have a slight effect on incubation, allowing this to be shorter and the embryos to develop without being flooded. The shorter duration of incubation associated with the low water period in the Xingu River allows *P. geoffroanus* incubation to take place in open areas of the beaches, with little or no risk of flooding and drowning the young.

The average incubation temperature in the nests was within the limits recorded in laboratory work, where incubation lasted longer (KARDON 1981, GUIX *et al.* 1989, LISBOA *et al.* 2004). Daily temperature variation influences the duration of incubation (GEORGES *et al.* 1994, 2004) and there may be a rela-

tionship between temperature and the shorter incubation time observed in the natural nests of *P. geoffroanus* along the Xingu. The shallowness of the nests and the wide temperature variations led to extremes in temperature, which reached 22 and 39 °C, and this may have affected hatching success. The distribution of the nests in various geomorphological sectors indicates great plasticity in *P. geoffroanus*, in terms of nesting environment. As sex determination of *P. geoffroanus* is genetic (EWERT *et al.* 2004) the species is free of incubation temperature's influence on the gender of its young, and may therefore make use of a greater variety of nesting sites.

The size of the litter and the dimensions and weight of young born on these beaches are similar to what has been described in other environments, such as the sandy beaches of the Guaporé River (VOGT 2008), clay riverbanks (MEDEM 1960) and urban rivers and creeks in areas changed by humans (SOUZA & ABE 2001). However, when only the beaches of the Xingu River are evaluated, it can be observed that the size and weight of the young differ between nests, and the incubation temperature is also influenced by nesting site. There is a well established connection between the effects of humidity and temperature on the phenotype of turtle hatchlings (PACKARD 1999, HEWAVISENTHI & PARMENTER 2001, FERREIRA JÚNIOR *et al.* 2007, BUJES & VERRASTRO 2009). More detailed studies are necessary to understand how environmental characteristics influence humidity and temperature, and how the fluctuating daily temperature regime (GEORGES *et al.* 2004) affects embryo development and hatchling phenotype in *P. geoffroanus*. As the species presents a wide geographical range with various ecological characteristics (SOUZA 2005), physiographic characteristics may play an important role in reproductive aspects, being reflected in population structure (SOUZA & ABE 2001).

The availability and type of predator are important components in reproductive success, mainly for freshwater turtles, which undergo the most critical moment of their lives in incubation. SCHNEIDER *et al.* (2009) reported predation of 92% (n = 39) of nests of *P. geoffroanus* by *Tupinambis teguixin* (Linnaeus, 1758) in the Guaporé River. FERREIRA JÚNIOR *et al.* (2010) reported predation of 97% of nests of *P. unifilis* by birds in the Javaés River. In the Xingu Indigenous Park, 26% of the nests (n = 31) completed incubation without any type of protection, and the main predator was *C. thous*. In this area there is no hunting pressure on *P. geoffroanus*, which is not consumed by the indigenous population. Capture may take place occasionally, and

the animal is sometimes kept as a pet in indigenous villages. The high predation rate on nests shows the importance of preserving the nesting areas (CONGDON *et al.* 2000) and, even more, the adult chelids along the Upper Xingu River.

This work presents data on the reproductive biology and incubation period of *P. geoffroanus* in its natural environment in the headwaters of the Xingu River basin. The incubation period in natural surroundings was shorter than in the laboratory. It remains to be investigated if this reduced incubation time is due to the genetic variation of the populations that were compared with those in laboratory experiments, taken from different areas in the broad distribution range of this species, or if they arise from local physiographic parameters. Only with further data obtained in natural habitats in other regions within the species' range will it be possible to make comparisons within the species as a whole.

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