



Neonatal biometry and characterization of Amazonian Turtle nests (*Podocnemis expansa*)

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ABSTRACT. The present study aimed to study the reproduction of the Amazon turtle (*Podocnemis expansa*) through the evaluation of the reproductive parameters of adult females. This study was carried out in the Crixás-Açu River, municipality of Mundo Novo, state of Goiás, Brazil. In September, the biometrics of 20 females were evaluated during the spawning period. Their nests were marked for subsequent evaluation of hatchlings, measuring the distances of each nest to the river and vegetation. The second stage consisted of the evaluation of hatchlings and characterization of nests after egg hatching. The data were tested using the Pearson Correlation to measure the degree of linear correlation between the variables analyzed, such as the parameters of females, hatchlings, nests, and eggs. Nest depth was positively correlated with the number of eggshells found. We also found a correlation between the number of eggshells and the distance of nests to the vegetation. Although moderate, this result indicates that as the distance to the vegetation increases, the number of eggshells found in nests decreases. The spawning site for Amazon turtle breeding is crucial to determine a higher number of eggs hatching and better development of hatchlings. However, the literature is still very scarce regarding the choice of spawning grounds and the influence that vegetation may have on the development of freshwater turtle hatchlings in Brazil.

Keywords: amazon turtle; reproductive parameters; biometrics; spawning period.

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Introduction

Brazil is one of the most biodiverse countries in the world, with more than 20% of the total number of species on Earth *Podocnemis expansa*, popularly known as the Amazonian turtle, suffers from predatory hunting, commercialization of shells, and capture of eggs to be used as oil since the discovery of Brazil (Ribeiro & Navarro, 2020).

Podocnemis expansa is the largest freshwater species in South America (Rodrigues, 1992). The reproductive activity of chelonians is strongly related to climatic conditions, where rainfall indexes and water and air temperatures are determinant factors for these animals' behavior (Souza, 2004). They are also selective concerning the egg position in comparison with *P. unifilis* (Castro & Ferreira Júnior, 2008), where they prefer fine sandbanks with little gravel and free of vegetation.

The spawning grounds of the Amazonian turtle are not randomly chosen. During the spawning period, the females walk along the beach, evaluating the characteristics of sandbanks to find the ideal nesting site (Vanzolini, 1967). Ferreira-Júnior, Castro, Addad, Lorenzo (2003) assessed the influence of the spawning site of sea turtles on hatching success and sex ratio of hatchlings. They found that the spawning site choice directly affects the reproductive success of these chelonian species. Therefore, the knowledge about this behavior is of great interest for understanding the reproduction of overall chelonians, including *Podocnemis expansa*.

Therefore, it is essential to contribute to the knowledge about the reproductive, biological, and behavioral patterns of these animals. Understanding which environmental parameters influence female reproductive success during nesting is also relevant for species management. This knowledge is crucial to guarantee the protection and preservation of species and ecosystems used for spawning by females, and thus, preventing the endangerment of Amazonian turtles. In this sense, the objective of this study was to evaluate the reproductive parameters of Amazonian turtle (*Podocnemis expansa*) females and hatchlings regarding the spawning site in the Crixás-Açu River, municipality of Mundo Novo, state of Goiás, Brazil.

Material and method

This study was carried out in the Crixás-Açu River (13 ° 32 '45.2 "13.5459 ° S, 50 ° 15' 19.9" 50.2555 ° W), which is a tributary of the Araguaia River, in the municipality of Mundo Novo, state of Goiás, Brazil. The beaches of this river are abundant from July to November and have a spawning history for the Amazonian turtle. This study consists of two stages: first, the evaluation of females during the spawning period and nest marking, and second, the evaluation of hatchlings and nest characterization after egg hatching.

We evaluated 20 females during the spawning period from September to October 2017. The shell width and length were measured using a 1 m length wood pachymeter. Their respective nests were marked for a subsequent evaluation of hatchlings, eggshells and eggs that did not hatch during the hatching period. The location of the nests was established considering the distance of each nest to the riverbank and vegetation. We measured the distance using a tape measure and identified the nests with wood stakes containing data regarding the female that spawned the nest.

We evaluated 20 nests and 10% of the hatchlings at different points along the spawning ground from September to November 2017. We measured the nest depth and width and the distance between each nest to the riverbank and vegetation using a tape measure. Besides, we measured the thermal conditions, humidity, and soil, in which the eggs were laid. We randomly chose 10% of the hatchlings from each nest and weighed them on a precision scale. Besides, we measured the length and width of the carapace and plastron with digital calipers, in addition to looking for irregular conformation patterns of the carapace and plastron. The turtles were released after measured (Salera-Junior, Malvasio, & Portelina, 2009). The collection was authorized by SISBIO No. 51602-2, with protocol 65/2017 from the animal ethics committee of the *Universidade de Brasília* (UnB).

Sand samples were collected from the bottom and surface of 16 nests in September and 22 nests in November. These samples with approximately 500 g each were frozen at 253.15 K and submitted to granulometric analysis (Folk, 1974) in the Soil Physics Laboratory of the Faculty of Agronomy and Veterinary Medicine (FAV) of the *Universidade de Brasília*. The granulometric analysis was performed by sieving, dividing the segments into fraction sizes of 4 mm, 2 mm, 1 mm, 0.5 mm, 0.25 mm, and bottom portion (amount passed in all sieves).

Mineralogical analyses to assess the demonstrated the concentration of calcium, aluminum, phosphorus, and pH of the sand samples were also performed in the Soil Physics Laboratory. For such mineralogical characterization of mineral species, fragments, and concretions, a stereoscope microscope, petrographic microscope, and occasionally the X-ray diffraction technique were used to analyze granules of a dubious or altered nature. Chemical microanalyses are used for manganese and carbonates when present in mineralogical constituents (Empresa Brasileira de Pesquisa Agropecuária [Embrapa], 2009).

The Pearson Correlation (r) analysis of reproductive parameters of the Amazonian turtle regarding the distance of each nest to the vegetation present in the beach was tested using the Pearson correlation function in the Excel software 2007. The Pearson correlation index measures the degree of linear correlation between two quantitative variables (Crespo, 2002).

In order to define the correlation intensities, we applied the proposal of Dancey and Reidy (2006), defined as: weak correlation ($0 < |r| < 0.4$), moderate ($0.4 \leq |r| < 0.7$) and ($0.7 \leq |r| \leq 1$) strong correlation.

Results

The females analyzed in September laid their eggs at different distances to the vegetation present on the beach of spawning in the Crixás-Açu River. We categorized these distance into two groups: far and near the vegetation. The mean and standard deviation values of the reproduction parameters of females according to the distance of laid eggs can be observed in Table 1.

Table 1. Biometry of females that lay their eggs near and far from the vegetation in September in the Crixás-açu river.

| | Near the vegetation | Away from the vegetation |
|-----------------------------|---------------------|--------------------------|
| Weight (g) | 25.75 ± 2.72 | 28.67 ± 6.72 |
| Length of the plastron (cm) | 51.58 ± 14.57 | 59.96 ± 3.97 |
| Plastron Width (cm) | 24.58 ± 0.64 | 25.62 ± 2.34 |
| Carapace length (cm) | 65.13 ± 4.48 | 67.10 ± 5.91 |
| Carapace width (cm) | 48.26 ± 2.01 | 47.88 ± 4.80 |
| Carapace curvature l (cm) | 67.44 ± 2.09 | 69.74 ± 5.07 |
| Width of the curvature (cm) | 55 ± 1.90 | 56.74 ± 5.15 |

Weight, plastron length, plastron width, carapace length, and carapace width of the 17 females analyzed weakly correlated with the vegetation distance ($r \leq 0.4$). These are not significant results regarding the location they deposited their eggs. Thus, female weight and other dimensions did not significantly correlate with the site choice for laying the eggs.

The correlation between egg weight ($r = -0.5821$), the number of broken eggs ($r = 0.10659$) and oil eggs ($r = 0.3636$) and distance to the vegetation showed that the distance to the vegetation influence weight of the eggs deposited by *P. expansa* (Figure 1). Therefore, the egg weight decreases as the distance to the vegetation increases.

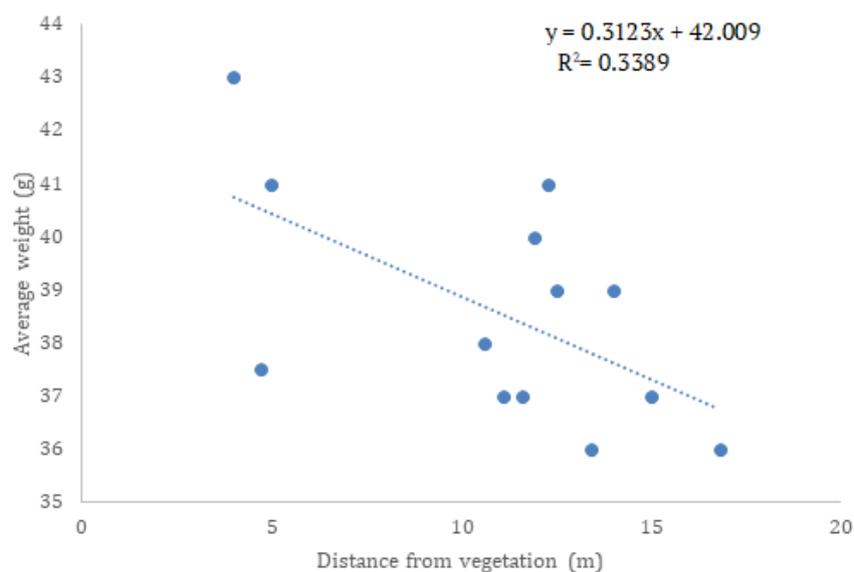


Figure 1. Relationship between the average weight of the eggs of the amazonian Turtle and the distance that the nests are from the vegetation.

We found a significant relationship between nest depth and background temperature ($r = -0.47069$). Therefore, nest depth moderately influences the nest bottom temperature, which is precisely the place where the eggs are laid.

The mineralogical analysis showed no significant results between sands collected in September and the distance to the vegetation. Thus, we found no significant relationship between the grain sizes of the nest bottom and nest sand samples in September and the number of eggs deposited there.

The mean and standard deviation values of nest parameters of the Crixás-Açu River analyzed in November are indicated in Table 2.

Nest depth and width, as well as eggs not hatched in each nest, weakly correlated with the distance to the vegetation. However, we found a significant correlation between nest depth and the number of unhatched eggs. Linear regression was performed between these two parameters, showing that deeper nests had a lower number of unhatched eggs (Figure 2).

The number of shells found in nests is also related to nest depth ($r = 0.5636$). The number of eggshells found in nests is indicative of how many hatchlings hatched in each nest and is widely used to calculate hatching success.

Table 2. Means and standard deviations of the parameters analyzed in *Podocnemis expansa* nests, Crixás-açu river, Goiás, Brazil.

| | Near the vegetation | Away from the vegetation |
|-----------------------------|---------------------|--------------------------|
| Depth (cm) | 62.28 ± 6.47 | 65.5 ± 6.56 |
| Width (cm) | 31.0 ± 3.95 | 205.0 ± 5.87 |
| Camera diameter (cm) | 32.28 ± 4.57 | 28.00 ± 12.42 |
| Distance of vegetation (cm) | 6.14 ± 2.54 | 25.16666 ± 7.30 |
| Eggs with oils (cm) | 1.57 ± 1.27 | 0.6666 ± 0.51 |
| Dead neonates (cm) | 0.57 ± 0.78 | 0.3333 ± 0.81 |
| Eggs hatched | 10.57 ± 13.91 | 4.66 ± 2.16 |
| Shells | 81.57 ± 24.90 | 71.83 ± 16.19 |

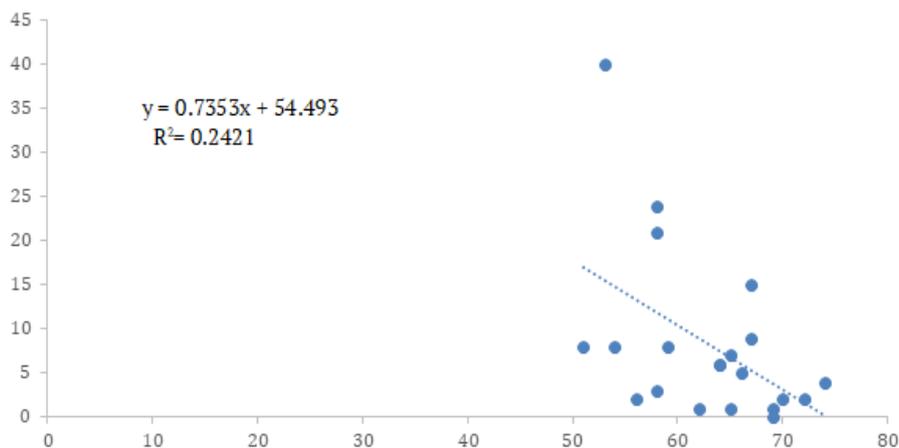


Figure 2. Number of eggs not hatched in relation to the depth of each nest.

The number of eggshells negatively correlated with the distance to the vegetation ($r = -0.4792$). Although moderate, this correlation indicates that as the distance to the vegetation increases, the number of eggshells found in nests decreases (Figure 3). Thus, we infer that nests removed from the vegetation had less hatching success since they had fewer eggshells.

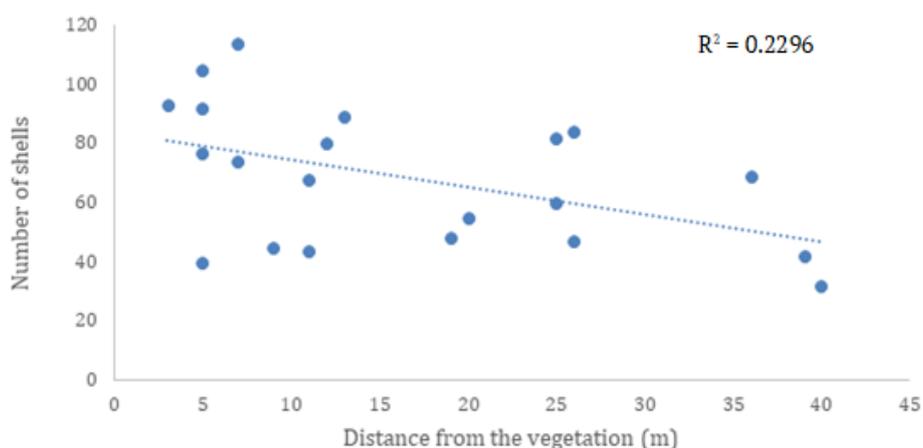


Figure 3. Number of egg shells in relation to vegetation distance.

In the November collection, the biometric parameters of *P. expansa* hatchlings were recorded, and their mean and standard deviation values were measured, which can be found in Table 3. We found no significant result when comparing the mineralogy analysis results of the sand samples with the distance of nests to the vegetation (Table 4 and 5). Besides, we did not find a significant result regarding the relationship between the distance to the nest with the granulometric composition of the sand samples collected in November.

Table 3. Analysis of mean and standard deviation of the biometric parameters of the newborns of Amazon Turtle, Crixás-açu River, Goiás.

| | Near the vegetation | Away from the vegetation |
|------------------------|---------------------|--------------------------|
| Weight | 27.22 ± 1.58 | 26.16 ± 1.98 |
| Amount of carapace | 24.0 ± 0.0 | 24.0 ± 0.0 |
| Carapace length | 5.81 ± 0.28 | 5.75 ± 0.27 |
| Carapace width | 5.80 ± 0.25 | 5.65 ± 0.27 |
| Length of the plastron | 5.39 ± 0.32 | 5.31 ± 0.19 |
| Plastron Width | 5.40 ± 0.41 | 5.38 ± 0.30 |
| Deformed | 2.00 ± 2.19 | 0.50 ± 0.54 |
| Number of puppies | 24.33 ± 16.63 | 19.33 ± 14.67 |

Table 4. Analysis of mean and standard deviation of mineralogy performed in the sand samples of the *Podocnemis expansa* nests in the Crixás- açu river, Goiás, in the month of September.

| | Near the vegetation | Away from the vegetation |
|-----------|---------------------|--------------------------|
| pH | 6.50 ± 0.12 | 6.41 ± 0.07 |
| Calcium | 0.72±0,36 | 0.43 ± 0.16 |
| Aluminum | 0.00±0.00 | 0.18 ± 0.40 |
| Potassium | 5.19±3.05 | 20.30 ± 29.77 |
| Phosphor | 8.28±1.32 | 8.47 ± 1.48 |

Table 5. Analysis of mean and standard deviation of mineralogy performed in the sand samples of the Amazonian turtle nests in the Crixás-açu river, Goiás, in the month of November.

| | Near the vegetation | Away from the vegetation |
|-----------|---------------------|--------------------------|
| pH | 6.28 ± 0.31 | 6.50 ± 0.33 |
| Calcium | 0.14 ± 0.05 | 0.13 ± 0.11 |
| Aluminum | 0.18 ± 0.40 | 0.33 ± 0.70 |
| Potassium | 23.60 ± 12.64 | 15.06 ± 7.27 |
| Phosphor | 9.67 ± 3.09 | 9.55 ± 3.53 |

Discussion

The preference for spawning near vegetation is not related to the size and weight of females in this research. However, Bustard and Greenham (1968) found that some turtle species prefer to spawn near vegetation because of the higher chance of success at the time they dig the nest since the plant roots would prevent the nest collapse.

The presence of vegetation may influence the turtle's choice of spawning location (Mortimer, 1995). However, Pantoja-Lima et al. (2009) found t stated that the Amazonian turtle females have a pattern of choice of nesting site, tending to spawn in higher beach sites. That is, it is possible that their choice is related to the beach height, avoiding the flood of nests, and consequently, choosing sites close to the vegetation.

The weight of eggs in nests closer to the vegetation was significantly different from those laid in more distant nests. Amazonian turtle females may have preferred to deposit their eggs close to the vegetation, attempting to incubate them in places of optimal temperature and humidity (Ferreira Júnior, 2009). That allowing the eggs to have better development, and as a consequence, a greater weight. These results were also observed by Packard et al. (1999).

The development of *Podocnemis expansa* eggs can be directly influenced by the water environment by the incubation site. According to Gutzke and Packard (1986), the eggs in a humid environment absorb water and generate larger hatchlings, whereas the eggs in a dry environment are dehydrated. Besides, the vegetation shading over the nests causes a reduced temperature and water evaporation from the eggs, allowing them to have a higher weight.

In this study, the number of eggshells correlated with nest depth ($r = 0.5636$). Nest depth is a parameter that influences the hatching success of hatchlings and also their sexual determination (Morreale, Ruiz, Spotila, & Standora, 1982; Spotila, Standora, Morreale, & Ruiz, 1987). A study suggests that more females are produced at the top portion of the nests, and as the depth increases and the temperature decreases, more males are generated (Ferreira Júnior, 2009). Thus, nest depth may cause the incubation temperature of the eggs to be adequate for the better development of the hatchlings, and thus, allow more eggs to hatch. Davenport (1997) and Ferreira Júnior & Castro (2006) found that the temperature of nests varies according to the depth. Besides, Pignati and Pezzuti (2013) found that nests close to the vegetation have lower temperatures than nests located in areas of lower vegetation cover. Finally, ++ Restrepo, Piñeros and Páez (2006) found that 82% of *Trachemys callirostris* nests were located next to herbaceous vegetation.

In this study, we found no significant relationship between the granulometric analysis and nest parameters, such as the distance from each nest to the vegetation and river. That is because the sand granulometry in every beach of Crixas-Açu River is homogeneous. Ferreira Junior and Castro (2003) observed that *Podocnemis expansa* has a preference for coarser sand.

Some studies with sea turtles suggest that the distance from the water provided higher hatching success. Hays and Spakman (1993) argue that the success of *C. Caretta* hatching increased in the Mediterranean as the distance from the sea increased. However, the influence of vegetation on the hatching success of chelonians is poorly discussed in the literature, and it is generally associated with the sexual determination of the neonates (Morreale et al., 1982; 82 Spotila et al., 1987).

The number of eggshells was correlated with the distance to the vegetation ($r=-0.4792$), indicating that the further the vegetation, the fewer eggs hatch in the nest. Therefore, we can infer that the relationship between vegetation distance and the number of eggshells indicates a tendency for nests more distant from the water and closer to the vegetation to be more successful since the effect of the tidal flood on nests is reduced (Ferreira-Júnior, Castro, Addad, & Lorenzo, 2003). Thus, laying eggs in nests closer to the vegetation may be a behavior adopted by females during nesting to adjust the incubation conditions of their eggs since nests close to the vegetation had more hatchlings. The same could be used for the Amazonian turtle, which had higher hatching success when the females laid their eggs in sites closer to the vegetation, a behavior that can avoid flooding the nests during the river flood.

Conclusion

We conclude that the highest hatching rate and egg weight were in nests close to the vegetation. The vegetation had a positive influence on the reproduction of the Amazon turtle.

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