Allometry of reproduction of *Podocnemis expansa* in Southern Amazon basin

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Abstract. *Podocnemis expansa* (giant Amazon freshwater turtle) is the biggest freshwater turtle of South America. Like in other reptiles, the nest of *P. expansa* is a hole in the sand deep enough to avoid predators and extremes of temperature and humidity. However, nesting habitat selection in reptiles has effects on incubation success and on hatchlings. Female body-size is also related to the size and shape (i.e., form) of the nest, which might in theory be related to the female reproductive success. In this study we established allometric relationships between female body size and nest form. Fieldwork was carried out in nesting beaches of Araguaia and Crixás-Açu Rivers in Southern Amazon basin, Brazil, from September to December 2000. As a result, we can establish that nest form does not affect reproductive success in the species, but female body length is positively related to egg mass and clutch size.

Introduction

Podocnemis expansa (giant Amazon freshwater turtle) is the biggest freshwater turtle of South America (Pritchard, 1979; Ernst and Barbour, 1989). The species breeds once a year and produces clutch sizes as large as 143 eggs (Alho et al., 1979; Von Hildebrand et al., 1988; Malvásio, 2001), which can represent as much as 16% of female body mass (V. Cantarelli, pers. comm.).

Like other reptiles, the nest of *P. expansa* is a hole in the sand deep enough to avoid predators and extremes of temperature and humidity (Seigel and Dodd, 2000). In general, the nesting habitats of the species are formed by seasonal exposure of sand banks due to a decrease in the river's water level (Vanzolini, 1967; Von Hildebrand et al., 1988; Soini, 1997). As a possible evolutionary response to the ephemeral nature of such sand banks, the species' incubation period (approximately 45 days) is one of the shortest among freshwater turtles (Pritchard and Trebbau, 1984). However, hatching success can be as high as 80 to 90% (Malvásio, 2001).

Nesting habitat selection in reptiles has effects on incubation success (Larriera and Piña, 2000), and on hatchling phenotype (Shine and Harlow, 1996). This is extremely important in species with temperature dependent-sex determination (TSD), such as *Podocnemis expansa* (Valenzuela et al., 1997; Bonach, 2003; Morjan, 2003) because incubation temperature would depend on nest characteristics (Vasconcelos, 2000).

Clutch size in many species of chelonians presents intra- as well as interpopulation variation (Congdon and Gibbons, 1985). As an example of the former, the bigger the female the bigger her clutch size (Gibbons, 1982). As an example of the latter, the higher the latitude the bigger the clutch size (Tinkle, 1961, but see Congdon and Gibbons, 1985 for discussion). Female body-size is also related to the size and shape (i.e., form) of the nest (Ehrhart, 1995; Morjan, 2003), which might in theory be related to female reproductive success. In this study we wish to establish the allometric relationships between female body size and nest form, considering their hatching success as an indicator of the female reproductive success.

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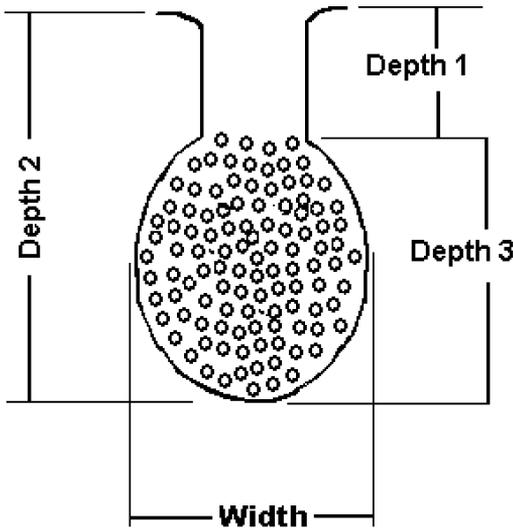Corresponding author; e-mail: lmv@esalq.usp.br

Figure 1. *Podocnemis expansa* nest measurements.

Materials and methods

This study was carried out in the Brazilian southern Amazon at sand beaches of the Araguaia River (from 13°21′S; 50°39′W to 13°30′S; 50°44′W; $n = 4$ beaches) and one of its tributaries, Crixás-Açu (13°34′S; 50°15′W; and 13°37′S; 50°14′W; $n = 2$ beaches) between September and December 2000, which coincides with the dry season, the time *Podocnemis expansa* nests in the area. Crixás-Açu connects the Araguaia River upstream. We sampled five nests per beach, located in both sides of both rivers, for a total of 30 nests. Fieldwork was done early in the morning (07:00 to 09:00) or late in the afternoon (16:00 to 18:00) in order to avoid high ambient temperatures that could harm the embryos. The following variables were taken for each nest: clutch size, hatching success (i.e., number of hatchlings / clutch size), egg-chamber bottom and roof depth, height and width, clutch mass, width in the sand of the female's plastron and foot tracks in the sand (an indicator of female body size), when unmistakably associated with a single nest. We measured female tracks in 12 nests at Araguaia River, and in two nests at Crixás-Açu River.

Prior to opening the nests, we recorded nest temperature with a calibrated thermometer and then opened the nests to record nest dimensions and count the eggs (Fig. 1). During egg counting, we measured egg masses with a digital scale (Scout, 0.1 g precision) and estimated embryo developmental stage (Yntema, 1968). Damaged eggs were discarded to avoid contamination, and healthy eggs were placed back in the egg chamber. After data collection nests were fenced and then reopened approximately 45 days after egg laying, in order to release the hatchlings.

All dataset was normally distributed (Kolmogorov-Smirnov Test: $P > 0.15$; Minitab, 2000). However, considering the unbalanced sample size between rivers, we compared nests from the two rivers with Kruskal-Wallis

Table 1. *Podocnemis expansa* nest dimensions from rivers Araguaia and Crixás-Açu in Brazilian southern Amazon (Mean value ± Standard deviation for each variable; $n =$ number of nests; P: $P_{\text{-value}}$ from One Way ANOVA).

	Araguaia River ($n = 10$)	Crixás-Açu River ($n = 20$)	P
Depth 1 (cm)	29.33 ± 8.38	41.10 ± 9.04	<0.01
Depth 2 (cm)	51.85 ± 7.23	63.50 ± 11.58	<0.01
Depth 3 (cm)	22.53 ± 4.54	22.40 ± 9.13	0.676
Eggs Chamber Width (cm)	24.23 ± 4.21	25.15 ± 5.00	0.965
Temperature (°C)	29.66 ± 0.81	30.81 ± 0.86	<0.001
Clutch Size	103.4 ± 18.25	104.10 ± 20.52	0.826

non-parametric test (Minitab 2000), where nest characteristics (i.e., nest depth and width, incubation temperature and clutch size) were the response variables and rivers were the factor. Then we used regressions to establish whether there was any relationship between female tracks and nest measurements considering the total sample pool (30 nests). We also regressed egg chamber depths (1 to 3) to clutch size, mean egg mass, number of hatchling produced by each nest, hatchlings with malformations, hatching success (number of hatchlings/number of eggs), and number of dead embryos for the total sample pool. In all the regressions where we excluded observations with leverage coefficient greater than $4/n$ and highly standardized residuals, data were reanalyzed (Sokal and Rohlf, 1995). In the text, means are followed ±1 SD (standard deviation).

Results

We found that nest characteristics were different between rivers Araguaia and Crixás-Açu. Depth 1 and Depth 3 were greater in Crixás-Açu River ($H_{1,30} = 8.7$, $P < 0.01$; and $H_{1,30} = 7.46$, $P < 0.01$). Nest temperature was also different between rivers (Araguaia, $x = 29.7 \pm 0.8$°C vs. Crixás Açu 30.8 ± 0.9°C; $H_{1,30} = 10.51$, $P < 0.001$); we did not detect any difference between rivers in relation to egg chamber width, clutch size, and Depth 3 ($P > 0.05$; Table 1). Thus Crixás-Açu River egg chambers were deeper, but with similar height. However, all nests were flooded at Crixás-Açu River. Therefore, we could not statistically compare rivers in terms of hatching success.

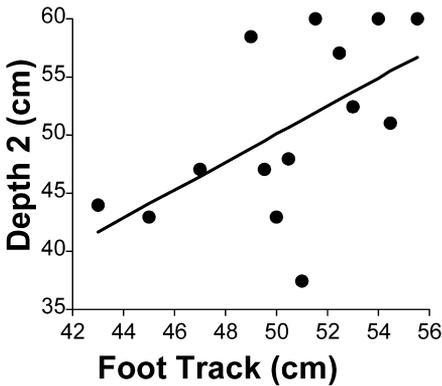In this study we found that clutch size was related to egg chamber width and Depth 2
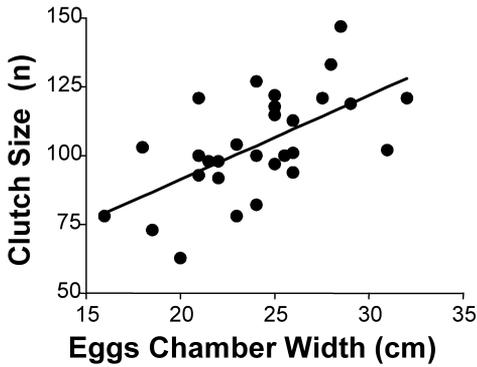
(a) y = 30.07 + 3.07x (r²$_{adj}$=34%; *P*<0.001; *n*=29)
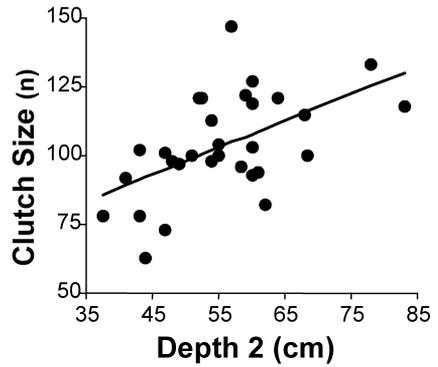
(b) y = 40.04 + 0.98x (r²$_{adj}$=27%; *P*<0.01; *n*=30)

Figure 2. Relationship between clutch size (number of eggs per nest) and egg chamber width (a), and Depth 2 (b) in *Podocnemis expansa*.

y = -10.22 + 1.21x (r²$_{adj}$=27%; *P*<0.05; *n*=14)

Figure 3. Relationship between female body size (based on tracks in the sand) and the bottom of the egg chamber (Depth 2) in *Podocnemis expansa*.

(F_{28} = 15.72, P < 0.001; F_{29} = 11.68, P < 0.01 respectively; Fig. 2). All the other measurements (clutch size, number of living hatchlings, number of hatchlings with malformations, number of dead hatchlings, number of infertile eggs, egg mass, or hatching success) were not related to egg chamber width (0.39 < P < 0.86), Depth 1, Depth 2, or Depth 3 (P > 0.05). Thus, as clutch size increases, the egg chamber gets deeper and wider.

We found foot track variation from 43 to 55.5 cm (x = 50.4 ± 3.6 cm, n = 14) and plastron tracks ranging from 18.5 to 26.5 cm (21.9 ± 2.5 cm, n = 14). Female tracks were positively related to Depth 2 (F_{13} = 5.92; P < 0.05; Fig. 3), but not with Depth 1 and 3 (0.19 < P < 0.57). Female plastron tracks were not related to egg chamber depth and width (P > 0.05). In this study clutch size was related to both plastron and foot track width, (F_{13} = 6.51, P < 0.05; F_{13} = 12.66, P < 0.01 respectively; Fig. 4). Egg mass was related to hatching success (F_{12} = 5.59, P < 0.05; Fig. 5), but not with clutch size (F_{23} = 0.12, P = 0.73). Thus, clutches containing bigger eggs had higher hatching success.

Nest temperature was related to Depth 1 and Depth 2 (F_{27} = 5.94, P < 0.05; F_{28} = 6.15, P < 0.05 respectively), but not to Depth 3 (P = 0.4456). Thus, the deeper the egg chamber, the warmer its temperature (Fig. 6).

Discussion

In this study we found nests between 37.5 and 83 cm deep, from the beach surface to the bottom of the egg-chamber (Depth 2). Previous studies report similar ranges (Vanzolini, 1967: 10-100 cm; Alho et al., 1979: 75-80 cm; Malvásio, 2001: 55-87 cm). In this study nest depth did not affect hatching success. Therefore, there seems to be a high phenotypic plasticity (sensu Via, 1994) in relation to this characteristic. Con-
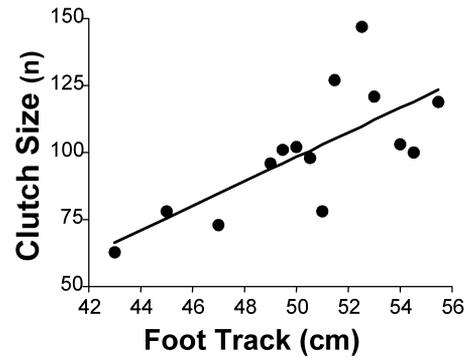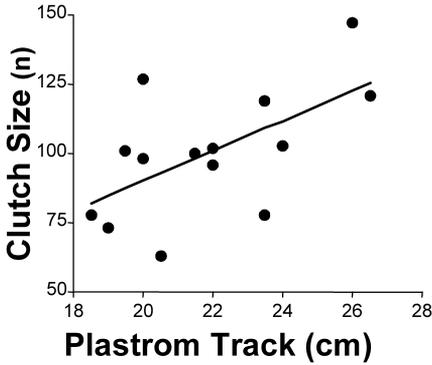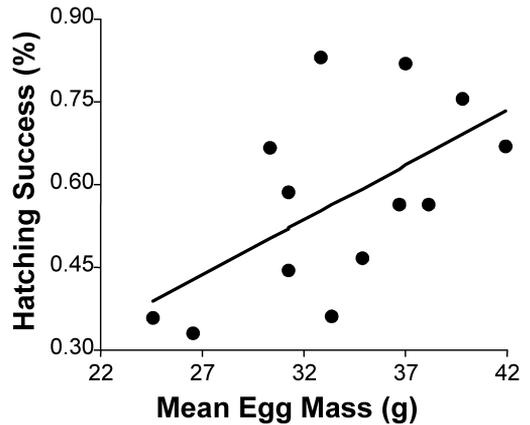
(a) y = -17.7 + 5.4x (r^2_{adj}=30%; P<0.05; n=14)   **(b)** y=-130.8 + 4.59x (r^2_{adj}=30%; P<0.05; n=14)

Figure 4. Relationship between clutch size and (a) plastron track (as an estimation of female body size), and (b) feet track (as an estimation of female body size) in *Podocnemis expansa*.

sidering the high diversity among sand beaches in terms of their physical characteristics (e.g., depth, sand grain size distribution, temperature, and humidity) such plasticity makes it possible for turtles to nest in a broader range of habitats. Egg-chamber height and width ranges found in this study (10-40 cm, 16-37.5 cm, respectively) are larger than those reported by Alho et al. (1979; 20-25 cm, 13-18 cm, respectively). This is possibly related to the females' body size and the physical limitations of small females to dig a hole wider and/or deeper (Ehrhart, 1995; Morjan, 2003). The significant relationship found in this study between Depth 2 and female body-size based on female tracks (Fig. 3) corroborates this assumption.

Even the deep nests from Crixás-Açu River were warmer than nests from Araguaia River. We also found a positive relationship between Depth 1 and 2 and temperature. Surprisingly, we found that the deeper the egg chamber, the warmer its temperature (Fig. 6a and b). According to Valenzuela et al. (1997), sex ratios vary among beaches. However nest depth was not measured in that study, which might have correlated temperature with egg chamber depth. Recently, Valenzuela (2001) reported that nest temperature was related to egg chamber depth, but the deeper the nest, the cooler its temperature, contrary to the present results. De-
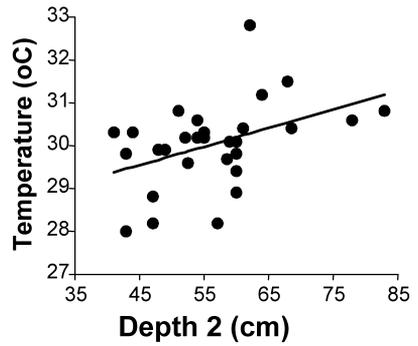

y=-0.1 + 0.02x (r^2_{adj}=28%; P<0.05; n=13)

Figure 5. Relationship between egg mass and hatching success in *Podocnemis expansa*.

spite the methodology used for recording nest temperatures [Valenzuela (2001) used data loggers, whereas we used a single measurement before opening the nests] it is also possible that both populations have different nesting patterns. Similar results to ours were reported for the jacky dragon (*Amphibolurus muricatus*, Agamidae), where the deeper the nest, the warmer the incubation temperature (Harlow and Taylor, 2000). These contradictory results should be considered for future studies comparing sand and thermal profiles from different sand beaches
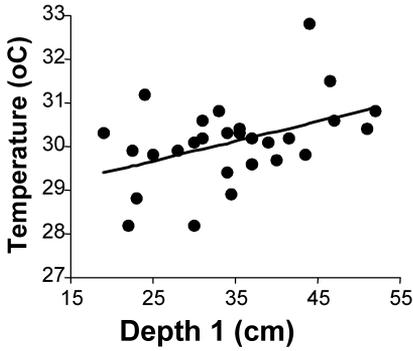
(a) y = 28.53 + 0.05x (r^2_{adj}=15%; *P*<0.05; *n*=28) **(b)** y=27.59 + 0.04x (r^2_{adj}=16%; *P*<0.05; *n*=29)

Figure 6. Relationship between mean incubation temperature and (a) Depth 1, and (b) Depth 2, in *Podocnemis expansa*.

where *P. expansa* nests. Whatever the reasons for these contradictory patterns, we agree with Valenzuela (2001) that females with similar body size could produce different sex ratios in different rivers, whereas females with different body size could produce biased sex ratios on the same river, because bigger females could dig deeper nests, thus incubating their eggs at different temperatures than shallow nests. Therefore, based on the present results, it would be possible for bigger females to produce a female biased sex ratio, since *Podocnemis expansa* has a TSD I pattern (Valenzuela et al., 1997) as described by Ewert et al. (1994). However, recent results suggest that the *P. expansa* TSD pattern might be type II (FMF, as described by Ewert et al., 1994), in which females are produced at low and high temperature, whereas males are produced at intermediate temperature (Bonach, 2003).

Podocnemis expansa produces big clutches. Alho et al. (1979) and Malvásio (2001) reported similar average clutch sizes of, respectively, 93 and 95 eggs at different regions of Brazilian Amazon. Von Hildebrand et al. (1988) reported an average of 105 eggs in Colombia, similar to that reported by Valenzuela (2001) of 103.1 ± 23.7 eggs / nest. Our results for clutch-size are larger than previous reports from Brazil, but similar to those reported from Colombia. These numbers are similar or even larger than

the values reported for marine turtles: *Caretta caretta* (90; Peters et al., 1994), *Chelonia mydas* (66; Márquez, 1995), and *Lepidochelys olivacea* (95; Márquez, 1995).

In this study there was a significant relationship between female body size – inferred from their plastron and foot tracks on the sand – and some clutch variables (clutch size, and Depth 2), similarly to what has been described for other freshwater turtles (Gibbons, 1982; Congdon and Gibbons, 1985; Dodd, 1997; Valenzuela, 2001). However, in order to have more precise information about the size class distribution of reproductive females in a certain population, future studies should establish regression equations between female tracks and body measurements (e.g., plastron width and body-mass) which is lacking for the species. Tracks are much easier (and cheaper) to measure than living animals. Reproductive females normally leave clear tracks on the sand, when returning back to water after laying the eggs. Determining body size of reproductive females at nesting habitats may provide valuable information about the fecundity curve (Begon and Mortimer, 1986) of the population, at least in terms of size classes. Tracks have been used in a previous work with this species (Valenzuela, 2001) and also with crocodiles (Thorbjarnarson and Hernández, 1993).

It is usually difficult to fit body-size data on growth curves of reptiles (Abercrombie and Verdade, 2002) because they are generally more plastic than mammals and birds. However, the assessment of the females' fecundity curve (based on size-class, not actual age) is possibly the most valuable information for management programs that involve egg collection (Abercrombie and Verdade, 1995), such as the Brazilian ranching program of the species (IBAMA, 1989). Moreover, if females of different sizes are producing different sex-ratios, or nesting in different beaches, this information would be essential for management purposes.

Small turtles usually produce smaller clutch sizes, but with relatively larger eggs (Pritchard, 1979). In addition, the bigger the species the larger its average clutch size (Gibbons, 1982). In this study, the same pattern was found intra-specifically for *Podocnemis expansa* as we found that bigger females laid larger clutches. However, we did not detect any relationship between female size and egg mass. Valenzuela (2001) reported that female body size (inferred from tracks) was related not only to clutch size but also to egg mass. The different patterns found in this study in relation to Valenzuela (2001) could be explained by the wider female track range (i.e., bigger females), observed by Valenzuela (2001). In addition, there could be differences among populations in such characteristic. However, besides female body size – and possibly age – other factors may possibly influence clutch size in chelonians, such as interval between nesting periods, sequence of nesting activity along the years, seasonality and health status of the female (Gibbons et al., 1978, 1982; Dodd, 1997).

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