



The life history of the Yellow-spotted Amazon River Turtle (*Podocnemis unifilis*) as told from the nests

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Abstract. Nesting site selection is of great ecological and evolutionary importance for turtles, because it is a key determinant of individual reproductive success. In this study we evaluated the effects of two types of nesting substrate (sand bank and clay banks) on the incubation success of a generalist nesting species, *Podocnemis unifilis*, in a floodplain area in the Brazilian Amazon. Nesting characteristics were recorded during the nesting seasons of 2012–2014, when 634 nests were monitored throughout the incubation period. Mean size of laying females did not vary between substrate types (sand = 24.71 cm; clay = 25.44 cm; $P = 0.2054$), nor was clutch size related to females' sizes. However, females that nested on sand bank exhibited a clearer pattern of nest positioning relative to distances to vegetation and water line than those laying eggs on clay banks. Mean egg size (40.24 ± 2.68 mm) and weight (21.09 ± 5.46 g) did not vary between types of nesting substrate, and there was no variation in clutch size across the reproductive seasons for either substrate. On the other hand, clutch sizes were higher in nests in clay banks (28 ± 8) than those that were deposited in sand bank (22 ± 7). Mean incubation time was shorter in sand bank (62 ± 5.98) than in clay banks (77 ± 4.33). Although eclosion success for sand and clay nests was high (84.82 and 72.97%, respectively), incubation success was relatively low in both substrate types (sand = 57.34%; clay = 25.80%). On sand bank, clutch losses were mainly due to human predation (16%), on clay banks, human predation also affected 16% of the nests, but floods compromised another 30%. Natural predation was mostly due to ovivory by the lizard *Tupinambis teguixin*, although fire ants, *Solenopsis geminata*, also contributed to egg predation on clay banks. Hatchlings incubated on sand bank were on average larger and heavier than those from clay banks nests. Our results suggest that differences found in the life histories of different populations of *P. unifilis* reflect flexible responses that are largely linked to the diversity of habitats this species utilizes for nesting. Furthermore, the presence of source-sink and habitat patches should be considered for short term population management, beyond the creation of networks of reserves that incorporate and protect a variety of nesting sites that these small local groups depend on for their survival.

Key words. Testudines, Central Amazon, clay bank, hatchling, incubation success, phenotypic plasticity, reproductive size, sandy bank, turtle.

Introduction

The basis for formulating any hypothesis or theory in ecology and evolution is formed by studies of natural history that describe the biology of organisms involved, their interactions, aspects of behaviour and use of the environment (RICKLEFS 2011). It is important to consider that when the life history of any organism is studied, in addition to the physiological, behavioural and morphological adaptations that are modified over time (ALCOCK 2011), there are also individual adjustments in response to environmental variations, and ecological interactions with the environment (ALCOCK 2011, RICKLEFS 2011). Of these, studies involving

the early stages of life, where the highest mortality occurs, are especially important for long-lived and late-maturing organisms, such as turtles (MYERS et al. 2007). Additionally, within the Chelonia, some ecological and behavioural processes are especially important for understanding the basic biological characteristics and changes in life history traits, including nesting habitat selection (CAPUTO et al. 2005, KOBAYASHI et al. 2017).

Nesting environment characteristics are key to an effective understanding of the nesting ecology and choice criteria used by females when selecting nesting sites (THORBJARNARSON et al. 1993, DOODY et al. 2003, MITCHELL & WALL 2013). In many cases, the choice of a nesting site

may represent a trade-off (SPENCER 2002, ESCALONA et al. 2009), due to the inherent risks of predation (SPENCER 2002, ERICKSON & BACCARO 2016), incubation substrate characteristics (PACKARD & PACKARD 1997, FERREIRA-JÚNIOR & CASTRO 2003), the phytophysionomy of the general nesting area (JANZEN 1994, KOLBE & JANZEN 2002), microclimate (MROSOVSKY & PIEAU 1991, HAYS et al. 2001), and the thermal properties of the nest (FERREIRA-JÚNIOR & CASTRO 2006, KOBAYASHI et al. 2017). In addition, with increasing global temperatures (IPCC 2013), changes to established water regimes have caused sudden floods (ALHO & PÁDUA 1982, ESCALONA & FA 1998) and, as a consequence, decreased the survival of embryos and hatchlings of turtles resulting from the flooding of nests (KASKA et al. 1998, EISEMBERG et al. 2016).

Attaining an effective understanding of the mechanisms and consequences of nesting decisions has been a focus of evolutionary ecology for a long time (KOLBE & JANZEN 2002). In an adaptive sense, female nesting site choice can influence the survival and/or phenotype of the offspring (ST. JULIANA et al. 2004, ESCALONA et al. 2009, KOBAYASHI et al. 2017), since selection depends on the ability of females to discriminate between sites that are favourable (or unfavourable) for their offspring's survival (HAYS et al. 2001, FERREIRA-JÚNIOR & CASTRO 2010). Different environmental conditions, along with incubation temperatures, may influence not only embryonic development (ACKERMAN 1997, MAGALHÃES et al. 2017) and sex determination in some species (EWERT & NELSON 1991), but can also significantly affect phenotype (SHINE & HARLOW 1996, BOOTH et al. 2004), offspring vigor (DOODY et al. 2003, KOBAYASHI et al. 2017) and, ultimately, hatchling survival (WILSON 1998, ESCALONA et al. 2009). In this sense, it is important to study other aspects of turtle reproductive ecology, including not only the spatio-temporal distribution of nests and reproductive success based on the number of emergent hatchlings, but also potential effects of maternal decisions (DOODY et al. 2003, ESCALONA et al. 2009, PÁEZ et al. 2009) on the morphological characteristics of hatchlings incubated in different nesting substrates (ERICKSON 2018).

In the freshwater Amazonian turtles of the genus *Podocnemis*, reproductive behaviour (i.e., maturation and sexual pairing, nesting and incubation) is synchronized with seasonal variations in water levels (ALHO & PÁDUA 1982, THORBJARNARSON et al. 1993). Populations of various species occur in a variety of hydrological systems (FERRARA et al. 2017), which may be associated with different nesting substrates (CAPUTO et al. 2005, CONWAY-GÓMEZ et al. 2014, ERICKSON 2018). The choice of nesting site does not appear to be random (FERREIRA-JÚNIOR et al. 2003), since it is associated with particular characteristics of the females involved (BONACH et al. 2006). Thus, incubation success may be affected differently between different species and populations of the genus *Podocnemis* (e.g., HALLER & RODRIGUES 2006: *P. sextuberculata*; RUEDA-ALMONACID et al. 2007: *P. vogli*; BATISTELLA & VOGT 2008: *P. erythrocephala*; PÁEZ et al. 2009: *P. lewyana*; VANZOLINI 2003: *P. expansa*; PIGNATI et al. 2013b: *P. unifilis*).

The Yellow-spotted Amazon River Turtle (*P. unifilis*) utilizes a wide variety of geomorphologically distinct substrates for nesting (e.g., including sandy and clay banks), probably because it does not tend to migrate as widely as its congeners. This is in agreement with the results of the recent work by AGOSTINI (2016), who through the sequencing of the mitochondrial DNA control region of several *P. unifilis* populations of the Amazon and Tocantins-Araguaia basins, suggested eight management units for the species, considering the high levels of population structure found (i.e., recognized as a resident species), and being the most generalist Amazonian turtle, (THORBJARNARSON et al. 1993, CAPUTO et al. 2005, RUEDA-ALMONACID et al. 2007). Due to its behavioural plasticity, of all species of the genus *Podocnemis*, *P. unifilis* seems to be especially able to deal with natural environmental variations, adjusting its nesting behaviour to the locally available substrates (FERREIRA-JÚNIOR et al. 2007, PIGNATI et al. 2013a).

Since nesting site selection is of great ecological and evolutionary importance in turtles, long-term studies are essential for understanding patterns and processes that influence incubation success. To do so, it is necessary to understand some of the aspects that constitute the life history and the development of the species, since several factors can affect its distribution and life history parameters (HAYS et al. 2010, SCHWANZ et al. 2013) and thus important population characteristics such as sex-ratio (e.g., ERICKSON 2018). In the current study we evaluated, across three sequential annual nesting seasons, (i) whether there are differences in nesting ecology between individual groups of *P. unifilis* that nest on sand bank (beach) or on clay banks, and (ii) the influence of such generalist nesting strategy on incubation success and hatchling characteristics.

Material and methods

Study area

The Piagaçu Purus Sustainable Development Reserve (PPSDR; 4°03'–5°25' S, 61°40'–63°30' W) is a protected area in the Brazilian state of Amazonas. It contains a variety of habitats, including seasonally flooded forests ('*igapó*'), floodplain lakes, and sandy beaches, connected to the lower part of the Purus River. The landscape is characterized by a mosaic of terra firme (upland) forests and lowland floodplain forests (JUNK et al. 1989, WALDEZ et al. 2013). This riverine floodplain system is maintained by regular and predictable annual flood pulses, which seasonally change the landscape and act as the main driver in the ecology of the rivers, canals and lakes of this region (JUNK et al. 1989). The annual water level fluctuation involves four hydrological periods (BITTENCOURT & AMADIO 2007): rising season (December–April), flooding season (May–August), followed by a reduction in water volume (receding season: August–October), and then the lowest-water period, (dry season: October–December), when sand banks (beaches) and clay banks are exposed.

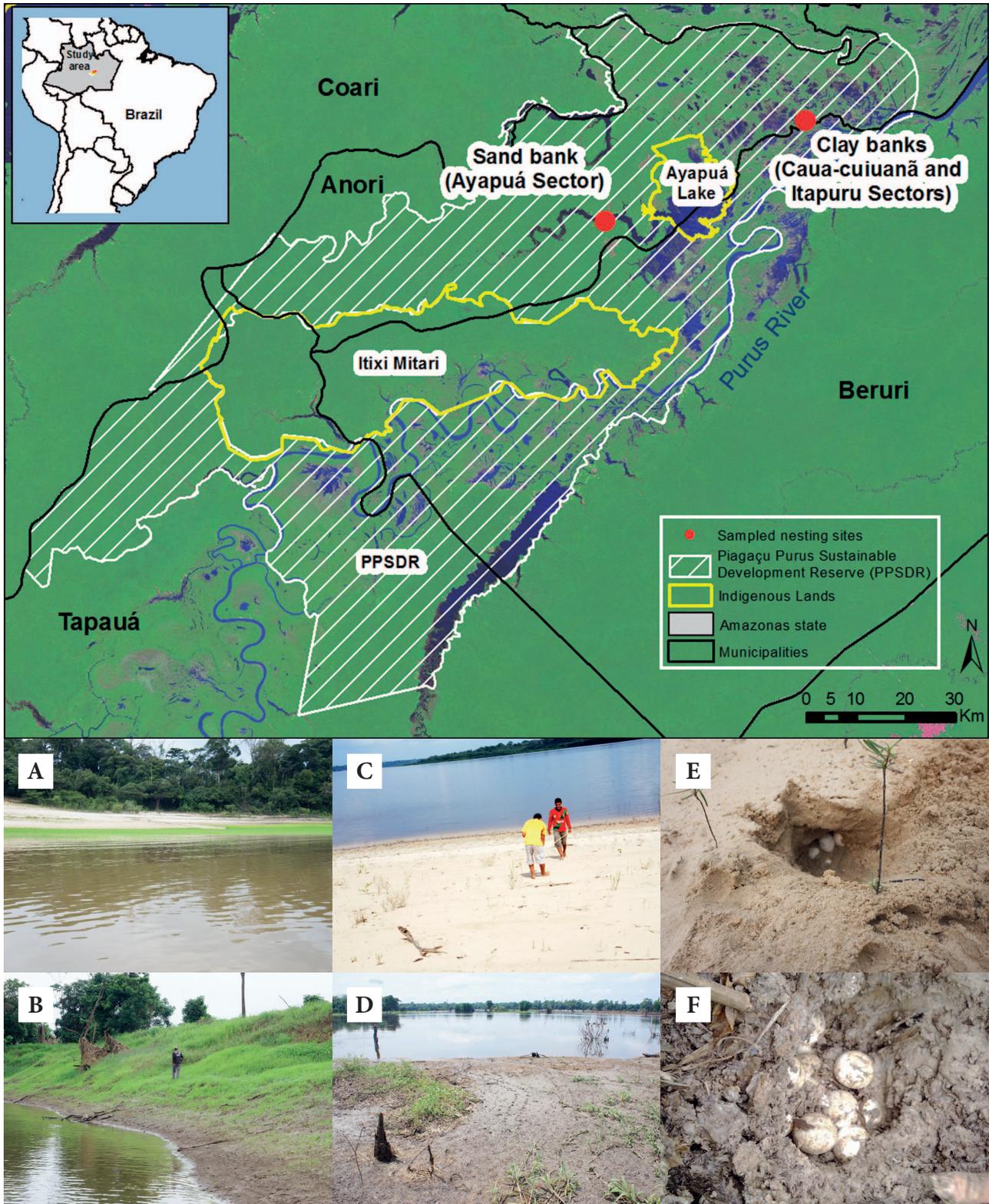


Figure 1. Two nesting sites of *Podocnemis unifilis* in the Piagaçu-Purus Sustainable Development Reserve (PPSDR), located in the border area between the municipalities of Anori, Beruri, Coari, and Tapauá, state of Amazonas, Brazil. A) Rising season ending with the emergence of the sand bank in the Ayapuá sector, B) clay banks in the sector Caua-Cuiuanã and Itapuru. C) Patrolling the beach for locating nests, D) tracks left by nesting females. E) Nest on the sand bank, and F) nest on the clay bank. Arrows indicate where a female ascended the bank for oviposition and where she descended after oviposition.

Fieldwork procedures

Nesting areas were monitored at two sites in the PPSDR (Fig. 1) between the end of the receding season and the beginning of the rising season in the years 2012, 2013, and 2014. The first site is located in the Ayapuá sector of the reserve, where there is a relatively small stretch of sand bank approximately 250 metres long that becomes covered only sparsely with vegetation during the low-water season (04°26'03.0" S, 62°17'42.7" W). The second study site consists of a series of clay banks with abundant grass vegetation, variable declivity, ranging from metres to kilometres in length along streams, canals and lakes, all connected to the Purus River (04°15'28.1" S, 61°55'52.9" W) in the Caua-Cuiuanã and Itapuru sectors of the reserve.

Patrolling of nesting sites began when the first sand (Fig. 1A) or clay banks (Fig. 1B) were exposed and was continued during the receding and dry seasons of each year. Nests were located by following female's tracks (Figs 1C, D) and signs of female nest-digging activity on the sand (Fig. 1E) and clay banks (Fig. 1F). Whenever possible, the approximate date of nesting, the distance of the nest to the nearest stand of vegetation, least distance from nest to water, and width of female body track were recorded for each nest found. As the reproductive cycle of *Podocnemis* spp. is generally synchronized with the flood pulse, the level of the Purus River was checked daily during the three years of monitoring as per readings from a local limnometric ruler.

In the 2013 breeding season, some of the nests at each site were opened (sand bank n = 14 nests, clay banks n = 14 nests) to provide replicate subsampling data on egg biometry at each study site (GOTELLI & ELLISON 2011). Biometric measurements included egg length and width (Digimess® digital caliper: precision 0.01 mm) and weight (Pesola® digital scale: precision 0.1 g). Selected nests were analyzed during the first 72 hours following oviposition. Any disturbances occurring during the incubation period were recorded. Whenever possible, predators were identified based on the type of disturbance that had been inflicted on the nest, and knowledge of the potential predator species of Podocnemididae turtles as reported in the literature (WALDEZ et al. 2013, ERICKSON & BACCARO 2016).

Nests were monitored and protected across the incubation period, which was defined as the time between oviposition and the emergence of the first hatchling. The incubation period on a sand bank is about 60 days (PIGNATI et al. 2013a, ERICKSON 2018), whereas on clay banks it is around 75 days (RUEDA-ALMONACID et al. 2007, ERICKSON 2018). Nests were inspected frequently, and on first emergence, the following hatchling biometrics were taken (as straight-line distances): Maximum Carapace Length (MCL), Maximum Carapace Width (MCW), Maximum Plastron Length (MPL), Maximum Plastron Width (MPW), Maximum Shell Height (MSH), and Weight (WEI).

Statistical analysis

Statistical analysis was performed using SPSS Statistics 22.0 and R 3.3.2 (R Development Core Team 2018), using the vegan (OKSANEN et al. 2018) and other, associated packages. For all the sections that follow, the descriptive statistics are expressed as the mean ± standard deviation of the recorded variables. Variance homogeneity for predictor variables and responses were evaluated with an F test, and data normality with a Shapiro-Wilk test.

Female size

The sizes of nesting females (FS) at each nesting site were estimated by measuring the width of individual tracks (equivalent to the Straight-line Maximum Plastron Width). We used a Mann-Whitney U test to verify whether there were differences in the sizes of the females that nested at the two nesting sites. For each nesting substrate, we used simple linear regression analysis to test whether female size had an effect on nest location in relation to its distances from water or vegetation, and clutch size.

Egg metrics

Egg shape was calculated by the ratio of length to width. Egg volume was obtained via the equation $v = \pi \times x \times (y)^2/6$, where 'x' is the length, and 'y' is the width of the egg (VANZOLINI 1977), with data being log-transformed. Association between the dependent variables (egg length, width, weight, shape and volume) and the independent variable (female size) was tested via simple linear regressions for each nesting substrate. Comparisons between the means of biometric variables of eggs from the two nesting substrates were performed with Student's t-tests.

Clutch size

Mean clutch size (CS) at each nesting site was estimated from the total number of eggs and/or hatchlings found in the nests in the three breeding seasons. To test whether clutch size varied between nesting seasons for each substrate, we used a two-way ANOVA, and between incubation substrates, we used a one-way ANOVA.

Incubation period

Estimated mean incubation periods for eggs were calculated from the difference between the date on which the nest was found and the date when at least one hatchling emerged. This was done for each nest sampled on each substrate type. To compare if mean hatchling incubation times differed between the two substrates during the three nesting seasons, we used a two-way ANOVA.

Variation in incubation periods in each nesting substrate over the three nesting seasons was tested with a one-way ANOVA.

the “Omnibus Tests of Model Coefficients”. We used a one-way ANOVA to test whether incubation success was different for each substrate type between reproductive seasons.

Eclosion and incubation success

Eclosion success was estimated only for those nests that were unaffected by external disturbances (i.e., biotic and abiotic factors). Eclosion success was calculated by the mean number of emergent hatchlings, and the size of their respective clutches in each substrate type. In turn, incubation success was calculated considering all sampled nests, including those impacted by various external factors during the incubation period. Factors that potentially influenced nest losses were sorted into three categories: human predation, natural predation (i.e., other animals), and abiotic factors (i.e., flooding or drought). We tested the hypothesis that incubation success differed between nesting substrates using Fisher’s Exact test. To test whether incubation success depended on substrate type a generalized linear regression model was incorporated in our analysis, where dependent and independent variables were categorical and binary. In the first model, a binary logistic regression was generated without considering substrate type, and treating all individuals as though they were in the highest frequency category (i.e., nests with incomplete incubation). The second model included substrate type, and the quality of our logistic regression model was assessed with

Hatchling biometry

The relationships between the average sizes of hatchlings (dependent variable) Maximum Carapace Length – MCL, Maximum Carapace Width – MCW, Maximum Plastron Length – MPL, Maximum Plastron Width – MPW, Maximum Shell Height – MSH, and Weight – WEI and Female Size – FS (independent variable) were tested with simple linear regressions. Mean values for hatchling biometrics for each nest, from each nesting substrate, were compared with a Student’s t-test, with a Mann-Whitney U test being used for the data that did not follow a normal distribution.

Results

Across the three nesting seasons, 634 *P. unifilis* nests were monitored, 142 on the sand bank, and 492 on the clay banks. Oviposition of *Podocnemis unifilis* was concentrated at the end of the receding season and during the dry season in the three nesting seasons monitored, with some nesting activity being recorded on the clay banks during the first weeks of the rising season (Figs 2A, B).

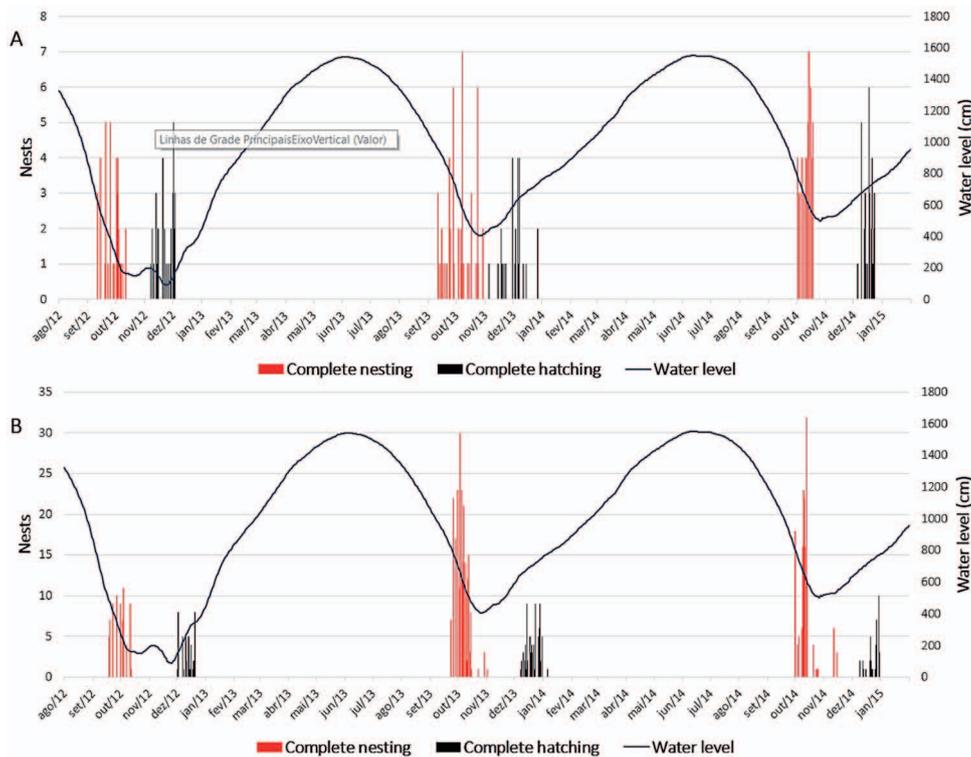


Figure 2. Daily water levels in the lower River Purus, Amazonas, Brazil, during the years 2012, 2013, 2014 and their relation to the nidification and success of incubation of *Podocnemis unifilis*: A) on the sand bank and B) on the clay banks.

Female size

The tracks of 297 *P. unifilis* females were measured at the two nesting sites, 56 on the sand bank and 241 on the clay banks. Overall, the mean size of nesting females was 25.30 ± 4.89 cm. The mean size of females nesting on the sand bank was 24.71 ± 5.8 cm (14–37 cm), and 25.44 ± 4.6 cm (20–40 cm) for those utilizing the clay banks. There was no significant difference in female size between the two substrate types ($W = 6017.5$, $P = 0.2054$). Although marginally significant, there was no relationship between nesting female size and nest distance to water ($F = 3.737$, $P = 0.0584$), nor between female size and clutch size ($F = 0.2385$, $P = 0.627$) on the sandy substrate. However, larger females tended to nest closer to the vegetation on the sand bank ($F = 6.851$, $P = 0.0114$; Fig. 3A). On the clayey substrate, there was no relationship between female size and nest positioning relative to vegetation ($F = 0.0087$, $P = 0.9256$), nor to water ($F = 1.828$, $P = 0.177$), but there was a positive relationship between clutch size and nesting female size ($F = 10.65$, $P < 0.0015$; Fig. 3B).

Egg biometrics

Biometrics of 762 eggs from 14 *P. unifilis* sand bank nests ($N = 346$ eggs) and 14 nests on clay banks ($N = 416$ eggs) were taken. Elliptical in shape and with a lightly porous shell, the average egg lengths and widths were 40.24 ± 2.68 and 27.90 ± 2.57 mm, respectively. For egg weight, shape, and volume, means were 21.09 ± 5.46 g, 1.45 ± 0.11 , and 9.69 ± 0.23 cm³, respectively (Supplementary document S1). There were no differences in sizes or weights of eggs between sand and clay banks (Table 1). Only egg weight from sand bank nests was influenced by nesting female size. The size of the female seemed to exert some influence

Table 1. Summary of statistical analysis comparing different parameters related to the size of females, biometrics of eggs and hatchlings, clutch sizes and incubation periods between nests of *Podocnemis unifilis* deposited in sandy substrate (sand bank) and clayey substrate (clay banks). MCL – Maximum Carapace Length, MCW – Maximum Carapace Width, MPL – Maximum Plastron Length, MPW – Maximum Plastron Width, MSH – Maximum Shell Height, and WEI – Weight.

Sand bank × Clay banks	Test	N	F, W, t	df	P
Female size	Mann-Whitney U	297	6017.5	1	0.2054
Egg metrics					
Number of eggs	t-test	762	2.076	25.21	0.048
Length (mm)	t-test	762	1.690	22.79	0.104
Width (mm)	t-test	762	0.169	22.44	0.866
Weight (g)	t-test	762	1.014	25.69	0.319
Shape	t-test	762	1.754	25.92	0.091
Volume (cm ³)	t-test	762	0.547	21.04	0.590
Clutch size	two-way ANOVA	280	50.01	1	0.0001
Incubation period	two-way ANOVA	273	18.68	1	0.0001
Hatchlings biometry					
MCL	t-test	3083	3.581	107.1	0.0005
MCW	Mann-Whitney U	3083	13.584	81.927	0.1781
MPL	t-test	3083	4.044	117.8	0.0005
MPW	t-test	3083	3.702	100.93	0.0003
MSH	t-test	3083	2.576	121.89	0.0111
WEI	t-test	3083	3.861	118.29	0.0001

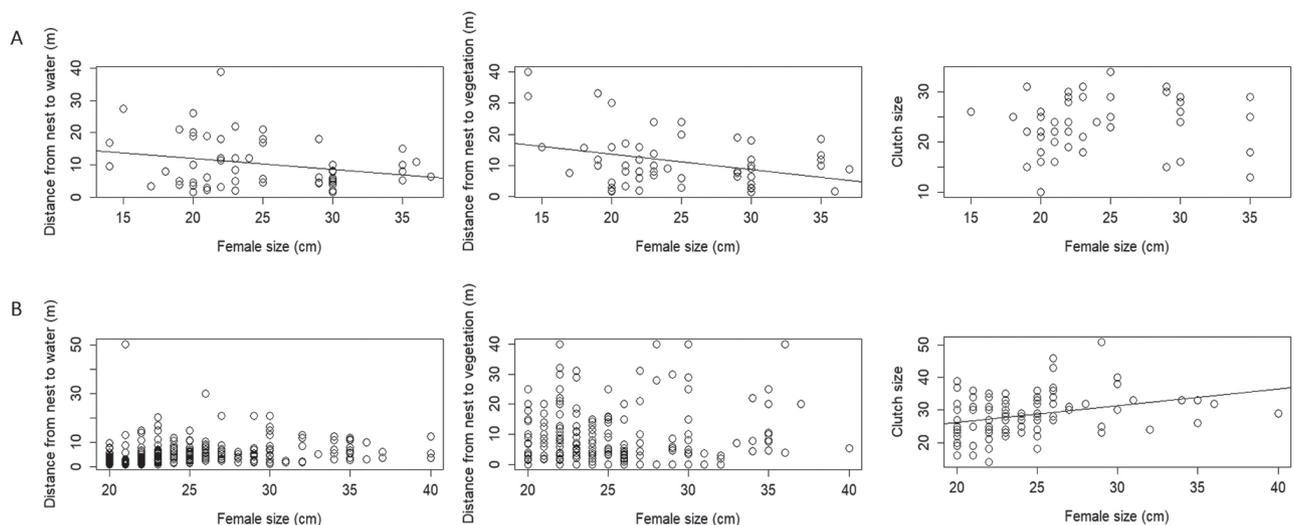


Figure 3. Linear relationship between response variables (distance from nest to water, distance from nest to the nearest vegetation and clutch size) and predictor variable (female size) for each nesting substrate utilized by *Podocnemis unifilis*. A) Sand bank and B) clay banks.

only on the average weights of eggs on the sand bank ($F = 17.44$, $P = 0.001$). For all other biometrics, there was no evidence that nesting female size and clutch size influenced the morphological characteristics of the eggs in either substrate type (Supplementary document S2).

Clutch size

Clutch size was recorded for 280 *P. unifilis* nests across the three nesting seasons monitored. We recorded clutch sizes of 100 nests on the sand bank, and 180 nests on clay banks. Mean clutch size on the sand bank (22 ± 7 , 4–40 eggs) was smaller than that on clay banks (28 ± 8 , 13–51 eggs) ($F = 50.01$, $df = 1$, $P < 0.0001$). There were no differences in clutch size between nesting seasons 2012:2013 ($P = 0.5315$), 2012:2014 ($P = 0.3358$), 2013:2014 ($P = 0.9585$) for nests deposited on the sandy bank (Fig. 4A) or for nests on clay banks between nesting seasons of 2012:2013 ($P = 0.5451$), 2012:2014 ($P = 0.9972$), 2013:2014 ($P = 0.6351$) (Fig. 4B).

Incubation period

The incubation periods of 273 *P. unifilis* nests were recorded for the three nesting seasons monitored (99 from the sand bank and 174 from clay banks). The mean incubation peri-

od on clay banks (76.83 ± 4.33 days) was significantly longer than those on the sand bank (62.2 ± 5.98 days) ($F = 18.68$, $P < 0.0001$). For sand bank nests, there were differences in the mean incubation period between the three nesting seasons ($P < 0.0001$; Fig. 4C), and this was also the case in two of the breeding seasons at the clay banks: 2012:2013 ($P < 0.0001$) and 2012:2014 ($P < 0.0001$). Only the 2013:2014 breeding seasons did not differ regarding their mean incubation periods ($P = 0.9502$; Fig. 4D).

Eclosion and incubation success

On the sand bank, overall eclosion success reached nearly 85% or 96 nests ($N = 1851$ emergent hatchlings) over the three nesting seasons, however, incubation success was considerably smaller (57.34%). On the clay banks, 174 nests hatched successfully ($N = 3581$ emergent hatchlings), equivalent to 72.97% of the nests over the three nesting seasons, whereas incubation success was only 25.80%.

On both substrate types, a variety of biotic and abiotic factors reduced incubation success during the three nesting seasons (Supplementary document S3). On the sand bank, natural predation (ants: *Solenopsis geminata*; birds of prey: Falconidae; lizards: *Tupinambis teguixin*) attacked 7% of the nests ($N = 10$), human predation caused 15% losses ($N = 21$), while abiotic factors (i.e., flooding or overly

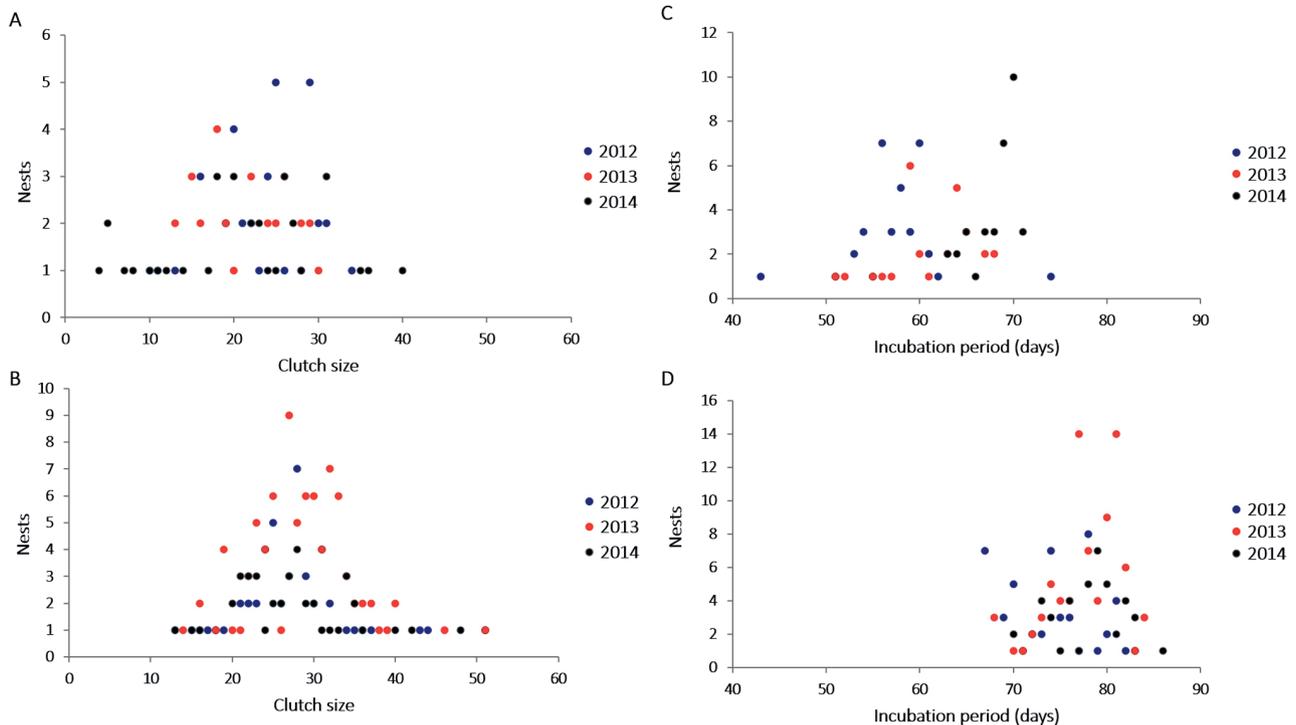


Figure 4. Average clutch sizes of *Podocnemis unifilis*. A) Nests on the sand bank, B) nests on the clay banks, and average incubation periods in sand (C), and in clay (D) across three reproductive seasons.

dry substrate) were responsible for the loss of little more than 8% of nests ($N = 12$). On the clay banks, 19% of the nests ($N = 95$) were impacted by natural predation events (*S. geminata*, *T. teguixin*, monkeys: *Sapajus apella*), while another 16% ($N = 77$) were lost to human predation, and 30% ($N = 146$) to flooding or overly dry substrate.

The results of the binary logistic regression showed that the first model correctly predicted observed incubation success in only 43.1% of sampled nests. The second model, including substrate type, increased reliability to 65.8% ($OR = 0.238$; $CI = 95\% = 0.1159-0.356$). Thus, nesting substrate was considered to have a significant influence on *P. unifilis* egg incubation success [$\chi^2(1) = 53.212$; $P < 0.000$; R^2 Nagelkerke = 0.108], with a higher incubation success on the sand bank (Fisher's Exact test, $P < 0.001$). The incubation success was similar only between the 2013:2014 nesting seasons for nests deposited on clay banks ($P > 0.2058$) and the sand bank ($P > 0.4416$).

Hatchlings biometry

A total of 3083 *P. unifilis* hatchlings from 152 nests were measured immediately post-emergence, with 1202 hatchlings from 56 sand bank nests and 1881 hatchlings from 96 clay banks nests. Overall, mean biometric values of hatchling size and weight were: MCL: 41.3 ± 3.23 mm, MCW: 36.47 ± 3.41 mm, MPL: 38.03 ± 2.96 mm, MPW: 29.63 ± 2.51 mm, MSH: 19.55 ± 1.76 mm, and WEI: 14.68 ± 2.44 g (Supplementary document S3). For hatchlings incubated on the sand bank, mean MCL was 42.22 ± 3.22 mm and WEI was 15.41 ± 2.35 g. Hatchlings from the clay banks had MCL and WEI means of 40.71 ± 3.1 mm and 14.21 ± 2.39 g, respectively. Hatchlings incubated on the sand bank were larger than those from clay banks in most biometric variables (Table 1). Only MCW did not differ between substrates ($P = 0.1781$). Female size did not affect the biometric characteristics ($P > 0.01$) of hatchlings from sand bank or clay banks nests either, when evaluated by Linear regression analysis (Supplementary document S2).

Discussion

Several studies have shown the ability of turtle species to adapt to different environmental conditions (PRICE et al. 2003, REFSNIDER & JANZEN 2012). Such adaptations are often associated with different reproductive strategies within a group, which can be observed between populations and even between genders (e.g., LICHT 1984, CEBALLOS et al. 2013, LIU et al. 2013). In the case of *P. unifilis* females, reproductive behavioural plasticity seems to be closely associated with their capacity to select nesting sites that are most likely to increase the incubation success for their offspring. The results of the present study show that the choice of nesting substrate by *P. unifilis* females influences hatching and incubation success rates, number of eggs and biometric characteristics (i.e., sizes of eggs and offspring). This sug-

gests that nesting site choice may have profound effects on the breeding success and fitness of a generalist species such as *P. unifilis*. Alternatively, source-sink dynamics could too explain some of these differences observed in our study, since reproduction in source patches usually can be much higher than in those habitats considered sink patches.

For *Podocnemis unifilis*, the track that a female leaves on the ground provides a good size estimator, as was already observed by ESCALONA et al. (2018) for a population studied in the Orinoco River basin in Venezuela. At our study sites, the average post-nest trail width left by *P. unifilis* females was 25 cm, with an estimated average size for nesting females close to 34 cm MCL (23–49 cm) for both substrates. Such results for female reproductive size agree with those from other studied *P. unifilis* populations, where MCL size class intervals varied between 27 and 51.8 cm (e.g., Guaporé River in the western Amazon: FACHÍN-TERÁN & VOGT 2004; Colombia: ESCALONA et al. 2012; northeastern Bolivia: CONWAY-GÓMEZ et al. 2014; Orinoco River basin in Venezuela: ESCALONA et al. 2018). Effects of female size or age on egg morphology and clutch size have been observed in some species of turtles (KOLBE & JANZEN 2001, FERREIRA-JÚNIOR et al. 2007, ESCALONA et al. 2018). In the current study, the magnitude of the association between egg and clutch sizes and female size varied with the substrate, with the effect of female size on egg shape and size being more pronounced in clutches from the sand bank than in those from the clay banks. ESCALONA et al. (2018) suggested that the *P. unifilis* nesting on sandy beaches in the Orinoco basin might be following the Optimality Theory. This theory suggests that clutch characteristics (i.e., egg size and shape, number of eggs) may be linked to female size, with individual females adjusting the numbers and sizes of their eggs in response to environmental variables (SMITH & FRETWELL 1974). Our results seem to corroborate two contrasting results predicted by the Optimality Theory: the “non-optimal egg size pattern”, where egg size could be linked to female size, and the “no trade-off” that links individual egg size to the number of eggs in a clutch. However, we point out that in our study, some observed associations may deviate from the predictions of the theory, as well as from the results of ESCALONA et al. (2018), since their results focused on the size variation of females that nest solely on sandy beaches. Furthermore, observed associations between female size and egg biometrics could be constrained by factors other than maternal sizes, such as the predominant incubation substrate in the study area, and energetic costs associated with a second clutch in the same season (ERICKSON 2018). In the event of environmental changes, natural selection is generally expected to favour new adaptations and behavioural adjustments of local groups to available habitats (e.g. nesting sites).

It is worth pointing out that in some localities, overlaps with more specialized beach-nesting turtles species (e.g., *P. expansa*: Giant South American River Turtle, *P. sextuberculata*: Six-tubercled Amazon River Turtle; PANTOJA-LIMA et al. 2009, FERREIRA-JÚNIOR & CASTRO 2010) may result in a differential selection of which parts of the beaches are to be utilized for nesting. *Podocnemis expansa*, for

example, prefers more elevated sites whereas *P. unifilis* uses lower ones (FERREIRA-JÚNIOR & CASTRO 2003, PANTOJA-LIMA et al. 2009). *Podocnemis unifilis* is the main species nesting in our study area, with only four nests of *P. sextuberculata* having been recorded, i.e., two in 2012 (ERICKSON et al. 2015), and two in 2013, and none of *P. expansa*. It is probable that these beach-nesting specialists nest in other areas, even if adults are locally present. When we analysed nest positioning relative to vegetation and water line, sandy-bank-nesting females seemed to be more selective than those that were nested on clay banks. Larger females appeared to nest earlier in the breeding season, and their nests were always closer to water and vegetation. Nests of smaller females were distributed along the sand bank, but always in intermediate positions between the vegetation and water. It is likely that younger females avoid competition with dominant and older individuals in the population. More mature individuals may occupy the best territories in the source so that the next best territories available may be in sinks. This alternative has been called the “ideal preemptive distribution” because a breeding site can be preempted if it has already been occupied (PULLIAM & DANIELSON 1991). Younger, subordinate individuals may then be able to take territories in the source, but new subordinate juveniles from the source will have to move to the sink. As fitness can vary with potential breeding sites within habitat patches, individuals of various reproductive ages (in this case, size of *P. unifilis*) must select the best available nesting sites. *Podocnemis unifilis* seems to follow this behavioural trend for groups that nest in sandy substrate. In the clayey substrate, evidently the criteria of choice seem to be related to other factors, with the choice of nesting site being apparently related more to the availability of substrate than to the proximity of the nest to water or vegetation, as seen in the group nesting on the sandy bank.

The choice of nesting substrate type is an important factor in the determination of potential female breeding success, especially in terms of investment into clutch size (VALENZUELA 2001, DOODY et al. 2003, KOBAYASHI et al. 2017). Our results demonstrate that *P. unifilis* nesting on sand banks deposited, on average, fewer eggs than those using clay banks, with no differences in clutch size between substrate types across the three nesting seasons monitored (Table 1). On the sand bank, clutches consisted of 4 to 40 eggs per nest (mean 22), and on clay banks, ranged from 13 to 51 eggs (mean 28), which are values that are similar to those reported for the same species from the Orinoco (ESCALONA et al. 2018) and Amazonas River basins (VANZOLINI 2003), although neither of these studies analysed substrate-related variations in clutch size. For the Tocantins-Araguaia, mean clutch size ranged from 12 to 13 eggs (FERREIRA-JÚNIOR et al. 2007, FERREIRA-JÚNIOR & CASTRO 2010), which is smaller than the values reported from the Amazon and Orinoco basins (VANZOLINI 2003, ESCALONA et al. 2018). A potential driver for the small size of Yellow-spotted Amazon River Turtle clutches in the Tocantins-Araguaia is niche overlap with *P. expansa*, because it is an abundant species in that area. Competitive exclu-

sion could, therefore, be selecting groups of females that would typically produce smaller clutches. Like most studies on *Podocnemis* spp., that in the Tocantins-Araguaia focused on sandy beaches, so that clutch sizes for that population of *P. unifilis* may have been underestimated, because clay banks host larger clutch sizes, but were not considered in the above-mentioned studies.

Most turtles show high sensitivity to thermal conditions (EWERT & NELSON 1991, FUENTES et al. 2010), which may be the result of ecological mechanisms associated with the clade’s life history and were adjusted in response to environmental changes (ERICKSON 2018), as well as of evolutionary processes that have undergone natural selection (DOODY et al. 2003, HULIN et al. 2009). In our study, we found a mean difference of 15 days in the egg incubation periods between *P. unifilis* nests deposited on clay banks (77 days) and on the sand bank (62 days) (Table 1), which is in line with the wide-range trend already observed in this species (RUEDA-ALMONACID et al. 2007). Clay-rich soils retain less heat than sandy ones and this could explain both the longer incubation period and the sex ratios generated in different substrates (ERICKSON 2018), as well as other factors impacting on hatchling growth and survivorship (HULIN et al. 2009, FUENTES et al. 2010, REFSNIDER & JANZEN 2012). Despite the substrate-linked pattern observed in this species, studies of *P. unifilis* nests on a complex of sandy banks on Bananal Island in the Tocantins-Araguaia basin reported little sensitivity to granulometric variation of the sandy substrate (FERREIRA-JÚNIOR & CASTRO 2003). This is in contrast to species that are more specialised in utilizing sandy banks for nesting, such as *P. expansa*, where incubation times are known to be influenced by granulometric differences (FERREIRA-JÚNIOR & CASTRO 2003). Turtle species nesting in substrates with different granulometric properties could vary their responses depending on the local environmental conditions (PACKARD & PACKARD 1997, KAMEL & MROSOVSKY 2005, ERICKSON 2018). It is highly likely that part of the variation observed in *P. unifilis* incubation periods is linked to the nature of its life history and observed physiological plasticity in the pre-nesting period (ERICKSON 2018), plus possible genetic differences at the population level (AGOSTINI 2016).

Variations in egg incubation period observed between species and between populations could provide a partial explanation for the relatively low survival rates reported for turtles (WILSON 1998, ESCALONA et al. 2009, DELGADO et al. 2010). Nests are located in fixed positions during incubation (MITCHELL & WALLS 2013), which renders them more susceptible to external influences (PACKARD & PACKARD 1997, HULIN et al. 2009, ERICKSON & BACCARO 2016). Although hatching success was higher on the sand bank (84.82%) than on the clay banks (72.97%), *P. unifilis* hatchling emergence rates can be considered high for both incubation substrates. At a first glance, our results on hatching success do not explain the low survival rates commonly reported for incubating turtle eggs. However, when the joint influence of several external factors that reduced incubation success on both substrates was considered, survival of hatchlings incubated

in sand fell to 57.34%, and to 25.80% in clay, which better explains the low survival rates reported for the group.

Observed rates of natural predation were higher for nests in clay banks (19%) than on the sand bank (7%). For the latter, lizards of the family Teiidae (*Tupinambis teguixin*: BATISTELLA & VOGT 2008, PIGNATI et al. 2013b; *Ameiva ameiva*, *Crocodilurus lacertinus*: BATISTELLA & VOGT 2008), birds of the family Falconidae (*Daptrius ater*: BATISTELLA & VOGT 2008; *Caracara plancus*: FERREIRA-JÚNIOR & CASTRO 2010), and Cathartidae (*Cathartes aura*, *Coragyps atratus*: FERREIRA-JÚNIOR & CASTRO 2010) are most commonly reported as natural predators of *Podocnemis* spp. nests. For nests on clay banks, the literature is scarce and limited to a few localities, with the main predation records being associated with *T. teguixin* (BATISTELLA & VOGT 2008, WALDEZ et al. 2013, ERICKSON & BACCARO 2016), and two species of ants (*Solenopsis saevissima*: PIGNATI et al. 2013b; *S. geminata*: ERICKSON & BACCARO 2016). As the nesting behavior of *Podocnemis* spp. is influenced by water level variation (ALHO & PÁDUA 1982, THORBJARNARSON et al. 1993), some populations could change the timing of nesting each year. However, recent studies focusing on the nesting success in *P. unifilis* and other species of the genus have indicated that flooding is still the main abiotic factor responsible for clutch failure (CAPUTO et al. 2005, PANTOJA-LIMA et al. 2009, PIGNATI et al. 2013b, EISEMBERG et al. 2016).

The consumption of turtle eggs by humans living close to nesting areas is still commonly reported in the literature (CAPUTO et al. 2005, NORRIS & MICHALSKI 2013, PANTOJA-LIMA et al. 2014, FIORI & SANTOS 2015). In our study, overall human predation compromised the successful incubation of 16% of *P. unifilis* nests. In general, the levels of human predation at the study sites were low compared to other localities (ESCALONA & FA 1998: Venezuela; PIGNATI et al. 2013b: Brazilian Amazon). This is likely related to the fact that the current study was carried out in a Sustainable Use Conservation Unit, where management and conservation education programs are conducted annually within the riparian communities living in the Reserve, both for turtles (WALDEZ et al. 2013) and other taxa (see: MARIANI et al. 2013, ARANTES & FREITAS 2016, PETERSEN et al. 2016). Consequently, it is likely that the research operations and the awareness activities of the Amazonian Chelonian Conservation Program together contributed to a reduction of local egg collection (ERICKSON 2018). This is notable, considering that this sector of the Purus River is still considered one of the main Amazonian turtle trafficking routes (PANTOJA-LIMA et al. 2014).

Life history differences between populations may also be reflected in hatchling morphological characteristics. Some studies have indicated that hatchlings' morphological characters could be maternally influenced (DOODY et al. 2003, ESCALONA et al. 2009). In line with this, we recorded a potential effect of female size on the morphological characteristics of *P. unifilis* hatchlings. However, a female's choice of nesting substrate has an evident impact. Hatchlings incubated in the sand were significantly larger and heavier than

those incubated in clay. In Ecuador, sizes (MCL and MCW) of Yellow-spotted Amazon River Turtle hatchlings from sandy-banks nests (41.8 and 39.2 mm, resp.; CAPUTO et al. 2005) were similar to those from the sandy soil we monitored (42.22 and 38.91 mm, resp.). In the Tocantins-Araguaia, hatchlings from nests in the sand were smaller (e.g., FERREIRA-JÚNIOR et al. 2007: MCL = 40.10 mm, WEI = 14.8 g) than the beach-incubated hatchlings in our study, even though mean sizes and weights were very similar to values for hatchlings from the clay-bank nests in our study, (MCL = 40.71 mm, WEI = 14.21 g). Considering that turtle nest ecology is still poorly understood, it is premature to make generalizations on the observed differences between hatchlings from the two substrate types, or on potential maternal effects on offspring size. However, since differences between substrate types were found for all reproductive aspects tested herein, we consider that physical characteristics of the nesting substrate are the most important factor correlated with *P. unifilis* hatchling size at our study site.

The present study has shown that incubation success may vary between substrates due to different biotic and environmental factors related to egg incubation. The availability of suitable substrates may drive nesting behaviour adjustments, which may then affect offspring survival and, hence, female fitness. Generalist species that nest on different substrate types, such as *P. unifilis*, could be less vulnerable to the effects of environmental change, but are still under constant threat from the illegal commercial trade of their meat and eggs (NORRIS & MICHALSKI 2013, PANTOJA-LIMA et al. 2014), and possibly by the consumption for the subsistence of riverine human populations in certain areas of the Amazon, including the lower Rio Purus (PANTOJA-LIMA et al. 2009, WALDEZ et al. 2013). Being among the main species of endangered turtles in Brazil (Turtle Taxonomy Working Group 2017), conservation strategies should no longer be limited to sites with beach-based nesting areas. The use of different substrates for nesting may have provided a greater resilience of the populations of *P. unifilis* to climatic changes, and a higher incidence of females hatching from sandy substrates apparently has been compensated by a predominance of males incubated in the clayey substrate (ERICKSON 2018). Understanding patterns of behavioural plasticity and habitat use at different temporal and spatial scales should be considered in conservation strategies since species may respond differently to both varying conditions and extreme events. Long-term studies of the demographic parameters (i.e., related to life history) of the populations in each patch are necessary since misguided decision-making might result in rapid population declines. Ecological traps can be created unintentionally by giving a site the appearance of quality habitat, but which in fact has not yet developed all the functional elements necessary for an organism's survival and reproduction (LAMB et al. 2016). Therefore, we strongly recommend focusing on ecological and behavioural studies to test hypotheses associated with life history variation, as does the data presented in this study. This multi-character approach of the nesting ecology of this generalist species ends up with be-

ing fundamental, so that future biodiversity-related actions and those essential for the management and conservation of other threatened turtle species can be more effective.

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References

- ACKERMAN, R. A. (1997): The nest environment and the embryonic development of sea turtle. – pp. 83–106 in: LUTZ, P. L. & J. A. MUSICK (eds): *The biology of sea turtles*. – Boca Raton, Florida.
- AGOSTINI, M. A. P. (2016): Padrões genético-populacionais do “tracajá” *Podocnemis unifilis* (Troschel, 1848) (Testudines: Podocnemididae) na Amazônia brasileira. – Unpubl. MSc thesis Federal University of Amazonas.
- ALCOCK, J. (2011): Comportamento animal: uma abordagem evolutiva. – Artmed, Porto Alegre.
- ALHO, C. J. R. & L. F. M. PÁDUA (1982): Reproductive parameters and nesting behaviour of the Amazon turtle *Podocnemis expansa* (Testudinata: Pelomedusidae) in Brazil. – Canadian Journal of Zoology, **60**: 97–103.
- ARANTES, M. L. & C.E.C. FREITAS (2016): Effects of fisheries zoning and environmental characteristics on population parameters of the tambaqui (*Colossoma macropomum*) in managed floodplain lakes in the Central Amazon. – Fisheries Management and Ecology, **23**: 133–143.
- BATISTELLA, A. M. & R. C. VOGT (2008): Nesting ecology of *Podocnemis erythrocephala* (Testudines, Podocnemididae) of the Rio Negro, Amazonas, Brazil. – Chelonian Conservation and Biology, **7**: 12–20.
- BITTENCOURT, M. M. & S. A. AMADIO (2007): Proposta para identificação rápida dos períodos hidrológicos em áreas de várzea do Rio Solimões-Amazonas nas proximidades de Manaus. – Acta Amazonica, **37**: 303–308.
- BONACH, K., C. I. PIÑA & L. M. VERDADE (2006): Allometry of reproduction of *Podocnemis expansa* in southern Amazon basin. – Amphibia-Reptilia, **27**: 55–61.
- BOOTH, D.T., E. A. BURGESS, J. McCOSKER, J. M. LANYON (2004): The influence of incubation temperature on post-hatching fitness characteristics of turtles. – International Congress Series, **1275**: 226–233.
- CAPUTO, F. P., D. CANESTRELLI & L. BOITANI (2005): Conserving the terecay (*Podocnemis unifilis*, Testudines: Pelomedusidae) through a community-based sustainable harvest of its eggs. – Biological Conservation, **126**: 84–92.
- CEBALLOS, C. P., D. C. ADAMS, J. B. IVERSON & N. VALENZUELA (2013): Phylogenetic patterns of sexual size dimorphism in turtles and their implications for Rensch's rule. – Evolutionary Biology, **40**: 194–208.
- CONWAY-GOMEZ, K., M. REIBEL & C. MIHIAR (2014): A predictive model of yellow spotted river turtle (*Podocnemis unifilis*) encounter rates at basking sites in lowland eastern Bolivia. – Applied Geography, **53**: 332–340.
- DELGADO, C., A. V. M. CANARIO & T. DELLINGER (2010): Sex ratios of loggerhead sea turtles *Caretta caretta* during the juvenile pelagic stage. – Marine Biology, **157**: 979–990.
- DOODY, J.S., P. WEST & A. GEORGES (2003): Beach selection in nesting pig-nosed turtles, *Carettochelys insculpta*. – Journal of Herpetology, **37**: 178–182.
- EISEMBERG, C. C., R. A. M. BALESTRA, S. FAMELLI, F. F. PEREIRA, V. C. D. BERNARDES & R. C. VOGT (2016): Vulnerability of giant south American turtle (*Podocnemis expansa*) nesting habitat to climate-change-induced alterations to fluvial cycles. – Tropical Conservation Science, **2016**: 1–12.
- ERICKSON, J., D. P. OLIVEIRA, B. MARIONI & I. P. FARIAS (2015): Analysis of the mating system of *Podocnemis sextuberculata* in the lower Purus River of the Brazilian Amazon: another record of multiple paternity in chelonians. – Salamandra, **51**: 215–220.
- ERICKSON, J. & F. BACCARO (2016): Nest predation of the yellow-spotted Amazon River turtle (*Podocnemis unifilis*, Troschel 1848) by the fire ant (*Solenopsis geminata* Fabricius, 1804) in the Brazilian Amazon. – Herpetological Journal, **26**: 83–186.
- ERICKSON, J. (2018): Explorando informações multicaráter da ecologia do tracajá (*Podocnemis unifilis* Troschel, 1848): a história de vida contada através dos ninhos. – Unpubl. PhD thesis National Institute of Amazonian Research.
- ESCALONA, T. & J. E. FA (1998): Survival of nests of the terecay turtle (*Podocnemis unifilis*) in the Nichare-Tawadu Rivers, Venezuela. – Journal of Zoology, **244**: 303–312.
- ESCALONA, T., N. VALENZUELA & D. C. ADAMS (2009): Nesting ecology in the freshwater turtle *Podocnemis unifilis*: spatio-temporal patterns and inferred explanations. – Functional Ecology, **23**: 826–835.
- ESCALONA, T., K. CONWAY-GOMEZ, M. MORALES-BETANCOURT, F. ARBELAEZ & R. ANTELO (2012): *Podocnemis unifilis*. – pp. 386–398 in: PÁEZ, V. P., M. A. MORALES-BETANCOURT, C. A. LASSO, O. V. CASTAÑO-MORA & B. C. BOCK (eds): *Biología y conservación de las tortugas continentales de Colombia. Series recursos hidrobiológicos y pesqueros continentales de Colombia*. – Instituto de Investigación de los Recursos Biológicos Alexander von Humboldt, Bogotá.
- ESCALONA, T., D. C. ADAMS & N. VALENZUELA (2018): A lengthy solution to the optimal propagule size problem in the large-bodied South American freshwater turtle, *Podocnemis unifilis*. – Evolutionary Ecology, **32**: 29–41.
- EWERT, M. A. & C. E. NELSON (1991): Sex determination in turtles: diverse patterns and some possible adaptive values. – Copeia, **1991**: 50–69.
- FACHÍN-TERÁN, A. & R. C. VOGT (2004): Population structure, size and sex ratio in *Podocnemis unifilis* (Testudines, Podocnemididae) in the Guaporé river (RO), northern Brazil. – Phyllomedusa, **3**: 29–42.
- FERRARA, C. R., C. K. FAGUNDES, T. Q. MORCATTY & R. C. VOGT (2017): Guia de distribuição e identificação dos quelônios Amazônicos. – Wildlife Conservation Society Brasil, Manaus.

- FERREIRA-JÚNIOR, P. D. & P. T. A. CASTRO (2003): Geological control of *Podocnemis expansa* and *Podocnemis unifilis* nesting areas in Rio Javaés, Bananal Island, Brazil. – *Acta Amazonica*, **33**: 445–468.
- FERREIRA-JÚNIOR, P. D., A. MALVASIO & O. S. GUIMARÃES (2003): The influence of geological factors on reproductive aspects of *Podocnemis unifilis* (Testudines, Pelomedusidae), on the Javaés River, Araguaia National Park, Brazil. – *Chelonian Conservation and Biology*, **4**: 626–635.
- FERREIRA-JÚNIOR, P. D. & P. T. A. CASTRO (2006): Thermal environment characteristics of *Podocnemis expansa* and *Podocnemis unifilis* nesting areas the Javaés river, Tocantins, Brazil. – *Chelonian Conservation and Biology*, **5**: 102–107.
- FERREIRA-JÚNIOR, P. D., A. Z. CASTRO & P. T. A. CASTRO (2007): The importance of nidification environment in the *Podocnemis expansa* and *Podocnemis unifilis* phenotypes (Testudines: Podocnemididae). – *South American Journal of Herpetology*, **2**: 39–46.
- FERREIRA-JÚNIOR, P. D. & P. T. A. CASTRO (2010): Nesting ecology of *Podocnemis expansa* (Schweigger, 1812) and *Podocnemis unifilis* (Troschel, 1848) (Testudines, Podocnemididae) in the Javaés River Brazil. – *Brazilian Journal of Biology*, **70**: 85–94.
- FIORI, M. M. & C. F. M. SANTOS (2015): A carne, a gordura e os ovos. – *EdiPUCRS*, Porto Alegre.
- FUENTES, M. M. P. B., M. HAMANN & C. J. LIMPUS (2010): Past, current and future thermal profiles of green turtle nesting grounds: implications from climate change. – *Journal of Experimental Marine Biology and Ecology*, **383**: 56–64.
- GOTELLI, N. J. & A. M. ELLISON (2011): Princípios de estatística em ecologia. – *Artmed*, Porto Alegre.
- HALLER, E. C. P. & M. T. RODRIGUES (2006): Reproductive biology of the six-tubercled Amazon river turtle *Podocnemis sextuberculata* (Testudines: Podocnemididae), in the Biological Reserve of Rio Trombetas, Pará, Brazil. – *Chelonian Conservation and Biology*, **5**: 280–284.
- HAYS G. C., J. S. ASHWORTH, M. J. BARNESLEY, A. C. BRODERICK, D. R. EMERY, B. J. GODLEY, A. HENWOOD & E. L. JONES (2001): The importance of sand albedo for ternal conditions on sea turtle nesting beaches. – *Oikos*, **93**: 87–94.
- HAYS, G. C., S. FOSSETTE, K. A. KATSELIDIS, P. MARIANI & G. SCHOFIELD (2010): Ontogenetic development of migration: Lagrangian drift trajectories suggest a new paradigm for sea turtles. – *Journal of the Royal Society Interface*, **7**: 1319–1327.
- HULIN, V., V. DELMAS, M. GIRONDOT, M. H. GODFREY & J.-M. GUILLON (2009): Temperature-dependent sex determination and global change: are some species at greater risk? – *Population Ecology*, **160**: 493–506.
- IPCC (2013): *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.* – Cambridge University Press, Cambridge, New York.
- JANZEN, F. J. (1994): Vegetational cover predicts the sex ratio of hatchling turtles in natural nests. – *Ecology*, **75**: 1593–1599.
- JANZEN, F. J. & C. L. MORJAN (2001): Repeatability of microenvironment-specific nesting behavior in a turtle with environmental sex determination. – *Animal Behaviour*, **62**: 73–82.
- JUNK, W. J., P. B. BAYLEY & R. E. SPARKS (1989): The flood pulse concept in river-floodplain systems. – pp. 110–127 in: DODGE, D. P. (ed.): *Proceedings of the International Large River Symposium.* – Canadian Special Publication of Fisheries and Aquatic Sciences, Ottawa.
- KASKA, Y., J. R. DOWNIE, R. TIPPETT & R. FURNESS (1998): Natural temperature regimes for loggerhead and green turtle nests in the eastern Mediterranean. – *Canadian Journal of Zoology*, **76**: 723–729.
- KOBAYASHI, S., M. WADA, R. FUJIMOTO, Y. KUMAZAWA, K. ARAI, G. WATANABE & T. SAITO (2017): The effects of nest incubation temperature on embryos and hatchlings of the loggerhead sea turtle: Implications of sex difference for survival rates during early life stages. – *Journal of Experimental Marine Biology and Ecology*, **486**: 274–281.
- KOLBE, J. J. & F. J. JANZEN (2001): The influence of propagule size and maternal nest-sites selection on survival and behavior of neonate turtles. – *Functional Ecology*, **15**: 772–781.
- KOLBE, J. J. & F. J. JANZEN (2002): Impact of nest site selection on nest success and nest temperature in natural and disturbed habitats. – *Ecology*, **83**: 269–281.
- LAMB, C. T., G. MOWAT, B. N. MCLELLAN, S. E. NIELSEN, & S. BOUTIN (2016): Forbidden fruit: human settlement and abundant fruit create an ecological trap for an apex omnivore. – *Journal of Animal Ecology*, **86**: 55–65.
- LICH, P. (1984): Reptiles. – pp. 206–282 in: LAMMING, G. E. (ed.): *Marshall's Physiology of Reproduction: reproductive cycle of vertebrates.* – Churchill Livingstone, Edinburgh.
- LIU, Y., C. M. DAVY, H.-T. SHI & R. W. MURPHY (2013): Sex in the half-shell: a review of the functions and evolution of courtship behavior in freshwater turtles. – *Chelonian Conservation and Biology*, **12**: 84–100.
- MAGALHÃES, M. S., R. C. VOGT, A. SEBEN, L. C. DIAS, M. F. OLIVEIRA & C. E. B. MOURA (2017): Embryonic development of the giant South American river turtle, *Podocnemis expansa* (Testudines: Podocnemididae). – *Zoomorphology*, **136**: 523–537.
- MARIONI, B., R. BOTERO-ARIAS & S. FONSECA-JÚNIOR (2013): Local community involvement as a basis for sustainable crocodilian management in Protected Areas of Central Amazonia: problem or solution? – *Tropical Conservation Science*, **6**: 484–492.
- MITCHELL, J. C. & S. C. WALLS (2013): Nest site selection by Diamond-backed terrapins (*Malaclemys terrapin*) on a Mid-Atlantic Barrier Island. – *Chelonian Conservation and Biology*, **12**: 303–308.
- MROSOVSKY, N. & C. PIEAU (1991): Transitional range of temperature, pivotal temperatures and thermosensitive stages for sex determination in reptiles. – *Amphibia-Reptilia*, **12**: 169–179.
- MYERS, E. M., J. K. TUCKER & C. H. CHANDLER (2007): Experimental analysis of body size and shape during critical life-history events of hatchling slider turtles, *Trachemys scripta elegans*. – *Functional Ecology*, **21**: 1106–1114.
- NORRIS, D. & F. MICHALSKI (2013): Socio-economic and spatial determinants of anthropogenic predation on yellow-spotted Amazon river turtle, *Podocnemis unifilis* (Testudines: Pelomedusidae), nests in the Brazilian Amazon: implications for sustainable conservation and management. – *Zoologia*, **30**: 482–490.
- OKSANEN, J., F. G. BLANCHET, M. FRIENDLY, R. KINDT, P. LEGENDRE, D. MCGLINN, P. R. MINCHIN, R. B. O'HARA, G. L. SIMPSON, P. SOLYMOS, M. H. H. STEVENS, E. SZOECES & H. WAG-

- NER (2018): Vegan: Community Ecology, R package. Available from: <https://CRAN.R-project.org/package=vegan>.
- PACKARD, G. C. & M. J. PACKARD (1997): Type of soil affects survival by overwintering hatchlings of painted turtle. – *Journal of Thermal Biology*, **22**: 53–58.
- PÁEZ, V. P., J. C. CORREA, A. M. CANO & B. C. BOCK (2009): A comparison of maternal and temperature effects on sex, size, and growth of hatchlings of the Magdalena river turtle (*Podocnemis lewyana*) incubated under field and controlled laboratory conditions. – *Copeia*, **4**: 698–704.
- PANTOJA-LIMA, J., J. C. B. PEZZUTI, A. S. TEIXEIRA, D. FÉLIX-SILVA, G. H. REBÊLO, L. A. S. MONJELÓ & A. KEMENES (2009): Seleção de locais de desova e sobrevivência de ninhos de quelônios *Podocnemis* no baixo Rio Purus, Amazonas, Brasil. – *Revista Colombiana de Ciencia Animal*, **1**: 37–59.
- PANTOJA-LIMA, J., P. H. R. ARIDE, A. T. OLIVEIRA, D. FÉLIX-SILVA, J. C. B. PEZZUTI & G. H. REBÊLO (2014): Chain of commercialization of *Podocnemis* spp. turtles (Testudines: Podocnemididae) in the Purus River, Amazon basin, Brazil: current status and perspectives. – *Journal of Ethnobiology and Ethnomedicine*, **10**: 1–10.
- PETERSEN, T. A., S. M. BRUM, F. ROSSONI, G. F. V. SILVEIRA & L. CASTELLO (2016): Recovery of *Arapaima* sp. populations by community-based management in floodplains of the Purus River, Amazon. – *Journal of Fish Biology*, **2016**: 1–8.
- PIGNATI, M. T., L. F. FERNANDES, P. S. MIORANDO, P. D. FERREIRA & J. C. B. PEZZUTTI (2013a): Effects of the nesting environment on embryonic development, sex ratio, and hatching success in *Podocnemis unifilis* (Testudines: Podocnemididae) in area of várzea floodplain on the lower Amazon river in Brazil. – *Copeia*, **2**: 307–315.
- PIGNATI, M. T., L. F. FERNANDES, P. S. MIORANDO, P. D. FERREIRA & J. C. B. PEZZUTTI (2013b): Nesting site and hatching success of *Podocnemis unifilis* (Testudines: Podocnemididae) in a floodplain area in lower Amazon river, Pará, Brazil. – *South American Journal of Herpetology*, **8**: 175–185.
- PRICE, T. D., A. QVARNSTROM & D. E. IRWIN (2003): The role of phenotypic plasticity in driving genetic evolution. – *Proceedings of the Royal Society B*, **270**: 1433–1440.
- PULLIAM, H. R. & B. J. DANIELSON. (1991): Sources, sinks, and habitat selection – a landscape perspective on population-dynamics. – *American Naturalist*, **137**: S50–S66.
- R Development Core Team (2018): R: A language and environment for statistical computing. – The R Foundation for Statistical Computing, Vienna, Austria. Available from: <http://www.R-project.org/>.
- REFSNIDER, J. M. & F. J. JANZEN (2012): Behavioural plasticity may compensate for climate change in a long-lived reptile with temperature-dependent sex determination. – *Biological Conservation*, **152**: 90–95.
- RICKLEFS, R. E. (2011): *Economia da Natureza*. – Guanabara Kooogan, Rio de Janeiro, 546 pp.
- RUEDA-ALMONACID, J. V., J. L. CARR, R. A. MITTERMEIER, J. V. RODRÍGUEZ-MAHECHA, R. B. MAST, R. C. VOGT, A. G. J. RHO-DIN, J. O. VELÁSQUEZ, J. N. RUEDA & C. G. MITTERMEIER (2007): Las tortugas y los cocodrilianos de los países andinos del trópico. – Editorial Panamericana, Bogotá.
- SCHWANZ, L. E., T. EZAZ, B. GRUBER & A. GEORGES (2013): Novel evolutionary pathways of sex-determining mechanisms. – *Journal of Evolutionary Biology*, **26**: 2544–2557.
- SHINE, R. & P. S. HARLOW (1996): Maternal manipulation of offspring phenotypes via nest-site selection in an oviparous lizard. – *Ecology*, **77**: 1808–1817.
- SMITH, C. C. & S. D. FRETWELL (1974): The optimal balance between size and number of offspring. – *The American Naturalist*, **108**: 499–506.
- SPENCER, J. R. (2002): Experimentally testing nest site selection: fitness trade-offs and predation risk in turtles. – *Ecology*, **83**: 2136–2144.
- ST. JULIANA, J. R., R. M. BOWDEN & F. J. JANZEN (2004): The impact of behavioral and physiological maternal effects on offspring sex ratio in the common snapping turtle, *Chelydra serpentina*. – *Behavioral Ecology and Sociobiology*, **56**: 270–278.
- THORBJARNARSON, J. B., N. PEREZ & T. ESCALONA (1993): Nesting of *Podocnemis unifilis* in the Capanaparo river, Venezuela. – *Journal of Herpetology*, **27**: 347–351.
- Turtle Taxonomy Working Group (2017): Turtles of the world: Annotated checklist and atlas of taxonomy, synonymy, distribution, and conservation status (8th ed.). – *Chelonian Research Monographs*, **7**: 1–292.
- VALENZUELA, N. (2001): Constant, shift, and natural temperature effects on sex determination in *Podocnemis expansa* turtles. – *Ecology*, **82**: 3010–3024.
- VANZOLINI, P. E. (1977): A brief biometrical note on the reproductive ecology of some South American *Podocnemis* (Testudines, Pelomedusidae). – *Papéis Avulsos de Zoologia*, **31**: 79–102.
- VANZOLINI, P. E. (2003): On clutch size and hatching success of the south American turtles *Podocnemis expansa* (Schweigger, 1812) and *P. unifilis* Troschel, 1848 (Testudines, Podocnemididae). – *Anais da Academia Brasileira de Ciências*, **75**: 415–430.
- WALDEZ, F., L. GAMA-E-ADÁRIO, B. MARIONI, F. ROSSONI & J. ERICKSON (2013): Monitoramento participativo da caça de quelônios (Podocnemididae) por comunitários no baixo Rio Purus e proteção de sítios de desova na RDS Piagaçu-Purus, Brasil. – *Revista Colombiana de Ciencia Animal*, **5**: 4–23.
- WILSON, D. S. (1998): Nest-site selection: microhabitat variation and its effects on the survival of turtle embryos. – *Journal of Herpetology*, **22**: 88–96.

Supplementary data

The following data are available online:

Supplementary document S1. Descriptive statistics of the biometrics of *Podocnemis unifilis* eggs sampled in the sandy substrate and clayey substrate.

Supplementary document S2. Summary of linear regression analyses between response and predictor variables for each nesting substrate used by *Podocnemis unifilis*.

Supplementary document S3. Total number of nests monitored from *Podocnemis unifilis* that have completed the incubation phase, and the abiotic and biotic factors who compromised the hatchlings during the incubation period.

Supplementary document S4. Descriptive statistics of the morphological measurements of the *Podocnemis unifilis* offsprings sampled from 152 nests in the nesting area of the Piagaçu Purus SDR.