

# Nesting ecology of *Podocnemis expansa* (Schweigger, 1812) and *Podocnemis unifilis* (Troschel, 1848) (Testudines, Podocnemididae) in the Javaés River, Brazil

Ferreira Júnior, PD.<sup>a</sup> and Castro, PTA.<sup>b</sup>

<sup>a</sup>Programa de Mestrado em Ecologia de Ecossistemas, Centro Universitário Vila Velha,  
Rua Comissário José Dantas de Melo, 21, Boa Vista, CEP 29102-770, Vila Velha, ES, Brazil

<sup>b</sup>Departamento de Geologia, Universidade Federal de Ouro Preto – UFOP,  
Campus Morro do Cruzeiro, CEP 35400-000, Ouro Preto, MG, Brazil

\*e-mail: pdfj@hotmail.com

Received October 6, 2008 – Accepted November 2, 2008 – Distributed February 28, 2010

(With 2 figures)

## Abstract

Nest site has influence on incubation duration and hatching success of two Neotropical turtles, the Giant Amazon River Turtle (*Podocnemis expansa*) and Yellow-Spotted Side-Neck Turtle (*Podocnemis unifilis* – “Tracajá”). The 2000 and 2001 nesting seasons have been monitored at the Javaés River in Bananal Island, Brazil. Although they nest on the same beaches, there is a separation of the nesting areas of *P. unifilis* and *P. expansa* nests on the upper parts of the beach. The incubation duration for *P. expansa* is influenced by the nesting period, the height of the nest from the river, the clutch size, and the grain size in the site of the nest. Nests of *Podocnemis expansa* placed in coarse sediments have shorter incubation duration than those placed in finer sediments. The hatching success in *P. expansa* is influenced by grain size, incubation duration, and nesting period. The grain size is negatively correlated with hatching success, indicating that the nests situated in finer-grained sand have better chances of successful egg hatching than those in coarser-grained sand. Nests of the end of the reproductive season have lower hatching success and incubation duration than those at the start of the season. For *P. unifilis*, the nesting period and nest depth influence the incubation duration; moreover, the river dynamics significantly affect the hatching success.

The oscillation of the river level and the moment of initial increase, the height of the nest from the river level, and the nesting period are all decisive components for hatching success. The results of this research show the importance of protecting areas with great geological diversity, wherein the features of the environment can affect the microenvironment of nests, with consequences on incubation duration and hatching success.

**Keywords:** turtle, incubation, reproduction, yellow-spotted side-neck turtle, giant amazon river turtle.

## Ecologia reprodutiva de *Podocnemis expansa* (Schweigger, 1812) e *Podocnemis unifilis* (Troschel, 1848) (Testudines, Podocnemididae) no Rio Javaés, Brasil

### Resumo

O local da desova influencia a duração da incubação e o sucesso de eclosão de duas tartarugas neotropicais brasileiras, a tartaruga-da-amazônia (*Podocnemis expansa*) e o tracajá (*Podocnemis unifilis*). Monitoramos as estações reprodutivas de 2000 a 2001 nas praias do Rio Javaés na Ilha do Bananal. Apesar de as duas espécies desovarem nas mesmas praias, existe uma separação entre as áreas de desova, com *P. expansa* desovando nas partes mais elevadas das praias. A duração da incubação de *P. expansa* foi influenciada pelo período da desova, pela altura do ninho em relação ao nível do rio, pelo número de ovos e pelo tamanho do sedimento. Ninhos de *P. expansa* localizados em sedimentos mais grossos apresentaram uma menor duração da incubação. O sucesso da eclosão de *P. expansa* foi influenciado pelo tamanho do sedimento, duração da incubação e período da desova. O sucesso da eclosão se correlacionou negativamente com o tamanho do sedimento indicando que ninhos situados em areias mais finas têm maiores chances de eclodirem. Ninhos do final da temporada reprodutiva têm menor sucesso de eclosão e menor duração da incubação. Para *P. unifilis*, a duração da incubação é influenciada pelo período da desova e a dinâmica fluvial exerce grande efeito no sucesso da eclosão. A oscilação do nível do rio Javaés e o período da desova foram decisivos para o sucesso da eclosão.

Os resultados ressaltam a importância da proteção de áreas com maior diversidade geológica onde as variáveis ambientais podem afetar o microambiente dos ninhos com reflexos na duração da incubação e no sucesso da eclosão dos filhotes.

**Palavras-chave:** tartaruga, incubação, reprodução, tracajá, tartaruga-da-amazônia.

## 1. Introduction

Incubation duration is frequently used as an indicator of metabolic activity in the embryonic development of turtles, which show temperature-dependent sex determination. Several investigators have reported that the incubation duration in turtles is negatively correlated with the incubation temperature (Ewert et al., 2004; Valenzuela, 2001). The incubation duration is considered the easiest and least invasive method to estimate the sex ratio of the sea turtle hatchlings because the definition of their sex depends on environmental conditions (Marcovaldi et al., 1997; Mrosovsky et al., 1999). In turtles, low temperatures induce low metabolic activity, longer incubation durations, and a greater proportion of males. These authors, who suggest the use of incubation duration as an indicator of sex ratio, are unanimous in pointing out their concern and need for a broad model to absorb climatic variations. Incubation temperature and consequently incubation duration, may be affected by the mineralogical composition of the sediment (Milton et al., 1997), grain size (Souza and Vogt, 1994; Ferreira-Júnior and Castro, 2006a), albedo (Hays et al., 2001), nesting date (Baptistotte et al., 2003), nest depth (Souza and Vogt, 1994), and nest shade (Vogt and Bull, 1984; Alho et al., 1985), which affect the sex ratio of the hatchlings. Both *Podocnemis expansa* (Alho et al., 1985; Valenzuela et al., 1997; Valenzuela, 2001; Malvasio, 2001) and *Podocnemis unifilis* (Souza and Vogt, 1994) have temperature-dependent sex determination, indicating the importance of the nest site on the resulting sex ratios.

Environmental factors may differently affect the hatching success of different populations of the same species. In general, factors such as flooding (Alho and Pádua, 1982; Pezzuti and Vogt, 1999; Ferreira-Júnior and Castro, 2005; 2006b), shade cover, nest depth (Wilson, 1998; Valenzuela, 2001), incubation temperature (Bobyne and Brooks, 1994; Öz et al., 2004), soil moisture (Packard et al., 1987), and type of soil (Packard and Packard, 1997) may affect hatching success.

We tested the hypothesis that both the incubation duration and the hatching success of *P. expansa* and *P. unifilis* can be affected by the geological characteristics of the nesting place. The grain size, height of the nest from the river level and moment of increasing river level were correlated to incubation duration and hatchling success to test if the nesting period and nest site influence the turtle's reproductive success.

## 2. Materials and Methods

Both *P. expansa* and *P. unifilis* are widespread in South America, occupying principal rivers and affluents in the Amazon Region. *Podocnemis expansa* and *P. unifilis* lay their eggs in sandbanks (denominated beaches) that appear throughout the margins and inside channels during the dry seasons (Pritchard and Trebbau, 1984). Ferreira-Júnior and Castro (2003) report that al-

though both species nest in the same beaches, there is a spatial separation of their nests. In the Javaés River, *P. expansa* lays its eggs in the highest point of the beach, usually 300 cm above the river level, whereas *P. unifilis* concentrates its nests in areas less than 150 cm above the river level. The clutch size, nesting period, and incubation duration also vary between the species (Pritchard and Trebbau, 1984).

Data concerning nests located along the Javaés River, in the Araguaia National Park, located in the central-eastern region of Brazil in Tocantins State were collected. The study site (50° 05' W and 9° 59' S) was located in the northern part of Bananal Island, approximately 25 km from the mouth of the Javaés River in the Araguaia River. This area is one of the main nesting areas for *P. expansa* and *P. unifilis* and harbours the chelonian monitoring and protection headquarters of the RAN/IBAMA (Reptile and Amphibian Conservation and Management Centre, an organisation associated with the Brazilian Institute of Environment and Natural Renewable Natural Resources). The dimensions of the beach vary in length from 800 to 3,450 m and in width, from 160 to 470 m.

Geomorphologically, all the beaches monitored in the area can be classified as sandbanks. The nests receive direct sunlight during a major portion of the day and only some nests located near the riparian forest are shaded in the late afternoon. The beaches are deprived of vegetation, except for rare and scattered bushes.

The mean river level was calculated based on data provided by the National Electric Energy Agency (ANEEL) from 1972 to 1999. During the years 2000 and 2001, the level of the Javaés River along the beaches was determined and it was monitored only during the nesting and hatching periods. Every day, the river level was recorded on the beach opposite the camp, which was located between the beaches under study.

In 2000, the Canguçu, Jaburu, Comprida, Murici, Coco, and Marreca beaches were monitored; and in 2001, the Canguçu, Comprida, Coco, Goiaba, and Bonita beaches were examined. The beaches studied were changed from one year to the next due to the territorial demands of the local Indian tribes inhabiting Bananal Island. A detailed description of the main beaches may be found in the report by Ferreira-Júnior and Castro (2003).

Data in relation to incubation duration and hatching success were collected during the months of August and December in the reproductive seasons of 2000 and 2001. The incubation duration is defined as the time between egg-laying and emergence of the first hatchling from the nest. Hatching success is defined as the ratio of the number of hatchlings to the number of eggs laid. Nesting period is the time of egg-laying (Julian dates). The beaches were monitored each morning to search for tracks that would indicate the presence of nests. To obtain the data on hatching success and incubation duration of *P. unifilis*, it was necessary to protect the nests from at-

tacks by vultures (*Coragyps atratus* and *Cathartes aura*) and hawks (*Carcara plancus*) with wire-screened shelters. For calculating the hatching success in *P. unifilis*, nests lost due to bird predation were excluded because this analysis was focused on how the physical environment affects incubation. All *P. unifilis* nests considered herein were protected by wire-screened shelters.

The height of the nest from the river level consisted of two categories: a) height during the nesting time, which reflected the conditions encountered by the turtles during nesting activity, and b) maximum height of the nests, to establish a common standard level along the beaches. Height of the nest from river level was measured with a 5-mm-accuracy level. The maximum height of the nests was recorded on November 1, 2000, and September 30, 2001. On these two days, the Javaés River reached its lowest level, which was considered as a reference, allowing the comparison of nest heights along the various beaches in the following years.

Sedimentary samples, weighing around 200 g, were collected from each *P. expansa* and *P. unifilis* nest. The sediments were collected at a depth of 10 cm during the collection of hatchlings. The sediments were sieved and classified according to the Folk/Wentworth (Folk, 1974) scale, which involves the following divisions: pebble (diameter >4 mm), granule (4-2 mm), very coarse sand (2-1 mm), coarse sand (1-0.5 mm), medium sand (0.5-0.25 mm), fine sand (0.25-0.125 mm), very fine sand (0.125-0.063 mm), and mud (<0.063 mm).

Comparisons of incubation duration, hatching success, nesting period, nest depth, and nest height between the different species were carried out using the Student-*t* tests. Analysis of Variance (ANOVA one-way, followed by the Bonferroni post hoc test) was used to compare these variables among the beaches when the data showed a normal distribution. For datasets that significantly strayed from the average, differences were examined by the Kruskal-Wallis nonparametric ANOVA on Ranks, followed by the Dunn post hoc test. The variables expressed in percentage (hatchling success and grain size) were transformed to arcsin before being submitted to the ANOVA. The effects of nesting period, nest depth, nest height from the river level, and clutch size on the incubation duration and hatching success for each species were analysed through multiple linear regression. All data are provided as mean  $\pm$  standard deviation (SD).

Analysis of grain-size influence on the incubation duration and hatching success required a separate treatment. Each of the granulometric portions was considered a quantitative and independent variable. To avoid colinearity among the granulometric fractions, some portions were removed from the data analysis. Kruskal-Wallis nonparametric ANOVA on Ranks, followed by the Dunn post hoc test, was used to analyse the effects of the grain size of *P. expansa* and *P. unifilis* nests along the beaches. The influence of grain size on incubation duration and hatching success was also tested by multiple linear regressions.

### 3. Results

#### 3.1. Basic parameters of the nests

During the nesting seasons in 2000 and 2001, there were 447 egg-laying activities of *P. expansa* and 1068 of *P. unifilis* (Table 1). At Coco Beach in 2000 two nests of *P. expansa* hatched after only 38 days of incubation and a success rate of 91.8% and 93.4%, higher than average of all other nests (85.4%).

The nesting period differed between different species in 2000 ( $n = 623$ ,  $t = 72.9$ ,  $p < 0.001$ ) and 2001 ( $n = 503$ ,  $t = 27.9$ ,  $p = 0.005$ ). The concentrated nesting in *P. expansa* was a consequence of the gregarious habit of the species, members of which usually climb up the beach in large groups during nesting activity (Alho and Pádua, 1982). Although *P. unifilis* does not form groups to lay its eggs, nesting peaked in August, when 50% of the eggs were laid within nine days (Figure 1)

Mean height of egg placement from the level of the Javaés River differed between the two species ( $n = 860$ ,  $p < 0.001$ ). *Podocnemis expansa* nested in the upper parts of the beach ( $301.4 \pm 86.74$  cm above the river level, range 71-625 cm,  $n = 359$ ), whereas *P. unifilis* nested in the lower sites ( $104.8 \pm 80.19$  cm, range 9-467 cm,  $n = 501$ ) (Tables 1 and 2).

For *P. expansa*, the incubation duration, the clutch size, the clutch depth, and the height of the nest from the river level varied significantly along the monitored beaches (Table 1). For *P. unifilis*, the incubation duration, the clutch depth, the hatching success, and the height of the nests from the river level varied significantly along the beaches (Table 2).

#### 3.2. Basic physical parameters

In the period from 1972 to 1999, the river reached its lowest level between 29 September (in 1995) and 14 November (in 1993). In the years 2000 and 2001, the lowest level was reached between these dates, thus permitting important observations regarding the influence of the dynamics of the river on the hatching success of *P. unifilis*. In August 2000, when *P. unifilis* nested high on the beaches, the river level lowered slowly at a rate of 1.5 cm/day. In 2000, the peak nesting of *P. expansa* occurred in September, coinciding with a slight increase in the river level, which started to subside during the following days (Figure 2). In 2000, all the protected *P. unifilis* nests completed incubation without flooding. In 2001, the increase in the river level on 30<sup>th</sup> September flooded 72 of the protected nests (18% of the total) (Figure 2).

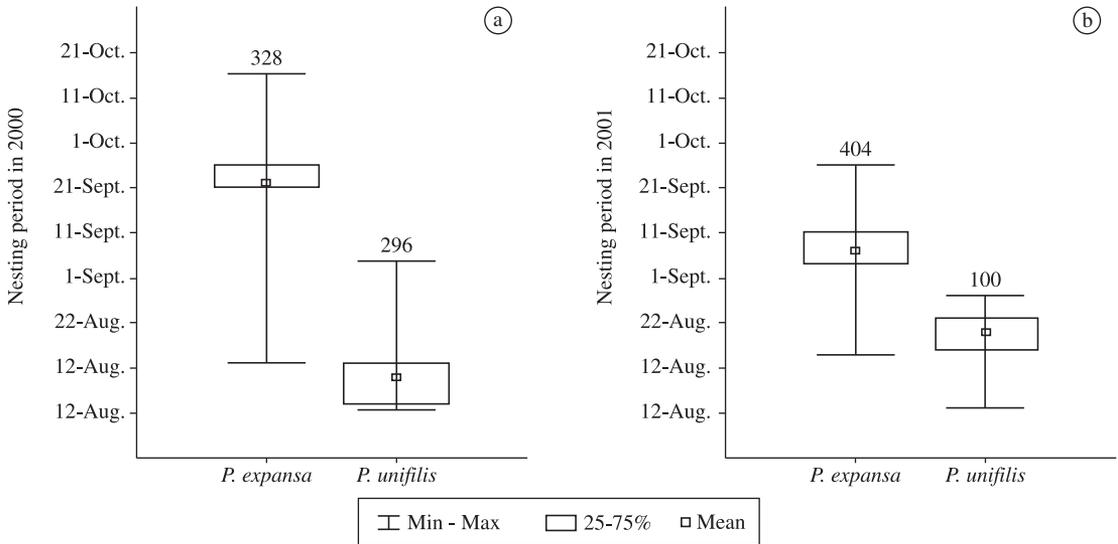
The grain size varied significantly among the beaches and the granulometric variation of *P. expansa* nests ( $n = 244$ ) was greater when compared to that of *P. unifilis* nests ( $n = 189$ ). For *P. expansa*, only the pebble and mud proportions remained fairly constant for all beaches, probably due to the small ratio of these components in comparison to the whole sample (<0.5% of both fractions). For *P. unifilis*, the proportions of very coarse sand, coarse sand, fine sand, and very fine sand

**Table 1.** Summary of reproductive and variation in the reproductive characteristics of *P. expansa* during 2000 and 2001 nesting seasons. Mean  $\pm$  SD (sample size). (only for n > 6). Dunn's test was performed to evaluate the differences among the beaches. For clutch depth in 2000 the Tukey test was used.

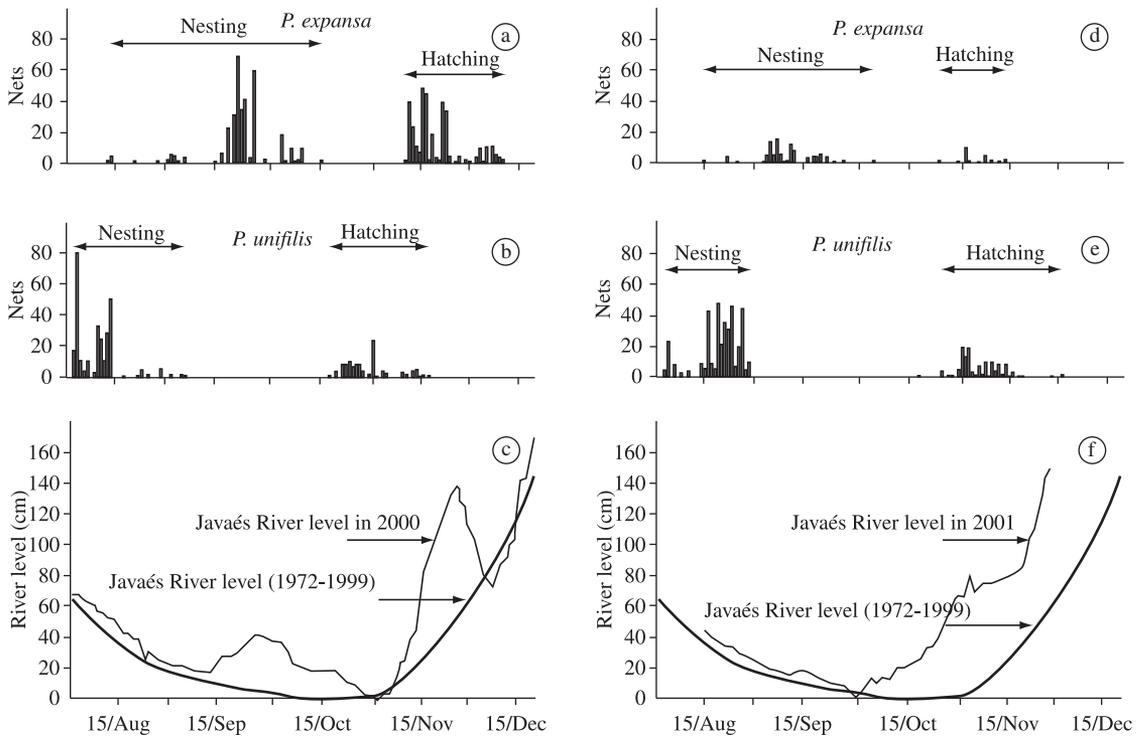
Beach	Incubation duration	Clutch size	Hatching success	Nest height in nesting	Maximum nest height	Clutch depth
Canguçu 2000	55.8 $\pm$ 7.60 (31) <sup>a</sup>	104.6 $\pm$ 21.02 (48) <sup>a</sup>	84.7 $\pm$ 24.22 (29)	281.8 $\pm$ 55.69 (50) <sup>a</sup>	313.0 $\pm$ 58.17 (50) <sup>a</sup>	64.6 $\pm$ 8.08 (29) <sup>a</sup>
2001	58.8 $\pm$ 6.06 (5)	56 (2)	74.0 $\pm$ 22.98 (2)	282.1 $\pm$ 83.71 (18)	307.2 $\pm$ 84.50 (19) <sup>a</sup>	58.0 (2)
Coco 2000	54.2 $\pm$ 4.48 (185) <sup>ac</sup>	104.0 $\pm$ 25.65 (185) <sup>bc</sup>	84.3 $\pm$ 22.25 (150)	263.6 $\pm$ 50.76 (163) <sup>ac</sup>	296.4 $\pm$ 51.02 (163) <sup>ac</sup>	60.1 $\pm$ 8.11 (189) <sup>a</sup>
2001	59.7 $\pm$ 5.86 (3)	121.3 $\pm$ 20.23 (3)	82.4 $\pm$ 12.35 (3)	275.1 $\pm$ 89.25 (7) <sup>a</sup>	299.0 $\pm$ 74.33 (9) <sup>a</sup>	56.67 $\pm$ 2.08 (3)
Comprida 2000	67.6 $\pm$ 7.51 (11) <sup>bde</sup>	108.1 $\pm$ 25.34 <sup>-c</sup>	86.7 $\pm$ 14.35 (11)	334.8 $\pm$ 67.29 (16) <sup>ade</sup>	363.6 $\pm$ 63.80 (16) <sup>-de</sup>	64.1 $\pm$ 7.68 (14) <sup>a</sup>
2001	66.5 (2)	(16) 124 (2)	92.6 (2)	359.8 $\pm$ 47.72 (23) <sup>a</sup>	379.5 $\pm$ 49.55 (24) <sup>bc</sup>	69.0 (2)
Murici 2000	61.4 $\pm$ 7.07 (21) <sup>bdf</sup>	110.2 $\pm$ 19.71 (24)	78.6 $\pm$ 26.89 (18)	511.1 $\pm$ 76.27 (23) <sup>bdfg</sup>	539.1 $\pm$ 73.14 (23) <sup>bdfg</sup>	57.8 $\pm$ 6.23 (20) <sup>b</sup>
Marreca 2000	62.5 $\pm$ 6.77 (19) <sup>bdf</sup>	118.2 $\pm$ 16.03 (18) <sup>a</sup>	87.1 $\pm$ 23.13 (18)	359.1 $\pm$ 61.17 (20) <sup>bd, g</sup>	389.7 $\pm$ 58.45 (20) <sup>-d, g</sup>	57.3 $\pm$ 7.70 (20) <sup>b</sup>
Jaburu 2000	68 $\pm$ 7.70 (4)	103.5 $\pm$ 16.24 (6)	51.2 $\pm$ 43.11 (5)	376.0 $\pm$ 38.06 (6) <sup>bde</sup>	405.0 $\pm$ 48.77 (6) <sup>ad</sup>	56 $\pm$ 10.14 (6) <sup>a</sup>
Bonita 2001	60.2 $\pm$ 5.95 (9)	114.1 $\pm$ 26.50 (9)	75.6 $\pm$ 29.72 (9)	253.6 $\pm$ 79.20 (18) <sup>bc</sup>	265.1 $\pm$ 75.55 (20) <sup>ade</sup>	66 $\pm$ 6.08 (3)
Goiaba 2001	67.2 $\pm$ 3.54 (6)	101.2 $\pm$ 27.29 (5)	74.4 $\pm$ 36.70 (5)	315.1 $\pm$ 34.92 (16) <sup>-c</sup>	334.7 $\pm$ 37.98 (16) <sup>acf</sup>	55.8 $\pm$ 3.77 (5)
2000	H = 58.477; p < 0.001*	H = 11.576; p = 0.041*	H = 2.93; p = 0.569	H = 111.75; p < 0.001*	H = 108.72; p < 0.001*	F = 3.67; p = 0.003*
2001	H = 2.64; p = 0.064	H = 2.76; p = 0.599	H = 20.71; p < 0.001*	H = 20.71; p < 0.001*	H = 23.74; p = 0.001*	
All beaches 2000	56.3 $\pm$ 6.68 (271)	105.9 $\pm$ 23.73 (263)	84.3 $\pm$ 22.25 (231)	300.1 $\pm$ 89.62 (278)	332.4 $\pm$ 88.48 (278)	60.4 $\pm$ 8.18 (278)
2001	62.0 $\pm$ 5.98 (25)	107.5 $\pm$ 30.87 (21)	77.7 $\pm$ 26.38 (21)	303.5 $\pm$ 76.65 (82)	321.5 $\pm$ 76.92 (88)	60.1 $\pm$ 6.31 (15)

**Table 2.** Summary of reproductive and variation in the reproductive characteristics of *P. unifilis* during 2000 and 2001 nesting seasons. Mean  $\pm$  SD (sample size). (only for n > 6). Dunn's test was performed to evaluate the differences among the beaches. For duration incubation in 2001 the Tukey test was used.

Beach	Incubation duration	Clutch size	Hatching success	Nest height in nesting	Maximum nest height	Clutch depth
Canguçu 2000	79 $\pm$ 5.28 (30)	13.8 $\pm$ 4.16 (31)	94.1 $\pm$ 11.33 (36)	147.4 $\pm$ 84.26 (67) <sup>a</sup>	182.2 $\pm$ 78.05 (133) <sup>a</sup>	15.6 $\pm$ 2.00 (48) <sup>a</sup>
2001	80.2 $\pm$ 6.37 (53) <sup>-c</sup>	13.1 $\pm$ 4.49 (35)	74.9 $\pm$ 40.91 (54) <sup>a</sup>	109.0 $\pm$ 74.28 (70) <sup>a</sup>	140.1 $\pm$ 66.51 (123) <sup>a</sup>	15.6 $\pm$ 2.79 (10)
Coco 2000	85.5 $\pm$ 4.95 (2)	12.7 $\pm$ 2.98 (23)	90.7 $\pm$ 21.24 (34)	55.7 $\pm$ 36.25 (113) <sup>bc</sup>	148.4 $\pm$ 32.71 (180) <sup>bc</sup>	15.3 $\pm$ 1.86 (81) <sup>bc</sup>
2001	80.0 $\pm$ 8.55 (24) <sup>a, -</sup>	13.4 $\pm$ 2.78 (25)	42.7 $\pm$ 47.32 (73) <sup>bc</sup>	88.6 $\pm$ 74.05 (98) <sup>ac</sup>	122.1 $\pm$ 62.74 (167) <sup>ab</sup>	16.0 $\pm$ 2.66 (68)
Comprida 2000	79.7 $\pm$ 4.09 (22)	14.4 $\pm$ 4.11 (28)	94.0 $\pm$ 9.17 (29)	154.2 $\pm$ 92.63 (42) <sup>-d</sup>	197.1 $\pm$ 92.77 (53) <sup>ade</sup>	16.3 $\pm$ 2.16 (41) <sup>ac</sup>
2001	84.7 $\pm$ 5.28 (7) <sup>bd</sup>	12.1 $\pm$ 3.19 (16)	77.7 $\pm$ 34.94 (20) <sup>-c</sup>	141.5 $\pm$ 94.42 (30) <sup>b</sup>	167.9 $\pm$ 94.10 (52) <sup>-cd</sup>	14.8 $\pm$ 1.93 (16)
Jaburu 2000	81.7 $\pm$ 13.20 (8)	12.7 $\pm$ 4.37 (10)	87.5 $\pm$ 30.13 (15)	132.6 $\pm$ 100.2 (22) <sup>-d</sup>	154.8 $\pm$ 88.37 (31) <sup>adef</sup>	15.6 $\pm$ 1.89 (22) <sup>-d</sup>
Bonita 2001	84.2 $\pm$ 2.68 (5)	12.8 $\pm$ 3.06 (6)	48.4 $\pm$ 49.79 (20) <sup>a</sup>	105.2 $\pm$ 65.39 (45)	135.7 $\pm$ 52.25 (106) <sup>-c</sup>	15.4 $\pm$ 2.08 (15)
Goiaba 2001	83.7 $\pm$ 8.36 (8) <sup>a</sup>	9.9 $\pm$ 4.91 (8)	63.2 $\pm$ 45.64 (13)	119.2 $\pm$ 85.35 (14) <sup>d</sup>	135.3 $\pm$ 73.24 (27) <sup>-d</sup>	16.4 $\pm$ 2.45 (9)
2000	H = 5.49; p = 0.240	H = 1.98; p = 0.738	H = 1.45; p = 0.693	H = 108.6; p < 0.001*	H = 127.8; p = < 0.001*	H = 2.01; p = 0.079
2001	F = 3.37; p = 0.02*	H = 2.75; p = 0.599	H = 20.26; p = 0.001*	H = 15.24; p = 0.004*	H = 17.36; p = 0.002*	H = 4.71; p = 0.319
All beaches	79.8 $\pm$ 6.43 (62)	13.6 $\pm$ 3.88 (92)	92.2 $\pm$ 17.56 (100)	104.8 $\pm$ 83.43 (244)	148.4 $\pm$ 75.65 (403)	15.6 $\pm$ 1.96 (207)
	81.0 $\pm$ 7.03 (97)	12.7 $\pm$ 3.86 (90)	58.3 $\pm$ 46.54 (180)	104.9 $\pm$ 77.16 (257)	135.6 $\pm$ 67.47 (475)	15.7 $\pm$ 2.57 (150)



**Figure 1.** Nesting period of *P. expansa* and *P. unifilis* in Javaés River in 2000 and 2001. The nesting period varied between the two species in 2000 and in 2001. a) For *P. expansa*, 50% of nesting in 2000 was concentrated between the 21<sup>st</sup> and 26<sup>th</sup> of September. For *P. unifilis*, 50% of the nesting in 2000 was concentrated in nine days, between the 4<sup>th</sup> and 13<sup>th</sup> of August. and b) In 2001, for *P. expansa*, nesting period was concentrated between the 4<sup>th</sup> and 11<sup>th</sup> of September, and for *P. unifilis*, it was mainly between the 16<sup>th</sup> and 23<sup>rd</sup> of August. Number above bar indicates sample size.



**Figure 2.** Nesting and hatching periods of *Podocnemis expansa* and *P. unifilis* in relation to the Javaés River level in 2000 and 2001: a) 328 *P. expansa* nests and 312 of these nests hatched; the peak nesting activity occurred when the Javaés River level had increased slightly. b) 296 *P. unifilis* nests and 121 of these nests hatched. c) Javaés River reached its lowest level on November 1<sup>st</sup>. The thin line represents the Javaés River level in 2000 and the thick line, the Javaés level for 27 years, between 1972 and 1999. d) 100 *P. expansa* nests and 25 of these hatched. e) 404 *P. unifilis* nests and 123 hatched. f) Javaés River level reached its lowest level on September 29<sup>th</sup>.

varied among the beaches. In general, the beaches could be divided into sandbanks consisting of well-selected medium sand (Jaburu, Comprida, Murici, Marreca, and Goiaba Beaches) and poorly selected medium and coarse sand (Canguçu, Bonita, and Coco Beaches). The beaches with finer sediments were more elevated than those with coarser sediments.

### 3.3. Factors related to the incubation duration

For *P. expansa*, significant correlations were found between incubation duration, nest height from river level, nesting period, and clutch size (Table 3). There was a negative correlation between sediment size and incubation duration of *P. expansa* in the years 2000 and 2001. Coco Beach, where the mean incubation duration was  $54.2 \pm 4.48$  days, contained the coarsest sediments in the study area, and Jaburu Beach (mean incubation duration of  $68.0 \pm 7.70$  days) constituted the sandbanks with the finer sediments.

Only the nesting period and the clutch depth affected the incubation duration of *P. unifilis* (Table 3). *Podocnemis expansa* and *P. unifilis* nests, formed in the end of the reproductive season, had higher incubation duration. None of the granulometric portions were correlated to the incubation duration of *P. unifilis*, suggesting that the grain size did not affect the incubation duration.

### 3.4. Factors related to hatching success

Hatching success of *P. expansa* was affected by the incubation duration, nesting period, and grain size. The incubation duration and nesting period were negatively correlated with hatching success of *P. expansa* (Table 4). Nests structured at the end of the nesting season, and with shorter incubation duration, had lower rates of hatching success. Nests placed in coarser sediments had lower rate of hatching success than those placed in finer sediments.

Hatching success of *P. unifilis* depended directly on the nesting period and the height of the nest in rela-

tion to the river level. In 2000, maximum hatching of *P. unifilis* eggs occurred before November when the level of the Javaés River was still decreasing (Figure 2). In 2001, the hatching occurred mostly in the beginning of November, when the river level was high (Figure 2). The nests most affected by flooding were those laid at the end of the nesting period and those situated in the lowest parts of the beaches. The significant difference in hatching success of *P. unifilis* between 2000 (92.2%) and 2001 (58.3%) was due to beach flooding. The grain size did not influence the hatching success of *P. unifilis*.

The small depth of *P. unifilis* clutches facilitates attacks by vultures (*Coragyps atratus* and *Cathartes*) and hawks (*Carcara plancus*). Predation occurs, mainly, during the first hours after egg-laying. There is also high predation of the *P. unifilis* hatchlings because predators are able to locate the nests after hatching takes place, attacking the nests before the hatchlings can leave the nest. In the two years under study, 1068 *P. unifilis* nests were found, and 462 were protected and/or managed during the experiments conducted by research teams working in the area. Of the remaining 606 nests, only seven (1.16% of the total) concluded the incubation without any protection efforts.

## 4. Discussion

Geological characteristics of the nesting sites of *P. expansa* and *P. unifilis* were significantly different. The two species nested in different areas of the beaches in Javaés River, with very little overlap. *Podocnemis expansa* nested in the upper areas of the beach near the riparian forests, whereas *P. unifilis* nested in areas closer to the river channel, preferably close to the base of the dunes (Ferreira-Júnior et al., 2003). Lowest river level is a basic requisite for *P. expansa* nesting activity on the Trombetas River, as pointed out by Alho and Pádua (1982) and Ferreira-Júnior and Castro (2003) for the

**Table 3.** Influence of biological, physical, and geologic factors on the incubation duration of *P. unifilis* and *P. expansa* in 2000 and 2001. (*P. expansa* in 2000,  $R^2 = 0.431$  and 2001,  $R^2 = 0.797$ ; *P. unifilis* in 2000,  $R^2 = 0.405$  and 2001,  $R^2 = 0.118$ ).

Variable	2000			2001		
	n	t	p	n	t	p
<i>Podocnemis expansa</i>						
Nesting period	193	-4.888	<0.001*	13	-2.525	0.036*
Clutch depth		-0.246	0.806		0.659	0.528
Maximum nest height from river level		8.356	<0.001*		3.043	0.016*
Nest height in nesting period		7.803	<0.001*		3.029	0.016*
Clutch size		-2.167	0.043*		0.0345	0.973
<i>Podocnemis unifilis</i>						
Nesting period	84	-2.574	0.012*	60	-5.345	<0.001*
Clutch depth		0.0161	0.987		-2.044	0.046*
Maximum nest height from river level		1.748	0.084		1.674	0.101
Nest height in nesting period		1.419	0.16		1.809	0.079
Clutch size		-0.887	0.378		-0.804	0.426

**Table 4.** Influence of biological, physical, and geologic factors on *P. unifilis* and *P. expansa*'s hatching success in 2000 and 2001. (*P. expansa* in 2000,  $R^2 = 0.123$  and 2001,  $R^2 = 0.155$ ; *P. unifilis* in 2000,  $R^2 = 0.045$  and 2001,  $R^2 = 0.353$ ).

Variable	2000			2001		
	n	t	p	n	t	p
<i>Podocnemis expansa</i>						
Nesting period	211	-2.306	0.022*	21	-0.891	0.399
Incubation duration		-3.372	<0.001*		0.0499	0.962
Clutch depth		0.784	0.434		0.758	0.47
Maximum nest height from river level		1.219	0.224		-0.335	0.746
Nest height in nesting period		0.119	0.905		-0.342	0.741
Clutch size		1.184	0.238		0.193	0.852
<i>Podocnemis unifilis</i>						
Nesting period	62	-0.33	0.743	78	-2.856	0.006*
Incubation duration		0.914	0.365		-0.837	0.405
Clutch depth		0.388	0.699		-0.156	0.877
Maximum nest height from river level		0.799	0.427		5.184	<0.001*
Nest height in nesting period		0.755	0.453		5.329	<0.001*
Clutch size		0.995	0.323		1.697	0.094

Javaés River. Eggs laid during the months of August and September had a higher incubation temperature in both *P. expansa* and *P. unifilis*, because embryonic development would occur during the warmest season of the year. For these turtles, an approximate one-month nesting phase and distinct incubation periods (Figure 1) made it possible for their hatching to coincide with an increasing river level. *Podocnemis unifilis* hatched earlier, at the end of October, when the Javaés River began to flood the lower parts of the beaches. *Podocnemis expansa* hatched later, in the months of November and December; however, because its nests are usually located higher on the beach, there is usually sufficient time for the hatchlings to leave their nests.

One of the principal causes for the loss of freshwater turtle nests is associated with changing river levels over the years. Flooding is common in the case of *P. expansa*, *P. unifilis*, and *P. sextuberculata* nests found along the beaches of Peruvian (Mitchell and Quiñones, 1994; Soini, 1995), Colombian (Hildebrand et al., 1988), and Brazilian rivers (Alho and Pádua, 1982; Pezzuti and Vogt, 1999; Ferreira-Júnior and Castro, 2003; 2006b). Floods are also the main cause of nest loss of *Trionyx muticus* (Plummer, 1976) and *Emydoidea blandingii* (Standing et al., 1999). Considering the Javaés River, the beaches are high enough to assure the hatching of *P. expansa* nests, and the river-level oscillation has no significant impact on the hatching success of this population. In contrast, *P. unifilis* nests are greatly influenced by changing river levels.

The timing of nesting affects embryonic development because, indirectly, it is responsible for the climatic conditions during the incubation period (Mrosovsky and Provancha, 1992; Valenzuela, 2001). In 2000, *P. expansa* nests laid at the end of the nesting period had shorter incubation duration, suggesting that they were subject

to higher temperatures (Spotila et al., 1987; Eendebak, 1995; Marcovaldi et al., 1997). Both higher and lower incubation temperatures can cause embryonic death (Krause et al., 1982; Wilson, 1998; Valenzuela, 2001). As *P. expansa* nests filled at the end of the reproductive period had shorter incubation duration with concurrent higher temperature, embryonic death increased.

In the area under study, *P. expansa* has nests only in sandy open beaches. On the contrary, *P. unifilis* is less selective and nests in open beaches, lake margins, and in vegetated areas. Data on *P. unifilis* is only partially representative, and it should not be generalised for the entire population. This fact should be especially considered when analysing the nest predation, which is higher than 98% (Ferreira-Júnior and Castro, 2003). Other turtles, such as *Chrysemys picta* (Tinkle et al., 1981), *Malaclemys terrapin* (Burger, 1976), and *Tryonix muticus* (Plummer, 1976), which lay eggs in shallower nests, show high nest predation. However, in none of these examples is the predation higher than that along the studied area. It is difficult to maintain a stable population under this intense predation (Congdon et al., 2000). In the vegetated areas, the predation should be less, according to the observations of other freshwater turtle species (Escalona and Fa, 1998; Spencer, 2002). The intense predation along the Javaés River beaches is attributed to the type of predator. Vultures and hawks patrol the beaches daily during the incubation and hatching periods, detecting nests of several ages. Even nests without any evidence of their existence on the surface are found and predated. In contrast to the observations related by Pezzuti and Vogt (1999) for *P. sextuberculata*, predation is independent of the density of *P. unifilis* nests. Both types of nests, those dispersed along the beaches and concentrated nests, were similarly attacked.

Therefore, management projects that involve egg collection from *P. unifilis* nests localised on the lower beach (Mitchell and Quiñones, 1994; Páez and Bock, 1998) should be analysed with caution. Nest flooding does not seem to follow a rigid pattern and its magnitude depends on difficult-to-predict factors such as the start of the rainy season and its intensity (Ferreira-Júnior and Castro, 2003; 2005; 2006b). The nests located in the lower portions of the beach are submitted to greater soil moisture and a more stable temperature than those in the higher portions of the beach (Ferreira-Júnior and Castro, 2006a). As such, the reproductive strategy of *P. unifilis* can be altered by selective egg collection. In species with a long reproductive period, the loss of some seasons does not result in significant effects on population stability (Congdon et al., 2000). The management projects that involve *P. unifilis*-transfer hatcheries should also consider the height of the nests from river level. Generally, the hatcheries are located in the higher parts of the beach, altering the characteristics of the hydric environment. It is suggested that *P. unifilis* hatcheries should be located in an area near the river, within a height of around one metre. The inconvenience of this position is that a rising river level might put the nests at risk, thus requiring another transfer. Nevertheless, this is the most recommended alternative, because the physiographic conditions of the nests will then be preserved.

Nests of *P. expansa* situated in fine sediments (Jaburu, Comprida, Murici, Marreca, and Goiaba beaches) have longer incubation duration than those located in coarser sediments (Canguçu and Coco beaches). This fact has been well illustrated with a difference of 14 days between the average incubation durations of *P. expansa* on the Coco and Jaburu beaches in 2000. This observation has important consequences for projects that involve nest transfer. As the incubation duration of *P. expansa* is affected by the grain size of the nest, the nests should not be transferred into a single and homogeneous hatchery. As presented by Milton et al. (1997) and Naro-Maciél et al. (1999), variations in the characteristics of hatchery sediments (such as grain size and mineral composition) could cause alteration in temperature during incubation and consequently affect the sex ratio of the hatchlings. As can be judged, it is desirable for the hatcheries to maintain the sediment characteristics similar to those of the origin of the nest.

The importance of the nesting place, mainly of the grain size, is enhanced for *P. expansa* when comparing distinct beaches in geological terms. Internally, the beaches show homogeneity in the grain sizes, which can little influence the incubation duration and the hatching success. But when one compares the effect among various beaches, the influence increases, reflecting the importance of evaluating all the nesting sites and more than one reproductive season. While extending the area of the research and different geological environments, the differences among the physiographic characteristics and its influence on the reproduction of the turtles are reflected,

which cannot occur in the case of only one beach or one reproductive season being considered.

*Acknowledgements* — This article is part of the PhD Thesis of P. D. Ferreira Júnior, Departamento de Geologia da Universidade Federal de Ouro Preto, with the financial assistance of a CAPES scholarship. Fieldwork was accomplished with the aid of RAN/IBAMA, the Instituto Ecológica, and Earthwatch Institute. We thank D. Rezende and M. Messias of the Instituto Ecológica and A.L.M. Carmo and Y.S.L. Bataus of RAN/IBAMA. Data collection was possible due to the collaboration with RAN/IBAMA agents; Gonzaga and Alfreu, students from the Universidade Federal do Tocantins; Giovanni, Eliene, Odimar, Fernando, Jackson, Edileila, Marcos, Valderico, and Ranieri, from the Universidade Federal de Ouro Preto; Ariadne and Luís Gustavo; and Leonardo and Julimar, from the Centro Universitário do Leste de Minas Gerais. We thank all the volunteers from the Earthwatch Institute - Theresa, Tessa, Stanley, Robert, Mika, Max, Maura, Laura, Helen and Tiffany, who actively participated in the data collection of 2001. Fieldwork and hatchling handling were authorised by RAN/IBAMA (license 02010.000932/00-79 – DIFAS/DIREC).

## References

- ALHO, C.J.R. and PÁDUA, LFM., 1982. Reproductive parameters and nesting behaviour of the Amazon turtle *Podocnemis expansa* (Testudinata: Pelomedusidae) in Brazil. *Canadian Journal of Zoology*, vol. 60, no. 2, p. 97-103.
- ALHO, C.J.R., DANNI, TMS. and PÁDUA, LFM., 1985. Temperature-dependent sex determination in *Podocnemis expansa* (Testudinata: Pelomedusidae). *Biotropica*, vol. 17, no. 1, p. 75-78.
- BAPTISTOTTE, C., THOMÉ, JCA. and BJORDNAL, KA., 2003. Reproductive biology and conservation status of the loggerhead sea turtles (*Caretta caretta*) in Espírito Santo State, Brazil. *Chelonian Conservation and Biology*, vol. 4, no. 3, p. 523-529.
- BOBYN, ML. and BROOKS, R.J., 1994. Incubation conditions as potential factors limiting the northern distribution of snapping turtles, *Chelydra serpentina*. *Canadian Journal of Zoology*, vol. 72, no. 1, p. 28-37.
- BURGER, J., 1976. Temperature relationships in nests of the northern diamondback terrapin, *Malaclemys terrapin terrapin*. *Herpetologica*, vol. 32, no. 4, p. 412-441.
- CONGDON, J.D., NAGLE, RD., KINNEY, OM., OSENTOSKI, M., AVERY, HW., LOBEN, CRS. and TINCKLE, DW., 2000. Nesting ecology and embryo mortality: implications for hatchling success and demography of Blanding's turtles (*Emydoidea blandingii*). *Chelonian Conservation and Biology*, vol. 3, no. 4, p. 569-579.
- EENDEBAK, BT., 1995. Incubation period and sex ratio of Hermann's tortoise *Testudo hermanni boettgeri*. *Chelonian Conservation and Biology*, vol. 1, no. 3, p. 227-231.
- ESCALONA, T. and FA, JE., 1998. Survival of nests of terecay turtle (*Podocnemis unifilis*) in the Nichare-Tawadu Rivers, Venezuela. *Journal of Zoology*, vol. 244, no. 2, p. 303-312.
- EWERT, MA., ETCHEBERGER, CR. and NELSON, CE., 2004. Turtle sex-determination modes and TSD patterns, and some TSD patterns correlates. In VALENZUELA, N. and

- LANCE, VA. (Eds.). *Temperature dependent sex determination in vertebrates*. Washington: Smithsonian Books. p. 21-32.
- FERREIRA Jr., PD. and CASTRO, PTA., 2003. Geological control of *Podocnemis expansa* and *Podocnemis unifilis* nesting areas in Rio Javaés, Bananal Island, Brazil. *Acta Amazonica*, vol. 33, no. 3, p. 445-468.
- \_\_\_\_\_, 2005. Nest placement of the giant Amazon river turtle, *Podocnemis expansa*, in the Araguaia River, Goiás State, Brazil. *Ambio*, vol. 34, no. 3, p. 212-217.
- \_\_\_\_\_, 2006a. Thermal environment characteristics of *Podocnemis expansa* and *Podocnemis unifilis* nesting areas on the Javaés River, Tocantins, Brazil. *Chelonian Conservation and Biology*, vol. 5, no. 1, p. 102-107.
- \_\_\_\_\_, 2006b. Geological characteristics of the nesting areas of the giant Amazon river turtle (*Podocnemis expansa*) in the Crixás-Açu River in Goiás State, Brazil. *Acta Amazonica*, vol. 36, no. 2, p. 249-258.
- FERREIRA Jr., PD., GUIMARÃES, OS. and MALVASIO, A., 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*, vol. 4, no. 3, p. 626-634.
- FOLK, RL., 1974. *Petrology of sedimentary rocks*. Austin: Hemphill Publication. p. 182.
- HAYS, GC., ASHWORTH, JS., BARNSLEY, MJ., BRODERICK, AC., EMERY, DR., GODLEY, BJ., HENWOOD, A. and JONES, EL., 2001. The importance of sand albedo for the thermal conditions on sea turtle nesting beaches. *Oikos*, vol. 93, no. 1, p. 87-94.
- HILDEBRAND, P., SAENZ, C., PEHUELA, MC. and CARO, C., 1988. Biología reproductiva y manejo de la tortuga Charapa (*Podocnemis expansa*) en el bajo río Caqueta. *Colombia Amazonica*, vol. 3, no. 1, p. 89-102.
- KRAUSE, L., GOMES, N. and LEYSER, KL., 1982. Observações sobre a nidificação e desenvolvimento de *Chrysemys dorbigni* (Dumeril & Bibron, 1835) (Testudines, Emydinae) na Estação Ecológica do Taim, Rio Grande do Sul. *Revista Brasileira de Zoologia*, vol. 1, no. 1, p. 79-90.
- MALVASIO, A., 2001. *Aspectos do Mecanismo Alimentar e da Biologia Reprodutiva em Podocnemis expansa (Schweigger, 1812), P. unifilis (Troschel, 1848) e P. sextuberculata (Cornalia, 1849) (Testudines, Emydidae)*. São Paulo: Universidade de São Paulo. [Tese de Doutorado].
- MARCOVALDI, MA., GODFREY, MH. and MROSOVSKY, N., 1997. Estimating sex ratios of loggerhead turtles in Brazil from pivotal incubation durations. *Canadian Journal of Zoology*, vol. 75, no. 5, p. 755-770.
- MILTON, SL., SCHULMAN, AA. and LUTZ, PL., 1997. The effect of beach nourishment with aragonite versus silicate sand on beach temperature and loggerhead sea turtle nesting success. *Journal of Coastal Research*, vol. 13, no. 3, p. 904-915.
- MITCHELL, C. and QUIÑONES, L., 1994. Manejo y conservación de la taricaya (*Podocnemis unifilis*) en la Reserva de Biofera del Manu, Madre de Dios. *Boletín de Lima*, vol. 16, no. 91-96, p. 425-436.
- MROSOVSKY, N. and PROVANCHA, J., 1992. Sex ratio of hatchling loggerhead sea turtles: data and estimates from a 5-year study. *Canadian Journal of Zoology*, vol. 70, no. 3, p. 530-538.
- MROSOVSKY, N., BAPTISTOTTE, C. and GODFREY, MH., 1999. Validation of incubation duration as an index of the sex ratio of hatchling sea turtles. *Canadian Journal of Zoology*, vol. 77, no. 5, p. 831- 835.
- NARO-MACIEL, E., MROSOVSKY, N. and MARCOVALDI, MA., 1999. Thermal profiles of sea turtle hatcheries and nesting areas at Praia do Forte, Brazil. *Chelonian Conservation and Biology*, vol. 3, no. 3, p. 407-413.
- ÖZ, M., ERDOGAN, A., KASKA, Y., DUSEN, S., ASLAN, A., SERT, H., YAVUZ, M. and TUNC, MR., 2004. Nest temperatures and sex-ratio estimates of loggerhead turtles at Patara beach on the southwestern coast of Turkey. *Canadian Journal of Zoology*, vol. 82, no. 1, p. 94-101.
- PACKARD, GC. and PACKARD, MJ., 1997. Type of soil affects survival by overwintering hatchlings of painted turtle. *Journal of Thermal Biology*, vol. 22, no. 1, p. 53-58.
- PACKARD, GC., PACKARD, MJ., MILLER, K. and BOARDMAN, TJ., 1987. Influence of moisture, temperature, and substrate on snapping turtle eggs and embryos. *Ecology*, vol. 68, no. 4, p. 983-993.
- PÁEZ, VP. and BOCK, BC., 1998. Temperature effect on incubation period in the yellow-spotted river turtle, *Podocnemis unifilis*, in the Colombian Amazon. *Chelonian Conservation and Biology*, vol. 3, no. 1, p. 31-36.
- PEZZUTI, JCB. and VOGT, RC., 1999. Nesting ecology of *Podocnemis sextuberculata* (Testudines, Pelomedusidae) in the Japurá River, Amazonas, Brazil. *Chelonian Conservation and Biology*, vol. 3, no. 3, p. 419-424.
- PLUMMER, MV., 1976. Some aspects of nesting success in the turtle, *Trionyx muticus*. *Herpetologica*, vol. 32, no. 4, p. 353-359.
- PRITCHARD, PCH. and TREBBAU, P., 1984. *The turtles of Venezuela*. Venezuela: Society for the Study of Amphibians and Reptiles. p. 403.
- SOINI, P., 1995. *Investigaciones en la Estación Biológica Cahuana*. Peru: Universidad Nacional Agraria La Molina. (Reporte Pacaya-Samiria). p. 435.
- SOUZA, RR. and VOGT, RC., 1994. Incubation temperature influences sex and hatchling size in the neotropical turtle *Podocnemis unifilis*. *Journal of Herpetology*, vol. 28, no. 4, p. 453-464.
- SPENCER, JR., 2002. Experimentally testing nest site selection: fitness trade-offs and predation risk in turtles. *Ecology*, vol. 83, no. 8, p. 2136-2144.
- SPOTILA, JR., STANDORA, EA., MORREALE, SJ. and RUIZ, G., 1987. Temperature dependent sex determination in the green turtle (*Chelonia mydas*): effects on the sex ratio on a natural nesting beach. *Herpetologica*, vol. 43, no. 1, p. 74-81.
- STANDING, KL., HERMAN, TB. and MORRISO, IP., 1999. Nesting ecology of Blanding's turtle (*Emydoidea blandingii*) in Nova Scotia, the northeastern limit of the species' range. *Canadian Journal of Zoology*, vol. 77, no. 10, p. 1609-1614.

- TINKLE, DW., CONGDON, JD. and ROSEN, PC., 1981. Nesting frequency and success: implications for the demography of painted turtles. *Ecology*, vol. 62, no. 6, p. 1426-1432.
- VALENZUELA, N., 2001. Constant, shift, and natural temperature effects on sex determination in *Podocnemis expansa* turtles. *Ecology*, vol. 82, no. 11, p. 3010-3024.
- VALENZUELA, N., BOTERO, R. and MARTÍNEZ, E., 1997. Field study of sex determination in *Podocnemis expansa* from Colombian Amazonian. *Herpetologica*, vol. 53, no. 3, p. 390-398.
- VOGT, RC. and BULL, J., 1984. Ecology of hatchling sex ratio in map turtle. *Ecology*, vol. 65, no. 2, p. 582-587.
- WILSON, DS., 1998. Nest-site selection: microhabitat variation and its effects on the survival of turtle embryos. *Journal of Herpetology*, vol. 22, no. 1, p. 88 -96.