

# *Trachemys dorbigni* in an anthropic environment in southern Brazil: II) Reproductive ecology

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The biology of the turtle *Trachemys dorbigni* is little known. To contribute to knowledge of its reproductive ecology, we studied a population in southern Brazil from September 2006 to January 2007. Nesting occurred from 22 October to 4 January. Mean clutch size was 8.2 eggs and mean egg size was 37.3 × 20.2 mm, the lowest values reported for the species. Egg dimensions showed no relationship to clutch size or female size. Only 35.3% of the females in the population nested during the breeding season; 11.9% of females nested twice. The incubation temperature in the nests ranged from 16.1 to 35.5 °C (mean 27.3 °C). The distance of the nests from an adjacent marsh ranged between 0.4 m and 160.5 m (mean = 60.5 m) and predation on the nests was more intense closer to the marsh. The study illustrates the importance of understanding habitat use and the potential for human disturbance to modify reproductive parameters of turtles.

**Key words:** D'Orbigny's slider turtle, clutch, Emydidae, incubation temperature, interesting, nesting, predation, Testudines

## INTRODUCTION

Studies of reproductive ecology are important in assessing population parameters of a species. In neotropical freshwater turtles, reproductive traits such as clutch and body size, nesting season, interesting interval and nest location can vary according to geographical distribution, characteristics of each environment and food availability (Iverson & Smith, 1993; Iverson et al., 1997; Aresco, 2004; Spinks et al., 2003).

The genus *Trachemys* has one of the widest distributions of all vertebrate genera (Seidel, 2002). For *T. dorbigni*, the southernmost species of the genus, little information on its reproductive biology is available (Gonçalves et al., 2007; Fagundes et al., 2010) and only Krause et al. (1982) and Bager et al. (2007) have studied its reproduction in nature, in one protected area. Vanzolini (1997) analysed one gravid female and suggested that two clutches are laid per breeding season.

The present study examined the reproductive ecology of *T. dorbigni* in a human-impacted environment in southern Brazil, to 1) characterize reproductive season, clutch size and egg size; 2) determine the percentage of gravid females and the occurrence of multiple nesting; 3) characterize the daily nesting period and nest-site selection; 4) identify incubation regimes; and 5) determine nest predation rates. We analysed differences in some of these characteristics in comparison to predation and reproductive data for the species in a protected area. This descriptive information can serve as a baseline for more thorough investigations of the effects of urbanization.

## MATERIALS AND METHODS

The study area is situated on the coastal plain of the state of Rio Grande do Sul, Brazil (Rambo, 2000; for more de-

tails see Fagundes et al., 2010). The study was carried out on the banks of a marsh (52°22'82.1"S, 31°46'94.7"W) formed by the waters of Santa Barbara Creek, with an approximate area of 6.8 ha, near the city of Pelotas, Rio Grande do Sul, Brazil (see Fagundes et al., 2010).

The study area was traversed once a day from 17 September 2006 to 11 January 2007, to search for turtles carrying out nesting activity, nests and/or signs of predation. A total of 306 h of sampling (214 h in the morning and 95 h in the afternoon from 0600 to 1900) was carried out. After females had laid their eggs, they were captured manually, marked by individual notches on the marginal scutes (modified from Cagle, 1939) and their straight-line carapace length (CL) was measured with a vernier caliper to a precision of 1 mm. Clutch frequency and the interval between nesting events were estimated by recapturing females. To determine the temporal frequency of nesting activity, the number of females nesting in the morning and in the afternoon was divided by the number of hours that we spent searching for them in each period.

In addition to manual captures in the field, turtles were collected by manual capture in water and by eight traps baited with fish (Vogt, 1980) set in the marsh from February 2006 to January 2007. Captured individuals that did not exhibit male secondary sexual characteristics according to Bager et al. (2007) were considered to be mature females (these individuals were longer than 127 mm CL; see also Fagundes et al., 2010). All the captured females were examined by palpating the inguinal region to determine the presence of eggs. The proportion of reproductive females was calculated by dividing the number of gravid females by the total number of mature females.

The nests were classified as intact, partially predated or totally predated. Intact nests were identified with numbered stakes. The nests were handled only after they were covered by the turtles. Of approximately every three nests

**Table 1.** Linear regression analyses between egg dimensions, clutch size and carapace length (CL) of *Trachemys dorbignii* females in southern Brazil.

	Regression parameters					
	<i>n</i>	<i>a</i>	<i>b</i>	<i>r</i> <sup>2</sup>	<i>F</i>	<i>P</i>
Egg length – egg width	705	3.00	0.20	8.54	66.72	<0.01
Clutch size – egg length	89	3.71	–0.04	2.48	3.24	0.07
Clutch size – egg width	89	2.89	0.06	0.64	1.56	>0.1
Clutch size – female CL	37	–11.67	2.57	26.15	13.75	<0.01
Egg length – female CL	37	5.82	–0.14	–1.13	0.6	>0.1
Egg width – female CL	37	4.94	0.13	1.99	1.73	>0.1

found in the field, one was not opened ( $n=25$ ). These were used to measure incubation temperature. In the opened nests, eggs were counted and their lengths and widths measured with a vernier caliper to the nearest 0.1 mm. The eggs were then returned to the original nest. We protected 42 intact and opened nests by covering each one with a steel screen secured with iron hooks, to analyse the efficiency of this method for combating predation.

The locations of the nests and the perimeter of the study area were determined and outlined by GPS. The positions of the nests were plotted on a map of the locality using Trackmaker® 13.0 GPS (Ferreira Jr., 2005) to calculate the distance of each nest from the water.

In 25 of the protected or unprotected nests, a plastic access tube measuring 1 cm in circumference and with the inner end open and the outer end closed was carefully inserted among the eggs, as close as possible to the bottom of the nest (mean depth 10 cm) and left in place until the end of the study. The access tube was opened once a day (in the morning or in the afternoon, at different times) and a type “T” digital thermometer was inserted into the tube to measure the incubation temperature. This tube allowed the thermometer to be inserted into the nest without damaging the eggs. The temperatures recorded were grouped in months for analysis of their temporal variation, and in two-hour segments for analysis of their daily variation.

### Statistical analysis

The data were tested for normality by the Shapiro–Wilk method. Non-normal data were transformed using natural logarithms. The relationships between egg length and width and between these dimensions and clutch size were assessed by linear regression, which was also used to evaluate the relationship of female size (CL) to the length, width and number of eggs in each nest. Regression analysis was also employed in the correlation of mean nest temperature and mean ambient temperature. Ambient temperature data were obtained from the Agroclimatological Station at Universidade Federal de Pelotas. Chi-square tests were applied to determine the nesting-period frequency (by month), the temporal nesting frequency (morning/afternoon) and the distribution of nest distances from water. Nearest-neighbour analysis was used (Clarck & Evans, 1954) to evaluate nest dispersion. The Kaplan–Meier estimate was used to calculate nest survival as a function of the time since nesting (Gonçalves et

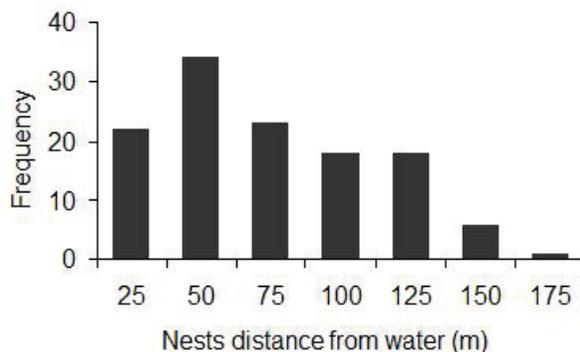
al., 2007). Kruskal–Wallis tests was employed to evaluate nest predation rates in relation to distance from the marsh and to evaluate monthly and daily variation in incubation temperature.

## RESULTS

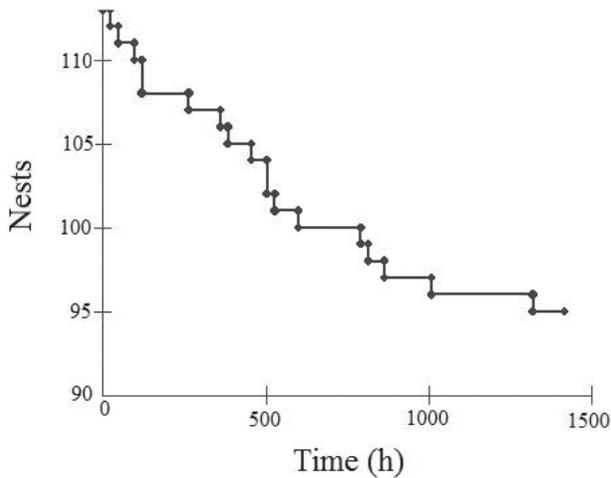
In total, 122 nests of *T. dorbignii* were found: 113 intact, six totally and three partially predated. In the intact and partially predated nests, we counted 705 elliptical, parchment-like eggs. Intact clutches ranged from 4–15 eggs per nest (mean =  $8.2 \pm 2.16$ ). The mean egg length was  $37.3 \pm 2.84$  mm (28.6–47 mm) and the width was  $20.2 \pm 2.21$  mm (12.1–29.5 mm). We found 93 reproducing females of between 176 mm and 239 mm in carapace length (mean =  $205.9 \pm 12.53$  mm).

Egg length was positively and significantly related to width ( $F_{1,703}=66.72$ ,  $P<0.05$ ). There was no significant relationship between clutch size and egg length ( $F_{1,87}=3.24$ ,  $P=0.07$ ) or egg width ( $F_{1,87}=1.56$ ,  $P=0.21$ ); egg length ( $F_{1,35}=0.60$ ,  $P=0.55$ ) and egg width ( $F_{1,35}=1.73$ ,  $P=0.19$ ) were also unrelated to female size. However, clutch size ( $F_{1,35}=13.75$ ,  $P<0.05$ ) was positively related to carapace length (Table 1).

The reproductive period occurred from 22 October to 4 January and 40.9% of nesting females were observed in the first two weeks of December ( $n=122$ ,  $\chi^2_5=74.03$ ,



**Fig. 1.** Frequency of nests of *Trachemys dorbignii* as a function of distance from water in southern Brazil in the 2006–2007 breeding season.



**Fig. 2.** Kaplan–Meier estimates for the nest survival of *Trachemys dorsbigni* in southern Brazil in the 2006–2007 breeding season.

$P < 0.05$ ). Of all females captured in the study locality ( $n = 286$ ), only 35.3% ( $n = 101$ ) were found to be gravid in this breeding season. Most females observed on land in the pre-nesting phase returned to water without nesting (55.1%). Of these females, 37.9% returned to the field for a new attempt within two days and 17.2% within three to eight days. A minimum of 11.9% of females deposited two clutches, at intervals of ten to 43 days.

Nest construction was observed from 0600 to 1800, but occurred most frequently in the morning ( $n = 114$ ,  $\chi^2_1 = 21.55$ ,  $P < 0.05$ ). The distance of terrestrial nests from the marsh ranged between 0.4 m and 160.5 m (mean = 60.5 m); 27.9% of the nests were found between 25 m and 50 m from the water ( $n = 34$ ,  $\chi^2_6 = 41.75$ ,  $P < 0.05$ , Fig. 1).

Nest incubation temperatures ranged between 16.1 and 35.5 °C (mean = 27.3 °C). The warmest incubation temperatures were observed during afternoons ( $H_6 = 238.11$ ,  $P < 0.05$ ), reaching a mean of 30.9 °C at 1700. The monthly incubation temperatures were warmest during December (28.9 °C) and January (30.7 °C,  $H_3 = 344.3$ ,  $P < 0.05$ ). The mean daily nest incubation temperature was positively related to the mean daily ambient temperature ( $F_{1,66} = 90.03$ ,  $P < 0.05$ ).

Nests protected with screens ( $n = 42$ ) were not predated. Of the unprotected nests, 18.1% ( $n = 21$ ) were destroyed between one and 57 days after the eggs were laid. The only predator identified was a lizard, *Tupinambis merrianae*. In the 24-h period after nesting, nests had a 3% predation risk and survival rates again decreased towards the end of the reproductive season. At 59 days after the beginning of the nesting period, survival was 82% (Fig. 2).

Nests were constructed in an aggregated pattern ( $R = 0.73$ ). The highest rate of nest predation was close to water ( $H_1 = 8.87$ ,  $P < 0.05$ ); of the predated nests, 52.4% were deposited between 20 m and 30 m from the marsh (mean = 39.4 m). Although we found 42 nests further

than 75.6 m away from water, only one of them was destroyed.

Besides natural predation, in mid January (after the study ended), more than 90% of the nests had their eggs removed for wild-animal trafficking. Local residents reported that this practice is common in the region and occurs every year. Juveniles are sometimes collected from the marsh as well. Unfortunately, the numbered stakes used to identify the nests of *T. dorsbigni* seem to have helped the traffickers to find the nests.

## DISCUSSION

The mean clutch size of 8.2 eggs is the lowest reported so far for *T. dorsbigni*, for which Krause et al. (1982) and Bager et al. (2007) reported means of 11.6 and 12.1 eggs, respectively. The mean size of *T. dorsbigni* eggs is also the smallest recorded (Freiberg, 1971; Krause et al., 1982; Vanzolini, 1997); the data presented by Freiberg (1971) and Vanzolini (1997) should however be considered with care, as they are derived from observations of a single nest. The studies of Krause et al. (1982) and Bager et al. (2007) were carried out less than 100 km from the present study. Thus, the differences in egg and clutch size between the populations probably do not result from latitudinal variation, but may have resulted from differences in food quality and availability in each locale, which influence turtle size, growth rates and time to maturity (Moll & Legler, 1971; Gibbons & Greene, 1990).

In spite of the large amounts of organic matter available in our marsh, females in a more natural area investigated nearby matured at a larger size and reached a larger mean body size (Bager et al., 2007). The lower egg and clutch sizes may be attributable to maternal growth rates (Gibbons & Greene, 1990) and to limitations of the females' bodily capacity (Wilbur & Morin, 1988). A positive relationship between egg width and egg length was also demonstrated by Bager et al. (2007). This presumably occurs because the bones of the female's pelvic aperture limit egg width (Congdon et al., 1983). There was no relationship between female size and egg measurements, but a positive correlation between clutch size and female size. This relationship is generally found in turtles (Congdon et al., 1987; Tucker et al., 1998; Aresco, 2004), including *T. dorsbigni* (Bager et al., 2007).

The nesting period was similar to that observed by Bager et al. (2007) and Gonçalves et al. (2007). In some chelonian populations, less than 50% of the females nest in a single breeding season (Congdon & Tinkle, 1982; Congdon et al., 1983; Wilson & Ernst, 2005). For *T. dorsbigni*, on average up to 30% of adult females nested in each year in a study by Bager et al. (2007). We estimated a similarly low rate (35.3%). However, the sex-determination method used (secondary sexual characteristics) may have overestimated the number of mature females, and our data might suggest that females of this species nest biannually.

Of the females analysed by Bager et al. (2007), 16% were capable of producing two nests and 14% three nests in the same breeding season. Clutch frequency is related to resource acquisition and lipid storage (Gib-

bons & Greene, 1990), so the lower incidence of multiple clutches documented in this study may result from physiological stress (Aresco, 2004) or insufficient resources. In our study, no individual was found to nest three times. Nesting may occur at different times of the day (Congdon & Gibbons, 1987; Feinberg & Burke, 2003). For *T. dorbigni*, the highest frequency of nesting behaviour was observed in the morning (see also Krause et al., 1982; Bager et al., 2007).

Turtles show wide within- and between-species variation in the distance of nests from water (Congdon et al., 1987; Horne et al., 2003; Baldwin et al., 2004; Rowe et al., 2005). The availability of suitable nesting sites may affect nest location (Rowe et al., 2005). However, even with a much smaller nesting area, the majority of the nests of *T. dorbigni* constructed in the marsh were placed at similar distances to those observed at another study site (A. Bager, pers. obs.). The concentration of nests suggests that nesting areas are not selected randomly (Ferreira & Castro, 2005). The distance of a nest from water may optimize the development of eggs and offspring (Congdon et al., 1983), control the sex ratio of the offspring (Janzen & Morjan, 2001) and reduce nest and female predation (Tucker et al., 1999; Kolbe & Janzen, 2002). Human disturbance also may alter nest-site choice in freshwater turtles (Spencer, 2002).

*Trachemys dorbigni* eggs do not develop at incubation temperatures lower than 25 °C or higher than 35 °C (Molina & Gomes, 1998; Moll & Legler, 1971). In our study locality, the nest-chamber temperature of *T. dorbigni* varied from 16.1 °C to 35.5 °C, an amplitude which is wider than the 22–30 °C observed by Moll & Legler (1971). These comparisons must be considered with care, because prior studies on captive turtles used constant temperatures. The highest temperatures that we measured could potentially damage the embryos, but the emergence of hatchlings could not be observed because pet-trade traffickers removed the eggs. The higher mean incubation temperature in the afternoon and in the last two months of the breeding season (December and January) may be explained by the daily and seasonal increase in the substrate temperature (Hewavisenthi & Parmenter, 2002). Nests of other species such as *Chelydra serpentina* and *Emydoidea blandingii* are also warmest in the afternoon (Congdon & Gibbons, 1990).

In turtles, nest predation levels are generally high (Congdon et al., 1987; Burke et al., 1998; Aresco, 2004). In a conservation area, the predation rate recorded for *T. dorbigni* was about 98% (Gonçalves et al., 2007), whereas only 18.1% of the unprotected nests that we studied were destroyed (see also Rowe et al., 2005). We identified only one predator (*T. merianae*), although the locality contains many domestic dogs that may feed on *T. dorbigni* eggs (Fowler, 1979). Nest predation often occurs in the first 48 h after the eggs are laid (Tinkle et al., 1981; Christens & Bider, 1987; Congdon et al., 1987) because predators locate the nests by smell and the odour of the nests gradually decreases (Legler, 1954). The liquid that *T. dorbigni* releases from its bladder during nest construction gradually dries (Clark & Wobeser, 1997). However, our nests continued to be destroyed for many weeks; handling of

the access tube may have facilitated later predation by releasing odours. The higher nest-predation rate close to the marsh may be associated with the higher density of nests in this area, or with the linear search mode used by predators (Congdon et al., 1983). Christens & Bider (1987) and Kolbe & Janzen (2002) also reported that larger numbers of *Chrysemys picta* nests were destroyed in locations near water. Future studies should focus on variations in reproductive rates and the recruitment of juveniles into the population. Human modifications that occur faster than a turtle population can respond result in a population that is not adapted to the altered habitats (Kolbe & Janzen, 2002). Fecundity parameters and information about the pet traffic of *T. dorbigni* should be collected in different areas, in order to evaluate the species' status as a whole.

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