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Physical Characteristics of Giant Amazon Turtle (*Podocnemis expansa*) Nests

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ABSTRACT. – **We determined the relationship between incubation success and physical characteristics of *Podocnemis expansa* nests on 4 sand beaches of the Araguaia River in the southern Amazon basin, Brazil. Sand samples measuring grain size, humidity, and density were collected during the first 7 days after egg laying. The choice of nest site seems to be more relevant for the reproductive success of a *P. expansa* than the female's capacity to build a nest.**

The habitat of the giant Amazon turtle (*Podocnemis expansa*) includes large rivers as well as meanders formed by clay–sand unconsolidated alluvium (Cantarelli and Alves 1999). In these environments sand beaches are normally formed by the exposition of sand banks (Vanzolini 1967; Von Hildebrand et al. 1988; Soini 1997).

Chelonians may use a combination of multiple factors for nest site selection, such as beach height in relation to the water surface and sand depth, consistency, and humidity (Pritchard and Trebbau 1984; Ratterman and Ackerman 1989; Mortimer 1990; Speakman et al. 1998; Seigel and Dodd 2000; Wood and Bjorndal 2000). Embryogenesis is significantly affected by those factors (Tucker and Warner 1999). Gaseous exchange is also affected by the substrate physical structure (Prange and Ackerman 1974; Milton et al. 1997) and can determine embryo survivorship (Bobyn and Brooks 1994; Milton et al. 1997; Packard et al. 1999).

Studies concerning the physical substrate structure at freshwater turtle nest sites should guide chelonian management programs based on egg collection (Ojasti 1967; IBAMA 1989; Martinez and Rodriguez 1997) either for artificial incubation (Plummer et al. 1994; Rostal et al. 1994; Souza and Vogt 1994; Molina and Gomes 1998) or egg translocation (Alho et al. 1979; Von Hildebrand et al. 1988; Stancyk 1995). In the present study we determined the relationship between incubation success and some physical characteristics of *Podocnemis expansa* nests in the southern Amazon basin, Brazil.

Materials and Methods. — This study was carried out on 4 sand beaches of the Araguaia and Crixás-Açu rivers in the southern Amazon basin on the border between the states of Goiás (GO) and Mato Grosso (MT) in Brazil. We sampled 5 nests per beach for a total of 20 nests in both rivers.

Sand samples were collected 0700 to 0930 hours or 1600 to 1830 hours during the first 7 days after egg-laying. The nests were carefully excavated until the egg chamber appeared. The first egg layer was then taken off the nest and a cylindrical metal ring (volume 18.64 cm^3) was introduced on the border of the egg chamber in order to collect sand as close as possible to the eggs. Two samples were taken from each nest, weighed on a digital balance (0.1 g/400 g), and stored in zip-lock plastic bags.

The remaining eggs were then removed from the egg chamber for counting, and then reintroduced randomly. After this procedure, the eggs were manually recovered with sand and the nests were protected by a surrounding plastic net and tapped by a metallic fence. Two control samples were taken by the same metallic cylinder by the side of each nest at the same depth, weighed, and returned to the nest. Nests were reopened at day 45 after egg laying for hatching monitoring.

The following physical characteristics of the sand were analyzed at the Laboratório de Física do Solo from Escola Superior de Agricultura "Luiz de Queiroz", University of São Paulo, according to Camargo et al. (1986) and Gee and Bauder (1986): 1) sand grain distribution (in percent): very fine ($<$0.106 mm), fine (0.107–0.25 mm), medium (0.25–0.4 mm), gross (0.5–1.17 mm), very gross ($>$1.18 mm), and total sand; 2) moisture in the sand (%) = (humid sand weight − dry sand weight) × 100/(dry sand weight); 3) apparent density (D) (g/cm^3) = dry sand weight/18.64.

For data that did not present normal distribution we used nonparametric statistics (Kruskal-Wallis and Mann-Whitmann) in order to test the relationships between the variables studied (Sokal and Rholf 1995). Otherwise, we used parametric tests.

Results. — Results are presented in Table 1. Sand varied among beaches in terms of all kinds of grain size (Kruskal-Wallis: $p < 0.05$, df = 5), but not for fine-grained and total sand percentages (Kruskal-Wallis: $p > 0.05$, df = 5). There was no consistent relationship between hatching success and the physical characteristics of the nests, the sand beach where the female laid eggs, and the sand apparent density, relative humidity, and grain size distribution (Kruskal-Wallis: $p > 0.100$, $n = 20$). There was no consistent relationship between hatching success and incubation period (ANOVA: $P = 0.196$, $n = 20$) either. However, there was a consistent relationship between the sand beach where the females

Table 1. Physical characteristics of *Podocnemis expansa* nests.

	Araguaia River (n = 10 nests)	Crixás-Açu River (n = 10 nests)
Total sand (%)	98.06 ± 4.51	96.96 ± 4.01
Very gross sand (%)	2.86 ± 4.04	15.42 ± 19.11
Gross sand (%)	6.84 ± 6.88	23.58 ± 15.89
Medium sand (%)	55.52 ± 23.10	40.79 ± 23.32
Fine sand (%)	33.60 ± 26.47	19.06 ± 14.07
Very fine sand (%)	1.28 ± 2.75	1.15 ± 0.94
Moisture at egg chamber (%)	4.39 ± 3.06	4.09 ± 1.79
Moisture external to egg chamber (%)	2.54 ± 1.55	7.44 ± 9.17
Apparent density at egg chamber (g/cm^3)	1.40 ± 0.05	1.29 ± 0.04
Apparent density external to egg chamber (g/cm^3)	1.41 ± 0.04	1.24 ± 0.09
Hatching success (Araguaia River) (%)	54.43 ± 19.97	-

nested and clutch size (ANOVA: $p = 0.004$; 18GO: 100.15 ± 14.09; 6MT: 76 ± 17.84 eggs, $n = 20$), and incubation period (ANOVA: $p < 0.001$; 18GO: 54.769 ± 0.725; 6MT: 57 ± 0.756 days, $n = 20$).

There was no significant difference in apparent density between egg chamber and the sand around the nest at the same depth (Mann-Whitney: $p = 0.094$, $n = 30$). However, moisture at egg chamber was significantly higher than at the sand around the nest at the same depth (Mann-Whitney: $p = 0.044$, $n = 30$) during the first week after egg-laying. The apparent density of sand at the egg chambers consistently varied among sand beaches (Kruskal-Wallis: $p = 0.006$, df = 5); however, sand moisture levels did not (Kruskal-Wallis: $p < 0.1$).

Discussion. — The Araguaia river basin has innumerous sand-belts, sandbanks, and sand beaches formed along its course by deposition of suspended materials carried by the water (Cantarelli and Alves 1999). Topography of the river borders and water speed determine the deposition pattern of large-grained sand particles along the beaches, whereas fine-grained particles are more easily carried away even by slow currents (Stancyk and Ross 1978). This is possibly the reason we did not find significant differences among beaches in terms of the fine-grained sand, but only in terms of the larger sand particles.

We found a relatively high rate of fine-medium sand grains, which is similar to the pattern described by Malvásio (2001) at the southernmost region of Araguaia River at Araguaia National Park. However, it differs from the pattern found in nesting beaches of the species at Trombetas, state of Pará, by Alho et al. (1979), where there is a higher rate of coarse grains, similarly to what has been described for marine turtles (*Chelonia mydas*) by Stancyk and Ross (1978). The percentage of very fine sand grains was the smallest component in this study as well as the other studies above.

Even though there was a significant variation among sand beaches in terms of apparent sand density of egg chambers, sand moisture levels did not significantly vary. There may be a significant variation among populations of the same species in terms of how temperature and humidity affect egg incubation (Bobyn and Brooks 1994; Packard et al. 1999).

The lack of significant variation in terms of sand apparent density between the egg chamber and the sand around for both rivers suggests that the egg chamber is not a less compacted "air-chamber."

In laboratory experiments under controlled conditions, moisture affects chelonian hatching success (Packard et al. 1991, 1999; Bobyn and Brooks 1994). However, in this study this relationship was not significant. A possible explanation for this is that we only measured humidity at the initial period of incubation, not recording possible later variation. On the other hand, laboratory experiments normally do not consider physical variation suffered by nests under natural conditions related for instance to rainfall or sudden temperature drops (Wood and Bjorndal 2000). In the present study the egg chambers were significantly more humid than the substrate around the nest at the same depth. This is possibly due to the presence of mucus (or water) expelled by the female during egg-laying (Iverson 1990). The ecological implications of this are still unclear, but it is possibly related to egg protection against desiccation.

The site wherein the artificial nest is built can affect the incubation period of translocated clutches (Bonach et al. 2003). The present results suggest that the sand beach—and consequently the female capacity to choose it—can be more relevant to her reproductive success than her capacity to build an elaborate nest. Contrary to seashore sand beaches used by marine turtles to nest, sand beaches used by freshwater turtles are geologically unstable, possibly lasting shorter than one single turtle generation. This possibly prevents the occurrence of philopatry in this group as it more likely occurs in marine turtles (FitzSimmons et al. 1997).

The giant Amazon River turtle occurs over a vast geographic area. People have exploited it for its meat and eggs for centuries. Its conservation depends on the protection of its nesting sites. Future studies about the relationship between physical characteristics of the nest (i.e., sand texture, temperature, and soil moisture), and the reproductive success of the species can help accomplish this mission. Temperature sex-determination (TSD) patterns can also be influenced by the physical characteristics of the nest substrate and—besides reproductive success—should also be considered for future studies.

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LITERATURE CITED

ALHO, C.J.R, CARVALHO, A.G., AND PÁDUA, L.F.M. 1979. Ecologia da Tartaruga da Amazônia e Avaliação de Seu Manejo na Reserva Biológica do Trombetas. Revista Brasil Florestal 29–47.

BOBYN, M.L. AND BROOKS, R.J. 1994. Incubation conditions as potential factors limiting the northern distribution of snapping turtles, *Chelydra serpentina*. Canadian Journal of Zoology 72:28–37.

BONACH, K., MIRANDA-VILELA, M.P., ALVES, M.C., AND VERDADE, L.M. 2003. Effect of translocation on egg viability of the giant Amazon river turtle (*Podocnemis expansa*). Chelonian Conservation and Biology 4(3):712–715.

CAMARGO, O.A., MONIZ, A.C., JORGE, J.A., AND VALADARES, J.M.A.S. 1986. Métodos de análise química, mineralógica e física de solos do Instituto Agronômico de Campinas. Boletim técnico do Instituto Agronômico 106:55–87.

CANTARELLI, V.H. AND ALVES, W.R. 1999. The meanders of the Araguaia River Environmental Protection Área. Boletim Goiano de Geografia 19(1):158–165.

FITZSIMMONS, N.N., LIMPUS, C.J., NORMAN, J.A., GOLDIZEN, A.R., MILLER, J.D., AND MORITZ, C. 1997. Philopatry of male marine turtles inferred from mitochondrial DNA markers. Proceedings of the National Academy of Science 94:8912–8917.

GEE, G.W. AND BAUDER, J.W. 1986. Methods of Soil Analysis. Second edition. Madison, WI: American Society of Agronomy-Soil Science Society of America.

IBAMA. 1989. Projeto Quelônios da Amazônia 10 Anos. Brasília, Brasil: Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis, 119 pp.

IVERSON, J.B. 1990. Nesting and parental care in the mud turtle, *Kinosternon flavenscens*. Canadian Journal of Zoology 68: 230–233.

MALVÁSIO, 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, Pelomedusidae). Thesis, University of São Paulo, São Paulo, Brasil. 199 pp.

MARTINEZ, E. AND RODRÍGUEZ, E. 1997. Manejo Participativo de la Tortuga Charapa *Podocnemis expansa* en la Zona de Influencia de un Area Protegida Amazonica Colombiana. In: Fang, T.G., Bodmer, R.E., Aquino, R., and Valqui, M.H. (Eds.). Manejo de Fauna Silvestre en la Amazonía. La Paz, Bolivia: Instituto de Ecología, pp. 175–179.

MILTON, S.L., SCHULMAN, A.A., AND LUTZ, P.L. 1997. The effect of beach nourishment with aragonite versus silicate sand on beach temperature and loggerhead sea turtle nesting success. Journal of Coastal Research 13(3):904–915.

MOLINA, F.B. AND GOMES, N. 1998. Incubação artificial dos ovos e processo de eclosão em *Trachemys dorbigny* (Duméril & Bibron) (Reptilia, Testudines, Emydidae). Revista Brasileira de Zoologia 15:135–143.

MORTIMER, J.A. 1990. The influence of beach sand characteristics on the nesting behavior and clutch survival of green turtles (*Chelonia mydas*). Copeia 1990:802–817.

OJASTI, J. 1967. Consideraciones sobre la ecologia y conservación de la tortuga "*Podocnemis expansa*" (Chelonia, Pelomedusidae). Atas do Simposio sobre a Biota Amazônica 7:201–206.

PACKARD, G.C., MILLER, K., PACKARD, M.J., AND BIRCHARD, G.F. 1999. Environmentally induced variation in body size and condition in hatchling snapping turtles (*Chelydra serpentina*). Canadian Journal of Zoology 77:278–289.

PACKARD, G.C., PACKARD, M.J., AND BENIGAN, L. 1991. Sexual differentiation, growth, and hatching success by embryonic painted turtles incubated in wet and dry environments at a fluctuating temperatures. Herpetologica 47(1):125–132.

PLUMMER, M.V., SHADRIX, C.E., AND COX, R.C. 1994. Thermal limits of incubation in embryos of softshell turtles (*Apalone mutica*). Chelonian Conservation and Biology 1(2):141–144.

PRANGE, H.D. AND ACKERMAN, R.A. 1974. Oxygen consumption and mechanisms of gas exchange of green turtle (*Chelonia mydas*) eggs and hatchlings. Copeia 1974:758–763.

PRITCHARD, P.C.H. 1979. Encyclopedia of Turtles. Neptune, NJ T.F.H. Publications, pp. 18–71.

PRITCHARD, P.C.H. AND TREBBAU, P. 1984. The Turtles of Venezuela. Oxford: Society of the Study of Amphibians and Reptiles, pp. 43–57.

RATTERMAN, R.J. AND ACKERMAN, R.A. 1989. The water exchange and hydric microclimate of painted turtle (*Chrysemys picta*) eggs incubating in field nests. Physiological Zoology 62(5): 1059–1079.

ROSTAL, D.C., GRUMBLES, J.S., LANCE, V.A., AND SPOTILA, J.R. 1994. Non-lethal sexing techniques for hatchling and immature desert tortoises (*Gopherus agassizii*). Herpetological Monographs 8:83–87.

SEIGEL, R.A. AND DODD, C.K., JR. 2000. Manipulation of turtle populations for conservation. In: Klemens, M.W. (Ed.). Turtle Conservation. Washington, DC and London: Smithsonian Institution Press, pp. 227.

SOINI, P. 1997. Ecologia y Manejo de Quelonios Acuáticos en la Amazonia Peruana. In: Fang, T.G., Bodmer, R.E., Aquino, R., and Valqui, M.H. (Eds.). Manejo de Fauna Silvestre en la Amazonía. La Paz, Bolivia: Instituto de Ecología, pp. 167–173.

SOKAL, R.R. AND RHOLF, F.J. 1995. Biometry. Third edition. New York: W.H. Freeman, 887 pp.

SOUZA, R.R. AND VOGT, R.C. 1994. Incubation temperature influences sex and hatchling size in the neotropical turtle (*Podocnemis unifilis*). Journal of Herpetology 28(4)453–464.

SPEAKMAN, J.R., HAYS, G.C., AND LINDBLAD, E. 1998. Thermal conductivity of sand and its effect on the temperature of loggerhead sea turtle (*Caretta caretta*) nests. Journal of the Marine Biology Association of the United Kingdom 78(4): 1337–1352.

STANCYK, S.E. 1995. Biology and Conservation of Sea Turtles. Third edition. Washington, DC: Smithsonian Institution Press, 615 pp.

STANCYK, S.E. AND ROSS, J.P. 1978. An analysis of sand from green turtle nesting beaches on Ascension Island. Copeia 1978(1):93–99.

TUCKER, J.K. AND WARNER, D.A. 1999. Microgeographic variation in response of red-eared slider (*Trachemys scripta elegans*) embryos to similar incubation environments. Journal of Herpetology 33(4):549–557.

VANZOLINI, P.E. 1967. Notes on the nesting behaviour of

Podocnemis expansa in the Amazon Valley (Testudines, Pelomedusidae). Papéis Avulsos de Zoologia 20(17):191–215.

VON HILDEBRAND, P., SÁENZ, C., PEÑUELA, M.C., AND CARO, C. 1988. Biologia Reprodutiva y Manejo de la Tortuga Charapa (*Podocnemis expansa*) em el Bajo Rio Caqueta. Colômbia Amazônica 3:89–111.

WOOD, D.W. AND BJORNDAL, K.A. 2000. Relation of temperature, moisture, salinity and slope to nest site selection in loggerhead sea turtles. Copeia 2000(1):119–128.

ZUG, G.R. 1993. Herpetology, an introductory biology of amphibians and reptiles. San Diego, CA: Academic Press, pp. 189–190.

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Excavation is a Nondeleterious Method for Obtaining Fecundity and Morphometric Data from Small-Sized Eggs of Freshwater Turtles

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ABSTRACT. – **We tested the hypothesis that handling turtle eggs decreases embryo survival in a well-studied population of midland painted turtle (*Chrysemys picta marginata*) by comparing embryo survival in handled and nonhandled natural nests during 3 nesting seasons. All nests were protected from mammalian predators. Upon excavation of the nests in the following spring, we found no differences in survival between the 2 treatments, suggesting that the benefits in knowledge gained from nest excavation far outweigh the possibility of a small increase in mortality that could arise from handling the eggs.**

Egg morphometric data are important for the study of questions relating to freshwater turtle life history, such as temperature-dependent sex determination (Roosenburg 1996), optimal egg size theory (Congdon and Gibbons 1985), and the relationship between hatchling size and survival (Brooks et al. 1991; Bobyn and Brooks 1994; Congdon et al. 1999; Packard et al. 1999; Janzen et al. 2000; Packard and Packard 2001). However, freshwater turtles have a cryptic reproductive cycle in which mating occurs in water (Ernst et al. 1994) and eggs are oviposited in terrestrial nests during a short period in early summer. Methods that do not require nest excavation, such as x-radiation or ultrasound, have been used to estimate clutch size and egg size (Gibbons and Greene 1979; Hinton et al. 1997; Kuchling 1998), but these methods cannot be used to measure egg mass and are prone to inaccurate measurements of egg size because they provide only 2-dimensional views of 3-dimensional eggs. Kuchling (1998) further noted that the number of oviductal eggs, measured with x-radiation or ultrasound, may not be an accurate measure of fecundity, as some eggs may be retained after laying and subsequently discarded in water. Excavating turtle nests immediately after oviposition is thus a preferred method to obtain fecundity data and accurate egg morphometrics.

Handling of turtle eggs has been criticized because it may have adverse effects on the survival of the embryos: *Chrysemys picta*, *Trachemys scripta*, and *Terrapene carolina* (Drajeske 1974; Simon 1975; Ewert 1979); *Chelonia mydas* (Parmenter 1980); *C. picta*, *Malaclemys terrapin*, and *Chelydra serpentina* (Feldman 1983; Ewert 1984); *Dermochelys coriacea* (Chan 1989); and *Pelodiscus sinensis* (Chou and Choo 1995). Evidence of the acceptance of the hypothesis that handling eggs has deleterious effects can be found in recent literature (e.g., Bjurlin and Bissonette 2004): "Because jarring and reorientation of eggs may affect embryo survival (Ewert 1979), we did not manipulate or uniquely mark eggs prior to incubation." Previous work on effects of handling and manipulating eggs has been conducted in laboratories under artificial incubation conditions and cannot be extrapolated with confidence to wild nests. Additionally, eggs in previous studies were exposed to levels and types of handling (e.g., single 90° rotations at varying times throughout incubation [Chou and Choo 1995]) that were not representative of the manipulations performed by researchers collecting morphometric data. And, even though Drajeske (1974), Feldman (1983), and Ewert (1984) found no effect of rotation on painted turtle eggs, Ewert (1984) stated that in painted turtle eggs, adhesion of the vitelline membrane to the egg (the basis for movement sensitivity) sometimes occurs as soon as the first hour following laying.

We assessed the effect of egg handling on the survival of the embryos in a population of midland painted turtle (*Chrysemys picta marginata*) where, from 1983 until the present study, all observed nests were excavated and the eggs measured and reburied immediately following oviposition.

Methods. — A long-term mark–recapture study of midland painted turtles has been conducted at Wolf Howl Pond (WHP) and West Rose Lake (WRL) which are less than 1000 m apart in Algonquin Park, Ontario (45°34′N, 78°41′W), Canada, for 21 summers between 1978 and 2003, and a study of reproductive ecology was initiated in 1983. The probability of observing most nesting females is very high because this population is located in a coniferous habitat that has very few suitable nesting sites. More precisely, all nests are found on a sandy railway embankment (now a popular hiking trail) a few meters