

- MARCELLINI, D.L. AND DAVIS, S.W. 1982. Effects of handling on reptile egg hatching. *Herpetological Review* 13:43–44.
- 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. AND PACKARD, M.J. 2001. Environmentally induced variation in body size and condition in hatchling painted turtles (*Chrysemys picta*). *Functional Ecology* 15:481–489.
- PARMENTER, C.J. 1980. Incubation of the eggs of the green sea turtle, *Chelonia mydas*, in the Torres Strait, Australia: the effect of movement on hatchability. *Australian Wildlife Research* 7:487–491.
- ROOSENBURG, W. 1996. Maternal condition and nest site choice an alternative for the maintenance of environmental sex determination. *American Zoologist* 36:157–168.
- SAMSON, J. 2003. The life history strategy of a northern population of midland painted turtles, *Chrysemys picta marginata*. MSc Dissertation, University of Guelph, Ontario, Canada.
- SCHWARZKOPF, L. AND BROOKS, R.J. 1985. Application of operative environmental temperatures to analysis of basking behavior in *Chrysemys picta*. *Herpetologica* 41:206–212.
- SEIGEL, R.A. AND DODD, C.K., JR. 2000. Manipulation of turtle populations for conservation: halfway technologies or viable options? In: Klemens, M. (Ed.). *Turtle Conservation*. Washington, DC: Smithsonian Institution Press, pp. 218–238.
- SIMON, M.H. 1975. The green sea turtle *Chelonia mydas*: collection, incubation and hatching of eggs from natural rookies. *Journal of Zoology* 176:39–48.
- SPINKS, P.Q., PAULY, G.B., CRAYON, J.J., AND SHAFFER, H.B. 2003. Survival of the western pond turtle (*Emys marmorata*) in an urban California environment. *Biological Conservation* 113: 257–267.

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Estimate of Trade Traffic of *Podocnemis* (Testudines, Podocnemididae) from the Middle Purus River, Amazonas, Brazil

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ABSTRACT. – The trade traffic estimate was made by using real data from 2 years of seizures (2000 and 2001), combined with river stage-level data. Abufari Reserve is located about 600 km south of Manaus,

where the largest stretch of the Amazonian floodplain is protected by a federal conservation unit. In the minds of the local people, the chelonian populations are still abundant and inexhaustible. However, records show that in 2000 and 2001, 3992 chelonians and 122 *Podocnemis* nets were confiscated, which represent only a small part of the total catch from this river. Consequently, these natural stocks could be decreasing because of trafficking by animal peddlers who invade the area and escape with animals to the detriment of the ecosystem and the local people.

For hundreds of years, naturalists have described the extraordinary abundance of river chelonians, especially *Podocnemis expansa* (Schweigger 1812), which was a very common species at the end of the 19th century (Silva-Coutinho 1868). This species is threatened because of blatant exploration and the lack of conservation measures (Smith 1979). Bates (1863) estimated that, during the 19th century, approximately 48 million *P. expansa* eggs were removed annually from the middle Solimões and Madeira Rivers. Today, there is scant occurrence of egg deposition by this species on beaches in most Amazonian rivers, although large populations still survive on the Purus River (Smith 1979). Nevertheless, chelonian eggs and adults are still important sources of protein and serve as currency in commerce for the local human population on most of the Amazonian rivers. (Cavalcanti 1999; Pezzuti and Vogt 1999; Soares 2000). A good example of continued abundance can be found on Abufari Beach of the Purus River, considered to be the last large egg-laying refuge for *P. expansa* in the state of Amazonas, where about 4000 females annually lay their eggs (A. Kemenes, *direct obs.*). However, the means required to protect this kind of phenomena is a considerable challenge. Because of predacity abetted by the adults and eggs linked to the demand for these items, combined with the poverty of riverside populations only increases the danger of extinction en masse. According to reports from the oldest fishermen, the Purus River has historically been a river of intense and continuous exploration of mainly chelonians and alligators. Its floodplain lakes and immense flooded area form an extended aquatic labyrinth, parallel to the Purus River and the Solimões River, uniting the local centers of commerce with consumers and middlemen in the cities of Manacapuru, Tapauá, and Manaus. The illegal trade of wild fauna species in these cities has become normal. For many riverine families, it is the only means of survival. Various kinds of wild animal meat and live chelonians are commonly available at municipal markets and sometimes even in other public places in these interior cities of Amazonas State.

The Amazonian floodplain is a complex aquatic system that has become one of the main areas of operation of well-organized animal smugglers in Amazonas State. The Abufari Reserve, which was once erroneously considered by the riverside people as an inexhaustible

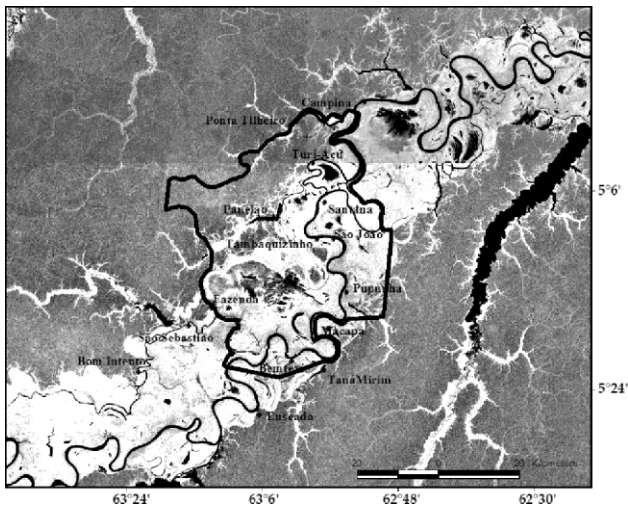


Figure 1. Geographic localization of Abufari Reserve (black line) and riverine communities (black points) along the Middle Purus River, 20 km near the Tapauá city, with other relevant places (radar JERS – 1, SAR, L band, at dry season).

source of hunting and fishing, is systematically losing its natural riches. Therefore, the objective of the present study was to evaluate the traffic activity of *Podocnemis* in the middle Purus River, with the hope that this research could be useful for environmental officials to aid them in inspection missions during critical periods of year.

METHODS

Study Area. — The Abufari Reserve is located at 4°50'–5°30'S and at 63°20'–62°50'W, encompassing a total area of 288,000 ha. It is situated 30 km from the city of Tapauá, between the beaches of Beabá and Camaleão (Fig. 1). This reserve is drained by the Purus River, a right

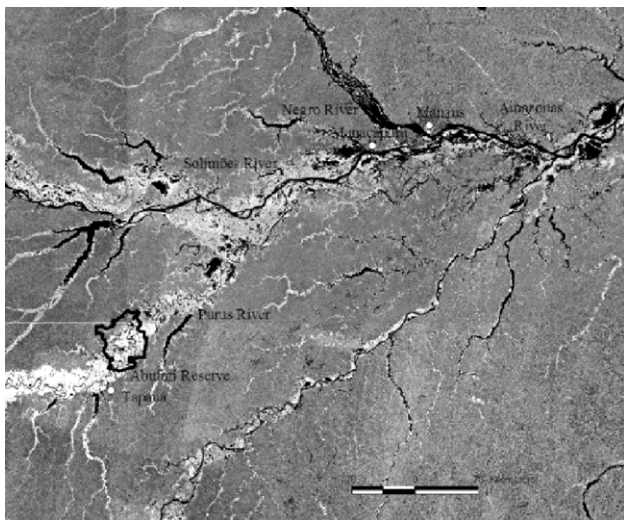


Figure 2. Abufari with black line along the Purus River, 500 Km of Manaus city, and 300 km near the Manacapuru city (radar JERS – 1, SAR, L band, at wet season).



Figure 3. *Podocnemis expansa* die in a capa-saco trawl net, Abufari Reserve, Amazonas, Brazil.

margin tributary of the lower Solimões River, which extends for 200 km (Fig. 2). The Purus River has a series of large white sand beaches of constant, firm, and uniform texture. These beaches are all of a possessing a saliencies

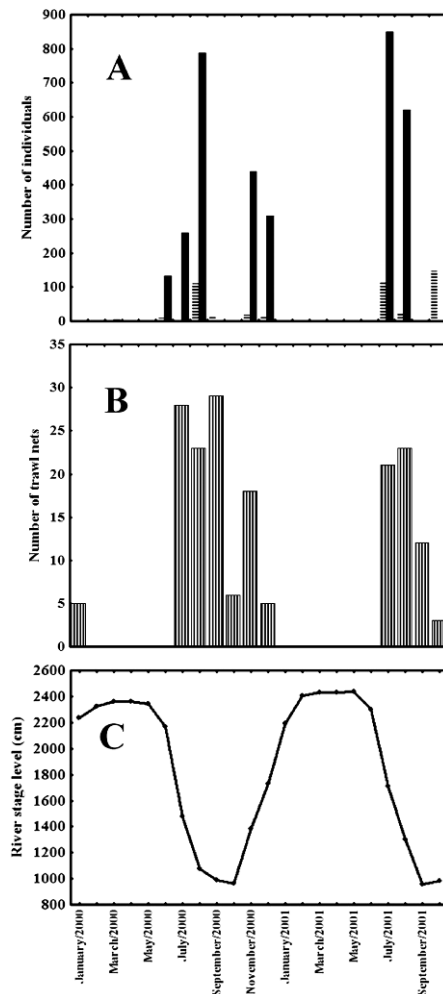


Figure 4. Diagrams detailing the abundance of *Podocnemis expansa* (■), *Podocnemis sextuberculata* (▨) A, and trawl nets (||) B confiscated, in relation to the seasonal Purus River stage level (cm) C.

Table 1. Most significant seizures apprehended on the Abufari Reserve in 2000 and 2001: the date, period, locale, method of transportation or hideout, *Podocnemis* species and quantity of animals.

Date	Period	Locale	Method	Species	Quantity
6/30/00	Night	Fluctuating Base (Purus River)	Boat	<i>P. expansa</i>	11
				<i>P. unifilis</i>	1
				<i>P. sextuberculata</i>	97
7/17/00	Morning	Samaúma Lake (Abufari River)	Hideout	<i>P. expansa</i>	3
				<i>P. unifilis</i>	14
				<i>P. sextuberculata</i>	88
7/30/00	Afternoon	Parati Beach (Purus River)	Canoe	<i>P. expansa</i>	4
8/16/00	Night	Furo Grande (Abufari River)	Hideout	<i>P. sextuberculata</i>	165
				<i>P. expansa</i>	112
				<i>P. unifilis</i>	2
11/25/00	Afternoon	Três Bocas (Purus River)	Canoe	<i>P. sextuberculata</i>	788
				<i>P. sextuberculata</i>	250
				<i>P. expansa</i>	7
12/3/00	Afternoon	Três Bocas (Purus River)	Canoe	<i>P. sextuberculata</i>	287
7/29/01	Night	Capitari Lake (Abufari River)	Hideout	<i>P. expansa</i>	120
				<i>P. unifilis</i>	8
				<i>P. sextuberculata</i>	850
8/12/01	Night	Abufari River	Hideout	<i>P. expansa</i>	4
8/13/01	Morning	Chapéu Complex	Canoe	<i>P. sextuberculata</i>	120
				<i>P. expansa</i>	21
				<i>P. sextuberculata</i>	421
10/20/01	Morning	Tapauá City (Purus River)	Boat	<i>P. expansa</i>	156

that oscillates between gambols and sand banks, including water wells and various water springs that disappear in the middle of October when the river level drops and the beaches become larger.

Monitoring. — In 2000 and 2001, the Brazilian Environmental Institute (IBAMA) base, located in front of Abufari Beach, confiscated animals and trawl nets in the possession of smugglers. The capa-saco (chelonian trawl net) was the principal tool used to capture the chelonians (Fig. 3). Without buoys, and being heavily weighted at the bottom, this trawl net opens up in the form of a sack and is placed across a medium-size stream that has a strong water flux. Consequently, this net is difficult to detect, because it lies well below the water line. When chelonians try to escape, the strong water flow captures them in this net. All of the chelonians confiscated belonged to only 3 registered aquatic species, most of them being *Podocnemis sextuberculata* (Cornelia 1849), which were as long as 28 cm and weighed more than 4 kg, then *P. expansa*, which

reached 60 kg and 80 cm in length; and finally *Podocnemis unifilis* (Troschel 1848), which usually weighed about 8 kg and were 40 cm in length.

RESULTS AND DISCUSSION

The Abufari Reserve is found within the Purus River waterway, which has constant traffic of boats, ships, and canoes. These vessels are routinely detained at the IBAMA base for a mandatory inspection of documents for fishing and equipment. During 2000 and 2001, 550 fishing vessels (iceboats: boats with ice on board to preserve fish), 120 regional transport vessels, and 92 wooden canoes were inspected, of which 6 iceboats, 8 regional boats, and 12 canoes were found transporting chelonians (Table 1). As well, in 2000 and 2001, 122 chelonian trawl nets were seized in Abufari Reserve. About 70% of these were on the Abufari River, and the rest were on the Purus River, its tributaries, and associated water channels. When consid-

Table 2. Morphometry of some *Podocnemis* species confiscated in many locales of Abufari Reserve in 2000 and 2001 with individual number (N).^a

Species	Locale	Date	N	CRC (cm)			CCC (cm)			CP (cm)		
				Max	Min	Med	Max	Min	Med	Max	Min	Med
<i>P. expansa</i>	Abufari Beach	9/1/00	18	78	40	68.8	81	42	71.3	68	36	59.2
<i>P. unifilis</i>	Bem-te-vi	9/4/00	12	39	35	37.1	42	38	39.7	37	33	34.1
<i>P. expansa</i>	Capitari Lake	7/29/01	120	77	26	67.3	80	28	70.1	67	22	58.3
<i>P. sextuberculata</i>	Capitari Lake	7/29/01	850	27	19	25.2	28	21	27.5	23	17	22.7
<i>P. expansa</i>	Abufari River	7/17/01	8	52	45	48.2	55	48	49.6	44	38	41.3
<i>P. sextuberculata</i>	Abufari River	7/17/01	19	26	18	21.9	28	19	23.1	22	15	18.9

^a Animal size is recorded: Max = maximum, Min = minimum; Med = medium; CRC = rectilinear carapace length; CCC = curvilinear carapace length; CP = plastron length.

ering the 3978 chelonians confiscated during these 2 years, 55% were taken from the Abufari River, *P. sextuberculata* (83.63%), *P. expansa* (10.41%), and *P. unifilis* (1.78%). Morphometry data of these same chelonians that were seized in the works are shown in Table 2. The more regular capture of these species occurred during the period when the water level of the Purus River was between 1000 and 1400 cm (Fig. 4A) (number of *P. expansa* = 91.98–0.038 [water level], $n = 10$, $p < 0.05$, $R^2 = 0.36$; and number of *P. sextuberculata* = 496.7–0.18 [water level], $n = 14$, $p < 0.05$, $R^2 = 0.3$).

These confiscates not only demonstrate their relation to the stage water level but also in accordance to the flood pulse of the river system in general. It was during these low phases that the large numbers of chelonians were confiscated. The trawl nets were subsequently seized during a period that coincided with the chelonians (Fig. 4B) (number of trawl nets = 31.57–0.01 [water level], $n = 54$, $p < 0.05$, $R^2 = 0.52$). The above values only indicate seizure estimates of chelonians, when, in reality, there are probably large variations in the number of animals taken from smugglers each year. In the single month of August of 1999, 38,000 chelonians were taken from 1 ferry that was used to transport cargo along the Solimões River (Cavalcanti 1999). The large number of seizures registered in this study may possibly represent only a small fraction of the total number of chelonians taken from the Purus River and the rest of Amazonas State. The number of animals apprehended in relation to the number of nets was considered low. This could indicate that the nets were taken away before the offenders made their catch or that they escaped from the reserve with many animals, without being caught by forest agents.

The amount of trawl nets confiscated on the Abufari River indicates the local migration and trajectory of chelonians in the reserve. However, the large number of animals confiscated and clandestine campsites along the margin of interior lakes belonging to the Chapéu Complex indicate that the perpetrators prefer not to remain on the margins of the Abufari River but rather in more protected sites that are removed and isolated (Table 1). Most seizures of chelonians occurred when the water level of the Purus River was between 1000 and 1400 cm. Most confiscates of *P. expansa* and *P. sextuberculata* occurred between the end of the dry season, during the months of June, August, October, and November (Fig. 4). However, catches of *P. sextuberculata* were confiscated over a much longer period than *P. expansa*, which shows that *P. sextuberculata* present a migratory behavior less selective than *P. expansa* in terms of water-level change, this is perhaps because of its smaller size, more solitary behavior, and a larger number of individuals in population.

The difficulty in inspecting vessels, combined with innumerable methods that smugglers have invented to conceal illegal material and elude the inspectors, has made it very challenging to control trafficking. However, without constant and effective policing, perpetrators will

continue to take advantage of these prohibitions to make an even greater profit. Unfortunately, in this Amazon paradise the animal hunting is facilitated by the sheer abundance of organisms and a lack of sustainable development resource policies, along with greater backing of solid investments in environmental education.

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LITERATURE CITED

- BATES, H.W. 1863. *The Naturalist on the River Amazon*. London: John Murray, 407 pp.
- CAVALCANTI, K. 1999. "Matança Amazônica". *Revista Veja* 25: 38–39.
- DOS SANTOS, C.A. 2001. A evolução populacional da cidade de Manaus. *Qualidade de Vida* 28. Universidade de São Paulo.
- PEZZUTI, J.C.B. AND VOGT, R. 1999. Nesting ecology of *Podocnemis sextuberculata* in the Japurá River, Amazonas, Brazil. *Chelonian Conservation on Biology* 3(3):419–424.
- SILVA-COUTINHO, J.M.D. 1868. Sur les tortues de L'Amazone. *Bull. Soc. Impér. Zool. Acclim.* (2)5:147–166.
- SMITH, N.J.H. 1979. Quelônios aquáticos da Amazônia: um recurso ameaçado. *Acta Amazonica* 9:87–97.
- SOARES, M.F.G.S. 2000. Distribuição, Mortalidade e Caça de *Podocnemis* (Testudinata, Pelomedusidae) no Rio Guaporé, Rondônia, Brasil. Master Dissertation, Instituto Nacional de Pesquisas da Amazônia. 61 pp.

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Deterioration of Green Sea Turtle (*Chelonia mydas*) Eggs After Known Embryo Mortality

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ABSTRACT. — To determine the time interval between embryonic death and physical alterations in appearance of green turtle (*Chelonia mydas*) eggs, one each of matched pairs of eggs were inverted after 7–10 days of incubation. Chalkiness of the white spot diminished after 44 hours as maintenance of the chorio-allantoic membrane, contributing to the opaque appearance of

viable eggs, ceased after embryo mortality. Results of this study will allow embryo mortality to be attributed to known specific events or conditions within the incubation period.

Prior to oviposition, embryonic development in the chelonian egg is suspended at gastrulation (Decker 1967; Mahmoud et al. 1973; Packard et al. 1977; Ewert 1985). The signal for the end of embryonic diapause and recommencement of development is unknown (Blanck and Sawyer 1981), but is likely to be due to one or more of the following: changes in oxygen tension, temperature, water uptake, or a lack of movement. After oviposition lack of movement allows differences in the density of the egg components to become patent, causing the yolk to rise through the liquefied albumen to rest at the top (Fisk and Tribe 1949). In the yolk, denser granules within the vitelline membrane settle, allowing the blastodisc to rotate to the top of the egg (Miller 1985).

Formation of the subembryonic fluid and changes in the eggshell's optical and structural properties are presumed to be similar to those described for other reptiles and birds. Water is drawn from the albumen by the ectodermal surface of the blastodisc and secreted via its endodermal surface beneath the embryo into the vitelline sac above the yolk (New 1956). As the volume of this subembryonic fluid increases, so does that of the vitelline sac, allowing it to occupy space created by dehydration of the albumen. As water is initially drawn from albumen immediately adjacent to the embryo (i.e., at the top of the egg) (New 1956) the vitelline membrane and blastodisc are pushed into close proximity of the shell membrane with only a thin layer of dehydrated albumen separating them (Webb et al. 1987a, 1987b). The *area vasculosa* of the yolksac is, therefore, in close proximity to the eggshell, and acts as a respiratory avenue until the chorio-allantois develops (New 1956).

These events result in a chalky white area that initially appears at the top of the egg (the location of the embryo) soon after oviposition (Ewert 1979; Blanck and Sawyer 1981). This opaque white spot enlarges to encompass the entire egg surface, and is often used as an indication of viability (Yntema 1981). In addition, the egg surface may appear mottled, displaying dark pink or purple areas indicative of blood vessel formation (Blanck and Sawyer 1981).

The change in hard-shelled crocodylian egg appearance from translucent at oviposition to opaque as the white spot forms is due to physical and structural changes of the eggshell associated with progressing dehydration (Webb et al. 1987a). At oviposition, the pores or matrix of all reptilian eggshells are filled with fluid (Deeming and Thompson 1991) that is probably of oviductal origin. The spread of the white spot to encompass the entire egg occurs in synchrony with or slightly beyond the expansion of the chorio-allantoic membrane (Thompson 1985). The displaced albumen allows the chorio-allantois to form a thin blood-gas barrier (Wangensteen and Weibel 1982) which

probably contributes to eggshell dehydration since there is a simultaneous increase in allantoic fluid (Ferguson 1982, 1985). Opacity of the eggshell is due to changes in its optical properties following dehydration (Webb et al. 1987a). There is also the possibility that opacity may be engendered by structural changes through loss of calcium carbonate crystals as the embryo begins incorporating calcium (Ferguson 1982). Moist, translucent sea turtle eggshell becomes opaque when dried and translucent again when rehydrated (A.D. Phillott, *pers. obs.*), supporting the contention that it is primarily water loss from the shell that engenders an opaque appearance. Sahoo et al. (1998) measured negligible calcium depletion in olive ridley sea turtle (*Lepidochelys olivacea*) eggshell until day 40 (embryological stage and incubation duration not described). The white spot encompasses the entire egg much earlier, further indicating that the depletion of calcium from turtle eggshell has little role in creating opacity (in contrast to *Alligator mississippiensis*, Ferguson 1982).

Fluid loss from the shell may occur by osmotic drag to the albumen (Kutachai and Steen 1971; Lomholt 1976; Seymour and Piiper 1988) or loss to the atmosphere (Kayar et al. 1981). Webb et al. (1987a) suggested the relationship observed between changes in albumen volume and opaque banding patterns in crocodile eggs implied an intrinsic rather than extrinsic dehydration pathway. Furthermore, since subembryonic fluid formation ceases with crocodile embryo mortality, there must be an active embryonic role in albumen dehydration (Manolis et al. 1987). The allantoic fluid (sequestering excretory or nitrogenous wastes) originates from moisture derived from the eggshell and albumen, in combination with the subembryonic fluid of the yolk (Manolis et al. 1987).

Unhatched eggs with dead embryos from emerged nests usually appear yellow and display a slight loss of turgor and deterioration of the eggshell. No work has been conducted to determine the time lapse between embryonic death and the occurrence of physical alterations in the egg's appearance. Early detection of nonviable eggs by visual inspection is likely to be advantageous in artificial incubation studies manipulating incubation conditions (e.g., temperature, moisture, microbial load) so that embryonic death may be attributed to specific events within the incubation period. In this study, we experimentally inverted eggs during early development and recorded changes of eggshell appearance after embryonic death.

Methods. — Two successive midclutch eggs were collected from 5 individual green turtles (*Chelonia mydas*) nesting at Heron Island (23°26'S, 151°55'E), eastern Australia. Eggs ($n = 10$) were weighed and measured and then incubated at ambient laboratory temperature (Heron Island Research Station) as matched pairs, with each pair in a separate transparent plastic container (16 × 10 × 7 cm) covered in plastic film. They were set on a 2-cm substrate of autoclaved sand collected at a depth of 55 cm (average nest depth) from the nesting beach. A subsurface trickle irrigation of sterile water (see Phillott

Table 1. Dimensions and weight of control and experimental eggs.

Replicate no.	Egg weight, maximum diameter × minimum diameter at oviposition		Egg weight at inversion of experimental egg	
	Control egg	Experimental egg	Control egg (g)	Experimental egg (g)
1	58.3 g, 4.74 × 4.60 cm	50.4 g, 4.64 × 4.40 cm	58.2	50.1
2	52.3 g, 4.71 × 4.45 cm	53.2 g, 4.71 × 4.49 cm	51.7	52.5
3	58.0 g, 4.76 × 4.59 cm	57.9 g, 4.74 × 4.70 cm	57.6	57.6
4	50.3 g, 4.54 × 4.43 cm	50.3 g, 4.55 × 4.44 cm	50.4	50.2
5 ^a	54.4 g, 4.76 × 4.54 cm	53.7 g, 4.67 × 4.54 cm	54.4	53.5

^a Data subsequently discarded as experimental egg remained viable after inversion.

2002) maintained sand moisture to a standard, predetermined by the “pinch method” (Blanck and Sawyer 1981).

Eggs were observed until white spot development covered approximately half of the surface, indicating embryonic viability (7–10 days). Eggs were then removed, weighed, and replaced, one in its original orientation, the other inverted. Inversion of green (Parmenter 1980) and loggerhead (Limpus et al. 1979) sea turtle eggs at this time during incubation is known to cause embryo mortality. Control (noninverted) eggs were incubated until hatching; experimental (inverted) eggs were opened at the same time to determine embryonic stage at mortality. During incubation, visual observations of white spot development and other changes in eggshell color were recorded.

Results. — Egg diameter and weight at oviposition (Table 1) were within the range previously recorded for green sea turtles at Heron Island (Limpus et al. 1984). During the 7–10 days prior to manipulation, pairs of eggs showed similar signs of development, i.e., progression of white spot and weight change.

White spot development ceased immediately after inversion in 4 of the 5 experimental eggs. In the exception, the white spot expanded slightly but at a slower rate than its unrotated control for a further 84 hours. Observations of this egg were subsequently discarded as the exact time of embryonic death was uncertain (it later failed to hatch). Sixteen hours after rotation, the remaining inverted eggs displayed a pale yellowing tinge to the eggshell outside the white spot. This color increased in intensity as time progressed, spreading from the top of the egg downwards. The entire egg displayed a distinct yellowing after 20–24 hours. The chalkiness of the white spot faded significantly after 44 hours. Shell pliability increased slightly. None of the control eggs displayed any alteration in eggshell appearance other than normal expansion of the white spot, and there was no change in shell pliability.

All of the control eggs produced hatchlings, while none of the inverted eggs hatched. When opened, unhatched eggs revealed development consistent with embryonic Stage 18–19 (after Miller 1985), suggesting death synchronous with egg rotation.

Discussion. — In the green sea turtle, egg inversion and subsequent embryonic death results in alterations to egg appearance. As eggshell structure is similar in green

(Solomon and Baird 1976; Baird and Solomon 1979), leatherback (*Dermochelys coriacea*; Solomon and Watt 1985; Chan and Solomon 1989), olive ridley (*Lepidochelys olivacea*; Sahoo et al. 1996a, 1996b), Kemp’s ridley (*L. kempii*; Packard et al. 1982), flatback (*Natator depressus*; Phillott 2002), and hawksbill (*Eretmochelys imbricata*; Phillott 2002) sea turtle eggs, postmortem changes in appearance are also likely to be similar. Although Carthy (1992) described pore-like structures on the inner surface of loggerhead (*Caretta caretta*) turtle shell membrane, these were not detected by Packard et al. (1982), Schleich and Kästle (1988), or Phillott (2002), hence loggerhead eggshell is presumed to display similar deterioration to that described here.

Blanck and Sawyer (1981) described 2 temporary extra-embryonic membranes during the first 2.5 weeks of development in loggerhead turtle eggs at 28°C (approximately the first trimester of the 57-day incubation period). The posterior amniotic tube and the “attachment membrane” (an extension of the amnion that fuses with the chorionic membrane adjacent to the eye region) aid in positioning and maintaining the embryo at the top of the egg. The physical support role of the extra-embryonic membranes is superseded after this time by the thickening of the yolk stalk and increase of the chorion–shell membrane adherence area.

Prior to this time, egg inversion results in the disruption of egg contents and tearing of extra-embryonic membranes and blood vessels (Blanck and Sawyer 1981). Miller (1985) attributed movement-induced early embryo mortality to rupturing of the vitelline membrane. The embryo remains attached to the shell, but is sheared from the embryonic disc (Ferguson 1985). Yolk and fluids within the vitelline sac and subgerminal space are then able to mix with the albumen (Ewert 1979). Postmortem discoloration of eggs in the experiment would likely result from similar mixing. Yellowing was first observed at the inverted northern or nonwhite pole, spreading downwards and finally encompassing the entire egg. This would be due to rearrangement of the egg contents after egg inversion. As the yolk moves through the albumen and rearranges itself to its preferred orientation, maximal leakage of the vitelline sac contents would occur at the new top surface of the egg, hence the initial site of yellowing. As the chalkiness of the white spot relies on shell dehydration

(and subsequent opacity) maintained by embryonic metabolic activity, it fades after embryonic death.

The loss of turgor by nonviable eggs occurs as they lose their water holding capacity (Ewert 1979), potentially acting as water donors, with a similar role to that presumed for yolkless leatherback eggs (Hall 1990). Viable eggs maintain a water potential of -900 kPa (Ackerman 1991). Death would result in the loss of metabolic sustenance and a less negative water potential, closer to the -5 to -50 kPa of the substrate (Ackerman 1991), allowing moisture transfer from the egg to the nest environment. Alternatively, water bridges may form between nonviable and adjacent viable eggs for direct exchange.

Unlike nonviable eggs in natural nests examined at full term, the experimentally killed eggs did not exhibit severe flaking of the shell. The slight increase in pliability that occurred was probably due to turgor loss, though it may have been indicative of postmortem degradation of the shell structural integrity. Sea turtle eggshell consists of variable sized aragonite crystals that are not organized into individual shell units (i.e., with intervening discrete pores) but with numerous open spaces allowing gas and water exchange (Solomon and Baird 1976; Baird and Solomon 1979; Packard et al. 1982; Schleich and Kästle 1988; Solomon and Watt 1985; Chan and Solomon 1989; Sahoo et al. 1996a, 1996b). When the underlying shell membrane decays postmortem, it dissociates from the calcareous crystalline eggshell (Hirsch 1983), probably leading to the subsequent degradation of the eggshell. It is possible that the relatively low levels of soil microbiota (when compared to those of a natural nest) were insufficient for complete flaking of the eggshell to be observed in this experiment. Flaking (exfoliation) of viable eggs in the week prior to hatching (Miller 1985) is a result of calcium mobilization from the eggshell by the rapidly maturing embryo (Simkiss 1962).

This experiment caused 2 major alterations to the interior of the egg: yolk displacement and embryonic death. It is unclear whether changes in eggshell appearance were due to these occurrences individually, or in combination. However, other laboratory experiments (to be reported separately) demonstrate that eggs dying without human intervention present similar signs of faded chalkiness, increased yellowing, and increased shell pliability. As yellowing is likely due to mixing of the yolk (and/or subgerminal fluids) with the albumen in combination with embryo autolysis, it would normally occur (in a noninverted egg) with breakdown of the vitelline sac by protein degradation or microbial action and be observed as a gradual stain of the entire egg rather than initiating at either pole. Fading chalkiness (due to rehydration of the shell interstices as metabolic activity ceases) is likely to be a direct result of embryonic death rather than inversion, and hence be the better indicator of egg mortality.

The time lapse between embryonic death and the occurrence of physical alterations in eggshell appearance has relevance to the interpretation of manipulative results derived from the artificial incubation of sea turtle eggs.

Unless the white spot has faded substantially within 2 days of a particular incubation event or mishap (e.g., temperature fluctuation or increased microbial load), egg death should not be ascribed to that event with any certainty. Eggs that demonstrate a loss of chalkiness and/or develop a yellow discoloration should be considered nonviable and removed from the turtle nest or incubation container to prevent their becoming a source of microbial infection.

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LITERATURE CITED

- ACKERMAN, R.A. 1991. Physical factors affecting the water exchange of buried reptile eggs. In: Deeming, D.C. and Ferguson, M.W.J. (Eds.). *Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles*. Cambridge: Cambridge University Press, pp. 193–211.
- BAIRD, T. AND SOLOMON, S.E. 1979. Calcite and aragonite in the egg shell of *Chelonia mydas* L. *Journal of Experimental Marine Biology and Ecology* 36:295–303.
- BLANCK, C.E. AND SAWYER, R.H. 1981. Hatchery practices in relation to early embryology of the loggerhead sea turtle, *Caretta caretta* (Linne). *Journal of Experimental Marine Biology and Ecology* 49:163–177.
- CARTHY, R.R. 1992. Scanning electron microscopy (SEM) of loggerhead (*Caretta caretta*) eggshell structure. In: Salmon, M. and Wyneken, J. (Comps.). *Proceedings of the 11th Annual Workshop on Sea Turtle Biology and Conservation*. NOAA Technical Memorandum NMFS-SEFC-302. pp. 143–144.
- CHAN, E.H. AND SOLOMON, S.E. 1989. The structure and function of the eggshell of the leatherback turtle (*Dermochelys coriacea*) from Malaysia, with notes on attached fungal forms. *Animal Technology* 40:91–102.
- DECKER, J.D. 1967. Motility of the turtle embryo, *Chelydra serpentina* (Linne). *Science* 157:952–953.
- DEEMING, D.C. AND THOMPSON, M.B. 1991. Gas exchange across reptilian eggshells. In: Deeming, D.C. and Ferguson, M.W.J. (Eds.). *Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles*. Cambridge: Cambridge University Press, pp. 277–284.
- EWERT, M.A. 1979. The embryo and its egg: development and natural history. In: Harless, M. and Morlock, H. (Eds.). *Turtles: Perspectives and Research*. New York: John Wiley and Sons, pp. 333–413.
- EWERT, M.A. 1985. Embryology of turtles. In: Gans, C., Billett, F., and Maderson, P.F.A. (Eds.). *Biology of the Reptilia*. Vol. 14. New York: John-Wiley and Sons, pp. 76–267.
- FERGUSON, M.W.J. 1982. The structure and composition of the eggshell and embryonic membranes of *Alligator mississippiensis*. *Transactions of the Zoological Society of London* 36: 99–152.
- FERGUSON, M.W.J. 1985. Reproductive biology and embryology

- of the crocodylians. In: Gans, C., Billett, F., and Maderson, P.F.A. (Eds.). *Biology of the Reptilia*. Vol. 14. New York: John-Wiley and Sons. pp. 330–491.
- FISK, A. AND TRIBE, M. 1949. The development of the amnion and chorion of reptiles. *Proceedings of the Zoological Society of London* 119:6–114.
- HALL, K.V. 1990. Hatching success of leatherback turtle (*Dermochelys coriacea*) clutches in relation to biotic and abiotic factors. In: Richardson, T.H., Richardson, J.I., and Donnelly, M. (Comps.). *Proceedings of the 10th Annual Workshop on Sea Turtle Biology and Conservation*. NOAA Technical Memorandum NMFS-SEFC-278, pp. 197–199.
- HIRSCH, K.F. 1983. Contemporary and fossil chelonian eggshells. *Copeia* 1983:382–396.
- KAYAR, S.R., SNYDER, G.K., BIRCHARD, G.F., AND BLACK, C.P. 1981. Oxygen permeability of the shell and membranes of chicken eggs during development. *Respiration Physiology* 46: 209–221.
- KUTACHAI, H. AND STEEN, J. 1971. Permeability of the shell and shell membranes of hens' eggs during development. *Respiration Physiology* 11:265–278.
- LIMPUS, C.J., BAKER, V., AND MILLER, J.D. 1979. Movement induced mortality of loggerhead eggs. *Herpetologica* 35: 335–338.
- LIMPUS, C.J., FLEAY, A., AND GUINEA, M. 1984. Sea turtles of the Capricornia Section, Great Barrier Reef Marine Park. In: Ward, W.T. and Saenger, P. (Eds.). *The Capricornia Section of the Great Barrier Reef. Past, Present and Future*. Brisbane, Australia: The Royal Society of Queensland and Australian Coral Reef Society (Incorporating The Great Barrier Reef Committee), pp. 61–78.
- LOMHOLT, J.P. 1976. The development of the oxygen permeability of the avian egg shell and its membranes during incubation. *Journal of Experimental Zoology* 198:177–184.
- MAHMOUD, I.Y., HESS, G.L., AND KLIČKA, J. 1973. Normal embryonic stages of the western painted turtle, *Chrysemys picta bellii*. *Journal of Morphology* 141:269–280.
- MANOLIS, S.C., WEBB, G.J.W., AND DEMPSEY, K.E. 1987. Crocodile egg chemistry. In: Webb, G.J.W., Manolis, S.C., and Whitehead, P.J. (Eds.). *Wildlife Management: Crocodiles and Alligators*. Chipping Norton, Australia: Surrey Beatty and Sons and the Conservation Commission of the Northern Territory, pp. 445–472.
- MILLER, J.D. 1985. Embryology of marine turtles. In: Gans, C., Billett, F., and Maderson, P.F.A. (Eds.). *Biology of the Reptilia*. Vol. 14. Development A. New York: Academic Press, pp. 270–328.
- NEW, D.A.T. 1956. The formation of sub-blastodermic fluid in hens' eggs. *Journal of Embryology and Experimental Morphology* 4:221–227.
- PACKARD, G.C., TRACY, C.R., AND ROTH, J.J. 1977. The physiological ecology of reptilian eggs and embryos, and the evolution of viviparity within the class Reptilia. *Biological Reviews* 52:71–105.
- PACKARD, M.J., PACKARD, G.C., AND BOARDMAN, T.J. 1982. Structure of eggshells and water relations of reptilian eggs. *Herpetologica* 38:136–155.
- PARMENTER, C.J. 1980. Incubation of the eggs of the green sea turtle, *Chelonia mydas*, in Torres Strait, Australia: the effect of movement on hatchability. *Australian Wildlife Research* 7: 487–491.
- PHILLOTT, A.D. 2002. *Fungal Invasion of Sea Turtle Nests in Eastern Australia*. PhD Thesis, Central Queensland University, Rockhampton, Queensland, Australia.
- SAHOO, G., MOHAPTRA, B.K., SAHOO, R.K., AND MOHANTY-HEJMADI, P. 1996a. Contrasting ultrastructures in the eggshells of olive ridley turtles, *Lepidochelys olivacea*, from Gahirmatha, India. *Current Science* 70:246–248.
- SAHOO, G., MOHAPTRA, B.K., SAHOO, R.K., AND MOHANTY-HEJMADI, P. 1996b. Ultrastructure and characteristics of eggshells of the olive ridley turtle (*Lepidochelys olivacea*) from Gahirmatha, India. *Acta Anatomica* 156:261–267.
- SAHOO, G., SAHOO, R.K., AND MOHANTY-HEJMADI, P. 1998. Calcium metabolism in olive ridley turtle eggs during embryonic development. *Comparative Biochemistry and Physiology A* 121:91–97.
- SCHLEICH, H.H. AND KÄSTLE, W. 1988. *Reptile Egg-shells*. SEM Atlas. New York: Gustav Fischer.
- SEYMOUR, R.S. AND PIPER, J. 1988. Aeration of the shell membranes of avian eggs. *Respiration Physiology* 71: 101–116.
- SIMKISS, K. 1962. The sources of calcium for the ossification of the embryos of the giant leatherly turtle. *Comparative Biochemistry and Physiology* 7:71–79.
- SOLOMON, S.E. AND BAIRD, T. 1976. Studies on the eggshell (oviducal and oviposited) of *Chelonia mydas* L. *Journal of Experimental Marine Biology and Ecology* 22:145–160.
- SOLOMON, S.E. AND WATT, J.M. 1985. The structure of the eggshell of the leatherback turtle (*Dermochelys coriacea*). *Animal Technology* 36:19–27.
- THOMPSON, M.B. 1985. Functional significance of the opaque white patch in eggs of *Emydura macquarii*. In: Grigg, G., Shine, R., and Ehmann, H. (Eds.). *Biology of Australasian Frogs and Reptiles*. Sydney, Australia: Royal Zoological Society of New South Wales, pp. 387–395.
- WANGENSTEEN, D. AND WEIBEL, E.R. 1982. Morphometric evaluation of chorioallantoic oxygen transport in the chick embryo. *Respiration Physiology* 47:1–20.
- WEBB, G.J.W., MANOLIS, S.C., DEMPSEY, K.E., AND WHITEHEAD, P.J. 1987a. Crocodilian eggs: a functional overview. In: Webb, G.J.W., Manolis, S.C., and Whitehead, P.J. (Eds.). *Wildlife Management: Crocodiles and Alligators*. Chipping Norton, Australia: Surrey Beatty and Sons and the Conservation Commission of the Northern Territory, pp. 417–422.
- WEBB, G.J.W., MANOLIS, S.C., WHITEHEAD, P.J., AND DEMPSEY, K.E. 1987b. The possible relationship between embryo orientation, opaque banding and the dehydration of albumen in crocodile eggs. *Copeia* 1987:252–257.
- YNTEMA, C.L. 1981. Characteristics of gonads and oviducts in hatchlings and young of *Chelydra serpentina* resulting from three incubation temperatures. *Journal of Morphology* 167: 297–304.

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