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Reproduction of the Cabeçudo, *Peltocephalus dumerilianus*, in the Biological Reserve of Rio Trombetas, Pará, Brazil

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The conservation of most species of freshwater turtles in Brazil is important not only to preserve biological diversity, but also as a sustainable resource and important protein source for people living in Amazonia. One of the first steps in the management and conservation of any species is an understanding of its reproductive biology. The reproductive biology of the cabeçudo, or big-headed Amazon river turtle, *Peltocephalus dumerilianus* (Fig. 1), is less known than that of the rest of the Amazonian Pelomedusid turtles. Some of the closely-related *Podocnemis* species make rather conspicuous nests, often on communal sand beaches where thousands of nests can be found on a single beach, whereas *Peltocephalus* has been reported to nest solitarily within the forest or at the forest edge (Silva Coutinho, 1868; Medem, 1983). Medem (1983) published the only available data regarding reproduction of *Peltocephalus* in Colombia, where he found 7 natural nests in December 1980 and December 1981. During the course of the conservation and management program of Centro Nacional dos Quelônios da Amazônia – Instituto Brasileiro do Meio Ambiente (CENAQUA/IBAMA), we had the opportunity to document nests and incubate the eggs of *Peltocephalus* in 1992 and 1993. The purpose of this study was to document nesting habitat, clutch size, incubation period, and effect of nest temperature on sex determination as base line data to begin a long term study to be used in preparing a management program for this species, because cabeçudos are harvested for food in the Amazon.

Methods. — During August and September 1992 nests were located by CENAQUA workers by searching the shoreline along Igarapés Mungubau and Candieiro. These two small blackwater streams are within the tropical rainforest in areas known as "tierra firme" within the Biological Reserve of Rio Trombetas, Oriximiná, Pará, Brazil (1°20'S, 56°41'W) (Fig. 2). These two streams are tributaries to Lago do Erepecu, which drains into Rio Trombetas. In August and September Igarapé Mungubau is 3-4 m wide and 1-2 m deep within rainforest with a nearly closed canopy (Fig. 3). The nests were excavated and taken back to the Santa Rosa field station where they were incubated at ambient temperature. Each nest was kept in its original substrate and placed in baskets woven from palm leaves. After hatching the hatchlings were maintained in captivity on a mixed diet of fish and rice. On 10 November all surviving yearlings were measured with a digital caliper to the nearest 0.1 mm and weighed with a digital electronic balance to the nearest 0.1 and individually marked by injecting microchip PIT tags (Avid, Norco, CA, USA) in the musculature between the carapace and tail.

The senior author searched along the shoreline of Igarapé Mungubau with CENAQUA workers 4-6 September 1993. One of the workers had previously marked six nests in August. We excavated all nests found and counted and measured the eggs (length and width to the nearest mm with a dial caliper) and weighed them (to the nearest g with a Pesola spring scale). After recording nest measurements the eggs from each nest were packed in plastic buckets in their original nesting substrate and transported to the station at Santa Rosa on 6 September, where the eggs and their original nesting substrate were transferred to woven palm baskets. Nine nests were placed on a wooden table 1 m off the ground in an open exposed clearing. Two nests were placed on a wooden table 1 m off the ground in the shade 20 m within the forest. On 11 November all eggs were remeasured with a digital caliper to the nearest 0.1 mm and reweighed with a digital electronic balance to the nearest 0.1 g. Nest temperatures were taken at 0600, 1200, 1800, and 2200 hrs with copper-constantin thermocouples attached to a Bailey digi-

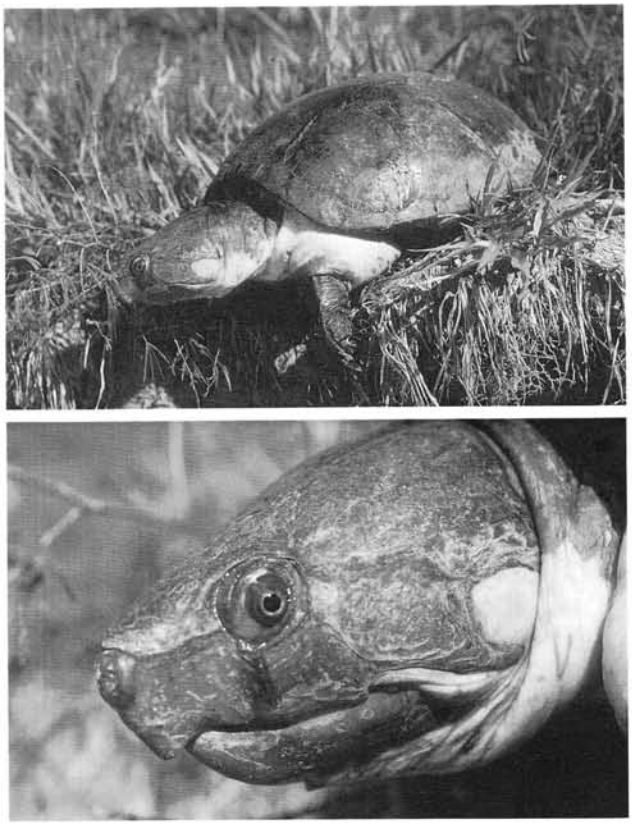

Figure 1. Adult *Peltocephalus dumerilianus* from Igarapé Mungubau, Rio Trombetas, Pará, Brazil, weighing 11 kg and measuring 430 mm in straightline carapace length.

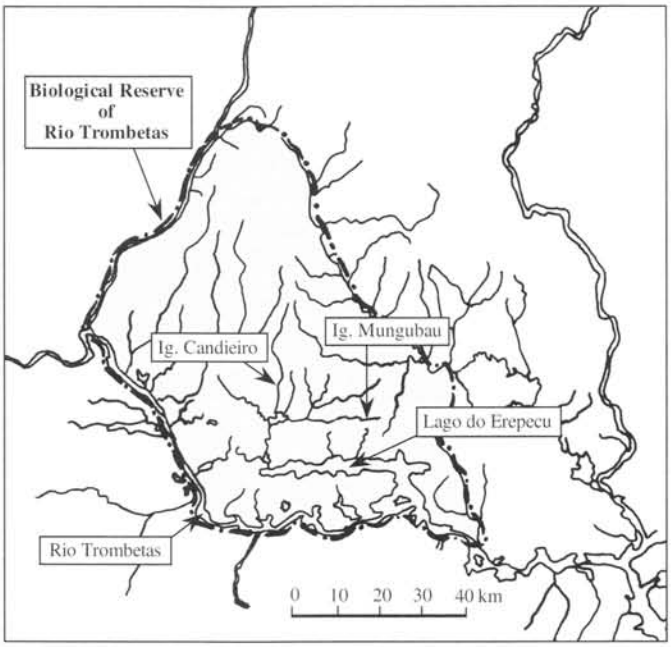

Figure 2. Map of the Biological Reserve of Rio Trombetas, Pará, Brazil (shaded, heavy dashed border, 385,000 ha). Nests of *Peltocephalus dumerilianus* were found along Igarapés Candieiro and Mungubau.

tal thermometer from 9 September until hatching. The thermocouples were permanently placed in the center of two of the nests at each site. Hatching was considered to occur when the egg developed longitudinal slits, not when the hatchling left the egg or the nest. Hatchlings were transferred to nest-specific plastic buckets immediately after pipping to prevent fly predation on the large amount of exposed vitelline yolk. Previous survivorship in 1992 was severely reduced by fly predation when the pipped eggs were left in the nests. Once the yolk had been absorbed the hatchlings were measured with a digital caliper to the nearest 0.1 mm and weighed with a digital electronic balance to the nearest 0.1 g and permanently and uniquely marked by injecting PIT tags (Avid) between the tail and carapace. Hatchlings from one clutch with high survivorship were euthanized with sodium phenobarbital after two months and sexed by gross examination of the gonads under a dissecting microscope (conducted independently by two persons). The ovary was elongated and cylindrical with a well developed Müllerian duct; the male gonad was shorter and medially thicker with gradually tapering ends and a less well developed Müllerian duct. Hatchlings were preserved in neutral buffered 10% formalin and deposited in the collection at INPA.

Results. — During 1992, 16 nests were collected from Igarapé Mungubau (mean clutch size=15; sd=6.24; range=3-25) and 19 nests from Igarapé Candieiro (mean clutch size=15.3; sd=4.64; range=7-23) for a total of 526 eggs. All nests occurred within 2 m of the shoreline in a mixture of leaves and soil. Two hundred eight eggs hatched after a mean of 90 days incubation; the low survivorship (39.5%) was attributed to fly predation. Twenty-one of these hatchlings died and 187 were maintained in captivity. After one year the surviving hatchlings had a mean mass of 47.2 g and mean carapace length of 68 mm (Table 1).

During 1993, three nests were marked on 13 August and three on 18 August, an additional five nests were located on 5 September and one on 6 September. The nest sites (n=11) varied from .1 to 2.5 m from the shoreline (mean=.98 m; sd=.641) and .3 to 1.8 m above the river level (mean=1.05 m; sd=.497). Nests were constructed in a variety of conditions: most commonly (n=6) they were found at bends in the river in exposed areas; two were on small (1-2 m in diameter) islands; one adjacent to, and under, a fallen log. Two were dug in termite nests and one was in an ant mound. Four were in clay soil, two in pure sand, and five in a mixture of sand

Table 1. Morphometrics (mean, one standard deviation, and range) of *Peltocephalus dumerilianus* hatchlings and yearlings. Measurements are total maximum straightline measurements in mm, except mass in g. CL=carapace length, CW=carapace width, CH=carapace height, PL=plastron length, FSL=femoral scute length, HW=head width.

	Hatchlings n=109	Yearlings n=187
Mass (g)	32.6 ± 2.2 (26.6-39.6)	47.2 ± 9.1 (27.2-90.3)
CL	56.1 ± 2.7 (44.6-60.5)	68.0 ± 4.4 (57.4-86.4)
CW	48.2 ± 1.5 (42.9-52.2)	57.8 ± 3.3 (48.8-69.9)
CH	24.9 ± 0.96 (22.9-27.3)	25.7 ± 1.8 (21.3-33.1)
PL	46.7 ± 1.5 (40.4-50.0)	56.3 ± 3.8 (46.1-69.6)
FSL	10.5 ± 1.1 (8.0-13.1)	12.5 ± 1.4 (9.1-17.8)
HW	14.9 ± 0.53 (13.6-16.8)	17.5 ± 0.85 (15.5-20.6)

and leaves. The top four cm or more of each nest was a mixture of leaves and humus. The opening of the nest cavity ranged from 12-14 cm in diameter (mean=12.5 cm; sd=0.91), the cavity was loosely plugged with leaves and soil, with no distinct hard plug as in the nests of *Phrynops geoffroanus*, which also nest in "tierra firme" in other regions of the Brazilian Amazon. The nest chambers were for the most part horizontally elliptical, with the eggs sometimes packed partially separated under both ends while in others the eggs were packed all together. Depth to the first egg ranged from 6 to 12 cm (mean=10.8; sd=1.8). Total nest depth varied from 12 to 24 cm (mean=16.9; sd=3.2).

Egg number varied from 12-24 (mean=18.1; sd=3.6). Freshly laid eggs were elliptical, brittle, and somewhat translucent. Eggs which were 18 days old were pliable and had a 25% increase in weight, presumably due to water absorption. This trend was apparent in one nest which was laid in clay soil. Nineteen of the eggs weighed 50 g each and were pliable with no soil clinging to the egg shell while one egg remained brittle, weighed 40 g, and had moist soil clinging to the shell (it was presumably dead at that time; it never developed). On 6 September eggs (n=142) ranged from 39 to 70.9 g (mean=51.7; sd=4.91), length varied from 50.2-59.0 mm (mean=54.2; sd=1.77), and width varied from 34.0-45.8 mm (mean=40.4; sd=2.12). The problem with these data is that some nests were recently laid while others were 18-23 days old. Measurements of 87 eggs from nests which were 1-2 days old weighed less, 39-50 g (mean=42.3; sd =3.12), and were narrower, 34-39 mm (mean=36.5; sd=1.11). The length of eggs, however, did not differ between the two groups, ranging from 50.2-58.5 mm (mean=54.6; sd=1.68). These same eggs were measured again 10 November and had a significant increase in mass with the mean change 8.17 g (sd=0.96; P=0.0000; t=-8.551) and the width had a mean increase of 3.54 mm (sd=0.357; P=0.000; t=-9.30). There was no significant change in egg length (sd=7.87; P=0.01; t=3.085).

Temperature in forest nests and exposed nests did not differ greatly (Table 2). Temperatures ranged between 23 and 33.7°C. Mean temperature at the coolest part of the day (0600 h) was 25.1°C in the forest and 25.3°C in the sun. Mean temperatures at 1800h were 29.03 and 29.42°C respectively.

Figure 3. *Peltocephalus dumerilianus* nesting habitat in rainforest under closed canopy of Igarapé Mungubau.

Table 2. Incubation temperatures (mean, one standard deviation, and range) in °C for two forest and two exposed artificial nests from 8 September - 18 December, the middle third of development when temperature is known to affect the sex-determination of species with temperature-dependent sex determination (TSD).

Hour	Forest	Exposed
0600	25.1 ± 0.92	25.3 ± 0.97
	(23.1-27.9)	(23.2-28.5)
1200	27.9 ± 1.71	27.9 ± 1.43
	(24.8-32.0)	(25.3-30.7)
1800	29.4 ± 1.87	29.7 ± 1.34
	(24.4-33.7)	(25.3-33.7)
2200	30.0 ± 1.65	29.7 ± 0.49
	(27.4-33.4)	(29.1-30.2)

Hatching began to occur after 124 days of incubation, with eggs from the earliest clutches hatching on 15 December, and all eggs having hatched by 15 January. The hatchlings (n=109) varied from 26.6 to 39.5 g (mean=32.6; sd=2.19) and had straight line carapace lengths of 44.6 to 60.5 mm (mean=56.1; sd= 2.74). Comparison of hatchling and yearling sizes are noted in Table 1. The caruncle was distinct at hatching and was gradually absorbed or worn down, not shed as suggested by Medem (1983). In one clutch where survivorship was high (14 of 16 eggs) the hatchlings were dissected to determine if incubation temperature was responsible for sex determination. There were nearly equal numbers of males (8) and females (6), not significantly different from 1:1, so it was not deemed prudent to sacrifice hatchlings from additional nests.

The carapace of the hatchlings was mottled brown with black sutures. The distal edges of the marginal scutes were fringed with cream-yellow; proximal to the yellow the outer third of the marginal was black. The plastron was yellow with brown mottling and the head and limbs were a uniform dark gray with the exception of a light yellow area above and anterior to the tympanum. A distinct keel was present on the third, fourth, and fifth vertebrals. The upper jaw was distinctly hooked, there was one chin barbel present, and the iris was brown with the conjunctiva black.

The hatchlings were highly aggressive even before complete emergence, and attempted to bite anything put in front of them. When grasped and particularly when they were injected with pit tags, they gave off a strong musky odor.

Discussion. — Clutch and egg size was similar to that found by R.A. Mittermeier (*pers. comm.*, cited in Pritchard and Trebbau, 1984) for *Peltocephalus dumerilianus* in the Rio Cuiuní, a tributary of the Rio Negro in Brazil (7-25 eggs). Measurements of 9 eggs from a clutch of 12 were 53.2-56.3 mm (mean=55.5) by 35.1-37.3 mm (mean=36.2). Medem (1983) reported similar nesting conditions on the Río Alto Tomo, Colombia, on 29-31 December 1980; these nests were found on sand beaches up to 12 m from the shoreline where the sand was mixed with leaf litter. Medem stated that Colombian clutches had 8-16 eggs, however, he

reported on data from only two complete natural nests of 12 and 13 eggs. His reported egg dimensions were within the range of those we measured in Brazil: 50-62 mm (mean=56.6; sd=3.96) by 35-43 mm (mean=38.5; sd=2.2) and weighed 36.5-51 g (mean=44.3; sd=5.09). Eggs from one clutch averaged 7 mm longer and 10 g heavier than those from the other. Twenty-eight eggs collected 5-7 December 1981 from four nests on the Río Tomo were incubated under artificial conditions. Nine hatched after 100-101 days under an ambient temperature regime of 27-32°C. The hatchlings were within the range of the ones we measured: 25-33 g (mean=30.6; sd=2.6) and 47-53 mm in carapace length (mean=51.2; sd=1.7). The most striking difference between the two studies was the difference in nesting season, this being three months later in Colombia than in Brazil.

Since the mean temperatures between the nests in the shade and sun were not significantly different (*t* test) there was no reason to sex hatchlings from both areas. The one clutch sexed from the shade was not significantly different from 1:1 (Fisher's exact test). Since these temperatures were low enough to produce 100% males in all other species of turtles studied it suggests that this species could have genetic sex determination (GSD). However, it is also possible that this species could have a low threshold temperature so it is necessary to incubate eggs at higher temperatures (31-32°C) to make certain that sex is genetically determined. It would not be surprising that sex may be determined genetically in this species, since it nests along forest streams, often under closed canopy, where temperatures are relatively low and not extremely variable. Many other neotropical species with extended incubation times (*Phrynops geoffroanus*, *Staurotypus triporcatus*, *Claudius angustatus*) also have genetic sex determination (Vogt, 1992; Vogt and Flores, 1992), so it would not be surprising to find GSD in this species as well. Before any massive transplantation of nests to protected areas is undertaken we plan to perform controlled incubation experiments under laboratory conditions at temperatures of 26, 30, and 32°C to be sure that we did not coincidentally use the threshold temperature in this species. Due to high predation of natural nests by humans, tegus (*Tupinambis*), and other natural predators, the success in artificial incubation, and the dispersed conditions of nesting impeding the guarding of natural nests, it is recommended that all located nests be removed and incubated under guarded conditions to enhance the eggs' survivorship. A long-term mark-recapture program of the hatchlings and yearlings released in 1994 may give insight as to the age at which hatchlings should be released. We are not recommending headstarting as a sole conservation measure, but are testing its feasibility by incubating eggs under protected conditions at natural temperatures and testing the survivorship of the hatchlings when released at different ages.

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Maximum Size and Clutch Size Records for Eastern Painted Turtles, *Chrysemys picta picta*, from Mid-Coastal Maine

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The eastern painted turtle, *Chrysemys picta picta* (Cryptodira: Emydidae) is a relatively small North American freshwater turtle. According to Conant and Collins (1991) adults of the subspecies have an average range of 115 to 152 mm carapace length, with a maximum recorded size of 181 mm. According to Ernst and Barbour (1972) clutch size varies from 2 to 11 eggs. The midland painted turtle, *C. p. marginata*, reaches an average range of 115 to 140 mm carapace length, with a maximum recorded size of 195 mm, and clutch size is 3 to 10 eggs (Conant and Collins, 1991; Ernst and Barbour, 1972). New England represents a zone of partial hybridization between these two subspecies (Conant