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Reproductive Biology and Conservation Status of the Loggerhead Sea Turtle (*Caretta caretta*) in Espírito Santo State, Brazil

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ABSTRACT. – The reproductive biology of loggerhead sea turtles nesting in Espírito Santo, Brazil, was evaluated for six nesting seasons (1991–92 through 1996–97), through data gathered by Projeto TAMAR-IBAMA, the Brazilian sea turtle conservation program. Mean curved carapace length of nesting females was 102.7 cm ($n = 198$). Mean clutch size for clutches with more than 50 eggs was 119.7 ($n = 3664$), and clutch size was significantly correlated with female body size. Management practices had significant effects on hatching success and incubation time. Mean hatching success of nests left *in situ* was 68.3% ($n = 879$), of undepredated nests left *in situ* was 79.9% ($n = 751$), and of nests moved to hatcheries and not depredated was 67.7% ($n = 2786$). For nests moved to hatcheries, hatching success declined significantly with increasing time interval between oviposition and transfer to the hatchery. Mean incubation time was 59.5 days for nests left *in situ* ($n = 572$) and 57.2 days for nests moved to hatcheries ($n = 2179$). Incubation time declined significantly throughout the nesting season as temperatures increased; we conclude that sex ratio of hatchlings also shifts to more females as the season progresses. There was significant annual variation for all parameters.

KEY WORDS. – Reptilia; Testudines; Cheloniidae; *Caretta caretta*; sea turtle; reproduction; nesting; conservation; management; Brazil

Five species of sea turtles nest in Brazil: loggerheads (*Caretta caretta*), green turtles (*Chelonia mydas*), hawksbills (*Eretmochelys imbricata*), olive ridleys (*Lepidochelys olivacea*), and leatherbacks (*Dermochelys coriacea*). The loggerhead accounts for about 80% of the nesting on Brazilian continental beaches. The beach along northern Espírito Santo State and the contiguous extreme southern Bahia State is the second largest nesting area for loggerheads in Brazil, next to the northern coast of Bahia State. In Espírito Santo State, apart from Trindade island, 1200 km offshore, which has a relatively large green turtle nesting colony (Moreira et al., 1995), loggerheads account for approximately 95% of sea turtle nesting.

Projeto TAMAR, the Brazilian sea turtle conservation program, is affiliated with IBAMA (Brazilian Institute of Environment and Renewable Natural Resources, a branch of the Brazilian government) and co-managed by Fundação Pró-TAMAR, an NGO. Projeto TAMAR began its activities in 1980 and now has 22 stations along the Brazilian coast and on oceanic islands, monitoring both nesting beaches and feeding areas. Projeto TAMAR started working in Espírito Santo State in 1982, initially at Comboios beach and gradually extending its activities in that state. Now, Projeto TAMAR has five stations in Espírito Santo, monitoring 194 km of nesting beaches. Besides beach monitoring, Projeto TAMAR conducts environmental conservation and educational activities with coastal communities.

This study focuses on loggerhead nesting biology in Espírito Santo State using data gathered from the year 1991–92 through the year 1996–97. We describe the spatial and temporal distribution of nests; evaluate annual variation in female body size, clutch size, incubation time, and hatching success; and assess the effect of management practices on incubation time and hatching success. Finally, a review of the conservation status of loggerheads in Espírito Santo State is presented.

METHODS

Study Area and Duration. — The study area is located on the north coast of Espírito Santo State, Brazil, runs in a north-south direction, and has a total length of 194 km between Barra do Riacho River (19°40'S) and Riacho Doce River (18°20'S) (Fig. 1). The area is divided into five sections, each monitored by a TAMAR station: Comboios (CB), Povoação (PV), Pontal do Ipiranga (PG), Guriri (GU), and Itaúnas (IA) (Fig. 1). The nearest other significant nesting beaches for loggerheads in Brazil are located 1000 km north in northern Bahia State and 400 km south in Atafona, Rio de Janeiro State.

According to Koeppen's classification (de Blij and Muller, 1993), the climate in the study area is predominantly "Aw," (i.e., tropical with hot and rainy summers), except for the northernmost beaches (Itaúnas and Conceição da Barra),

Figure 1. Map of the study area in Espírito Santo State, Brazil.

which have an "Am" climate (i.e., tropical with drier climate than "Aw"). In the coolest month (July), the mean air temperature is 21.2°C (mean minimum 17.1°C, mean maximum 25.5°C). In the warmest month (January), the mean air temperature is 25.4°C (mean minimum 21.5°C, mean maximum 29.7°C). Precipitation in the area is between 950 and 1380 mm/yr and is higher in summer than in winter. The coastline is covered by halophyllous-psammophyllous plant communities, composed mainly of *Mariscus pedunculatus*, *Panicum racemosum*, *Ipomoea pes-caprae*, *Ipomoea littoralis*, and *Blutaparom portucaloides* (Thomaz, 1991). Most of the beaches in the area, which is part of the Rio Doce coastal plains, are high energy beaches with steep profiles and coarse sand. Those in the northernmost area are lower energy beaches with finer sand.

The nesting season for loggerheads in Brazil is from September to March, and so each year is denoted by a two-year code, e.g., 1991–92. Although Projeto TAMAR began its activities in Espírito Santo in Comboios in 1982, the entire region has only been monitored since 1991. Therefore, only data from 1991–92 through 1996–97 will be presented here.

Monitoring Activities. — The goal was to leave every nest *in situ*. However, some nests were transplanted for several reasons: risk of beach erosion or tidal flooding, risk of human or animal predation, and difficulty or impossibility of completely monitoring the beach due to difficult access or limited financial resources. The northernmost 15 km of the beach monitored by the Comboios station is an Intensive Study Area (Marcovaldi and Laurent, 1996), where monitoring is carried out daily both at night and early in the morning by Projeto TAMAR technical personnel. In this area, which has been declared a biological reserve (Biological Reserve of Comboios), all nests are left *in situ*, except those threatened by beach erosion or tidal flooding. In other parts of the study area, called "conservation areas" (Marcovaldi and Laurent, 1996), monitoring is carried out early in the morning by TAMAR technical personnel or by local fishermen who are hired by TAMAR and work under the supervision of TAMAR's technical personnel. In these areas, some nests are kept *in situ*, but most clutches are transferred either to open-air beach hatcheries or, infrequently, to another spot on the beach if a problem occurs during transport of the clutch to the hatchery.

Hatcheries are designed to emulate natural conditions as closely as possible. Transferred eggs are moved and reburied carefully. For transferred nests, relocation time—the time interval between original oviposition and reburial—was classified as A (< 6 h), B (6–12 h), C (12 –24 h), or D (> 24 h).

All nests were excavated within 24 h after the majority of hatchlings had emerged. For *in situ* nests, the number of eggs was determined by counting egg shells, and the species was determined by examining dead or live hatchlings remaining in the nest. Clutch size could not be determined for *in situ* nests that were depredated. TAMAR's field methodology is described in detail by Marcovaldi and Laurent (1996).

The entire area was marked with stakes at each kilometer, and the location of each nest was recorded. The geographic location of the nests was not recorded at Campo Grande, a 12 km beach located between km 108 and 120 (306 nests in the six years).

Females encountered when nesting were double tagged on the front flippers with monel tags, and curved carapace length and width were recorded to ± 0.1 cm (Bolten, 1999). Due to the extent of the beaches and limited resources for night patrolling, not all nesting females were intercepted on the beach.

Data Analysis. — Data were collected for 3898 clutches. Data from 235 clutches (6.0%) were excluded from the analyses of clutch size, hatching success of undepredated nests, and incubation time, although they were included in the total number of nests in the area (Table 1, Figs. 2 and 3). These clutches were excluded from the above analyses for four reasons: partial or total depredation (n = 144), unrecorded clutch size (n = 18), unrecorded placement of nest (*in situ*, hatchery, or transferred to another spot on the beach; n = 1), or clutches that had fewer than 50 eggs (n = 72). Clutches with fewer than 50 eggs were excluded because these nests may have lost eggs to unrecorded predation events or oviposition may have been interrupted by activities of people or animals on the beach. The 50-egg minimum was chosen based on visual inspection of clutch size distribution (Fig. 4) and is consistent with the minimum clutch size recorded for other Atlantic loggerhead populations (Dodd, 1988).

Hatching success is the percentage of eggs that produced live hatchlings, including those hatchlings unable to

Table 1. For each year, number of clutches, clutch size (for clutches with at least 50 eggs), hatching success of undepredated nests, incubation time, and curved carapace length (CL) of nesting loggerheads at Espírito Santo, Brazil. Values are mean ± standard deviation, range, and sample size. Means in a row with the same letter superscript are not significantly different (ANOVA, $\alpha = 0.05$, Tukey post hoc test).

	Year						
	91-92	92-93	93-94	94-95	95-96	96-97	All
No. of clutches	520	749	589	625	735	680	3898
Clutch size	123.2^{a}	120.1^{ab}	118.2^{bc}	121.3^{ab}	119.7^{ac}	116.3^{c}	119.7
	± 22.6	± 22.4	± 22.4	± 23.4	± 22.8	± 21.9	± 22.7
	57-177	50-175	52-184	54-214	54-173	55-178	50-214
	n = 491	*n* = 693	*n* = 546	*n* = 604	*n* = 693	*n* = 637	*n* = 3664
Hatching success of	78.1^{ab}	76.2^{b}	76.9^{b}	79.4^{ab}	81.1^{ab}	84.2^{a}	79.9
undepredated nests (%)	± 22.9	± 22.7	± 23.3	± 19.2	± 17.8	± 16.3	± 20.0
In situ	0-98.0	0-98.1	0-100	0-100	2.4-100	0-100	0-100
	n = 69	*n* = 101	*n* = 103	*n* = 143	*n* = 190	*n* = 145	*n* = 751
Hatchery	73.0^{a}	63.9^{b}	67.5^{bc}	66.0^{bc}	67.6^{bc}	70.0^{ac}	67.7
	± 22.6	± 26.1	± 27.5	± 25.1	± 22.5	± 21.4	± 24.6
	0-100	0-99.3	0-100	0-100	0-98.1	0-100	0-100
	n = 422	*n* = 592	*n* = 443	*n* = 450	*n* = 449	*n* = 430	*n* = 2786
Incubation time (days)		62.8^{a}	57.2^{b}	58.7^{bc}	59.8^{c}	60.2^{c}	59.5
In situ		± 8.1	± 4.1	± 6.3	± 5.5	± 6.2	± 6.2
		51-96	49-68	45-76	45-74	45-80	45-96
		n = 66	*n* = 93	*n* = 134	*n* = 175	*n* = 104	*n* = 572
Hatchery		59.0^{a}	55.9^{b}	56.4^{bc}	56.8^{bc}	57.4^{c}	57.2
		± 7.0	± 3.9	± 6.1	± 4.7	± 6.2	± 5.8
		41-103	40-74	44-74	46-71	48-84	40-103
		n = 509	*n* = 426	*n* = 425	*n* = 420	*n* = 399	*n* = 2179
CL (cm)	104.4^{a}	105.0^{a}	102.5^{ab}	102.4^{ab}	100.8^{b}	102.5^{ab}	102.7
	± 4.6	± 6.0	± 4.1	± 5.0	± 5.6	± 3.7	± 5.3
	95-116	95-123	96-109	92-120	83-114	97-108	83-123
	n = 27	*n* = 34	*n* = 25	*n* = 53	*n* = 49	*n* = 10	*n* = 198

leave the nest. Hatching success was arcsin transformed for the statistical analyses (Zar, 1996). To evaluate effect of management practices, hatching success was analyzed in this paper for undepredated nests with 50 or more eggs under two of the three management practices—nests left *in situ* and nests transferred to open-air beach hatcheries (*n* = 3537, or 90.7% of the total number of nests). Hatching success was not analyzed for nests transferred to another spot on the beach (*n* = 126 clutches with 50 or more eggs); this management practice has only been employed since 1994–95. Hatching success of depredated and undepredated *in situ* nests was also calculated, assuming hatching success of depredated nests was 0%.

Incubation time was calculated as the number of days between oviposition and emergence of the first hatchlings. Incubation time was only analyzed for 1992–93 through 1996–97 because the staff at station Pontal do Ipiranga did not record incubation times for nests left *in situ* in 1991–92 and for undepredated nests with 50 or more eggs under the two management practices—*in situ* nests and open-air beach hatcheries. Some nests in other years were excluded from the analyses because dates of either nesting or hatchling emergence were not recorded. In all, 2751 nests were included in the analyses of incubation time. To evaluate the relationship between incubation time and nesting date, July 1 was set as day 1 of the nesting season.

A total of 267 curved carapace length (CL) measurements was obtained in 1991–92 through 1996–97. Only the first CL measurement of each turtle in each year (*n* = 198) was used in ANOVA of CL among years. Note that remigrants are included in this analysis in more than one year. When analyzing the dependence of clutch size on CL, all CL measurements were included (provided that clutch size was greater than 50 eggs).

In the statistical analyses, alpha = 0.05. Analyses of variance were followed by Tukey post hoc tests (Zar, 1996). All statistical analyses were carried out with the software Systat 7.0 (SPSS Inc., 1997).

RESULTS AND DISCUSSION

Spatial and Temporal Distribution of Nests. — From 1991–92 through 1996–97, 3898 nests were recorded with a range of 520 to 749 nests each year (Table 1). Nesting was more dense in the southern half of the study area (Fig. 2), in the area of the Comboios and Povoação stations, especially around the mouth of the Rio Doce at km 37.

Nesting activity (Fig. 3) was greatest in November with 89.4% of the clutches deposited between October and December. This temporal distribution is quite similar to the temporal distribution of loggerheads nesting at Praia do Forte, Bahia, Brazil (Marcovaldi and Laurent, 1996).

Clutch Size. — The mean (119.7) and range (50-214) of clutch size for clutches with 50 or more eggs (*n* = 3664; Table 1; Fig. 4) are within the range of reported values for other Atlantic loggerhead populations (Dodd, 1988) except for

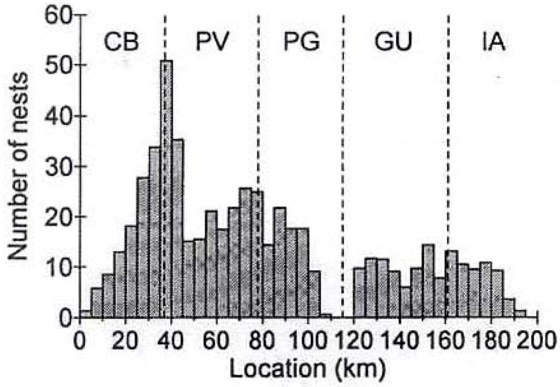

Figure 2. Geographic distribution of loggerhead nests (n = 3336) in Espírito Santo State from south to north for years 1991–92 through 1996–97 expressed as average number of nests per 5 km of beach per year. Exact position was not recorded for 306 nests (average 51.0/year) laid on a 12-km beach located around km 110. Dashed vertical lines indicate the boundaries of each station.

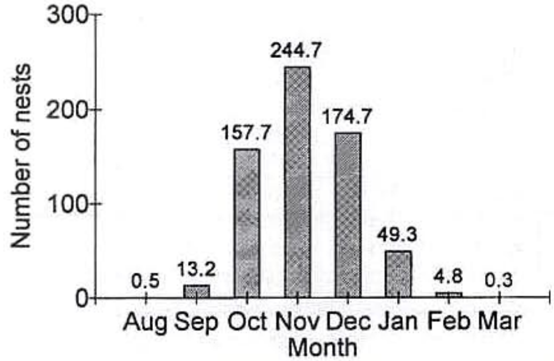

Figure 3. Mean number of loggerhead nests per month in Espírito Santo State for years 1991–92 through 1996–97 (n = 3871).

one exceptionally large clutch (see Fig. 5; value was reconfirmed) of 214 eggs that exceeds reported values. Mean clutch size of loggerheads nesting in Bahia State has been reported as 126.7 (n = 1921, SD = 25.0; Marcovaldi and Laurent, 1996) and 130.5 (n = 28, SD = 20.2; Tiwari and Bjorndal, 2000).

Mean clutch size was significantly different among years (ANOVA, F = 6.396, $p < 0.001$, Table 1). Although poaching has been nearly eliminated in Espírito Santo State, partial poaching of eggs at a low level cannot be ruled out and may explain, at least in part, the differences observed among the years. Other variables, such as nesting date, could influence clutch size. The number of eggs per nest decreased as the season progressed (n = 3639, F = 84.36, $p < 0.001$), but nest date only explained 2.3% of the variation in clutch size (Fig. 5), so the relationship is quite weak. In a 19-year study of nesting loggerheads on Little Cumberland Island, Georgia, USA (Frazer and Richardson, 1985a,b), mean clutch size was significantly different only for the year with the largest mean clutch size (127.5 eggs) and the smallest mean clutch size (114.4 eggs), and mean clutch size was significantly smaller in the last month of the season compared with earlier in the season.

Hatching Success. — From 1991–92 to 1996–97, 3537 clutches that were either left *in situ* or transferred to the

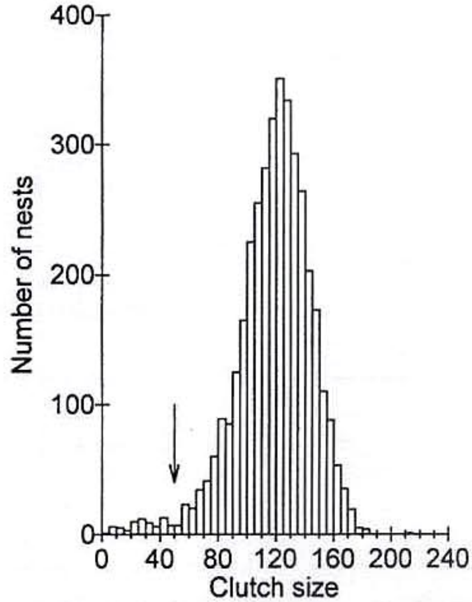

Figure 4. Clutch size distribution for undepredated loggerhead nests in Espírito Santo State for years 1991–92 through 1996–97 (n = 3736). Arrow marks the cut-off point (50 eggs), below which clutches were excluded from statistical analyses.

beach hatchery were monitored for hatching success (Table 1). Mean hatching success of *in situ* nests was 68.3% (range 0–100, n = 879) based on 0% hatching success for depredated nests. There was no interaction between management practice and year (two-way ANOVA, F = 1.875, $p = 0.095$) for hatching success. However, mean hatching success was significantly different among years for both undepredated *in situ* nests and transferred nests (one-way ANOVA, F = 9.970, $p < 0.001$) and among management practices (one-way ANOVA, F = 166.2, $p < 0.001$). Mean hatching success of undepredated *in situ* nests (79.9%) was higher than that of nests transferred to the beach hatchery (67.7%), which was similar to hatching success of all *in situ* nests (68.3%), although mean hatching success varied significantly among years.

Hatching success of loggerhead clutches in Bahia State was 73.1% for *in situ* nests and 63.2% for hatchery nests

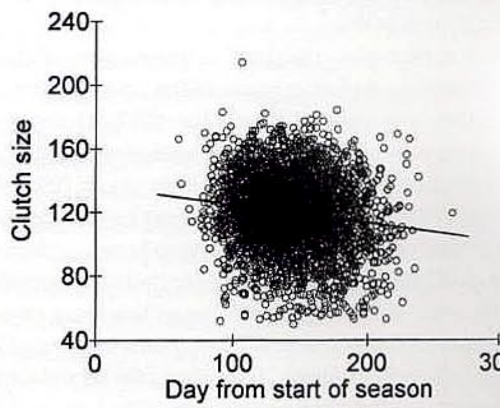

Figure 5. Clutch size of loggerhead nests (n = 3639) by day of oviposition (day 1 = July 1) in Espírito Santo State for years 1991–92 through 1996–97. The line represents a linear regression y = 136.88 - 0.1198x.

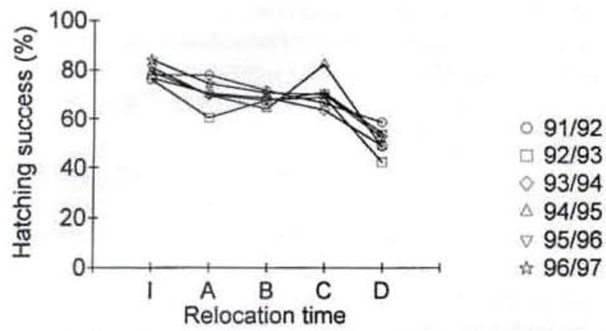

Figure 6. Mean hatching success of loggerhead nests ($n = 3490$) by relocation time in Espírito Santo State for years 1991–92 through 1996–97. I = *in situ* nests (not transferred, no relocation time); relocation times = A < 6 h, B = 6–12 h, C = 12–24 h, D > 24 h.

(Marcovaldi and Laurent, 1996). *In situ* nests in Bahia were protected from predators by screening the nests in areas of high predation. The values from Bahia (Marcovaldi and Laurent, 1996) are lower than those reported for Espírito Santo State, but have the same relative relation between *in situ* and hatchery nests.

Hatching success of transferred nests is affected by movement or rotation of eggs during relocation, and the effect of egg movement increases greatly with time after oviposition (Limpus et al., 1979; Miller, 1997). For hatching success, there is a significant interaction between relocation time (time elapsed between oviposition and reburial) and year (two-way ANOVA, $n = 3490$, $F = 2.494$, $p < 0.001$, Fig. 6). A comparison of hatching success of undepredated *in situ* nests and transferred nests by relocation time by year (Fig. 6) indicates that relocation time can, at least in part, explain the observed differences in mean hatching success between *in situ* and transferred nests (Table 1).

Previous observations have suggested that the finer sand in northern Espírito Santo beaches improved the hatching success of loggerhead nests. To evaluate this hypothesis, we examined the relationship between hatching success for undepredated *in situ* nests and geographic location (Fig. 7). A significant, positive relationship was found (linear regression, $n = 740$, $F = 5.670$, $p = 0.0172$, $r^2 = 0.008$), but geographic location accounted for less than 1% of the variation in hatching success. Thus, for *in situ* nests, there was not a biologically significant geographic trend in hatching success, and our results do not support the hypothesis.

Incubation Time. — Incubation times were analyzed for 2751 clutches (1992–93 through 1996–97). Incubation time of nests in Espírito Santo State exhibited great variability (Table 1). The range of incubation times for *in situ* nests (45–96 days) was greater than the range reported for other loggerhead populations (Dodd, 1988). For incubation time, there was no interaction between years and management practices (two-way ANOVA, $F = 1.744$, $p = 0.138$). Mean incubation time varied significantly among years (one-way ANOVA, $F = 23.61$, $p < 0.001$) and between management practices (one-way ANOVA, $F = 72.25$, $p < 0.001$). Mean incubation time of *in situ* nests was 2 to 3 days longer than that of transferred nests (Table 1). A similar relationship, although with a smaller difference of only 0.5 day, was reported for *in situ* and hatchery nests in Bahia State (Marcovaldi and Laurent, 1996).

Incubation time of nests (*in situ* and hatchery nests combined) decreased as the season progressed (linear regression, $n = 2751$, $F = 3878.3$, $p < 0.001$, $r^2 = 0.59$, Fig. 8). The horizontal line in Fig. 8 indicates the estimated pivotal incubation time (i.e., the incubation time of nests with a 1:1 sex ratio) for loggerheads in Brazil (59.3 days; Marcovaldi et al., 1997). Thus, we suggest that the sex ratio of hatchlings also changes during the season, with more males produced during the early season when incubation times are longer as a result of lower temperatures. The effect of management practices on the sex ratio of hatchlings produced in Espírito Santo State is now being investigated.

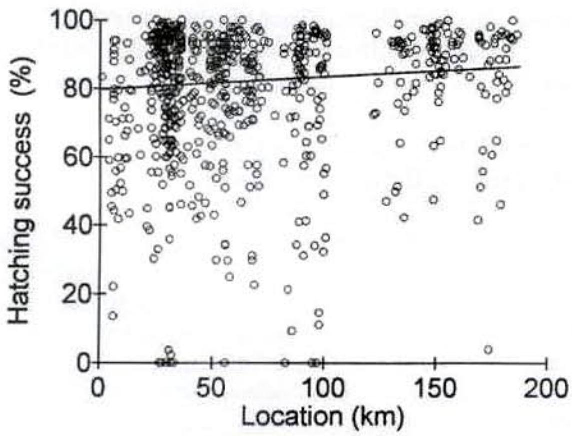

Figure 7. Hatching success of undepredated *in situ* loggerhead nests ($n = 740$) by geographic location in Espírito Santo State from south to north for years 1991–92 through 1996–97. The solid curve (nearly a straight line) represents the linear regression $y' = 1.105507 + 0.00048702x$ (where y' is arcsin-transformed hatching success and x is geographic location) transformed back to the hatching success scale.

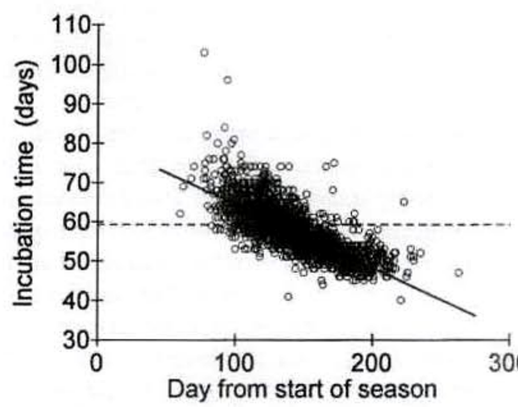

Figure 8. Incubation time of loggerhead nests ($n = 2751$) by day of oviposition (day 1 = July 1) in Espírito Santo State for years 1992–93 through 1996–97. The dashed horizontal line represents the estimated pivotal incubation time (59.3 days, Marcovaldi et al., 1997). The solid line represents a linear regression $y = 80.65 - 0.1618x$.

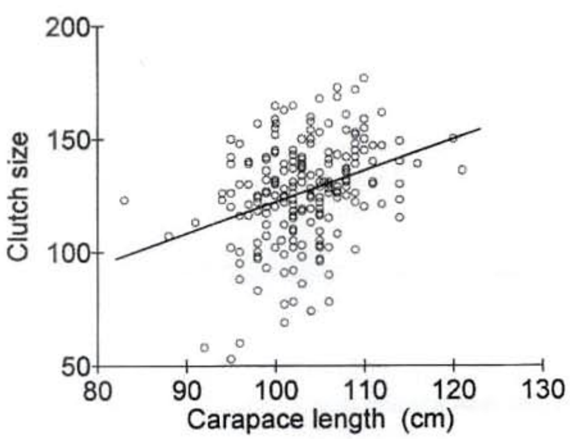

Figure 9. Clutch size by curved carapace length for adult female loggerheads ($n = 210$; first measurement for each turtle in each year) in Espírito Santo State for years 1991–92 through 1996–97. The line represents a linear regression $y = 1.3988x - 17.70$.

No relationship was found between incubation time of *in situ* nests and geographic location (linear regression, $n = 566$, $F = 0.692$, $p = 0.406$). However, farther north in Bahia State, mean incubation time for *in situ* loggerhead nests is 53.2 days ($n = 432$, SD = 4.3; Marcovaldi and Laurent, 1996), a significantly shorter interval than the mean of 59.5 days for Espírito Santo State (t-test, df = 1002, $t = 18.1$, $p < 0.001$).

Carapace Length. — Including only the first measurement of each turtle in each year, we have a total of 198 CL measurements between 1991–92 and 1996–97 (Table 1). The mean CL, although within the range of reported mean values for other Atlantic loggerhead populations, is greater than that of most populations, and the maximum CL, 123 cm, is very close to the maximum CL reported for other Atlantic populations (124 cm, in Florida, USA) (Dodd, 1988). The mean CL calculated for loggerheads nesting in Espírito Santo State is similar to the mean values reported for loggerheads nesting in Bahia State, Brazil (102.8 cm, $n = 176$, Marcovaldi and Laurent, 1996; 101.2 cm, $n = 29$, Tiwari, 1998). Mean CL varied significantly among years (one-way ANOVA, $n = 198$, $F = 3.311$, $p = 0.007$). No significant difference in mean CL among years was found for loggerheads nesting in Australia (Limpus, 1985).

A positive relationship was found between clutch size and female CL in our study ($n = 210$, $F = 26.07$, $p < 0.001$, $r^2 = 0.111$), but it only accounted for 11.1% of the variation (Fig. 9). A significant, positive relationship between clutch size and body size has been reported for a number of marine turtle populations (Hirth, 1980; Van Buskirk and Crowder, 1994), although other studies have not found such a relationship (Ehrhart, 1982). Female CL accounted for 30% ($n = 77$) of the variation in clutch size in loggerheads nesting on Little Cumberland Island, Georgia (Frazer and Richardson, 1986), and for 40% ($n = 48$) and 19% ($n = 27$) for loggerheads nesting in Florida, USA, and Bahia, Brazil, respectively (Tiwari and Bjorndal, 2000).

Conservation Status. — Several management strategies can be employed in conservation programs for sea turtles, some of which have already been tested and recommended. Any conservation program should take local conditions into account and should include local inhabitants, if any, in their formulation and execution (Marine Turtle Specialist Group, 1995).

In the case of Projeto TAMAR, the commitment of local people who in former times used sea turtles for commerce or subsistence provided a good basis for the relationship between our conservation program and the communities located in the nesting areas in Espírito Santo State. As our program has developed, the knowledge we have obtained of the habits of the local people and their socio-economic condition has allowed us to develop management alternatives that have improved the socio-economic and cultural status of local people. These developments have also been positive for the sea turtles, which, as "flagship species," contribute to the conservation of coastal and marine environments. In Espírito Santo State, commerce in sea turtle products has ceased, the number of nests has been relatively stable among years, there is an increasing trend in the proportion of nests that have been left *in situ*, and a decreasing numbers of nests are harvested by humans. The activities of Projeto TAMAR also increase the potential for ecotourism in the area, as nesting turtles are attractive to tourists.

Relocation of clutches to protected hatcheries is a common management practice (Marcovaldi and Laurent, 1996). However, Projeto TAMAR has attempted to leave as many nests as possible *in situ* to avoid possible effects on hatching success and natural sex ratio of hatchlings.

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