Insect Infestation of Hawksbill Sea Turtle Eggs in Rio Grande do Norte, Brazil

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Insect Infestation of Hawksbill Sea Turtle Eggs in Rio Grande do Norte, Brazil

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ABSTRACT. – We describe infestation of hawksbill turtle (*Eretmochelys imbricata*) nests by insects on Pipa beach in the municipality of Tibaudo Sul, Rio Grande do Norte, Brazil in January and June 2011. The mean number of live hatchlings (83.96 ± 43.31) was higher in nests unassociated with vegetation, although it is important to consider that a number of parameters other than proximity to vegetation may also affect nest success.

Resumo. – Neste trabalho, foi descrito o fenômeno de infestação por agentes da entomofauna em ninhos da tartaruga-de-pente (*Eretmochelys imbricata*) na praia de Pipa no município de Tibau do Sul, Rio Grande do Norte, Brasil; no período de janeiro a junho de 2011. Os valores médios de filhotes vivos por ninho (83,96 ± 43,31) demonstram que os ninhos não associados à vegetação apresentaram uma média mais alta de filhotes vivos, porém, é preciso considerar que existem diversos outros parâmetros que predispõem o sucesso ou insucesso dos ninhos.

The Brazilian state of Rio Grande do Norte is an important nesting area for the hawksbill sea turtle, *Eretmochelys imbricata* (Linnaeus 1766), which nests in the area from September to March (Marcovaldi et al. 2007). The TAMAR Project (Tartarugas Marinhas, the Sea Turtle Project), affiliated with the Chico Mendes Institute for Biodiversity (ICMBio, a Brazilian government agency for environmental protection), patrols 42 km of coastal beaches of which 9 km are located in the municipality of Tibau do Sul within an area designated as an intensive monitoring zone. A total of 813 nests were located on Pipa beach between the 2001–2002 and 2005–2006 nesting seasons (Marcovaldi et al. 2007), most of which were nests of *E. imbricata*.

The hawksbill turtle is listed as critically endangered, both in Brazil (Ministério do Meio Ambiente 2008) and worldwide (International Union for Conservation of Nature [IUCN] 2012). This reinforces the need to understand the life cycle of this species, in particular the ecological conditions that determine its embryonic development (nests), to inform conservation planning (Katilmis et al. 2006). During incubation, the eggs of sea turtles are vulnerable to a diversity of predators including armadillos (*Dasypus novemcinctus*) (Drennen et al. 1989), crocodiles (*Crocodylus porosus*) (Whiting and Whiting 2011), mongooses (*Herpestes javanicus*) (Leighton et al. 2011), and foxes (*Vulpes vulpes*) (Yerli et al. 1997) as well as to infestation by invertebrates such as insects of the orders Orthoptera (Maros et al. 2005), Diptera (Vogt 1981; Broderick and Hancock 1997; McGowan et al. 2001; Hall and Parmenter 2006; Katilmis et al. 2006; Bolton et al. 2008), Hymenoptera (Parris et al. 2002), and Coleoptera (Donlan et al. 2004; Katilmis et al. 2006). During egg laying, cloacal excretions as well as the eggs themselves may attract many insects (Bolton et al. 2008). Turtle nests may be attacked by insects at a number of different stages in their development (Broderick and Hancock 1997).

There are few data on the factors that lead to nest infestation; however, Ozdemir et al. (2004) identified the granulometry of the sand and the distance of the nest from the nearest vegetation as the primary factors correlated (negatively) with the presence of invertebrates. Given the urgent need for data on the natural history of the marine turtles of the South Atlantic, the present study investigated the infestation of *E. imbricata* nests by insects in the study area on Pipa beach in Tibau do Sul, Rio Grande do Norte, Brazil.

Study Area. — The TAMAR Project's intensive monitoring zone in Rio Grande do Norte extends for 42 km along the coast of the state, of which 9 km are located in the municipality of Tibau do Sul, located 80 km south of the state capital, Natal. The present study was conducted in the northern extreme of this zone on the beaches of Cacimbinhas, Madeiro, and Baía dos Golfinhos (Fig. 1), which extend along the coast for 5.9 km from lat 6°11′17″S, long 35°05′01″W in the north to lat 6°13′38″S, long 35°03′23″W in the south. The landscape is composed of cliffs, interspersed with dunes which back up a narrow beach (Santos et al. 2010).

Methods. — The study was conducted during the 2010–2011 breeding season, between January and June, simultaneousy with surveys conducted by the TAMAR Project. The nests were initially located and identified by Project team members and, only after the confirmation of hatchling emergence (~ 2–10 d), each nest was excavated to determine infestation status and hatching success.

Upon excavation, nest material was sorted to separate the empty shells (representing live, emerged hatchlings), unhatched eggs, and dead hatchlings. We determined the total number of eggs per nest when possible. The unhatched eggs and dead hatchlings were collected together with the insects found in the nest. In cases where infestation was found, the developmental stage of the insects was recorded. Insect infestation was recorded in terms of presence–absence within a nest, not per egg. Specimens recovered from nests were examined with a microscope and identified to the lowest taxonomic unit possible based on appropriate identification keys.

Insect specimens were collected, counted, recorded, and placed in plastic bags labeled with the nest code number. Adult insects found in the nests were preserved in 70% alcohol for identification. Larvae and pupae were placed in well-ventilated glass jars with abundant food and were reared until the adult stage for taxonomic identification. During the examination of each nest we measured nest depth (DN) and distance to the nearest vegetation (DV) with a surveyor's tape. When a nest was located within the vegetation, DV was considered to be negative.

For statistical comparisons among beaches, Baía dos Golfinhos was excluded due to the small number of nests ($n = 3$) found at this location during the study period in comparison with Cacimbinhas ($n = 30$) and Madeiro ($n = 19$). Following confirmation of the normality of the data (Kolmogorov-Smirnov test and Shapiro-Wilk W test), Cacimbinhas and Madeiro beaches were compared in relation to the mean number of eggs per nest and the mean incubation period using a Student t-test. The variation in the number of live hatchlings in relation to the distance of the nest from vegetation (within vegetation, at the vegetation line, and in the open sand) was tested using 1-way analysis of variance (ANOVA). The potential

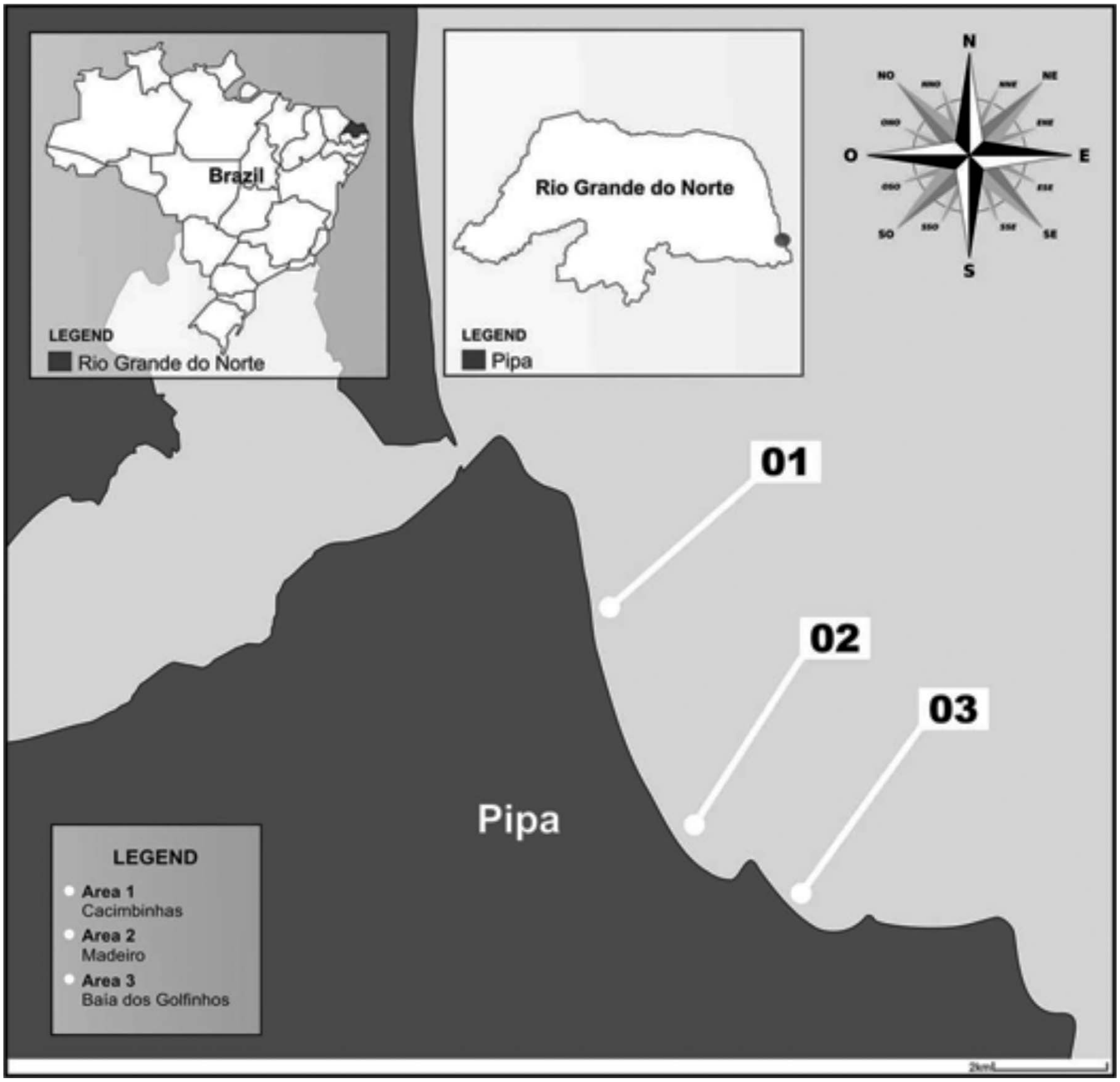

Figure 1. Map showing Cacimbinhas (01), Madeiro (02), and Baía dos Golfinhos (03) beaches in the municipality of Tibau do Sul, Rio Grande do Norte, Brazil.

relationship between survival rate and hatching time, and between survival rate and nest depth, were evaluated using Pearson's correlation coefficient. The analyses were run in STATISTICA® v. 4.0, and an $\alpha = 0.05$ significance level was considered for all tests.

Results. — Between January and June 2011, 52 hatched nests of *E. imbricata* were investigated. Overall, 24 (46.15%) of the nests presented signs of infestation by insects, and infested nests were observed on all the beaches (Fig. 2). Diptera was the most-common taxon and the infestation of *E. imbricata* nests by dipterans was most prevalent in unhatched eggs and dead hatchlings. Larvae and adults specimens were also encountered (Fig. 3A–C). The taxonomic orders of insects found represents the families Tachinidae (Fig. 3B), Piophilidae (Fig. 3C), Sarcophagidae, Calliphoridae (Fig. 3D), and Phoridae (Fig. 3E–F). The phorids were the most abundant of these taxa. The families Formicidae (Order: Hymenoptera; Fig. 3G–H) and Staphylinidae (Order: Coleoptera; Fig. 3I) were also recorded, although the staphylinids were only found in the sand above the nests and did not appear to have been feeding on any part of their contents. Damaged eggs, recent hatchlings, and viable hatchlings attacked by ants were also found within the nests. No significant difference was found between Cacimbinhas and Madeiro beaches in the number of eggs per nest, incubation period, or infestation rate (Table 1). Because infestation reduces hatchling viability, the number of live hatchlings was considered to be an indicator of favorable environmental conditions, thus representing reproductive success.

Our data show a tendency for infestation to be greatest in nests closest to vegetation (Fig. 2A–B), and hatching success (83.96 ± 43.31 SD) was greater in the nests located away from vegetation (Fig. 2C). However, there was no significant difference in infestation rates ($F = 1.68$; df = 2; $p > 0.05$) in the 3 different beach sections (within the vegetation, at the vegetation line, and in the open sand; Fig. 4). No correlation was found between incubation period and

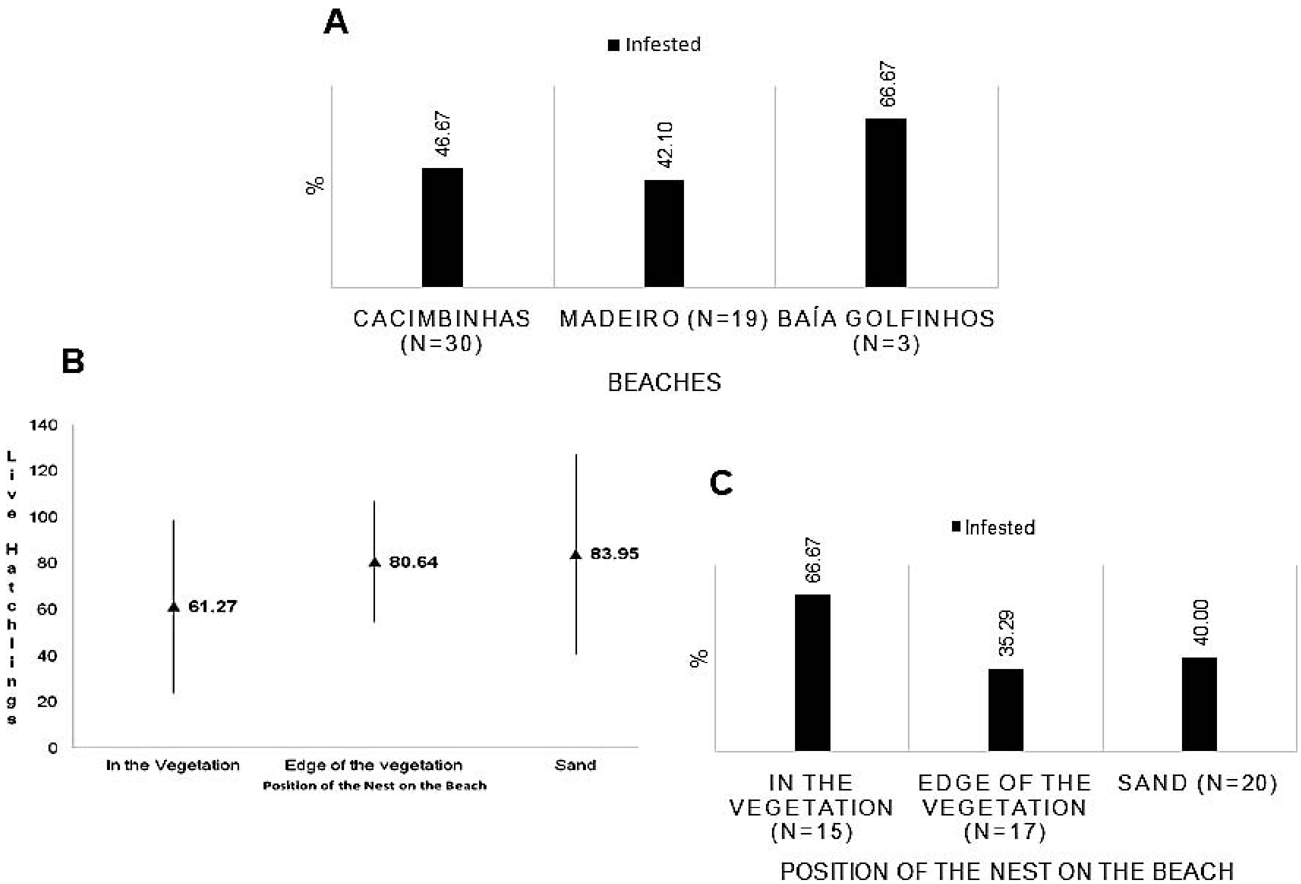

Figure 2. (A) Proportion of infested and uninfested *Eretmochelys imbricata* nests on the 3 study beaches in Rio Grande do Norte, Brazil, in 2011. (B) Mean (± SD) number of live *Eretmochelys imbricata* hatchlings found in nests in the different sectors of the study beaches in Pipa, Rio Grande do Norte, Brazil, in 2011. (C) Proportion of infested and uninfested *Eretmochelys imbricata* nests in the different sectors of the study beaches in Pipa, Rio Grande do Norte, Brazil, in 2011.

survival ($r = 0.01$; $p > 0.05$) nor between nest depth and the survival of the eggs ($r = 0.18$; $p > 0.05$).

Discussion. — The presence of dipterans has been recorded in the nests of a number of species of marine turtles (Fowler 1979; Lopes 1982; Bjorndal et al. 1985; Andrade et al. 1992; Broderick and Hancock 1997) and freshwater turtles (Vogt 1981; Iverson and Perry 1994; Bolton et al. 2008). Broderick and Hancock (1997) found dipterans in the nests of *Chelonia mydas* and *Caretta caretta* on the island of Cyprus in the eastern Mediterranean. Lopes (1982) investigated the damage caused by these invertebrates to *C. mydas* nests on the Pacific coast of Mexico. Bjorndal et al. (1985) found sarcophagid and phorid larvae in *E. imbricata* nests in Costa Rica. Bolton et al. (2008) concluded that variation in nest temperatures and associated physiological and ontogenetic differences may result in asynchronous incubation and that the chemical signals associated with early hatching may attract adult flies. The evidence was inconclusive on the role of the fly larvae in this context, and it remains unclear whether larvae gather necrotic material from the nest or actually prey on the eggs and hatchlings.

Hall and Parmenter (2006) have suggested that dipteran larvae preferentially infest dead embryos, but they may also attack hatchlings that are emerging from their eggs. These authors also concluded that the dipterans cause minimal damage to the nests, although Legler (1971) observed dipteran larvae attacking viable hatchlings of the freshwater species *Pseudemys scripta*. Lopes (1982) reported that sarcophagids caused a 30% reduction in hatching success in *C. mydas* nests on the east coast of Mexico. In the present study, the infestation of *E. imbricata* nests by dipterans was most prevalent in unhatched eggs and stillborn hatchlings.

Recently hatched turtles may be especially vulnerable to attacks from ants (Parris et al. 2002), given the time it takes them to emerge from the nest and reach the sea. Even when these attacks are not fatal, they may cause irreparable damage to the flippers or the head, in particular the eyes, with the animals often being left blind. The exact timing of the ant attacks remains unclear, given that Parris et al. (2002) found evidence of the presence of these insects at all stages of development.

In the present study, there was clear evidence of attacks by ants on viable hatchlings in addition to stillborn animals and damaged eggs. In some cases, entire colonies of ants were observed in *E. imbricata* nests, with some individuals guarding larvae. In one nest infested with ants on Cacimbinhas beach, no live hatchlings were found. In this nest, the ants were observed attacking living

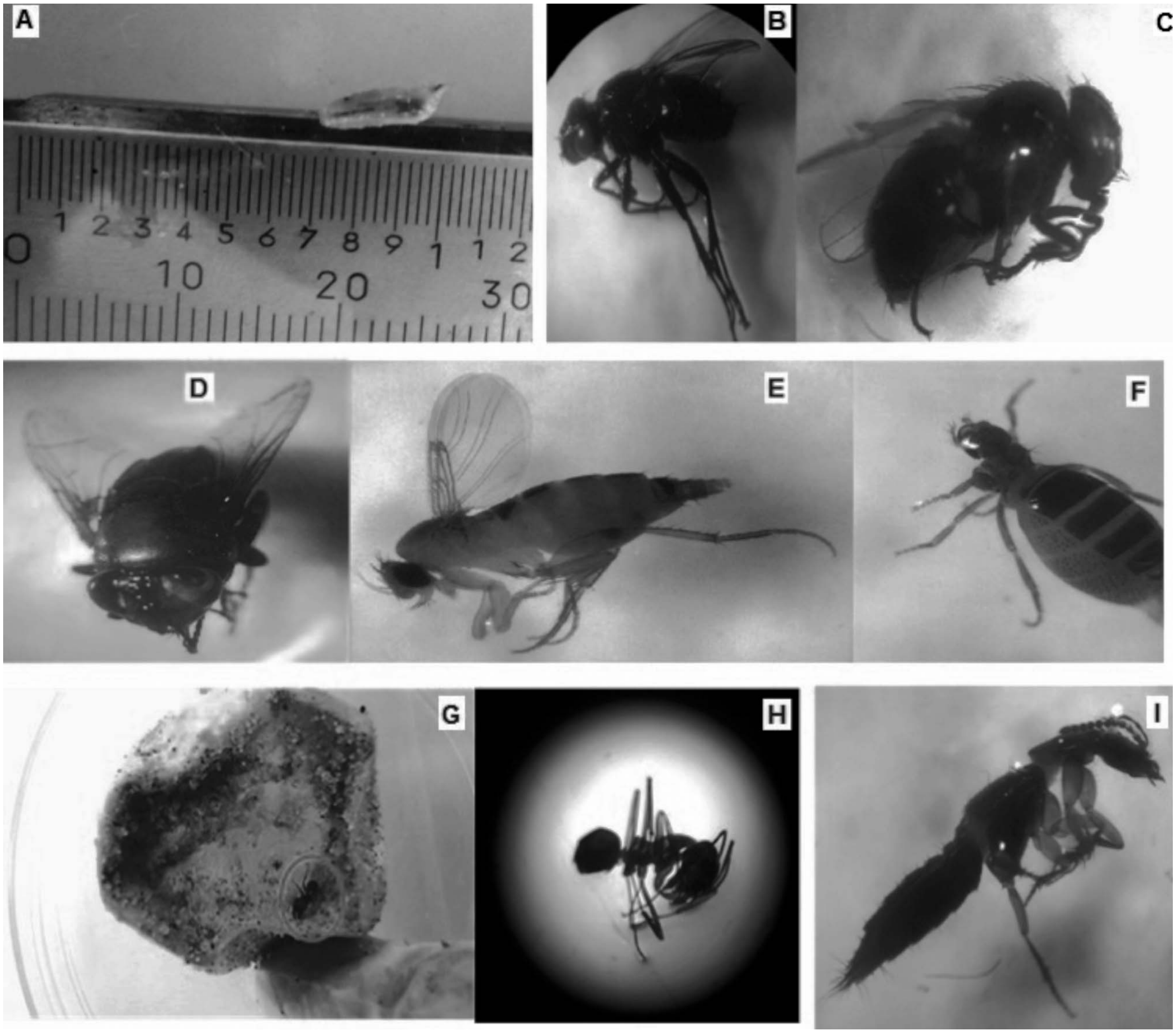

Figure 3. (A) Dipteran larva found in an *Eretmochelys imbricata* nest. Adult flies of the families (B) Tachinidae and (C) Piophilidae found in the nests of *Eretmochelys imbricata*. Adult flies of the families (D) Calliphoridae and (E–F) Phoridae found in the nests of *Eretmochelys imbricata*. Ant found in a damaged *Eretmochelys imbricata* egg (G) in situ and (H) under the microscope. (I) Beetle of the family Staphylinidae found in the nests of *Eretmochelys imbricata*. Photos by Paula Fonseca da Silva, 2011 and Márcio Frazão Chaves, 2011.

specimens newly hatched as well as damaged eggs. Even in relation to Formicidae, ant larvae were found throughout the nest, inside and outside the shells, while the adults presented defensive behavior.

Two contradictory selective forces are observed in relation to the location of the nests. While nests close to the edge of the water have the advantage of reducing the risk of the hatchlings becoming disoriented, they are also more vulnerable to inundation (Eckert 1987). At the opposite extreme, while nests higher up the beach are safe from flooding, roots may destroy the eggs (Wood and Bjorndal 2000), and the long distance to the water's edge

Table 1. Characteristics of *Eretmochelys imbricata* nests encountered on Cacimbinhas and Madeiro beaches, Rio Grande do Norte, Brazil, in 2011.[a]

Variable	Cacimbinhas, mean ± SD (n = 30)	Madeiro, mean ± SD (n = 19)	df	t	p
No. of eggs	119.50 ± 29.64	117.89 ± 26.50	47	0.17	0.85 (ns)
Hatching time (days)	39.53 ± 28.67	41.16 ± 28.85	47	−0.19	0.85 (ns)
Infestation (% of eggs)	0.47 ± 0.51	0.42 ± 0.51	47	0.31	0.76 (ns)

[a] df = degrees of freedom; ns = not significant.

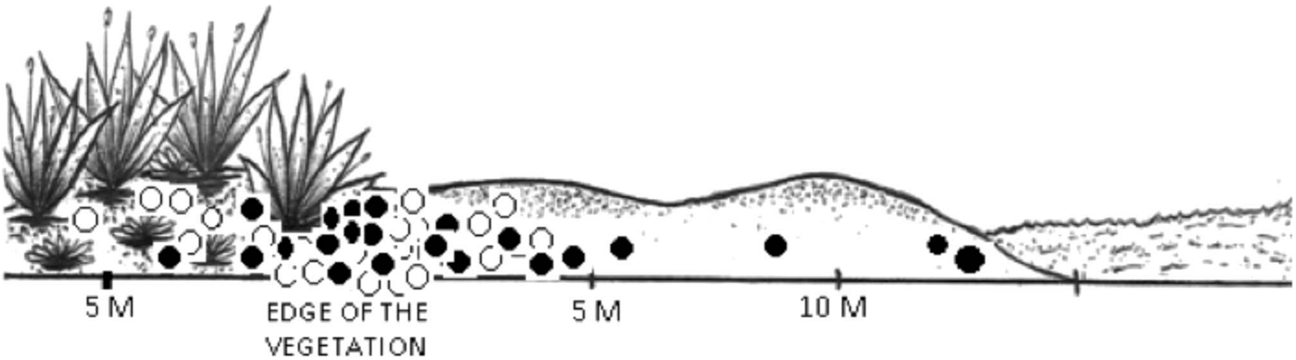

Figure 4. Diagram representing the locations of the *Eretmochelys imbricata* nests recorded in the present study relative to the vegetation and high-tide line on the study beaches in Pipa, Rio Grande do Norte, Brazil. The white dots represent infested nests, and the black dots represent uninfested nests. Drawing by Israel Macedo de Sousa.

significantly increases the vulnerability of the hatchlings to predation and the chances of disorientation. The numerical tendency for lower rates of infestation in the nests located in the open sand indicates that the selection of nesting sites may represent an important reproductive strategy in *E. imbricata*.

In Barbados, Horrocks (1991) found that female *E. imbricata* preferred to dig their nests in locations with vegetation, although Wetterer et al. (2007) concluded that nests close to the vegetation cover in the dunes are significantly more exposed to infestation by ants. In Turkey, Ozdemir et al. (2004) and Katilmis and Urhan (2007) found that the closer the nests were to vegetation, the higher their susceptibility to infestation by dipterans. A similar pattern was observed in the present study. Turtle populations are exposed to a wide range of potential dangers, both on the beach and in the sea. In the case of endangered species, it is important to protect each developmental stage, and for migratory species the conservation of nesting beaches and breeding and foraging grounds should be of the highest priority (Katilmis et al. 2006). All efforts designed to guarantee that the hatchlings are able to reach the ocean are of the utmost importance and should be intensified.

The results of the present study have clearly shown that ants (order Hymenoptera, family Formicidae) can be found in *E. imbricata* nests, where they attack hatchlings and viable embryos. These findings reinforce the need for a more-detailed, long-term study of this phenomenon in order to evaluate more systematically its effects on the reproductive success of the local *E. imbricata* population.

While infestation rates did not appear to be related to the depth of the nests, the distance from the vegetation appeared to be a decisive factor in the process. The mean number of live hatchlings found per nest was much lower in the nests associated with vegetation, although it is important to consider a number of other parameters that may contribute to the success of a nest.

The present study identified some of the factors that determine the infestation of *E. imbricata* nests by insects on Pipa beach in the Brazilian state of Rio Grande do Norte. The importance of this nesting area for the survival of the species emphasizes the need for more-detailed research on the local population, reinforcing the database available for the planning of effective conservation strategies. In future research, quantification of the number of infested eggs and the level of infestation of each egg found will increase the accuracy of others comparative studies on the same area.

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Hematologic, Morphometric, and Biochemical Analytes of Clinically Healthy Green Sea Turtles (*Chelonia mydas*) in Peru

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ABSTRACT. – **Hematologic and biochemistry ranges were established for 31 clinically healthy green sea turtles (*Chelonia mydas*) incidentally captured in artisanal fisheries in Sechura Bay, Peru. Postcapture stress may have influenced heterophil values and glucose concentration. Sechura Bay provides abundant dietary protein affecting urea and glucose values.**

The green sea turtle (*Chelonia mydas*) is the most abundant of the 5 sea turtle species that use the Peruvian coast as a foraging and developmental habitat (Hays-Brown and Brown 1982). Because of fisheries interactions and other threats throughout their life cycle, *C. mydas* is listed as endangered on the IUCN Red List (Alfaro-Shigueto et al. 2011; International Union for Conservation of Nature [IUCN] 2004). In addition, *C. mydas* is the most incidentally captured sea turtle species in Peru especially