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Helminth Component Community of the Loggerhead Sea Turtle, *Caretta caretta,* From Madeira Archipelago, Portugal

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ABSTRACT: The helminth fauna of pelagic-stage loggerhead sea turtles, *Caretta caretta*, is still poorly known. Here, we describe the helminth-component community of healthy, free-ranging juvenile loggerhead sea turtles captured in the waters around Madeira Island, Portugal. Fifty-seven were used in this study. The esophagus, stomach, intestine, liver, gallbladder, spleen, kidneys, trachea, bronchi, urinary bladder, heart, left and right aortas, and coelomic cavity were macroscopically inspected; organs and tissues were removed and washed through a sieve. A search for parasites was made using a stereoscopic microscope; recovered parasites were fixed and stored in 70% alcohol until staining and identification. Prevalence, mean intensity, and mean abundance values were recorded. In total, 156 parasite specimens belonging to 9 species were found: nematodes included *Anisakis simplex* s.l. (larvae) and an unidentified species; digenetic trematodes present were *Enodiotrema megachondrus*, *Rhytidodes gelatinosus*, *Pyelosomum renicapite*, and *Calycodes anthos*; acanthocephalans included *Bolbosoma vasculosum* and *Rhadinorhynchus pristis*; a single cestode, *Nybelinia* sp., was present. Parasite infections were found to have both low prevalences and intensities. Possible reasons for this include the oligotrophic conditions of the pelagic habitat around Madeira; a 'dilution effect' because of the vastness of the area; and the small size, and thus ingestion rate, of the turtles. Results are discussed in terms of the various turtle populations that may use the waters surrounding Madeira. This work provides valuable information on the parasite fauna of a poorly known stage in the life of loggerhead sea turtles, thereby filling a fundamental gap with regard to features of the parasite fauna in this species.

The loggerhead sea turtle, *Caretta caretta* (Linnaeus, 1758), is an omnivorous and opportunistic feeder, with a worldwide distribution throughout the Atlantic, Pacific, and Indian Oceans as well as the Mediterranean, Red, and Black Seas (Dodd, 1988). This species is endangered, primarily due to high incidental mortality as an accidental catch during fishing activities (Aguilar et al., 1995; Tudela, 2000; Deflorio et al., 2005). Most data on the occurrence of parasites of the loggerhead sea turtle were reported before the 1990s and are summarized by Dodd (1988). The helminth fauna of pelagic loggerhead sea turtles is still poorly known.

In Australian waters, Blair and Limpus (1982) have reported 6 species of digenetic trematodes in the loggerhead sea turtle. The report did not, however, include the age stage of the turtles examined. Aznar et al. (1998) examined the gastrointestinal helminths of 54 juvenile and subadult loggerhead turtles in the western Mediterranean. *Enodiotrema megachondrus*, *Calycodes anthos*, and a hemiuroid sp. accounted for 99% of all helminth specimens found. The infracommunities were numerically dominated by *E. megachondrus*, which showed the highest prevalence and intensity (96% and 121.9 worms/host, respectively). In the Adriatic Sea, Manfredi et al. (1998) found the nematode *Sulcascaris sulcata* and the digenean *Rhytidodes gelatinosus* as the most frequent and abundant species in the stomach and intestine, respectively. In a study of diseases and causes of mortality among loggerhead sea turtles stranded in the Canary Islands, Spain, Orós et al. (2005) found several larval anisakid nematodes causing gastritis in 15 of 88 turtles examined (16.1%).

In recent decades, the occurrence of parasites has been used not only to assess the health status of their hosts, but also as an important tool in understanding important features of host biology, i.e., their migratory behavior, distribution, and feeding ecology (Williams et al., 1992; MacKenzie, 2002). Studies regarding the parasite infrapopulation dynamics and parasite biogeography in relation to host dispersal patterns are recommended to provide a useful source of information regarding the evolution and ecology of turtles (Esch et al., 1990). As a consequence of their wide geographical distribution, habitat, and biological characteristics, marine turtles are open to a different intensity and diversity of parasitic infections (Gregory et al., 1995).

The Macaronesian region comprises 3 archipelagos, the Azores, Madeira, and Canaries, which represent important developmental areas in the North Atlantic for loggerhead sea turtles born in the southeastern United States, the adjacent Yucatan Peninsula, Mexico (Bolten et al., 1998), and probably Cape Verde Island (T. Dellinger, pers. comm.). However, parasite data from loggerhead sea turtles in the archipelago areas have not been acquired. The objective of the present work was to describe the helminth-component community of the juvenile loggerhead sea turtles accidentally captured in waters around Madeira Island. Prevalence and intensity data are compared with previous data from other geographical areas.

Fifty-seven specimens of *C. caretta* were used in the present study. They were accidentally caught by the long-line black-scabbard fish (*Aphanopus carbo*) fishery or found dead offshore of the Madeira Archipelago (32.6°N, 16.9°W). Most turtles were obtained during the summer months (June to September 1996–2005). The ranges of the curved carapace lengths (CCL_{nt}) and weights were 153–615 mm (437.68 ± 98.86 mm, median 442 mm) and 0.243–27.97 kg (10.87 ± 6.39 kg, median 9.36 kg), respectively (Fig. 1). The turtles were frozen at −20 C immediately after capture and stored until the necropsy. Twenty-two necropsied turtles were sexed, and the esophagus, stomach, intestine, liver, gallbladder, spleen, kidneys, trachea, bronchi, urinary bladder, heart, left and right aortas, and coelomic cavity of each was macroscopically inspected; organs and tissues were removed and examined. Contents were washed in a sieve (mesh = 180 μm). A search for parasites was performed using stereoscopic microscopy.

For the remaining turtles, only the esophagus plus stomach and/or intestine were examined (intestines, n = 35; stomachs, n = 6; esophagus, n = 2). In these turtles, the same procedure was used for searching of parasites. Collected parasites were fixed and stored in 70% alcohol. Digeneans, acanthocephalans, and cestodes were stained in acetic carmine or modified Harris' hematoxylin, cleared in beechwood creosote, and mounted in Canada balsam. Nematodes were cleared in lactophenol and temporarily mounted in glycerin jelly. Prevalence, mean intensity, and mean abundance values were calculated according to Bush et al. (1997), using the Quantitative Parasitology program (QP 3.0) (Rózsa et al., 2000). Digeneans belonging to *Pyelosomum* and *Enodiotrema* were compared with specimens borrowed from the Harold W. Manter Laboratory (HWML), University of Nebraska, Lincoln, Nebraska.

Of the 57 turtles examined, 45.6% were infected with at least 1 parasite species. Of 49 turtles sexed (41 females/8 males), 3 males were infected only with nematode larvae (intensity 1, 1, and 6 parasites). In total, 156 parasite specimens belonging to 9 species (Table I) were found in the 57 turtles. Nematodes, mainly *Anisakis simplex* s.l. type-I larvae, were the parasite group with highest prevalence (35.7%). These larvae were recovered from the stomach, intestine, and coelomic cavity where, in 2 turtles, the worms were attached to the liver parenchyma, suggesting no site specificity. Larval stages of *Anisakis simplex* s.l. type II were found in 2 turtles (intensities of 1 and 5). Thirteen specimens of an unidentified nematode species, together with 2 larvae of *A. simplex* s.l. type I, were found in the stomach of 1 turtle.

Digeneans were the most diverse helminths group. The 4 digenean species present included only mature individuals. *Enodiotrema megachondrus* (Plagiorchiidae) was the most dominant digenean species in the component community, accounting for 69% of total parasites recovered. In most cases (n = 11/13), this parasite occurred in the intestine, being present in the stomach in only 2 of the turtles. Although with a lower prevalence (24.6%) than the *Anisakis* larvae, *E. megachondrus* had the highest mean intensity (7.71 worms/host). *Rhytidodes gelatinosus* (Rhytidodidae), *Pyelosomum renicapite* (Pronocephalidae), and *Calycodes anthos* (Calycodidae) were found in the intestines, but

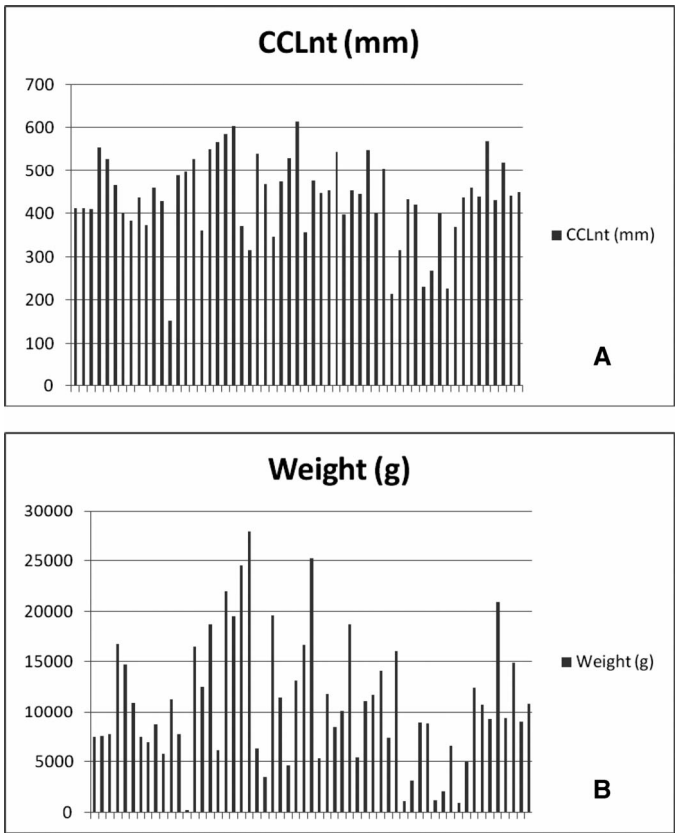

FIGURE 1. Distribution of curved carapace length (CCL_{nt}) (A) and weight (B) of Loggerhead sea turtles analyzed to parasites in the Madeira Archipelago, Portugal.

in very low prevalences (3.5%, 1.8%, 1.8%, respectively) and mean intensities (1.5, 1.0, 1.0, respectively). Individual hosts had only 1 or 2 of these digenean species.

Cystacanths of *Bolbosoma vasculosum* (Polymorphidae) and *Rhadinorhynchus pristis* (Rhadinorhynchidae) were found in the coelomic cavity and intestine, respectively, both with a prevalence of 1.8%. A post-larva of *Nybelinia* sp. (Eucestoda: Trypanorhyncha) was found in the stomach of 1 turtle.

No helminths were found in the esophagus, spleen, kidneys, trachea, bronchi, urinary bladder, heart, or left and right aortas. No tissue damage associated with the occurrence of parasites was macroscopically observed, except in 2 turtles where larvae of the anisakids produced a small ulceration in the gastric mucosa.

Loggerhead sea turtles, *C. caretta*, like other species of sea turtles, occupy 3 different habitats during their lives, i.e., the terrestrial zone, the oceanic zone, and the neritic zone (Dodd, 1988). After hatching, loggerhead turtles swim offshore and enter the oceanic zone where they spend the first 6–9 yr of their lives (Bjorndal et al., 2003). Within the North Atlantic, these immature loggerheads have been found, and primarily studied, in the waters around the Azores and Madeira (Bolten, 2003). Since most marine parasites are transmitted through the ingestion of infected prey (Marcogliese, 2002), the low prevalence of infections found in this study can be explained by a 'dilution effect' and the oligotrophic condition of the pelagic habitat and, consequently, by low availability of the intermediate hosts necessary to complete the parasites' life cycles. In this context, the small size and age of the examined turtles may be an important factor, due to the low prey consumption and low density of parasites in prey populations, respectively.

From the 8 parasite species found in our study, only the 4 digenean species are typical of marine turtles, namely, *E. megachondrus*, *Rhytidodes gelatinosus*, *Calycodes anthos*, and *Pyelosomum renicapite*. The first of these 3 species had been previously recorded from loggerhead sea turtles in the Mediterranean Sea (Aznar et al., 1998; Manfredi et al., 1998). At the component-community level, turtles from Madeiran waters have a higher number of parasite species than those from the western Mediterranean population (Aznar et al., 1998) and a similar number as those from the central Mediterranean (Manfredi et al., 1998). Overall, however, their infracommunities were particularly poor. This scant harvest of parasites is likely related to differences in the size distribution of the turtles examined in both areas. Turtles from the Madeiran Archipelago ranged from 135–557 mm in length and 0.24–28 kg in weight, whereas those from Mediterranean ranged from 340–690 mm and 5–52 kg. The CCl_{nt} mean from turtles studied by Aznar et al. (1998) was apparently lower than that of our study; however, those authors did not present size–distribution data. Smaller loggerheads have smaller feeding rates and, thus, lower probabilities of infection. Furthermore, loggerhead life histories undergo ontogenetic shifts that are reflected in their diet (Tomas et al., 2001). As loggerhead turtles increase in size, they appear to feed at progressively higher trophic levels (Godley et al. 1998) that may possess more parasite larval stages than smaller prey (Price et al., 1986). The young age of the turtles should, therefore, reflect the depauperate infracommunities seen in this study. Nestling turtles from Atlantic populations are larger in size than those from the Mediterranean Sea (Dodd, 1988). Accordingly, turtles from Madeira of the same size as those analyzed by Aznar et al. (1998) may actually be

TABLE I. Prevalence, mean intensity, and mean abundance of helminths in juvenile loggerhead sea turtles (*Caretta caretta*) in the Madeira Archipelago, Portugal.

Parasite species	Turtles (n)	% Prevalence (95% C.L)	Mean abundance (mean ± SD)	Mean intensity (range)	Total no. of worms
Nematoda					
Anisakis simplex s.l. (larvae)	28	35.7 (18.6–55.9)	0.96 ± 1.29	2.6 (1–6)	27
Unidentified nematodes	28	3.6 (0.09–18.3)	0.46 ± 2.46	13 (13)	13
Digenea					
Enodiotrema megachondrus	57	24.6 (14.6–37.8)	1.89 ± 5.37	7.71 (1–31)	108
Rhytidodes gelatinosus	57	3.5 (0.4–12.11)	0.04 ± 0.19	1.5 (1–2)	3
Pyelosomum renicapite	57	1.8 (0.04–9.4)	0.02 ± 0.13	1 (1)	1
Calycodes anthos	57	1.8 (0.04–9.4)	0.02 ± 0.13	1 (1)	1
Acanthocephala					
Bolbosoma vasculosum	57	1.8 (0.04–9.4)	0.02 ± 0.13	1 (1)	1
Rhadinorhynchus pristis	57	1.8 (0.04–9.4)	0.02 ± 0.13	1 (1)	1
Cestoda					
Nybelinia sp.	28	3.6 (0.09–18.3)	0.04 ± 0.19	1 (1)	1

younger. The low species richness of parasite infracommunities in juvenile and subadult *C. caretta* found in the western Mediterranean population (Aznar et al., 1998) was attributed to constraints on parasite acquisition and other host factors that could limit parasite recruitment. A fundamental difference in Madeira is that 4 of the 8 species (50%) are not specialists in marine turtles, contrary to what Aznar et al. (1998) predicted. Based on our findings, we question the extent to which the phylogenetic constraints could affect parasite recruitment. Could the differences in species richness be a question of lower availability of infecting parasites in the western Mediterranean?

It is clear that substantive differences exist between the parasite infection levels in both ecosystems. In 54 turtles from the western Mediterranean waters, a total of 7,098 individual digeneans belonging to 5 species was present (Aznar et al., 1998), whereas in Madeiran waters, only 113 individual digeneans (4 species) were found in 55 turtles. In both areas, *E. megachondrus* was the most frequent digenean species in the component community. In the western Mediterranean, this fluke had a mean abundance of 121.9 worms/host, which contrasts with the 7.7 worms/host in the present study. This digenean has also been recorded from olive ridley sea turtles (*Lepidochelys olivacea*) from the Pacific coasts of Costa Rica (Santoro and Morales, 2007) and Mexico (Vivaldo et al., 2006).

The component community of turtles in the Adriatic Sea (eastern Mediterranean) possessed several parasite species not found in Madeiran turtles, i.e., the digeneans *Orchidasma amphiorchis*, *Pachypsolus irroratus*, *Pleurogonius trigonocephalus*, and *Plesiochorus cymbiformis*, and the anisakid nematode *Sulcascaris sulcata* (Manfredi et al., 1998). In that geographic area, the component community was dominated by *S. sulcata* and the digenean *Rhytidodes gelatinosus*. In a survey of helminth parasites of 28 loggerheads from Egyptian waters conducted by Sey (1977), the digeneans *E. megachondrus* and *Pachypsolus irroratus* were not found. Several factors affect the geographical distribution of the parasites, including the availability of infected prey and the migration patterns of their hosts. Based on the biogeographical distribution of digenean species in the loggerhead turtle, Manfredi et al. (1998) suggested that the low exchange of parasites between Mediterranean and Atlantic turtles requires reconsideration as an explanation. Movement of loggerhead turtles near, and through, the Straits of Gibraltar has been reported in both directions (Camiñas, 1997), indicating that the Atlantic and Mediterranean loggerhead populations share developmental habitats in the western Mediterranean (Margaritoulis et al., 2003) and the northeastern Atlantic.

In the present study, the presence of immature stages of other helminths seems to reflect the opportunistic feeding habits of loggerhead turtles. Thus, larval nematodes, cystacanths of acanthocephalans, i.e., *B. vasculosum* and *Rhadinorhynchus pristis*, and the post-larva of the trypanorhynch *Nybelinia* sp. are typical parasites of fish and squid hosts and reflect the low host-specificity of these parasite species. For the larval parasites, the loggerhead sea turtles are most likely an accidental host. *Bolbosoma vasculosum* and *Nybelinia* sp. have been recorded from several fish species along the Madeira coast (Costa et al., 2000; Costa et al., 2003b). The definitive hosts of *B. vasculosum* are marine mammals (Meyer, 1932; Pendergraph, 1971); cystacanths have been found in the visceral cavity of the black-scabbard fish (*A. carbo*) and oceanic horse mackerel (*Trachurus picturatus*) from Madeira (Costa et al., 2000), as well as the silver scabbard fish (*Lepidopus caudatus*) from the Great Meteor Seamount in the east-central Atlantic (Klimpel et al., 2006). In the same areas, adults of *Rhadinorhynchus pristis* have been found in the intestine of chub mackerels (*Scomber japonicus*) and silver-scabbard-fish (Costa et al., 2004; Klimpel et al., 2006). Of 8 parasite species found in the loggerhead turtles, 4 are also present in black-scabbard fishes captured in the same area (Costa et al., 2000; Costa et al., 2003a). There is a high accidental capture of loggerheads in the deep-drifting, pelagic long-line fishery that targets this fish species (Dellinger and Encarnação, 2000). The co-occurrence of half of their parasite species suggests that juvenile turtles and black-scabbard fish prey on the same intermediate hosts. According to Costa et al. (2004), the presence of *Rhadinorhynchus pristis* in chub mackerel from Madeiran waters provides evidence of the relationships between the Madeiran parasite fauna and those of the western tropical Atlantic and western Mediterranean regions.

Larval *Anisakis simplex* s.l. are frequently found in marine invertebrate and vertebrate hosts throughout the Atlantic Ocean, with cetaceans as final hosts (Brattey and Stenson, 1993; Mattiucci et al., 1997; Herreras et al., 2004; Mattiucci and Nascetti, 2006). High prevalences (58–100%) of these larvae have been recorded from fishes captured in Madeira waters (Costa et al., 2003a). The Madeira Archipelago is an area with seasonal high concentrations of marine mammals, including 6 species of true whales (Mysticeti), 14 dolphin and porpoise species (Odontoceti), and the threatened monk seal (*Monachus monachus*) (Freitas et al., 2004; Cabral et al., 2005). The high prevalence of *Anisakis simplex* larvae in the loggerhead sea turtles and fish is probably a result of the common presence of these final hosts in the area.

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