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Research Note

Helminth Fauna of Juvenile Green Sea Turtles (*Chelonia mydas*) from Rio de Janeiro State, Brazil

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Summary

The helminth fauna of juvenile green sea turtles (*Chelonia mydas* Linnaeus, 1758) is still poorly known. Herein, we study the gastrointestinal helminths of 28 juvenile green sea turtles found stranded on the north coast of Rio de Janeiro state, Brazil. All turtles were infected showing a rich helminth fauna. In total, 14802 trematodes belonging to 30 species and 5 families including Microscaphidiidae, Plagiorchiidae, Pronocephalidae, Hapalotrematidae, and Telorchiidae were recovered. An unidentified nematode specimens was also found. The mean intensity was 536 (95% CI = 362 – 853) (range: 1 – 2831), and the species richness was 7.86 (95% CI = 6.46 – 9.21) (range: 1 – 17). The coast of Rio de Janeiro state represents new locality records for *Angiodictyum posterovitellatum, Microscaphidium aberrans, M. warui, Octangium hyphalum, O. sagitta, Enodiotrema reductum* and *Pleurogonius laterouterus*. This study confirms that the green sea turtle harbors the richest helminth fauna among sea turtle species and provides useful information on the gastrointestinal helminths of a poorly known stage in the life cycle of this endangered chelonian.
Keywords: *Chelonia mydas*; green sea turtle; helminths; trematodes; Rio de Janeiro; Brazil

Introduction

The green sea turtle (*Chelonia mydas*) is considered the largest among the hard-shell sea turtles (Márquez, 1990). It has a global distribution, with most nesting and feeding sites located in the tropics, being considered a highly migratory species (Miller, 1997). The diet of green sea turtles is closely associated with the spatial and temporal use of the habitat and the ontogenic changes experienced during the life cycle (Bjorndal, 1997), being in the early stage of their life omnivores and later become herbivores.

The green sea turtle is the most prevalent species on the Brazilian coast (Reis *et al*., 2009; ICMBio, 2011), with juvenile individuals being the prevalent life stage along the coast of the Rio de Janeiro state, where they forage in shallow-water seagrass beds (Marcovaldi & Marcovaldi, 1999; Tagliolatto *et al*., 2020).

In Brazil, there are still few studies detailing on the parasite fauna of juvenile stages of sea turtles. Only recently, Werneck and Silva (2015) in the state of São Paulo and south of Rio de Janeiro, and Binoti *et al*. (2016) and Gomes *et al*. (2017) in the state of Espírito Santo investigated the parasite fauna of juvenile green sea turtles. Aiming the fulfillment of knowledge gaps in the field, we report the gastrointestinal parasite fauna of juvenile green sea turtles from the coast of Rio de Janeiro, Brazil.

Material and Methods

From September to December 2020, 28 juvenile green sea turtles were found dead, stranded on the beach during monitoring activities (Werneck *et al*., 2018). The sea turtles were obtained from the municipalities of São Francisco de Itabapoana (21° 26’ 3.5874” S

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41° 6’ 54.9354” W), Búzios (22° 44’ 53.1276’’ S 41° 52’ 52.644’’ W), Casemiro de Abreu (22° 35’ 36.708”S 41° 59’ 39.084”W), Macaé (22° 22' 18" S 41° 47' 9" W), Rio das Ostras (22° 31’ 36.8364’’ S 41° 56’ 34.872’’ W) and Unamar (22° 38’ 34.08” S 41° 59’ 55.7874” W). All sites are located on the north coast of Rio de Janeiro, Brazil.

Before necropsy, all sea turtles were weighed and the curved carapace length notch-to-tip (CCL) was measured to the nearest cm. Visual assessment of the body condition for each turtle was measured according to Thomson *et al.* (2009). In brief, turtle condition was classified as good when the plastron was convex, fair when it was flat, and poor when it was concave.

At post mortem examination, the esophagus, stomach, small and large intestines were studied for helminth parasites following the methods described by Greiner *et al*. (1980). Briefly, the viscera were opened and surfaces grossly examined, then they were washed through a 60 µm mesh screen. The remaining washed material from each organ was examined under a dissecting microscope and parasites were collected, counted, washed in physiological saline solution, and preserved in 70 % ethanol. For morphological identification trematodes were stained with carmine and cleared with eugenol, then were mounted on glass slide using Canada balsam, and studied using an optical microscope equipped with the S-EYE software program.

Prevalence, mean intensity of infection and mean abundance followed Bush *et al*. (1997) using Quantitative Parasitology Program (QP 3.0; Reiczigel *et al*., 2013). The 95 % confidence intervals (CI) of prevalence were calculated using Sterne’s exact method and those for mean intensity of infection and mean abundance were calculated using bootstrapping with 2,000 replications.

Species richness, mean abundance and mean intensity of infection were compared among turtle body condition classes and between turtles from different geographical areas using the non-parametric Mann -Whitney U test. The significance level adopted in the statistical tests was 5 %. Statistical analyzes were calculated using SigmaStat 3.1 (Jandel Scientific Corporation, San Jose, California).

Representative samples of the helminths collected during this study were deposited in the Helminthological Collection of the Instituto Oswaldo Cruz (CHIOC – numbers requested) in the state of Rio de Janeiro, Brazil.

Results and Discussion

Out of 28 green sea turtles, 21 were females, 4 were males and 3 were of undetermined sex. All were juveniles (CCL: 36.9 ± 5.6 ranging from 27.9 to 51.2 cm; weight: 4.5 ± 1.2 ranging from 2.6 to 6.4 kg). All green sea turtles were positive for helminths. A total of 14802 trematodes belonging to 30 species and 5 families, including Microscaphidiidae (9 species), Plagiorchiidae (3 species), Pronocephalidae (15 species), Hapalotrematidae (2 species) and Telorchiidae (1 species), were recovered (Table 1). In addition, an unidentified nematode larva was found in two turtles. The mean intensity was 536 (95% CI = 362 – 853) (range: 1 – 2831), the mean abundance was 536 (95% CI = 364 – 850), and the species richness was 7.86 (95% CI = 6.46 – 9.21) (range: 1 – 17).

The present study reports the highest prevalence, mean intensity of infection and species richness when compared to other studies on juvenile green sea turtles in Brazil (Werneck & Silva, 2015; Binoti *et al*., 2016; Gomes *et al*. 2017). The present results were also similar to those found in adult females of green sea turtles studied in Costa Rica (Santoro *et al*., 2006).

This finding together to different component community than previous studies (Werneck & Silva, 2015; Binoti *et al*., 2016; Gomes *et al*. 2017) suggest that the northern region of the state of Rio de Janeiro could have different environmental conditions that make richer the parasite fauna of the juvenile green sea turtles. That in the study area there is the coastal upwelling system with the highest intensity of the Brazilian coast (Coelho-Souza *et al*., 2012). This region represents important sources of marine productivity, generating high levels of primary productivity that propagate through the food chain. Consequently, the concentration of organisms at this site is high, possibly also including the concentration of parasitic species (Costa & Fernandes, 1993; Valentin, 2001; Coelho-Souza *et al*., 2012; Reis *et al*., 2017). Therefore, the present study supports the idea that in addition to host ontogenic factors and age, some abiotic conditions related to the specific geographical area can also directly influence the parasite fauna in these hosts (Santoro *et al*., 2006; Valente *et al*., 2009; Santoro et al., 2010; Gracan *et al*., 2012; Werneck & Silva, 2015; Werneck *et al*., 2015).

Trematodes were the predominant class of parasites in the juvenile green turtles, as expected. All species identified have been previously reported in the same host. However, *Angiodictyum posterovitellatum* Chattopadhyaya, 1972, *Microscaphidium aberrans* Looss, 1902, *Microscaphidium warui* Blair, 1986, *Octangium hyphalum* Blair, 1987, *Octangium sagitta* (Looss, 1899) Looss, 1902, *Enodiotrema reductum* Looss, 1901 and *Pleurogonius laterouterus* Fischthal & Acholonu, 1976 have not been previously reported in Brazil, representing new locality records. In fact, *A. posterovitellatum* and *P. laterouterus* had not yet been identified in this host from the Neotropical region (Central and South America), according to Werneck and Silva (2016).

Only 4 species of parasites were found in more than 50 % of the animals analyzed: *Cricocephalus albus* (Kuhl & van Hasselt, 1822) Looss, 1899, *Metacetabulum invaginatum* Teixeira de Freitas & Lent, 1938, *Pronocephalus obliquus* Looss, 1899, and *Ruicephalus minutus* (Ruiz, 1946) Skrjabin, 1955. *Cricocephalus albus* was the most prevalent species (89.3 %), followed by *M. invaginatum* (82.1 %), *P. obliquus* (82.1 %), and *R. uterocrescens* (53.6 %). Similar results were observed from the coast of the state of Espírito Santo (northernmost state) (Binoti *et al*., 2016; Gomes *et al*., 2017), and from São Paulo (southernmost state) and the south of the state of Rio de Janeiro (Werneck & Silva, 2015).

Sixteen (57 %) carcasses were in poor body condition; the remaining turtles were in good or fair condition. In this latter group

Table 1. Prevalence, mean intensity, and mean abundance of helminths parasites identified in Chelonia mydas (n=28) from the Rio de Janeiro State, Brazil.

Species	Number of parasites	Site of infection	% Prevalence (95% CI)	Mean abundance (95% CI)	Mean intensity (95% CI)
Microscaphidiidae					
Angiodictyum posterovitellatum	33	Si	3.6 (0.2-17.5)	1.18 (0-3.54)	33
Deuterobaris intestinalis	63	Si, Li	25 (11.9-44.6)	2.25 (0.5-6.45)	9 (2.86-19)
Microscaphidium aberrans	87	St, Li	7.1 (1.3-22.9)	3.11 (0-11.9)	43.5 (14-73)
Microscaphidium reticulare	22	Li	7.1 (1.3-22.9)	0.786 (0-2.18)	11 (9-11)
Microscaphidium warui	2	Li	3.6 (0.2-17.5)	0.0714 (0-0.214)	2
Neoctangium travassosi	207	Si, Li	39.3 (22.9-59.1)	7.39 (3.5-13.8)	18.8 (10.6-29.1)
Octangium hyphalum	124	St, Si, Li	14.3 (5-31.9)	4.43 (0.179-20.9)	31 (2-87.5)
Octangium sagitta	9	Si	3.6 (0.2-17.5)	0.321 (0-0.964)	9
Polyangium linguatula	335	St, Si, Li	28.6 (14.2-48.2)	12 (3.14-46.4)	41.9 (14.6-116)
Plagiorchiidae					
Enodiotrema sp.	1	Si	3.6 (0.2-17.5)	0.0357(0-0.107	1
Enodiotrema megachondrus	3	Si	7.1 (1.3-22.9)	0.107 (0-0.321)	1.5 (1-1.5)
Enodiotrema reductum	39	St, Si	25 (11.9-44.6)	1.39 (0.357-4.76)	5.57 (1.86-15)
Pronocephalidae					
Charaxicephaloides polyorchis	38	Es, St, Li	17.9 (7.3-35.7)	1.36 (0.214-5.68)	7.6 (1.8-23.4)
Charaxicephalus robustus	2	St	3.6 (0.2-17.5)	0.0714 (0-0.214)	2
Cricocephalus albus	4,275	Es, St, Si, Li	89.3 (71.8-97)	153 (93.9-300)	171 (105-334)
Cricocephalus megastomum	120	St, Si	32.1 (17.5-51.8)	4.29 (1.61-11.6)	13.3 (6.22-29.4)
Desmogonius baldassinae	5	St	14.3 (5-31.9)	0.179 (0.0357-0.357)	1.25 (1-1.5)
Metacetabulum invaginatum	1,333	Es, St, Si, Li	82.1 (64.3-92.7)	47.6 (30.3-81.8)	58 (37.5-95.1)
Pleurogonius sp.	51	Si	10.7 (3-28.2)	1.82 (0.214-7.97)	17 (3-29.3)
Pleurogonius laterouterus	533	Es, St, Si, Li	35.7 (19.3-55.4)	19 (6.46-48.4)	53.3 (18.1-117)
Pleurogonius linearis	22	Si	7.1 (1.3-22.9)	0.786 (0-3.75)	11 (1-11)
Pleurogonius lobatus	72	Es, St, Si	25 (11.9-44.6)	2.57 (0.786-9.58)	1033 (4-27.5)
Pleurogonius longiusculus	339	St, Si, Li	46.4 (28.2-64.5)	12.1 (3.89-30.6)	26.1 (9.46-59.9)
Pronocephalus obliquus	974	Es, St, Si, Li	82.1 (64.3-92.7)	34.8 (22.3-63.2)	42.3 (27.3-72.4)
Pyelosomum crassum	79	St, Si, Li	14.3 (5-31.9)	2.82 (0.643-7.5)	19.8 (9-31.5)
Rameshwarotrema uterocrescens	3,472	Es, St, Si, Li	53.6 (35.5-71.8)	124 (19.8-534)	231 (36.1-945)
Ruicephalus minutus	2,493	Es, St, Si, Li	75 (55.4-88.1)	89 (42.3-183)	119 (60.9-229)
Hapalotrematidae					
Amphiorchis solus	1	Li	3.6 (0.2-17.5)	0.0357 (0-0.107)	1
Hapalotrema postorchis	3	Li	3.6 (0.2-17.5)	0.107 (0-0.321)	3
Telorchiidae					
Orchidasma amphiorchis	9	Si	14.3 (5-31.9)	0.321 (0.0714-0.821)	2.25 (1-3.25)
Nematode larvae	3	St, Si	7.1 (1.3-22.9)	0.107 (0-0.357)	1.5 (1-1.5)

the cause of death was drowning. This data confirms that accidental capture remains one of the main threats for this species in the coast of Rio de Janeiro state, as described in a recent study (Tagliolatto *et al*., 2020).

Out of 16 carcasses in poor condition, five had gross esophageal pathological changes characterized by diffuse ulcers with yellowish necrotic caseous exudate obstructing the esophageal lumen. These lesions were associated with massive infection by *R. uterocrescens*. The present results confirm that this trematode species may be responsible of severe disease as previously reported (Santoro *et al*., 2007; Ribeiro *et al*., 2017; Jerdy *et al*., 2019).

No significant statistical differences were found for species richness, abundance, and mean intensity of infection among carcasses of different body condition classes. The present results also suggest that all green sea turtles here studied belonged to the same population, as there were no differences in composition of parasite fauna or statistical values.

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Conflict of Interest

Authors state no conflict of interest.

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