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An extensive survey on helminth community of *Caretta caretta* from the neritic feeding grounds of Northwestern Adriatic sea

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The opportunistic diet of loggerhead sea turtles, *Caretta caretta*, their long life-span and migratory behavior contribute to shaping the diversity of the gastrointestinal helminthic community. Heteroxenous helminths are also sentinels of marine environmental health, reflecting any perturbation of the trophic chain and, indirectly, of abiotic components of the ecosystem. With the aim of studying the helminth community of a top predator such as the loggerhead sea turtle *C. caretta*, parasites were collected from the digestive tract of 157 individuals, stranded dead along NW Adriatic Sea in the period 2009–2023, and morphologically identified. Prevalence, intensity, abundance, relative abundance and importance index were calculated for each taxon. Ontogenetic stages and sex of the hosts, seasons and observation periods were compared to unveil any difference in parasite community structure. Overall, richness and diversity were similar to other neritic grounds in the Mediterranean Sea; helminths from 9 species were recovered, with the trematode *Rhytidodes gelatinosus* and the nematode *Sulcascaris sulcata* having the highest importance. Prevalence, intensity and abundance of helminthiases increased significantly in the period 2015–2023. Helminthic community composition and richness generally overlap with what observed in other similar neritic areas, confirming the role of the ecosystem in shaping *C. caretta* gastrointestinal helminthic community. Increase in prevalence and intensity of helminthiases and composition of the parasitic community throughout the observation period suggests potential alterations within the Adriatic ecosystem in its biotic and/or abiotic components, potentially associated with underlying global climate change.

Keywords *Caretta caretta*, Helminths, Adriatic sea, Ecosystem

Observations on communities of heteroxenous parasites offer an indirect view on the integrity of the trophic web and environmental health. A number of drivers may affect the patterns of trematode infections at the top of the food chain. Abiotic factors influence both trematode and intermediate host fitness and reproductive rate (temperature, water flow, pollution), but also definitive host population dynamics can modify abundance of infective forms[1–3]. Alteration of the marine environment may therefore be mirrored by changes in the helminth community structure, though the specific underlying mechanisms are not always completely understood. Loggerhead sea turtles, with their omnivorous diet and opportunistic feeding behaviour, act as optimal "host-model" for studies on heteroxenous parasites ecology in the marine environment.

The Northern Adriatic Sea represents one of the major neritic feeding grounds for loggerhead sea turtles, *Caretta caretta*, in the Mediterranean Sea[4–6]. The rich benthos of its shallow waters is exploited by this species all-year round, with population density increasing during the warm season, reaching the highest numbers in July-September[4,7–9]. The life history of the species has been described as made up of two ontogenetic stages which rely on the development of proper diving ability[10–12]. During the "pelagic" life stage, hatchlings and small juveniles get dispersed in the open waters and feed on the surface of the water column. After attaining a certain body size which allows submersion to the sea bottom, a "neritic" stage is reached, in which turtles shift to the continental shelf and feed mainly on benthic prey[4,10]. Carapace length at the time of dietary shift differs among *C. caretta* populations worldwide and is by far the smallest in the Mediterranean Sea, i.e. about 30 cm[10], reflecting a very early switch to benthic prey in Mediterranean loggerheads. Nevertheless, individuals with CCL of 25 cm have

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been found already feeding on benthic prey in the Adriatic Sea[13,14]. This shift in feeding behaviour is slowly progressive during a long period of life[10,15] and not definitive, as epipelagic prey are also included in the diet of adult sea turtles, though in smaller proportions[16]. Thanks to the ecological and bathymetric characteristics, the Northern and Central Adriatic Sea represent one of major neritic feeding grounds for loggerhead sea turtles in the Mediterranean. Main items in their diet are represented by benthic preys, such as Mollusca (Bivalvia, Gastropoda), Crustacea and Echinoidea[13,15].

The varied diet of benthic feeders and their migratory behavior predictably increase values of diversity of the parasitic community of loggerheads in neritic ecosystems if compared with pelagic ones, favoring the contact and infestation with larval forms of different heteroxenous parasite species. Furthermore, larger turtles and higher host density further enhance the probability of parasite recruitment by sea turtles at their neritic life stage compared with pelagic small juveniles, which live in the "diluted" oceanic habitat and feed at lower rates[17,18]. Indeed, gastrointestinal parasitic communities of loggerhead sea turtles have been described in the Mediterranean Sea highlighting a strong connection between turtle ontogenetic stage, ecosystem and parasite diversity[17]. Overall, results of the analysis of parasitic communities of loggerhead sea turtles from pelagic areas, namely the Madeira archipelago, Western Mediterranean Sea and Northern Ionian coasts, report particularly depauperate gastrointestinal helminthofauna, with the digeneans *Enodiotrema megachondrus* and *Calycodes anthos* composing the bulk of isolated individuals, with infections characterized by low intensity levels[18,19]. A predominant pelagic life cycle has been indeed hypothesized for these two species[19]. On the counterpart, loggerhead turtles sampled along the coasts of neritic grounds, including the Italian Tyrrhenian and Eastern Adriatic coasts, display much higher species richness of the helminthic community[17,20]. Other small-scale studies on loggerhead parasites have been conducted along Northwestern Adriatic Sea[21–23]. Results of these studies report a varied parasitofauna, mainly composed of digenetic trematodes including the ubiquitarian *Enodiotrema* spp., *Calycodes anthos*, and the species *Orchidasma amphiorchis*, *Pachypsolus irroratus*, *Pleurogonius trigonocephalus*, *Rhytidodes gelatinosus*, adding also the nematodes *Kathlania leptura* and *S. sulcata*. It is thus supposed that benthic invertebrates are involved in the life cycle of these parasitic taxa which are exclusively present in neritic grounds[19], although only the intermediate hosts of *S. sulcata* have been identified to species level by now; indeed, benthic species of gastropods and mollusks infected with *S. sulcata* L3 have been found in Adriatic and Tyrrhenian Sea[24,25].

The studies carried out in the Northern Adriatic Sea by now are mostly focused on documenting species and analysis of infection indices. Host-related and environmental factors affecting community composition and epidemiology have been scarcely explored, and the limited sample included unavoidably limit comprehension of the epidemiology of helminthiases in the host population. Given the high host density and the importance of the Northern Adriatic Sea as hotspot of biodiversity, further studies on the diversity and composition of the gastrointestinal helminthofauna of this host species are advocated in the area. We therefore carried out a large-scale study on loggerhead sea turtles stranded along Northwestern Adriatic coast aimed at evaluating (i) infection indices, diversity and composition of the gastrointestinal helminthic community, and (ii) the drivers of helminth community variation, including intrinsic and extrinsic factors, i.e. sea turtle ontogenetic stage and sex, period of sampling and season. Considering the heteroxenous nature of almost all helminths of loggerheads, a period of fifteen years of sampling was considered in order to gain a worthwhile perspective on the stability of trophic networks throughout time.

Results

Overall, 157 turtles were recovered, temporally distributed as shown in Table 1. Larger turtles within the benthic development stage were more prevalent and a greater proportion of turtles were recovered in the warm season. Sex was determined for 137 turtles, of which 44 were male and 93 were female.

Overall, 103/157 animals were found infected with one or more helminth taxa ($P = 65.6\%$, 95%CI = 57.5–72.7%). A total number of 6139 helminths were collected belonging to 9 species, specifically to two nematode species, *Sulcascaris sulcata* and *Kathlania leptura* ($n = 1504$), and 7 digenean species, *Rhytidodes gelatinosus*, *Pleurogonius trigonocephalus*, *Enodiotrema megachondrus*, *Pachypsolus irroratus*, *Orchidasma amphiorchis*, *Calycodes anthos* and *Cymatocarpus solearis* ($n = 4635$) (Fig. 1). Calculated mean intensity was 59.6 (95%CI: 35.5–83.7) helminths per infected host, while mean abundance was 39.1 (95%CI: 24.6–74.4) per host (Table 2). *Rhytidodes gelatinosus* was the most prevalent and abundant parasite species (RA = 23.4), followed by the nematode *S. sulcata* (RA = 14.3); these also had the highest importance index ($I = 48.4$ and 17.3, respectively). A high value of RA (= 20.8) was calculated for *C. solearis*, as consequence of its considerable intensity in the sole

	Period 1 (2009–2014)		**Period 2 (2015–2023)**		
	Cold	Warm	Cold	Warm	**overall**
SCL < 30 cm epipelagic/transitional stage	1	10	4	8	**23**
SCL > 30 cm benthic stage	12	66	12	43	**133**
overall	**89**		**68***		**157***

Table 1. Loggerhead sea turtles *Caretta caretta* ($n = 157$) from the Northwestern Adriatic Sea: distribution into categories used for statistical analysis. * The SCL was not available for one turtle collected in the cold season of the year 2019 and therefore categorization into feeding stage was not possible.

Fig. 1. Photomicrographs of trematodes isolated from gastrointestinal tract of *Caretta caretta* in the present study. *Pleurogonius trigonocephalus* (**a**), *Pachypsolus irroratus* (**b**), *Orchidasma amphiorchis*, immature specimen (**c**), *Enodiotrema megachondrus* (**d**), *Cymatocarpus solearis* (**e**), *Rhytidodes gelatinosus*, immature specimen (**f**) and *Calycodes anthos* (**g**). Scale bar = 1 mm.

positive animal. Indeed, *C. anthos* and *C. solearis* had the lowest prevalence (*P* = 1.9% [95%CI: 0.5–5.6%] and 0.6% [95%CI: 0–3.7%]) and importance index (*I* = 0.01 and 0.7) (Table 2).

Individual hosts harboured between 1 and 6 species (mean = 1.39), with the majority of animals hosting one (35.9%) or two (31.1%) taxa. Shannon-Wiener's diversity index was 1.95.

	P (%) (95%CI)	I (95%CI)	A (95%CI)	RA (%)	*I*
Nematoda					
Sulcascaris sulcata	22.3 (19.1–33.8)	25.1 (12.3–53.7)	5.61 (2.62–13.3)	14.3	17.3
Kathlania leptura	7.0 (3.7–12.3)	56.7 (19.6–120)	3.97 (1.13–10.8)	10.2	3.9
Trematoda					
Orchidasma amphiorchis	15.9 (10.7–22.6)	10.8 (6.04–19)	1.73 (0.90–3.49)	4.4	3.8
Enodiotrema megachondrus	16.6 (11.4–23.2)	21.4 (12.3–39.3)	3.54 (1.83–7.02)	9.1	9.1
Pachypsolus irroratus	18.5 (13–25.4)	9.93 (7.16–14.6)	1.83 (1.11–2.97)	4.7	4.7
Rhytidodes gelatinosus	38.2 (30.8–46.2)	23.9 (14.8–41.9)	9.14 (5.66 -17)	23.4	48.4
Pleurogonius trigonocephalus	18.5 (13–25.4)	27.6 (11.7–55.4)	5,1 (2.33–12)	13	13.1
Calycodes anthos	1.9 (0.5–5.6)	2.33 (1–2.75)	0.04 (0.01–0.12)	0.1	0.01
Cymatocarpus solearis	0.6 (0–3.7)	1280	8.13 (0–24.4)	20.8	0.7

Table 2. Prevalence (P), mean intensity (I) and abundance (A) levels of gastrointestinal parasites of loggerhead sea turtles *Caretta caretta* ($n = 157$) from the Northwestern Adriatic Sea, with confidence interval (95%CI). Relative abundance (RA) and importance index (*I*) are also reported.

	P%		A		*I*	
	< 30 cm	> 30 cm	< 30 cm	> 30 cm	< 30 cm	> 30 cm
Overall helminths	56.5	66.9	16.7	43.3	-	-
Nematoda						
Sulcascaris sulcata	16.7	26.5	0.3	7.5	0.7	18.0
Kathlania leptura	0.0	8.3	0	4.6	0.0	4.6
Trematoda						
Orchidasma amphiorchis	4.3	18.0	0.7	1.9	0.6	4.1
Enodiotrema megachondrus	34.8*	13.5*	13.1	1.91	89.9	3.0
Pachypsolus irroratus	17.4	18.8	1.2	1.9	4.2	4.3
Rhytidodes gelatinosus	21.7	40.6	0.9	10.6	3.7	50.8
Pleurogonius trigonocephalus	8.7	20.3	0.3	5.9	0.5	14.3
Calycodes anthos	8.7*	0.8*	0.3	0.01	0.45	0.0
Cymatocarpus solearis	0.0	0.8	0	9.6	0.0	0.9

Table 3. Comparison of helminths prevalence (P), abundance (A) and importance index (*I*) between epipelagic (SCL < 30 cm) and neritic stage turtles (SCL > 30 cm) among loggerhead sea turtles *Caretta caretta* ($n = 157$) from the Northwestern Adriatic Sea. *p-value at Chi-square test < 0.05.

Group comparison

Intrinsic factors

Comparison of infection indexes between epipelagic/transitional and benthic stage turtles marked a significant difference in the prevalence of *E. megachondrus* ($p = 0.01$, $\chi^2 = 6.37$) and *C. anthos* ($p = 0.01$, $\chi^2 = 6.56$) among the two groups, showing higher values in epipelagic/transitional stage (Table 3). No significant differences in intensity or abundance were detected for any parasitic species (Table 3). *Enodiotrema megachondrus* also had very high relative abundance and importance in the epipelagic/transitional stage turtles, while for benthic turtles the distribution of species RA and *I* were similar to the overall sample (Table 3 and Supplementary Table S1). Shannon-Wiener's diversity index was 0.86 and 1.90 for the two groups, with benthic turtles demonstrating greater diversity.

No differences in infection indices of helminthiases were found among males and females except for *P. trigonocephalus*, which showed significantly higher prevalence in males ($p = 0.046$, $\chi^2 = 3.96$) (Supplementary Table S2).

Extrinsic factors

Comparing the two periods of sampling considered, general abundance and intensity of helminthiases marked a significant increase in *period2* (Table 4), and also the increase of prevalence of helminthiases in *period2* almost reached significativity ($p = 0.06$, $\chi^2 = 3.34$). Specific species also showed significant increase in prevalence (i.e. *S. sulcata* and *P. trigonocephalus*, $p = 0.01$, $\chi^2 = 5.93$ and $p < 0.01$, $\chi^2 = 9.53$), intensity (i.e. *E. megachondrus* and *R. gelatinosus*) and abundance (i.e. *P. trigonocephalus*) in the second period (Supplementary Table S3). Finally, importance index of *E. megachondrus* and of *P. trigonocephalus* markedly raised in *period2* (Table 4). Shannon-Wiener's diversity index was similar for the two periods, being 1.74 and 1.85 for *period1* and *period2*, respectively.

	P%		**A**		***I***	
	period1	*period2*	*period1*	*period2*	*period1*	*period2*
Overall helminths	59.6	73.5	17.1°	67.9°	-	-
Nematoda						
Sulcascaris sulcata	19.1*	26.5*	4.9	6.6	25.4	12.6
Kathlania leptura	5.6	8.8	2.4	6.4	3.8	3.8
Trematoda						
Orchidasma amphiorchis	19.1	11.8	2.1	1.3	10.7	1.1
Enodiotrema megachondrus	12.4	22.1	0.7	7.3	2.2	11.7
Pachypsolus irroratus	18.0	19.1	1.6	2.1	7.9	2.9
Rhytidodes gelatinosus	37.1	39.7	4.7	14.9	48.0	42.8
Pleurogonius trigonocephalus	10.1*	29.4*	0.7°	10.9°	2.0	23.1
Calycodes anthos	2.2	1.5	0.0	0.0	0.03	0.0
Cymatocarpus solearis	0.0	1.5	0.0	18.8	0.0	2.0

Table 4. Comparison of helminths prevalence (P), abundance (A) and importance index (*I*) between periods of stranding (*period1*, 2009–2014 and *period2*, 2015–2023) of loggerhead sea turtles *Caretta caretta* ($n = 157$) from the Northwestern Adriatic Sea. *p-value at Chi-square test < 0.05; °p-value at Mann-Whitney test < 0.05.

Finally, overall prevalence, abundance and intensity of helminthic infections were higher in the cold season than in the warm one; the same trend was found for selected helminth species, namely *S. sulcata*, *O. amphiorchis*, *E. megachondrus* and *P. trigonocephalus* (Supplementary Table S4).

Discussion

The strict connection between parasite diversity and marine ecosystem makes the host-parasite interaction a useful tool for the evaluation of marine health and the integrity of trophic webs. This wide-scale study on the gastrointestinal parasitic community of *C. caretta* in the Adriatic Sea offered a worthwhile view on the infection indices and helminth community structure in this species, highlighting the relationship between parasitic communities and ecosystem types. General results on the diversity confirm the assumption and evidence that benthic feeders in neritic areas show high diversity level, with typical species exploiting benthic intermediate hosts, such as the anisakid nematode *S. sulcata*, having high prevalence and abundance. Indeed, diversity and prevalence are already reported to be higher in neritic grounds when compared with oceanic developmental zones populated by smaller, epipelagic stage turtles, both within and outside the Mediterranean Sea[17,26]. The wide diversity of prey composing the diet of benthic stage turtles stands for higher probability of diverse parasites acquisition, and higher host density in these foraging grounds increases the probability for infective forms to reach their target host, increasing prevalence and abundance[17,27]. In this study, parasite diversity and species richness was similar to other neritic areas in the Mediterranean, with slightly higher diversity ($H = 1.95$) but a comparable number of species composing the community. Richness was similar to that reported in a coastal area off Campania region in Italy by Santoro et al.[17]. Eleven species of helminths constituted the parasitic community in that area, with nine of them being sea turtle specialists. Similarly, Gračan et al.[20] reported eight helminth taxa, in a survey carried out on the Eastern part of the Central portion of the Adriatic Sea, here including two non-specific nematode genera, i.e. *Anisakis* and *Hysterothylacium*. The high values of species richness found in both previous surveys as well as the present study were not reflected by diversity at the infracommunity level: a mean value of 1.60–1.89 was calculated by Santoro et al.[17], while Gračan et al.[20] report most turtles (67.5%) being infected with one or two parasitic taxa. Our survey reports a mean individual species richness of 1.39 per turtle and 66.0% of the animals hosting one or two helminth species. Being that the great majority of helminths are acquired by sea turtles through consumption of invertebrate prey, this low individual richness has been coupled with low food intake due to heterothermy of the host[16,17,19], resulting in a low recruitment rate. Additionally, we speculate that the probability of infection may be further reduced if consumption of a specific prey item is carried out with low abundance and sporadically in a highly diversified diet[16], considering that trematodes are often distributed with low prevalence among intermediate hosts populations in aquatic environments[28,29]. In this case, encounters with the infective larval form may come as a scarcely probable event during feeding.

A comparison of diversity and species richness with other neritic grounds outside the Mediterranean Sea is not easily conducted, as most studies in literature focus on single species reporting or include limited samples. An interesting comparison can be made with a study by Greiner et al.[26] in Florida, which reports infection indices and describes the diversity of the entire helminthic community of *C. caretta* in the area. Parasite community of loggerheads in our study, although among the highest in the Mediterranean, appears quite depauperate when compared to northwestern Atlantic neritic grounds, both at component and infracommunity levels. Indeed, Greiner et al.[26] reported 16 different taxa from the gastrointestinal tract of loggerheads, here including four nematode and thirteen trematode species, and overall species richness per turtle was 8.7. Overall prevalence for helminthiases and species-specific values, as well as intensity values, were also markedly higher in that survey - almost double for some species also present in Mediterranean grounds. Diversity of the tropical ecosystem both at biotic and abiotic factors, coupled with the co-occurrence of several different species of sea turtles may enhance parasite diversity, as the exchange of parasites with broad host spectrum appear as a much more probable event in that context than in central Mediterranean waters, where *C. caretta* is almost the exclusive species[19].

A look at the composition and structure of the parasitic community reported in this survey also remarks its similarity to other neritic grounds mostly populated by large-sized animals at their benthic stage. The highest importance was indeed assigned to the species *S. sulcata* and *R. gelatinosus*, both taxa typically reported with moderate to high prevalence in loggerhead sea turtles in other surveys in neritic grounds within the Mediterranean[20,21,30–32] and outside[26,33,34]. Intermediate hosts have been extensively described only for the nematode *S. sulcata* within the groups of the Bivalvia, mostly in the family Pectinidae, and of gastropods, accounting for the high prevalence of this nematode in turtles foraging on the benthos[24–26,32,35]. A comparison with the loggerhead population sampled in the central-eastern Adriatic Sea marks a relevant difference in the prevalence of *Anisakis* spp., which was the dominant species in the study by Gračan et al.[20] but was completely lacking in this survey. A lower prevalence of this heteroxenous nematode in the Northern Adriatic Sea, compared to the Central and Southern regions, is reported also in intermediate/paratenic hosts[36,37]. High freshwater loads coming from rivers and the sandy, shallower bottom in the Northernmost part of the basin indirectly affect faunal composition, decreasing the abundance of definitive and intermediate hosts of *Anisakis* spp[38,39]. Nevertheless, such difference in the presence of *Anisakis* sp. larvae between Northwestern and Central-Eastern regions is noteworthy, as migration patterns of loggerheads in the Adriatic Sea are supposed to include overwintering movements of both juveniles and adults between the two subregions[4,7,40,41]. Indeed, as the survival of *Anisakis* spp. in paratenic hosts overcomes the timing for seasonal movements of sea turtles[42], the presence of *Anisakis* sp. would be expected in our sample from the Northwestern region as well. Whether the two feeding grounds may host different, non-mixing stocks of loggerhead sea turtles should deserve further studies, but the employment of *Anisakis* spp. as a biological tag would represent an interesting perspective in this species, being already used as such for other paratenic and definitive host species[43–45]. Another difference in community composition between Central and Northern Adriatic Sea concern the nematode species *Kathlania leptura* and the trematodes *Cymatocarpus solearis* and *Pleurogonius trigonocephalus*, which were only isolated in the present survey but were absent in the study by Gračan et al. 2012 along the central Eastern coast[20]. *Kathlania leptura* and *P. trigonocephalus* are reported with variable frequency from other neritic grounds worldwide[17,32,34,46,47] and from the Adriatic Sea as well in previous surveys on the Western coast[21,23]. *Cymatocarpus*, a monotypic genus including the species *solearis*, synonimised with *C. undulatus*, is rarely reported in the Mediterranean Sea[21,48] and at low prevalence rates (3.3–1.5%), although other sporadic reports do exist from the Adriatic Sea[30,49]. As the second intermediate host of *C. solearis* has been identified in decapods of the species *Panulirus argus*[50] and the sandy bottom of Northern Adriatic Sea does not represent a suitable habitat for members of this genus, a low prevalence of *C. solearis* in the area is expected, and its sporadic presence in the hosts may help trace a past foraging activity in rocky grounds, including the nearby regions of Southern or Eastern part of Adriatic Sea[51].

The results of the analysis of the effect of intrinsic and extrinsic drivers on the community structure partially overlap with findings in literature but also report some previously undetected differences between groups. Comparison of epipelagic/transitional stage turtles (CCL < 30 cm) and benthic feeders highlighted higher prevalence of *E. megachondrus* and *C. anthos* in the former group, confirming the overall trend of higher recruitment rate of these parasitic species in animals with a "pelagic" lifestyle, as reported in literature[17]. Indeed, these two cosmopolitan trematodes are extensively reported in the parasitic community of loggerhead sea turtles in pelagic areas, together making up more than 90% of total helminth abundance[17–19]. In the Eastern Adriatic Sea, *E. megachondrus* was detected with a 4.3% rate and exclusively in small juvenile turtles undergoing the ontogenetic habitat shift (CCL < 30 cm)[20]. Although in our study the prevalence of *E. megachondrus* was significantly higher in smaller, epipelagic/transitional stage turtles, it was also detected in large juveniles and adults (CCL range 34–80 cm) with a 13.5% rate. A reason for this could be found in migratory behavior of large juvenile and adult loggerhead sea turtles, which may acquire a diverse parasitic fauna during seasonal or reproductive migrations in oceanic grounds[4,5,8,52]. Alternatively, the high dietary plasticity of loggerheads may also account for life-long consumption of ubiquitarian pelagic and epipelagic prey[15,16,53] conveying larval trematodes of this species also in neritic grounds.

Differences in the prevalence of *P. trigonocephalus* between male and female sea turtles are not yet well explored within the literature. Different migratory routes are possible for mature males and females during the reproductive season[6], but a relatively low number of individuals included in this study already attained the size for sexual maturity, i.e. >70 cm CCL[4]. Other differences in dietary habits between males and females have not yet been evidenced in the Mediterranean Sea[15]. We may suspect a *bias* effect due to the differing sample sizes of the two sex groups (see Table 1), nevertheless, further investigations including a multivariate analysis may be advocated in this topic.

Trematode prevalence in molluscan intermediate hosts generally shows a peak in late summer/autumn, decreasing gradually during the coldest season[54,55]. A combination of long prepatent period of trematodiases and decreased immune function in cold temperatures in reptiles[56] may account for an increase in parasite prevalence and burden in winter. On the other side, considering that sea turtle feeding rate slow down with low temperatures[57], an increased parasite recruitment in this period should be excluded. Our results on seasonal variations in infection indices for helminthiases somehow differ with previous findings in literature and report such an increase during the cold season. Gracan et al. 2012[20] and Santoro et al.[17] report no significant difference in prevalence and abundance of helminthiases in loggerheads between cold and warm seasons, while in this study overall prevalence, intensity and abundance of helminths were significantly higher during the colder months, and specific species also followed the same trend. We could advocate the aforementioned ecological reasons for such a seasonal trend, nevertheless this topic requires a deeper understanding of infection dynamics and knowledge of specific parasites species biology to better interprete these results.

Due to their complex life cycles, involving two to three hosts, and free-living forms, marine trematode communities are considered particularly sensitive to environmental changes, and can act as useful sentinels for any variations in biotic and abiotic components of the ecosystem[1,58,59]. During the 15 years of observation, a remarkable increase in abundance and intensity of general helminthiases was reported, and also general prevalence increased, with this difference almost reaching statistical significativity ($p = 0.06$). Moreover, two trematode species, i.e. *E. megachondrus* and *P. trigonocephalus*, markedly raised in importance within the community in the second period. The relevant environmental changes to which the Mediterranean Sea is particularly subject as a result of global warming, have led to indisputable perturbations of the food web, starting from phytoplanktonic and zooplanktonic communities up to the benthic community[60–64] and to predator population distribution[65]. More in detail, water temperature is well-known to influence parasite egg developmental rate, survival of free living cercariae and larvae and, finally, helminth infection patterns. Although little is known about biology and life cycles of sea turtle trematode species, their worldwide distribution at tropical latitudes let us speculate a good adaptation to higher marine temperatures, which may facilitate their spread in the scenario of a warming Mediterranean Sea. As to the sea turtle population, relevant changes of the distribution within the Mediterranean have been observed as a consequence of global warming, with a west and northward shift of nesting sites occurring at an unexpectedly fast rate[65,66]. Moreover, as the warming of Mediterranean waters offers a progressively more suitable habitat for the green turtle, *Chelonia mydas*, outside its historical range distribution[67], changes in helminth distribution may also come as a consequence of a newly available host species in the central Mediterranean. Although environmental changes are generally object of larger time-scale studies, this study highlights relevant changes in the helminth community of loggerhead sea turtles in a relatively short period of observation, which may shed a light on the current ongoing massive and fast environmental change and its indirect effects at the top of the marine food chain.

Conclusions

Data collected in this survey bring new evidence to the assumption that the parasitic community of loggerhead sea turtles is strongly influenced by ecosystem type. A look at the diversity of the parasitic community reflects the high faunal biodiversity and overall integrity of food webs in the area, accounting for the maintenance of the life cycle of several heteroxenous parasite species. Observation of parasitic infection at the top of the food chain prove as a valuable tool to monitor perturbations of the entire trophic web undergoing at a surprisingly fast rate as a consequence of global warming. Changes in the helminth community and patterns of infection here observed in a relatively short period, shed light on possible perturbations of parasites ecology, eventually associated to environmental change. Knowledge on life cycles of most of the helminths considered here are still too scarce to achieve a complete understanding of the mechanisms behind the changes in prevalence, abundance and intensity of helminthiases and of community structure. Nevertheless, the fast changes in abiotic and biotic components of the ecosystem, undergoing as a consequence of global warming in the Mediterranean, could probably account for a relevant part of the phenomenon. How temperature actually influence helminth biology and infection patterns would deserve further studies, as a notable interseasonal discrepancy on infection parameters was also observed here for the first time. This also would help foresee how host-parasite dynamics may be altered in the next decades under the scenario of an increasingly warming sea.

Materials and methods

Analysis of the gastrointestinal helminthic community was performed on loggerhead sea turtles *Caretta caretta*, stranded dead along the northwestern Adriatic coast in a fifteen year period (2009–2023). Following recovery, carcasses were transported and submitted to necropsy at the Department of Comparative Biomedicine and Food Science of the University of Padova (Legnaro, Italy). Samples were obtained from a CITES-approved scientific Institution (the Mediterranean Marine Mammal Tissue Bank) (IT 020) based at the Department, that works in agreement with the Italian Ministry of the Environment. Curved carapace length was measured from nuchal scute to supracaudal tip to allow classification in age categories; a cut-off of 30 cm was set to define epipelagic/transitional stage and benthic stage turtles[10,14]. Sex determination was performed following direct observation of gonads[68].

During necropsy, the gastrointestinal tract was isolated after firmly tightening the esophagus in its most cranial portion, and the rectum. After longitudinal opening, the contents of esophagus and stomach, and intestinal tract were separately collected and filtered with 1.0 and 0.5 mm mesh sieves for the isolation of helminths. All parasites recovered were washed in physiological saline (NaCl 0.9%), counted and fixed in 70% ethanol, and subsequently identified using a stereomicroscope or optic microscope by their morphometric features, following specific literature[69,70].

Data analysis

To describe parasite population structure, prevalence (P) (number of hosts infected over the total number of hosts examined), mean abundance (mA) (mean number of parasites in each host examined, regardless of whether or not the host was infected) and mean intensity (mI) (mean number of parasites in the infected hosts) were calculated for each taxon[71]. The 95% confidence intervals (95%IC) of prevalence were calculated by Sterne's exact method, while for the mean intensity and mean abundance, bootstrapping with 2000 bootstrap replications was used. Component community structure was described by relative abundance (RA) (proportion of individuals in a species among the total number of helminths), species richness (total number of helminth species), and importance index (*I*), which was calculated as follows: $I_j = A_j x B_j / A_i x B_i$, where A_j=number of parasite for species $_j$; B_j= Number of host infected with species $_j$; A_i and B_i represent the total number of parasite observed and the total number of infected hosts with any parasite species, respectively[72]. Finally, Shannon-Wiener's diversity index (H) was calculated with the formula $H=-\sum[(p_i)\times\ln(p_i)]$, in which p is the proportion of individuals of the species i compared to the total number of helminths in the population.

The influence of intrinsic factors - ontogenetic stage (epipelagic/transitional or benthic) and sex - and extrinsic factors - period of sampling (*period1*, 2009–2014 and *period2*, 2015–2023) and season (*cold*, November to April; *warm*, May to October) - on each parasitic species was assessed for prevalence, mean intensity and mean abundance using the Chi-square test (df = 1) and the Kruskal-Wallis test, respectively, which were run on the online software Quantitative Parasitology[73]. Furthermore, differences in community structure among groups were assessed through calculation of RA and I for each group separately and descriptively evaluated. Shannon-Wiener's diversity index (H) was also compared between the groups belonging to the two ontogenetic stages and periods of sampling.

Data availability

Data is provided within the manuscript and supplementary information files.

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Author contributions

E.M.: conceptualization; investigation; methodology; formal analysis; writing - original draft. R. C.: conceptualization; formal analysis; writing - review and editing. E.S.: formal analysis. C. C.: Investigation; F.M.: conceptualization; investigation; methodology; writing - review and editing.

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Declarations

Competing interests

The authors declare no competing interests.

Human and animal participants

All animals involved in the research were found already dead at the time of stranding. Samples were obtained from a CITES-approved scientific Institution (the Mediterranean Marine Mammal Tissue Bank) (IT 020) based at the Department of Comparative Biomedicine and Food Science, that works in agreement with the Italian Ministry of the Environment.

Additional information

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