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First multicenter coprological survey on helminth parasite communities of free-living loggerhead sea turtles *Caretta caretta* (Linnaeus, 1758) from the Adriatic Sea and Northern Ionian Sea

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ABSTRACT

The prevalence of endoparasite infections in 83 free-living specimens of *Caretta caretta*, classified as vulnerable species, from the Adriatic Sea and Northern Ionian Sea was investigated by coprological examination. Thirty-seven (44.6%) turtles were found to be infected with helminths. The helminth infections found were: *Rhytidodes gelatinosus* and *Sulcascaris sulcata* (18.1% each), *Hapalotrema mistroides* (13.2%), *Cymatocarpus solearis* (9.6%), *Eniodotrema megachondrus* (7.2%), *Kathlania/Tonaudia* sp. (3.6%), *Neospirorchis* sp., *Octangium sagitta* and *Plesiochorus cymbiformis* (1.2% each). There were no significant differences in the total prevalence of helminth infections between sexes, size classes, and seasonal periods. Conversely, the prevalence of helminth infections was significantly higher ($P < 0.01$) in accidentally caught turtles than in stranded turtles. Highly significant differences in prevalence of helminthiases were also seen among marine sampling areas. This report provides important baseline information about the helminth fauna of free-living *C. caretta* in the examined geographical region. This is also the first report of *O. sagitta* infection in *C. caretta* thus broadening the host range of the parasite.

1. Introduction

The marine turtle *Caretta caretta* (Linnaeus, 1758), commonly named loggerhead sea turtle, is widely distributed in temperate and tropical waters throughout the world, including the Atlantic, Pacific and Indian Oceans (Wallace et al., 2010). In addition, *C. caretta* is the most common sea turtle species in the Mediterranean Sea, nesting on beaches of Greece, Turkey, Libya, and Cyprus (Casale and Margaritoulis, 2010) as well as on some beaches of Southern Italy (Mingozzi et al., 2007; Carlino et al., 2019). Unfortunately, this sea turtle species is strongly threatened throughout the Mediterranean basin. Indeed, the accidental capture in fishing gears, usually referred to as bycatch, is a serious problem for loggerhead turtles because they frequently come in contact with fisheries (Cambie et al., 2012; Pulcinella et al., 2019). Moreover, many of their nesting areas are threatened by fishing activity (Cambie et al., 2010) and tourism development (Poland et al., 1995; Taylor and Cozens, 2010). Therefore, according to the International Union for the Conservation of Nature (IUCN), *C. caretta* is considered as vulnerable species within the Mediterranean basin, including the Italian territorial waters (Casale and Tucker, 2017).

The role of parasites has attracted great attention in conservation biology (Preston and Johnson, 2010) and parasitism can be considered a primary factor underlying the dynamics of wild animal populations (Irvine, 2006). In particular, parasites can regulate host population size through their impact on the host reproductive potential (Anderson and May, 1978) and on the rate of host mortality (Anderson and May, 1978). Parasites can also spread more and more in wild animal populations, particularly when they act together with ecological, biological, and anthropogenic factors (Kołodziej-Sobocińska, 2019), which could be a particularly critical aspect in conservation of threatened species (Thompson et al., 2010). Therefore, it is essential to determine the

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prevalence and species composition of parasite communities in wild animal populations, in order to better understand the management of their conservation status.

There have been some previous studies investigating the helminth fauna of loggerhead turtles in Brazil (Werneck et al., 2018), Portugal (Valente et al., 2009), and Mediterranean countries such as Spain (Aznar et al., 1998), Croatia (Gračan et al., 2012), and Tunisia (Karaa et al., 2019). Additionally, there have only been a limited number of published studies regarding the prevalence of helminths in *C. caretta* populations in Italy. Two were necropsy surveys on a small number (n = 14 each) of loggerhead turtles found stranded or found dead following incidental capture by fisheries (Manfredi et al., 1998; Piccolo and Manfredi, 2001). Three comprehensive investigations focused on the helminth communities of loggerhead turtles (Santoro et al., 2010) or on the presence and pathological findings caused by cardiovascular flukes (Marchiori et al., 2017) and *Sulcascaris sulcata* (Santoro et al., 2019). To date, there is only one recent coprological study providing baseline data to determine the occurrence of helminths in 30 loggerhead sea turtles in Italy (Pace et al., 2019).

Given the diversification of the marine and coastal environment across the Mediterranean Sea, the composition of parasite communities may differ between various loggerhead turtle populations. Therefore, it is possible that populations of Mediterranean loggerhead sea turtles with different range of foraging and nesting areas can harbor different endoparasite patterns. In order to give further insights, the aims of the present coprological survey were *i)* to establish a baseline of endoparasites of *C. caretta* in the Adriatic Sea and Northern Ionian Sea, *ii)* to evaluate whether some epidemiological factors may affect *C. caretta* endoparasite prevalence, and *iii)* to discuss the potential relevance of the parasite infections detected to the health of this sea turtle species.

2. Material and methods

2.1. Study area

From August 2018 to August 2019, 83 loggerhead sea turtles were hospitalized at four Sea Turtle Rescue Centres located along the Italian coast of the medium low Adriatic Sea and the Northern Ionian Sea: Pescara Rescue Centre (Site A, 42°52′41.88″ N 13°55′23.33″ E - 41°55′40.72″ N 15°8′12.62″ E), Manfredonia Rescue Centre (Site B, 42°03′54.22″ N 14°47′16.45″ E - 41°13′50.14″ N 16°32′21.88″ E), Torre Guaceto Rescue Centre (Site C, 40°53′27.4″ N 17°23′21.3″ E - 40°24′33.2″ N 17°12′27.5″ E) and Calimera Rescue Centre (Site D, 40°30′56.15″ N 18° 5′52.21″ E - 40°18′3.10″ N 17°45′54.35″ E) (Fig. 1).

This specific stretch of coast is located in the central Mediterranean Sea between the Italian and the Balkan Peninsula. It extends between the regions of Abruzzo and Apulia, along a total of approximately 700 km of coastline characterized for about 70% by sandy beaches and the remaining by pebbles or rocks. The stretch of sea reaches a surface of 132.000 km^2 and a depth of 1.222 m. Due to the presence of shallow sandy beaches and high density of edible fish species, many fishing activities are widely practiced, such as bottom trawls and small-scale fishing with longline or gillnets. In particular, Sites A and B are characterized by an intensive bottom trawling that presumably takes place over 3 nautical miles beyond a bathymetric line of 50 m. Sites C and D are characterized by fishing methods using gillnets and longlines.

2.2. Sampling

Out of 83 turtles, 37 were found stranded along the coast but still alive and 46 were accidentally caught by fishermen. Twenty-three of them had a mean curved carapace length notch-to-tip (CCL) > 70 cm, 36 had a mean CCL ≤70 cm - > 35 cm and 24 had a mean CCL ≤35 cm, according to the classification of Margaritoulis et al. (2003).

Once in the Sea Turtle Rescue Centre, each turtle was subjected to the first clinical examination according to the Ministerial Guidelines of the Italian Institute for Environmental Protection and Research, ISPRA (Mo et al., 2013), received, if needed, rehydration with fluidic therapy and vitamin administration, and was kept in an individual basin with salt water.

In addition to CCL, other biometric parameters such as total tail length and weight as well as health parameters (i.e., vitality, sensory evaluation, muscle tone or flaccidity, state of nutrition and appetite, absence or presence of injuries or trauma to hard tissues, fins and head, immersion capacity and floating position) were individually annotated. Sex identification was made with considerable certainty only in 28 turtles (21 females and 7 males) showing evident external characteristics of sexual dimorphism (Casale et al., 2005).

Since the main method to diagnose endoparasite infections is based on faecal examination to detect eggs, a faecal sample was collected, as soon as possible, from each turtle after the first spontaneous voiding. After collection in a sterile falcon, the sample was labelled for turtle identification, kept refrigerated, and delivered to the Parasitology Lab of the University of Foggia within 24 h for further analysis.

2.3. Qualitative and quantitative copromicroscopic analyses

Each faecal sample was subjected to microscopic investigation by using the Mini-FLOTAC® technique, slightly modified, in combination with the Fill-FLOTAC® and a flotation solution of ZnSO4 (specific gravity 1360). The method has an analytic sensitivity of 5 eggs per gram (EPG) of faeces, as reported originally in Cringoli et al. (2017). For the purpose of this study, the technique was adapted to the small amount of faecal material available from turtles, as described by Pace et al. (2019). Eggs were identified by their morphologic and morphometric characteristics as reported by Greiner (2013). The number of EPG of faeces was calculated according to the practical technical instructions presented at the manufacturer's website (https://www.parassitologia.unina.it/wp-content/uploads/2015/07/001-Dog-and-Cat-3.pdf).

2.4. Statistical analysis

The prevalence of each helminth parasite was determined as number of coprologically positive turtles/number of examined turtles X 100 together with the corresponding 95% confidence interval (95% CI). Mean (M), standard error of the mean (SEM), and range of EPG of faeces were also calculated (Table 1). In addition, prevalence and 95% CI of multiple infections were determined as above described (Table 2).

Samples were stratified according to the following categorical variables: CCL size class (> 70 cm, ≤70 cm - > 35 cm, ≤35 cm), type of finding (stranding vs. bycatch), seasonal period (spring-summer vs. autumn-winter), and marine study area (A, B, C, D). Differences in prevalence values between groups were compared by chi-square test (Table 3). Odd-ratios with the corresponding 95% CI were also calculated as a measure of the risk. The Student's t-test or the ANOVA test were used to determine the statistical significance between differences in mean EPG counts according to the variables mentioned above (Table 4). *P* values < 0.05 and < 0.01 were considered significant and highly significant, respectively.

3. Results

Overall, parasite eggs were detected in 37 (44.6%) out of 83 faecal samples from loggerhead sea turtles examined by the modified Mini-FLOTAC® technique. Eggs of nine helminth parasites were identified. These included eggs of six species and one genus of trematodes as well as one species and one genus of nematodes. In decreasing order of frequency, the most prevalent helminths were *Rhytidodes gelatinosus* and *S. sulcata* (18.1% each) followed by *Hapalotrema mistroides* (13.2%), *Cymatocarpus solearis* (9.6%), *Eniodotrema megachondrus* (7.2%), *Tonaudia/Kathlania* sp. (3.6%), finally *Neospirorchis* sp.,

Fig. 1. Map of the four turtle rescue centres located along the medium low Adriatic Sea and Northern Ionian Sea of the Italian Mediterranean coast.

Octangium sagitta and *Plesiochorus cymbiformis* (1.2% each). The mean number of EPG of faeces ranged from 5 for *O. sagitta* and *P. cymbiformis* to 104.5 for *S. sulcata*. Eggs of *R. gelatinosus*, *H. mistroides*, and *C. solearis* were detected in faeces from turtles of all four examined areas, while *Neospirorchis* sp., *O. sagitta* and *P. cymbiformis* infections occurred in one area and the remaining parasite infections in two areas. The list of helminth infections identified, number of positive samples, prevalence values with 95% CI, Ms with SEMs and ranges of EPG of faeces, and distribution of parasites in the examined areas are shown in Table 1.

Single parasitic infections were detected in 20 (24.1% [95% CI: 14.9–33.3%]) faecal samples while 11 (13.2% [6–20.5%]), 5 (6% [0.9–11.1%]), and 1 (1.2% [0–3.5%]) turtles showed infections with

Table 1
Eggs of helminths detected in individual faecal samples from sea turtles (n = 83) by coprological analysis: number of positive samples, prevalence (%), 95% confidence interval (in brackets), mean ± standard error of the mean (M ± SEM), range of eggs per gram (EPG), and parasite distribution in four sampling areas in the Adriatic Sea and Northern Ionian Sea.

Helminths	No. positive samples	EPG		Distribution			
		M ± SEM	Range	Area A	Area B	Area C	Area D
Rhytidodes gelatinosus	15 18.1% (9.8–26.3%)	29.4 ± 5.5	5–70	+	+	+	+
Sulcascaris sulcata	15 18.1% (9.8–26.3%)	104.5 ± 24.6	10–280	+	+		
Hapalotrema mistroides	11 13.2% (5–20.5%)	14.2 ± 2.3	5–30	+	+	+	+
Cymatocarpus solearis	8 9.6% (3.3–16)	68.7 ± 29	5–200	+	+	+	+
Eniodotrema megachondrus	6 7.2% (1.6–12.8%)	10.5 ± 4.9	5–35	+	+		+
Tonaudia/Kathlania sp.	3 3.6% (0–7.6%)	51.6 ± 24.9	5–90	+	+		
Neospirorchis sp.	1 1.2% (0–3.5%)	5*	ND	+			
Octangium sagitta	1 1.2% (0–3.5%)	115*	ND		+		
Plesiochorus cymbiformis	1 1.2% (0–3.5%)	5*	ND		+		

*Single sample.

Table 2
Number of positive samples, prevalence, and 95% confidence interval of infections with one, two, three and four parasites species detected by coprological analysis in sea turtles (n = 83) from four sampling areas in the Adriatic Sea and Northern Ionian Sea.

Infections	Helminths and their associations	Positive samples	Prevalence (%)	95% CI (%)
One species	*Cymatocarpus solearis*°	7	8.4	2.5–14.4
	Hapalotrema mistroides °	4	4.8	0.2–9.4
	*Sulcascaris sulcata**	4	4.8	0.2–9.4
	Rhytidodes gelatinosus°	2	2.4	0–5.7
	Enodiotrema megachondrus°	1	1.2	0–3.5
	Neospirorchis sp.°	1	1.2	0–3.5
	Plesiochorus cymbiformis°	1	1.2	0–3.5
Two species	*R. gelatinosus*° – *S. sulcata**	5	6	0.9–11.1
	H. mistroides° – *R. gelatinosus*°	4	4.8	0.2–9.4
	E. megachondrus° – *H. mistroides*°	1	1.2	0–3.5
	E. megachondrus° – *R. gelatinosus*°	1	1.2	0–3.5
Three species	*E. megachondrus*° – *S. sulcata** – *Tonaudia/Kathlania**	2	2.4	0–5.7
	C. solearis° – *R. gelatinosus*° – *S. sulcata**	1	1.2	0–3.5
	E. megachondrus° – *R. gelatinosus*° – *S. sulcata**	1	1.2	0–3.5
	H. mistroides° – *R. gelatinosus*° – *S. sulcata**	1	1.2	0–3.5
Four species	*H. mistroides*° – *Octangium sagitta*° – *S. sulcata** – *Tonaudia/Kathlania**	1	1.2	0–3.5
Total		37	44.6	33.8–55.3

* nematodes, °trematodes.

two, three, or four helminth species, respectively. Helminth associations detected in concurrent infections along with number of positive samples, prevalence rates and 95% CI are shown in detail in Table 2.

With respect to the categorical variables taken into consideration, the prevalence of helminth infections was higher in males, in turtles with CCL size > 70 cm, and during the autumn-winter period (Table 3). Mean EPG counts were higher in males, in turtles with CCL size ≤35 cm, in accidentally caught turtles, during the autumn-winter period, and in the marina area D (Table 4). However, these differences did not reach statistical significance. Conversely, statistical analysis indicated that there were highly significant differences between the prevalence of helminth infections in turtles accidentally caught by gillnets or shrimp trawl nets vs. stranded ones and in the total prevalence values from areas A and B vs. area D (Table 3).

4. Discussion

Results of our study provide baseline information on helminth infections occurring in loggerhead sea turtles from the Adriatic Sea and Northern Ionian Sea. In this study, the modified Mini-FLOTAC® technique in combination with Fill-FLOTAC® was used for coprological detection of helminth eggs and counts of EPG of faeces. The FLOTAC® technique has successfully been used in previous coprological surveys for the detection of gastrointestinal parasites in *C. caretta* (Pace et al.,

Table 3
Number of positive samples/number of examined samples, prevalence (%) and 95% confidence interval (in brackets) of helminth infections in sea turtles (n = 83) found stranded or by caught in four sampling areas in the Adriatic Sea and Northern Ionian Sea according to sex, CCL size class, type of finding, and seasonal period, as determined by coprological analysis.

Variables		Areas				Total
		A	B	C	D	
Sex	Females	1/3	5/9	1/2	1/7	8/21
		33.3% (0–86.7%)	55.5% (23.1–88%)	50% (0-100%)	14.3% (0–40.2%)	38.1% (17.3–58.9%)
	Males	3/4	1/2	0/0	0/1	4/7
		75% (32.6–100%)	50% (0-100%)	0% (0-0%)	0% (0-0%)	57.1% (20.5–93.8%)
	Unknown	10/18	9/16	3/7	3/14	25/55
		55.6% (32.6–78.5%)	56.2% (31.9-80-6%)	42.9% (6.2–79.5%)	21.4% (0–42.9%)	45.4% (32.3–58.6%)
CCL size class	> 70 cm	5/8	6/9	1/2	1/4	13/23
		62.5% (28.9–96%)	66.7% (35.9–97.5%)	50% (0-100%)	25% (0–67.4%)	56.5% (36.3–76.8%)
	≤70 cm –	5/12	7/15	2/3	1/6	15/36
	> 35 cm	41.7% (13.7–69.6%)	46.7% (21.4–71.9%)	66.7% (13.3–100%)	16.7% (0–46.5%)	41.7% (25.6–57.7%)
	≤35 cm	4/5	2/3	1/4	2/12	9/24
		80% (44.9–100%)	66.7% (13.3–100%)	25% (0–67.4%)	16.7% (0–37.5%)	37.5% (18.1–56.9%)
Finding	Stranded	5/12	2/5	1/5	2/15	10/37[a]
		41.7% (13.7–69.6%)	40% (0–82.9%)	20% (0-55%)	13.3% (0–30.5%)	27% (12.7–41.3%)
	Accidentally caught	9/13	13/22	3/4	2/7	27/46[a]
		69.2% (44.1–94.3%)	59.1% (38.5–79.6%)	75% (32.6–100%)	28.6% (0-62%)	58.7% (44.5–72.9%)
Season	Sping/Summer	3/8	4/7	0/1	1/7	8/23
		37.5% (3.9–71%)	57.1% (28.5–93.8%)	0% (0-0%)	14.3% (0–40.2%)	34.8% (15.3–54.2%)
	Autumn/Winter	11/17	11/20	4/8	3/15	29/60
		64.7% (42-87-4%)	55% (33.2–76.8%)	50% (15.3–84.6%)	20% (0–40.2%)	48.3% (35.7–61%)
Total		14/25 [b]	15/27[c]	4/9	4/22 [b,c]	37/83
		56% (36.5–75.5%)	55.6% (36.8–74.3%)	44.4% (12–76.9%)	18.8% (2.1–34.3%)	44.6% (33.8–55.3%)

[a] Highly significant difference.
[a] P = 0.0039, chi-square = 8.32, odd-ratio = 3.84 (1.51–9.76).
[b, c] Highly significant differences.
[b] P = 0.0078, chi-square = 7.08, odd ratio = 5.73 (1.50–21.89).
[c] P = 0.0076, chi-square = 7.13, odd ratio = 5.63 (1.50–21.12).

Table 4
Mean numbers of helminth eggs per gram (EPG) of faeces ± standard error of the mean (SEM) and range observed in faecal samples from 37 Mediterranean Sea turtles (*Caretta caretta*) found coprologically positive by modified Mini-FLOTAC® technique according to sex, CCL size class, type of finding, seasonal period, and marine area.

Variables		Mean EPG ± SEM	Range
Sex*	Females	33.3 ± 22.6	5–280
	Males	68.2 ± 20.4	5–195
CCL size class	> 70 cm	61.3 ± 18	5–280
	≤70 cm - > 35 cm	35.2 ± 9.7	5–270
	≤35 cm	63.7 ± 19.1	15–200
Finding	Stranded	41.8 ± 11	5–200
	Accidentally caught	50.6 ± 10.5	5–280
Seasonal period	Spring/summer	21.4 ± 5.9	5–75
	Autumn/winter	57.1 ± 10.3	5–280
Marina area	A	44.3 ± 10.2	5–195
	B	51.7 ± 13.4	5–280
	C	12 ± 3.4	5–20
	D	89 ± 45.5	5–200
Total		48-8 ± 8.3	5–280

*Sex could be identified in 28 animals.

2019) and in different species of tortoises (Dipineto et al., 2012). The Mini-FLOTAC® can be considered as one of the most accurate methods for coprological diagnosis of endoparasite infections and egg counting nowadays available in veterinary medicine (Bosco et al., 2014). Therefore, it allowed an accurate and reliable interpretation of the coprological results of the present survey. However, lower detection of trematode eggs is a limitation of the Mini-FLOTAC®, as stated by Cringoli et al. (2017). To our knowledge, only the results of a single similar investigation are currently available in the literature, focused on the coprological analysis of 30 specimens of *C. caretta* with the FLOTAC® technique (Pace et al., 2019). The present detection of coinfections in 17/83 (20.5% [11.8–29.2%]) of the analysed faecal samples is in contrast with data previously reported by Pace et al. (2019), since these authors detected just one parasite species in most of the turtles (9/11) and only two cases of coinfection. Conversely, they found mean counts of EPG of faeces substantially higher (range from 35 to 180 EPG) than those detected in the present survey for each helminth infection (from 5 to 104.5 EPG). This might be due to higher parasite fecundity or higher parasite burdens. Wide variations in parasite burdens can occur depending on several factors such as the parasite life cycle, the availability of hosts necessary to complete the life cycle, the interactions between parasite species, the host immune response, and the host population density (Pace et al., 2019). It is reported that faecal egg counts might not allow an accurate estimation of parasite burdens in reptiles (Jorge et al., 2013). In this survey, it was not possible to assess the true relation between EPG of faeces and helminth infection intensity.

Among the helminth infections detected in the examined loggerhead turtles, the two trematode species *E. megachondrus* and *R. gelatinosus* have frequently been reported in previous studies carried out on different Mediterranean populations of *C. caretta* in Italy (Manfredi et al., 1998; Piccolo and Manfredi, 2001; Santoro et al., 2010; Pace et al., 2019), Croatia (Gračan et al., 2012), Portugal (Valente et al., 2009), and Spain (Aznar et al., 1998). Similarly, the nematode species *S. sulcascaris* has commonly been reported in loggerhead turtles from Italy (Manfredi et al., 1998; Piccolo and Manfredi, 2001; Santoro et al., 2010), Croatia (Gračan et al., 2012), and Brazil (Werneck et al., 2018). Reported ranges of prevalence rates are 4.3–96.3% for *E. megachondrus*, 0–74.1% for *S. sulcascaris*, and 0–51.5% for *R. gelatinosus* (Santoro et al., 2010; Gračan et al., 2012). Eggs of the nematode species *Kathlania leptura* are practically indistinguishable from those of another nematode species, *Tonaudia tonaudia* (Greiner, 2013). *K. leptura* was quite commonly found in studies carried out on loggerhead turtles in Brazil (Werneck et al., 2018) and Italy (Piccolo and Manfredi, 2001; Santoro et al., 2010) with reported prevalence range of 0–18.3% (Santoro et al., 2010). *T. tonaudia* has recently been reported in a specimen of *C. caretta* found stranded in Tunisia (Karaa et al., 2019). Therefore, the prevalence values observed in the present study (18.2%, 18.2%, 13%, and 3.6%) fall within the previously reported prevalence ranges for the above-mentioned parasites. *Cymatocarpus* spp. eggs and adult parasites of *C. solearis* were detected, respectively, in 3.3% (Pace et al., 2019) and 7.2% (Piccolo and Manfredi, 2001) of *C. caretta* sampled along the Italian coasts. Thus the prevalence of 9.6% found for *C. solearis* infection in our survey is higher than values previously reported in Italy. The prevalences of *H. mistroides*, *Neospirorchis* sp., and *P. cymbiformis* found in this survey (13.2%, 1.2%, and 1.2%) are slightly or considerably lower than those previously reported (15.5%, 6%, and 4.5%) for these trematode species in Mediterranean loggerhead sea turtles (Marchiori et al., 2017; Santoro et al., 2010). *O. sagitta* was recorded in 74 dead green sea turtles (*Chelonia mydas*) from Florida (Greiner, 2013). To the best of our knowledge, this is the first report of *O. sagitta* in the loggerhead sea turtle *C. caretta*. The infected turtle was an adult male from area B, with 115 EPG of *O. sagitta* in its faecal sample. In addition to *O. sagitta*, the infected turtle presented a mixed infection with *H. mistroides*, *S. sulcata* and *Tonaudia/Kathlania* sp. The occurrence of *Neospirorchis* sp., *O. sagitta*, or *P. cymbiformis* eggs only in single specimens of *C. caretta* suggests limited sources of infections with *Neospirorchis* sp. and *P. cymbiformis* and occasional infection with *O. sagitta*.

Results of the statistical analysis revealed that loggerhead sea turtles could harbour helminth parasites irrespective of the gender, CCL size class, and sampling period. However, comparing the total prevalence of helminth infections in animals from the four examined areas, there is evidence that prevalence values were higher in areas A and B, reaching highly significant differences in comparison to area D. These results suggest the hypothesis that different geographical areas can be related to different helminth prevalence and distribution. Since areas A and B are located in the medium Adriatic Sea, it is likely that these areas represent important neritic foraging grounds for loggerhead sea turtles where they spent most of the time and thus where they have a greater chance of getting some types of helminth infections. This hypothesis is corroborated by the finding of 7–8 types of helminth infections in areas A and B vs. 3-4 types in areas C and D. Surprisingly, the prevalence of helminth infections in loggerhead sea turtles accidentally caught by gillnets or shrimp trawl nets was higher than in stranded ones, showing a highly significant level. It is reported that parasites, especially spirorchiids, can represent a major threat for sea turtle health and are considered as one of the most important causes of turtle stranding and mortality worldwide (Santoro et al., 2017). However, although many stranded *C. caretta* were infected, results of the present survey suggest that helminth infections were not the main cause of stranding in loggerhead sea turtles. This is in agreement with the findings of Chen et al. (2012) in green turtles stranded on Taiwan. Further investigations, comparing the prevalence and composition of helminth fauna in stranded sea turtles vs. accidentally caught ones, are needed to better understand if parasites may play a role in stranding of sea turtles and in their conservation status.

To our knowledge, no data concerning the potential pathogenic role, if any, of *E. megachondrus*, *R. gelatinosus*, *C. solearis*, *T. tonaudia* and *K. leptura* in *C. caretta* are reported in literature. Similarly, no data are available about the pathogenic role of *O. sagitta* in *C. mydas*. These parasites seem to be just components of the helminth fauna naturally present in marine turtles. Conversely, *H. mistroides*, *Neospirorchis* sp., *S. sulcata*, and *P. cymbiformis* have been reported as agents responsible for serious parasitic diseases in loggerhead sea turtles. *H. mistroides* and *Neospirorchis* sp. are cardiovascular flukes. Their eggs act as emboli and can be responsible for mild to moderate arteritis in the heart and great vessels of *C. caretta* along with multifocal granulomas widespread in the spleen, lung, thymus, and pancreas (Marchiori et al., 2017). *H. mistroides* has also been reported as a confirmed cause of death in a specimen of *C. caretta* (Santoro et al., 2017). Reported pathological changes caused by *S. sulcata* in loggerhead sea turtles are mucous gastritis with ulcerous lesions, atrophic gastritis with heterophilic infiltration in the lamina propria, destruction of the mucosal and submucosal surfaces, and necrosis (Santoro et al., 2019). *P. cymbiformis* has been associated with a case of chronic cystitis in a female loggerhead sea turtle from Brazil (Werneck et al., 2018). In the present survey, no one of the examined loggerhead sea turtles showed clinical signs such as cachexia and digestive disorders referable to severe damages caused by parasitic diseases, thus helminth infections did not have any apparent impact on the general health status of the animals. However, the lack of histopathological data precludes any definitive conclusion about the impact of injuries caused by *H. mistroides*, *Neospirorchis* sp., *S. sulcata*, and *P. cymbiformis* on the health status of the examined animals. Our findings draw attention to the presence of these parasites and to the risk of parasitic diseases that these parasites can cause among loggerhead sea turtles inhabiting the Adriatic Sea and the Northern Ionian Sea.

5. Conclusion

Currently, *C. caretta* is listed as vulnerable species. We detected widespread infections of *C. caretta* with helminth parasites at four rescue centres in the Adriatic Sea and the Northern Ionian Sea. Although helminth infections were not associated with clinical signs, baseline knowledge of the helminth fauna of the loggerhead sea turtle is necessary to better understand parasitic diseases that may emerge in the future as potential conservation threats. This survey provides new data on the prevalence of helminth infections in a population of Mediterranean loggerhead sea turtles and reports for the first time the presence of *O. sagitta* in this host species. Thus, it contributes to the knowledge of the host range of this parasite of sea turtles.

Declaration of competing interest

The authors declare that they have no conflict of interest and that they have no actual or potential competing financial interests.

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