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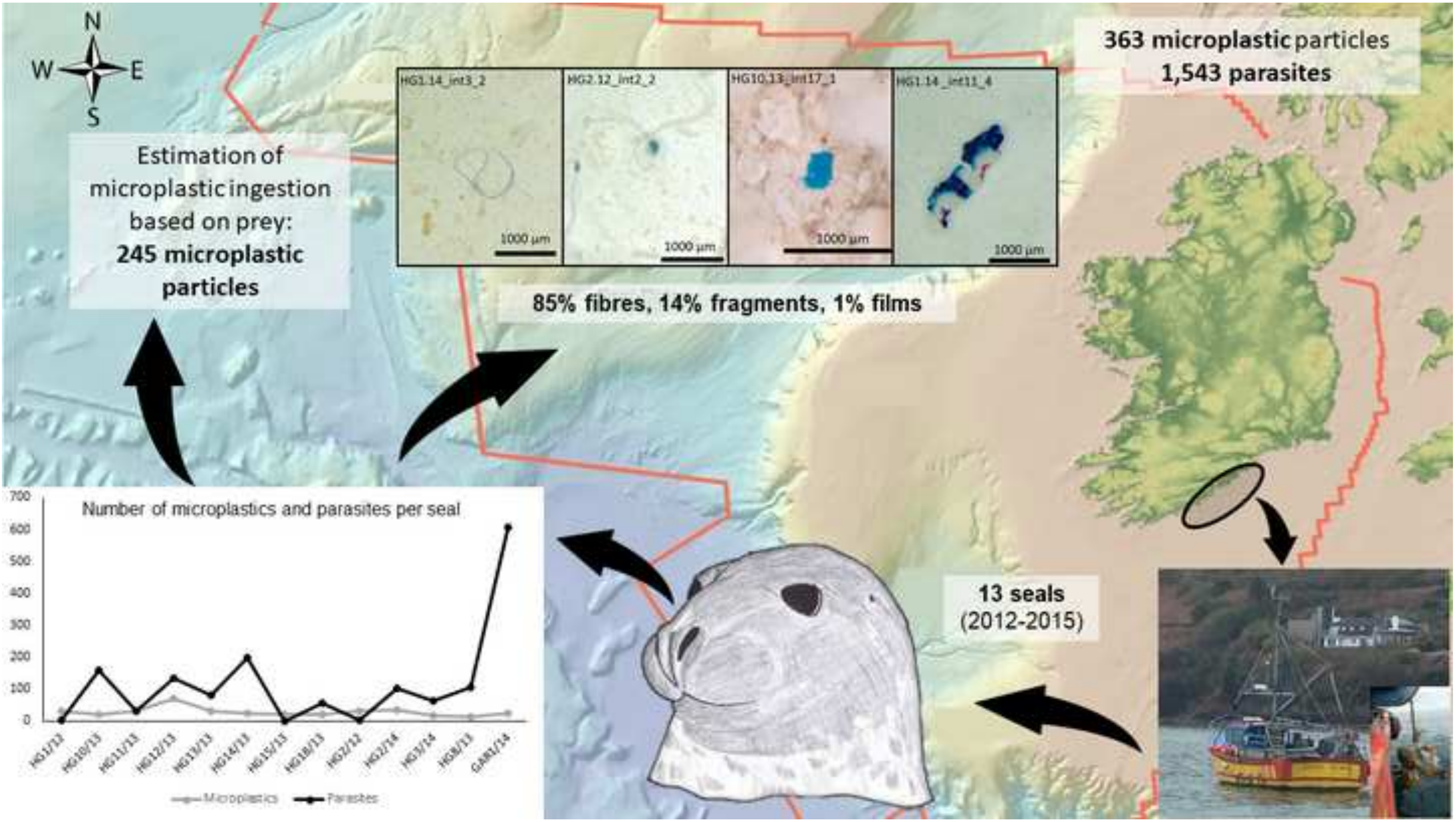
Graphical Abstract



***Highlights**

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- Knowledge on trophic transfer of microplastics is still scarce
- By caught seals may provide information on microplastic uptake via prey
- All seals had microplastics but no macroplastics were found
- microplastic retention in the intestines may be related to parasite aggregations
- By caught seals are a good source of data to monitor the incidence of microplastic pollution within coastal food webs

Microplastics in grey seal (*Halichoerus grypus*) intestines: are they associated with parasite aggregations?

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ABSTRACT

Between 2012 and 2015, 13 grey seals were recovered from trammel nets targeting monkfish and rays off the south coast of Ireland. Incidence and distribution of microplastics were investigated along the intestines of bycaught seals. No macrodebris items were found, whereas microplastics were detected in all seals. A total of 363 microplastics items were identified (85% fibers, 14% fragments, 1% films). Estimation of microplastic ingestion based on prey ingestion (245 particles) was lower than the observed data. Acantocephala parasites (n=1,543) were found in 12 seals, with an average of 74.5 ± 67.7 parasites per seal. Distribution of microplastics varied between seals, although microplastics tended to accumulate in areas where more parasites were aggregated; however, there was no significant relationship between the number of parasites and microplastics was found. Seals recovered from nets appear to be a good source to monitor the incidence of microplastic pollution within the coastal food webs.

Keywords: grey seals, microplastics, Ireland, parasites, bycatch

INTRODUCTION

Many different forms of marine pollution have been identified, including oil spills, chemicals, and marine debris (e.g. Islam & Tanaka 2004). Over the last few decades, marine debris has emerged as a major threat to the environment and biota. Persistent anthropogenic debris which consists primarily of plastic items is of particular concern (Bergmann et al. 2015). Explosive development of the plastic industry and consumer use has consequently produced a considerable amount of plastic waste (Jambeck et al. 2015). Plastics may ultimately end up in the marine environment, if disposal methods are not appropriate. It is therefore vital to understand the environmental implications of this long-lived pollutant. The importance of this issue is reflected in the implementation of Descriptor 10 of the EU 'Marine Strategy Framework Directive (MSFD)' which monitors marine litter, including plastics, for a 'Good Environmental Status (GES)'. As plastics and other anthropogenic debris move through the marine environment over large areas, they may become dangerous for marine organisms either because debris entering a new ecosystem/habitat could have consequences on the habitat itself, transport species which might behave as invasive organisms or directly impact upon biota themselves. Many species of biota have been reported to interact with, and be affected by, marine debris. Monitoring and detecting marine debris is necessary to understand the implications to different groups of biota (GESAMP 2016; Galgani et al. 2013). Several review publications compiled information on marine debris, and highlighted impacts including entanglement and ingestion by marine mammals which sometimes lead to fatalities (Baulh & Perry 2014, Kühn et al. 2015, CBD 2016). Marine mammals may be affected through direct interaction, or secondary interaction following the ingestion of contaminated prey (e.g. Fossi et al. 2018, Lusher et al. 2018, Panti et al. 2019). It is particularly important to understand the effects of marine debris on megafauna with life histories characterized by slow growth, late maturity, and low fecundity (e.g. Panti et al. 2019).

Microlitter particles are more conspicuous than macrolitter and have many different origins (GESAMP 2016). For instance, plastic items can persist in the environment as large (macrolitter) but over time they are exposed to environmental and mechanical processes which cause them to become brittle and fragment (Andrady 2015). A substantial number of marine species from invertebrates to vertebrates were found to ingest microplastics (e.g. Fossi et al. 2018). Within vertebrates, sea turtles and seabirds are the most commonly studied groups (e.g. Schuyler et al. 2014; van Franeker & Law 2015), although more recently marine mammals have been receiving increased attention (e.g. Eriksson & Bruton 2003, Lusher et al. 2015, Besseling et al. 2015).

It has been hypothesized that microplastics may facilitate the transfer of other pollutants either from the manufacturing process (e.g. plasticers and flame-retardant additives) or from the environment (e.g. organochlorates) (Rochman 2015). Sub-lethal effects associated with microplastics are still unclear (Rochman et al. 2016), and the impacts of microplastics on top predators require further investigation. Therefore, studying the ingestion of microplastics directly through investigation of digestive tracts may be considered as an appropriate method.

Bycatch in commercial fisheries can be an important source of carcasses for different analyses including dietary studies (e.g. Auttila et al., 2014; Lundström et al. 2007) and interactions with marine debris (Bravo-Rebolledo et al. 2013; Lusher et al. 2018). In contrast to stranded animals, where cause of death is often unknown, seals bycaught in fisheries are usually disease free, feeding normally in the marine environment, and therefore should provide a good overview of any effects of pollution in these animals, thus providing indication of the exposure to microplastics for the entire population. In general, the behavior of microplastics and plastics within the digestive tracts of marine mammals are difficult to predict, including how long they may remain in the digestive tract or whether they are egested with feces. The presence of substances in the intestine, such as parasites, may influence the retention of microplastics as they can increase the surface area for adhesion and retention.

The aims of this study were to (1) report the incidence and characteristics of microplastics within the last part of the digestive tracts (small and large intestines) of grey seals by caught in southern Ireland; (2) investigate the implications of parasite prevalence to the retention of microplastics and their aggregation; and (3) investigate the relationship between microplastic presence and prey items

METHODS

Sample collection

Grey seals by caught in fishing trammel nets targeting monkfish (*Lophius* spp) and rays (Rajaidae) off the Irish south coast (Co. Cork) were recovered for post mortem examination (Table 1). General measurements (body length and maximum girth), sex, age and any marks and external abnormalities were noted. Postmortem examinations were performed following the standard methodology (Dierauf, 1994), including measurement of blubber thickness, and sampling of different organs. Full digestive tracts, from oesophagus to anus, were recovered for all seals. Stomachs were analyzed for dietary purposes (Gosch et al. 2017), and intestines were stored frozen (-20ºC) for parasite infection and microplastic studies.

Laboratory analysis

Stomachs were analyzed prior to the development of a microplastics protocol (Lusher et al. 2015); consequently, they have not been included in this study. Intestines were thawed, measured and washed with pre-filtered tap water to avoid external contamination. Each intestine was then divided into 20 sections of equal length. Each section was washed with pre-filtered tap water through three nested sieves (from 250 µm on the bottom to 1,000 µm on the top). Food remains and parasites were transferred into ethanol for further analysis (MacGabban 2015). The remains on the smallest mesh sieve were frozen for microplastic analysis.

A pre-made solution of 10% KOH (Foekema et al. 2013) was used to dissolve organic matter. After the samples were defrosted, the solution was added in a proportion of three times solution to one part sample and left for about three weeks. The remaining solution was filtered using a GF/C microfiber filter paper using a Büchner funnel with a vacuum pump. Identification and measuring of microplastic items were carried out under a microscope (Olympus SZX10) with a mounted camera (Q-imaging Retiga2000R), and classification followed Lusher et al. (2014).

For the purpose of this study we used the term microplastic for both anthropogenic particles, up to five millimeters; FTIR was not used to confirmation synthetic polymers which it was confirmed to be important for particles below one millimeter (Isobe et al. *in press*). Nevertheless, this study sought to investigate the relationship between parasites and anthropogenic particles, therefore the specific polymer identification is not required and visual identification following Lusher et al. (2014) deemed sufficient.

Contamination conditions were avoided during the full process and intestine sections were covered to minimize air exposure, following Lusher and Hernandez-Milian (2018) protocol guidelines. Due to the limited ability to prevent airborne contamination the size limit was set to 200µm, airborne microplastics were monitored with wet filter papers in petri dishes and procedural blanks of replicate filtered distilled water and 10 % KOH were included to monitor procedural contamination.

Data analysis

The number of microplastics and parasites per individual were calculated. In parasite studies, level of aggregation is usually investigated using different indexes (Poulin 2013, MacGabban 2015). These aggregation indexes can also be used to investigate aggregations of microplastics. In this study, two statistical indexes were used to investigate microplastics aggregation; firstly, we used the Corrected Moment Estimate (1) as is the most common statistical index used in parasite aggregation studies; secondly we used the Poulin's Discrepancy Index, because it provides information when aggregation of parasites varies between hosts.

(1) Corrected Moment Estimate of the negative binomial distribution (k) quantifies aggregations (Sherrard-Smith et al. 2015), where $\bar{x}$ is the average abundance of microplastics, S2 is the variance and 'n' is the sample size (number of seals). The index shows that if 'k' increases, the level of aggregation also increases.

$$k = (\bar{x}^2 - s^2 / n) / (s^2 - \bar{x}) \qquad \text{Eq. 1}$$

(2) Poulin's Discrepancy Index (D) measures the degree of inequality between a hypothetical equal distribution and that of the observed distribution, where 'x' is the total number of microplastics in the individual 'j', and 'N' is the total number of individuals investigated (number of seals). D ranges from 0 to 1, where 1 implies all microplastics are aggregated.

$$D=1-2i=1N(j=1ixj)xN(N+1) \qquad \text{Eq. 2}$$

In order to investigate the relationship between microplastics and parasites the Kendall's Tau correlation test was used; this test allows parasite concentrations between seals to be compared, using ranks when data is scarce. In addition, Kendall's Tau test was used to investigate the relationship between microplastics and the number of prey ingested by seals. Theoretical ingestion of microplastic of seals was estimated using the average value of 1.9 microplastics ingested per fish given by Lusher et al. (2013). These values were compared with the empirical results.

RESULTS

Sample composition

Thirteen grey seals ranging from 107.5 cm to 240.0 cm (Table 1) were recovered dead from trammel nets operating between Crosshaven, Co. Cork (51.79° N, 8.28° W) and Kinsale, Co. Cork (51.66°N, 8.78°W) between 2012 and 2014. Most of the seals were juveniles (77%, n=10), two were subadults (15%) and one an adult (8%). Four of the seals (31%) were juvenile females. Postmortem examinations indicated that seals died due to drowning. Signs of interactions with fisheries (e.g. abrasions, cuts) were also identified.

Intestines of the juvenile and sub-adult seals ranged in length from 15.0 m to 22.4 m (with an average of 18.9 m) and the adult seal intestine measured 25.9 m. Average intestine section length ranged from 75.0 cm to 111.7 cm with an average of 94.4 cm (excluding the single adult measurement).

3.2 Ingested items

Food remains in the stomachs were partially digested. Most of the prey found in seals stomachs were demersal prey (96.9% by number), which was composed primarily of gadoids (70.4% by number, Table 2). Comparatively, Low numbers of food remains were found in the intestines which corresponded to prey found in the stomachs.

There were no macroplastics present in any grey seal stomachs. However, all seal intestines contained microplastics. A total of 363 particles were identified, 85% of which were fibers, 14% fragments and 1% films (Fig 1). The average number of particles per seal was 27.9 ±14.7 (range 13 to 71) and the number of microplastics per section ranged from 0 to 19 particles (Fig. 2). Considering an average value of 1.9 (± 0.1 SE) microplastic particles per prey item (see Lusher et al. 2013), a total of 245 particles for all seals in this study were estimated based on the dietary results (Table 1), this was lower than the observed count (363 particles). Most of the seals had a lower estimated microplastic count when compared to the actual count (Table 1). There were four seals where the estimation was higher based on the stomach contents, in one particular seal (HG 3/14) the estimation value was three times greater than the actual value.

3.3 Aggregation of microplastics and parasites

All seals had parasites in their intestines apart from one juvenile male (HG 15/13) Most of the parasites (n=1,543) were identified as Acantocephala (MacGabban 2015), with one unidentified Cestode. Total numbers of acantocephalan parasites ranged from two to 199 per seal (when the adult seal was not included), with an average prevalence of 74.5 ± 67.7 parasites per seal. A total of 606 parasites were counted in the adult seal which accounted for 40% of the overall parasite burden. When the adult seal was included in the analysis, the average prevalence of parasites was 115.0±116.1. (Fig. 3, Table 1).

Both the corrected moment estimate (k = -0.06) and Poulin's discrepancy index (D = 0.857) detected microplastic aggregation. We hypothesized that aggregations of microplastics may be related to parasite aggregations; however, Kendall's tau correlation test showed that the number of microplastics were not significantly related to parasites (Table 3). Gadoid prey was significantly correlated to microplastic incidence (r_{τ}= -0.469, p=0.034) however no correlation was found when all the dietary items were considered (Table 3).

DISCUSSION

Marine debris is considered as a major threat to the marine environment, and interactions with marine mammals have been reported to reach 56% either due to entanglement or ingestion (Baulch & Perry 2014, Simmonds & Baulch 2016). Recently, Lusher et al. (2018), found that all cetaceans investigated from Irish coasts contained microplastics. In this study, all seal intestines analyzed presented microplastics but none of them contained macroplastics in either their stomachs or their intestines.

Microplastic presence in cetacean digestive tracts have already been confirmed in a humpback whales (Besseling et al. 2015) and some Odontoceti (Lusher et al. 2018). Plastic items were found in pinnipeds stomachs, such as in harbor seals (Bravo-Rebolledo et al. 2013), but also microplastics were reported in the scats of fur seals (Eriksson & Burton 2003). Ingestion of microplastics in marine mammals, especially on pinnipeds, is mainly related to consumption, as they target prey by hunting and directly consuming whole prey which might content microplastics. Therefore, it can be assumed that the ingestion of microplastics in pinnipeds may come from prey, as originally suggested by Eriksson & Burton (2003). Estimating microplastic ingestion based on occurrence in prey items is a valuable resource when anthropogenic debris information is not accessible; however, it is necessary to use these calculations with caution as under and overestimations may occur. Interestingly, the estimated number of microplastics based on prey ingestion in one of the seals was 40% less than the number of microplastic found in the intestines. However, the values increased twice (105%) and even three times (213%) when looking at the estimations obtained from two other individual seals. For the remaining individuals, the equation (Lusher et al., 2013) underestimated the observed number of microplastics.

Despite the large amount of microplastics that these top predators may ingest annually, and based on the data presented, most of them, if not all, will be egested following digestion and particles will be released back into the marine environment. Some studies have suggested that plastics, in particular microplastics, might act as vectors of other chemicals such organochlorates, heavy metals and plastic additives (e.g. Rochman 2015; Fossi et al. 2012). These chemicals may be released within the digestive tracts and absorbed into tissues. In fact, some researchers have suggested that if plastics facilitate the transference of chemicals to biota it may be possible to monitor their levels through biopsies (e.g. Fossi et al. 2012). However, this is yet to be verified as any observed levels of contaminants may be related to macrodebris, microplastic or consumed prey items.

Relationships between microplastics presence in prey items, as mentioned above, has been used to estimate levels of ingestion in other studies (Lusher et al. 2016). Prior to this study microplastics or anthropogenic particles smaller than 5mm, have not been studied in relation to other elements of the digestive system, or pathologies, e.g. the presence of parasites. Aggregations of helminth parasites are commonly observed within hosts due to both intrinsic and extrinsic factors, such as sex and site, respectively (e.g. Behnke et al. 2001; Poulin 2013). These aggregations may decrease the intestinal lumen and increase the contact surface within the intestinal lumen, therefore microplastics may have more chances to be retained in these areas. MacGabban (2015) found that most of the Acantocephala parasites in seal intestines tended to aggregate between the 9th and the 15th section. Although no statistical relationship with such aggregations was found in this study, microplastics were found to be more abundant before the 14th section of the intestine. The low sample size (n= 13) and the lack of information regarding microplastic presence in corresponding seal stomachs should may alter the results.

It is notoriously difficult to obtain biopsy samples from marine mammals due to their diving behavior; therefore, stranding and bycaught animals can be a good source of data. In cetaceans, the predicted recovery rate of stranded carcasses ranges from 8 to 30 % of dead dolphins (e.g. Peltier et al. 2012; Carretta et al. 2016**)**. With regard to pinnipeds, strandings are rarely reported unless in extreme cases of disease (Bravo-Rebolledo et al. 2013), or deliberate kills or bycatch (Pierce et al. 2011). Post mortem examinations of bycaught animals provide a good source of data for studies of microplastics, pollutant and their trophic transfer within marine mammals. At the time of death these animals were actively feeding and might provide information on healthy organisms that stranded and dead organisms cannot provide, especially when they are found with signs of illness or starvation. This study presents the incidence of microplastic on seals incidentally caught in fisheries activities. Samples were only obtained from south of Ireland, leading to restricted spatial coverage. It will be necessary to investigate stranded and bycaught seals (both adults and juveniles) as well as scats from other areas to obtain a better understanding of the incidence of this type of pollution and the effects at a population level. In addition, in this research only intestines were available for microplastic analysis, while stomachs were open previously for dietary analysis only. We believe that the use the full digestive tract will provide us a more realistic picture of the incidence of microplastics in the individuals analyzed, however, the result showed us the importance of considering the full digestive tracts and not only the stomach of these species when studying both microplastics and diet.

Nowadays, marine microplastic pollution assessments are primarily carried out analyzing non-biota samples (e.g. sediments and water), algae, invertebrates, or fish (GESAMP 2016). Organisms may act as bioindicators or sentinels (e.g. Fossi et al. 2018), because they provide information on the quality of the environment and changes over time. These species can be use as monitoring tools for ecosystem health; marine litter (macro- and microplastics) is one of the recent threats that these species are confronted with. Management strategies for microplastics are difficult to implement because the information available is still patchy, and the toxicological effects of microplastics are still unclear. Islam & Tanaka (2004) reviewed different issues on marine pollution management including governmental decisions, lack of communication among scientists, and low participation of different sectors; however, these authors found it difficult to address the problems as marine pollution is defined by characteristics such as uncertainty, conflicts, and complicated interactions.

Seals can provide further data on the incidence of microplastic pollution within higher trophic levels of the food web, especially in coastal ecosystems where the highest input occurs (more than 80% of marine debris come from land sources). However, there are three factors to be further considered; i) the effect of microplastic pollution should be studied using different age groups; ii) the stranding rate of seals is low, it is necessary to include long-term sampling, and iii) analysis of additional tissues (e.g. muscle, liver) to investigate the possible effect of chemicals associated with microplastics is required. In addition, the analysis of seal feces may provide scientists with the opportunity to obtain information without the need for carcasses. However, analyzing seal scats needs to be taken with caution because external airborne contamination may occur.

CONCLUSION

Research on the implications of large marine debris has increased during the last decade, including evaluating the interactions between predators (e.g. marine mammals, seabirds, sea turtles, sharks) and ghost nets, plastic bags, and other type of marine debris. More recently, studies on microplastic interactions have highlighted the potential effects on invertebrates and fish. Based on initial results, researchers point out that top predators might also been affected by microplastic pollution; however, incidence and accumulation of microplastics within digestive tracts are important variables needed to understand any adverse effects. Different parameters should be taken into consideration when top predators are being used in studies of pollution. Distribution and accumulation of microplastics, effects of trophic transfer and the presence of parasites are variables to be considered. Trophic transfer has been suggested as one of the main factors of microplastic incidence in top predators, and estimation of microplastic ingestion by prey has been used. The current study shows that these may be underestimates of the incidence of microplastics in top predators, although it is a valuable tool when microplastics analysis cannot be carried out. It is necessary to understand that microplastic distribution varied from one individual to another, however some kind of aggregation may occur. Aggregation can be associated with the retention effect where the reduction of lumen is higher with parasite aggregation, which may also affect the aggregation of microplastics. In this study, aggregation could not be statistically confirmed but there appeared to be some form of aggregation occurring. It is essential for future research to investigate microplastic incidence related to other factors such as prey and parasite prevalence. The knowledge of these associations will be paramount in enabling the use of these predators as monitoring tools in ecosystem management.

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TABLES

Table 1. By caught seals in south of Ireland (Co. Cork: 51.79N, 8.28W - 51.66N, 8.78W). M: male, F: female, J: juvenile, SA: subadult, A: adult. Length of seals in cm. Blubber: ventral blubber thickness in mm. T.B.: total burden of parasites. MP: number of microplastics. MPe: number of microplastics estimated using Lusher et al. 2013 value of 1.9 MP/fish.

Table 2. Number of prey ingested by seals

Table 3. Kendall's rank correlation (tau) output. * adult seal was not included. r_t correlation coefficient tau

Table 1. By caught seals in south of Ireland (Co. Cork: 51.79N, 8.28W - 51.66N, 8.78W). M: male, F: female, J: juvenile, SA: subadult, A: adult. Length of seals in cm. Blubber: ventral blubber thickness in mm. T.B.: total burden of parasites. MP: number of microplastics. MPe: number of microplastics estimated using Lusher et al. 2013 value of 1.9 MP/fish, where (+) and (-) indicates underestimated and overestimated percentages of microplastics when comparing with data obtained from seals.

	Year	Sex	Age	Length	Blubber	T.B.	MP	MPe	Variation
HG1/12	2012	M	J	119	35	2	29	5.7	-80%
HG2/12	2012	M	J	115	20	3	30	1.9	-94%
HG8/13	2013	M	J	130	18	105	13	23	+63%
HG10/13	2013	F	J	145	25	159	19	27	+40%
HG11/13	2013	F	J	147	25	32	32	1.9	-94%
HG12/13	2013	M	SA	156	40	135	71	7.6	-89%
HG13/13	2013	M	SA	162	35	84	30	7.6	-75%
HG14/13	2013	M	J	137	8	199	24	19	-21%
HG15/13	2013	M	J	130	18	0	20	15	-24%
HG16/13	2013	F	J	138	28	8	19	7.6	-60%
HG18/13	2013	M	J	144	21	56	NA	7.6	NA
GAR1/14	2014	M	A	240	25	606	25	-	NA
HG2/14	2014	F	J	124	15	102	36	76	+105%
HG3/14	2014	M	J	140	13	62	15	53	+213%

Table 2. Number of prey ingested by seals.

	HG12/13	HG13/13	HG1/12	HG16/13	HG10/13	HG11/13	HG2/12	HG8/13	HG15/13	HG2/14	HG3/14	HG14/13
Merlangius merlangus				2				2		2	6	
Melanogrammus aeglefinus	1				1			5				6
Gadus morhua					5					12	2	0
Pollachius pollachius					1							
Trisopterus sp.	1	3	1	1		1	3	6	12	7	1	
Gaidropsaurus vulgaris	1											
Molva molva								1	1			
Merluccius merluccius	1	1										1
Trachurus trachurus	1											
Clupea harengus	1											
Belone belone						1						
Ammodytidae	1				5					14	9	1
Callionymus sp.									1		1	
Labrus sp.											1	
Flatfish								1			2	
Unknown fish												1
Total	4	4	3	4	14	1	1	12	8	40	28	10

Table 3. Kendall's rank correlation (tau) output. * adult seal was not included. r_τ correlation coefficient tau

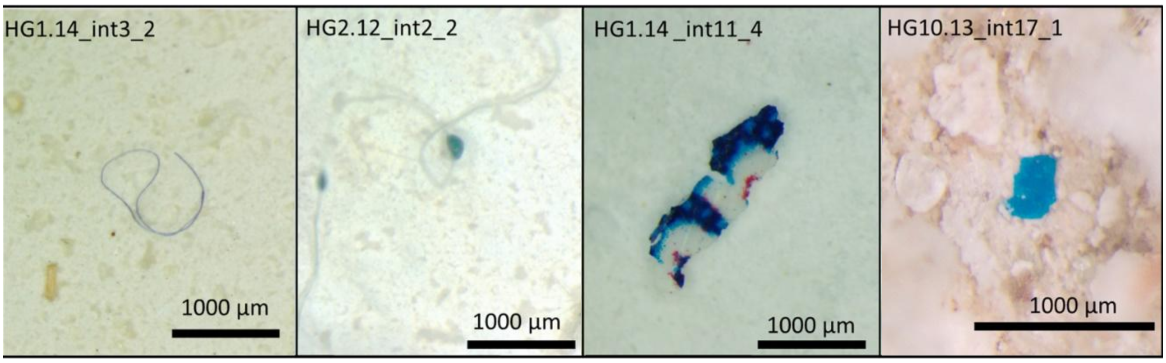
	r_τ	p-value	z
Microplastics vs parasites	0.0260	0.9025	0.11247
Microplastics vs parasites*	0.1378	0.8904	0.13779
Microplastics vs diet	-0.3493	0.1254	-1.5324
Microplastics vs gadoid prey	-0.4688	0.0379	-2.0765
Microplastics vs demersal prey	-0.4252	0.0606	-1.8762
Parasites vs diet	0.3127	0.1655	1.3868
Parasites vs gadoid prey	0.1846	0.4084	0.8268
Parasites vs demersal prey	0.2326	0.2995	1.0376

FIGURES

Figure 1. Photographs of fibers (a, c), films (b), and fragments (d) within the seal intestines.

Figure 2. Average number of microplastics per section of intestines. Error bars show the standard error.

Figure 3. a) number of microplastics (GREY) and parasites (BLACK) in all seals except the adult seal (GAR 1/14)



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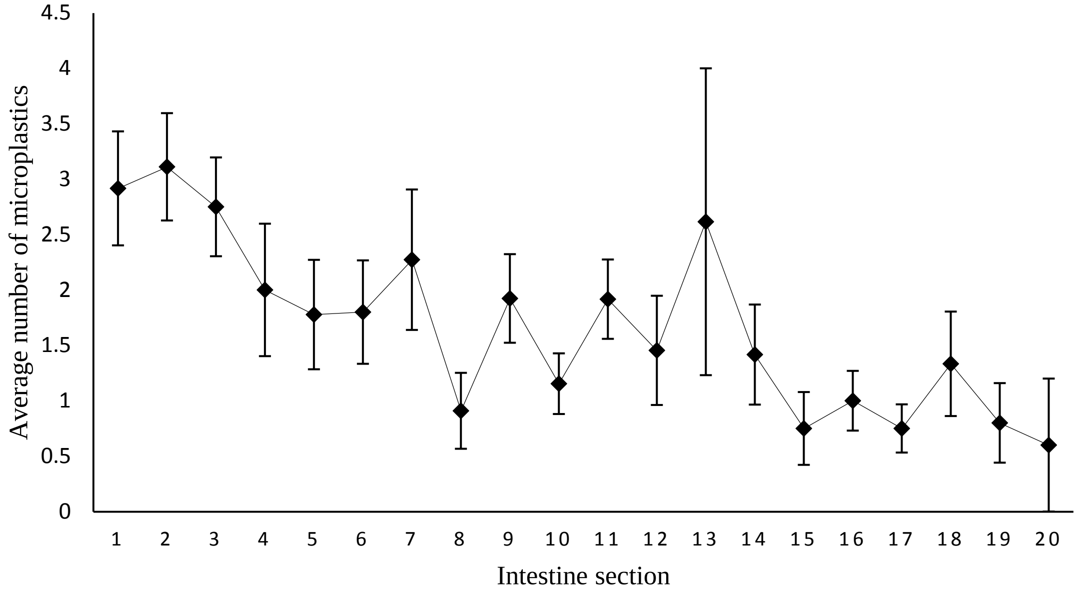

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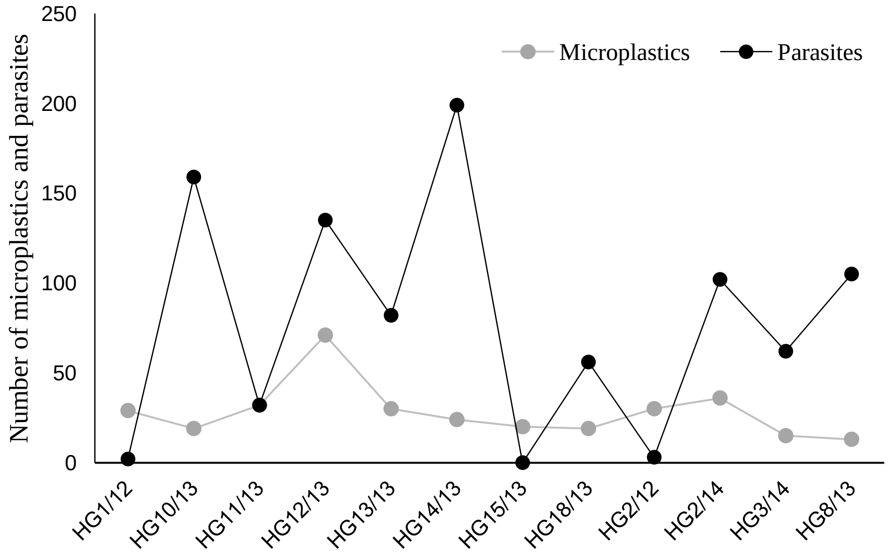

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