Investigating Microplastic Presence Amongst Grey Seals (Halichoerus Grypus) of the North Sea

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Abstract

Plastic pollution is of increasing concern to marine ecosystems worldwide. Specifically, microplastics (<5mm) may interact with a variety of biota with potential to cause harm to organism health. Studies concerning microplastics are increasing, yet their occurrence within live marine mammals remains largely unexplored. Here, faecal samples collected from a haul-out site in the North Sea, were used to investigate microplastic pollution within grey seals (Halichoerus grypus). 71 microplastic particles, consisting of both fibres and fragments in a variety of colours and sizes, were identified across 66 scat subsamples analysed. This indicates that marine mammals are ingesting microplastics and that faecal material can be used to indirectly and humanely record microplastic uptake data in pinnipeds. Since the current paper is the first to document microplastic exposure amongst wild, living and free-ranging grey seals in the North Sea, further research is needed to begin to understand the biological significance of these findings.

Introduction

Plastic is a material of such societal benefit[1] that production has soared in recent decades – as

has the quantity of marine plastic pollution[2]. With current estimates predicting that oceanic

plastic litter could reach 250 million metric tonnes by 2025[3], this anthropogenic debris is considered a major threat to marine biodiversity[4]. Recently, increased research interest has focussed on plastic debris at the micro-[5], and nano-scale[6], with particles less than 5mm in size being collectively referred to as microplastics[7,8]. Whether originally manufactured as microscopic in size (for instance, fibres), or formed through the degradation of larger items of plastic debris[9], microplastics have accumulated in our oceans for over 40 years[10] and now pervade even some of the most remote environments[5,11]. As such, these synthetic particles are believed to be ubiquitous within marine ecosystems worldwide[7,9,12]. Consequently, better understanding of the potential ecological ramifications of microplastic pollution is a globally recognised high-priority research area in Marine Biology[13].

Owing to their small size, microplastics are bioavailable for ingestion by a range of marine fauna including; seabirds[14], fish[15], invertebrates[16] and marine mammals[17,18]. For mammalian species, microplastic ingestion has been hypothesised to occur unintentionally during prey capture in microplastic-polluted areas, particularly during filter feeding[19,20]. Furthermore, indirect microplastic consumption following the ingestion of microplastic-contaminated prey, termed trophic transfer, and this has been proven to occur under captive conditions[21] and is widely speculated to occur *in natura*[17,18,22].

Microplastics are known to be associated with toxic substances including adsorbed Persistent Organic Pollutants (POP's) and/or harmful additives contained by the debris [23]. Following the ingestion of plastic particles by marine biota,[24–27]. For marine mammals, exposure to these lipophilic, microplastic-associated toxins may be related to adverse health effects, including cancer-associated mortality in California sea lions (*Zalophus californianus*)[28] and reproductive toxicity in dolphins[29].

Although several papers report the presence of microplastics amongst marine mammals[5,17,19,30–32], research typically relies on necropsies performed on stranded or by-caught individuals[33] – providing few occasions to study anthropogenic contaminants[32] and with relatively small sample sizes from individuals that have died, and may have been feeding abnormally pre-mortem[12]. Alternatively, scat analysis offers the opportunity to obtain data on microplastic exposure in live, wild and free-ranging pinnipeds through a non-invasive and humane manner[18,21]. This could permit the analysis of larger sample sizes, which may provide more representative estimates of microplastic pollution amongst wider populations[12]. Nonetheless, scat-based microplastic investigations on wild pinnipeds are sparse and none exist for grey seals inhabiting the North Sea.

In the current study, faecal material collected from 66 wild, free-ranging grey seals was analysed for microplastic pollution. We strove not only to gain preliminary data on microplastic ingestion within a grey seal population of the North Sea, but also to characterise the particles identified according to type (fibre or fragment), colour and size.

Results

Microplastic presence. A total of 71 microplastics were found in 43 of the 66 scat subsamples examined (supplementary table S1), with a mean of 1.08 ± 1.01 particles identified per subsample. Of the microplastics detected, fibres (n=43; 61%) were more numerous than fragments (n=28; 39%). Amongst fragments, light blue was the main colour observed (n=10; 36%), then clear (n=8; 29%), blue (n=4; 14%), white (n=3; 11%), yellow, black and red (all three n=1; 3%; Figure 1. i.). Conversely, black and blue were the most common colours of fibre (both n=18; 42%), followed by red (n=5; 11%) and light blue (n=2; 5%; Figure 1. ii.). Clear coloured fibres were also seen, but were excluded from our analysis as consistent separation of these and the thread-like structures present on the filter was not possible. Therefore, the figures shown under present plastic occurrence, as clear fibres were not included. Fragment sizes ranged from 30µm to 1400µm, with a mean of 248 ± 264µm. Whilst fibres had a mean length of 1212 ± 811µm, the length varied between 100µm and 3400µm (Figure 1. iii.). Photographs of microplastics from the present study are included in Figure 1. iv.

Data analysis. No significant difference was found between the incidence of one colour from any other. A statistical difference ($p<0.0001$) was shown, where fragments were significantly smaller than fibres.

Laboratory controls. Microplastics were not identified in any of the procedural blanks or airborne contamination controls used, validating the efficacy of the measures employed to reduce the risks of contamination.

Discussion

Whilst contamination between defecation and sample collection cannot be excluded, microplastics were absent from all our blanks and controls, providing confidence that particles observed were endogenous to the scats and did not arise from laboratory-based sources. Conversely, controls were not exposed to the clear plastic sample collecting bags and this may have potentially contaminated the scats – although the range of fibre/particle types observed and exclusion of clear coloured microplastics from our results excludes clear

plastics derived from any source from the analysis. Given that each filter paper analysed represented 44.4 □% of the initial mass of the faecal subsample, we estimate an average microplastic abundance within these individuals to be 0.81 particles per gram of dried scat. Contrary to other studies reporting both particle number per scat[18,22,33] and per gram of wet scat [18], we consider particle number per gram of dried scat to be a robust 'relative measure' – an 'index' independent of original scat sample weight, and a useful way to present the detection rate data, as it accounts for variation in scat mass and water content. However, microplastic egestion rates in grey seals are unknown, and thus whether plastic particles would be intermittently or continually shed remains unknown.

Placing these findings into context is problematic, since only a handful of studies have analysed wild pinniped faeces for microplastic presence[18,22,33,37,38], and only two have examined grey seal scat, and none from Seals in the North Sea: Hudak and Sette[39] isolated two microplastic particles from 129 scats in south-eastern Massachusetts, USA, and Nelms *et al.*[12] found 17 microplastic particles, in 15 subsampled scats collected from Skomer Island, Wales. While caution should be applied when interpreting the preliminary work of Nelms *et al.*[12] due to the small sample size analysed (15 subsamples), the microplastic abundance (53%) was similar to that observed here (66%) and considerably higher than that reported by Hudak and Sette[39] (<2%). Although spatial variations may potentially influence environmental and/or prey-based microplastic uptake in marine mammals [20], along with disparities which may be caused by the different prey species ingested [40] , these differences in microplastic abundance may also be due to dissimilarities in the detection methods used in our study and these previous studies. Firstly, absence of an enzymatic digestion technique in the method adopted by Hudak and Sette[39], but employed by Nelms *et al.*[12] and in our study, may have caused biological material to obscure microplastics during identification. Secondly, particles <500µm were excluded in the work of Hudak and Sette[39]. This size category constituted a great proportion (37/71) of all microplastics identified in our study, and the entirety of those identified by Nelms *et al.*[12]. The exclusion of certain sized particles – often through disregarding the fluid collected after sieving in any analyses, which may contain small microparticles – is a procedural bias evident within other microplastic investigations on pinniped species[22,37,39]. For the present study, all material (including all fluid) was collected after sieving and analysed for microplastics. While very small microplastics <500µm may still be under reported due to the limited detectability of these small particles and the presence of pores (20µm) within the filter papers used[17], the analysis of all material collected gives confidence that the true number of particles present was recorded. Therefore, it is difficult to draw comparisons between the microplastic abundance observed here and in previous studies [22,37,39], since these studies potentially under report the presence of small (<500µm) particles. This emphasises the need for future studies to employ methods which can detect and report all grades of microplastic extracted, such as that utilised here and by Nelms *et al.*[12,17,21], to facilitate increased comparability between datasets.

Investigations into microplastic presence amongst pinnipeds[12,18,21,31], and marine mammals in general[17,30,32], largely report a higher number of fibre particles when compared to fragments, and this corresponds with our finding of 61% of our samples findings being fibres. Thus could be explained by two principal factors. Firstly, the fibres could be disproportionately represented, as they have been suggested to have an increased predisposition for methodological contamination during sampling and analyses[19,41]. Yet, the strict contamination measures employed, and absence of any microplastics present on our controls, and the mix of

colours and fibre types seen in our samples, makes this seem an unlikely explanation. More likely, the finding of higher levels of fibres is likely to be reflective of the high relative levels of environmental fibres[7,42,43]. Domestic washing, and the textiles industry expel large quantities of microfibres into water, which often evade filtration systems and/or municipal water treatment plants, and enter the ocean[44]. It could however be the case that pinnipeds ingest these fibres in higher proportions along with prey in water ingested at the time of prey capture[18]. However, what appears to be more probable is that the seals are ingesting fibres indirectly via trophic transfer[21,45]. Previous studies have shown fibres to have long body residence times in low-trophic level organisms, which would suggest an increased chance of secondary fibre ingestion by predators[43,46–48]. Microplastic fibres may be transferred along food chains and reflected in greater proportions among high-trophic level species such as marine mammals[43,46,49]. Whether the higher relative number of fibres compared to fragments observed here is representative of a biological process such as trophic transfer, or purely an incidental finding, is unknown.

Previous studies examining microplastic pollution amongst marine mammals commonly describe a variety of colours, including colours identified here[17,18,30,31]. Conversely, some investigations report a predominance of white [22,33] and blue microplastics [18,50]. Notably, after oxidation and density separation treatments, Donohue *et al.*[33] only recovered white microplastic fragments from northern fur seal (*Callorhinus ursinus*) scats. Hydrogen peroxide has been employed in some studies to digest organic debris, and this may have bleached the particles observed. Donohue *et al.*[33] hypothesised that the prey which seals were ingesting had disproportionally taken in white particles due to enhanced observability of white particles in the water column. Other authors have postulated that certain coloured microplastics may resemble the prey of predatory fish, thus causing visual confusion and a propensity for the ingestion of specific particle colours by prey species[51,52]. Although the exact mechanism remains unclear, selective ingestion by prey species could result in a disparity in the microplastic colours subsequently found in seal scat[21,33]. Nevertheless, the range of particle colours in our study, combined with the lack of any significant difference in the frequency of any one colour against another, suggests that active selection (based on colour) by grey seal prey was not occurring in the sea area where the study was carried out. Instead, microplastics could have been visually targeted based on size[22] or selected through olfactory signals, as is seen in foraging seabirds[53]. Alternatively, direct accidental microplastic uptake could have occurred – potentially during bottom-feeding, as suggested for harbour seals of the North Sea[37] – which would reflect a reasoning for the diversity of colours found in reported environmental microplastics[35].

A significant difference was present between particle sizes – fragments were found to be significantly smaller than fibres, with all but two being <500µm in size. This correlates with other reports on marine mammals that have employed similar protocols for the extraction of a wide size range of microplastics[5,17,33] and this may be biologically consequential. Smaller sized particles are assumed to be increasingly bioavailable, facilitating uptake by lower trophic level organisms[7,54]. Therefore, the predominance of fragments <500µm detected in our study, suggest that small synthetic particles may have a high bioavailability to ingestion throughout numerous trophic guilds[33]. Consequently, microplastics could be contaminating multiple trophic tiers of the grey seal food chain (as shown in **Figure 2.**), which may not only assist the widespread trophic transfer of microplastics to these top marine predators, but also to various compartments of the marine food web[7]. However, it is not

possible to determine whether the microplastics recovered here originate from direct ingestion, or through trophic transfer from contaminated prey and/or organisms lower in the food chain.

The size and abundance of particles observed in the current study are unlikely to present a physical hazard (e.g. gastrointestinal obstruction) to grey seals in the same way they may do for lower trophic level organisms[9]. Equally, our microplastic recovery from faecal matter, alongside previous plastic particle identification from post mortems conducted on grey seal intestinal tracts[31], indicates that microplastics are transitory, i.e.. not retained in the gut, for Grey seals. However, the potential for microplastics to cause localised harm to the digestive tract[5] cannot be excluded, since particle retention times and egestion rates in Grey seals are undetermined. Furthermore, while microplastics have been proposed to be transitory in marine mammals[5,17,55], their presence in faecal matter is still a concern. Defecation releases particles back into the environment where they are made re-available for uptake by other organisms[18,54], and this cycle may continue to very long periods, potentially for hundreds of years.

Microplastics are known to adsorb and concentrate hydrophobic toxins (e.g. POPs) present in the ocean[56,57] – perhaps due to their large surface area to volume ratio and the natural affinity of these compounds for the hydrophobic surface of plastic[23,26,58]. These substances, along with chemical additives used during plastic manufacture (e.g. phthalates and Bisphenol A), may leach into organisms tissues following microplastic ingestion[24–27]. Owing to the small size, and thus high bioavailability of this synthetic debris by low-trophic level species[33], ingested microplastics could act as a carrier for transporting these substances throughout the aquatic food web[7,25,30]. The long-life spans, high trophic level status, and dense lipid stores of marine mammals, may leave them potentially vulnerable to the bioaccumulation of lipophilic contaminants[59]. Chronic microplastic ingestion by marine mammals, and/or elsewhere in the food web, may expose these megafauna to high concentrations of these chemicals with the potential to influence population viability through immune, endocrine and/or reproductive system disruption[28,29], but, studies demonstrating whether microplastics may facilitate increased contaminant uptake in marine mammals directly, or elsewhere in the food chain, remain absent.

Our identification of microplastics within Grey seal scat, collected non-invasively, and with no direct disturbance of seals, not only verifies that grey seals in the North Sea are ingesting these synthetic particles, but also indicates the pervasiveness of this issue. Currently, the health, animal welfare, and conservation implications of microplastic, and associated chemical ingestion, remain unclear and do appear to warrant further investigation. The present study shows that faecal material can be used to humanely (without disturbance) and indirectly record levels of microplastic exposure – particularly important since opportunities to conduct necropsy are infrequent, and collection of biopsies for phthalate analysis[20] would be challenging due to the diving behaviour of seals[31], and inadvisable from an animal welfare perspective. Our method has produced results delivered as a relative measure ‘particles per gram’ of faecal material, and this ‘metric’ of environmental contamination could be used as a reference for comparison across different species and geographical areas. Plastics are relatively new agents present in the evolution of the wild animal world, which we may ignore or explore – time will probably illustrate whether monitoring of sentinel markers of animal plastic uptake should raise, or should have raised, concerns about human derived environmental change.

Methods

Sample collection. On the 27th February 2015, researchers from the Sea Mammal Research Unit, St Andrews, collected 66 grey seal faecal samples from a haul-out site in Donna Nook, Lincolnshire. The low density of seals at this site ensured that multiple scats were not gathered from the same individual. The low density of seals, and collection of scat from areas where seals were not concurrently present, ensured that seals were not disturbed during collection. Whole scats were picked by hand using clear plastic bags 'over then hand'. The bags were then inverted, tied and transported to the University of St Andrews before being frozen at 20^{O}C. In May 2019, scat samples were brought to the University of Bristol, where they were further stored at 20^{O}C before the commencement of laboratory analysis.

Sample preparation. Scats were thawed within their sample collection bags at room temperature (21^{O}C) overnight. Material from each sample bag was then broken apart by passing through a stainless-steel sieve of mesh size 1180µm, facilitated by dilution with Milli-Q water and a agitation with a metal spatula. Faecal matter retained by the sieve, along with all liquid contained after sieving, was then dried at 60^{O}C until no moisture remained.

Enzymatic digestion. To remove any biological material that may have concealed microplastics during later identification, a methodology involving enzymatic digestion was employed. This protocol was introduced by Lindeque and Smerdon[34], modified by Cole *et al*.[35] and revised by Nelms *et al*.[21] for use in seal scats.

Firstly, a 3g (dry mass) subsample was placed into a pre-rinsed glass Duran bottle, with 45ml of a homogenising solution containing: 400ml Tris-HCl buffer, 120ml ethylenediaminetetraacetic acid, 30ml sodium chloride, 100ml Sodium Dodecyl Sulphate and 350ml Milli-Q water. Each mixed aliquot was then physically homogenised by stirring with a metal spatula rapidly for 30 seconds, before heating at 50^{O}C for 30 minutes. Following this, 2250 µl of 20mg/ml Proteinase-K was added and samples were incubated at 50^{O}C for 24 hours. 9ml of 5M sodium perchlorate was then added and samples were shaken at 500rpm for one hour at room temperature. Mixtures were stirred again with a metal spatula for 60 seconds and heated at 60^{O}C for 30 minutes. Once cooled, a 25ml subsample was taken to prevent excess biological debris from obscuring later microplastic visualisation. Subsamples were passed through Whatman® filter papers (grade 41, pore size 20 µm) using a vacuum pump. Finally, filter papers were dried at room temperature within a positive pressure laminar flow hood and then placed within sealed petri-dishes for later microscope analysis.

Microplastic identification. Material retained on the filter papers was examined under a Leica Wild M3Z microscope. To better distinguish synthetic particles from any organic debris present, microplastic was only recorded if all the following criteria proposed by Norén[36] were met: 1.) No organic structures should be visible within the particle; 2.) if the present particle is a fibre, there should be no tapering towards one/both ends and it must have three-dimensional bending; 3.) each particle should be homogeneously coloured.

Once identified, microplastics were classified according to their type (fragment or fibre) and colour, which was visually determined. The length of each microplastic's longest axis (irrespective of type) was also measured using the eyepiece graticule. Limitations of funding, and time available to conduct the study, restricted our ability to conduct comprehensive Fourier transform infrared (FTIR) spectrometer analysis on any recovered

microplastics which would have indicated the plastic polymer type present, and it is recommended that future studies of scat analysis apply methods to determine the polymer type of the plastic microparticles. This study is a proof of concept study – 'could we detect particles in Grey seal scat from the North Sea'.

Data analysis. A Kruskal-Wallis test was carried out to determine if there was a significant difference in the incidence of any one particular microparticle colour. Secondly, a D'Agostino-Pearson omnibus normality test and Mann-Whitney U test were performed to establish if a significant difference was present between the particle sizes observed. Following this, a Chi-Squared test was completed to ascertain if there were significant differences in the frequency distribution of particle sizes overall, and then whether there were differences between individual size grade categories. All analyses were undertaken using GraphPad Prism 7®.

Contamination prevention. During laboratory procedures, contamination of samples by microplastics risks generation of inaccurate results[17]. Therefore, several measures were implemented throughout the present study to minimise, and assess the risk of, external microplastic contamination from equipment and/or atmospheric sources.

Prior to any work commencing, a cotton lab coat was worn over synthetic clothing and all work surfaces were cleaned using 70% ethanol and paper towels. Glass or metal equipment was used alternatively to plastic wherever possible and all equipment was washed meticulously with Milli-Q water before use. Likewise, the sieves used were soaked in detergent and hot water, cleaned, rinsed with Milli-Q water and then visually examined for any lasting debris and/or microplastics between samples.

Sample bags were closed following scat collection and remained closed throughout sample storage and thawing. Lids were placed on Duran bottles wherever possible and when required to be removed, such as during the addition of reagents, this was performed for as little time as possible and within a positive pressure laminar flow hood.

Our ability to minimise external microplastic contamination was appraised using two types of laboratory control. Four procedural blanks (comprising 50ml Milli-Q water) were processed identically and concomitantly to the scats, to reveal contamination by reagents, equipment and/or other procedural sources. Two filter papers soaked in Milli-Q water were placed within the laminar flow hood to serve as airborne controls. Filter papers from all blanks and controls were visually inspected under a microscope for any microplastic contamination.

Declarations

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CRediT authorship contribution statement.

L.D.D and A.B conceived the idea of this paper. L.D.D and T.C performed laboratory analyses. L.D.D and T.C discussed the results and analysed the data collected. L.D.D wrote the full manuscript while A.B contributed substantially to revisions.

Additional information.

Supplementary information. The data that support the findings of this study are available in the supplementary information that accompanies this paper.

Competing interests. The authors declare no competing interests.

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Figures

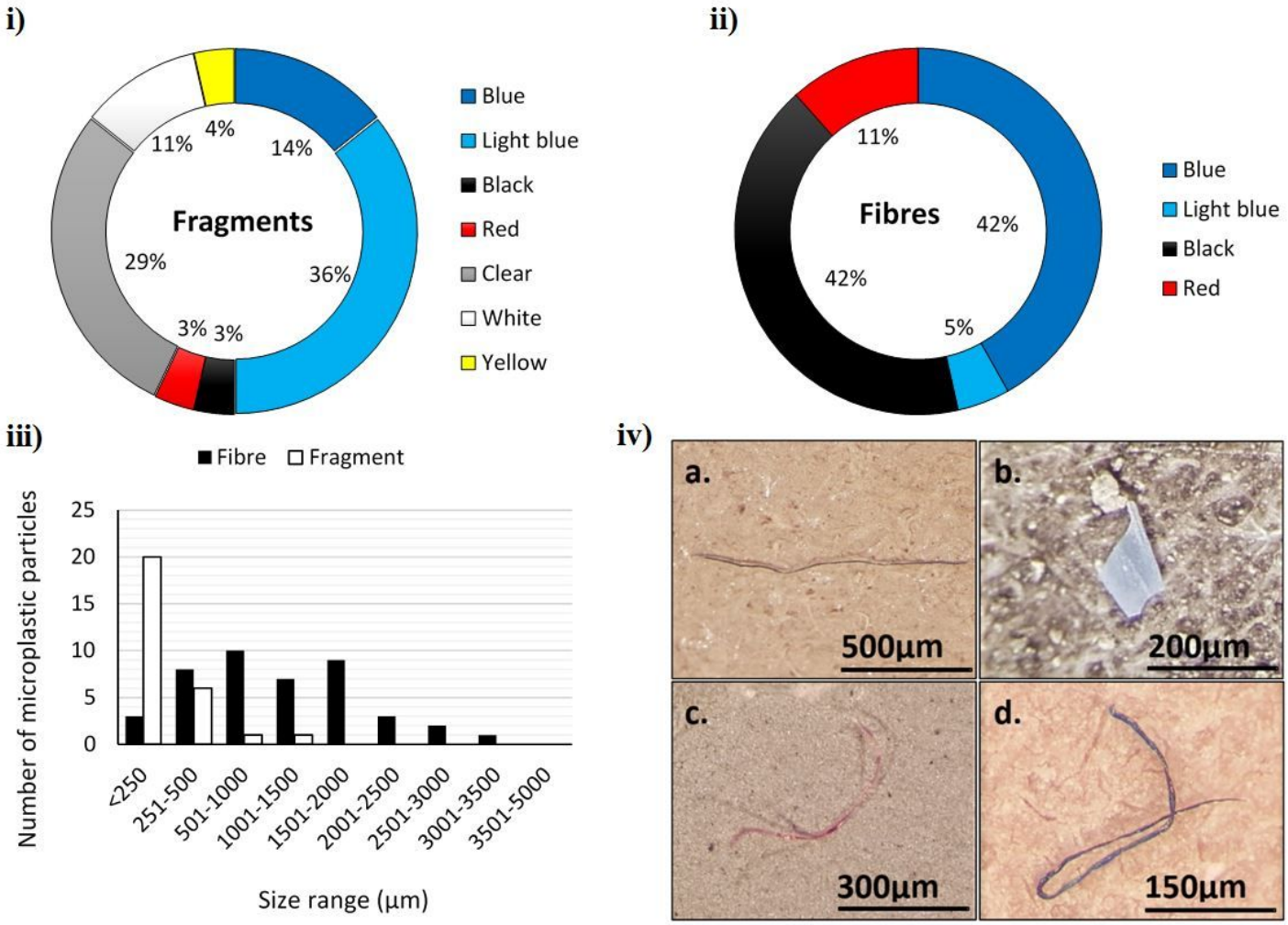

Figure 1

i) Proportion of fragment colours detected throughout all samples. ii) Proportion of fibre colours detected throughout all samples. iii) Size ranges of microplastics detected throughout all samples. iv) Photographic examples of microplastic particles identified including (a.) blue fibre; (b.) light blue fragment; (c.) red fibre and (d.) black fibre.

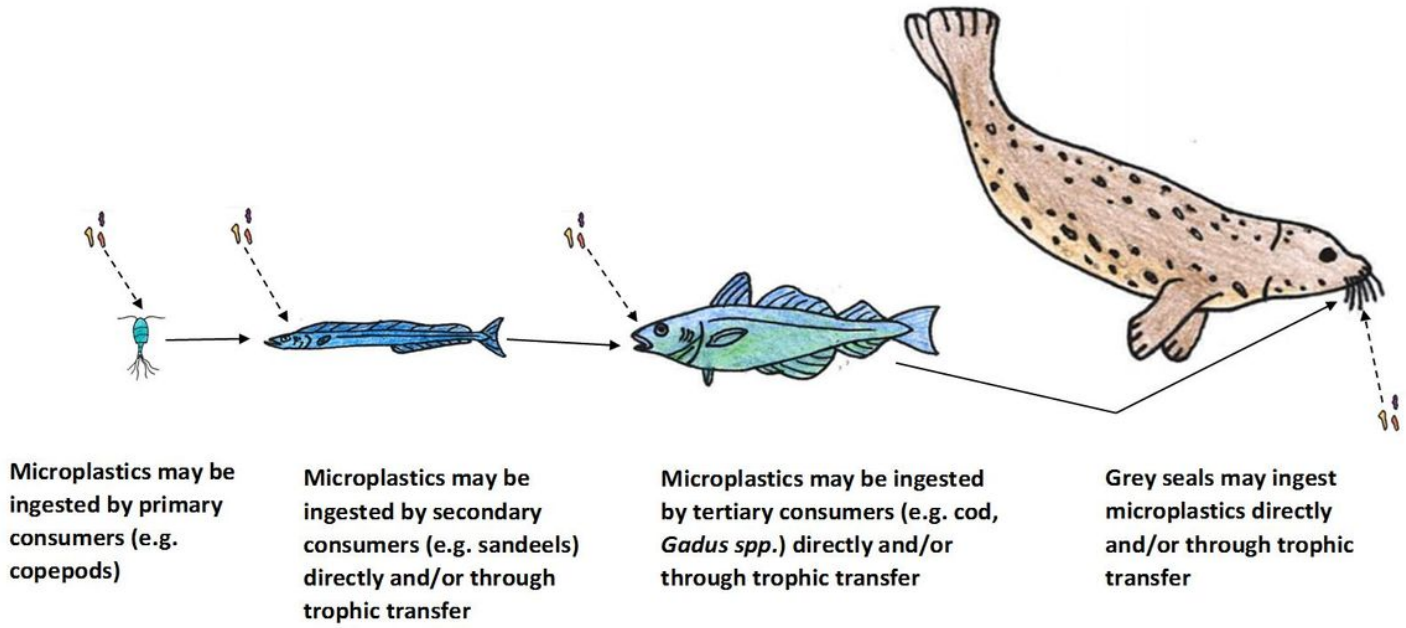

Figure 2

Diagram showing the potential routes for microplastic entry into the grey seal food chain

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