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Investigating microplastic trophic transfer in marine top predators☆

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ABSTRACT

Microplastics are highly bioavailable to marine organisms, either through direct ingestion, or indirectly by trophic transfer from contaminated prey. The latter has been observed for low-trophic level organisms in laboratory conditions, yet empirical evidence in high trophic-level taxa is lacking. *In natura* studies face difficulties when dealing with contamination and differentiating between directly and indirectly ingested microplastics. The ethical constraints of subjecting large organisms, such as marine mammals, to laboratory investigations hinder the resolution of these limitations. Here, these issues were resolved by analysing sub-samples of scat from captive grey seals (*Halichoerus grypus*) and whole digestive tracts of the wild-caught Atlantic mackerel (*Scomber scombrus*) they are fed upon. An enzymatic digestion protocol was employed to remove excess organic material and facilitate visual detection of synthetic particles without damaging them. Polymer type was confirmed using Fourier-Transform Infrared (FTIR) spectroscopy. Extensive contamination control measures were implemented throughout. Approximately half of scat subsamples (48%; $n = 15$) and a third of fish (32%; $n = 10$) contained 1–4 microplastics. Particles were mainly black, clear, red and blue in colour. Mean lengths were 1.5 mm and 2 mm in scats and fish respectively. Ethylene propylene was the most frequently detected polymer type in both. Our findings suggest trophic transfer represents an indirect, yet potentially major, pathway of microplastic ingestion for any species whose feeding ecology involves the consumption of whole prey, including humans.

1. Introduction

Microplastics (<5 mm in size) are ubiquitous in a wide range of marine habitats (GESAMP, 2015) and research interest is growing to better understand their impacts on the health of the marine environment and the organisms within it. These synthetic and persistent particles originate from a variety of sources, which include the fragmentation of larger macro-plastics (e.g. fishing gear, packaging) by UV photo-degradation, wave action and physical abrasion; shipping spills of pre-production pellets (nurdles) and polystyrene beads; the discharge of waste water containing microbeads used in cosmetics and microfibers released during the washing of textiles; and run-off from land containing road marking paint and vehicle tyre fragments (Andrady, 2011; Barnes et al., 2009; Boucher and Friot, 2017; Browne et al., 2011; Napper and Thompson, 2016; UNEP, 2009). Their small size means that microplastics are bioavailable to ingestion by a variety of taxa including zooplankton, marine invertebrates, fish, seabirds, and marine mammals (Amélineau et al., 2016; Cole et al., 2013; Lusher et al., 2015, 2013). Reasons for *direct* ingestion include accidental consumption of particles through indiscriminate feeding strategies (e.g. filter-feeders; Besseling et al., 2015; Cole et al., 2013); or active selection due to misidentification of microplastics for food (de Sá et al., 2015; Hall et al., 2015; Neves et al., 2015), based on sensory signals, such as visual or olfactory cues (Hoarau et al., 2014; Savoca et al., 2016). Once ingested, microplastics can cause a reduction in feeding capacity, energy reserves and reproductive output as well as detrimental alterations to intestinal function as shown in a

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number of low trophic level organisms (Cole et al., 2015; Pedà et al., 2016; Sussarellu et al., 2016; Wright et al., 2013a). Microplastics may also be ingested *indirectly* as a result of trophic transfer, whereby contaminated prey items are consumed by predators (Farrell and Nelson, 2013).

To date, empirical studies have demonstrated that trophic transfer occurs under laboratory conditions for low trophic level organisms, such as crabs (Batel et al., 2016; Farrell and Nelson, 2013; Setälä et al., 2014; Watts et al., 2014), but the extent to which this occurs in the wild and in higher trophic level organisms, is as yet unknown. Studies have recorded microplastic particles within the gastro-intestinal tracts (GIT) of various wild-caught fish species (Lusher et al., 2013; Neves et al., 2015; Rummel et al., 2016), highlighting the potential for transfer to predators to occur. Marine mammals that employ a raptorial feeding strategy, where prey is captured using the jaws and teeth alone, may be more likely to experience trophic transfer as primary route of microplastic ingestion than through direct intake (Hocking et al., 2017). For example, Lusher et al. (2016) found that 11% of mesopelagic fish investigated contained microplastics and calculated that ~463 million microplastics could be ingested by one striped dolphin (*Stenella coeruleoalba*) through the consumption of contaminated prey. This, however, remains to be demonstrated by empirical research. In seabirds, pellets (regurgitate) from great skuas (*Stercorarius skua*) containing remains of Northern fulmars (*Fulmarus glacialis*) exhibited the highest plastic prevalence, leading the authors to surmise that plastic burden is related to prey type and is therefore a result of trophic transfer (Hammer et al., 2016). Eriksson and Burton (2003) found that scats (faeces) collected from an Antarctic fur seal (*Arctocephalus tropicalis* and/or *A. gazella*) colony contained plastic particles, ranging from 2 to 5 mm (<0.5 mm were not included in the analysis). The authors suggest that, as the fur seals are unlikely to have ingested plastic of this size directly, the observed microplastic presence could be explained by a 'plastics concentrating stage', whereby a species of fish (*Electrona subaspera*) consume plastic particles from the water column and are in turn predated upon by the fur seals (Eriksson and Burton, 2003). Similar inferences were made for observations recorded for Hooker's sea lions (*Phocarctos hookeri*; Goldsworthy et al., 1997; McMahon et al., 1999). Another study analysed stomachs, intestines and scats of harbour seals (*Phoca vitulina*) and found the incidence of plastic to be 11%, 1% and 0% respectively (Bravo Rebolledo et al., 2013). The methods used to locate and identify plastic particles, were not appropriate for microplastics and the authors acknowledge the risk of losing 'small and poorly visible' particles and overlooking small particles (0.12–0.3 mm) during microscopic sorting. Though deemed unlikely by Eriksson and Burton (2003), the possibility that microplastics found in scat is a result of direct plastic consumption (either accidentally or through naivety) cannot be excluded. For example, twelve of 32 seal species have been documented to ingest marine debris (Kuhn et al., 2015; Ryan et al., 2016) and Hoarau et al. (2014) inferred that small plastic pieces found within marine turtles resulted from fragmentation of larger plastic pieces within the gastro-intestinal tract (GIT). This indicates that microplastics detected in GITs may have originally been directly ingested as macro-plastics. Furthermore, external contamination of the scats *in situ*, cannot be discounted. The ethical constraints of subjecting large organisms, such as marine mammals, to laboratory investigations, hinder the resolution of practical issues, such as contamination, experienced by *in natura* studies. Here, we analysed scats from captive seals (residents of a rehabilitation centre) and the wild-caught fish they are fed upon. As a result, the issue of contamination and the likelihood of direct plastic consumption were significantly lessened. The aims of this study were to; a) assess the abundance of microplastics in both scats and fish prey and characterise them by type (fragment or fibre), colour, size and polymer b) evaluate the efficacy of the methods utilised to isolate and identify microplastic particles and c) determine whether microplastic presence can be attributed to trophic transfer.

2. Materials and methods

2.1. Sample collection

2.1.1. Seal scats and fish

Seal scats were collected from an outdoor enclosure at the Cornish Seal Sanctuary in Gweek, Cornwall (United Kingdom) containing four resident adult grey seal males. The animals, which are of wild origin, have been residing at the Seal Sanctuary for at least four years and are not exposed to anthropogenic litter items, which may be encountered by wild animals. A plastic enrichment toy, however, is provided. As such, samples were taken from the toy to compare with any particles found in the scats. Two scat samples (approx. 100 ml) were collected per week for 16 weeks, approximately three or four days apart ($n = 31$).

To examine the trophic link and possible transfer of microplastics, fish usually fed to the seals ($n = 31$) were retained. These were mackerel (mean weight ± SD = 130 ± 22 g; mean length ± SD = 23 ± 2 cm) obtained from a local supplier, caught within the local region (Celtic Sea/English Channel/Western Approaches).

2.1.2. Water samples

Water for the enclosure pool is pumped from the Helford River via a sediment trap, and though filtered, is a potential source of microplastic contamination. To control for this, water samples (50 ml; $n = 31$) were collected alongside the scats.

All samples were stored at −20 °C prior to further examination.

2.2. Sample preparation: fish prey items

2.2.1. Gastro-intestinal tract and content extraction

Whole mackerel were thawed at room temperature. An incision was made at the anus, along the ventral side of the fish to the gill covers to expose the internal organs. The gastro-intestinal tract (oesophagus, stomach, pyloric ceca and intestines) was located, removed and rinsed with Milli-Q water (ultrapure and filtered). A syringe was used to flush approximately 50 ml of Milli-Q water through the GIT from the entrance of the oesophagus and the resulting fluid was collected. On a clean metal surface, an incision was made along the length of the GIT. Milli-Q water and a metal spatula were used to extract the GIT content which was collected and contained with the flushed fluid from the previous step. The resulting liquid was then passed through a 40 μm mesh disc using a vacuum pump. The mesh disc was placed inside a Petri dish and dried.

2.3. Sample preparation: seal scats

2.3.1. Sieving

Scats were thawed at room temperature before being passed through a stack of fractionating sieves (mesh sizes: 2000 μm, 1000 μm, 500 μm and 200 μm) using Milli-Q water and a metal spatula. The material was collected at each level, including 50 ml of liquid contained in the beaker in which the sieves were held, to ensure particles of <200 μm in size were also captured. The collected material was dried at 60 °C until no moisture remained to optimise concentrations of solutions used during enzymatic digestion.

2.3.2. Enzymatic digestion

Microplastics present in environmental samples may be masked by biological material. Some methods of removing this material, such as the use of strong oxidizing agents (e.g. acids) can damage or degrade the microplastic particles (Lusher et al., 2017; Lusher et al., 2018). The use of enzymes, however, is considered a more appropriate method as it does not alter the properties of plastic (Lusher et al., 2017; Lusher et al., 2018). As such, an enzymatic digestion protocol developed by Lindeque and Smerdon (2003) and adapted by Cole et al. (2014), was further adapted for application to seal scats. A 3 g subsample (50% ± 15% SD of total scat sample dry weight) of the desiccated material was digested. 15 ml of homogenizing solution (400 ml Tris-HCI buffer, 120 ml ethylenediaminetetraacetic acid (EDTA), 30 ml sodium chloride (NaCl), 100 ml Sodium Dodecyl Sulphate (SDS), 350 ml Milli-Q water) per gram of dried scat was added to a clean (acid washed and rinsed with Milli-Q water) Duran bottle. Samples were physically homogenized by stirring rapidly with a metal spatula for 30 s and incubated at 50 °C for 30 min. 750 µl of 20 mg ml^{-1} Proteinase-K was added to each gram of dried scat and incubated for up to 24 h at 50 °C. Following this, 3 ml 5 M sodium perchlorate ($NaClO_4$) was added per gram of dried scat and samples shaken at 20 °C (room temperature) for >30 min. Samples were again physically homogenized for a longer period of 1 min and then incubated a final time at 60 °C for 30 min. Each sample was then split across three 40 µm mesh discs using a vacuum pump and subsequently left to dry.

2.4. Microplastic identification

The physical characteristics of microplastics can facilitate an understanding of their possible sources and reasons for ingestion. As such, material retained on the mesh discs (for fish GITs and seal scats) was visually inspected under a microscope (Olympus SZX16) and any potential plastic particles were classified (type - fragment or fibre; colour; size and description), photographed (using a microscope mounted Canon EOS 550D DSLR camera) and retained separately for further analysis using Fourier-Transform Infrared (FTIR) spectroscopy (Agilent Cary 630 FTIR spectrometer; Agilent FTIR Spectral Library – Poly 8). Microplastic colour was determined by eye.

When interpreting FTIR output, only readings with confidence levels of 70% or greater (Lusher et al., 2013) and those considered to have reliable spectra matches (after visual inspection) were accepted. Only these particles were included for further analysis. All confirmed synthetic polymer particles were included in our results.

2.5. Contamination and microplastic loss prevention

Contamination of samples by microplastics present on equipment and within the atmosphere risks producing inaccurate results and should therefore be minimised. In addition, their small size means that microplastics present within the samples may be lost during processing. A number of measures, listed below, were implemented to limit these risks and control for any contamination that did occur.

2.5.1. Sample collection

Sample collection pots were thoroughly rinsed with Milli-Q water in a clean environment. Scat collection was carried out using a metal scraping instrument and sample pot caps were removed for as limited time as possible.

2.5.2. Sample preparation

Throughout the sample preparation process, a cotton lab coat and gloves were worn. All work surfaces were wiped down with 70% ethanol prior to any work commencing and all equipment was thoroughly rinsed with Milli-Q water.

2.5.2.1. Sieving. Work was carried out inside a positive pressure laminar flow hood. Prior to use and between scats, the sieves were scrubbed using a natural fibre brush and veterinary detergent and then rinsed thoroughly with Milli-Q water. Damp filter paper in a Petri dish was used to control for any air-borne contamination inside the flow hood where the work was carried out. In addition, a procedural blank (20 ml Milli-Q water) was run through the sieves and filtered using a mesh disc to control for any contamination at this stage of processing. Both the mesh disc and filter paper were inspected under a microscope for any particles at the beginning and end of this step respectively.

2.5.2.2. Enzymatic digestion. Prior to any work, all equipment was rinsed with Milli-Q and all pipettes and syringes were flushed with Milli-Q. Lids were removed from Duran bottles for as limited time as possible. Scats were weighed in an enclosed balance. After homogenizing, the metal spatula was rinsed with homogenizing solution to avoid loss of particles from samples. The vacuum pumping process was carried out inside the laminar flow hood. Prior to vacuum pumping all mesh discs were visually inspected for contamination under a microscope and any particles removed. A procedural blank was run through the vacuum pump and the mesh disc inspected before samples were filtered. If contamination was found, the vacuum pump and mesh disc were cleaned until no particles were detected.

3. Results

3.1. Microplastic presence in fish prey

Of the individual fish examined ($n = 31$), 10 (32%) contained 18 confirmed microplastic particles (Table 1). The number per fish ranged from 0 to 4 (mean ± SD = 0.58 ± 1.05 particles; Fig. 1a). The majority were fibres ($n = 13$; 72%) and the remaining 28% comprised of fragments ($n = 5$). The most prevalent colours were red and blue (both 28%), black (22%), orange and green (both 11%; Fig. 1c). Fibres ranged from 0.5 to 6.0 mm in length. The largest fragment found was 0.7 × 0.2 mm and the smallest 0.1 × 0.1 mm in diameter. The mean particle length was 2.0 mm (±SD = 1.8 mm). The most prevalent polymer types as confirmed by FTIR were ethylene propylene and polyethylene (both 28%) followed by neoprene (11%), polypropylene, ethylene propylene diene monomer (EPDM), nitrile butadiene rubber (NBR), aramid woven fabric, poly (butylene terephthalate), polyacrylamide (all 6%; Fig. 1d). See Fig. 2a for photographic examples of microplastics found in fish.

3.2. Microplastic presence in scats

Of the 31 scat subsamples analysed, 15 (48%) contained a total of 26 confirmed microplastic particles (Table 1). The number of particles per scat ranged from 0 to 4 (mean ± SD = 0.87 ± 1.09 particles; Fig. 1b). Of these, 18 were fragments (69%) and eight were fibres (31%). Black particles were most commonly found (27%) followed by clear (transparent) and red (both 23%), blue (15%) and orange (12%; Fig. 1c). Particle size varied with fragments ranging from 0.4 × 0.3 mm to 5.5 × 0.4 mm. Fibres ranged from 0.6 to 3.5 mm in length. The mean particle length was 1.5 mm (±SD = 1.2 mm). The most common polymer types identified by FTIR were ethylene propylene and polypropylene (both 27%)

Table 1
Characteristics of particles found in fish and seal scats.

Sample	Type	Colour	Size (μm)	Polymer	FTIR confidence	Spectra match
Fish	Fibre	Blue	5000 × 30	NBR	0.808	Reliable
	Fibre	Black	4200 × 50	Polyacrylamide	0.888	Reliable
	Fibre	Red	2000 × 30	Neoprene	0.845	Reliable
	Fibre	Orange	2500 × 30	Polyethylene terephthalate	0.893	Reliable
	Fragment	Red	700 × 200	Aramid woven fabric	0.702	Reliable
	Fragment	Red	300 × 100	Polyethylene	0.849	Reliable
	Fibre	Red	2000 × 100	Polyethylene	0.834	Reliable
	Fragment	Orange	100 × 100	EPDM	0.865	Reliable
	Fragment	Green	100 × 100	Polyethylene	0.823	Reliable
	Fibre	Red	700 × 50	Ethylene Propylene	0.832	Reliable
	Fibre	Black	4000 × 30	Poly (butylene terephthalate)	0.814	Reliable
	Fibre	Blue	50 × 50	Neoprene	0.874	Reliable
	Fibre	Black	1200 × 30	Polyethylene	0.851	Reliable
	Fibre	Black	2500 × 30	Ethylene propylene	0.768	Reliable
	Fibre	Blue	6000 × 30	Ethylene Propylene	0.881	Reliable
	Fibre	Blue	3300 × 50	Ethylene propylene	0.838	Reliable
	Fibre	Blue	1800 × 50	Ethylene Propylene	0.859	Reliable
	Fragment	Green	200 × 150	Polypropylene	0.875	Reliable
Seal scats	Fragment	Red	500 × 500	Polypropylene	0.81	Reliable
	Fragment	Clear	2600 × 400	Polypropylene	0.81	Reliable
	Fragment	Clear	800 × 600	Polypropylene	0.93	Reliable
	Fibre	Black	600 × 50	Ethylene propylene	0.88	Reliable
	Fragment	Red	1000 × 400	Polyethylene	0.91	Reliable
	Fibre	Black	1200 × 50	Ethylene propylene	0.88	Reliable
	Fibre	Black	2100 × 10	Ethylene propylene	0.89	Reliable
	Fibre	Black	1300 × 10	Ethylene propylene	0.92	Reliable
	Fragment	Red	1200 × 900	Polyethylene	0.77	Reliable
	Fibre	Black	600 × 50	Ethylene propylene	0.95	Reliable
	Fragment	Black	2500 × 100	Polyacrylamide	0.84	Reliable
	Fragment	Red	500 × 600	Polyurethane	0.83	Reliable
	Fragment	Clear	5500 × 400	Polypropylene	0.71	Reliable
	Fragment	Blue	400 × 300	Ethylene propylene	0.84	Reliable
	Fragment	Orange	1800 × 1200	Ethylene propylene	0.85	Reliable
	Fragment	Black	700 × 100	Polyaramid Kevlar	0.77	Reliable
	Fragment	Orange	3500 × 2300	EPDM	0.87	Reliable
	Fragment	Red	600 × 300	Polypropylene	0.89	Reliable
	Fibre	Clear	3500 × 100	Polyethylene	0.84	Reliable
	Fibre	Blue	600 × 500	Styrene butadiene rubber	0.83	Reliable
	Fragment	Clear	2300 × 1500	Neoprene	0.86	Reliable
	Fragment	Blue	1000 × 800	Styrene butadiene rubber	0.88	Reliable
	Fibre	Red	2300 × 50	Polypropylene	0.82	Reliable
	Fragment	Clear	20 × 800	NBR	0.78	Reliable
	Fragment	Orange	1100 × 700	Polyacrylamide	0.86	Reliable
	Fragment	Blue	500 × 400	Polypropylene	0.86	Reliable

followed by polyethylene (12%), polyacrylamide and styrene butadiene rubber (both 8%), neoprene, EPDM, NBR, polyaramid Kevlar, polyurethane (all 4%; Fig. 1d). These results are from scat subsample representing ~50% of total dry weight. See Fig. 2b for photographic examples of microplastics found in scats.

3.3. Contamination levels

3.3.1. Water samples and enrichment toy

Black ethylene propylene fibres ($n = 4$) were detected in water samples taken from the enclosure pool but as these were also found in the fish GITs, those detected in the scats were included within the results. It is likely that the seals defecated in the pool and so introduced the particles themselves. No particles matching the enrichment toy were detected.

3.3.2. Sample preparation

No evidence of contamination was found in any of the procedural controls or blanks. Blue polypropylene fragments ($n = 5$) matching FTIR output for the bottle lids used during sample preparation were found in two of the samples. These were excluded from the results as these were considered to be a possible result of contamination. Aluminium foil lids were used for the remaining samples to avoid any further possibility of contamination.

4. Discussion

This study is the first to investigate and demonstrate empirical evidence for the trophic transfer of microplastics from fish to a marine top predator. Studies on microplastics and pinnipeds are scarce (Bravo Rebolledo et al., 2013), making it challenging to draw comparisons with our results. A wild study found the number of particles per scat ranged from 0 to 4 and the majority of those containing microplastics had one particle (Eriksson and Burton, 2003). It is not clear whether the whole scat or a subsample was examined, or what methods were employed to do so. In this study black, clear and red were the most frequently found colour particles in scats which differs from Eriksson and Burton (2003) where white, brown, blue, green and yellow were most common. Additionally, the mean particle length was 4.1 mm which differs from our result (1.5 mm; Eriksson and Burton, 2003). It is possible that methodological techniques employed in our study allowed for smaller particles to be detected. Though not discussed explicitly, it seems that all particles found were fragments, which is similar to

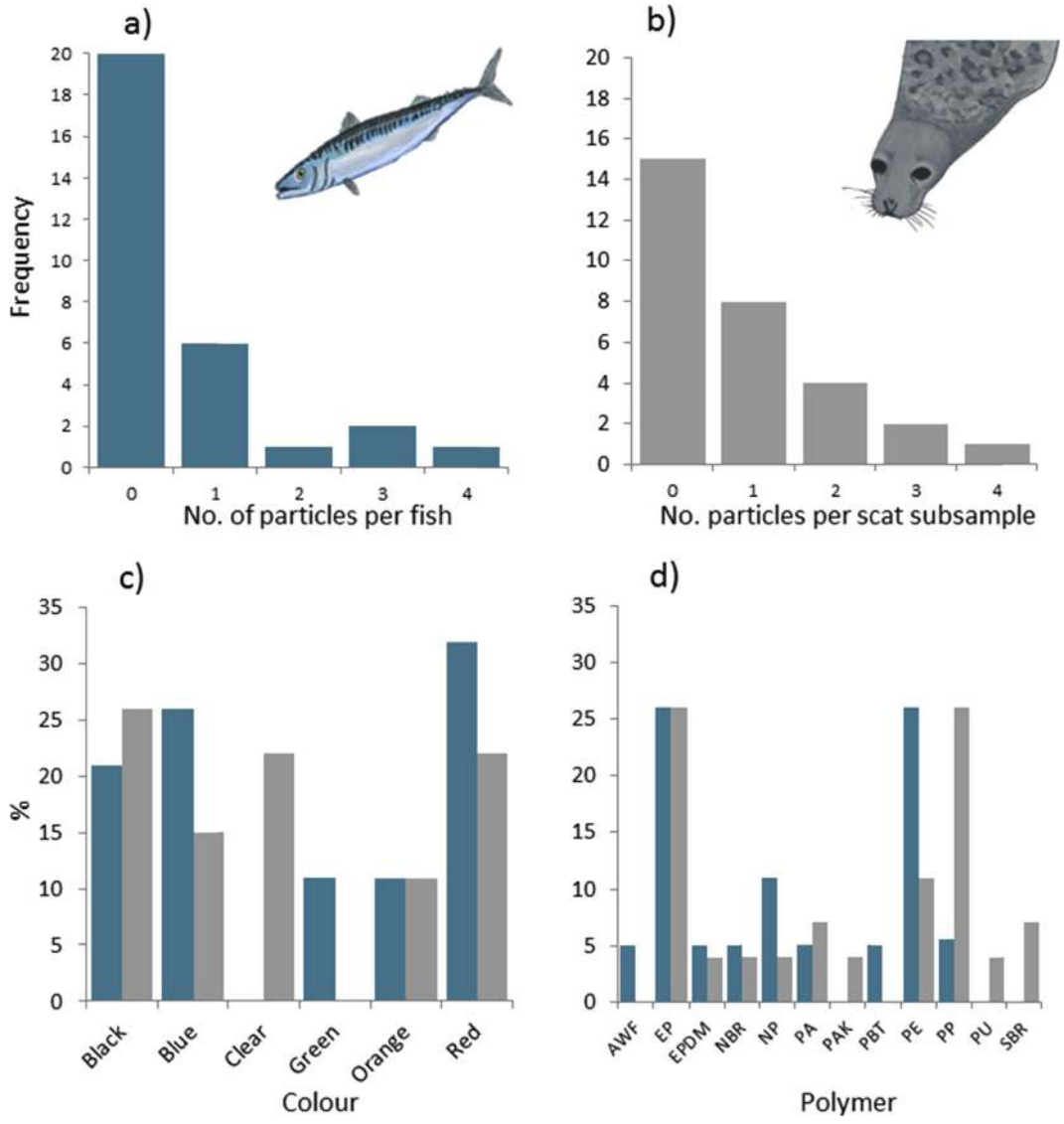

Fig. 1. a) Frequency histogram showing number of particles per fish **b)** Frequency histogram showing number of particles per scat subsample **c)** Barplot showing percentage of particles for each colour in fish and scats **d)** Barplot showing percentage of particles per polymer type for fish and scats (AWF = aramid woven fabric; EP = ethylene propylene; EPDM = ethylene propylene diene monomer (M-class) rubber; NBR = nitrile butadiene rubber; NP = neoprene; PA = polyacrylamide; PAK = polyaramid Kevlar; PBT = poly (butylene terephthalate); PE = polyethylene; PP = polypropylene; PU = polyurethane; SBR = styrene butadiene rubber). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the results of our study, though some fibres were identified.

Ingestion rates of microplastics by fish prey could not be accurately assessed in this study because samples were obtained from the fishing industry and not collected using the necessary sampling protocols. This is important because some species of fish are known to regurgitate stomach contents during capture as a result of handling stress which may result in the loss of microplastics and so bias the results of any analysis (Bromley, 1994; Lusher et al., 2017; Lusher et al., 2018). Conversely, during capture, fish may ingest microplastics that accumulate in the net, or originate from the net itself (Lusher et al., 2013). Nevertheless, Neves et al. (2015) found that 31% Atlantic mackerel sampled had ingested microplastics, with a mean of 0.46 (±0.78) microplastics per individual. This corresponds with the results of this study, whereby 32% of mackerel contained microplastics with mean of 0.58 particles per fish. Our finding that fibres were more commonly detected (72%) than fragments corresponds with findings from other research on environmental microplastic concentrations (Claessens et al., 2011; Lusher et al., 2013; Neves et al., 2015; Wright et al., 2013b) and two studies investigating fish found approximately 66% and 68% of microplastics were fibres (Lusher et al., 2013; Neves et al., 2015). One study reported particles of various colours with the black being the most common at 45% (Lusher et al., 2013). We found black to be the third most common colour (22%) after red and blue. Neves et al. (2015) found the size of particles generally ranged from 0.217 to 4.81 mm (mean 2.11 ± 1.67 mm) and Lusher et al. (2013) reported a larger range of 0.13–14.3 mm the most common size class to be 1–2 mm. The mean particle length detected in fish in our study was 2 mm.

In total, 12 polymer types were detected in the fish and scats analysed in this study. The most common for both was ethylene propylene, indicating a clear link between the seals and the fish they consumed. The particles detected in scats by Eriksson and Burton (2003) comprised five major polymer groups;

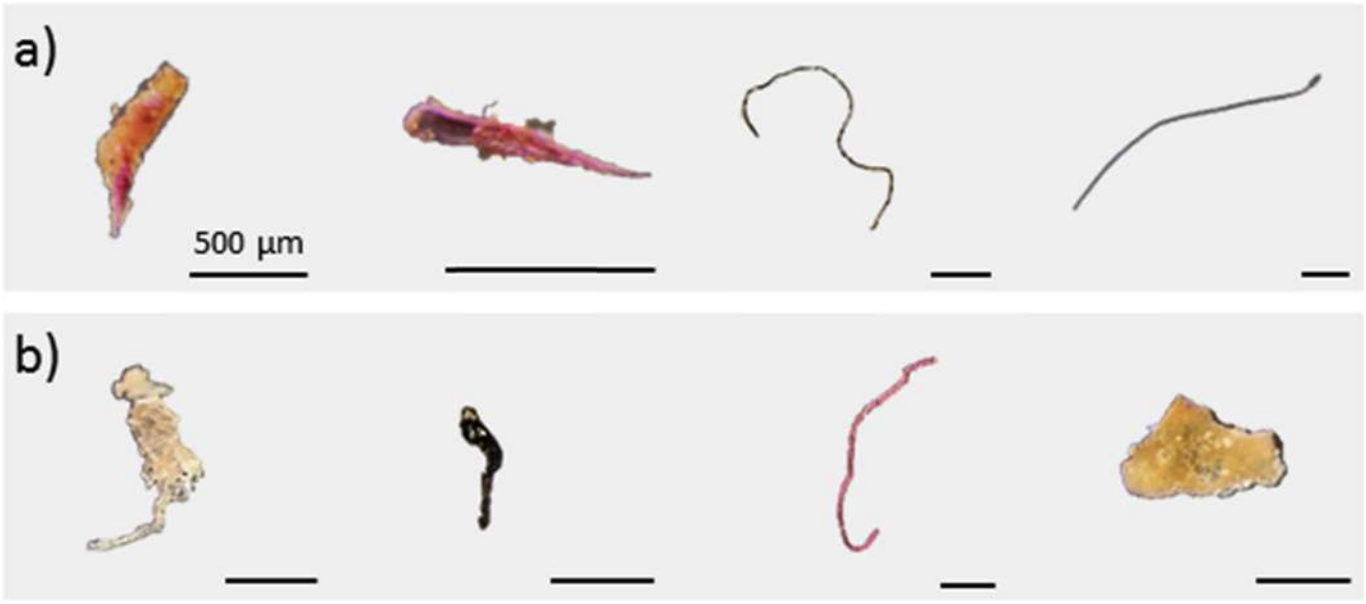

Fig. 2. Photographic examples of particles found in a) fish (from l–r: aramid woven fabric, polyethylene, ethylene propylene, polyacrylamide) and b) scat subsamples (from l–r: polyethylene, polyaramid Kevlar®, polypropylene, polyacrylamide). Scale bars represent 500 µm.

polyethylene (93%), polypropylene (4%), poly(1-Cl-1-butenylene) polychloroprene (2%), melamine-urea (phenol) (formaldehyde) resin (0.5%) and cellulose (0.5%). The polymer types detected in the scats of our study were more varied (10 polymer types), which may be as a result of diversity within the marine environment. The animals investigated by Eriksson and Burton (2003) were located on Macquarie Island, a remote island in the southwest Pacific Ocean. As such, they are likely exposed to different microplastic inputs from those in our study, which are fed on fish from the north-east Atlantic, caught near the British coast. The two most common polymers detected in fish by Neves et al. (2015), polypropylene and polyethylene, were also commonly detected in the scat and fish analysed in this study.

Our findings indicate some disparities between the type, colour and size of microplastics detected in fish compared with those found in scats. For example, the majority of particles detected in scats were fragments while the reverse is true for the fish with fibres being most common. Though black, red and blue particles featured prominently in both fish and scats, and they contained the same proportion of orange particles, the latter also contained a high proportion of clear particles which were not detected in fish. A range of sizes of fragments and fibres were detected. These variations may be due to several reasons;

Diversity within the system: The fish examined for microplastics may not have been caught concurrently with those fed to (and excreted by) the seals. As a result of the considerable diversity in microplastic abundance, type (fragment/fibre), size, colour and polymer observed not only among fish individuals, populations and species (Lusher et al., 2013; Neves et al., 2015; Rummel et al., 2016) but within the marine environment generally (Amélineau et al., 2016; Cózar et al., 2015; Woodall et al., 2014), we would not expect to see a complete match between the particles found in the scats and the fish.

Methodological constraints: The differing methods of microplastic extraction and isolation from fish GITs and scat may have contributed to some of the observed variation. For example, though efforts were made to minimise microplastic loss, it is possible that the protracted processing involved in enzymatic digestion of the scats, increased the risk of losing some particles. In addition, microplastic detection relies on human ability so it is likely that particles that are 'natural' in colour (i.e. brown, beige) are under reported in some cases. The colour of background substrate may influence which colours are more likely to be detected. For example, clear/transparent particles are less obvious in fish than scat because the substrate is translucent. The relatively small sample sizes are also likely to have contributed to some of the observed variation.

Biological implications: One study found more plastic in the stomachs of harbour seals (*Phoca vitulina*) than elsewhere in the GIT or scat (Bravo Rebolledo et al., 2013). This suggests that the stomach may act as a trap for non-food items, such as microplastics. To investigate this further, it would be necessary to examine the GITs of dead animals, preferably those known to have died as a result of physical trauma, such as by-catch, whereby normal feeding behaviour prior to death can be assumed.

It has been suggested that atmospheric microplastics may be a source of particles found in the gut content/faeces of marine mammals (Lusher et al., 2017; Lusher et al., 2018). Though this is possible in some cases, it is unlikely in this study for a number of reasons. Firstly, most atmospheric microplastics are fibres (Dris et al., 2015) and the majority of particles found in the scats were fragments. Secondly, the animals investigated in this study reside in a rural area, with very low levels of air pollution (www.uk-air.defra.gov.uk/air-pollution; last accessed 16 October 2017). Lastly, the strong correlation between polymer type in both fish and seal scats indicates that the microplastics found in scats were a consequence of ingestion as opposed to inhalation or contamination. It is unknown to what extent wild animals are exposed to atmospheric microplastics but examination of the lungs and airways of stranded animals could be a worthy aspect for future research efforts, alongside the monitoring of atmospheric microplastic levels at sea.

The methods of microplastic extraction and contamination control used in this study were effective for determining the presence and characteristics of microplastic particles in fish and scat. In addition, the use of captive seals significantly reduced the possibility of direct plastic consumption. As such, we attribute the

presence of microplastic particles in seal scats to the occurrence of trophic transfer from prey to a marine top predator. Whether these particles were directly consumed by the fish or underwent trophic transfer from ingestion of contaminated zooplankton is not known. Mackerel in the north east Atlantic, though opportunistic, feed primarily on calanoid copepods (Bachiller et al., 2016), which are approximately 2 mm in length (Lindeque et al., 2006). Zooplankton can consume microplastic particles of 0.4–30.6 μm in size (Cole et al., 2013) but all microplastics found in the fish were considerably larger than this (>100 μm) with a mean size of 2 mm. This indicates that microplastics found within the mackerel were most likely consumed directly from the water column, possibly because they were mistaken for prey items. Similarly, Amberstripe scad (*Decapterus muroadsi*) have been shown to readily ingest microplastics resembling their copepod prey in colour and size (Ory et al., 2017). The authors surmise that planktivorous fish are more likely to consume microplastics directly because of their feeding ecology as visual predators (Ory et al., 2017). Further investigation is needed to understand selectivity and its impacts on trophic transfer.

The occurrence of microplastic trophic transfer may have a number of impacts for top predators;

Physiological implications: Microplastic ingestion has been shown to cause a number of detrimental physiological impacts resulting in a reduction in feeding capacity, energy reserves and reproductive output for smaller low-trophic level organisms (Cole et al., 2013; Sussarellu et al., 2016; Wright et al., 2013a). It is not yet known whether this occurs in larger animals, such as marine mammals. Furthermore, very little information exists regarding the retention time of microplastics in marine mammal GITs. A study investigating the prey passage time of grey seals found that the majority of fish otoliths (ear bones) could be recovered from scats ~88 h after consumption (Grellier and Hammond, 2006; Lusher et al., 2016). The feeding trial also found that all polystyrene beads (3 mm) were recovered after 6 days. This suggests that, although they may take longer, microplastics are egested alongside indigestible dietary items (Lusher et al., 2016). It is not known, however, what effect this partial retention has on digestive processes and whether fibres behave differently within the digestive tract to the beads used by Grellier and Hammond (2006).

Prey availability: The known impacts for low trophic level organisms may have secondary implications for predators in the form of reduced food availability, i.e. Increased mortality of prey species as a result of microplastic ingestion. Further research is needed to assess whether this is the case.

Microplastics and chemical contaminants: Biomagnification and bioaccumulation of chemical contaminants, such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs), are known to occur at higher trophic levels, particularly affecting marine top predators (Jepson et al., 2016; Tsygankov et al., 2015). Whether a similar mechanism occurs for microplastics is unknown. For example, does the abundance of microplastic particles increase through and up marine food webs, and with the age of the animal? Further research is needed to investigate whether animals at higher trophic levels experience higher plastic loads than those at lower levels and whether older animals experience higher abundances than younger ones of the same species/population. In addition, microplastics may act as a vector for transporting chemicals, both trophically (Teuten et al., 2007) and spatially. For example, population declines in some marine mammal species have been linked to elevated burdens of OCs as a result of their presence within the marine environment (Murphy et al., 2015). The large surface area to volume ratio of microplastics can lead to the adsorption and concentration of such hydrophobic toxicants (Teuten et al., 2007). If consumed, they may desorb into biological tissues, potentially leading to detrimental endocrine and/or immune system effects with implications for reproductive success (Jepson et al., 2016; Murphy et al., 2015; Teuten et al., 2009). The ingestion of microplastics may represent an additional pathway by which these chemicals enter marine mammals, aside from the usual dietary input.

Human health: Our finding that microplastics can be transferred from fish to top predators has implications for human health. For instance, seafood that is consumed whole (i.e. including the GIT), such as shellfish, has been found to contain microplastics (Murray and Cowie, 2011; Rochman et al., 2015; Van Cauwenberghe and Janssen, 2014). Further work is required to better understand the extent of exposure to and impacts of microplastic ingestion on humans.

5. Conclusion

We present empirical evidence that microplastic particles can be transferred across trophic levels, from fish to a marine mammal top predator. Our findings suggest that trophic transfer represents an indirect, yet potentially major, pathway of microplastic ingestion for any species whose feeding ecology involves the consumption of whole prey.

Notes

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