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Microplastic and macroplastic ingestion by a deep diving, oceanic cetacean: The True's beaked whale *Mesoplodon mirus*

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ABSTRACT

When mammals strand, they present a unique opportunity to obtain insights into their ecology. In May 2013, three True's beaked whales (two adult females and a female calf) stranded on the north and west coasts of Ireland and the contents of their stomachs and intestines were analysed for anthropogenic debris. A method for identifying microplastics ingested by larger marine organisms was developed. Microplastics were identified throughout the digestive tract of the single whale that was examined for the presence of microplastics. The two adult females had macroplastic items in their stomachs. Food remains recovered from the adult whales consisted of mesopelagic fish (*Benthosema glaciale*, *Nansenia* spp., *Chauliodius sloani*) and cephalopods, although trophic transfer has been discussed, it was not possible to ascertain whether prey were the source of microplastics. This is the first study to directly identify microplastics <5 mm in a cetacean species.

1. Introduction

Pollution in the form of marine debris is recognized as a major threat to marine life, and can affect marine species through ingestion and entanglement (Derraik, 2002). Ingestion of large quantities of plastic threatens organisms through blocking the passage of food or reducing nutritional intake, which can potentially lead to starvation, malnutrition and ultimately death (Derraik, 2002). It is estimated that about 10% of the 230 million tonnes (by weight) of plastic produced globally every year ends up in the marine environment (PlasticEurope, 2012). Subsequently, as plastics breakdown, degradation and fragmentation leads to smaller and smaller particles (Barnes et al., 2009). These microplastics (defined as <5 mm in longest diameter, Arthur et al., 2009) can persist and accumulate throughout the marine environment, and are difficult, if not impossible, to remove. The indiscriminate input of microplastics in the form of nurdles (raw plastic pellets and beads used in the production of plastics), abrasive scrubbers from cosmetics and air blasting, and fibres from clothing are further sources of microplastics in the marine environment (e.g. Browne et al., 2011). Microplastics have been shown to float in surface waters and are transported by ocean currents to regions of low circulation or washed onto shores (Ivar do Sul and Costa, 2014). When plastics are denser than sea water they sink and can accumulate in deep-sea sediments (Van Cauwenberghe et al., 2013; Woodall et al., 2014).

Once in the marine environment, marine biota can interact with plastics and associated chemicals Macroplastic ingestion has been reported in several groups of marine organisms, such as seabirds, fish, turtles and mammals (e.g. Derraik, 2002). However, due to their small size microplastics are more difficult to detect during dissection procedures. Whilst they have been detected in wild organisms (as reviewed in Wright et al., 2013), the level of exposure by marine organisms' to microplastics requires greater understanding. Plastics can also be associated with chemicals including those incorporated during production (Lithner et al., 2011), and those sorbed from the environment (e.g. Mato et al., 2001). It has been suggested that chemicals such as plasticisers can leach from polymer matrices into organisms' tissues after ingestion (Teuten et al., 2007). For example, the same chemical tracers were identified in the blubber tissue of short-tailed shearwaters (*Puffinus tenuirostris*) and isolated from plastics found in their stomachs

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(Tanaka et al., 2013). It has been suggested that marine organisms exposed to these chemicals could suffer adverse effects, for example Browne et al. (2013) showed diminished ability to engineer sediment burrows and remove pathogenic bacteria in the lugworm (*Arenicola marina*) when exposed to Triclosan and nonylphenol respectively.

Microplastics are frequently ingested by marine fish (e.g. Boerger et al., 2010, Lusher et al., 2013) and invertebrates (e.g. Murray and Cowie, 2011; Braid et al., 2012). However, for marine mammals, the only records are from seals, where small plastic fragments were reported in stomachs of harbour seals (*Phoca vitulina*, L. 1758) from the North Sea (Bravo-Rebolledo et al., 2013) and in the scats of fur seals (*Arctocephalus* spp.) from Macquarie Island, Australia (Eriksson and Burton, 2003). It was suggested that these microplastics might have been ingested through their prey as a result of trophic transfer. Lantern fish (Myctophids) were reported to be the target prey of fur seals (Eriksson and Burton, 2003), and studies have shown that mesopelagic fish including Myctophids (Boerger et al., 2010; Davison and Asch, 2011) and Lampriformes (Choy and Drazen, 2013) had microplastic in their stomachs. Although direct trophic transfer has not been seen in larger marine mammals, laboratory feeding studies on invertebrates reported *Nephrops* fed fish seeded with microplastic strands, ingested but did not excrete these strands (Murray and Cowie, 2011). To the authors knowledge there are currently no published studies directly identifying microplastics in cetaceans.

Beaked whales from the family Ziphiidae are some of the rarest and least understood animals on the planet (Dalebout et al., 1998). They live in oceanic offshore waters where they forage at great depths on squid and fish. Little is known about the ecology of beaked whales, which have inconspicuous surfacing behaviour; most sightings are very brief and some species are only known from stranded individuals and have never been identified at sea. The *Mesoplodon* genus is one of the lesser known groups among the beaked whales, and information about their distribution, behaviour and biology is incomplete (Dalebout et al., 1998). True's beaked whales (*Mesoplodon mirus*, T. 1913) occur in the Northwest Atlantic and less frequently off the coasts of Ireland, France, the Iberian Peninsula and the Canary Islands, which suggests a probable relationship to the Gulf Stream (MacLeod, 2000). Information on the diet of *Mesoplodon* spp. can help to elucidate their behaviour, ecology and interaction with marine pollution, especially in offshore waters which are more difficult to study.

It is important to understand how rare species such as beaked whales are affected by interactions with marine pollution, especially in areas such as the North Atlantic where microplastics are ubiquitous (Lusher et al., 2014). The purpose of this study was to describe the plastics found in the digestive tracts of True's beaked whales and to develop a novel methodology with which to study microplastic ingestion in marine mammals. This is the first study to specifically target microplastic ingestion in a cetacean species.

Table 1
Stranding information related to the three stranded True's beaked whales in Ireland, 2013.

Code	Date	Location	Length (cm)	Sex	Cause of death
TBW/2013_077	12th May 2013	Five fingers strand, Co. Donegal, Ireland 55°19′N 7°20′W	476	Female	Unknown
TBW/2013_080	14th May 2013	Inishowen, Co. Donegal, Ireland 55°17′N 7°17′W	250	Female	Unknown
TBW/2013_088	27th May 2013	Ballyconneely, Connemara, Co. Galway, Ireland 53°25′N 10°4′W	500	Female	Unknown

2. Materials and methods

In May 2013, three beaked whales stranded on the north and west coast of Ireland (Table 1). An adult, female beaked whale stranded at Five Fingers' Strand, Co. Donegal and two days later a female beaked whale calf was found washed ashore inside the same bay around 2 km from the adult. Two weeks later, a second adult, female beaked whale was reported stranded at Ballyconneely Co. Galway.

2.1. Handling procedure and post-mortem examination

Post-mortem examinations of all animals were carried out following standard protocols (Kuiken and Garcia-Hartmann, 1993). Animals were measured and sexed, and examined for external lesions and other anomalies. Dissections followed standard procedure and were carried out in a veterinary laboratory in the case of the two whales from Donegal and *in situ* on the beach in Co. Galway due to the animals' large size and accessibility. Biopsies were taken and the digestive tract removed. Skin samples were sent to the University of Copenhagen for species confirmation and genetic relationship. For this study, the full digestive track (oesophagus, stomachs and intestines) were obtained during the post-mortem dissections. Digestive tracts were frozen (−20 °C) prior to analysis. Of the three individuals, one was analysed for microplastics and macroplastics, and two were only analysed for macroplastics as sufficient controls were not in place for them to be checked for microplastics.

2.2. Laboratory procedure

Gut contents were washed through a set of three nested sieves (118 μm, 500 μm and 1,000 μm), using pre-filtered tap water. Intestines were divided into 20 separate pieces and washed individually as well as each stomach chamber and oesophagus. Food remains, including hard structures, were collected and identified. Material retained in the sieve was frozen in sterilized glass containers and retained for microplastic analysis.

For microplastic analysis, once defrosted, the remaining suspension was dissolved using a pre-made solution of 10% KOH following Foekema et al. (2013). Three times the amount of solution (by mass) was added to the suspension and left for three weeks until biological material had dissolved. The remaining solution was filtered under vacuum using a Buchner Filter. Particles retained on GF/C microfiber filter paper were identified under a microscope (Olympus SZX10 with a mounted Q-imaging Retiga2000R camera). Visual classification was carried out using existing criteria (Lusher et al., 2014), and a subsample of plastics ($n = 80$) was retained for polymer classification using Fourier Transform Infrared spectroscopy (FT-IR). FT-IR determines the structure of molecules through the analysis of their absorption spectra (for more information, refer to Lusher et al., 2013). A Bruker IFS 66 Spectrometer with a Bruker Hyperion 1000 microscope was used to obtain the spectra which were then compared to a polymer library in OPUS v6.5 software. Polymers which matched reference spectra with a Euclidian Distance that had a high level of certainty (>70%) were accepted as microplastics whilst those with a lower level of certainty (60–70%) were subjected to further visual examination of spectra characteristics before being accepted or rejected.

2.3. Contamination control

All work was carried out under contamination controlled conditions (following Lusher et al., 2014). All manipulation instruments and equipment were cleaned and checked under a microscope for contamination with airborne fibres before use, as previous studies have commented on the abundance of textile fibres found in biological samples (e.g. Foekema et al., 2013). Finally blank samples were processed in the same way as gut samples. To check for sufficient contamination control, recently published EU guidelines suggest that background levels of microplastics in control samples should be less than 10% of the overall microplastic average throughout all samples (Hanke et al., 2013). All gut sections were covered prior to analysis and were exposed to air for the minimum time possible.

3. Results

Skin samples of all three beaked whales confirmed the identification as True's beaked whales. Based on a sequence of a 401 base pair (bp) fragment of the mtDNA control region, the first two animals were identical indicating they might be mother and calf. The sequence of a 400 bp mtDNA fragment from the individual from Co. Galway differed from the mother-calf pairing at four positions (217, 218, 257, 374) which indicated she belonged to different maternal lineage.

During post-mortem examination, all three animals (TBW/2013_077, TBW/2013_080 and TBW/2013_088) presented signs that were characteristic of live strandings. Macroscopic examination of their organs showed that many of them were collapsed with haemorrhagic areas; acoustic fat also presented haemorrhagic areas and bruises. There was nothing conspicuous in terms of external injury. Cause of death could not be determined.

On observation of the digestive tracts of both adult True's beaked whales, macroplastic fragments were found. A 4.2 × 3.1 cm fragment was found in the connecting chambers of TBW/2013_077 and a polyethylene shotgun cartridge, 7.1 × 2.2 cm, was found lodged in the accessory main stomach of TBW/2013_088 (Fig. 1). FT-IR spectroscopy confirmed that the plastic fragments were both polyethylene (Figure S1 in supplementary information).

Microplastics were identified in all stomach compartments and in 17 of 20 sections (85%) of the intestine of TBW/2013_088 (Fig. 2). Out of the four stomach compartments examined, 29 particles (58% fibres; 42% fragments) with a mean of 7.25 particles per compartment (±2.63, range 5–11) were isolated. Most particles were found in the main stomach (38%), followed by the accessory main stomach (24%), connecting chambers (21%) and pyloric stomach (17%). On average there were 2.95 microplastic particles (±2.09, range 0–7, Fig. 2A) in each section of the intestine. Of the 59 particles identified in the intestine, the majority were fibres (89%). In total, 88 particles were identified in the whole digestive tract with a mean length of 2.16 mm (±1.39, range 0.3–7 mm, Fig. 2B).

All filters left in the laboratory during the dissection procedure were free of contamination. Blank samples (10% KOH solution) were also free of contamination. As the controls were free of contamination, no corrections were made when calculating concentrations.

FT-IR was used to determine the polymer structure of a subsample (n = 80) of the plastics isolated from the digestive tracts. Polymers were identified as Rayon (53%), polyester (16%), acrylic (10%), polypropylene (6%), polyethylene (4%) and nine (11%) could not be identified from the absorbance spectra produced (Figure S2 in supplementary information). Euclidian distance was used to accept or reject particles based on their percentage match to reference spectra. Of the best matches, polyester, polypropylene and polyethylene all had hits of over 70% and were accepted based on Euclidean Distance alone, whereas many Acrylic and Rayon spectra were accepted based on visual analysis of the spectra.

At the time of the stranding, TBW/2013_077 had an unidentified cephalopod in its mouth. A total of 50 individual fish were identified within its digestive tract corresponding mainly to mesopelagic fish including *Benthosema glaciale* (R. 1837), *Nansenia* spp. (J.&E. 1896) and *Chauliodius sloani* (B.&S. 1801), and the presence of five squid were detected by the presence of eye lenses. Prey identified in the digestive tract of TBW/2013_088 also consisted primarily of the mesopelagic fish *B. glaciale* and squid eye lenses belonging to two different individuals, although no cephalopod beaks were found. The stomach of the calf beaked whale (TBW/2013_080) contained

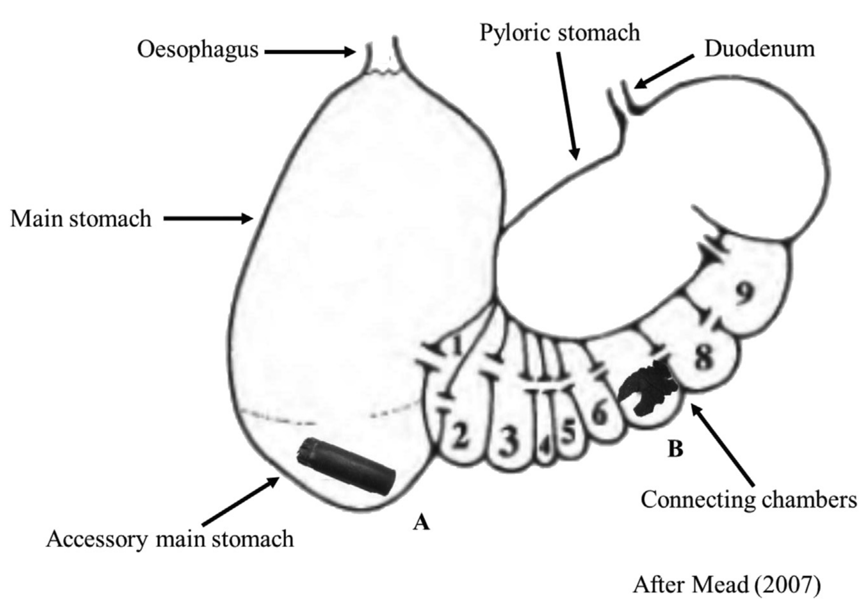

Fig. 1. Diagram of True's beaked whale stomach (after Mead, 2007), showing the positions of macroplastic items found in both adult females. (A) 7.1 × 2.2 cm shotgun cartridge found in the accessory main stomach of TBW/2013_088 and (B) 4.2 × 3.1 cm fragment found in the connecting chambers of TBW/2013_077.

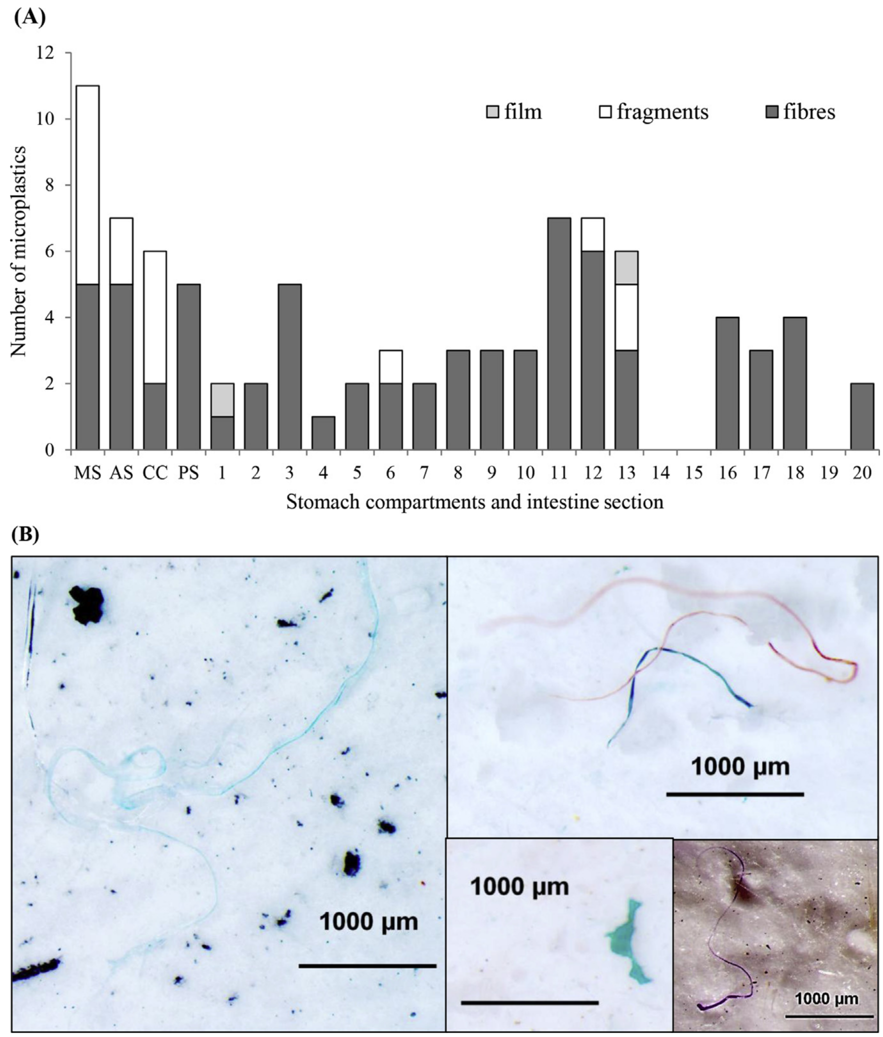

Fig. 2. (A). Microplastic distribution, according to type along the whole intestine (22.17 m) when divided into 20 equal sized subsections. MS = main stomach, AS = accessory main stomach, CC = connecting chambers, PS = pyloric stomach. Intestine Section 1 = closest to stomach and section 20 = closest to anus (B). Images of microplastics found in the intestine and stomach of TBW/2013_088.

an off-white substance that had the appearance of milk. No sign of macroplastics were found and the presence of microplastics was not explored in the calf.

4. Discussion

This is the first study to directly identify microplastics in the digestive tracts of a cetacean species. The study is novel because it applied a new technique for the detection and identification of microplastics in the digestive tracts of marine vertebrates. We were able to collect the full digestive tract and carry out a full analysis of its contents under contamination controlled conditions. Microplastic particles were found throughout the whole digestive tract suggesting that marine vertebrates can pass microplastics through their digestive system and egest them.

Macroplastic ingestion is not uncommon in beaked whales (as reviewed in Baulch and Perry, 2014) and it is clear that there is a potential problem of plastic ingestion in this group of marine mammals. It was previously reported that a True's beaked whales stranded in Brazil had three pieces of macroplastic in its stomach (Souza et al., 2005), but no food items were present. Furthermore, a Cuvier's beaked whale (*Ziphius cavirostris*, C. 1823) which stranded in the Canary Islands contained a plastic sheet but no food items in its stomach, in this case the authors inferred that the plastics could not be excreted by the whale (Santos et al., 2001).

The cause of death of all three whales could not be determined. Strandings of True's beaked whales in Ireland are very rare with only 13 records to date (O'Connell and Berrow) so, it is not impossible that the three stranding events were connected. In the case of the adult and calf in Donegal these were linked as the adult was lactating, and the calf still feeding on milk. Although the close temporal and spatial occurrence of these events does warrant concern about a possible common cause, reasons for strandings are multi-factional, and it is beyond the scope of this paper to speculate further.

As this is the first study to identify microplastics in the stomachs of a cetacean, the levels of microplastic reported in this study cannot be directly compared to other studies, such as invertebrates and fish, because of the novel methodology used. However, small plastic fragments have previously been reported in the stomach and intestines of harbour seals (*P. vitulina*, Bravo-Rebolledo et al., 2013). Direct microplastic ingestion by other species of marine mammals has not been observed. Thus, the frequency of microplastic uptake by marine mammals is hitherto unknown. However, there is no evidence at present that microplastics have detrimental effects on marine mammals and further research is necessary.

Microplastics were found throughout the intestine which suggests that egestion might be occurring, which would be in accordance with the identification of microplastics in seal scats (Eriksson and Burton, 2003). The small number and size of microplastics identified in TBW2013_088 did not appear to have lethal consequences however the origin of such contamination is intriguing. The source of the microplastics has not been identified; but ingestion could have occurred through targeted feeding, inhalation at the water–air interface, or via trophic transfer from prey items. Cetaceans could directly ingest microplastics from the water column; however this is less likely for this piscivorous predator which would instead be targeting prey. Microplastics are small enough not to be detected by cetaceans, and they could be ingested if they are present in the water column at the same time at which the cetaceans swallow prey. Beaked and toothed whales, which are active predators of squid and fish, may be less susceptible to direct microplastic ingestion than baleen whales as they capture prey directly from sea water (Pauly et al., 1998). Furthermore, it has been suggested that marine mammals are exposed to microplastic via trophic transfer from prey species. Such would be the case if they ingest organisms which already have microplastics present in the gut. For example, microplastics were recovered from the scats of fur seals (*Arctocephalus* spp.) believed to originate from lantern fish (*Electrona subaspera*) (Eriksson and Burton, 2003). Respiration has been seen as a method of uptake in invertebrates (Watts et al., 2014), although in the case of mammals, they breathe at the surface and their digestive system is more complex than invertebrates and this would be less likely.

Interestingly, the True's beaked whales from this study showed a preference for meso- and bentho-pelagic fish, and cephalopod prey. Mesopelagic fish, including Mycophiids (lanternfish), have been found to ingest microplastic in previous studies (Boerger et al., 2010; Davison and Asch, 2011; Choy and Drazen, 2013) which suggests that they could subsequently be consumed by larger predatory species, including True's beaked whales. As mesopelagic fish undergo diel migration, feeding at night in the surface waters and descending to depths during the day, there is a high possibility that they feed on microplastics floating in the surface waters acting as a link for microplastic transport between different depths of the water column. In the Northeast Atlantic, microplastics appear to be ubiquitous in the surface waters (Lusher et al., 2014), and thus there is a high likelihood that mesopelagic fish feed on them, however further work is required to validate this statement. Trophic transfer has been studied under laboratory conditions, and diet appears to be a route for trophic transfer of microplastics between marine biota, for example, between zooplankton and mysid shrimps (Setälä et al. 2014) and from food to *Nephrops* (Murray and Cowie, 2011). It is more likely that the route of exposure of marine mammals, including True's beaked whales, is through ingestion of prey species rather than direct uptake of microplastics from the water column or breathing on the surface. Furthermore, this may suggest that animals feeding on prey which are regularly exposed to high levels of microplastics may be more vulnerable to ingesting microplastics.

Both adult female True's beaked whales ingested a small amount of macroplastic but in insufficient quantities to have caused a false sense of satiation, and there were no signs of reduced appetite or malnutrition. The macroplastics identified in this study could have been from a land based, rather than marine origin, partially in the case of the shot-gun cartridge, which would commonly be used on land to target large animals. It is possible that people have used them to kill seals, although there has been no reported event like this in the UK and Ireland. Macroplastics can make their way into the sea through direct input to the marine environment or surface run off and transport through the riverine systems (Derraik, 2002) to estuaries where they can be distributed by wind and water currents. Ireland has several large rivers, and it is likely that the large rain fall and stormy conditions of early 2013 contributed to the input of land based materials to the sea. Beaked whales are an oceanic species and based on their stomach contents it is likely the stranded animals might have been feeding in offshore upwelling areas such as the Rockall Trough. This area is also an area where microplastics are abundant (Lusher et al., 2014) and thus microplastics may interact with prey species as well as True's beaked whales themselves.

It has been suggested that mistaken identity could be the cause of macroplastic ingestion; however, echolocation by odontocetes is highly efficient and they should be able to differentiate between prey items and marine debris if using echolocation for hunting (Thomas et al., 2004). It is possible that if marine debris is close to potential prey items, it may be harder to differentiate between marine debris and potential prey items. A recent review marine organisms interacting with marine debris found that at least 663 species were affected, and that plastic ingestion was seen in 26% of all marine mammals (CBD, 2012). This shows that plastic ingestion is not uncommon amongst marine organisms, and in particular, marine mammals. Detection of plastic ingestion by cetaceans depends on data collected from small sample sizes, mainly stranded animals which only provide a snapshot (Baulch and Perry, 2014) and represent only a fraction of stranded organisms that undergo full necropsy.

Plastic polymers identified by FT-IR in this study include substances which are commonly used in the textile industry such as polypropylene, acrylic and polyester; whereas polyethylene rope is often used in the marine sector. The polymers identified in this study are similar to that reported in fish (Lusher et al., 2013), marine sediments (Dekiff et al., 2014), surface waters (Reisser et al., 2013) and sewage effluent (Browne et al., 2011). In the case of the Rayon, a semi-synthetic fibre manufactured from cellulose, the spectra produced with FT-IR is extremely hard to differentiate from pure cellulose. The chemical structure of Rayon (Figure S2 in supplementary information) is the same to that of cellulose, or in our example, sea grass. This implies that additional processing steps such as visual identification methods are important in the analysis of fibres, including the separation of matt fibres from shiny fibres which are essential in microplastics determination. As such, the classification of Rayon should be aired with caution. Although we are unable to speculate further the origins of the fibres, we are confident that the fibres did not come from the stomach analysis procedure, both from laboratory exposure to airborne fibres and contamination through methodology, as controls were free of contamination.

Other alternative methods, to our study, for identifying potential microplastic ingestion in marine mammals include the identification of phalates in the tissue samples of large filter feeding organisms (such as basking sharks and fin whales) (Fossi et al., 2012, 2014). Phalates are assumed to be tracers for the accumulation of microplastics during feeding. Fossi et al. (2014) infer a toxicological effect of microplastic ingestion when phalate tracers are found in muscle tissue, although the presence of microplastic was not confirmed through ingestion. In future studies it is suggested that muscle tissue and blubber is collected and analysed for the presence of microplastic tracers and further compared to stomach analysis results, as was previously carried out by Tanaka et al. (2013) with birds. The analysis of blubber samples could provide a proxy for exposure to pollutants especially in the case of large stranded organisms where stomach samples are difficult to collect. However, the potential for some microplastic associated chemicals to be assimilated in different tissue cannot be neglected.

In conclusion, this novel study has identified the presence of microplastic and macroplastic in stranded True's beaked whales, highlighting that top oceanic predatory species are interacting with plastic as a marine pollutant on macro- and micro-scales. The routes of exposure are, at present, unclear and further research on ingestion by the prey species is required to explore trophic transfer as the probable cause.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2015.01.023.

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