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Microplastic in a macro filter feeder: Humpback whale *Megaptera novaeangliae*

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ABSTRACT

Marine filter feeders are exposed to microplastic because of their selection of small particles as food source. Baleen whales feed by filtering small particles from large water volumes. Macroplastic was found in baleen whales before. This study is the first to show the presence of microplastic in intestines of a baleen whale (*Megaptera novaeangliae*). Contents of its gastrointestinal tract were sieved, dissolved in 10% potassium hydroxide and washed. From the remaining dried material, potential synthetic polymer particles were selected based on density and appearance, and analysed by Fourier transform infrared (FTIR) spectroscopy. Several polymer types (polyethylene, polypropylene, polyvinylchloride, polyethylene terephthalate, nylon) were found, in varying particle shapes: sheets, fragments and threads with a size of 1 mm to 17 cm. This diversity in polymer types and particle shapes, can be interpreted as a representation of the varying characteristics of marine plastic and the unselective way of ingestion by *M. novaeangliae*.

1. Introduction

Microplastic (i.e. particles with a synthetic origin <5 mm, Barnes et al., 2009) is present in the marine environment due to direct disposal and degradation of larger plastic items (Barnes et al., 2009) and was first emphasised in the 1970's (Carpenter et al., 1972). Because of its small size and wide spread occurrence, microplastic is now thought to be available to species throughout the marine food web (Cole et al., 2011). Only a few studies about possible negative effects of microplastic on organisms have been published (Lee et al., 2013; Besseling et al., 2013; Browne et al., 2013; Wright et al., 2013; Rochman et al., 2014). So far reported possible negative effects of microplastic are on survival, feeding, oxidative status and uptake of persistent organic pollutants (Besseling et al., 2014).

Due to their feeding behaviour, filter feeders are thought to collect microplastic particles from the water column. Microplastic has indeed been encountered in bivalves (De Witte et al., 2014; Van Cauwenberghe and Janssen, 2014) and in planktivorous fish (Boerger et al., 2010; Foekema et al., 2013). By filtering a size range from plankton up to small fish (Deméré, 2014; Nemoto, 1970), baleen whales can potentially ingest microplastic directly from the water column as well as via prey species. Exposure of baleen whales to microplastic has therefore been hypothesised recently (Fossi et al., 2012; Fossi et al., 2014). Phthalates in the blubber tissue as indirect indication of microplastic in a fin whale have been suggested by Fossi et al. (2012), although this does not differentiate between phthalate uptake from food items (zooplankton, small fish) and microplastic. Baleen whales are suggested to be useful as a monitoring species in the implementation of Descriptor 10 (Marine litter) of the Marine Strategy Framework Directive (MSFD, Fossi et al., 2012; Fossi et al., 2014; Galgani et al., 2014), even though direct measurement of microplastic in baleen whales has not yet been reported.

Mesoplastic (i.e. items with a synthetic origin of 5–20 mm) is often included in the macroplastic size category (i.e. items with a synthetic origin >20 mm, Barnes et al., 2009). This includes plastic lids, bags and fishing lines and has been found in 31 marine mammal species, including baleen whales (Simmonds, 2012). Records of macroplastic in Cetacean species are increasing. While being reported in at least 26 Cetacean species before (Denuncio et al.,

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2011), macroplastic is reported in 48 (56% of) Cetacean species by 2014 (Baulch and Perry) and in 61.5% in the review by Kühn et al., (2015). Examples are 28% of examined Franciscana dolphins (*Pontoporia blainvillei*) having plastic in their stomach, including microplastic (Denuncio et al., 2011), micro- and mesoplastic in True's beaked whales (*Mesoplodon mirus*, Lusher et al., 2015), severe incidences of large macroplastic quantities causing starvation and death in a beaked whale and several sperm whales (*Mesoplodon densirostris*, *Physeter microcephalus*, Secchi and Zarzur, 1999; De Stephanis et al., 2013) and marine debris in two baleen whale species, Minke and Sei whale (*Balaenoptera acutorostrata*, *B. borealis*, Baulch and Perry, 2014). Raised hypotheses based on these incidences are that (1) chances of micro- and macroplastic ingestion are higher for relatively passive feeders, as compared to active predators (Di Beneditto and Awabdi, 2014), (2) even small amounts of macroplastic can cause obstruction of the digestive tract (Simmonds, 2012; De Stephanis et al., 2013), and (3) microplastic might be of special concern as it may clog the filtering apparatus of organisms (Simmonds, 2012). Theoretically, all of these hypotheses apply to baleen whales.

The non-selective feeding mode of many baleen whale species by ingesting material surrounding the intended prey in the water with a size large enough to be retained by their baleens (Johnson and Wolman, 1984), might result in exposure to microplastic. The ratio between microplastic and zooplankton (Collignon et al., 2012) indicates a possible daily intake of 3.7 thousand microplastic particles in fin whales in the Mediterranean (Fossi et al., 2014). Negative effects of microplastic uptake on organisms in the marine environment might occur, though the information about effects is still limited. Meanwhile, microplastic is already present in the marine environment (Cole et al., 2011). This is why we studied the occurrence of microplastic in a stranded baleen whale, a humpback whale. Our study describes the first reported case of microplastic ingested by a humpback whale, and discusses it within the context of microplastic uptake related to ecological traits.

2. Materials and methods

2.1. Animal

At December 12th 2012, a 10.34 m long, ca. 16 thousand kg juvenile female humpback whale (*Megaptera novaeangliae*) stranded on a sandbank between harbour city Den Helder and the island Texel in The Netherlands, and was publically called 'Johanna'. Four days later, it died.

2.2. Sampling

Two days post-mortem, necropsy was performed on the severely autolytic carcass. Wood shreds were used around the humpback carcass for absorption of body fluids. Multiple tissue samples were preserved, including part of the gastrointestinal tract for content analysis. Gastrointestinal tract samples were stored at −18 °C till further processing. After thawing, samples were sequentially sieved over two sieves with a mesh size of 1 mm and 0.5 mm. Subsequently, the residues were dissolved in 10% potassium hydroxide (KOH) solution. The remainder was washed according to previous methods in a washing machine in double washing bags, the inner bag having a mesh size of 300 µm and the outer bag 120 µm (Bravo Rebolledo et al., 2013). After washing, the samples were dried for three hours at 70 °C. From the remaining material, possible synthetic polymer particles were selected based on density (floating/sinking in saturated NaCl dispersion) and appearance (Zeiss Stereo Discovery V8 microscope) according to previous procedures (Van Franeker et al., 2011), measured by marking gauge (for subsequent volume calculation) and subjected to Fourier transform infrared (FTIR) analyses. FTIR spectra of the samples were gained with a Varian Scimitar 1000 FT-IR spectrometer equipped with a DTSG-detector. Sample and reference spectra were obtained using a measurement resolution of 4 cm^{-1}, following Gonzalez-Contreras et al. (2010).

2.3. Data analysis

FTIR spectra of the particles were compared with reference polymer spectra (Thompson et al., 2004; Ng and Obbard, 2006) of the seven most produced polymers polyethylene (PE), polypropylene (PP), polyvinylchloride (PVC), polyethylene terephthalate (PET), polystyrene (PS) and nylon (PA) (Andrady, 2011). Additionally, comparisons with reference spectra of natural rubber and cellulose were made. Statistical analyses were performed with linear regression in 'RStudio' statistical software (Version 0.98.976, R Development Core Team). Particles where the quality index i.e. the correlation coefficient (R^2) of the comparison with reference spectra was >0.7 were classified as synthetic polymers.

3. Results

3.1. Post-mortem examination

According to the well-developed musculature and blubber thickness, the humpback whale was in good nutritional condition. Severe post-mortal decomposition of all internal organs prevented detailed macroscopic and microscopic evaluation. About a fifth to tenth of the total length of the gastrointestinal tract was sampled for content analysis. There were few contents in the gastrointestinal tract. Continued digestion of the contents of the gastrointestinal tract during the four days of stranding, might have resulted in fluid contents that were partly deflated from the gastrointestinal tract during sampling. The primary cause of the stranding could not be identified. However, prolonged stranding in itself caused deterioration and death of the animal.

3.2. Plastic

A total of 45 particles of possible synthetic origin was found in the gastrointestinal tract samples. Of these, 77.7% was large enough (>1 mm^2) to be analysed by FTIR. Of these particles,

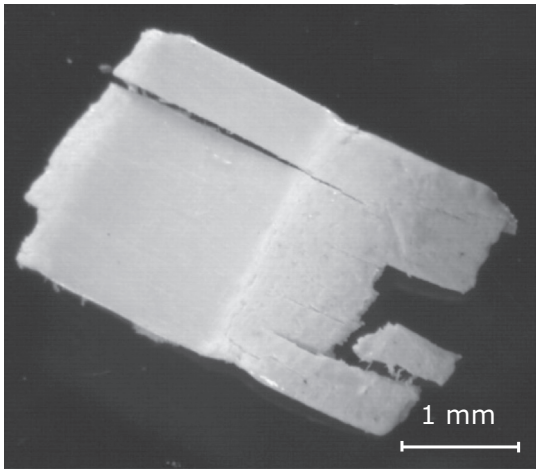

Fig. 1. Polypropylene (PP) particle found in the gastrointestinal tract samples of the studied humpback whale, $R^2 = 0.82$. Additional photos of other particles found in the gastrointestinal tract of the studied humpback whale are given in the supporting information of this article.

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