



Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

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

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ARTICLE INFO

Article history:

Available online xxxx

Keywords:

Microplastic
Filter feeders
Humpback whale
Ingestion

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*.

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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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<http://dx.doi.org/10.1016/j.marpolbul.2015.04.007>

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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 Benedetto 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 ($>1\text{ mm}^2$) to be analysed by FTIR. Of these particles,

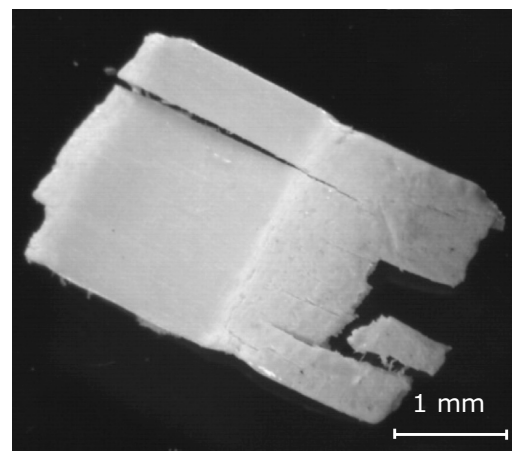


Fig. 1. Polypropylene (PP) particle found in the gastrointestinal tract samples of the studied humpback whale, $R^2 = 0.82$. Additional photos of other particles found in the gastrointestinal tract of the studied humpback whale are given in the supporting information of this article.

Table 1

Particle shape, number and size of particles and percentage of the total plastic volume found in the studied humpback whale, per polymer type with average R^2 values and ranges, together with the percentage of the total global production of these polymer types, PE (polyethylene), PA (nylon), PP (polypropylene), PVC (polyvinylchloride) and PET (polyethylene terephthalate) (Andrady, 2011).

Polymer type	Average R^2	Particle shape	Number of particles	Size (mm)	% Volume	% Global production
PE	0.90 (0.88–0.98)	Sheet	9	$3.3–12 \times 0.3–8.2 \times 0.04–0.2$	55.01	38
PA	0.80 (0.70–0.96)	Thread, fragment	4	$2.3–170 \times 0.1–1.5 \times 0.1–0.4$	37.64	<3
PP	0.82	Sheet	1	$3.6 \times 2.4 \times 0.1$	5.61	24
PVC	0.82	Sheet	1	$5.8 \times 3.3 \times 0.01$	0.97	19
PET	0.82	Fragment	1	$1.1 \times 0.8 \times 0.2$	0.77	7

45.7% had a synthetic origin (Fig. 1), 25.7% had a natural origin and for 28.6% no matching spectra were obtained. The identified polymer types were PE, PP, PVC, PET and PA (Table 1). For size categories, we follow the classification of Barnes et al. (2009). Of the synthetic particles, 12.5% had a maximum dimension of 2.5–17 cm (macroplastic). These were threads, with a diameter of 0.1–0.23 mm. 50% had a size of 5.8–12.0 mm by 0.3–8.2 mm (mesoplastic). Those were all sheets. The remaining 37.5% consisted of sheets and fragments with a size of 1.1–4.7 mm by 0.4–2.4 mm (microplastic). The found plastic particles all made up less than 3.5 mm³ per particle. As particles might fall apart during passage of the gastrointestinal tract, sampling or processing, we discuss particle volumes instead of particle numbers. A total plastic volume of 13.7 mm³ was encountered, of which most was PE and PA (Table 1), which might represent the worldwide most produced polymer (PE) as well as polymers used in the marine environment in fishing gear (Andrady, 2011).

There were few remainders of fish found in the gastrointestinal tract samples, being small fish bones, vertebra and otoliths of herring and sprat. Wood shreds were found in the gastrointestinal tract samples, most likely originating from the necropsy site. Twenty-five gram of comparable wood shreds was investigated by microscope. No plastic particles were found among these wood shreds.

The high variation in particle appearance and polymer type of the plastic found in the gastrointestinal tract samples is an indication that the particles originate from the marine environment. Studying synthetic fibres in the gastrointestinal tract samples was omitted, because of the high risk of fibres being caused by methodological contamination during sampling and analyses, i.e. clothes, washing bags (Foekema et al., 2013; Fries et al., 2013). As fragments, threads and sheets are less than fibres prone to contaminate samples during the used methods and additionally because of the eroded condition of the found particles, we render it likely that they were ingested by the humpback whale at sea.

Humpback whales have an intestine length of about 5.5 times their body length (Slijper, 1979), such that the sampled humpback whale was estimated to have an intestine length of about 57 m. As a fifth to a tenth of this length was sampled, it is likely that five to ten times as much plastic was present on a whole organism basis if we neglect gastrointestinal tract section type, than encountered in the subsample. That would result in an estimate of up to 160 small plastic particles or a volume of up to 137 mm³ of plastic in the whole humpback whale.

4. Discussion

By combining information on daily filtered water volume, gut passage time and plastic particle numbers in an organism, it is possible to calculate a plastic concentration in an organisms' foraging area from biological traits. The estimated concentration then can be compared with data on measured concentrations. Here we performed such a calculation by using plastic numbers of the

humpback whale we studied. The daily filtered water volume of humpback whales has to our knowledge not been reported yet. For baleen whale species with similar feeding type (Deméré, 2014; Nemoto, 1970), we here assume that daily filtered water volume is constant per unit surface of the baleen plate rows. We use the formula:

$$V_{w,y} = V_{w,x} \left(\frac{BPR_y}{BPR_x} \right) \quad (1)$$

with V_w (m³) the average daily filtered water volume and BPR (m²) the average surface of the baleen plate rows for species y and species x. Fossi et al. (2014) report a daily filtered water volume of 5.9 thousand m³ for fin whales. With the BPR of 4 m² of fin whales and 2.6 m² of humpback whales (Nemoto, 1970), we calculate a daily filtered water volume of 3.8 thousand m³ for humpback whales. The gut passage time (GPT) of baleen whales is to our knowledge unknown. We here make the assumption that the GPT can be assumed to be constant per unit length of the gastrointestinal tract, across Cetacean species. We use the formula:

$$GPT_y = GPT_x \left(\frac{BG_y}{BG_x} \right) \left(\frac{BL_y}{BL_x} \right) \quad (2)$$

with GPT (h) the average gut passage time, BG the body to gastrointestinal tract length ratio and BL the body length for species y and species x. A GPT_x of 4.2 h has been reported for (Cetacean species) Amazon river dolphins (*Inia geoffrensis*) with a BL_x of 2.22 m (Kastelein et al., 1999). By lacking the BG_x for the Amazon river dolphin species we use BG_x of another river dolphin (Gangetic dolphin, *Platanista gangetica*) of 7.3 (Slijper, 1979). We use a BG_y of 5.5 for humpback whales (Slijper, 1979) and BL_y of the studied humpback whale of 10.34 m, and calculate the GPT of the studied humpback whale to be ~14.6 h. For inert particles, the particle content of the water volume ingested within the GPT, can be seen as the steady state concentration of plastic in a filter feeding organism. We use the formula:

$$C_{SS} = \frac{NP}{V_w \left(\frac{GPT}{24} \right)} \quad (3)$$

with C_{SS} (plastics/m³) the steady state concentration of plastic in the humpback whale, NP (n), V_w (m³) and GPT (h) respectively the number of particles, daily filtered water volume and gut passage time of the studied humpback whale. With this formula we calculate the filtered water volume of the humpback whale within the gut passage time $V_w \left(\frac{GPT}{24} \right)$ to be 2.3 thousand m³ of water and C_{SS} to be 0.07 plastics/m³. We hypothesis that C_{SS} is directly related to the concentration of plastic in the foraging area of a filter feeding organism. We use the formula:

$$C_{SS} \approx C_{fa} \quad (4)$$

with C_{fa} the concentration of plastic in the foraging area of the humpback whale. The average microplastic particle concentration in the Northeast Atlantic is 0.1 microplastics/m³ excluding synthetic fibres (Lusher et al., 2014). This measured concentration in

water is thus very close to the calculated concentration based on the small plastic particles in the humpback whale, implying that formula 4 may hold. It must be noted that this calculation concerns only one humpback whale and includes several uncertain conversion factors. Further research is needed to confirm whether this relation between plastic concentration in organisms and foraging area applies more generically.

Microplastic has different characteristics based on the large surface to volume ratio compared to macroplastic (Mato et al., 2001; Barnes et al., 2009; Hidalgo-Ruz et al., 2012). As first studies on physical as well as chemical effects of microplastic on organisms have only recently appeared (Lee et al., 2013; Besseling et al., 2013; Browne et al., 2013; Wright et al., 2013; Rochman et al., 2014), there is still a large knowledge gap about possible negative effects of microplastic. Therefore, although not reported yet for most whale species, ingestion of microplastic might be of specific concern and is recommended to be studied alongside ingestion of macroplastic.

The estimated number of small plastic particles in the studied humpback whale may be lower than in other baleen whales. Humpback whales are mainly lunge feeders, that is, swallowing a mouth full of preferably concentrated masses of planktonic crustaceans or fish, subsequently retaining the prey by filtering the water through the baleens (Deméré, 2014; Nemoto, 1970; Slijper, 1979). This may result in lower plastic uptake compared to other preferably water or mud skimming baleen whales. Feeding by these strategies as well as filter feeding by making use of the water flow, which in general can be created by either hydrodynamics or organisms themselves, might result in a higher plastic intake compared to lunge feeding. Mortality at sea and decay of carcasses before necropsy diminishes the number of opportunities to study plastic occurrence in whales (Simmonds, 2012). Together with the likelihood of (micro)plastic ingestion, this indicates the importance of reporting also singular incidences of plastic in gastrointestinal tracts of examined baleen whales.

Acknowledgments

The authors like to thank Gerrit Hoornsman and André Meijboom (IMARES, Wageningen UR, The Netherlands) for their help at the sampling site and Wouter Teunissen (Food & Biobased Research, Wageningen UR, The Netherlands) for his help on the FTIR measurements.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.marpolbul.2015.04.007>.

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