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Evaluating the Ingestion of Microplastics in Blue Whales (Balaenoptera musculus) in the Northern Indian Ocean

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Evaluating the Ingestion of Microplastics in Blue Whales (*Balaenoptera musculus*) in the Northern Indian Ocean

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Abstract

Microplastics, an emerging contaminant in the marine environment, have attracted widespread attention due to their ubiquitous occurrence and harmful effects, however, little has been documented on the presence of microplastics in free-ranging cetaceans. In this study, we assessed methodologies that could be used in Sri Lankan University facilities to analyse mammalian faecal samples, using blue whale (*Balaenoptera musculus*) samples obtained from the northern Indian Ocean. Of the samples analysed, 45 microplastic particles were detected at an average of ~0.82 items/g dry weight. The predominant material was microfibre (68.9%) and the dominating colour was blue (55.6%). Fourier-transform infrared spectroscopy (FTIR) detected six polymer types, nearly half of which were polyethylene terephthalate (PET). The diversity of shapes, colours and polymer types of particles identified demonstrates the diversity of marine microplastic present and available for ingestion, by whatever means, in the top predators of the Indian Ocean. This study assists in establishing a baseline for microplastic presence in free-ranging blue whales and has also initiated a capacity building programme between a regional Centre of Excellence and a University new to this field. Future work will continue to build skills transfer and investigate the potential adverse effects of long-term exposure to microplastics in both marine and terrestrial megafauna.

Keywords

Microplastics; faeces; blue whale; cetacean; elephant; leopard; Sri Lanka; northern Indian Ocean

1. Introduction

Marine pollution is of worldwide concern, with plastics, which are lightweight, versatile and durable, highlighted as a high-risk contaminant due to its persistence and ubiquity (Donohue et al., 2019; Zantis et al., 2021). Plastics enter the ocean through both land-based and maritime activities, although it is often challenging to discern the initial source of this pollution (Carlsson et al., 2021; Sevwandi Dharmadasa et al., 2021). Plastic debris gradually decomposes in the marine environment into smaller particles due to aging and/or biological effects, and particles measuring smaller than 5 mm are classified as microplastics (MP) (McIvor et al., 2023; Perez-Guevara et al., 2021). The small size of MP enhances dispersion in marine ecosystems and transportation of these particles by surface currents and bottom water have resulted in the widespread contamination of large areas of the ocean, at all levels of the water column (Garcia-Garin et al., 2020; Huang et al., 2022; Liu et al., 2021). Marine organisms ingest microplastics through direct uptake and/or trophic transfer (Perez-Guevara et al., 2021; Xiong et al., 2018). The trophic transfer of microplastics represents an indirect but likely significant exposure pathway (Carlsson et al., 2021; Nelms et al., 2018; Ortega-Borchardt et al., 2023). In addition, ingested MP serve as carriers of persistent organic pollutants (POPs) and/or heavy metals and have a high potential to introduce toxic chemicals into organisms and nutrient networks, thereby causing multiple health issues to marine life (Alzugaray et al., 2020; Moore et al., 2020). MP have been recorded in myriad marine species (Garcia-Garin et al., 2020); zooplankton (Taha et al., 2021); fishes (Nelms et al., 2018); pinnipeds (Hudak and Sette, 2019; Perez-Venegas et al., 2020) and sea turtles (Caron et al., 2018). At the top of the oceanic trophic level, cetaceans likely accumulate more MP than other marine organisms and may face greater risks than other taxa (McIvor et al., 2023; Perez-Venegas et al., 2018; Yong et al., 2021).

Blue whales (*Balaenoptera musculus*) primarily feed on plankton and krill, with a worldwide distribution of habitats from the equator to the polar regions (Besseling et al., 2015; García-Vernet et al., 2021). Baleen whales may ingest MP through feeding bouts, where large amounts of water are taken into the mouth, from where both prey and MP can be filtered out and ingested (Alzugaray et al., 2020; Yong et al., 2021). As cetaceans occupy the top of the marine food chain, these species are reliable sentinels for monitoring MP pollution in the marine environment (Huang et al., 2022; Moore et al., 2020). Monitoring MP trends may provide advance warning of environmental stressors (e.g., marine contaminants and pathogens) and provide an indication of aquatic ecosystem health (Zantis et al., 2021). MP have been detected in whale skin (Fossi et al., 2016), stomach (Besseling et al., 2015), intestine

(Alzugaray et al., 2020) and faeces (Moore et al., 2020). Moreover, given the longevity of many whale species, prolonged exposure to MP may impair the viability of whale populations.

Currently, there is little research reported on the quantification and evaluation of MP in the faeces of marine cetaceans. In this study, MP were quantified and characterised from faecal samples obtained from free-ranging blue whales (*Balaenoptera musculus*) collected from waters of the northern Indian Ocean. The primary objectives of this study were to: (1) develop analytical techniques appropriate for use in our collaborators University in Sri Lanka; (2) assess the occurrence of microplastics in blue whale faeces; (3) characterise the features of microplastics detected; and (3) build local expertise in Sri Lanka so that the analytical techniques developed can be used for other mammalian species.

2. Methodology

2.1 Sample Collection

Faecal samples were collected opportunistically from whale watching vessels in the waters to the south of Sri Lanka and, as such, search effort was non-systematic. When any cetaceans were sighted, the date, time and the vessel's location were recorded using a handheld GPS device. The number of individuals, presence of mother-calf pairs and predominant behavior (e.g., feeding, traveling) were also recorded. When blue whales were observed defecating, faecal samples were collected using a fine-mesh plankton net. Each sample was drained of excess seawater, decanted into a sterile collection tube and placed into an ice box until the vessel returned to shore, where it was stored locally at −20°C. When full, the onsite freezer was emptied, and all samples were transported to the Sabaragamuwa University of Sri Lanka University and placed in a −20°C. Given the often unreliable electricity supply in Sri Lanka, and the long overland transport, there were periods when the samples may have been kept at temperatures higher than −20°C. For the purposes of this study, to develop techniques and to build capacity, 18 faecal samples from 18 separate encounters were used that were collected between September to December 2016, where complete data was available for the original collection event (Fig. 1)

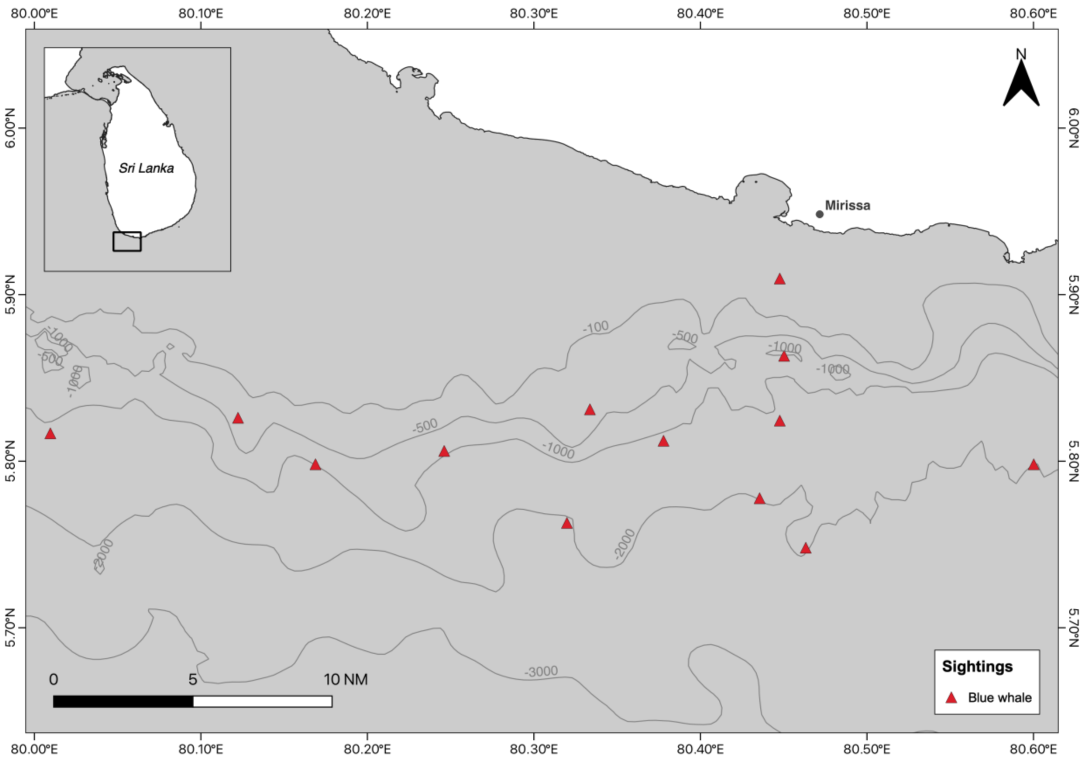

Fig. 1. Collection location of blue whale faecal samples, northern Indian Ocean, 2016.

2.2 Sample treatment

In the laboratory, faecal samples were weighed before and after the freeze-drying process to calculate moisture content. Following the addition of 200 ml H_2O_2 to each dried sample (1.08–19.94 g d.w.), hydrogen peroxide (30%) was used as a digestion agent. The solution was maintained at 60°C during the digestion process. To ensure the complete digestion of organic matter, it was determined that this process should be maintained for at least 12 hours. If organic matter was still observed after 12 hours, more 30% hydrogen peroxide solution was added, and the above procedure repeated.

After digestion, each solution was poured through a sieve stack, consisting of 300 µm and 20 µm mesh. As some faecal contained higher-density MP, sodium iodide solution (ρ=1.5 g/cm^3) was used to separate these particles out via floatation. The filtered content from both mesh surfaces was transferred to beakers and then transferred to glass separation funnels. The beakers were rinsed with a solution of sodium iodide to transfer all the solid matter to the funnel. The funnels were used to conduct density separation and, after visually checking the solid matter at the bottom of the funnel, the stopper was opened and the flow rate controlled so that the denser materials slowly flowed out. The supernatant was filtered using a glass fiber membrane (pore size = 1 µm). The funnels were rinsed several times with filtrated purified water to transfer all the samples to the membrane. The membrane was then placed in a clean culture dish and, after drying at 60℃, examined under a stereomicroscope for MP.

2.3 Microplastic classification and identification

MP were classified according to material and colour. Material categories included: line/fiber, film/sheet, pellet, foam and unidentified fragments. Colour categories included: white, black, blue, green, yellow, red and transparent. To identify the chemical compositions of the identified MP, chemical composition was analysed with μ-FTIR (Thermo Nicolet iS10 with Continuμm/iN5, Thermo Fisher Scientific, USA). The spectral range was set from 4000 cm^{-1} to 550 $cm^{-1,}$ and 16 scans were performed for each measurement. All suspected particles were analyzed with μ-FTIR, and their spectra were compared with standard spectra in open-access databases, thus chemical composition could be identified according to the presence of characteristic peaks and the similarity (minimum requirement match score > 60%) of the matched spectra.

2.4 *Quality assurance and quality control (QA/QC)*

All the containers were glass or stainless steel, and only stainless-steel sieves were used for sample sieving. Nitrile gloves were used during sample collection and cotton lab coats and nitrile gloves were used during laboratory analysis. The sample processing was conducted in a fume hood to minimize air-borne plastic particle contamination. Procedure blanks were used in the laboratory with every batch of sample treatment to examine any contamination apparent during sample analysis in the laboratory. Eight grams of quartz sand were used for faecal samples and procedure blank samples were treated and analysed similarly to the real samples. Results were corrected with the procedure blanks.

3. Results and discussion

3.1 Occurrence of microplastics in whale faeces

151 possible MP microplastic samples were identified by visualization and classification with a stereo microscope. Following FTIR detection, there were 45 microplastic items (29.8%) identified within these 151 possible MP samples. MP were identified in all but one of the faecal samples collected. MP abundance was not evenly distributed among the faecal samples (Fig. 2), with a range from not-detected to 2.4 items/g d.w. (mean 0.82 items/g d.w.). This distribution of microplastic abundance was consistent with the findings detected by Moore et al. (2020) in the intestinal faeces of beluga whales.

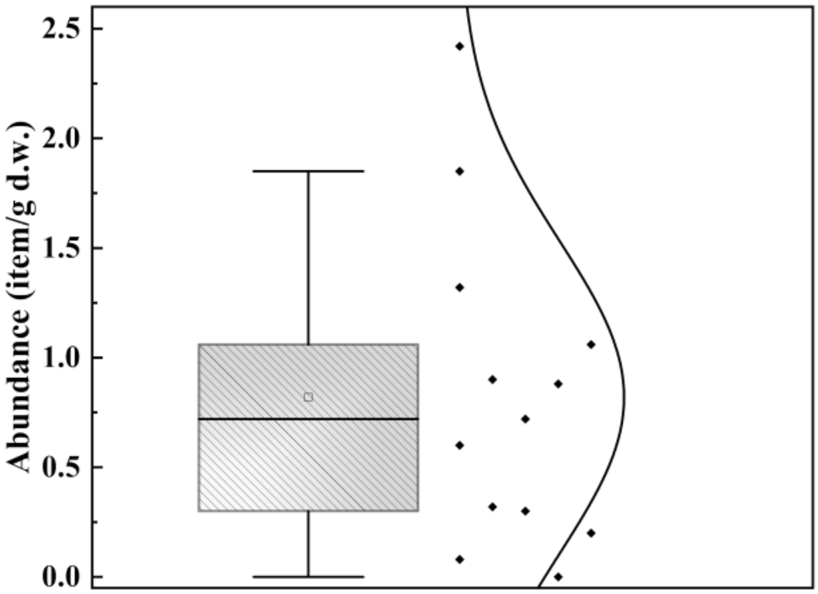

Fig. 2. The abundance of microplastics (MP) within the blue whale faecal samples analysed.

Compared with MP reported in the faeces of other marine mammal species, MP levels in this study were within the range recorded for multiple pinniped species in a variety of areas (e.g., McIvor et al., 2023; Nelms et al., 2018) but lower than that recorded in a study on beluga whales (an odontocete) (Table 1). Even though this study only used a few samples, obtained from a large area over 4 months, MP were present in all but one of the samples analysed, demonstrating the prevalence of microplastic contamination in the ocean (Carlsson et al., 2021; Donohue et al., 2019; Perez-Guevara et al., 2021). Identifying the quantity and type of MP within blue whale faeces may provide new insights to additional threats that this resident population may face. Baleen whales can ingest MP through multiple pathways, including direct or indirect ingestion (Donohue et al., 2019; Perez-Venegas et al., 2020; Xiong et al., 2018). Blue whales could ingest MP via the process of filtering water with free floating debris during feeding bouts (Moore et al., 2020) or indirectly through their prey, where MP occur in the prey itself (Nelms et al., 2018; Ortega-Borchardt et al., 2023; Perez-Venegas et al., 2018).

Table 1 Summary of presence of microplastics in marine mammal faeces/scats studies.

Organism	Samples	Microplastics abundance	Confirmed MPs	Particle Size (μm)	References
Monk seal	Scats	0.2–8.6 items/g d.w.	18	20–3251	(McIvor et al., 2023)
Fur seals	Scats	2.7–13.35 items/g w.w.	51	< 1000	(Perez-Venegas et al., 2018)
Pinniped species	Scats	0.1–3.7 items/g	205	-	(Perez-Venegas et al., 2020)
Sea lion	Scats	0.11–0.6 items/g	77	212–1000	(Ortega-Borchardt et al., 2023)
Walrus	Scats	0.034 items/g	7	-	(Carlsson et al., 2021)
Gray seal	Scats	0.87 ± 1.09 items/sample	15	1500	(Nelmset al., 2018)
Beluga whales	Faeces	0–2 items/100 mL	81	< 2000	(Moore et al., 2020)
Blue whale	Faeces	0.82 items/g d.w.	45	20–2000	*Present study*

3.2 Microplastic Morphology

Three types of MP shape were identified; fibre, fragments and sheets (Fig. 3). The morphology of MP identified in this study was consistent with other marine MP studies (e.g., Wang et al., 2021; Xiong et al., 2018). Fibre-shaped MP dominated (n=31; 68.9%), followed by fragments (n=12; 26.7%) and sheet (n=2; 4.4%). Xiong et al. (2018) reported that faecal samples of finless porpoise contained 70.1% fibre-shaped MP, 14.9% fragment MP and 13.4% sheet-shaped. The predominance of fibre may indicate that the textile industry, marine-related fisheries and shipping sources may be the main contributors to MP in the ocean (Perez-Venegas et al., 2018; Xiong et al., 2018). Fibre has been recorded as the dominant shape in coastal waters and in the atmosphere (Kwon et al., 2020; Qu et al., 2018). And fibres likely have higher bioavailability in the ocean for marine mammals (Donohue et al., 2019; Perez-Guevara et al., 2021; Perez-Venegas et al., 2020).

3.2 Microplastic Colour

Eight colours were observed in the 45 microplastic particles detected; blue (55.6%), purple (17.8%), yellow (6.7%), white (6.7%), red (4.4%), transparent (4.4%), black (2.2%) and green (2.2%) Blue was the most predominant fibre (51.6%) ands sheet (100%) colour (Fig. 3). This is similar to the findings of Ortega-Borchardt et al. (2023) and Perez-Venegas et al. (2018) where the majority of samples recorded in sea lion and fur seals faecal samples were blue. As MP originate from different products and have varying morphology and colours, the analysis of physical characteristics contribute to our understanding and identification of the potential sources of these contaminants (Perez-Guevara et al., 2021; Sevwandi Dharmadasa et al., 2021).

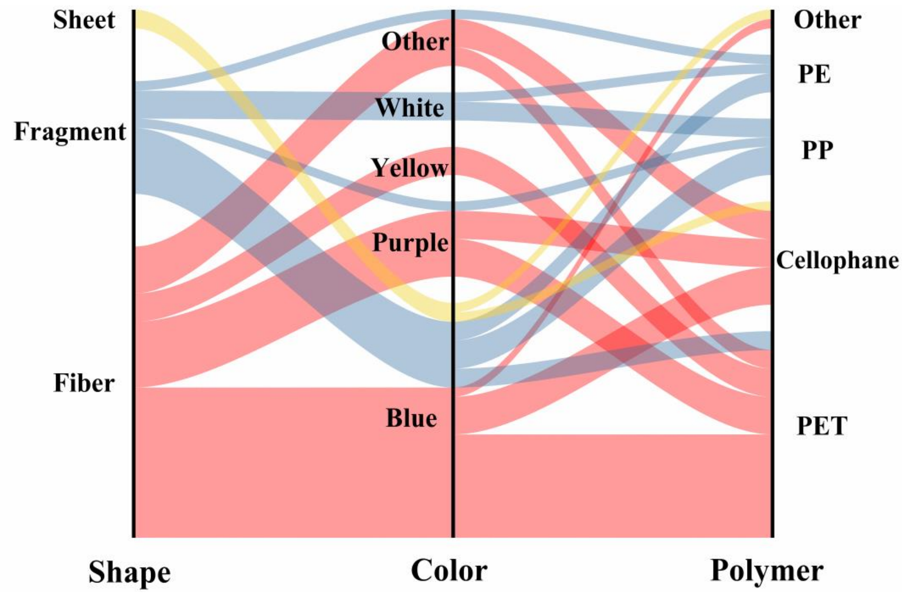

Fig. 3. Alluvial pattern summarizing the MP characteristics (PET, [polyethylene terephthalate]; PP [polypropylene]; PE [polyethylene]).

3.3. Polymer type

FTIR detected six polymer types; poly(ethylene terephthalate) (PET) (48.9%); cellophane (24.4%); polypropylene (PP) (13.3%); poly(ethylene) (PE) (8.9%); poly(ethylene: ST: acrylamide) (2.2%) and; poly(vinyl acetate: ethylene) 4:1 (2.2%) (Fig. 4). Of the PET particles, 90.9% were fibre-shaped and of the cellophane particles 90.8% were fibre-shaped (Fig. 3), Fragment-shaped particles were 100% PE/PP. The identified polymer types in the few samples analysed highlights the diversity of MP in the marine environment (Nelms et al., 2018). PET is used extensively in the textile industry, which can enter the marine habitat through industrial and domestic washing processes and PET is abundant in the atmosphere, thus can enter the marine environment via multiple pathways (Li et al., 2020; Liu et al., 2019). Cellophane is primarily utilized in packaging (Piskula and Astel, 2023), whereas both PP and PE have more widespread applications (Athapaththu et al., 2020; McIvor et al., 2023), e.g., fisheries activities, plastic films,etc. Potential sources of India Ocean plastic contamination include fishing and shipping activities (Sevwandi Dharmadasa et al., 2021; Zhang et al., 2021), garment manufacture, a major industry in Sri Lanka (Athapaththu et al., 2020; Bimali Koongolla et al., 2018). Sri Lanka's tourism industry also increases the amount of plastic waste entering the ocean, from additional waste created by the increase in people and a municipal refuse disposal system that cannot cope with this increase (Athapaththu et al., 2020; Jang et al., 2018).

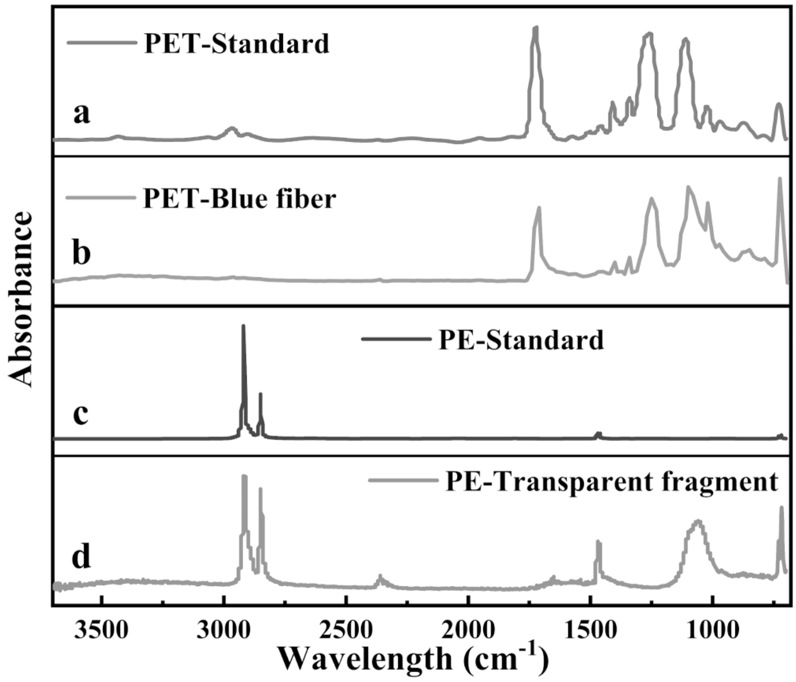

Fig. 4. FTIR Spectra; (a) PET standard spectra; (b) PET/blue fibre spectra (92.2% match); (c) PE standard spectra; and (d) PE/transparent fragment (87.4% match).

4. Conclusion

This study is preliminary in nature in that its primary purpose was to establish an analytical technique that could be used in Sri anka institutes and to build capacity in analytical expertise. Only a few samples were included in the study, however, a variety of MP were identified and quantified. A total of 45 MP particles were identified from 18 faecal samples, with an average abundance of 0.82 items/g dry weight. Three MP shapes were identified; fibre, fragment and sheet. Fibre-shaped particles dominated (n = 31; 68.9%). Six MP colours were observed,of which blue dominated (55.6%). Examination via FTIR identified xx polymers, the majority of which were PET (48.9%). This study provided some insight to the diversity of MP that occur within the northern India Ocean and that impact a blue whale population that is known to reside within the area. Sample collection has been ongoing since 2016, albeit with some interruption due to the cessation of whale watching during the Covid-19 pandemic. It is hoped that this study is the first step in a longer-term project that aims to better assess the health status of the blue whale population of the northern Indian Ocean, that will be managed by the local scientists that have collaborated in this work.

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