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Microplastics in beluga whales (*Delphinapterus leucas*) from the Eastern Beaufort Sea

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ABSTRACT

Microplastics (MPs, particles < 5 mm) represent an emerging global environmental concern, having been detected in multiple aquatic species. However, very little is known about the presence of MPs in higher trophic level species, including cetaceans. We worked with community based monitors and Inuvialuit hunters from Tuktoyaktuk (Northwest Territories, Canada) to sample seven beluga whales (*Delphinapterus leucas*) in 2017 and 2018. Microplastics were detected in the gastrointestinal tracts in every whale. We estimate that each whale contained 18 to 147 MPs in their GI tract (average of 97 ± 42 per individual). FTIR-spectroscopy revealed over eight plastic polymer types, with nearly half being polyester. Fibres made up 49% of MPs. The diversity of MP shapes and polymeric identities in beluga points to a complex source scenario, and ultimately raises questions regarding the significance and long-term exposure of this pollutant in this ecologically and culturally valuable species.

1. Introduction

Plastic has become one of the most useful materials in society today as it is lightweight, durable and highly versatile (Thompson et al., 2009). These attributes also make it extremely persistent in the environment. Recent estimates suggest that over five trillion plastic pieces are currently suspended in our oceans (Eriksen et al., 2014) and it has been projected that, by 2025, the weight of plastic litter in the ocean could reach 250 million metric tonnes (Jambeck et al., 2015).

The presence of plastic in the oceans poses a growing threat to marine biota. Larger plastic debris can cause physical external harm through entanglement or suffocation (Gregory, 2009). If ingested, plastic debris can cause internal harm by creating blockages and lesions within the gastro-intestinal tract (Gregory, 2009; Foekema et al., 2013) and also pose chemical toxicity concerns (Bradney et al., 2019; Holmes et al., 2012; Chen et al., 2019). As plastic fragments into smaller particles within the marine environment, it increases in bioavailability to more and smaller organisms (Cózar et al., 2014). Therefore, microplastics (MPs, particles < 5 mm) are of global concern. The current lack of understanding of how microplastics impact the health of organisms has catalyzed a surge of investigative research conducted on a variety of organisms and environments around the world.

Recent studies have documented microplastics in remote Arctic environments with particles having been found in seabirds (Trevail et al., 2015), fish (Morgana et al., 2018; Kühn et al., 2018; Rummel et al., 2016), and sea ice (Peeken et al., 2018; Obbard et al., 2014). It has even been suggested that the Arctic may be a sink for microplastic pollution due to long range transport via sea ice and thermohaline circulation (Peeken et al., 2018; Obbard, 2017; Cózar et al., 2017). There is, however, scant information on the uptake of these particles by top predators, including those inhabiting or frequenting the Arctic.

Investigating microplastics within animals often requires access to internal organs, therefore the animal must be euthanized and already deceased. The inherent ethical, legal and logistical constraints to studying microplastics in cetaceans underlie the shortage of relevant data on this topic. The few studies that are available have relied on opportunistic access to deceased stranded whales (Lusher et al., 2015a; Nelms et al., 2019). The subsistence harvest of beluga whales (*Delphinapterus leucas*) within the Inuvialuit Settlement Region (ISR) provided us with an invaluable opportunity to investigate this emerging

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contaminant on a healthy population of free-ranging cetaceans.

Beluga whales are iconic omnivorous toothed cetaceans, and the most abundant odontecete in the western Canadian Arctic (Loseto et al., 2009). Each year, belugas belonging to the Eastern Beaufort Sea (EBS) population are harvested for subsistence by Iñupiat and Inuvialuit communities along the coastline of northern Alaska (USA) and the western Canadian Arctic, respectively. Belugas represent an important traditional staple of Inuit diet (Wesche and Chan, 2010; DFO, 2000). For over 30 years, morphometric measurements, observations, and tissues of beluga have been collected throughout the Inuvialuit Settlement Region as part of a community-partnered harvest-based monitoring program. At Hendrickson Island, near Tuktoyaktuk, Northwest Territories, Canada, a team of community monitors and scientists work in close collaboration with local Inuvialuit harvesters to collect more intensive samples and measure the levels of a variety of organic pollutants, mercury levels, and various health parameters (Desforges et al., 2012; Noël et al., 2014; Loseto et al., 2008).

In this study, we worked with the Hendrickson Island Beluga Monitoring Program and the community of Tuktoyaktuk to secure samples from beluga whales with the goal of characterizing presence and degree of potential contamination by microplastics in this cetacean population.

2. Methods

2.1. Sample collection

In the summer of 2017 and 2018, biological samples were collected from seven adult male belugas in collaboration with the annual beluga harvest by Inuvialuit harvesters on Hendrickson Island, Northwest Territories (Fig. 1) located 25 km northwest of the community of Tuktoyaktuk. The sampling team wore natural fibres when possible, but collected a sample of clothing to control for potential contamination. Entire stomachs (n = 7) were removed from the body cavity, with the terminus of the esophagus and the duodenum was tied with leather string to contain stomach contents, and placed in 20 L pails. In addition, for each whale, six intestinal subsections (n = 42) measuring approximately 30 cm in length were collected in situ. The selection of the six sub-sections was randomized within tissue type, containing sections of small intestine (n = 3), large intestine (n = 2) and colon (n = 1) for every whale sampled. All intestinal segments were individually sealed in plastic Ziplock® (SC Johnson, Wisconsin, USA) bags and stored in the pails. Fecal samples were collected when available (n = 2) and stored in Whirl-Pak® bags (Wisconsin, USA). When fecal samples were collected, they were found in the intestinal segments within 24 in. from the anus. All samples were frozen at −20 °C and transported to the Ocean Wise Plastics Lab (Vancouver, BC) for further analysis.

2.2. Contamination control

The Ocean Wise Plastics Lab is specifically modified for microplastic research to take place with controlled airflow filtered with high-efficiency particulate air (HEPA) fans. The Plastics Lab follows a regular cleaning regime and contamination is rigorously monitored using air blanks. All researchers working in the lab wear low-shedding Tyvex suits, non-shedding rubber shoes, and nitrile gloves. These precautionary measures commonly used in forensics have been shown to significantly reduce laboratory contamination (Woodall et al., 2015).

Processing of intestinal segments was performed inside a laminar flow hood to restrict airborne contamination. Due to their large size, the stomachs could not be processed inside a laminar flow hood. A portable, particle controlled isolation area was created for the purpose of dissecting and rinsing the stomachs. This isolation area measured 6 ft. × 4 ft. × 4 ft. and was made of wood frame tightly sealed with thick vapor guard plastic sheeting. Prior to dissection, the floor, walls and ceiling of the isolation area were rinsed with filtered water, and thoroughly rinsed plastic sheeting was placed underneath to contain biological material.

All tools and glassware used for sample processing and analyses were rinsed three times with filtered water. Water used for all rinsing was filtered through a 1 µm borosilicate filter paper. All samples were kept covered with aluminum foil as much as possible to further reduce potential contamination.

2.3. Quality control and data correction

Within all dissection and filtration locations, a damp filter paper inside a petri dish was left exposed to monitor any background contamination. In addition to these “air blanks”, procedural blanks (PB) were used to monitor potential contamination of reagents and/or glassware. All PBs underwent the same treatment (exposure to air, addition of reagents, use of filtered water) as samples.

One PB per stomach and one per six intestinal sections were processed for each whale (total of two PBs per beluga, making a total of 14 PBs for all seven GI tracts, and two for feces). Filter papers from PBs and air blanks were visually inspected and all SMPs confirmed using FTIR spectroscopy. If particles found within the PB or air blanks matched the particle shape, colour and composition of any particle found within the corresponding sample, this particle would then be removed from final counts to correct the data.

2.4. Microplastic extraction

Microplastics were extracted from stomachs, intestinal segments and feces using methods adapted from Lusher et al. in which they require the use of metal sieves during sample processing (Lusher et al., 2015a). However, since more recent work has shown that using metal sieves may cause particles (notably fibres) to be lost through the mesh (Covernton et al., 2019), we did not use sieves to ensure minimal loss of particles and therefore more accurate particle count.

2.4.1. Stomach

Stomachs were thawed at room temperature for 48 h. Filtered water was used to externally wash the stomach to remove any potential contamination. The leather string was removed from one end of the stomach and filtered water was flushed thoroughly through all stomach compartments and directly collected in glass jars. Any prey items found were rinsed and set aside for further analysis. Dried potassium hydroxide (KOH) flakes were then added to the jars to obtain a 10% concentration solution to prepare samples for filtration. The use of a 10% KOH solution is effective for removing biological material in samples while having little effect on plastic polymers (Foekema et al., 2013). The solution was tightly sealed and left for 2 weeks at room temperature to digest. The solution was then vacuum filtered through a 20 µm polycarbonate filter paper. Filter papers were stored in petri dishes until further analyses.

2.4.2. Intestines

All subsections were thawed for 24 h at room temperature, and thoroughly rinsed using filtered water to remove any external sediment and associated contamination that may have occurred during collection. Subsections were then placed, one at a time, in a steel dissection tray. Leather strings were removed and filtered water was flushed through intestines using a syringe into a rinsed glass jar to collect intestinal contents. Dried KOH flakes were added to the intestinal liquid contents to create a 10% concentrated solution. The solution was left for 2 weeks at room temperature, and then vacuum filtered through a 20 µm polycarbonate filter paper. Filter papers were stored in petri dishes until further analyses.

2.4.3. Feces

Fecal samples (approximately 100 mL, liquid state) were thawed for

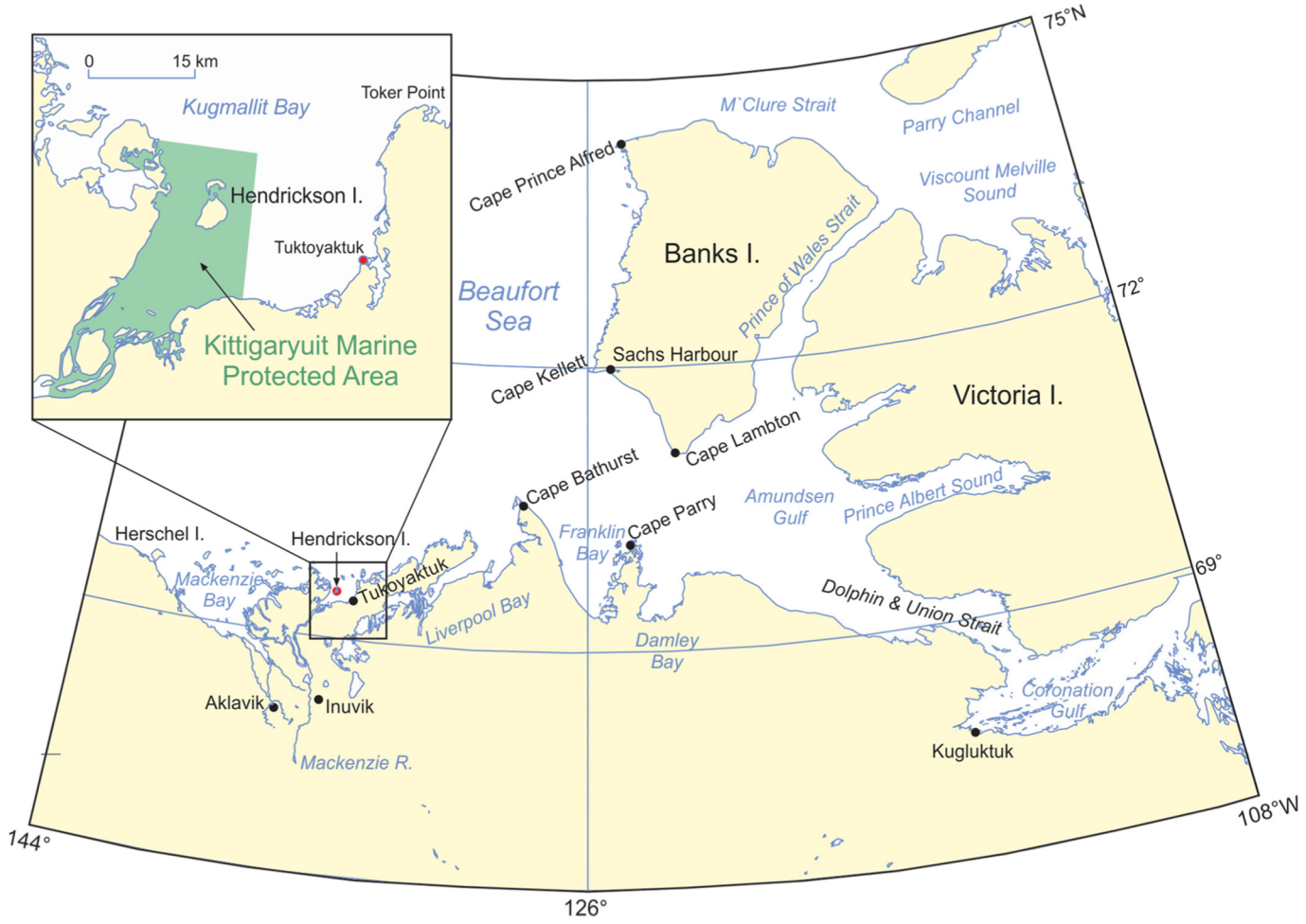

Fig. 1. Samples from seven beluga whales were collected on Hendrickson Island in 2017 and 2018 during an annual traditional harvest. The community of Tuktoyaktuk harvests beluga whales annually during the summer at this site.

8 h at room temperature, transferred to glass jars and treated with 10% KOH to digest organic material. After two weeks digestion time at room temperature, samples were vacuum filtered through a 20 µm polycarbonate filter paper. Filter papers were stored in petri dishes until further analyses.

2.5. Estimating intestinal length and total MP abundance

Due to the extensive length of cetacean intestines and challenging field conditions, it was impractical to collect and measure intestinal length of each whale. Therefore, we estimated intestinal length of each beluga sampled in this study based on their individual body lengths. Historical reports indicate that beluga intestines are approximately seven times the length of their body (Morrison Watson, 1880; Jefferson et al., 1994). Based on the intestinal sub-section MP counts, we estimated total MP abundance per whale by calculating the mean number of MP per meter of intestines and extrapolating this data.

2.6. Microplastic enumeration and polymer identification

Following filtration, filter papers were analyzed using a dissection microscope (Olympus SZX16 microscope with Olympus DP22 camera and DP2-SAL software, Shinjuku, Tokyo, Japan) without removing the lid of the petri dishes to avoid potential contamination. All suspected microplastics' (SMPs) size, shapes (fibre, fragment or sphere) and colours were catalogued, with photos and measurements of each particle taken. Once visual identification was complete, the petri dish lid was removed and each SMP was transferred using tweezers to a glass slide sprayed with a 2% dextrose solution, to affix the particle and decrease risk of particle loss during FTIR analysis.

Each SMP was then scanned using a Cary 670 Fourier Transform Infrared Spectrometer equipped with a Cary 620 microscope (Agilent Technologies, Mulgrave, AUS) using the micro-ATR accessory equipped with a Germanium crystal. Each SMP was scanned at a resolution of 8 cm^{-1} in the range of 3800 to 900 cm^{-1}. The resulting particle spectra were matched against a commercial polymer library with 250,000 polymer spectra entries (KnowItAll, BioRAd). Sample spectra were identified successfully if they met the following criteria: i) all peaks were present in both reference and sample spectra, and ii) the total overlap of the reference and sample spectra was > 80%.

3. Results and discussion

We identified a total of 350 suspected microplastic particles in the seven beluga whales sampled, of which 81 (23%) were confirmed through FTIR-spectroscopy to be plastic. Of the remaining (non-plastic) 269 particles, 55% were confirmed as semi-synthetic (e.g. modified cellulose), 14% as natural (e.g. minerals, protein, shells), and 7% of particles could not be identified because of weak spectral matches due to weathering or biofouling. The proportion of visually identified SMPs subsequently confirmed to be plastic using FTIR-spectroscopy in our study compares favourably to other studies and illustrates the importance of polymeric identification using FTIR or other analytical laboratory techniques (Bergmann et al., 2015; Eriksen et al., 2013; Li

Table 1
The number of FTIR-confirmed microplastic particles varied among whales. An average of 53% ± 15% of particles were found in the stomachs compared to particles found in intestinal sections. Fecal samples were available in two of the seven sampled whales. Estimated total MP concentrations in GI tracts are based on extrapolation from quantities of MPs encountered in intestinal subsections paired with estimated total intestinal length.

Beluga ID	Beluga body length (m)	#MPs encountered stomach	#MPs encountered intestine	#MPs encountered feces	Estimated total MPs in GI tract
ARHL-DL-17-12	4.2	14	8	2	147
ARHL-DL-17-11	4.1	2	1	n/a	18
ARHL-DL-17-15	4.2	7	7	n/a	121
ARHL-DL-17-17	4.1	5	5	0	85
ARHL-DL-18-02	4.4	7	6	n/a	110
ARHL-DL-18-04	4.3	4	3	n/a	54
ARHL-DL-18-07	4.5	2	8	n/a	143
				Mean ± SD	97 ± 47
				Range	18–147

et al., 2015).

All stomachs and intestinal segments were empty, containing no prey items other than several upper and lower cephalopod beaks belonging to armhook squids (*Gonatus fabricii*) and one upper beak of a smoothskin octopus (*Benthoctopus leioderma*) (William Walker, personal comm.).

We detected microplastics within every whale sampled (mean 11.6 ± SD 6.6 per individual, Table 1). Our results of observed particles within the intestinal sub-samples indicate that microplastic abundance can be unevenly distributed throughout the intestinal tract. Based on these results and this apparent variation across intestines, we estimate that the whales sampled contain between 18 and 147 microplastic particles (mean 97 ± 42).

These microplastic particles consisted of both fibres and fragments (photographic examples in Fig. 2), making up an average of 49% and 51%, respectively (Fig. 3). One sphere, confirmed to be polyolefin, was classified as a fragment (ARHI-DL-17-12). Given the small size scale of particles encountered (Fig. 4), it was not possible to reliably further categorize particles based on shape (e.g. foam, film).

More than eight types of plastic polymers were identified (Fig. 3). Polyester was the most prominent polymer, making up 44% of the particles found in GI tracts. The majority (83%) of these polyester particles were fibres, one of the primary polymers used in creating microfibre textiles (Henry et al., 2019). Plastic microfibres have been reported to be widespread in arctic seawater (Lusher et al., 2015b) and have been found in the GI tracts of numerous fish species around the globe (Morgana et al., 2018; Lusher et al., 2015c; Rochman et al., 2015; Zhang et al., 2019). Our results support the growing body of evidence that microfibers make up a significant part of microplastic pollution in the environment, and in aquatic food webs (Napper and Thompson, 2016; Browne et al., 2011; Dris et al., 2016; Murphy et al., 2016; Sun et al., 2019).

Our results indicate that microplastic pollution in EBS beluga whales is higher than counts reported in by Nelms et al. (2019) in their study on stranded cetaceans on the UK coast (n = 43). At first glance, this may appear surprising given that belugas feed in less industrialized areas than those sampled in the UK study. However, these studies are difficult to compare due to differences in sample size and methodology.

Our failure to find any meso or macro-plastic items in the beluga provides support for the notion that belugas are not directly or deliberately ingesting pieces of plastic. We suspect that the majority of MPs found in our beluga originated from trophic transfer from prey. Arctic cod (*Boreogadus saida*) are an important food source for belugas belonging to the EBS during the summer (Loseto et al., 2009). Arctic cod has previously been examined for microplastic ingestion by Kühn et al. (2018) Two of the 72 examined cod within their study contained MPs in their stomach. However, the authors excluded plastic microfibres from their study, so these results only account for fragments. An additional study by Morgana et al. which included plastic microfibres, revealed that 18% of 82 sampled Arctic cod had MPs in their stomachs, 88% of which were microfibres (Morgana et al., 2018). Although these studies sampled fish from outside of the Beaufort Sea (Svalbard, and Northeast Greenland, respectively) they confirm that Arctic prey species of belugas are ingesting microplastics.

As microplastics were found in all parts of the GI tracts sampled, as well as in feces, our results may indicate that particles of this size are transitory, as initially suggested by both Lusher et al. (2018) and Nelms et al. (2019). Based on the relatively small size of particles (Fig. 4) it is unlikely that these microplastic fibres and fragments would cause any physical obstructions, but the possibility that they become embedded or otherwise cause localized harm in the GI tract cannot be excluded.

The toxicological effects of microplastic particles on marine organisms remain unclear. Some evidence suggests that microplastic particles, when ingested, can act as vectors for toxic substances such as heavy metals and POPs, which have the ability to bioaccumulate in top predators (Bradney et al., 2019; Holmes et al., 2012; Gao et al., 2019; Rochman et al., 2013) and induce adverse health effects (Chen et al., 2019; Noël et al., 2014; Stow, 2005). It is also important to consider that the potential toxicity of microplastics could be size dependent, and also differ based on polymer type (Ma et al., 2016; Jeong et al., 2016;

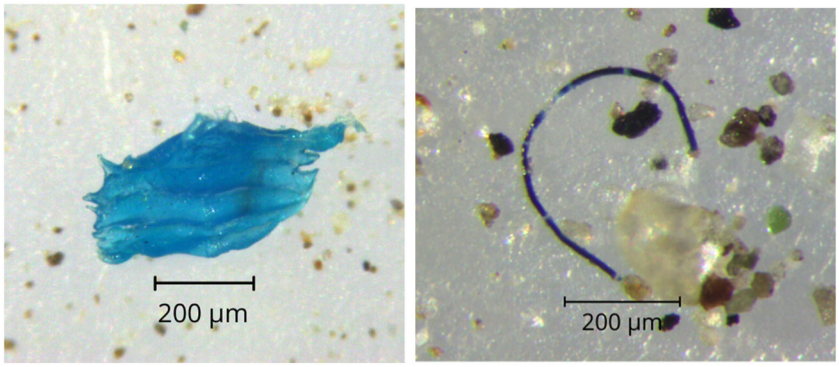

Fig. 2. Photographic examples of microplastic particles observed within beluga GI tracts (left: polystyrene fragment; right: polyester fibre).

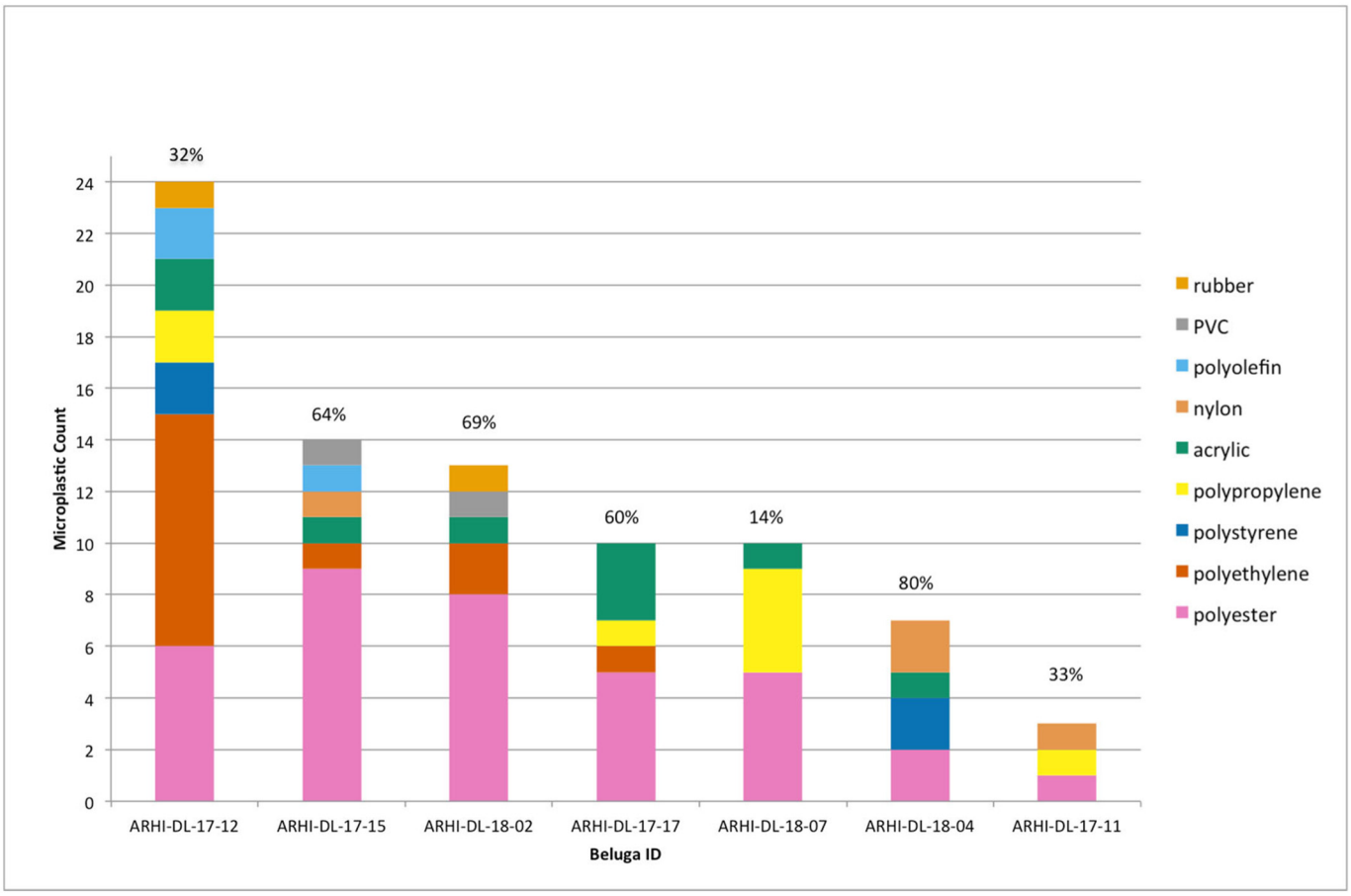

Fig. 3. Microplastic numbers and associated polymeric identities in all particles identified in gastrointestinal tracts of beluga whales from Hendrickson Island from the Beaufort Sea. The percentage of these particles that were fibres is denoted by the number on top each individual bar.

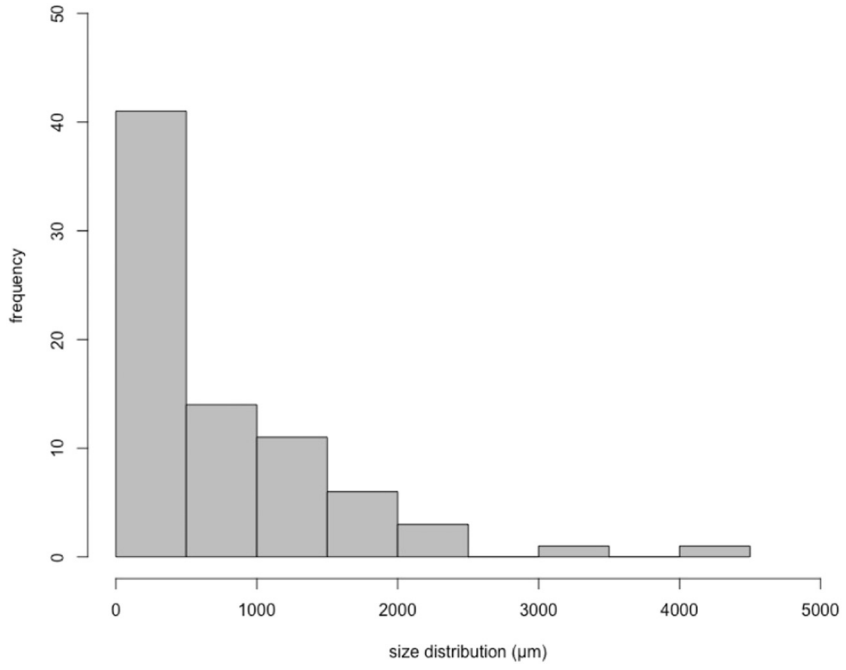

Fig. 4. Most microplastic particles found within beluga gastrointestinal tracts were smaller than 2000 µm in length. No particles > 5 mm in length were found.

Rochman et al., 2019). Given that belugas are known to bioaccumulate a variety of contaminants via the consumption of natural prey (Desforges et al., 2012; Noël et al., 2014), we expect the contribution to their burden arising from the ingestion of small amounts of microplastics to be minimal.

Plastic pollution, like many other pollutants, knows no boundaries. Our observation that that microplastics were present in every individual beluga we sampled underscores the global nature of this emerging pollutant, and the vulnerability of remote regions to contamination. Although these microscopic particles may be relatively inert at the moderate concentrations we encountered, significant questions remain about the potential for long-term harm associated with chronic exposure to synthetic particles. With the projected global increase in plastic pollution within marine environments, and continued access to these biological samples, these long-lived top predators could act as an important indicator for microplastic contamination within the Arctic. The presence of microplastics in this culturally and ecologically important species highlights a need for global action on understanding the source, impact and fate of this ubiquitous contaminant.

CRediT authorship contribution statement

R.C. Moore: Investigation, Methodology, Formal analysis, Data curation, Writing - original draft. **L. Loseto:** Conceptualization, Resources, Writing - review & editing. **M. Noel:** Conceptualization, Writing - review & editing, Supervision, Project administration, Funding acquisition. **A. Etemadifar:** Formal analysis. **J.D. Brewster:** Conceptualization, Resources. **S. MacPhee:** Conceptualization, Resources. **L. Bendell:** Supervision, Resources, Writing - review & editing. **P.S. Ross:** Conceptualization, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

None.

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