

Pinnipeds in Arctic Canada show no evidence of retained microplastic pollution

by

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Abstract

As the threat of plastic pollution continues to loom heavily over the global environment, the Arctic has drawn increasing research interest as a potential sink for debris. More specific to the Canadian Arctic, it is unclear whether marine mammals accumulate microplastics (MPs: $\leq 5\text{mm}$). Pinnipeds, like walrus (*Odobenus rosmarus*) and ringed seals (*Pusa hispida*), are both ecologically and culturally significant, which poses a risk to northern food security. Here, I present the first assessment of MPs in Canadian walrus by examining the stomachs of 36 animals from Nunavut. Additionally, I expand on existing literature by evaluating the stomachs of 10 ringed seals from the Northwest Territories, Canada. I detected no MPs $\geq 80\mu\text{m}$ in any of the animals. This result suggests that walrus and seals in the Canadian Arctic either do not retain MPs or are not exposed to them, which is consistent with studies from similar regions.

Preface and contribution statement

The samples used for the completion of this thesis were acquired through separate sources of government, academic, and non-government organization (NGO) funding and harvested in different geographical areas of Arctic Canada (Northwest Territories and Nunavut). Apart from the overall objectives of quantifying microplastics in Arctic mammalian stomachs, hunters collected the walrus and seals for different research projects. As a result, two separate manuscripts have been produced reflecting their respective contributors and focusing on each species, while I present the thesis as one unified project. I was the lead investigator and writer for both manuscripts, completed data collection and analysis, and helped with conceptualization.

The first manuscript (Alexander M. Jardine, Jennifer F. Provencher, Stephen J. Insley, Lila M. Tauzer, William D. Halliday, Madelaine Bourdages, Magali Houde, Derek Muir, Jesse C. Vermaire. 2022. No accumulation of microplastics detected in the stomachs of ten ringed seals (*Pusa hispida*) harvested in the Inuvialuit Settlement Region in northern Canada) has been submitted for publication in '*Marine Pollution Bulletin Baseline*' with alterations to the content and following the journal guidelines. Jennifer F. Provencher and Jesse C. Vermaire both provided supervision and guidance throughout the project and were involved in conceptualization, funding acquisition, and review & editing. Stephen J. Insley, William D. Halliday, and Lila M. Tauzer also helped with funding acquisition, project administration, and resource collection. Madelaine Bourdages assisted with the investigation, and Magali Houde and Derek Muir aided with resource collection, project administration, and review & editing. The Northern Contaminants Program (NCP) [M-65, M-04], the Weston Family Foundation, and the Inuvialuit Fisheries Joint

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The second manuscript (Alexander M. Jardine, Cory Matthews, Jennifer F. Provencher, Claire Hornby, Mary Gamberg, Madelaine Bourdages, David Alexander, Jesse C. Vermaire. 2022. “*No accumulated microplastics detected in the first assessment of walrus (*Odobenus rosmarus*) stomachs from Nunavut, Canada*” has been submitted for publication in '*Arctic*', with appropriate alterations to the content and following the journal's guidelines. Jennifer F. Provencher, Cory Matthews, and Jesse C. Vermaire supervised the project and contributed to conceptualization, funding acquisition, and review & editing. Madelaine Bourdages assisted with the investigation. Mary Gamberg, Claire Hornby, and David Alexander were involved in project administration and resource collection. Funding was provided by the Canadian Department of Fisheries and Oceans (DFO), World Wildlife Fund (WWF) Canada, Environment and Climate Change Canada (ECCC), and Acadia University, with support from Carleton University, Gamberg Consulting, and the Amaruq Hunters and Trappers Association.

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1.0 Introduction

Plastic pollution is a global, multidimensional threat that not only permeates the environment but also pervades human health, cultural, and economic geographies (Vethaak and Leslie, 2016; Nielsen et al., 2019; Alda-Vidal et al., 2020; AMAP, 2021; Bucci and Rochman., 2022). Since most user plastics are highly durable, they can persist in the environment for long periods, eventually breaking down into smaller microplastics (MPs: pieces $\leq 5\text{mm}$). In the ocean, microplastics are remarkably invasive since currents, surface winds, gyres, and biota can all act as 'source to sink' transportation mechanisms, allowing plastics to reach some of the most remote regions of the world (Suarua et al., 2020; Jones-Williams et al., 2020; Xiong Xiong et al., 2022). As one of the more vulnerable environments to anthropogenic disturbances, researchers have identified the Arctic as one such region and a potentially significant sink for marine plastic pollution (Cózar et al., 2017; Provencher et al., 2022). Ice floes and sea ice accumulate microplastic fibres, and researchers have documented plastic debris in Arctic waters and numerous species (Evangard, 2011; Bergmann et al., 2017; Collard et al., 2021). However, extensive research on the full reach of pollution in the region is not yet complete. As a result, many uncertainties remain surrounding how practitioners should conduct Arctic research, as well as the ultimate fates and consequences of microplastic pollutants on the environment and in animals.

To date, studies that have evaluated microplastic ingestion in Arctic fauna have focused mainly on seabirds (Collard and Ask, 2021). This circumstance is logical since seabirds are proven useful bioindicators of anthropogenic debris and readily ingest and accumulate it (Baak et al., 2020). However, they severely outclass other taxa in terms of plastic-specific studies,

representing a considerable knowledge gap in pan-Arctic research since many understudied species are more traditionally comestible in Inuit communities (Provencher et al., 2019; Baak et al., 2020; Collard and Ask, 2021; Bourdages et al., 2021). Mammals, for example, are a critical group for virtually all northern communities (Government of Canada, 2014). In the literature, however, there has been comparatively little attention paid to plastic ingestion in Arctic marine mammals, and only a handful focus explicitly on microplastics. Relatively few publications are specific to Arctic Canada.

Moreover, the results of what limited studies exist are so far mixed. For example, some have provided putative evidence that Arctic marine mammals both ingest and accumulate microplastic pollution, while others have reported no ingested plastics (Moore et al., 2020; Bourdages et al., 2020). Consequently, there is a lack of data assessing the potential risk to humans that rely on healthy wildlife, and the overall profile of plastic pollution in the region is relatively indistinct (Moore et al., 2020; Bourdages et al., 2020). Alarming, plastic pollution and related chemicals can also bioaccumulate in the food web, threatening food security in the region (UNEP, 2016).

Pinnipeds (*Phocidae*, *Otariidae*, and *Odobenidae*) are essential to Arctic communities (along with other marine mammals like cetaceans). Still, we know very little about their exposure to MPs or their ability to retain them (Bourdages et al., 2020). Globally, some evidence suggests that Phocid pinnipeds (true seals) can indeed retain MPs. However, most of these findings come from studies at sub-Arctic and southern latitudes (and may not reflect equivalent exposure levels specific to the Arctic) (Bourdages et al., 2020; Hernandez-Milian et al., 2019; Donohue et al., 2019; Lusher et al., 2022). Ringed seals (*Pusa hispida*) represent a considerable portion of

mammalian subsistence harvests in the Canadian Arctic (along with harp: *Pagophilus groenlandicus*, hooded: *Cystophora cristata*, and grey seals: *Halichoerus grypus*), and other pinnipeds like walrus (*Odobenus rosmarus*) are vital components of Inuit trade, culture, and diet (DFO, 2016). That withstanding, seals have been evaluated for microplastic ingestion in the eastern Canadian Arctic in only a single study (ringed seals, bearded seals: *Erignathus barbatus*, and a harbour seal: *Phoca vitulina*), though the researchers did not find any plastics in the stomachs of the animals at the chosen detection limit of 425 μ m, and no data yet exist from the western Canadian Arctic (Bourdages et al., 2020).

Similarly, there is only one known study assessing MPs in walrus, where Carlsson et al., (2021) evaluated feces from 15 walrus in the European Arctic. However, it is unlikely that this finding would represent the overall abundance of ingested MPs in walrus at the pan-Arctic level, as it does not provide further insight into the internal content in the walrus' gastrointestinal tracts (GITs) or their diets. The utility of studying faecal samples on their own to represent mammalian MP ingestion is also questionable since it yields little information regarding the initial intake volume and the retained MPs in the GIT. There are currently no GIT assessments on microplastic accumulation for walrus.

The primary objective of this thesis is to expand on existing literature by examining the stomachs of 10 ringed seals from the Northwest Territories, Canada, a previously unstudied region (western Canadian Arctic), with a lower detection limit (80 μ m) than earlier research. Additionally, using the same detection limit, this thesis provides the first assessment of accumulated microplastic in walrus by evaluating the contents of 36 hunter-harvested stomachs from five communities in Nunavut, Canada (eastern Canadian Arctic). The results of this thesis

contribute to a more robust understanding of MP ingestion in pinnipeds from Arctic Canada at both east and west geographies. Given the chosen detection limit (80µm) and the apparent susceptibility of mammals to MP exposure in the literature, I expected to find microplastics retained in the GITs of both the seals and walrus. Observing MPs of this size would be consistent with findings in Arctic Canadian beluga (*Delphinapterus leucas*) while simultaneously expanding on the results of Bourdages et al., (2020) and Carlsson et al., (2021) by demonstrating that MPs are transient in pinniped GITs, but that the animals retain them at specific sizes.

2.0 Literature Review

2.1 Plastics 101: Contextualizing Arctic plastic pollution

Marine plastic pollution has become a global crisis threatening ecological harmony for humans and wildlife alike (AMAP, 2021; UNEP, 2022). Virtually every environment on earth has been contaminated by plastic pollution in some form, principally through the ubiquity of microplastics (UNEP, 2022). Although global production began to soar as recently as the mid-20th century, plastic already appears in the fossil record in coastal ocean sediments (Brandon et al., 2019). Even though the plastic pollution hazard is serious, very little is known about the ultimate fates of these pollutants, even less so in the Arctic. In Canada, we often describe our Arctic regions as 'pristine' and 'untouched.' Unfortunately, there is mounting evidence suggesting that microplastics have permeated them through atmospheric, oceanic, and biological transport, representing a genuine health concern for people in the region who are accustomed to low anthropogenic disturbance (Hamilton et al., 2021; Ross et al., 2021; Hamilton et al., 2022). Evaluating anthropogenic pollutants in animals is especially important when healthy populations in the wild are relied on for harvest and can directly impact food security where few other options exist.

Moreover, critical species like ringed seals and walrus may risk exposure to these pollutants in the water column or when feeding. Walrus, for example, are benthic feeders and may be susceptible to microplastic pollution when sifting through sediment or eating contaminated bivalves. Plastic pollution is an ostensive, deeply anthropogenic issue (Brandon et al., 2019). Ultimately, its origins and consequences must be understood to appropriately

contextualize research within global and regional investigations to form appropriate hypotheses or consistent recommendations for further reviews.

2.2 From mass production to mass pollution

Polymerized hydrocarbon molecules, initially originating from fossil fuels, form the basis of most commercial user plastics (Su et al., 2022). Before the 20th century, semi-synthetic polymers like cellulose-nitrate and casein formaldehyde were commonly used to imitate high-value materials (like ivory) and are now considered to be early forms of plastic despite incorporating organic polymers into their structures (Cucci et al., 2016). In 1907, the Belgian-American chemist Leo Baekeland invented the first fully synthetic plastic (Bakelite), catalyzing a new era of efficient industrial manufacturing, improved tools of war, and advances in medical science and textile production, transportation, and consumer products (Chalmin, 2019).

Following the Second World War, leading into the 1950s, the utility and efficiency of plastics were incontestable, and various other fully synthetic polymers had since been developed (polychloride vinyl, cellophane, polyethylene, nylon, polypropylene) (Beall, 2009). This innovation escalated global plastic production to around 1.5 million metric tons per year and solidified synthetic plastics as titans of the manufacturing industry, particularly in the global west (Chalmin et al., 2019). However, without much regard for the long-term consequences of such unfettered mass production, negligent waste disposal practices, shipping, and unintended runoff soon began introducing new hazards into the natural world, including the ocean. The first scientific reports on marine plastic pollution were published in 1972 when Carpenter et al.,

(1972) and Carpenter and Smith (1972) found plastics adrift in the Sargasso Sea in the North-Atlantic Subtropical Gyre (Rochman, 2020).

In modern times, annual plastic production has increased exponentially to over 330 million metric tonnes (Thevenon, 2014; Weyler, 2017; Boucher and Friot, 2017). Approximately 8 million tonnes enter the marine environment annually, and experts have recently identified plastic pollution as one of the earth's most urgent environmental threats (Thevenon, 2014; AMAP, 2021). There has been a growing interest in plastic pollution research in the last few decades as researchers have attempted to tackle this multi-faceted issue. However, the inherently complex behaviours of plastic in the environment make their eventual consequences challenging to quantify (Provencher et al., 2017). Plastic pollution has become so widespread, mainly via microplastics, that it is ubiquitous in virtually all terrestrial and aquatic habitats (World Wildlife Fund, 2022).

2.3 The physical impacts of plastic pollution on animals in the marine environment

2.3.1 Mechanical injuries resulting from exposure to plastic pollution

Marine animals are vulnerable to several physical risks when exposed to plastic pollution. Common injuries range from external entanglement and lacerations to internal gastrointestinal blockages, ulcers, and lesions (Pierce et al., 2004; Ahrendt et al., 2020). The entanglement of marine animals occurs most often with macroplastics manufactured in long strands or loops, like fishing lines, ropes, plastic 6-pack rings, or nets (Canadian Wildlife Health Cooperative, 2019). When swimming or feeding in the water column, marine animals can become hooked on or have plastic wrapped around their limbs, neck, and body (Canadian Wildlife Health Cooperative, 2019; Kühn and van Franeker, 2020). Depending on the severity of the restriction, entanglement may kill the animal via laceration, traumatic amputation, or asphyxiation (Gregory, 2009). Generally, entanglement and external physical injuries can affect most taxonomic groups, though it has been reported in some taxa more often than others. The Blastic Project is an initiative funded by the European Union's Strategy for the Baltic Sea Region, focused on plastic pollution education and reducing marine litter (Blastic, 2018). In a 2018 review, The Blastic Project estimated that globally, researchers had detected 340 total marine species entangled in plastic (Blastic, 2018). Additionally, according to a more recent review by Kühn and van Franeker (2020), that number has increased to at least 354 marine species. In both reports, seabirds vastly outnumbered other marine taxonomic groups in terms of individual species studied; however, when considering the overall proportion of entangled species for each group, pinnipeds were the dominant class, with 22 of 33 (or 71%) species reported (Figure 1) (Blastic, 2018; Kühn and van Franeker, 2020).

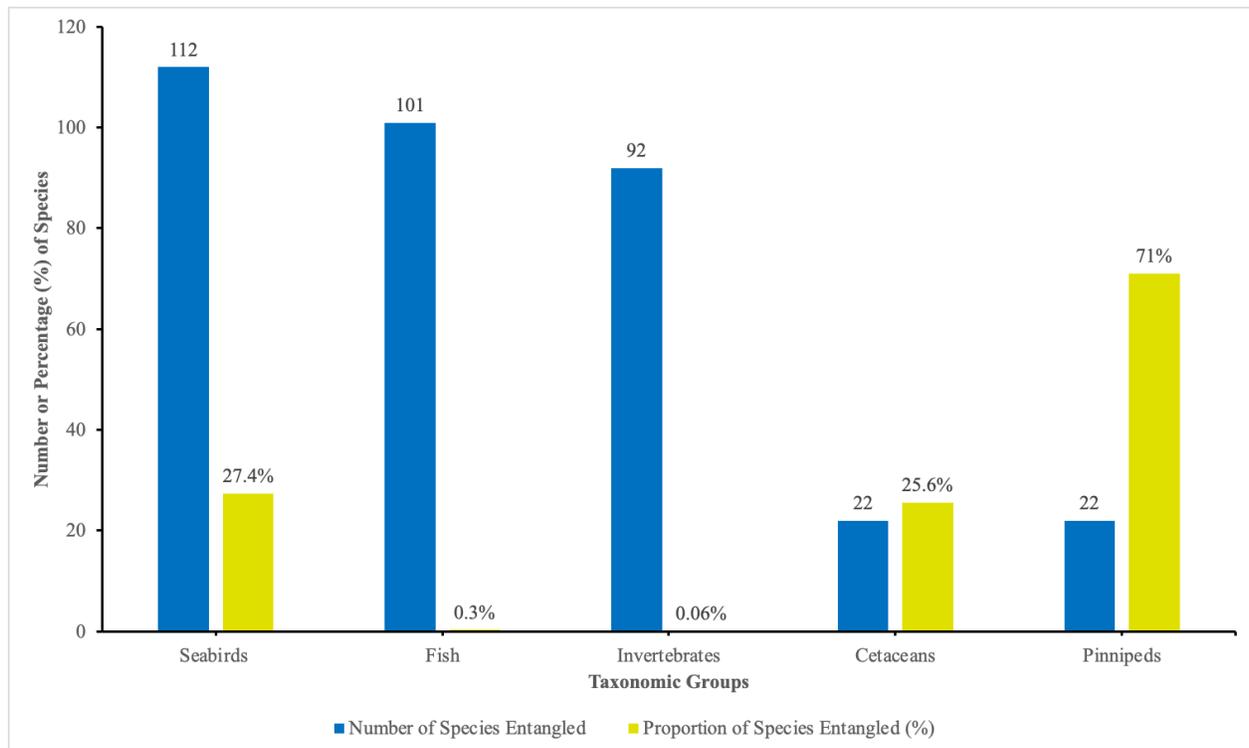


Figure 1. Global distribution of marine plastic entanglement in main taxonomic groups (groups with less than ten species in the wild are omitted, like sea turtles: $n = 7$), where the number of entangled species is blue, and the proportion (%) of species within a respective taxonomic group is yellow. This graph was produced based on data interpreted from Blastic (2018) and Kühn and van Franeker (2020) and are not representative of reviews conducted in the present research.

Surprisingly, pinnipeds represent the most proportionately significant group in terms of species that have become entangled in marine plastics, suggesting that it may be a global issue in that suborder; though, researchers have made no reports of entanglement in walrus (*Odobenidae*) (Butterworth, 2016; Kühn and van Franeker, 2020). Given that the number of species within any given taxonomic group is different, a direct comparison may not reflect an individual group's overall propensity for entanglement. Instead, predispositions based on regional factors and the animal's typical feeding and breeding ranges may influence their respective entanglement proportions. That withstanding, the harmful effects of entanglement are evident for all macro-vertebrate animals. They can act as barriers to population recovery as well as the decline of some

species (like the Hawaiian monk seal (*Monachus schauinslandi*) reported in Donohue and Foley, (2007)).

Although entanglement is probably the most apparent and outwardly distressing interaction plastics can cause, they can do just as much damage internally. Once ingested, plastic can perforate, lacerate, or clog the gastrointestinal tract of animals (Jâms et al., 2020). Physiological consequences of excessive plastic ingestion also include reduced fecundity, malnutrition, endocrine disruption, and potentially death (Ryan, 1989; Seif *et al.*, 2018; Thiel *et al.*, 2018; Jardine, 2021). Certain environmental factors may also lead some animals to be more inclined to ingest plastics, including high ambient concentrations and intersecting breeding and feeding ranges with commercial fishing or shipping routes (Čhulin and Bielić, 2016; Provencher et al., 2017). In addition, since many plastics are buoyant, they can be mistaken as prey and accidentally ingested when foraging (Provencher et al., 2017). Researchers may therefore consider predisposition to MP ingestion given a specific animal's feeding habits. On land, some seabirds also eat considerable amounts of plastic at anthropogenic landfills, where they can consume it alongside organic material when scavenging (Jardine, 2021).

The ingestion of marine plastic may also pose a severe risk to population conservation (Ryan, 2016). If enough plastic is consumed or retained in the gut, it can cause 'false satiation' in the animal, resulting in reduced dietary efficiency, malnutrition, and in some cases, starvation (Provencher et al., 2015; Galloway and Lewis, 2016; Ryan, 2016; Roman et al., 2020). Some animals, like sea turtles, are sensitive to changes in dietary structure and as a result suffer disproportionately because of improper nutrient uptake, causing losses in population and the further endangerment of species at risk (Wilcox et al., 2018). For this reason, direct ingestion of

plastics can cause severe and long-lasting damage to endangered or imperilled marine species and overall ecosystem health.

Marine mammals are high-level trophic predators and often get plastics through secondary ingestion more than through direct ingestion (Provencher et al., 2017). Consequently, when evaluating the risk of plastic ingestion in higher trophic level organisms, it may be more pragmatic to analyze plastic concentrations in prey items (Ryan, 2016; Provencher et al., 2017). Provencher et al., (2017) stated that researchers had already shown several marine prey species to retain plastic, particularly microplastic debris. Some of these species, like zooplankton, molluscs, cephalopods, and crustaceans, represent critical components of mammalian, seabird, and fish food webs (Provencher et al., 2017; Blanchet et al., 2019). Setälä et al., (2014) and Farrel and Nelson (2013) have also demonstrated trophic transfer of microplastics in zooplankton as basal organisms in the food web, as well as from mussels (*Mytilus edulis*) to crabs (*Carcinus maenas*). In megafauna, the risk of plastic retention (i.e., accumulation) is more severe through the repeated consumption of prey items that may be more inclined to consume large amounts of plastic. For Canadian beluga whales, Moore et al., 2021 found evidence that MP accumulation in the GIT was related to the overall abundance of plastics in their food web, particularly Arctic cod (*Boreogaidus saida*). They reached this conclusion after a previous analysis confirmed the presence of debris in the stomachs of the whales (Moore et al., 2020; Moore et al., 2022).

2.3.2 Chemical consequences: Plastics are multiple stressors to the marine environment

Apart from the mechanical injuries that plastics can cause, inconspicuous chemical consequences may also pose a significant risk to the health of biotic communities. For example, manufacturers make plastic using an array of additives that add desired properties like flexibility, rigidity, and heat resistance to virgin polymers (Hahladakis et al., 2018; Fred-Ahmadu et al., 2020). These additives include synthetic monomers like polyvinyl chlorides (PVC), Di(2-Ethylhexyl) phthalate (DEHP), PBDE, and Bisphenol-A (BPA), which have been documented as harmful to human and animal health, causing endocrine disruption, reproductive harm, and cancer (Hahladakis et al., 2018).

Prevalent types of plastic like PVC, polystyrene, and polycarbonate can release these harmful monomers into the environment (Browne et al., 2007). Microplastics have drawn increased attention in this regard due to their miniature sizes and their potential to adsorb, release and partition toxic chemicals in marine systems (Fred-Ahmadu et al., 2020). Sometimes referred to as Persistent Organic Pollutants (POPs), most additives can be grouped into four categories depending on their functionality (Table 1, with corresponding citations in Appendix A) (Hahladakis et al., 2018). Fred-Ahmadu et al., (2020) also state that the properties of plastic, such as surface area, molecular arrangement, and the functional group of a pollutant can also influence the sorption of chemicals under various conditions. The potential to release toxic chemicals in various media could be hazardous for aquatic life and humans through exposure and biomagnification of MPs in the ocean.

Table 1. General categorization of some chemical additives used in plastic production.

Category	Short description	Examples	General health effects
Functional Additives	Functional additives add characteristics to virgin plastics, like flexibility, heat resistance, and anti-oxidative properties. Functional additives can be further divided into <i>plasticizers, antioxidants, and heat stabilizers</i> and comprise most synthetic monomers used in plastic production.	DEHP Diketopyrrolopyrrole (DPP) PBDE	Cancer, congenital disabilities Respiratory complications Neurotoxicity, reproductive harm, liver, thyroid, and pancreas damage, cancer
Fillers	Fillers are usually added as powders to plastic products to reduce the costs of moulding compounds and can increase plastic's overall mass and stability.	Calcium carbonate Zinc Oxide Metal powders	Skin corrosion, organ toxicity, eye damage Irritation, mutagenic in mammalian somatic cells Heavy metal poisoning, general toxicity, liver damage
Structural Reinforcers	Reinforcers are fibrous additives incorporated into the plastic for increased structural integrity and rigidity.	Glass fibre Aramide fibre	Irritation, lung, and gastrointestinal damage Cytotoxicity, cancer
Colourants	Usually, metal compounds are added to change the colour of the plastic. Some are inherently toxic, like lead, while others become acutely toxic at high doses or when inhaled.	Cadmium Lead Cobalt Chromium	Kidney, bone, and lung disease, cancer Lead poisoning, anemia, renal and brain damage, cancer Cardiovascular damage, lung disease, sensory impairment, cancer Renal and liver damage

Some publications have demonstrated that synthetic monomers translocate into animal tissues in multiple taxa. For instance, Tanaka et al., (2013) analyzed the presence of PBDEs in the adipose tissue of short-tailed shearwaters concerning the anthropogenic contents in their GITs (Tanaka et al., 2013). As a result, the researchers could holistically quantify the presence of PBDE (and its congeners) in the birds. Furthermore, they determined a correlation between observed PBDE in the adipose and the plastic recovered from their GITs (Tanaka et al., 2013). Rochman et al., (2013) conducted a similar study on the health effects of Polybutylene Terephthalate (PBT) on a euryhaline species of fish (Japanese Medaka (*Oryzias latipes*)). Over two months, Rochman et al., (2013) dosed three control groups with (1) virgin polyethylene with unaltered concentrations of PBT, (2) polyethylene with additional PBT, and (3) no plastic. They found that during one month, not much change occurred; however, over longer time frames (2+

months), increased PBT concentrations caused more hepatic stress to the fish (Rochman et al., 2013).

Plastics do not only have direct interactions with elements of an ecosystem. Rather, they can potentially be multiple stressors to the environment via indirect influences (Rochman et al., 2013). Plastics move both vertically and laterally in aquatic food systems. Some researchers have studied these lateral and vertical transfers within marine systems to quantify the risk to human and wildlife communities (Kelly and Gobas, 2001; Kelly et al., 2007; Romero-Romero et al., 2017; Jamison et al., 2017). Essentially, consuming contaminated prey by marine animals (or humans) may lead to biomagnification and accumulation in food webs. This process can yield high concentrations of POPs in high trophic level organisms, like mammals (for instance, pinnipeds), as an indirect consequence of plastic pollution (Ren et al., 2017). In this sense, the vertical food web structure may also act as a mechanism for the transfer and increased risk of chemical pollution via plastic vectors since higher trophic level organisms tend to accumulate larger volumes of plastic than their prey (Carbery et al., 2018).

2.4 Marine litter and microplastics

Oceanic pollution is typically the result of improperly managed waste, runoff, and effluent from land-based sources (Ritchie and Roser, 2018). Lakes and rivers can also act as direct inputs, or 'transportation highways,' for plastics entering the ocean (Imhof et al., 2013). Lower-density plastics tend to emerge from freshwater systems (but are not limited to them) more commonly than large debris due to their smaller surface areas and shorter water residency times (Wessel et al., 2016; Hamid et al., 2018). Although most plastic contaminants originate from land and accumulate in coastal waters, marine sources contribute a substantial amount (around 20-30 percent of global marine plastic) from the fishing and shipping industries (Ritchie and Roser, 2018).

Generally, marine plastics are categorized into four sizes (Table 2). As the most direct input of terrestrial litter and dumping, macroplastics (> 10mm) form the bulk of debris in the ocean (> 65%) (Thushari and Senevirathna, 2020; Lebreton et al., 2020). These items are highly resistant to environmental degradation and are sometimes found in the open ocean several years or decades after their initial emission (Lebreton et al., 2020). In addition to their physical resilience, macroplastics are often buoyant and accumulate in offshore surface waters (Lebreton et al., 2018). Over time, weathering from the sun, physical abrasion and seawater deteriorates large items, fragmenting them into smaller mesoplastics (5mm to 10mm) and microplastics.

Table 2. Marine plastic debris is broadly categorized into four groups, represented here with approximate sizes and comparably sized objects (a plastic bottle, a dime, grains of rice, and a microscope for particles invisible to the naked eye).

Size Reference	Type	Size	Description	Examples
	Macroplastic	> 10mm	Larger pieces of litter that can typically be collected without specialized equipment	Plastic bags, bottles, large fragments, rope
	Mesoplastic	5mm to 10mm	Debris that is either slightly smaller than or derived from macroplastics	Plastic bags, bottles, large fragments, rope
	Microplastic	1µm to 5mm	Tiny debris that usually is the product of degradation from larger plastics	Microbeads, synthetic fibres, fragments < 5mm
	Nanoplastic	< 1µm	Microscopic plastic particles that require specialized equipment to observe and sample	Microscopic fibres and particles from synthetic polymers

Microplastics themselves are partitioned into two functional types: 'primary' and 'secondary' (Gomiero et al., 2018). Primary microplastics are intentionally fabricated at small sizes and enter the environment as such (Lehtiniemi et al., 2018; Gomiero et al., 2018). For example, personal hygiene and skin care products often contain exfoliating 'microbeads,' usually made of polyethylene, polyesters, and polypropylene; once discarded, these beads can be washed into the environment in wastewater (Government of Canada, 2015). Secondary microplastics come from bigger items that have broken down, fragmented, or otherwise deteriorated over time (Lehtiniemi et al., 2018). Some common examples of secondary microplastics include small fragments from plastic containers and bottles, fibres from textiles and clothing, or foamed pieces from polystyrene packaging (Baak et al., 2020; Jardine, 2021).

The effects of small plastics (MPs) are more challenging to quantify since their direct environmental consequences are less immediately noticeable than their larger macroplastic and

mesoplastic counterparts. In the marine environment, surface waters contain the most MPs, predisposing them to ingestion by surface feeders, including some marine mammals, fish, and seabirds (McCormick et al., 2016; Provencher et al., 2018; Savoca et al., 2021). Epipelagic and mesopelagic waters (depths between 200m and 600m) also contain significant concentrations of MPs, indicating that profound parts of the water column may act as reservoirs for marine debris (Choy et al., 2019). Even benthic sediments have large amounts of microplastic, typically concentrated near coastlines, which extends the risk of ingestion to invertebrates and other animals that feed on the ocean floor (Willis et al., 2017; Yang, 2021).

2.5 Drivers of marine microplastic transportation, how debris reaches the Arctic

2.5.1 Oceanic transport and thermohaline circulation

A circumnavigational study of global MP abundance estimated an average of fifty particles m^{-3} in open ocean surface waters (Tanhua et al., 2020). Microplastic concentrations also vary quite heavily spatially, accumulating predominantly in the West Tropical North Pacific Ocean and the southwest Pacific Ocean, with relatively high abundances in localized regions of southwestern European waters (Hamid et al., 2018; Tanhua et al., 2020). Comparatively, microplastics tend to be less abundant north of the equator and increasingly diffuse towards polar latitudes, like the north and the south Atlantic Ocean, the Barents Sea, and the Arctic (Tanhua et al., 2020; Hänninen et al., 2021). Though concentrations are lower in Arctic climates than in equatorial latitudes, they are nonetheless present in seawater, ice, and some animals (Provencher et al., 2018; Baak et al., 2020; Huntington et al., 2020). Roughly over the last two decades, researchers have attempted to establish the leading transportation mechanisms responsible for the universality of plastics (Ajith et al., 2020). However, we still do not yet understand the ultimate consequences it may bring to vulnerable ecosystems.

Principally, ocean and rotating currents (gyres) are responsible for the widespread distribution of marine plastics (Su et al., 2022). As they make their way from land-based, riverine, and estuarine sources, plastics enter these currents, concentrating them in subtropical gyres (Figure 2) (Su et al., 2022). The high Arctic would presumably be free of this mass pollution, given that most major currents fall below a latitude of 60°N (Figure 2). Nevertheless, plastics are also in the northernmost and easternmost areas of the Greenland and Barents Seas (Cózar et al., 2017). At the pan-Arctic level, such regions have been described as 'dead-ends' for

MPs, resulting from thermohaline circulation pathways also playing pivotal roles in the endangerment of polar waters (Cózar et al., 2017). Thermohaline circulation operates effectively as a 'conveyor belt', where, in the North Atlantic current systems (Figure 2), southern currents bring warm water over the top of the northern currents, which carry cold polar water (Toggweiler and Rey, 2001). Once it reaches the sub-polar Arctic, the temperature causes the water to cool and sink, resulting in an overturning motion in the deep ocean (Toggweiler and Rey, 2001; Rahmstorf, 2015). The Barents Sea contains the densest water in this interaction, causing the most significant 'sinking' effect in the North Atlantic, which extends its flow into the Arctic Ocean and back down through the Greenland Sea (Toggweiler and Rey, 2001). Since plastic is buoyant, as the northward water reaches the Arctic and sinks, it leaves the garbage behind, leading researchers to speculate that the Arctic acts as a substantial reservoir for pollution (van Sebille et al., 2015; Cózar et al., 2017; Onink et al., 2019; Cox et al., 2019).

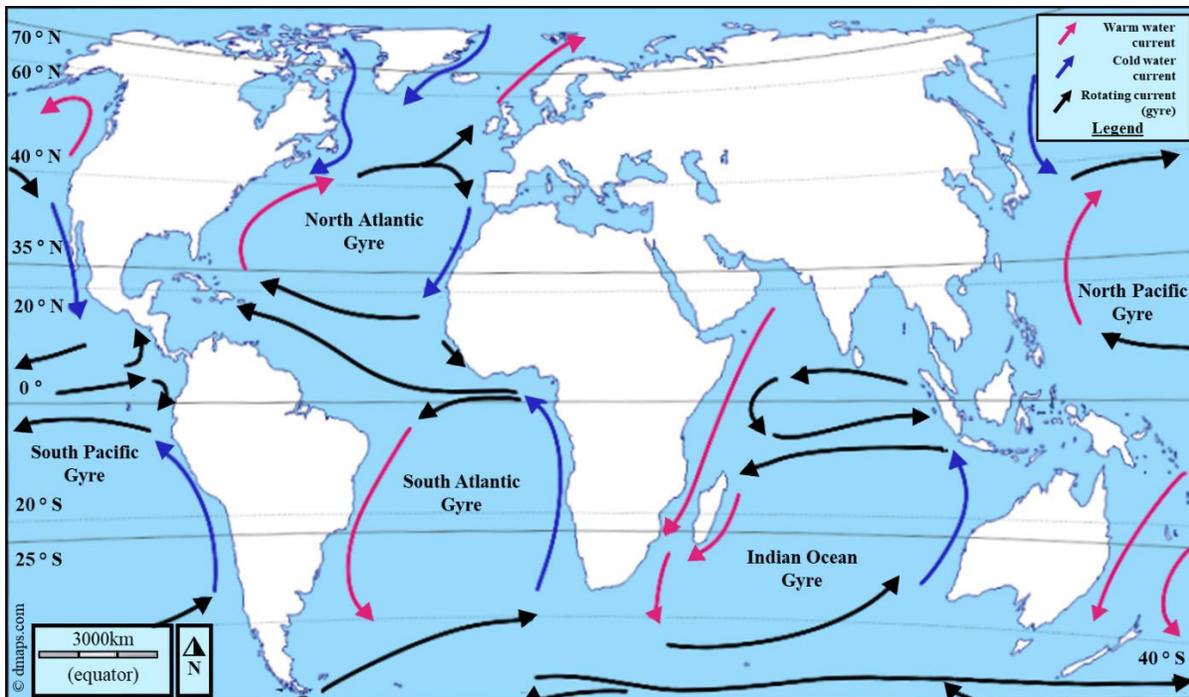


Figure 2. Major currents and gyres in the ocean sweep plastic debris into 'convergence zones.' Marine animals can also act as vectors for plastic pollution by becoming entangled or ingesting it.

High concentrations of plastic pollution are common in the Central and European Arctic and sub-Arctic. At 12 of 48 study stations (25%) in the Barents and East-Siberian Seas, surface water microplastics (0.004 m^{-3}) were detected by Yakushev et al., (2021), as well as in 50 out of 60 subsurface sites (0.8 m^{-3}). Lusher et al., (2015) also report a mean of $0.34 \text{ microplastics m}^{-3}$ in surface water at 95 percent of sampled sites near Svalbard, Norway, and $2.68 \text{ average particles m}^{-3}$ in 93% of subsurface sites. In the Arctic Ocean, surface water samples have presented between 0 and $27 \times 10^{-1} \text{ microplastics per km}^2$ of surface water and $2.4 \text{ particles m}^{-3}$ in subsurface water (Cózar et al., 2017; Morgana et al., 2018). The Central Arctic Basin also contains microplastics between depths of 8m and 4369m, with 0.7 m^{-3} recorded in 2018 (Kanhai et al., 2018). Although microplastics are generally buoyant, higher concentrations at sub-surface depths appear consistent in the literature. This feature of microplastic distribution in Arctic climates may be attributed to floating microplastics becoming trapped in sea ice after being transported to northern

regions during short ice-free seasons (Yakushev et al., 2021). Subsequently, once the ice melts and begins to release imprisoned MPs, they sink, which is consistent with observations near the Vilkitsy Strait in Russia, where MPs were absent in surface water but high in subsurface samples (Yakushev et al., 2021).

Huntington et al., published the first assessment of microplastic abundance in Canadian Arctic water in 2020 and found an average of 0.22 particles L⁻¹ in surface water from Hudson Bay to North Baffin Bay. They also reported 1.92 MPs g⁻¹ in benthic sediment (Huntington et al., 2020). These measurements were unrelated to nearby human settlements, which they interpret to indicate that eastern Canadian Arctic MP pollution is more likely the result of long-range transport (Huntington et al., 2020). In the same area, subsurface waters were later observed to contain an average of 0.031 MPs/L⁻¹, a lower concentration than previously reported (Jones-Williams et al., 2021). Further, microplastics exist throughout the water column in the Beaufort Sea (Ross et al., 2021). According to Ross et al., (2021), an average of 174 particles m⁻³ were present at six observation sites, with higher concentrations in near-surface waters in the Beaufort Gyre. These preliminary findings provide an essential understanding of the transport of microplastics in the Canadian Arctic; however, there are substantial knowledge gaps concerning the overall distribution of MPs in the area, as well as their sources, drivers, and ultimate fates.

2.5.2 Atmospheric deposition of microplastics transcends oceanic and continental barriers

An emerging driver of concern for microplastic transport is atmospheric deposition, though relatively few researchers have studied this mechanism extensively. Recent analyses have shown that the air can carry microplastic particles across continents and oceans (Evangelidou et al., 2020; Allen et al., 2021). Textiles, dust, and microfibres are all light and therefore easily transported, allowing them to reach remote and isolated regions of the world (Peeken et al., 2018). Pan-Arctic studies also show that ice cores and snow samples contain up to 14.4 million particles m^{-3} (Huntington et al., 2020; Bergmann et al., 2019; Bergmann et al., 2022).

Since sea ice forms from particles in the water column and the atmosphere, it can trap as much as 234 microplastic particles m^{-3} (even higher than concentrations observed in seawater: 0.12-0.34 particles m^{-3}) (Thomson et al., 2004; Goldstein et al., 2012; Obbard et al., 2014). As climate change continues to threaten polar climates, atmospheric deposition of MPs in the Arctic coupled with the melting of sea ice may release unprecedented amounts of plastic pollution into the ocean over the next decade (a minimum of 1 trillion pieces, given current warming trends) (Obbard et al., 2014).

2.5.3 Animals as vectors for microplastics, and microplastics as vectors for microorganisms

Marine animals may also play an essential part in the long-range transport of anthropogenic pollutants and their movement within Arctic systems. Animals that ingest plastic can potentially carry it to various new areas (Bourdages et al., 2020; Jardine et al., 2021). Seabirds, for example, eat considerable amounts of plastic, and many in the Arctic exhibit circumpolar breeding habits, allowing them to move plastic from one end of the Arctic to the other (Baak et al., 2020; Jardine, 2021). Further, many Arctic seabirds are pelagic surface and diving feeders. Little auks (*Alle alle*) dive between 0 and 50m below the surface in search of zooplankton which allows them to gather debris at both surface and sub-surface levels (Amélineau et al., 2016). In the Greenland Sea, little auks consume an average of 9.49 MP fragments per meal, indicating that MP dispersal in breeding colonies is due to the excretion of material eaten while feeding (Amélineau et al., 2016).

Microplastics are common in seabird guano (Gil-delgado et al., 2017; Provencher et al., 2018; Anderson et al., 2019; Wang et al., 2021; Hamilton et al., 2021). Colonial breeding behaviours of seabirds also contribute to the concentration of microplastic particles in colonies and breeding sites, some of which are far away from initial feeding grounds where ingestion may occur; an interaction that has been recently documented in the Canadian Arctic (Wang et al., 2021; Süring et al., 2022). Evidence suggests that some species may also exhibit selective feeding habits for microplastics based on the smell, colour and shape of debris, mistaking it for prey (Amélineau et al., 2016; Savoca et al., 2016). Whether this behaviour applies to other taxa is yet to be evaluated. Still, it is a potential trait that may lead to an increased risk of microplastic ingestion in some species.

Considering that most marine mammals in Arctic Canada have limited migration ranges, there is less evidence to suggest that groups like pinnipeds would act as important vectors for the direct transport of plastic to northern regions. However, the range of some mammalian species like killer whales in eastern Canada (*Orcinus Orca*) spans from Baffin Bay (75° N) to the Bay of Fundy (45° N) (occupying roughly 3,329,551 km²), which would allow them to ingest plastics from nearly the entirety of the eastern coastlines (COSEWIC, 2018). Unfortunately, researchers have not studied this interaction in eastern killer whales. Only speculative evidence based on models of Polybrominated Diphenyl Ether bioaccumulation suggests that killer whales in western Canada are potentially exposed to MPs through the food web (Alava, 2021). Otherwise, investigations on MP ingestion in cetaceans have seldom been completed, especially in Arctic Canada. For example, this literature review found only one study assessing microplastic ingestion in Canadian cetaceans (Moore et al., 2020). However, beluga whales do not migrate from cold polar waters during the summer and, therefore, would be unlikely to ingest debris not already present at Arctic latitudes. One reason that data are absent for many marine mammals is that sampling procedures are complex logistically, considering many species are dietarily essential or endangered (Zhu et al., 2019).

Inversely, microplastics (and plastic pollution, generally) can also introduce invasive species to the Arctic environment (Arctic Council, 2022). The *plastisphere* refers to microbial communities that have colonized plastic debris (Amaral-Zettler et al., 2021). Fungi, diatoms, and bacteria can adhere to plastics adrift at sea, leading to microcosmic communities populating anthropogenic litter (Amaral-Zettler et al., 2021; Du et al., 2022). While the plastisphere does not represent a mechanism for the distribution of microplastic itself, it does highlight the omnipresence of plastic in the marine environment and the multidimensional nature of its

consequences. Introducing invasive species to the Arctic can have surprisingly disastrous consequences since the Arctic is a harsh, highly adapted, and fragile ecosystem (National Park Service, 2021). Animals, microorganisms, and plants that have adapted to Arctic conditions are particularly vulnerable to the introduction of new species, which could result in the loss of critical food systems, the establishment of new pathogens, and reduced or extant populations (Higdon and Ferguson, 2009; Wernberg et al., 2012; Hyrring and Sejr, 2018).

2.6 Sampling and quantification methods

2.6.1 Standardization in an emerging field

Researchers from multiple disciplinary backgrounds have attempted to tackle the plastic pollution problem, blurring lines between human and physical geographies by incorporating analytical techniques applied in biology, geography, chemistry, geology, and environmental sciences. These multi-faceted approaches are possible due to the complicated array of physical interactions and anthropogenic consequences that plastics have in aquatic systems. For example, they can affect animal physiology, benthic sediment composition, environmental toxicology, and, from an anthropological perspective, even the degradation of tourist areas and human food systems (Tanaka et al., 2013; Waring et al., 2018; Franzellitti et al., 2019; Mendoza and Balcer, 2019; Pagter et al., 2020). Despite the gravity of this problem and long-standing concern from the scientific community, there remain prominent knowledge gaps surrounding the ultimate fates of these plastics, particularly in Arctic environments (Provencher et al., 2017). These gaps persist predominantly because the field of marine plastic pollution research is relatively young, maturing in its current form by the early 2000s (Wagner, 2014). In combination with the developing nature

of the field, plastics also exhibit several unique characteristics that make them challenging to study.

2.6.2 Sampling microplastics in animals and the environment

Standard protocols for sampling and quantifying microplastics are still at their outset, though there are currently some preferred techniques for gathering them depending on whether sampling occurs in aquatic media or animals (Razeghi et al., 2021). For surface waters, manta or neuston trawls (mantas) are commonly used to gather a representative sample of MPs in study areas and tend to be more accurate than some legacy techniques such as primary collection pumps (Pasquier et al., 2022). Manta nets can sample copious volumes of water by filtering debris from the first 15-25cm of the water column on long-distance trawls (Pasquier et al., 2022). While mantas are a valuable technique for gathering accurate MP samples in large volumes of water, some recent advancements have outperformed them in terms of affordability and abundance of MPs collected (Du et al., 2022). A trawl-underway pump combined with an *in-situ* filtration device and a stationary onboard pump coupled with a custom Y-shaped filter showed increased MP yield over standard manta trawl samples in the open ocean (Du et al., 2022). Both new techniques were able to collect notably more microplastic fibres ≥ 0.1 mm when compared to the manta trawl, which mainly yielded fragments (Du et al., 2022). The efficiency of these techniques is primarily due to the *in-situ* filtration systems, which are more sensitive to smaller items that may otherwise pass through a standard manta (Barrows et al., 2017). Ultimately, these techniques are not standard but push the science forward in identifying best practices and cost-efficient solutions for MP sampling in the open ocean.

For sub-surface and benthic MP sampling, there are fewer dependable options for collecting material (Balent et al., 2013). Due to the remoteness and inefficiencies of collecting small particles at benthic and pelagic depths, calculating MP transport and deposition estimates is difficult without modelling or intermediary samples (like animals and microorganisms) to infer baseline levels of pollution (Sfriso et al., 2020). Commonly, these samples are collected with benthic sediment grabs, which collect benthic deposits and organisms for further MP analysis (Razeghi et al., 2021). Both grabs and pumps tend to capture more MPs of a smaller size than surface trawls, but this comes at the expense of MP diversity, capturing mostly fibrous material (Hung et al., 2021). Ekman (3L – 12L) and Van Veen grabs (1.4L – 15L) help deploy in shallow waters and are therefore more applicable in freshwater environments (Pando et al., 2013; Aquatic Biotechnology, 2022). Very little is understood about benthic and sub-surface microplastic transport in the deep ocean. However, some techniques like grabs, pumps and cores may be applied to collect rudimentary samples under appropriate conditions (depth of grab, sedimentation profile, or suitability of the pumping system) (Pando et al., 2013).

In animals, microplastic sampling procedures are much more straightforward. However, some challenges exist when determining best practices for quality assurance and control (QA/QC), as well as which elements of an animal's physiology generate the most representative data for defining the overall burden of MPs in their system. Generally, most assessments of microplastic ingestion are completed via the necropsy of the gastrointestinal tract or stomach (Lusher et al., 2017). However, when internal assessments cannot be completed, either from coordination difficulties or to avoid invasive consequences for animals, scat (or guano, for birds) sampling can be used for collecting MPs (Nelms et al., 2019).

2.6.3 Quantifying retrieved microplastics

There are many ways to quantify the morphologies and abundance of debris once it has been recovered. It must be stated that high QA/QC standards should be met when performing microplastic-specific analyses since contamination from MPs in the air is inevitable in most circumstances (Hung et al., 2021). For this reason, sterilized equipment, thoroughly cleaned work surfaces, and procedural lab blanks are essential for ensuring productive and reliable data. In recent years, more standardized methods have slowly taken shape to quantify the morphologies and biotic abundance of MPs in aquatic environments and animals, which allows for more directly comparable results (Provencher et al., 2017; Mendoza and Balcer, 2019). The frequency of occurrence (FO) is a valuable unit for reporting the number of studied animals that contain ingested plastics and serves as a good reference point for comparing species (Provencher et al., 2019).

Visually identifying plastics is the most common technique for characterizing microplastics (Corradini et al., 2019). When quantifying microplastic items, qualitative and quantitative properties should be categorized in simple and descriptive groupings to limit ambiguous classifications. Provencher et al., (2017) have promoted some standard descriptors for recovered microplastic analysis, including functional types, sub-types, and colours (Provencher et al., 2017; Provencher et al., 2018). The research presented in this thesis adopts a similar set of qualifiers for recovered plastic and incorporates some morphological features like colour into more straightforward categories, with specific colours represented therein (Tables 3 and 4).

Table 3. Microplastic subtypes are used for categorizing retrieved debris in animal samples.

Subtype	Description
Fibre	User plastics (remains of ropes, nets, clothing, nylon line, packaging straps)
Bundle	Multiple fibres bundled together.
Fragment	Rigid plastic items used in a variety of products (bottles, boxes, toys, tools, equipment housing, toothbrush, lighters)
Film	Thin remains of plastic bags, wrappers, and films.
Foam	Remains of polystyrene cups or packaging or construction foams.
Microbeads	Tiny round beads that are typically used in personal hygiene and exfoliating products.
Other	Items that are 'plastic-like' or do not fit a clear category (rubber, elastics)

Table 4. Broad colour groupings for retrieved debris. Although colour proportions are reported using the broader category, specific colours were recorded for further analyses.

Colour Category	Black	Clear	White	Blue	Red	Green	Yellow	Brown
								
Included Colours	<i>Black, Grey</i>	<i>Clear</i>	<i>White</i>	<i>Light Blue, Blue, Dark Blue, Purple</i>	<i>Red, Pink</i>	<i>Light Green, Green, Dark Green</i>	<i>Yellow, Orange</i>	<i>Brown</i>

At the chemical level, there are two favoured techniques for identifying the polymeric structure of microplastic particles, Fourier Infrared Transform Spectroscopy (FTIR) and Raman Spectroscopy (Raman); other options like thermal analysis and differential scanning calorimetry (TGA and DSC) may be more prone to higher risks of false positives and negatives, or the partial or complete destruction of samples (Majewsky et al., 2016; Shim et al., 2017; Yu et al., 2019). FTIR is effective at identifying plastic polymers by capturing infrared spectra and is highly applicable for small-sized MPs ($\geq 10\text{-}20\ \mu\text{m}$) (Chen et al., 2020). FTIR is currently the most popular approach for identifying microplastics and can collect a spectrum from plastics in multiple modes of function (like refraction, reflection, and contact attenuated total reflection (ATR)) (Chen et al., 2020; Veerasingam et al., 2020). These spectra can be accurately compared to existing spectra in the FTIR database and matched based on statistical similarity. Particles below $10\text{-}20\ \mu\text{m}$ can instead be identified using micro-FTIR (μFTIR), though it requires higher

QA/QC practices since measurements can take extended amounts of time and the risk of sample loss or contamination is much higher (Löder et al., 2015; Veerasingam et al., 2020). It may be more appropriate to use Raman for these smaller items, as well as for nanoplastics (below 1 μm), since it can increase the 'signal noise' of a particle, allowing it to detect weak Raman spectra (light intensity versus frequency of scattered light) in the presence of background interference (Luo et al., 2022). This works by capturing measurements of a sample on a pixel by pixel basis and aggregating the resulting 'spectrum matrix' into an overall image, which can be processed using precise algorithms to delineate other signal noise (Frezzotti et al., 2012; Luo et al., 2022). A combination of visual identification, light microscopy, and chemical analysis will yield a detailed morphology for microplastics in any given sample. However, the results of each technique may not be directly comparable across studies that do not adhere to strict QA/QC or employ the wrong treatment to samples of an appropriate morphology.

2.6.4 Units and measurements: Quantifying the abundance of plastic in animals and the environment

As with any relatively new field, standardization across marine plastic research is yet to be fully realized. Although it is popular to employ well-established analytical techniques for sampling and plastic identification, like FTIR and Raman, few standardized procedures have emerged regarding reporting quantified values (Xu et al., 2019). For instance, separate studies may present the density of plastic in aquatic systems in different terms, usually as plastic per some volumetric unit (e.g., mg/m³, g/km³, mg/ml, g/L; Xu et al., 2019; Taipale et al., 2019; Mendoza and Balcer, 2019). Mendoza and Balcer (2019) discuss these discrepancies and explain that, in some cases, they may lead to the improper presentation of results within contexts relevant to specific research goals. They also state that manipulating reported units may produce more captivating or urgent headlines for practitioners, policymakers, and the media (for example, reporting values in cubic kilometres would appear vast and spatially important but would misrepresent the resolution of the data ascertained by the study) (Mendoza and Balcer, 2019).

For this reason, it would be best for practitioners to use standard units when reporting concentrations of plastics and associated chemicals. Typically, it is recommended that microplastic density in large water samples should be presented in particles *per* litre (L⁻¹), or *per* cubic metre (m⁻³) for smaller water samples, and *per* metre squared (m⁻²) if expressing data on an areal basis (Mendoza and Balcer, 2019). In addition, for the total plastic burden in a sampled population (number of affected animals in a sample), the result should be represented as the frequency of occurrence (FO). This approach is helpful for researchers seeking to present their findings accurately and for other researchers to interpret and compare them more easily.

2.7 The state of microplastic research in Canadian Arctic mammals

Mammals show some indication of microplastic ingestion, mainly via evidence from GIT necropsies and egestion; though, studies explicitly focused on this area are sparse in the peer-reviewed literature (Hernandez-Millian et al., 2019; Moore et al., 2020; Meaza et al., 2021; Pinzone et al., 2021; Carlsson et al., 2021). Cetaceans may be of particular interest for future research, given that many Arctic species, like bowhead whales (*Balaena mysticetus*), feed at both epipelagic and mesopelagic depths (Heide-Jørgensen et al., 2013). These multilateral feeding habits could imply that cetaceans in the Arctic ingest MP debris from multiple ocean layers, a quality already observed in beluga whales from the Beaufort Sea (Moore et al., 2020; Moore et al., 2022). In the literature review, this (Moore et al., (2020)) was one of the only two studies found regarding a direct assessment of microplastic litter in Canadian Arctic mammals, the other being an assessment of microplastic particles $\geq 425 \mu\text{m}$ in seals from Nunavut (Bourdages et al., 2020).

Moore et al., (2020) showed that beluga accumulated microplastics with an average of 97 ± 42 particles ($\geq 5\mu\text{m}$) in the feces and gastrointestinal tract (GITs) in their study that examined seven whales from the Inuvialuit Settlement Region (ISR). Based on their findings, they suggested that microplastics of this size are likely transient in the diet of beluga whales, meaning they pass through their GITs more readily than they accumulate within them; however, their study did show some evidence of retained microplastics in all seven of the studied whales (Moore et al., 2020). Contrastingly, Bourdages et al., (2020) found no evidence of plastic retention ($\geq 425\mu\text{m}$) in 142 seal stomachs harvested from four communities in Nunavut, Canada, but their detection limit was considerably larger than that of Moore et al., (2020). Beyond the discrepancy

between detection limits used for assessing microplastic retention, the samples used for analysis differed as well (scat and GIT versus stomach). The limited results of the existing literature render the overall profile of plastic pollution in the Canadian region relatively indistinct. Consequently, there is a lack of information needed to assess the potential risk for humans since mammals represent the most important sources of food to local communities (Moore et al., 2020; Bourdages et al., 2020).

2.8 Pinnipeds: *Odobenus rosmarus* and *Pusa hispida*

Pinnipeds are among the most critical mammals in Arctic communities. Globally, some evidence exists suggesting that *Phocidae* (true seals) are capable of retaining microplastics; however, the majority of these findings come from studies at sub-Arctic and southern latitudes and may not reflect equivalent exposure levels in the high Arctic and polar regions (hence the absence of plastic detected in Phocid pinnipeds by Bourdages et al., (2020) (Hernandez-Milian et al., 2019; Donohue et al., 2019; Lusher et al., 2022). Moreover, findings indicate that microplastic ingestion in pinnipeds tends to be concentrated in European waters (Rebolledo et al., 2013; Nelms et al., 2019; Wang et al., 2021; Desclos-Dukes et al., 2022). Seals are far more considered on a global scale, where at least 71% of species have been assessed for plastic ingestion in some form, while only a single study has commented on such interactions in walrus globally (Kühn and van Franeker, 2020; Carlsson et al., 2021)

2.8.1 Walrus: *Odobenus rosmarus*

Odobenus rosmarus is the only living species in the family Odobenidae and is endemic to the Arctic Ocean (Lyamin and Siegel, 2019). In the fossil record, odobenids are quite diverse, and during the Miocene and Pliocene, they inhabited southern latitudes beyond the high Arctic (VanBlaricom et al., 2001). However, the modern walrus is the only species to survive the late Pleistocene, when most other odobenids were lost to global-scale climate change and mass habitat loss (VanBlaricom et al., 2001). Today, walrus are a keystone species in Arctic marine ecosystems, migrating with moving ice floes, and are denoted as 'vulnerable' to climate change and habitat loss on the IUCN Red List (which is roughly equivalent to a 'threatened' designation by COSEWIC; Desforges et al., 2021) (WWF, 2022). Weighing between 400 and 1800kg, walrus can reach up to 3.6m in length once fully grown; there are an estimated 25 000 individuals in the Atlantic regions and 230 000 globally (Pacific, Atlantic, Laptev) (WWF, 2022).

In Canada, walrus are both dietarily and economically crucial to Nunavut communities, although in other countries (like Greenland), some populations have been over-exploited, prompting redesigned management strategies (Witting and Born, 2005). Well over 20 Canadian communities harvest walrus for subsistence and trade (Government of Canada, 2014). In addition, the ivory tusks of walrus are a valuable commodity that is often used for carvings or sculptures (Shadbolt et al., 2014). Beyond their profitability, walrus tusks are also efficient tools for animals. An average tusk can grow up to 76cm and is used to perforate breathing holes in the ice, defend against predators and prey, and help to haul the walrus out of the water (Stewart et al., 1993; WWF, 2022). Walrus are benthic feeders and eat bivalves primarily by sifting in shallow coastal sediment (Levermann et al., 2003). Since microplastics tend to accumulate either on the

surface or benthic layers of the water column, lower trophic level organisms accumulate higher amounts of plastic and simultaneously comprise the majority of *Odobenus rosmarus*'s diet (Sfriso et al., 2020; Walkinshaw et al., 2020; Bosker et al., 2022). As an important component of the Arctic benthic ecosystems, walrus may be particularly vulnerable to microplastic ingestion via secondary ingestion of contaminated bivalves (Sfriso et al., 2020; Walkinshaw et al., 2020; Bosker et al., 2022). Bivalves and other benthic invertebrates are well known to accumulate MPs even in remote polar environments like Antarctica, and Svalbard, Norway (Li et al., 2015; Covernton et al., 2019; Baechler et al., 2020; Sfriso et al., 2020; Teichert et al., 2021). Due to the high frequency of occurrence (FOs) of microplastics associated with bivalves, walrus (and humans) in the Canadian Arctic are likely to interact with marine debris, which is a genuine concern for Inuit communities that rely on healthy and safe harvests.

Previously, microplastic particles have been detected in walrus feces off the coast of Svalbard, Norway, in a study by Carlsson et al., (2021), who estimated that walrus scat might contain 34 particles per kilogram of feces. Although Carlsson et al., (2021) presented preliminary evidence of microplastic ingestion in walrus, their study region was limited to a single haul-out location (Poolepynten) in coastal Svalbard, Norway, and only a representative subsample of 100g of feces was used to determine the microplastic content of scat from 15 individual walrus. It is unlikely that this subsample would represent the overall abundance of ingested MPs in walrus, and it equally does not provide further insight into the internal content in the walrus' GITs or its diet (exposure). Furthermore, there have been no assessments of MPs in Canadian walrus, and no studies have evaluated it by examining the GIT.

2.8.2 Ringed seal: *Pusa hispida* and phocid pinnipeds

As the smallest Arctic phocid, ringed seals play a crucial role in food webs as important pelagic predators (National Oceanic and Atmospheric Administration (NOAA), 2022). Ringed seals are circumpolar in their range and spend most of their lives in ice-covered water, where sea ice and snow can act as a protective barrier to extreme temperatures (NOAA, 2022). Of the five ringed seal subspecies, *Pusa hispida* is the only one found in Canada (COSEWIC, 2019). As the primary source of food for polar bears, ringed seals are also essential to the diet of top marine predators and are traditionally harvested in Inuit communities for food and income (Harwood et al., 2012; COSEWIC, 2019). Average adults can live up to 30 years in the wild and weigh between 50 and 70kg (DFO, 2019). *Pusa hispida* is polyphagous and will consume a wide variety of small prey like shrimp (*Pandalus borealis*), Arctic cod (*Boreogadus saida*), small crustaceans, herring (*Clupea harengus*), and perch (*Perca flavescens*) (DFO, 2019).

Within Canada, ringed seals are broadly distributed in the Arctic and sub-Arctic waters, from the western Yukon and the Beaufort Sea, throughout the Arctic Archipelago and down to the Davis Strait and the northern Labrador Sea (COSEWIC, 2019). Species abundance is estimated to be high in Canada at 2 300 000 individuals (including adjacent regions with population overlap, like Alaska and Russia), but has not been as thoroughly assessed in western regions and was designated as 'Special Concern' by COSEWIC in 2019 (COSEWIC, 2019). Since ringed seals rely on sea ice as a platform for rest and breeding, their susceptibility to harmful consequences of climate change and the melting of polar ice is high (Reimer et al., 2019). Further, ringed seals are a suitable indicator species for detecting changes in Arctic marine ecosystems and can be used to assess relative system productivity and changes in population

trends or sea ice (Reimer et al., 2019; Ogloff et al., 2021). Where anthropogenic threats to the Arctic environment like plastic pollution are concerned, it is becoming increasingly important to identify indicator species that may be able to represent the overall health of marine ecosystems (Bergmann et al., 2022). Even though ringed seals have shown potential for representing indicators of ecosystem change, they have not been thoroughly assessed for microplastic ingestion.

Globally, other phocid pinnipeds have been the focus of several studies examining plastic pollution found in the peer-reviewed literature, most reporting it at more southern latitudes and primarily on entanglement and macroplastic ingestion (Derraik, 2002; Butterworth et al., 2016). For example, Boland and Donohue (2003) reported entanglement in 58% of all seal and sea lion species. They also suggested that entanglement by marine debris played a prominent role in the endangered Hawaiian monk seal' (*Monachus schauinslandi*) lack of population recovery. Lawson et al., (2015) removed 138 entanglement items from fur seals in southern Australia, and even near Antarctica, Waluda and Staniland (2013) reported low entanglement rates (1033 observed between 1999 and 2013) for fur seals from Bird Island.

There have been fewer studies focusing specifically on microplastic ingestion in phocid pinnipeds, though it has been reported. For example, Rebolledo et al., (2013) found that 12.2% of 107 seals had ingested microplastic near the Netherlands. Similarly, grey seals have also been shown to ingest microplastics in the North Sea, where 71 particles were identified from 66 fecal samples (Desclos-Dukes et al., 2022). In the sub-Arctic (latitudinal regions within 50°N and 70°N), northern fur seals (*Callorhinus Ursinus*) are potentially exposed to microplastics based on results from Donohue et al., (2019), where 55% of 44 scat samples contained either microplastic

fibres or fragments. Given the apparent susceptibility to MP ingestion in pinnipeds, they are presumably capable of ingesting plastic in southern latitudes and transporting it to the Arctic or ingesting it in Arctic latitudes if it is already present in their environment. Considering that some phocid species, like the harp seal, share ecological ranges with ringed seals but migrate from the Arctic to the Gulf of Saint Lawrence (49°N) and the coastal waters of Newfoundland and Labrador, Canada, in the fall, ringed seals may be at risk of ingesting debris in the water column or contaminated prey (Government of Canada, 2022). There is only a single study that has analyzed it in Canadian seals, where Bourdages et al., (2020) found no evidence of plastic retention of particles $\geq 425\mu\text{m}$ in the stomachs of 142 seals (135 ringed seals) harvested in Nunavut. However, they did not evaluate items more diminutive than that (Bourdages et al., 2020). No assessments have been made for phocid pinnipeds in the western Canadian Arctic.

3.0 Research Objectives

Based on the literature review, this thesis presents the first assessment of accumulated microplastic ingestion in Canadian walrus by evaluating the contents of 36 hunter-harvested stomachs from five communities in Nunavut, Canada, at a lower detection limit (80 μ m) than previous studies on Arctic Pinnipeds. Globally, this is the first study that directly addresses retained microplastics in walrus by examining internal gut contents. It is also the first analysis for microplastic ingestion in phocid pinnipeds in the western Canadian Arctic at the same detection limit (10 seals from two Northwest Territories communities). Additionally, this thesis aims to compare the evaluation of microplastic ingestion in pinnipeds to that of more thoroughly studied groups, namely seabirds, within a global context. In doing so, more information regarding the suitability of pinnipeds as indicator species for trends in global microplastic pollution can be evaluated. This thesis provides necessary context for microplastic pollution in Canadian Arctic marine mammals and prompts a more robust understanding of anthropogenic debris in *Odobenidae* and *Phocidae* in Arctic geographies. Due to the chosen detection limit (80 μ m), I hypothesize that smaller microplastics will be detected in the GITs of walrus and ringed seals, in contrast to the absence of debris recorded by Bourdages et al., (2020) at 425 μ m. The observation of microplastics of this size would be consistent with other mammals, as reported in Moore et al., (2020). It would simultaneously expand on the findings of Carlsson et al., (2021) by demonstrating that microplastics are transient in mammalian GITs but are retained within the animals at specific sizes.

4.0 Methods

4.1 Sample collection and QA/QC

During the annual walrus hunts in Iqaluit (n = 2), Coral Harbour (Salliq; n = 2), Naujaat (n = 2), Sanirajak (n = 17) and Pangnirtung (n = 4) (9 = NA), Nunavut, hunters collected the stomachs of 36 walrus specifically for examination of accumulated microplastics with sampling kits provided by the Canadian Department of Fisheries and Oceans (Figure 3). Sample sizes were based on local efforts in each community. The walrus were shot by hunters and tissues were removed in the field as part of a more extensive DFO research program focused on walrus populations and diet. Similarly, hunters in two communities in the Northwest Territories, Canada (Paulatuk; n=2, and Sachs Harbour (Ikaahuk); n=8) harvested ten ringed seal stomachs (Figure 4). In collaboration with Wildlife Conservation Society Canada (WCS), the Paulatuk and Sachs Harbour Hunters and Trappers' Committees (HTCs) collected the seals as part of a diet and condition study (Insley et al., 2021). The walrus and the seal stomachs were shipped frozen to the Aquatic Ecosystems and Environmental Change Lab (AEEC) at Carleton University for microplastic analysis.

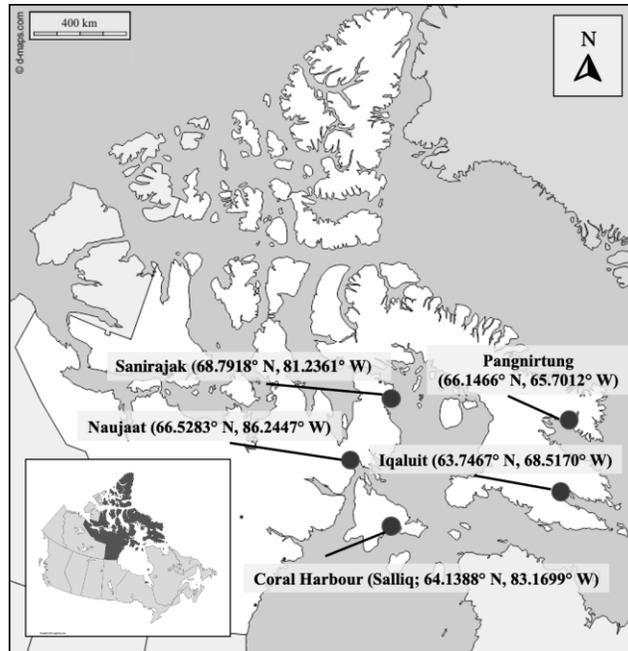


Figure 3. Map of Nunavut, Canada, where 36 walrus stomachs were harvested across five communities: Sanirajak (n = 17), Naujaat (n = 2), Pangnirtung (n = 4), Coral Harbour (Salliq; n = 2) and Iqaluit (n = 2) (9 = NA).

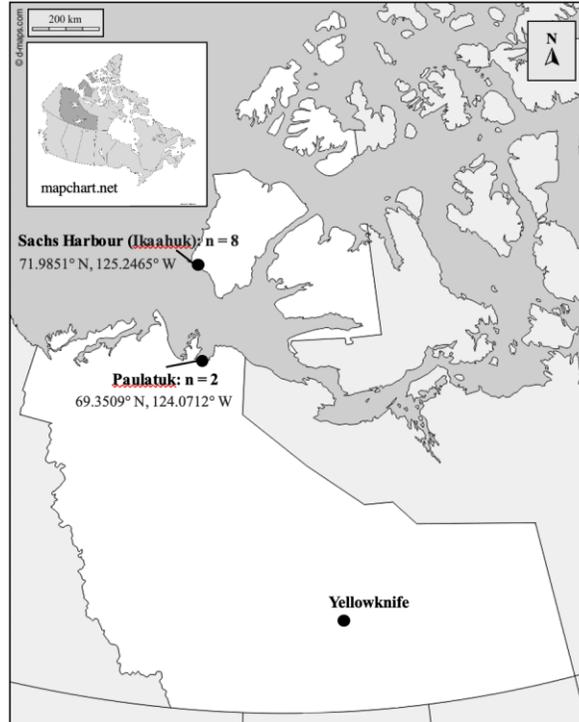


Figure 4. Map of the Northwest Territories, Canada, where ten ringed seal stomachs were harvested in two different communities: Paulatuk (n = 2) and Sachs Harbour (Ikaahuk) (n = 8)

Using the methods suggested by Provencher et al., (2017), which were also incorporated in the works of Moore et al., (2020) and Bourdages et al., (2020), all 46 stomachs were processed in the 2021 calendar year. The stomachs were kept frozen until dissection, at which point they were thawed at room temperature over 48h, then rinsed with deionized water (DI) that had been filtered through 80µm nylon membrane filters to ensure any external contamination had been removed. All the stomachs were dissected under a laminar flow hood (Design Filtration Microzone, model: CAW4-V) with sterilized and triple rinsed (DI) equipment. Before dissection, two identical beakers ($\leq 4000\text{mL}$ for walrus, $\leq 1000\text{mL}$ for seals; beaker size dependent on the stomach) were sterilized and rinsed in preparation for each sample. One beaker received the contents of the stomach, while the other served as a procedural lab blank exposed to identical conditions. Both beakers were tightly covered with triple rinsed (DI) aluminum foil before and after the dissection. The walrus (trial 1) and the seals (trial 2) were completed separately, but with identical methods.

4.2 Dissection and microplastic collection by funnel filtering

Beginning the dissection, an incision in the stomach was made with sterilized (DI) scissors, and liquid contents were guided into the sample beaker by squeezing. Once empty, the stomach was cut into smaller portions (roughly 20cm long), exposing the mucosa, and smaller items adhering to the inner membranes were scraped into the beaker with a clean, stainless-steel spatula. To remove and digest internal stomach contents, filtered (10µm fibreglass filter) 10% Potassium Hydroxide (KOH) was used to rinse each stomach's mucosa and then to fill each beaker to a 3:1 ratio of KOH to stomach content following the dissection. This method was chosen because KOH is effective for externally digesting organic materials while having little

effect on plastic polymers (Foekema et al., 2013; Kühn et al., 2017; Bourdages et al., 2020). The beaker acting as the procedural blank received an equal amount of KOH to ensure identical conditions under the flow hood. The sample and procedural blanks were tightly recovered with the aluminum foil and left to digest under a fume hood. After two weeks, each sample and blank were funnel-filtered (with 80 μ m nylon filters) under a fume hood to recover any microplastics and placed into clean Petri dishes. Large items like rocks were rinsed onto the filter and stored in clean petri dishes for further examination.

4.3 Visual microplastic identification

After the filters had dried, each one was individually inspected with a Leica Microsystems light microscope. First, filters were examined in a horizontal pattern, from top to bottom, to ensure that the entire surface had been observed. Then, using a pair of clean forceps, visible plastics were picked from the filter and placed onto a piece of clear double-sided tape, secured to a microscope slide, and placed inside a new petri dish. In the case of dried, thick, or solid content on the filter, forceps and small amounts (1-2 mL) of triple filtered DI water were used to gently break up the material so it could be sifted through to check for microplastics. Each plastic item on the double-sided tape was circled with a fine-tip permanent marker, and particle morphologies were recorded. Representative subsamples of all recovered particles (walrus: n = 46, 10% of the total; seals: n = 8, 25% of the total) were selected, and particles were transferred to new, clean microscope slide using a thin layer of liquid SkinTac, brushed on sparingly with a sponge applicator. The particles were again circled with a permanent marker after the adhesive had dried and stored in clean Petri dishes for further use.

4.4 Chemical identification using Fourier Infrared Transform Microscopy

Micro-ATR-FTIR analysis was completed using a ThermoFisher Scientific Nicolet iN10 Infrared Microscope (FTIR microscope) at Carleton University on each of the 54 particles. Spectral readings for each microplastic were recorded and matched to existing polymeric spectra using native databases (Hummel Polymer Library) provided in the 'OMNIC Picta' software. Before loading a slide containing microplastic subsamples, the stage was lowered on the FTIR microscope and wiped gently using a Kimwipe to remove any debris. Next, the glass slide was gently placed into the slot on the stage, with an additional glass slide directly underneath it to raise the sample to an appropriate height for the ATR-FTIR equipment.

After repositioning the stage, microplastics were located using the built-in focal depth and orientation controls in OMNIC Picta. Next, the focal point was readjusted until a microplastic particle was visible on the screen. Once the crosshairs of the microscope's camera were directly over a particle, the stage was lowered slightly, and the germanium-tipped ATR crystal attachment was carefully inserted into the FTIR aperture. Standard contact pressure was selected in the ATR dialogue box, and spectral readings for each particle were recorded. After a spectrum was recorded, it was compared to the Hummel Polymer Library entries and matched based on similarity. A threshold of $\geq 60\%$ for matches in the Hummel Polymer Library was applied to confirm the identity of material with relative certainty.

4.5 Data analyses

4.5.1 One-tailed *t*-test for statistical significance and power analysis

A one-tailed *t*-test determined if the difference between the means (\bar{x}) of the microplastics obtained from the procedural blanks (*Pb*) and the samples (*S*) was more significant at $p \geq 0.05$. Assuming that microplastics will be detected at this small detection limit means that the *t*-test should be able to accept or reject the null hypothesis (H_0) that the procedural blanks would contain an equal or higher proportion of MPs ($H_a: \bar{x}(S) > \bar{x}(Pb)$; $H_0: \bar{x}(S) \leq \bar{x}(Pb)$). Given the MPs observed across the 36 walrus and ten seal samples and blanks, power analyses completed in R-studio (version 1.2.1335; using the '*pwr*' package: version 1.3-0) calculated the sample size and number of MPs required to detect a statistically significant signal in the animals based on the result of the *t*-tests. The effect size (Cohen's *d*) demonstrated the magnitude of difference between the sample and *Pb* groups, indicating the overall strength of the relationship.

4.5.2 Principal Components Analysis (PCA) for colour distribution

The colour of each microplastic particle was recorded upon retrieval, and for the walrus trial, a principal components analysis (PCA) was completed (in R-studio version 1.2.1335, using the '*vegan*' package, version 2.6-2) to reduce the dimensionality of the data, allowing identification of any trends or relationships when observing particles of a particular colour in either walrus samples (S_w) or blanks (Pb_w). Before running the PCA, a Hellinger transformation was employed to account for redundancies or false correlations since Euclidean distance measures (the length of a line segment between two datum points) have difficulty with higher dimensions. In addition, the transformation allowed for assessing relative abundances of colours

rather than absolute ones. The data was subsequently down-weighted (< 1.0) to reduce the variability of more dominant colours.

4.5.3 Comparing plastic ingestion studies among seabirds and pinnipeds

Marine mammals are the group most harvested by Arctic communities, however, the bulk of the literature on plastic ingestion in Arctic fauna is focused on seabirds, who have multi-lateral foraging habits over wide-ranging habitats (Provencher et al., 2017; Mallory et al., 2010; Jardine et al., 2021; Hamilton et al., 2021). As culturally and dietarily important animals, it is inopportune that Arctic marine mammals have not been as thoroughly assessed concerning interactions with plastic and microplastic debris. Some marine mammals would presumptively make good indicators of microplastic concentrations in marine food webs, given that they obtain most MPs through secondary ingestion that could eventually work its way into humans.

Arctic seabird assessments severely outnumber those for mammals, representing the most ingested debris of any group (Baak et al., 2020; Collard and Ask., 2021). A brief review of existing literature was completed to illustrate the disparity between research conducted on microplastic ingestion in pinnipeds and seabirds. Given the disproportionate representation that seabirds already have in the literature, Procellariiformes (fulmars, shearwaters, petrels, albatross, and prions) were selected for direct comparison to pinnipeds since they are the dominant order of seabirds in this context and are established bioindicators for MPs in the Arctic. The literature review was conducted using academic search engines (ScienceDirect, Elsevier) with the following search terms: Pinnipeds, Seal, Sea Lion, Walrus, Seabirds, Plastic, Microplastic,

Procellariiformes, Fulmar, Petrel, Shearwater, Albatross, and Prion. Studies that fit these criteria were reviewed and classified based on the latitude at which the study occurred.

The earth's latitudes were roughly grouped into eight 'regions of latitude' to reflect global areas of study. The regions of latitude identified in this review are: Arctic (70°N - 90°N), Sub-Arctic (50°N - 70°N), North Temperate (25°N - 50°N), North Torrid (0°N - 25°N), South Torrid (0°S - 25°S), South Temperate (25°S - 50°S), Sub-Antarctic (50°S - 70°S), and Antarctic (70°S - 90°S). It should be noted that the cut-off for some latitudinal zones, like 50°N at the sub-Arctic, overlaps with conventional climatic zones (sub-Arctic conditions are not unilateral at 50°N). This is because the species in which ingestion studies have historically been conducted would likely fit more appropriately within only one of these regions.

A few existing reviews have aggregated microplastic ingestion studies in both seabirds and marine mammals, like Kühn and van Franeker (2020) and Collard and Ask (2021). These publications help collect some baseline data; however, there are a few shortcomings that these recent reviews (and similar studies) have that are specific to microplastic ingestion. Namely, the studies included in these reviews are often exclusively those that have been declared to have assessed microplastics of a particular size. One problem with this is that microplastic ingestion studies have only recently begun to sprout up in the literature as a mainstay of plastic pollution research. Older studies, in comparison, have evaluated plastic ingestion 'broadly' but report particles that would fall within the microplastic range (1µm to 5mm) as well as bigger debris.

For this reason, using the studies retrieved by the literature search, publications that do not explicitly report microplastics but that have discernable incidences of microplastic-sized

debris are still included in this review. Not all studies report the same metrics. For example, they were omitted if microplastic sizes were recorded but not described proportionately concerning their frequency of occurrence (FO) in animals compared to larger items. Moreover, studies that evaluate plastic ingestion in multiple species are recorded as the same number of assessments or MP ingestion incidences. For example, if a study assessed MP ingestion in three species, this review would indicate that three assessments had been conducted, one for each species. Duplicate assessments for species by the same or different authors are considered mutually exclusive.

As MP ingestion studies are still finding their footing, there are discrepancies in sampling techniques and reporting. Studies that have evaluated either scat or GIT samples are included in the review; however, the frequency of occurrence is often not reported for publications that use scat as their model vector. Although each scat study for a species counts as an assessment, no corresponding FO was often associated with it. As such, the mean FOs for each latitudinal region may be skewed for studies that report FO or that have been conducted using GIT samples. If a FO was not directly reported but was calculable based on the data therein, it was tabulated and included in this review.

The graph produced by this review visually compares seabirds to pinnipeds in terms of both ingested plastics and the number of studies at various latitudes. Comparing these two groups provides further insight as to whether pinnipeds make suitable indicator species for plastic ingestion in Arctic regions. Subsequently, it may highlight other more applicable roles they could fit within environmental management plans.

5.0 Results

5.1 Visual identification of microplastics

5.1.1 Walrus

The 36 walrus (11 females, 11 males, and 14 unknown) were shot by hunters across the five communities in Nunavut, Canada, between 2019 and 2020. The mean weight of the stomach contents was 334.69g, with a minimum weight of 47.84g and a maximum of 1363.33g. Most of the stomachs, upon visual inspection, contained only gastric juices and blood (n = 29). Some stomachs did, however, contain prey items like bivalves and some partially digested organic material (n = 5). Other contents included pieces of shell (n = 1), a small fly (n = 1), benthic sediment, sand, and rocks (n = 7) (Figure 5 A, B). Visible anthropogenic items were absent inside the stomachs, and the mucosa and inner membranes showed no evidence of previous ingestion or lesions from other materials. MPs were recovered from the filters in the samples (S_w) and the blanks (Pb_w) and were exclusively fibres (between 500 μ m and 5mm in length, total = 445MPs). 191 microfibre particles were recovered from the sample filtrate (min = 0, max = 27, sd = 6.93). In the procedural blanks, 254 microfibrils were recovered from the filters (min = 0, max = 31, sd = 9.30). The sd_{pooled} for both groups was 8.35. Nine walrus filters and seven blank filters had 0 MPs.

5.1.2 Ringed seals

The ten seals (9 males and one unsexed) were harvested during the spring (n = 2), summer (n = 6), and fall (n = 2) between 2016 and 2019. The weight of stomach contents ranged from 53.7g to 374g (mean = 141.27g). The contents of the stomachs were again primarily

composed of gastric juices and blood ($n = 10$), while occasionally, small rocks ($\leq 1 \text{ cm}^3$; $n = 3$), otoliths (likely Arctic cod, $n = 1$), and a parasitic roundworm ($n = 1$) were found (Figure 5 C, D). Like the walrus, no visible anthropogenic items were found inside the stomachs upon dissection, and there was no evidence of internal injuries. Following filtration and inspection with the stereomicroscope, 13 microplastic fibers were recovered from the samples (S_{rs}) (mean = 1.3, min = 0, max = 5, sd = 1.63), and 19 from the blanks (Pb_{rs}) ($n = 10$) (mean = 1.9, min = 0, max = 7, sd = 2.02). All 32 recovered microplastics were fibrous (between 1mm and 4mm in length) and were mainly black (34.4%), blue (21.9%), grey (18.8%), and clear (15.6%) in colour.

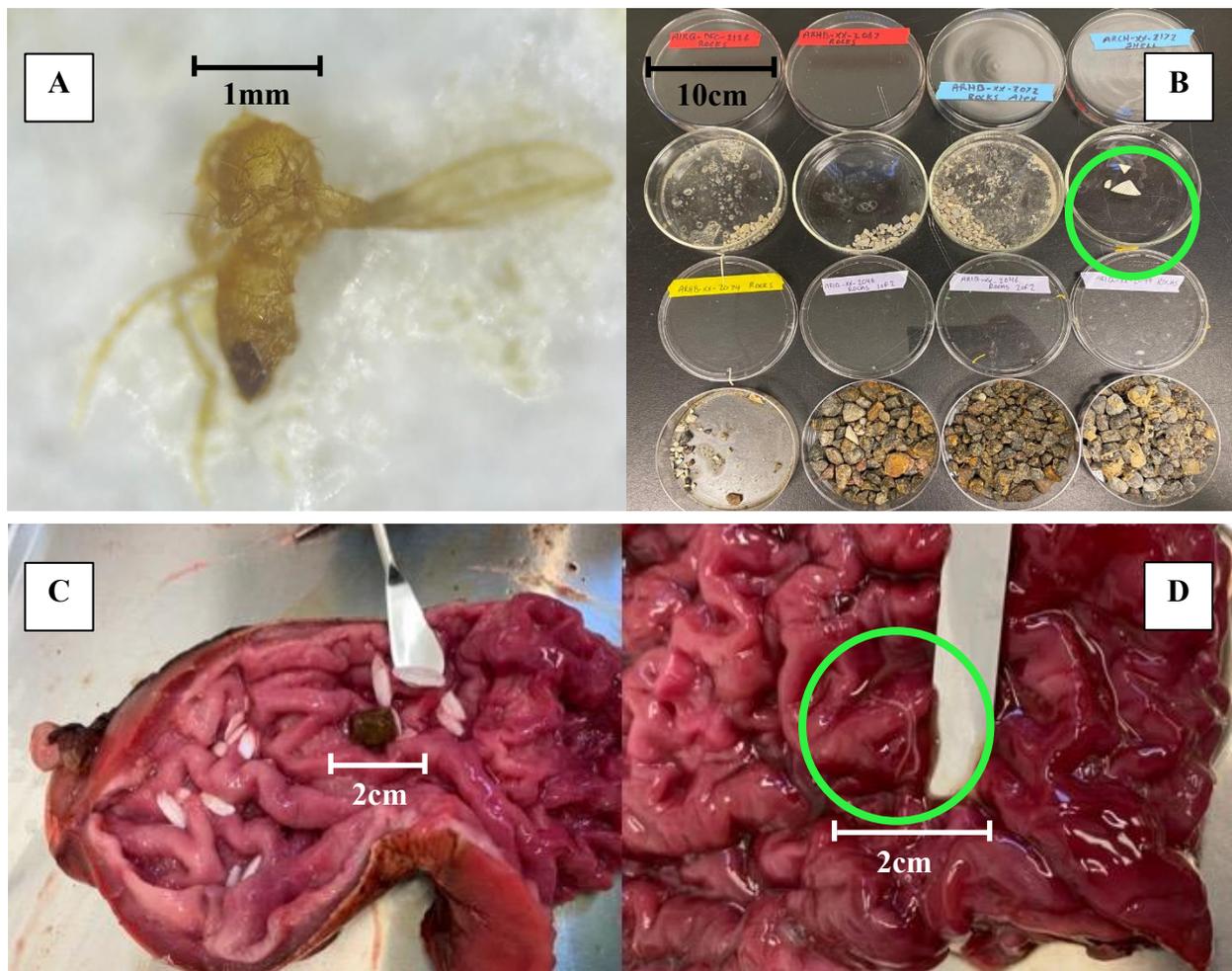


Figure 5. Items retrieved from walrus (A, B) and seal (C, D) stomachs. Green circles identify pieces of shell (B) and a parasitic roundworm (D).

5.2 One-sided t-tests and power analyses

5.2.1 Walrus

There was no difference between the means of the MPs found in the S_w versus the Pb_w filters. The one-sided t -test (significant at $p \leq 0.05$) was 0.184, meaning that the plastic particles were unlikely to have originated from the walrus since the procedural blanks contained nearly identical amounts of MPs. Furthermore, the observed power of the data was 0.49, and the effect size (*Cohen's d*, or ' d ') was 0.21. Given this result, the relationship between the mean number of MPs from the S_w filters and the Pb_w filters was inconsequential since the effect size was markedly small, meaning the standard deviations that separate the means were incidental. Given the number of microplastics observed, a power analysis was used to estimate a hypothetical effect size and sample size to determine what variables would have yielded a significant result.

With the observed d value (0.21), an appropriate sample size for detecting a statistically significant MP signal at a $p \leq 0.05$ and a power of ≥ 0.80 would be 281 walrus stomachs (Figure 6). Conversely, at the actual sample size for this study ($n = 36$), the required d value for a statistically significant result would have been 0.6 (Figure 6). For these findings to have conclusively demonstrated that walrus in the Canadian Arctic were exposed to significant amounts of MPs, the sample size would have had to be large enough to represent consistently high amounts of retained MPs in comparison to the ambient MP contamination from the lab, which was represented by the procedural blanks. Since the required d value calculated for a significant MP signal at $n = 36$ was higher than the observed d of 0.21, the relevance of the number of MPs we detected and the sample size for this study is inappreciable. Furthermore, the mean number of microplastics required to detect a significant signal at this effect size is 12 MPs

(compared to the mean of 4MPs retrieved during the analysis). Walrus, therefore, do not appear to be retaining or potentially not even encountering microplastic particles in the study region.

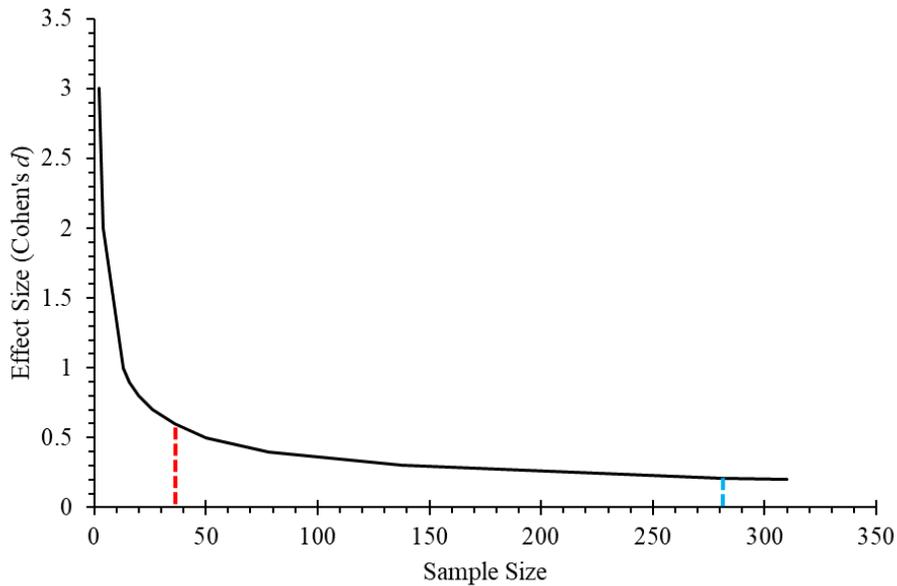


Figure 6. Given the observed effect size (0.21), the required sample size for statistical significance would have needed to be 281 (blue delimited), while at the actual sample size ($n = 36$), the required effect size would have needed to be 0.6 (red delimited).

5.2.2 Ringed seals

A one-sided *t*-test ($p = 0.24$, significant at $p \leq 0.05$) determined that the means were not significantly different between the samples from the seals and the procedural blanks. Therefore, the 13 microplastics likely had not originated from the stomachs. The power analysis calculated the power of the observed data as 0.55 and the effect size (Cohen's *d*) as 0.37 based on the significance level (*p*-value) obtained (0.24). The number of standard deviations separating the two means is again trivial at such a small effect size. Therefore, the relationship between the microplastics found in the S_{rs} versus the Pb_{rs} is inconsequential regardless of statistical significance. Given the effect size of 0.37, the sample size required to detect a statistically

significant difference between microplastic concentration in seals versus procedural blanks (with a $p < 0.05$ and power: > 0.8) is 91 stomachs (Figure 7). This result indicates that considerably larger sample size was required to detect a clear microplastic signal beyond the potential for contamination that can occur throughout the analysis (which the procedural blanks reflect). Additionally, at the actual sample size ($n = 10$) of this study, the required effect size for statistical significance (≤ 0.05) would be 1.16, a disproportionately large result that would require a larger number of recovered microplastics in the seals versus the procedural blanks (mean = 4MPs).

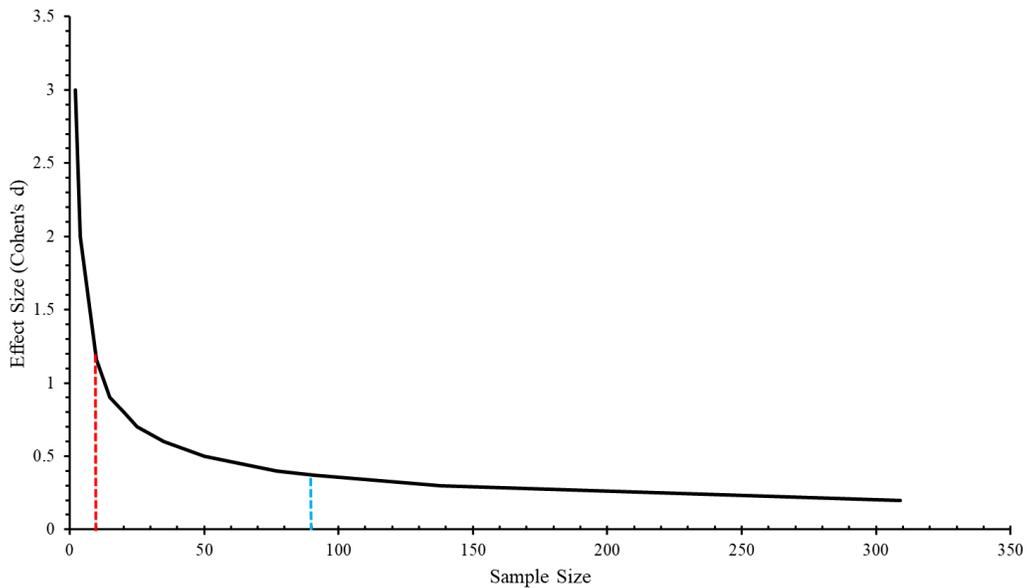


Figure 7. As the sample size increases, the required effect size for statistical significance decreases; the observed effect size (0.37) requires a sample size of 91 to be significant (blue delimited), while the actual sample size (10) requires an effect size of 1.16 (red delimited).

5.3 Colour distribution and Principal Components Analysis

5.3.1 Walrus

A collective 445 microfibrils were recovered from the walrus samples and blanks. The dominant colours were blue (32%), black (27%), clear (16%), and grey (10%) (Figure 9). The remaining 12 colours (white, light blue, dark blue, red, pink, purple, light green, green, dark green, yellow, orange, and brown) were all relatively uncommon ($\leq 5\%$). A PCA reduced the dimensionality of this data to just 13 colour variables. Dark green, orange and brown had 0 observed pieces in the walrus and the blanks. Principal components (PC1 and PC2) were selected based on the highest eigenvalues (PC1 = 0.1529, PC2 = 0.0973), although all principal components for the 13 colour variables were notably small, with the smallest being PC13 ($6.832e^{-6}$) (Appendix B). After ordinating the sample points ('site scores' in '.vegan') in a biplot and interpreting the absolute values of the coefficients calculated from the colour variables ('species scores,' in '.vegan'), no correlations were detected that would indicate meaningful relationships between colours (Figure 8, Appendix C). PC1 was most influenced by black (-0.807577) and blue (1.020069), while PC2 was predominantly influenced by clear (0.7921094), with minimal impact corresponding to the other colours (Appendix C). Although black, clear, and blue MPs comprised most of all microplastic colours (75% collectively), the prevalence of other colours was not dependent on their abundance. Similar colours (i.e., light green, green, dark green) were then grouped into more straightforward categories (i.e., green) to represent the overall distribution of colours across both the samples and the blanks, as described by the methods in Table 4 (Figure 9).

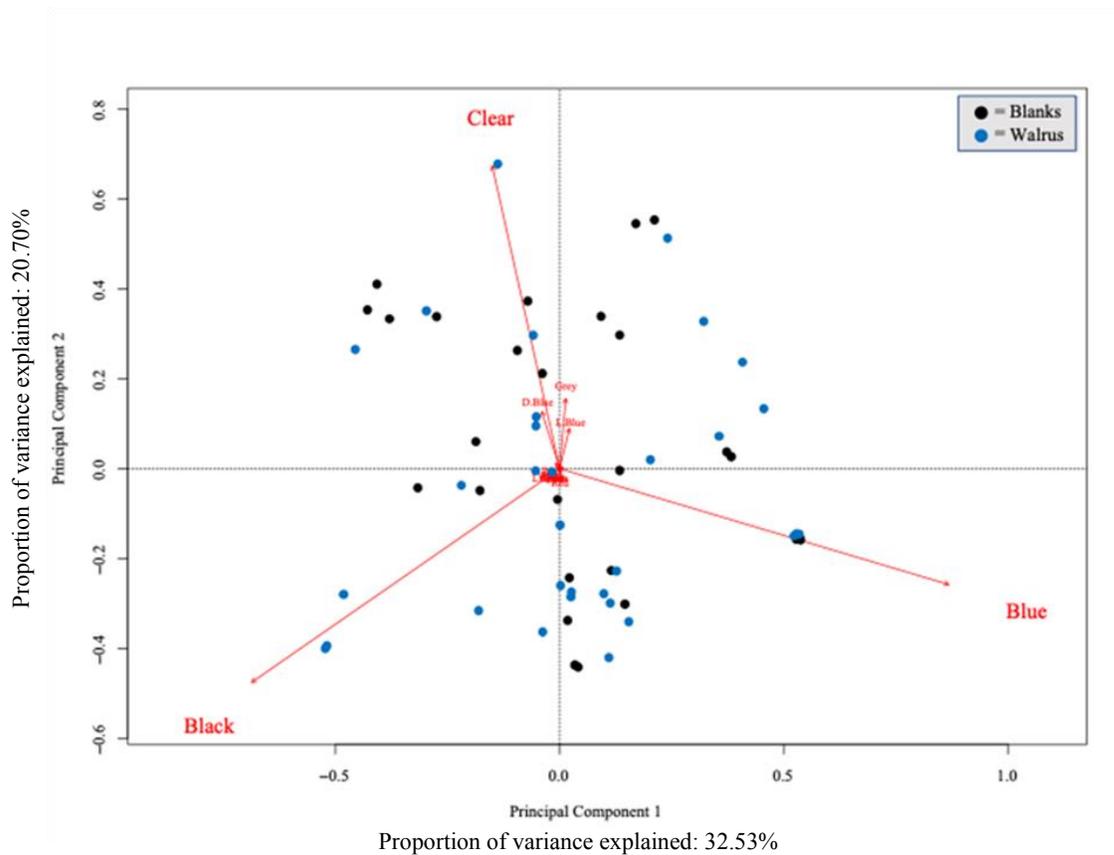


Figure 8. Ordination biplot of principal components (PC1 = 32.53% variance, PC2 = 20.70% variance) generated from the MP colours observed in the walrus (blue points) and the blanks (black points). Data points are not clustered and do not exhibit any noticeable trends.

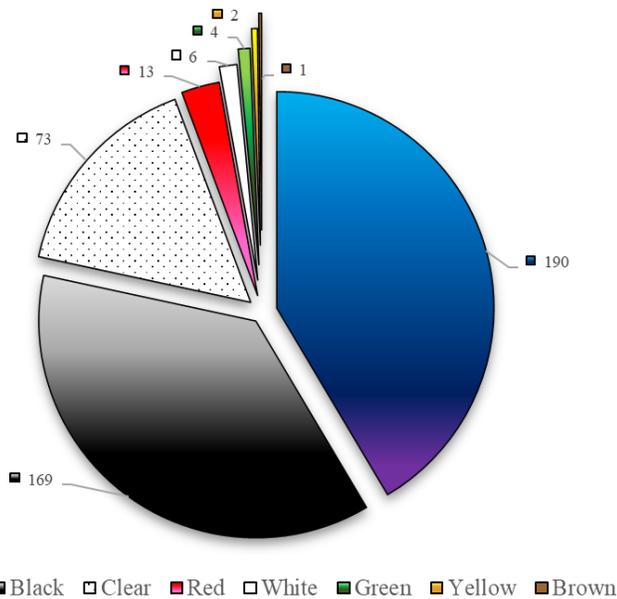


Figure 9. Colour distribution of MPs recovered in the walrus S_w and Pb_w , where similar colours have been grouped into more straightforward categories (as described in Table 4) to represent the proportions more generally.

5.3.2 Ringed seals

All 32 recovered microplastics were fibrous (between 1mm and 4mm in length) and were mainly black (53.2%), blue (21.9%), and clear (15.6%) in colour (Figure 10). A PCA was not completed for the seal trial for a few reasons. First, there were very few MPs recovered in comparison to the walrus, which may have resulted in false correlations or false negatives. Second, only three MPs were not black, clear, or blue (two red, one green), limiting the data's dimensionality significantly. Since PCAs are most helpful in interpreting data at higher dimensions, this approach is not fitting for the data. Finally, the seal MPs were not instead added to the PCA generated for the walrus since they were harvested in different locations, at different times, and by different participants. Additionally, they were processed entirely separately from the walrus stomachs at different times of the year. Therefore, although identical procedures and

equipment were applied to both trials, it is impossible to guarantee that the ambient concentrations and types of airborne contamination were also the same. For this reason, grouping similar colours into more straightforward categories helps represent the overall distribution of colours in the retrieved MPs, which can be compared to the distribution observed for the walrus in Figure 9.

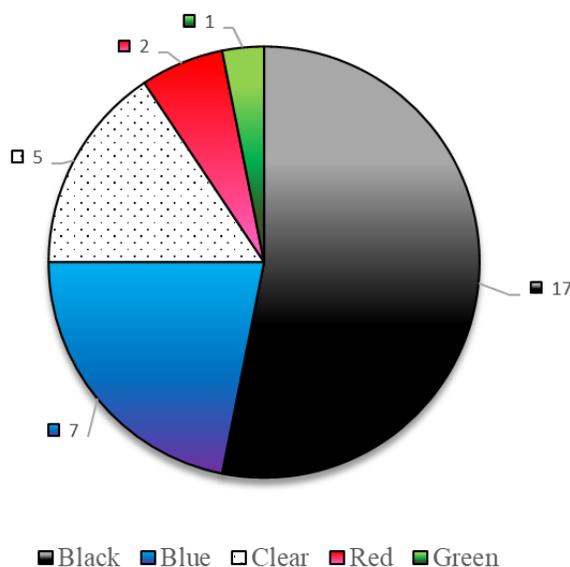


Figure 10. Colour distribution of MPs recovered in the seal S_{rs} and Pb_{rs} where more straightforward categories (Table 4) have been used to represent the proportions more generally.

5.4 Chemical identification of microplastics

5.4.1 Walrus

Micro-ATR Fourier Infrared Spectroscopy was completed on 46 microplastic particles from both groups. A threshold of $\geq 60\%$ for matches in the Hummel Polymer Library was applied to confirm the identity of material with relative certainty. The morphologies of MPs were consistent between groups (Figure 11). At the same time, spectral results were varied where more naturally occurring materials like cellulose, acetic acid, as well as bentonite (clay) and various hydrocarbons were detected (but loosely matched: 45% - 59%) in the walrus subsamples (n =

13). Some synthetic polymers ($n = 9$) were successfully identified at $\geq 60\%$, in both the Pb_w ($n = 4$), and S_w ($n = 5$). For successfully identified MPs, synthetic polymer IDs were consistent between both groups, where Polyethylene Terephthalate (PET) ($n = 3$) and Poly (ethyl acrylate) ($n = 6$) yielded strong matches (Figure 12). In the blanks, some other synthetic materials were weakly detected (39% - 57%) (Poly (N-methyl acrylamide), $n = 2$, Cellophane, $n = 1$, Chipboard, $n = 1$, PET, $n = 1$), but could not conclusively be identified since they fell below the criteria of a $\geq 60\%$ match. Wood Rosin was also detected at $\geq 60\%$ (8 blanks, two walrus) and is the primary ingredient in SkinTac. The detection of Wood Rosin is likely due to the micro-ATR apparatus contacting the microplastic particle when performing a reading and recording the transmittance spectra based on the first layer the instrument touches. Repeated attempts to identify materials coated in the Wood Rosin were largely unsuccessful due to the size and fragility of fibrous particles.

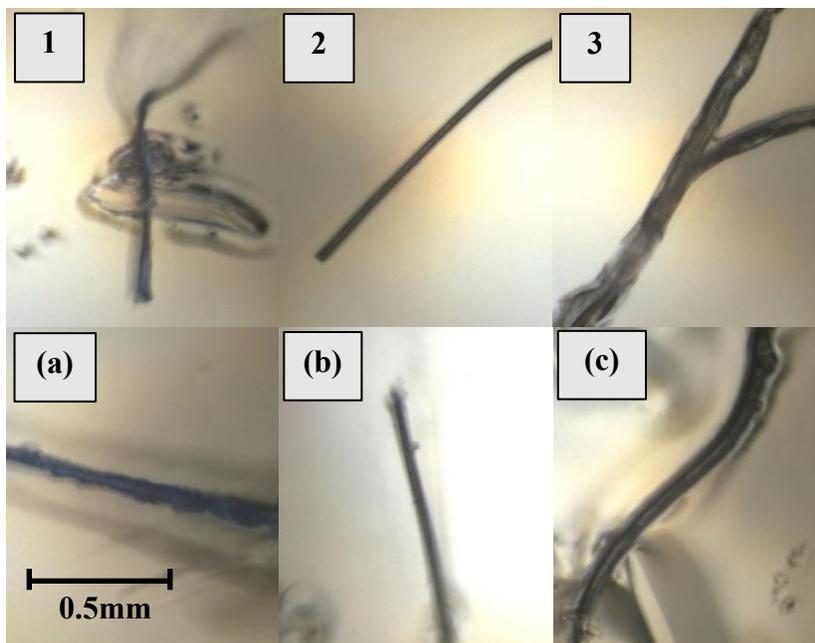


Figure 11. Examples of microfibrils recovered from the blanks (1,2,3) and walrus (a,b,c) filters. Morphologies were consistent between both groups. The size, shape, and colours were compared [1, (a); 2, (b); 3, (c)] and putatively attributed to ambient airborne contaminants.

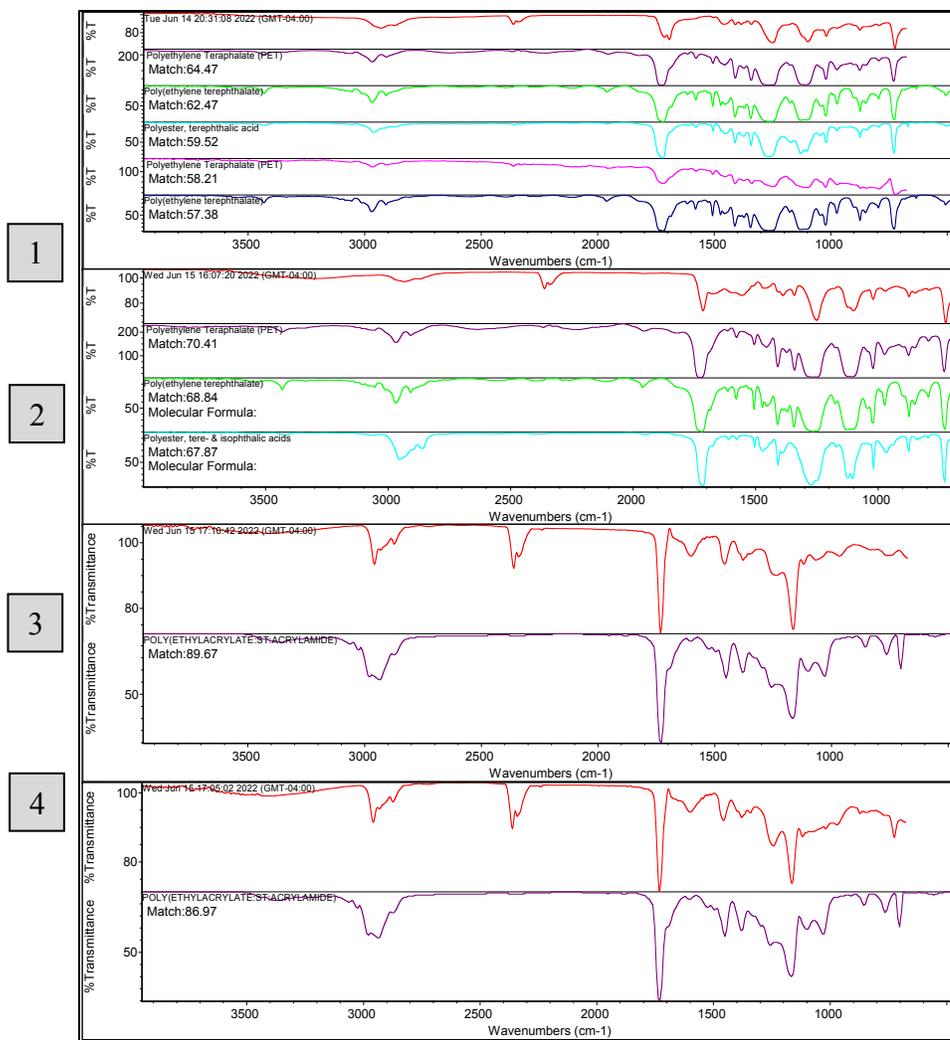


Figure 12. Examples of matching MP spectra for PET and Poly-ethyl-acrylate in walrus blanks (1,3) and samples (2,4). Red wavelengths represent the observed spectrum of a particle, while subsequent wavelengths are drawn from the polymer library for comparison.

5.4.2 Ringed seals

Similarly in the seals, Micro-ATR-FTIR analysis detected both organic and inorganic polymers. Like the walrus trial, Wood Rosin was the prominent organic polymer detected by the ATR-FTIR, and the overall morphologies of the MPs were similar across both groups (Figure 13). However, only one microplastic particle retrieved from a stomach filter was conclusively identified, with spectral readings of 72.76% to 78% consistent with Poly (ethyl) and Poly (butyl acrylate) (commonly used in the fabrication of textiles and clothing; Figure 4) (de Falco et al., 2019; Acharya et al., 2021). This finding fits with very few fibres detected in the seal stomachs and was likely due to ambient contamination within the lab (a known and recognized challenge in microplastic research) (Bogdanowicz et al., 2021).

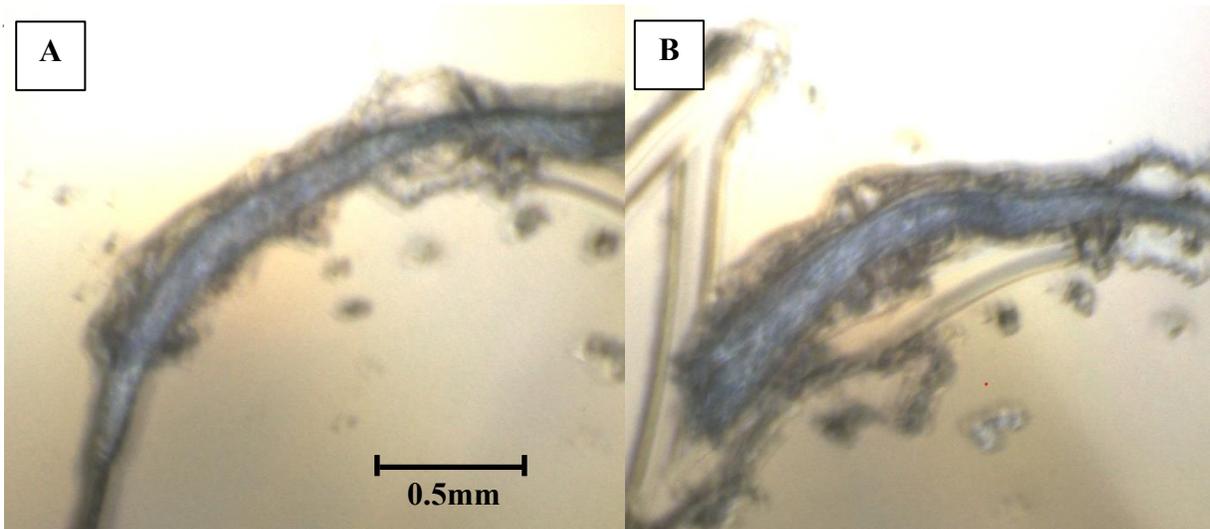


Figure 13. Examples of microfibres recovered from the seal S_{rs} (A) and Pb_{rs} (B), morphologies were consistent between both groups.

5.5 Global assessments and incidences of microplastic ingestion in Procellariiformes and pinnipeds

Of the 1009 studies identified by the search tools, 58 assessments were found to have included some incidence of microplastic-sized particles in either Procellariiformes or pinnipeds (Appendix D,E). For pinnipeds, the latitudinal region with the most individual assessments or reports of microplastic ingestion was the sub-Arctic (50°N - 70°N) (n = 7) (Figure 13). The south-Torrid region (0°S - 25°S) was the most abundant zone for assessments on Procellariiformes (n = 17). The highest mean frequency of occurrence was also in the sub-Arctic for both pinnipeds and Procellariiformes (FO_{pin} = 72%; FO_{pro} = 97%) (Figure 13). The sub-Antarctic was the least studied zone, where three assessments on pinnipeds had been conducted, but no FOs were reported.

Procellariiformes consistently outnumbered pinnipeds in terms of both assessments and incidences of MP ingestion and the mean frequency of occurrence in nearly all latitude regions. In the sub-Arctic and south-Temperate areas, however, more assessments had been completed for pinnipeds (Table 13). The mean FO for pinnipeds was also higher in the south-Temperate zone. The mean FO of MP ingestion in Procellariiformes generally increased north of the equator (0°), which is consistent with their status as indicator species for MPs in Arctic regions (Provencher et al., 2017). Interestingly, the FO and number of assessments or incidences of MP ingestion in pinnipeds spike near the middle of the northern and southern hemispheres but taper off at increasingly polar or equatorial latitudes.

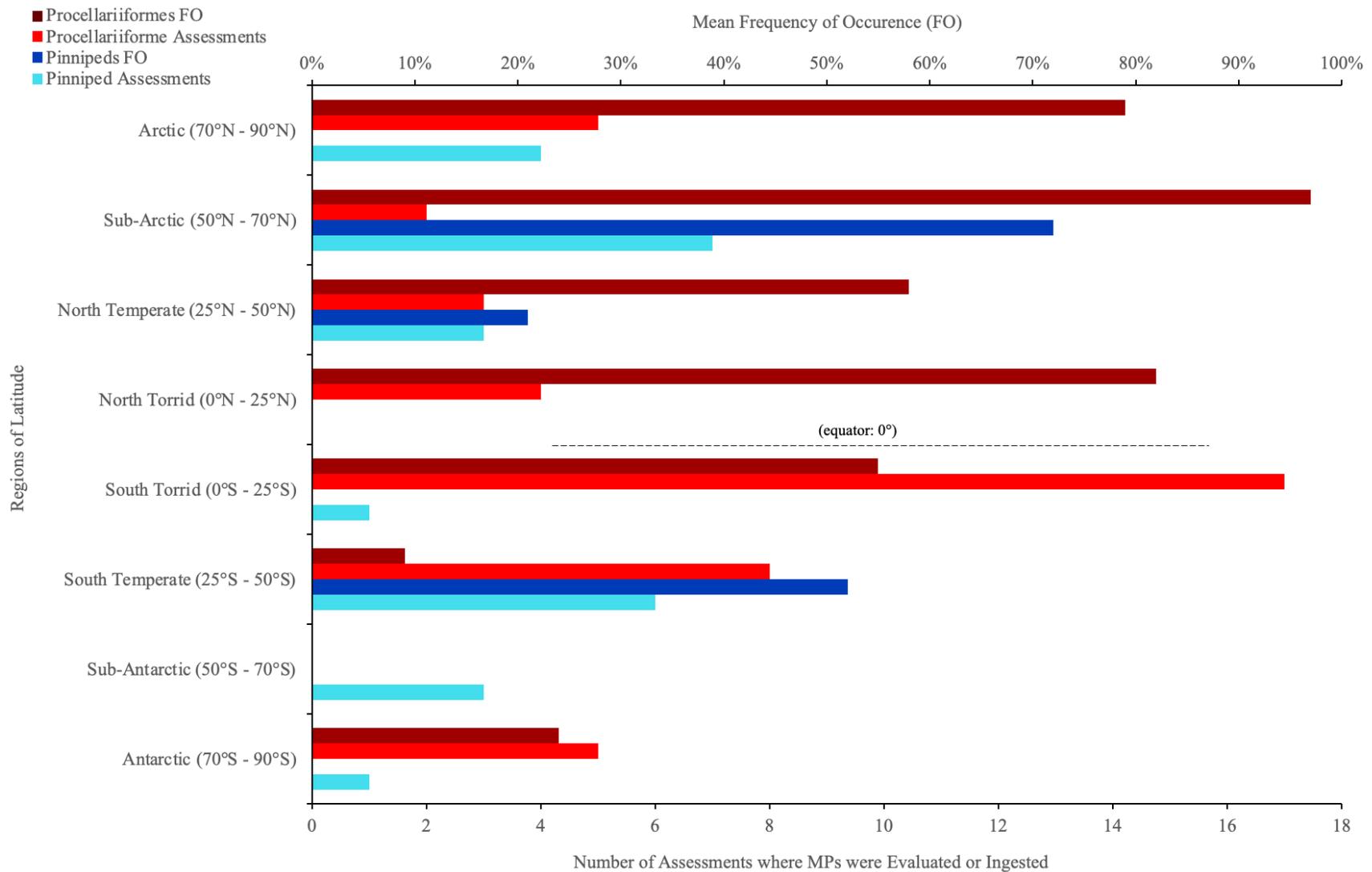


Figure 14. Number of MP (items $\leq 5\text{mm}$) assessments and incidences of ingestion and mean FOs for pinnipeds and Procellariiformes at different degrees of latitude. Latitude regions have been grouped to broadly represent general regions of study, globally.

6.0 Discussion

6.1 Walrus and ringed seals are not retaining MPs in the Canadian Arctic

With no substantial differences between the mean numbers of particles in the sample and blank filters, these results likely indicate that MPs are not retained in walrus in the eastern Canadian Arctic, or ringed seals from in western Canadian Arctic. The two trials completed in this study, in combination with the results of Bourdages et al., (2020) may also show that pinnipeds are not accumulating microplastic in the Canadian Arctic as a whole. Instead, these results suggest that if pinnipeds do ingest microplastics, they are not retained within the animal in substantive quantities and are likely excreted following consumption. This conclusion is consistent with both Bourdages et al., (2020), Carlsson et al., (2021), but does not provide further insight as to the potential exposure to MPs through prey items or environmental concentrations. Given that the stomachs examined in this thesis were mostly empty, it is difficult to predict whether MPs from secondary ingestion would have been present if scat was also collected. Given the absence of detectable particles, the current vulnerability of Canadian Arctic pinnipeds to microplastic ingestion is likely much lower than in other regions and taxonomic groups.

Since microfibres are regularly found in the air and were the only MP subtype present in both trials, it is highly likely that the collective 477 fibres in the filter membranes were the result of ambient contamination in the laboratory (Dris et al., 2016; Acharia et al., 2021). Fibers are the most abundant subtype of microplastic and are present in as much as between 2 and 355 particles per m² of air in urban areas (Dris et al., 2016; Acharia et al., 2021). Synthetic fibers are also predominantly associated with textiles and clothing, which is supported by the identification of Poly (ethyl acrylate), and Polyethylene Terephthalate (de Falco et al., 2019). In addition to the

polymer types, the colour distributions between both trials (Figures 9 and 10) were also consistent and showed no relationships between colours, indicating that the fibres had likely come from background sources like clothing. Despite the regular and thorough cleaning of laboratory utensils, lint rolling lab coats before processing samples, and the use of a laminar flow hood for all open-air procedures, low concentrations of airborne microplastic contamination in the laboratory are challenging to avoid and must be considered using procedural blanks. This precaution is necessary when analyzing samples with low microplastic levels predominantly composed of microfibers. Additionally, at the observed effect sizes ($d = 0.21$ and $d = 0.37$), the sample sizes required for a significant result were 281 walrus and 91 seals (or, at 36 and 10 stomachs, the required average MP yield would have needed to be 12 and 4) reemphasizing that any observed particles were probably the result of laboratory contamination.

The Principal Components Analysis for microplastic colours observed in the walrus trial showed that the presence of any singular MP did not have a significant bearing on the detection of another. This result could indicate a few things. First, that the source of the exposure was consistent, but not constant, or that there were multiple sources of contamination present during sampling at varying concentrations. Second, is that although there appeared to be some sources that emitted more blue and black MPs, they were not any more likely to be observed than less common colours, like red. Ultimately, the MPs found in the samples and blanks were not from sources that would predispose only certain colours to detection. Given this result, even if a significant MP signal had been detected in the walrus, they would not appear to have selective feeding habits regarding the ingestion of particular-coloured MPs.

6.2 Would pinnipeds make good bioindicators for Arctic microplastics?

Monitoring frameworks often identify indicator species to evaluate regional trends in plastic pollution (AMAP, 2021). Considering the lack of microplastics recovered in this study and the study by Bourdages et al. (2020), pinnipeds in the Canadian Arctic do not accurately represent levels of contamination that are present in other Arctic species or the environment, with little to no practical relationship between the number of animals studied and the number of MPs detected. Consequently, the suitability of pinnipeds for use as indicator species for Arctic MP contaminants is inadequate. The highest MP FOs in pinnipeds also occur where more assessments have been conducted. They are at their highest in the sub-Arctic, but spike again at south-Temperate latitudes (notably where seabird FOs are their lowest). The FO of MP ingestion in pinnipeds is also entirely absent in Arctic latitudes, with mixed levels between north and south Temperate regions (50°N - 50°S). Presumably, this could imply that pinnipeds are generally susceptible (potential to be affected by ingestion) to plastic ingestion if it exists in their environment, but do not exhibit selective feeding habits regarding its consumption (Nelms et al., 2018; Nelms et al., 2019). Further, pinnipeds in the Canadian Arctic do not appear to be currently vulnerable (exposed) to MP pollution in concentrations that would predispose them to ingestion.

Seabirds, on the other hand, show mixed but consistently much higher levels of microplastic ingestion throughout most latitudes regardless of regional concentrations or the number of MP assessments conducted. Even though more individual assessments for Procellariids have been completed in the southern hemisphere, the highest FOs of plastic ingestion occur almost exclusively north of the equator. Their highest average FO is also in the sub-Arctic zone, coinciding with typical breeding colony ranges for Northern Fulmars, which are

the most assessed Procellariid species. In addition, the mean FO for MP ingestion in Arctic latitudes is high (79%), indicating that they are highly sensitive to changes MP composition even in regions where less MPs are reported. Seabirds (and Procellariiformes more specifically) are therefore vulnerable (regularly exposed) and susceptible (likely to be affected by ingestion) to microplastics in all latitudes. In terms of identifying key indicator species for Arctic Canadian regions, it is more sensible to apply taxonomic groups and species that are both susceptible and vulnerable to MPs in their environment, like seabirds.

6.3 If Canadian pinnipeds don't accumulate microplastics, how can they help track pollution in the Arctic?

The absence of microplastic retention does not translate to an absent impact. While pinnipeds may not be suitable indicators of MP composition in Arctic Canada, it is still vital to maintain regular monitoring and evaluations of their health in relation to associated risks of plastic pollution. For instance, Canadian Arctic marine mammals may be more useful for tracking harmful chemical pollutants associated with plastics (additives like PBDE) since they have the potential to translocate from particles into the tissues of animals (Tanaka et al., 2013; Lusher et al., 2022). In fact, the transfer of harmful chemical additives to marine mammals has been recognized for over two decades (Ludovic et al., 2017). In 1998, de Boer et al., detected around $100 \mu\text{g kg}^{-1}$ of PBDE in sperm whale (*Physeter microcephalus*) blubber. Even within Canada, multiple seal species (grey and harbour) presented concentrations of $112 \pm 55.2 \text{ ng/g lipid}$ and $319 \pm 132 \text{ ng/g lipid}$ of PBDEs on both coasts (Nova-Scotia and British Columbia) (Ikonomu and Addison, 2008).

Although Canadian Arctic pinnipeds do not appear to actively ingest microplastic pollutants, their potential to display changes in marine chemical composition could be a highly valued attribute. In addition, chemical additives and POPs are known to biomagnify in food-webs (Robinson et al., 2018). High trophic level organisms like mammals and pinnipeds are also more likely to get microplastics through secondary ingestion of contaminated prey. Since high MP concentrations have already been detected in Arctic fish and invertebrates from the same regions, pinnipeds could be accumulating substantial amounts of chemicals even if MPs pass through them after eating an animal (Moore et al., 2021; Teichert et al., 2021). This interaction was proven in grey seal pups, where Robinson et al., (2018) found that concentrations of POPs were high enough to alter adipose function. Perilously, the risk of elevated POP concentrations extends to humans who consume contaminated prey.

6.4 New approaches to study design in mammalian MP monitoring

While it appears that the risk of ingesting MPs in the Canadian Arctic is minimal for pinnipeds, ongoing trends in global microplastic pollution are still worrisome (Zhang et al., 2020). The findings presented here support recent discussions on Arctic monitoring of plastic pollution, suggesting that researchers should consider others less vulnerable taxonomic groups (like marine mammals and pinnipeds) on a regional basis rather than at the pan-Arctic level (Provencher et al. 2022). Assessing these groups on a regional level would provide more relevant information addressing local concerns, which could vary based on the level of ambient concentration or the welfare of a certain population.

Given that this thesis calculated the necessary number of ringed seal and walrus stomachs required to detect a significant MP signal at existing levels (91 and 281), researchers could take this approach by asking different questions regarding the spatial resolution of monitoring studies. For example, if we wish to compare regional microplastic abundance in seals from multiple locations, considering how microplastic trends in aquatic systems may not be homogenous, the presumed necessary sample size (91) would apply to each site and individual time of harvest. However, if we want to explore whether seals from a general region contain more microplastics than what can be detected through ambient contamination in the laboratory, the 91 stomachs would come from all sampling locations combined. This approach could be useful for studies where the suspected concentration of MPs is low and would reflect a more directed assessment of regional impacts. It is important to point out, however, that the samples sizes recommended by this study are quite large (91 and 281 walrus). Harvesting this many individual animals, especially if each study site required that same amount, would be costly both in terms of human resources as well as to the animal populations. At the minimal to potentially non-existent levels at which pinnipeds appear to be ingesting microplastic in the Canadian Arctic, the validity of such analyses is questionable.

The comparison of populations and species across studies is vital to the identification of widespread trends in microplastic abundance. Throughout the literature there are noticeable inconsistencies in these metrics that have led to some vague and immaterial evidence for overall trends in many species. Most importantly, many studies do not report a frequency of occurrence, or decide to lump all sizes of debris together without describing how many of each type were present in the overall sample size. Consequently, these studies are difficult to compare to others, which muddles the overall representation of marine MPs within taxonomic groups, populations,

or species. It would be best for future studies to include the FO for all sizes of plastics within the sample size, which is a popular approach used in seabird studies (Provencher et al., 2018).

6.5 How this thesis supports emerging Arctic monitoring frameworks

As the first assessment of microplastic retention and ingestion in Canadian walrus, and the evaluation of microplastics in seals from the western Canadian Arctic, this thesis provides important baseline information to support the development of monitoring frameworks such as the ones proposed by AMAP (2021). The data obtained here contribute to AMAP's Litter and Microplastics Expert Group (LMEG) who are currently planning an assessment of plastics in the Arctic, including mammals, invertebrates, fish, and seabirds (AMAP, 2021). Given that microplastics and associated polymers in walrus and seal stomachs are likely to be of interest to many communities, the LMEG requires information on current levels, and how different methods may yield different results as the field moves towards standardized methods in microplastic analysis.

As researchers continue to work towards this robust understanding of global plastic pollution, identifying key indicator species will also be an important part of evaluating its full extent in remote and mostly uninhabited regions (Provencher et al., 2019). As discussed in this thesis, some species may be more applicable than others for specific contaminants. Seabirds for example are proven to be useful for detecting trends in plastic ingestion itself, while pinnipeds and mammals may be more appropriate for monitoring levels of associated chemical contaminants (Lu et al. 2019). Additionally, studying mammals at regional levels may provide more meaningful information to local communities that depend on healthy populations. The

recommended sample sizes for each respective species (91 seals and 281 walrus) are helpful in this regard for developing theoretical approaches to study design and could be compared to other species once baseline levels for statistical significance have been identified.

6.6 Suggestions for standardizing sample collection in marine mammals

In terms of the methodological approaches for sampling marine mammals, the evidence provided in this study favors a combination of techniques for further analyses. Although sampling scats has some advantages (non-invasive, easy to collect, fewer permits needed, and larger sample sizes are possible), it fails to provide critical information on retained microplastics within the GIT (Nelms et al., 2019). Researchers may also need to account for airborne contamination that could be deposited onto scat samples between the time they are produced and the time that they are sampled. Enough volume should be collected to identify MPs at least three times higher than in the field or in lab blanks to accommodate this shortcoming (Brander et al., 2020). In the case of Arctic pinnipeds, more assessments for benthic invertebrates in the region may also be helpful, since they are known to accumulate microplastic particles and could potentially serve as bioindicators for lower trophic level feeders in marine environments (Staichak et al., 2021). Sampling scats should therefore be completed in tandem with some form of GIT analysis; though, this is often difficult to accomplish given that sampling whole animals is logistically demanding (Nelms et al., 2019; Zantis et al., 2021).

Whole animals or mammalian GITs are in some cases difficult to obtain for research and are not as readily accessible as scat in field settings. Hunting communities are vital and pragmatic components of Arctic research for this reason, aiding with sampling and the logistical aspects of

studies. Much of northern research is successful due in part to the collaboration and support of Arctic communities, and providing important services and information encourages this relationship. Increased partnerships between these communities and researchers allow for better access to sought-after samples and benefit both parties through long-term monitoring of Arctic animals. With more comprehensive diet studies at various trophic levels, trends in microplastic transfer and retention within marine communities and food webs would be far less ambiguous, since contamination through secondary ingestion may pose a more significant risk to pinnipeds than exposure in the water column (Nelms et al., 2018). For animals that do not appear to heavily ingest microplastics, thorough assessments of their food webs would also be practical.

7.0 Conclusion

The Canadian Arctic is home to many ecologically and culturally important animals, which extends the plastic pollution problem to communities that inhabit the region who traditionally rely on wildlife as sources of food, income, and clothing. While walrus and ringed seals do appear to be susceptible to microplastic pollution if it is present in their environment, the findings presented in this study show that they are not ingesting or retaining it in substantive quantities in the eastern or western Canadian Arctic. This conclusion does not mean that the impacts of microplastic pollution are wholly absent, since MPs have been detected in other animals of the same region, and harmful chemicals are known to be present in some Arctic mammals, including seals (Ikonomu and Addison, 2008; Robinson et al., 2018; Baak et al., 2020).

While global trends in microplastic abundance increase, so does the risk of exposure to them in remote and polar regions. Although more of these pollutants tend to accumulate in southern latitudes, ocean transport and atmospheric deposition act as significant vectors for accessing the north (Mountford and Maqueda, 2020). As a highly susceptible region to environmental change, the Arctic is not threatened by microplastic pollution only in a vacuum. Rising sea levels and temperatures threaten animals that rely on the homeostasis of polar climates, which in and of itself is an imminent threat. Conjointly, microplastic pollution and climate change further exacerbate the deterioration of polar habitats, as the melting of sea ice releases enormous amounts of debris into the ocean; simultaneously harming animals and the environments that they rely on (Obbard et al., 2014).

The risk to human populations has also become more apparent in recent years (Smith et al., 2018). While some taxonomic groups like seabirds are proven to be useful for monitoring the composition of environmental MP pollution, little is known regarding trends in animals that are more traditionally harvested for consumption. It is important to ensure the health of animals that are relied on for human consumption, since clean and healthy wildlife are vital to many northern communities and are essential to food security in northern Canada. To address this important knowledge gap, this study provided the first assessment of internal microplastics in walrus and augmented the existing but limited literature by evaluating ringed seals in the western Canadian Arctic. Using a smaller detection limit of 80µm, the results presented in this thesis expand on the work of Bourdages et al., (2020), and Carlsson et al., (2021), by demonstrating that MPs do not appear to accumulate within the GITs of pinnipeds in Arctic Canada but may instead be transient if they happen to be ingested. Moreover, the ultimate roles that pinnipeds are suited to within the context of microplastic pollution research have been identified through comparison to an established taxonomic group (seabirds) for monitoring the spread of MPs.

The practical applications of this thesis are also useful for long-term monitoring programs. Such programs should look to diversify the species they assess since plastic pollution presents multi-dimensional threats to food webs at all trophic levels (Bucci and Rochman., 2022). In view of the lack of standardized techniques for microplastic analyses, this thesis also provided concrete recommendations for the progression of the field. Re-evaluating the roles and utility of Arctic marine mammals in the context of the stressors that are most relevant to them is important for implementing adaptive conservation and monitoring strategies. Additionally, this thesis compared sampling techniques and approaches to study design across the field of microplastic research more broadly. In doing so, new parameters for future research can be considered for

evaluating trends in environmental MP composition. For example, determining an appropriate sample size for detecting significant amounts of MPs in certain species would allow for the readjustment of physical sampling boundaries, as well as the refinement of hypotheses regarding the spatial distribution and sources of MPs in Arctic wildlife. Researchers may instead evaluate marine mammals to describe regional trends in MP abundance, which would appropriately reflect the concerns of northern communities.

By comparing the data reported in microplastic ingestion studies across pinnipeds and seabirds, the discrepancies in how researchers choose to report their data became glaring. Since most publications in this field are quite recent, there has been some historical ambiguity in relation to the importance of certain measurements. The frequency of occurrence is a prime example of this shortcoming. The FO of microplastics in a sample size is a critical value that allows for the direct comparison among species and studies (Provencher et al., 2017; Mendoza and Balcer, 2019). Unfortunately, much of the earlier literature fails to report it, or lumps all sizes of plastic debris into one general proportion. Marine plastics should alternatively be categorized into definitive sizes and morphological subtypes. Macroplastics, mesoplastics, microplastics and nanoplastics should be grouped according to size, and represented in their overall abundance within sample sizes (Provencher et al., 2017). Similarly, microplastics should be categorized based on subtype and colour, since morphometric data can help indicate the source of a pollutant (Miller et al., 2021).

Beyond the theoretical and logistical approaches to MP ingestion research, this thesis also provided recommendations for the techniques used in sample collection and processing. A combination of sampling both scats and whole animals is favoured, if administratively possible.

Whole animal samples do not provide an index for microplastic egestion and scat samples lack the same for initial ingestion. As such, the two should be viewed as opposite sides of the same coin and applied in tandem. The information yielded from both methods can be further augmented by incorporating evaluations of MP composition within the food web, since higher trophic organisms are likely to ingest MPs mainly in their prey. In the laboratory, it is essential to uphold strict QA/QC protocols. Ambient microplastics in the air are virtually unavoidable in most settings and should always be accounted for with the use of procedural blanks.

Plastic pollution is a profoundly anthropogenic issue. The long-term preservation of Arctic climates demands immediate and effective measures for remediating and preventing both climate change and pollution. In Canada, Arctic regions are extremely valuable and are central to our national identity (Government of Canada, 2021). The Arctic represents over 40% of Canadian territory and is home to over 200 000 people (Government of Canada, 2021). As an exceptional source of culture and biodiversity, the prospective impacts of further pollution in the Arctic are bleak should current trends progress. It is through the continued advancement and standardization of microplastic research as a field that actionable and informed policies can be developed to address this issue. We are at a decisive moment for understanding the fates and consequences of microplastic pollution in the Arctic, and a failure to act judiciously would likely exacerbate the effects other critical threats like climate change. In tandem, these issues may increase the risk of devastating loss of biodiversity, population overgrowth, and ecosystem collapse (Choy et al., 2019; Parker, 2020; World Wildlife Fund, 2022).

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9.0 Appendices

Appendix A. Safety Data Sheets for health risks associated with common plastic additives.

Substance	MSDS Fact Sheet References
Cadmium	<i>Cadmium Factsheet National Biomonitoring Program CDC. (n.d.). Retrieved July 31, 2022, from https://www.cdc.gov/biomonitoring/Cadmium_FactSheet.html</i>
Calcium Carbonate	<i>Calcium Carbonate SECTION 1: Identification. (n.d.). Retrieved July 31, 2022, from www.labchem.com</i>
Cobalt	<i>California - OEHHA, B. (n.d.). Cobalt Fact Sheet.</i>
DPP	<i>Cayman Chemical, (2019). Section 8. Exposure Controls/Personal Protection 8.1 Exposure Parameters: Multi-region format. (n.d.).</i>
DEHP	<i>Di(2-ethylhexyl)phthalate (DEHP) - Proposition 65 Warnings Website. (n.d.). Retrieved July 31, 2022, from https://www.p65warnings.ca.gov/fact-sheets/di2-ethylhexylphthalate-dehp</i>
Glass fibre	<i>Fiberglass Fact Sheet. (n.d.). Retrieved July 31, 2022, from http://www.idph.state.il.us/envhealth/factsheets/fiberglass.htm</i>
Chromium	<i>New Jersey Department of Health, (2009). Chromium - Right to Know Hazardous Substance Fact Sheet. (n.d.). Retrieved July 31, 2022, from www.cdc.gov/niosh/topics/ctrlbanding/.</i>
Lead	<i>Lead - information sheet - Canada.ca. (n.d.). Retrieved July 31, 2022, from https://www.canada.ca/en/health-canada/services/chemical-substances/fact-sheets/chemicals-glance/lead.html</i>
Aramide	<i>Marsh, J. P., Mossman, B. T., Driscoll, K. E., Schins, R. F., & Borm, P. J. A. (1994). Effects of Aramid, a high strength synthetic fiber, on respiratory cells in vitro. <i>Drug and Chemical Toxicology</i>, 17(2), 75–92. https://doi.org/10.3109/01480549409014303</i>
PBDE	<i>Polybrominated diphenyl ethers (PBDEs) - information sheet - Canada.ca. (n.d.). Retrieved July 31, 2022, from https://www.canada.ca/en/health-canada/services/chemical-substances/fact-sheets/chemicals-glance/polybrominated-diphenyl-ethers-public-summary.html</i>
Metal Powder	<i>Safety Data Sheet Iron metal powder, <75 microns. (2015). www.noahtech.com</i>
Zinc Oxide	<i>Science Lab Chemicals & Laboratory Equipment. (2013). Material Safety Data Sheet. MSDS, 1–6.</i>

Appendix B. All Eigenvalues and Proportions Explained by the Principal Components calculated in the PCA.

	Eigenvalue	Proportion Explained
PC1	0.1529	0.3253
PC2	0.0973	0.207
PC3	0.07896	0.16798
PC4	0.05742	0.12217
PC5	0.04457	0.09483
PC6	0.0268	0.05702
PC7	0.00977	0.02079
PC8	0.0009218	0.0019613
PC9	0.0008414	0.0017901
PC10	0.0002519	0.0005359
PC11	0.0002249	0.0004785
PC12	0.0000693	0.0001474
PC13	6.83E-06	1.45E-05

Appendix C. 'Species Scores' for the first 6 Principal Components of the 13 colour variables, calculated with '.vegan' in R-Studio, only PC1 and PC2 were selected.

	PC1	PC2	PC3	PC4	PC5	PC6
Black	-0.80758	-0.55869	-0.31639	0.162981	0.126816	0.198836
Grey	0.015886	0.183248	-0.40625	-0.62704	0.289147	0.048175
White	-0.00303	0.000961	0.010635	-0.00335	-0.00333	0.001873
Clear	-0.17713	0.792109	-0.33448	0.378398	0.105674	0.094518
L.Blue	0.02524	0.105076	0.642134	-0.02345	0.337787	0.277735
Blue	1.020069	-0.30309	-0.31812	0.194875	0.082135	0.181556
D.Blue	-0.04705	0.148894	-0.00535	-0.21632	-0.52027	0.315613
Red	0.003468	-0.02939	0.021883	-0.00793	-0.02733	-0.20119
Purple	-0.00741	0.002424	0.019452	-0.01065	0.009208	0.007579
Pink	-0.01164	0.014243	0.012578	-0.01649	0.010465	0.011499
L.Green	0.00069	-0.00193	0.002006	0.000995	0.001348	0.002003
Green	-0.00386	-0.00228	-0.00497	0.006761	0.001635	-0.00092
Yellow	-0.00197	-0.00366	0.01797	-0.00312	-0.01643	-0.04006

Appendix D. List of recovered studies on microplastic ingestion in Procellariiformes and Pinnipeds, with corresponding designated regions of latitude and frequencies of occurrence, if available.

Order / Suborder	Family	Spp. (Common name)	FO (%)	Region of Latitude	Reference		
<u>Procellariidae</u>	Fulmars	<i>Northern Fulmar</i>	31	North Temperate	Terepocki et al., (2017)		
			97	Sub-Arctic	Provencher et al., (2018)		
			90	Arctic	Sühring et al., (2022)		
			NA	Arctic	Hamilton et al., (2021)		
			74	Arctic	Bourdages et al., (2021)		
			72	Arctic	Baak et al., (2020)		
			NA	Arctic	Poon et al., (2017)		
			NA	Sub-Arctic	Avery-Gomm et al., (2018)		
			Shearwaters	<i>Southern Fulmar</i>	7	Antarctic	Van Franeker et al., (1988)
					<i>Wedge-tailed Shearwater</i>	10	South Temperate
	77	North Torrid				Kain et al., (2016)	
	50	South Torrid				Berr et al., (2020)	
	<i>Sooty Shearwater</i>	64			North Temperate	Terepocki et al., (2017)	
	<i>Newell's Shearwater</i>	50			North Torrid	Kain et al., (2016)	
	<i>Flesh footed shearwater</i>	7			South Temperate	Lavers et al., (2019)	
	<i>Cory's Shearwater</i>	80			South Torrid	Vanstreels et al., (2021)	
	<i>Manx Shearwater</i>	35			South Torrid	Vanstreels et al., (2021)	
	Petrels	<i>Mottled Petrel</i>			NA	South Temperate	Roman et al., (2019)
			<i>Tahiti Petrel</i>	0	South Torrid	Berr et al., (2020)	
			<i>Gould's Petrel</i>	74	South Torrid	Berr et al., (2020)	
			<i>Tristram's storm Petrel</i>	100	North Torrid	Youngren et al., (2018)	
			<i>Wilson's storm Petrel</i>	75	Antarctic	Van Franeker et al., (1988)	
			<i>Cape Petrel</i>	33	Antarctic	Van Franeker et al., (1988)	
			<i>White faced storm Petrel</i>	79	North Temperate	Furtado et al., (2016)	
		<i>Antarctic Petrel</i>	NA	South Temperate	Roman et al., (2019)		
			0	Antarctic	Van Franeker et al., (1988)		

		<i>Snow Petrel</i>	4	Antarctic	Van Franeker et al., (1988)
	Albatrosses	<i>Black footed Albatross</i>	100	North Torrid	Hyrenbach et al., (2017)
		<i>Yellow nosed Albatross</i>	60	South Torrid	Vanstreels et al., (2021)
	Prions	<i>Antarctic Prion</i>	NA	South Temperate	Roman et al., (2019)
		<i>Broad-billed Prion</i>	NA	South Temperate	Roman et al., (2019)
		<i>Salvin's Prion</i>	NA	South Temperate	Roman et al., (2019)
		<i>Slender-billed Prion</i>	NA	South Temperate	Roman et al., (2019)
<u>Otariidae</u>	Eared Seals	<i>Antarctic fur seal</i>	NA	Sub-Antarctic	Eriksen and Burton (2003)
			0	Sub-Antarctic	Ryan et al., (2016)
		<i>Hooker's sea lion</i>	NA	Sub-Antarctic	McMahon et al., (1999)
		<i>Northern fur seal</i>	55	North Temperate	Donohue et al., (2019)
		<i>Juan-Fernández fur seal</i>	NA	South Torrid	Perez-Venegas et al., (2020)
		<i>South American fur seal</i>	NA	South Temperate	Perez-Venegas et al., (2020)
			67	South Temperate	Perez-Venegas et al., (2018)
			NA	South Temperate	Perez-Venegas et al., (2020)
		<i>Australian sea lions</i>	65	South Temperate	Brooke (2019)
<u>Phocidae</u>	Earless Seals	<i>Grey seal</i>	100	Sub-Arctic	Hernandez Milian et al., (2019)
			100	Sub-Arctic	Nelms et al., (2019)
			48	Sub-Arctic	Nelms et al., (2018)
			1	North Temperate	Hudak and Sette (2019)
			NA	Sub-Arctic	Desclos-Dukes et al., (2022)
			80	Sub-Arctic	Hernandez Milian et al., (2017)
		<i>Ringed seal</i>	0	Arctic	Bourdages et al., (2020)
		<i>Bearded seal</i>	0	Arctic	Bourdages et al., (2020)
		<i>Antarctic fur seal</i>	0	Antarctic	Garcia-Garen et al., (2020)
		<i>Australian fur seals</i>	50	South Temperate	Brooke (2019)
		<i>Long-nosed fur seals</i>	21	South Temperate	Brooke (2019)
		<i>Harbour seal</i>	6	Sub-Arctic	Rebolledo et al., (2013)
			100	Sub-Arctic	Nelms et al., (2019)

		<i>Harbour seal</i>	0	Arctic	Bourdages et al., (2020)
			6	North Temperate	Hudak and Sette (2019)
Odobenidae	Walrus	<i>Atlantic Walrus</i>	NA	Arctic	Carlsson et al., (2021)

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Appendix F. R-code transcript for the Power Analyses; a detailed explanation is provided for the seals, and an identical procedure is followed for the walrus.

```
#1/2 RINGED SEALS
#Opening the .power package
>install.packages ("pwr")
#For a one sided 2 sample test
#Testing Ho: mu1 < / = mu2
#Ha: mu1 > mu2
#The following is a preliminary calculation with
#a significance level of 0.05
#power.t.test
      # n = 10,                = sample size for each group
      # delta = 0.6,          = the difference between the means
      # sd = 1.62,            = this is a pooled SD
      # sig.level = 0.05      = sig level or p-value
      # type = "two.sample",
      # alternative = "one.sided"
#Run this test to confirm it works
>power.t.test(n = 10, delta = 0.6, sd = 1.62, sig.level = 0.05, type = "two.sample",
alternative = "one.sided")
#Now plugging in my real data
#One sided t-test p-value: 0.24
#n = 10 for each group
#Delta xbar is still 0.6
#The pooled SD is 1.62
#Using the one sided p-val
>power.t.test(n = 10, delta = 0.6, sd = 1.62, sig.level = 0.24, type = "two.sample",
alternative = "one.sided")
Two-sample t test power calculation
      n = 10
      delta = 0.6
      sd = 1.62
      sig.level = 0.24
      power = 0.5461965
      alternative = one.sided
#The power is 0.55, let's use it to calculate effect size
>pwr.t.test(n=10,sig.level=0.24,power=0.55, alternative = greater)
>The effect size (d) is 0.37, now I can calculate the sample size
>pwr.t.test(d=0.37,sig.level=0.05,power=0.80,alternative="greater")
      n = 91.00624
      d = 0.37
      sig.level = 0.05
      power = 0.8
      alternative = greater
#To get a significant result at my actual effect size, I'd need 91 seal stomachs, I
can now calculate the effect size I'd need at my own sample size of 10
>pwr.t.test(n=10,sig.level=0.05,power=0.80,alternative="greater")
#The answer is d = 1.16
#To create the graphs, I manipulated the d value in the following command from 0.1 to
#3.0
>pwr.t.test(d=X,sig.level=0.05,power=0.80,alternative="greater")
```

```

#2/2 WALRUS
#Opening the .power package
>install.packages ("pwr")
#For a one sided 2 sample test
#Testing Ho:  $\mu_1 < / = \mu_2$ 
#Ha:  $\mu_1 > \mu_2$ 
#One sided t-test p-value: 0.18
#n = 36 for each group
#Delta xbar is 1.75
#The pooled SD is 8.35
>power.t.test(n = 36, delta = 1.75, sd = 8.35, sig.level = 0.18, type = "two.sample",
alternative = "one.sided")
      n = 36
      delta = 1.75
      sd = 8.35
      sig.level = 0.18
      power = 0.4884891
      alternative = one.sided
>pwr.t.test(n=36,sig.level=0.18,power=0.48, alternative = "greater")
d = 0.21
>pwr.t.test(d=0.21,sig.level=0.05,power=0.80,alternative="greater")
n = 281.0669
>pwr.t.test(n=36,sig.level=0.05,power=0.80,alternative="greater")
d = 0.5918161

```

Appendix G. R-code transcript for the Principal Components Analysis

```
#Setting a working directory
>setwd("~/Home/Documents/Carleton MSc/Thesis/Principal Components")
#Opening the .csv file and naming it "pca"
>pca <- read.csv("pca.csv", header=T)
#Make sure it loaded properly
>pca
>names(pca)
#I am using the PCA functions in the .vegan package
>library(vegan)
#Converting absolute abundance to relative abundance
>sum(pca[1,c(2:14)])
>sum(pca[2,c(2:14)])
#Using the decostand function to transform the data since Euclidean
#distance is not suited to data with many dimensions
#Hellinger transformation on the colour data
>pcaT<-sqrt(decostand(pca[,c(2:14)],method="total"))
#Checking it worked
>pcaT
#Running the PCA and downweighting the data to reduce the
#gravity of more prominent variables
>PCAdat<-rda(downweight(pcaT))
#Checking it worked
>PCAdat
#Detailed summary to show Eigenvalues and Proportions Explained
>summary(PCAdat)
#Rough plot
>plot(PCAdat,type="n",scaling=2)
>text(PCAdat,"sites", col="red")
>text(PCAdat, "species", col="blue", cex=0.8)
>plot(PCAdat,display="species",type="n")
>text(PCAdat, "species", col="red", cex=0.8)
#Show Site Scores
>Site_scores<-scores(PCAdat,"sites",choices=c(2,6),)
#Creating a biplot with the Site Scores and Principal Components
>biplot(PCAdat)
#The aesthetics of the biplot were subsequently edited in PDF
editing software (Adobe Acrobat) for presentation
```

Appendix H. Walrus samples and blanks processing data (PG = Pangnirtung; HB = Sanirajak; CH = Coral Harbour; RB = Naujaat).

Sample ID	Weight	Sample Fibres	Sample Bundles	Sample Total	Blank Total	Year	Community	Notes
arpg-xx-2002	1055.12	0	0	0	0	2020	PG	
arpg-xx-2000	85.74	13	0	13	6	2020	PG	
arhb-xx-2068	85.85	11	0	11	12	2020	HB	
arhb-xx-2078	125.48	5	0	5	0	2020	HB	
qwb-2017-2010	74.85	4	0	4	5	2019	HB	
ariq-dfo-2131	81.84	6	0	6	0	2019	HB	
arhb-xx-2072	232.77	1	0	1	0	2020	HB	Rocks
arhb-xx-2073	64.29	2	0	2	0	2020	HB	
arpg-xx-2004	47.84	0	0	0	10	2020	PG	No microplastics in sample
arhp-xx-2071	333.46	5	0	5	3	2020	HB	
arch-xx-2172	646.26	0	0	0	4	2020	CH	Small pieces of shell
arch-xx-2175	144.67	13	0	13	14	2020	CH	Rocks
ariq-dfo-2138	290.19	1	0	1	1	2020	HB	
arhb-xx-2075	176.95	0	0	0	0	2020	HB	No microplastics in sample
arhb-xx-2067	234.77	1	0	1	4	2020	HB	Rocks
arrb-xx-2047	423.68	11	0	11	7	2020	RB	
arhb-2017-2096	203.62	17	0	17	25	2020	HB	
ariq-dfo-2126	370.26	7	0	7	0	2020	HB	
arhb-xx-2068	368.36	6	1	7	3	2020	HB	Bundle found
arhb-xx-2074	554.47	0	0	0	6	2020	HB	Rocks
arhb-xx-2082	404.25	0	0	0	0	2020	HB	
arhb-xx-2079	765	3	0	3	31	2020	HB	
arrb-xx-2065	225.13	0	0	0	0	2020	HB	
arpg-xx-2008	1363.33	20	0	20	24	2020	PG	
ariq-xx-2049	400.34	1	0	1	1	NA	NA	Rocks
ariq-xx-2046	670.41	2	0	2	2	NA	NA	Lots of rocks (2 petri dishes full)

ariq-xx-2052	101.79	0	0	0	8	NA	NA	The samples from Iqualuit likely fall under the 'NA's, based on the 'iq' sample ID.
arrb-xx-2052	297.16	0	0	0	0	2020	RB	
arhb-xx-3004	60	5	0	5	30	NA	NA	Insect (Fly)
arhb-xx-3039	115	1	0	1	5	NA	NA	
arpg-xx-2042	315	0	0	0	3	NA	NA	
arhb-xx-3002	160	7	0	7	3	NA	NA	
arhb-xx-3007	360	20	0	20	7	NA	NA	
arhb-xx-3031	115	27	0	27	3	NA	NA	
arhb-xx-2060	850	1	0	1	7	NA	NA	
arhb-xx-3011	715	0	0	0	30	NA	NA	
arhb-xx-3006	NA	NA	NA	NA	NA	NA	NA	Unusable (Contaminated)
arhb-xx-3008	NA	NA	NA	NA	NA	NA	NA	Unusable (Contaminated)

Appendix I. Ringed Seals samples and blanks processing data (PT = Paulatuk; SH = Sachs Harbour).

Sample ID	Weight	Sample Fibres	Sample Bundles	Sample Total	Blank Total	Year	Community	Notes
2017-07	119	1	0	1	2	2017	PT	387.7-268.7
2019-006	59.2	2	0	2	2	2019	PT	323-263.8
010-2019	156.7	1	0	1	7	2019	PT	314.7-158
2017-03	374	0	0	0	1	2017	PT	550-176
2017-20	151.6	1	0	1	1	2017	PT	316-164.4
2016-03	53.7	0	0	0	0	2016	PT	309.9-256.2
sh-2019-16	133.8	3	0	3	0	2019	SH	357.9-224.1
028-18	77.9	0	0	0	2	2018	PT	309.6-231.7
sh-2019-13	210	0	0	0	1	2019	SH	439-229
2016-04	76.8	5	0	5	3	2016	PT	239.6-162.8

Appendix J. Colour data for the walrus samples and blanks where the number listed in each column represents the number of MPs of that colour.

<i>Sample ID</i>	Black	Grey	White	Clear	L Blue	Blue	D Blue	Red	Pink	Purple	L Green	Green	D Green	Yellow	Orange
arpg-xx-2002	3				1	2									
arpg-xx-2000				1	1	2									
arhb-xx-2068						2									
arhb-xx-2078						1									
qwb-2017-2010		1				2		1							
ariq-dfo-2131	2					3									
arhb-xx-2072	2	2		4		2									
arhb-xx-2073				2				3							
arpg-xx-2004		1		5	1	4									
arhp-xx-2071	6	4				6		1							
arch-xx-2172	4	1		1		1									
arch-xx-2175	1			1											
ariq-dfo-2138														1	
arhb-xx-2075	2	1		2				8							
arhb-xx-2067				1		6									
arrb-xx-2047	3				2	5				1		1			

arhb-2017-2096	1		1	5	1	
ariq-dfo-2126	1			1	1	
arhb-xx-2068	1			2		2
arhb-xx-2074	1					
arhb-xx-2082	5	2	2	4		
arhb-xx-2079	2					
arrb-xx-2065	2		2			
arpg-xx-2008	1					
ariq-xx-2049						1
ariq-xx-2046			1	3		
ariq-xx-2052	2	1		4	1	
arrb-xx-2052	1			1		
arhb-xx-3004	4				1	
arhb-xx-3039	1					
arpg-xx-2042						
arhb-xx-3002	4	1		1		1
arhb-xx-3007	7	3	1	7	1	
arhb-xx-3031	10	2	5	10		1

arhb-xx-2060					1				
arhb-xx-3011									
Blank ID									
arpg-xx-2002	1		1		3		1		
arpg-xx-2000				1					
arhb-xx-2068		1			1				
arhb-xx-2078	1			2	2				
qwb-2017-2010					1				
ariq-dfo-2131	2		3						
arhb-xx-2072		1	7		4				
arhb-xx-2073	2	2	10	2	8		1		
arpg-xx-2004	1		2						
arhp-xx-2071	1	1	1						
arch-xx-2172					1				
arch-xx-2175	1		6		3		1	1	1
ariq-dfo-2138	1	1	3		1				
arhb-xx-2075	2	5	2	1			1	1	

arhb-xx-2067					1			
arrb-xx-2047		1	3		2		2	
arhb-2017-2096	6	5	7	3	4		1	3
ariq-dfo-2126	1	2	4	1	5			
arhb-xx-2068	1	1			1			
arhb-xx-2074								
arhb-xx-2082								
arhb-xx-2079								
arrb-xx-2065							None	
arpg-xx-2008								
ariq-xx-2049								
ariq-xx-2046								
ariq-xx-2052								
arrb-xx-2052								
arhb-xx-3004	15				15			
arhb-xx-3039	1	2			1		1	
arpg-xx-2042	1	2						

arhb-xx-3002	1		1				1
arhb-xx-3007	2	1	4				
arhb-xx-3031		1	1				
arhb-xx-2060	3		1	1		2	
arhb-xx-3011	15		15				

Appendix K. Cumulative colour data for the ringed seal samples and blanks.

Sample ID	Black	Blue	Clear	Grey	Red	Green
2017-07	1	1	1	1	1	
2019-006	1	1	1	1	1	
010-2019	2	1	1	1		1
2017-03	1	1	1	1		
2017-20	1	1	1	1		
2016-03	1	1		1		
sh-2019-16	1	1				
028-18	1					
sh-2019-13	1					
2016-04	1					

