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Microplastic ingestion in zooplankton from the Fram Strait in the Arctic

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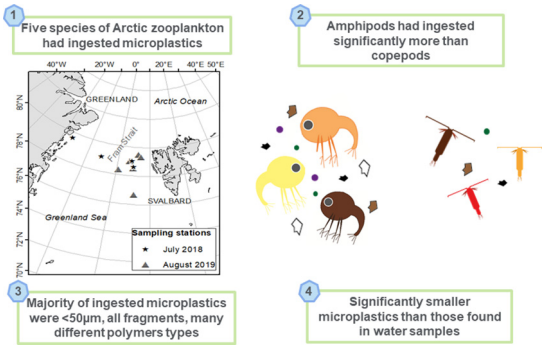
HIGHLIGHTS

- We investigate microplastic ingestion in several species of Arctic zooplankton.
- Novel technique allows microplastic identification to 6.25 μm and removes human bias.
- Amphipods had ingested significantly more microplastics than copepods.
- Ingested microplastics were all fragments and the majority below 50 μm in size.
- Comparison with water samples suggest selectivity of smaller-sized microplastics.

GRAPHICAL ABSTRACT



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ABSTRACT

Some of the highest microplastic concentrations in marine environments have been reported from the Fram Strait in the Arctic. This region supports a diverse ecosystem dependent on high concentrations of zooplankton at the base of the food web. Zooplankton samples were collected during research cruises using Bongo and MOCNESS nets in the boreal summers of 2018 and 2019. Using FTIR scanning spectroscopy in combination with an automated polymer identification approach, we show that all five species of Arctic zooplankton investigated had ingested microplastics. Amphipod species, found in surface waters or closely associated with sea ice, had ingested significantly more microplastic per individual (*Themisto libellula:* 1.8, *Themisto abyssorrum:* 1, *Apherusa glacialis*: 1) than copepod species (*Calanus hyperboreus*: 0.21, *Calanus glacialis/finmarchicus*: 0.01). The majority of microplastics ingested were below 50 μm in size, all were fragments and several different polymer types were present. We quantified microplastics in water samples collected at six of the same stations as the *Calanus* using an underway sampling system (inlet at 6.5 m water depth). Fragments of several polymer types and anthropogenic cellulosic fibres were present, with an average concentration of 7 microplastic particles (MP) L^{-1} (0–18.5 MP L^{-1}). In comparison to the water samples, those microplastics found ingested by zooplankton were significantly smaller, highlighting that the smaller-sized microplastics were being selected for by the zooplankton. High levels of microplastic ingestion in zooplankton have been associated with negative effects on growth, development, and fecundity. As Arctic zooplankton only have a short window of biological productivity, any negative effect could have broad consequences. As global plastic consumption continues to increase

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and climate change continues to reduce sea ice cover, releasing ice-bound microplastics and leaving ice free areas open to exploitation, the Arctic could be exposed to further plastic pollution which could place additional strain on this fragile ecosystem.

1. Introduction

Microplastic particles (microscopic plastic; 1 μm–5 mm) have been widely reported in the Arctic Ocean (Halsband and Herzke, 2019). They have been discovered in snow, sea ice, sea surface, water column, and deep-sea sediments (Obbard et al., 2014; Lusher et al., 2015; Bergmann et al., 2017, 2019; Tekman et al., 2020), and as such they are a contaminant of growing concern in this remote region (AMAP, 2021). Prevailing Atlantic and Pacific water and wind currents can transport nutrients, biota and marine debris including microplastics to this ocean basin (Zarfl and Matthies, 2010; Kanhai et al., 2018). Microplastics can become trapped in sea ice which can not only function as a temporary sink but act as a transport medium and a subsequent secondary source of microplastics upon melting (Peeken et al., 2018; Kanhai et al., 2020). Accelerated melting due to global warming would release an increased number of microplastics into the surrounding water, increasing the exposure to Arctic species (Obbard et al., 2014). In addition, reduced sea-ice coverage would lead to increased local anthropogenic activities such as fishing, tourism, shipping, and resource exploitation, potentially increasing the plastic burden and placing further stress on an already vulnerable ecosystem (Dalsøren et al., 2007; Bergmann and Klages, 2012; Melia et al., 2016; Rodríguez-Torres et al., 2020).

The Fram Strait, which lies between Greenland and Svalbard, is the only deep-water connection between the North Atlantic and Arctic Oceans (Thiede et al., 1990). Recent research has shown that the amount of plastic debris, particularly around the Svalbard region, has been steadily increasing over the last 15 years (Parga Martínez et al., 2020). Previous studies investigating microplastic concentrations have shown the Fram Strait to have some of the highest recorded concentrations (1.2×10^7 m^{-3}) in the Arctic and are amongst the highest records worldwide, with the majority of these microplastic particles being smaller than 50 μm (Peeken et al., 2018). These waters support highly productive food webs, which may be vulnerable to microplastic pollution (Lusher et al., 2015; Rist et al., 2020).

Due to the small size of microplastic particles, they are bioavailable to a wide range of species including many species of zooplankton (Cole et al., 2013). Zooplankton is a crucial food source and provides an important link in the marine food web between phytoplankton and higher trophic levels (Kiørboe, 2011). In the Arctic, amphipods and copepods are particularly important food sources found within the zooplankton. They are also essential for vertical export of organic matter and carbon sequestration (Dalpadado et al., 2008; Steinberg and Landry, 2017). Laboratory studies have shown that microplastics are readily ingested by several species of zooplankton and can cause a range of detrimental effects including reduced feeding behavior, growth and fecundity (Lee et al., 2013; Cole et al., 2015). Ingestion of microplastic in the field has also been documented, however, impacts in the field are difficult to assess due to major methodological obstacles in controlling experimental conditions and variables (i.e. contamination, biotransformation of microplastics, food availability and prior dietary history) (Botterell et al., 2019). Yet current knowledge gaps regarding occurrence of ingestion in the natural environment, where information is scarce, difficult to obtain and limited to a few geographical regions, still need to be addressed as they provide important data for exposure scenarios for use in laboratory experiments and help toward an assessment of risk to an individual, population, and species (Everaert et al., 2020).

Recent laboratory research has highlighted that factors, such as microplastic shape and size, can affect the bioavailability of microplastics to a species (Vroom et al., 2017; Coppock et al., 2019; Botterell et al., 2020; Isinibilir et al., 2020). To better understand the mechanisms behind microplastic ingestion, it is vital to identify which environmental microplastics are ingested in the field and whether this is representative of the microplastics available in the marine environment or demonstrates selectivity by a species. Previous studies have shown there is a close overlap to what is found ingested by the zooplankton and that present in the surrounding water (Desforges et al., 2015; Steer et al., 2017). However, these studies used a different methodology that would have been unable to detect the smallest microplastics.

In this study, we investigate microplastic ingestion in several species of Arctic zooplankton, i.e., the calanoid copepods *Calanus finmarchicus*, *C. glacialis* and *C. hyperboreus* and the amphipods *Themisto abyssorum*, *T. libellula* and *Apherusa glacialis*, collected from the Fram Strait. The *Calanus* species often dominate the Arctic mesozooplankton communities in terms of biomass (Astthorsson and Gislason, 2003; Aarflot et al., 2018). They are suspension feeders which accumulate large lipid reserves during spring/summer by feeding on the ice algae and/or phytoplankton bloom whereas they migrate to deeper water layers and enter a diapause state during winter (reviewed e.g. by Falk-Petersen et al., 2009). Their approximate prey size is ≤35 μm, but many prey species are chain forming and copepods are able to bite smaller pieces off of larger items (Nejstgaard et al., 1997; Cole et al., 2019; Coppock et al., 2019; B. Niehoff per comms). The copepods high nutritional value makes them an important prey species for higher trophic levels such as amphipods, fish, or seabirds (Wold et al., 2011; Kraft et al., 2013; Majewski et al., 2016; Bouchard and Fortier, 2020). The epi- to mesopelagic *Themisto* species *T. libellula* and *T. abyssorum* are dominant members of the Arctic amphipod communities (Dalpadado et al., 2001). They are omnivorous, feeding on dinoflagellates but primarily mesozooplankton species such as *Calanus* (approx. prey size ≤7–8 mm)(Auel et al., 2002; Dalpadado et al., 2008; Kraft et al., 2013; Leinaas et al., 2016). The sympagic (ice-associated) *A. glacialis* feeds on ice algae and small detritus particles (Poltermann, 2001). All three amphipod species are important food sources for Arctic fish including polar cod, marine mammals and seabirds, which are all suggested as indicators for biomonitoring in the Arctic (Lønne and Gulliksen, 1989; Mehlum and Gabrielsen, 1993; Dalpadado et al., 2001; Majewski et al., 2016; McNicholl et al., 2016; Collard and Ask, 2021). Therefore, amphipods together with the copepods, represent a route whereby microplastics could enter the food web. As high concentrations of microplastics have been reported in the Arctic surface waters and sea ice this could put certain species such as amphipods that inhabit these waters at an increased risk of encountering microplastic (Peeken et al., 2018; Tekman et al., 2020).

In this study, we seek to characterize in terms of size, shape, and polymer what types of microplastics are ingested and quantify the ingestion of microplastics in the copepod and amphipod species investigated. Previously, microplastics smaller than 30 μm have been difficult to characterize due to methodological constraints. However, using spectral imaging FTIR (Fourier Transform Infrared) spectroscopy combined with SIMPLE software analysis we were able to identify microplastic polymers down to the size of 6.25 μm whilst also removing human bias. We hypothesize that 1) the majority of the microplastics found ingested will be smaller than 50 μm, 2) those species of zooplankton, e.g. *Apherusa* and *Themisto* sp., that are closely associated with sea ice and surface waters will have ingested more microplastics than other species, and 3) that microplastics ingested by zooplankton will be representative of what is found in the surrounding water.

2. Materials and methods

2.1. Study area and sample collection

Zooplankton samples were collected from 10 stations in the Fram Strait during research cruises on board the research icebreaker RV Polarstern (expedition PS114, 2018) to HAUSGARTEN/FRAM observatory and the RRS

James Clark Ross (research cruise JR18007, 2019) (Fig. 1, see **Supplementary materials Table S1** for station list). The Fram Strait is characterised by a complex hydrographic regime with warm waters of Atlantic origin prevailing in the eastern parts (West Spitsbergen Current) and colder less saline water of polar origin carried with the transpolar drift to the western parts (East Greenland Current)(Beszczynska-Möller et al., 2012). During PS114, zooplankton was sampled with a Bongo net (150 μm mesh size, towed at 0.5 ms^{-1} for ~40 min) that was attached to the side of a multinet. During JR18007, we used Bongo nets (200 μm mesh size, towed at 0.3 ms^{-1} for ~25 min) and a MOCNESS multinet (330 μm mesh size, towed at 0.16 ms^{-1} for ~120 min, with nets opening and closing at set depths for 5–10 min). Back on board, the net content was released into plastic-lidded buckets (JR18007) or metal buckets (PS114). Using a 200 μm mesh sieve, a sample from the bucket was transferred to a Petri dish. Under a dissection microscope (Wild M5-4936; ×20 magnification - JR18007; or a Leica MZ9.5, ×20 magnification - PS114), individuals were carefully picked out using stork bill forceps and gently but thoroughly rinsed with Milli-Q water and visually examined to ensure that no plastic debris was attached to the external surface of any of the individuals. Specimens of the amphipod species *Themisto libellula*, *Themisto abyssorum*, *Apherusa glacialis,* and copepod species *Calanus hyperboreus, Calanus glacialis/finmarchicus* of similar life stage (adult amphipods and adult female or stage CV copepods) were collated into a glass vial and 5 mL 10% sodium dodecyl sulfate (SDS) homogenising solution (filtered over 0.45 μm cellulose nitrate filter) was added to begin enzymatic digestion immediately (**Supplementary materials Table S1**). The vials were then stored at room temperature, with regular manual shaking, until further analysis could be conducted at Plymouth Marine Laboratory (PML). Amphipod species were individually digested, and copepods digested in batches. To keep both sets of data constant, the amphipod species data were pooled to match those of the copepods.

Water samples were collected in plastic bottles from the underway system (inlet at 6.5 m water depth) at each of the stations sampled from the JR18007 (2019) cruise. One sample was taken from a cast of the CTD rosette at 5 m depth whilst in the ice. Plastic bottles were used for ease of handling on ship, but a sample from the bottles was scanned and added to the FTIR polymer library (polypropylene) to account for any potential contamination from the sample bottle. Two litres of water were filtered onto a 25 mm polycarbonate filter (5 μm pore size, Whatman, UK) at each station using a peristaltic pump and vacuum pump system with a 20 μm mesh covering the filtration vessel to minimise contamination from the environment. Filters were then retained in a Petri dish and frozen at -20 °C for analysis at PML.

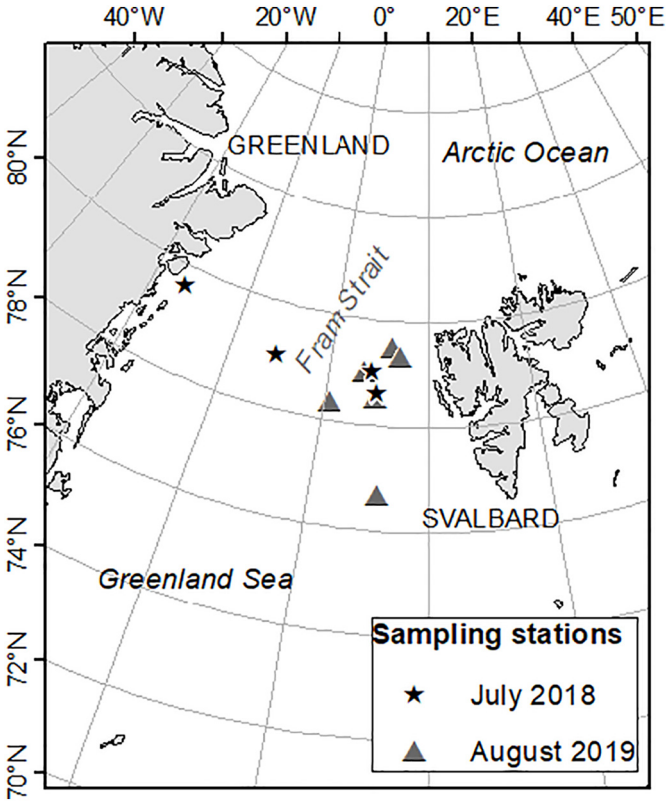

Fig. 1. Map of sampling locations in the Fram Strait in July 2018 (PS114) and August 2019 (JR18007).

2.2. Sample preparation

2.2.1. Enzymatic digestion

An enzymatic protocol, developed by Lindeque and Smerdon (2003) and further adapted by Cole et al. (2014) was used to digest the zooplankton samples and remove organic material. The 10% sodium dodecyl sulfate (SDS) homogenising solution consisted of 400 mL Tris-HCI buffer, 120 mL ethylenediaminetetraacetic acid (EDTA), 30 mL sodium chloride (NaCl), 100 mL sodium dodecyl sulphate (SDS) and 350 mL Milli-Q water.

2.2.2. Filtration of zooplankton samples

Prior to filtration, samples were simultaneously shaken and incubated at 50 °C for 24 h to help digest any remaining biological material. Each sample was then poured through a 500 μm mesh directly into the filtration vessel and rinsed thoroughly with Milli-Q water. The mesh was used to catch remaining empty carapaces from the zooplankton, which could interfere with FTIR analysis. Then the sample was filtered onto a 13 mm silver filter (5 μm pore size, Sterlitech, USA) using a vacuum pump. The filtration vessel was thoroughly rinsed with Milli-Q and a few drops of ethanol (30%) to remove any debris that may be adhered to the vessel. The filter was transferred to a sterile Petri dish and left to dry at room temperature. Filters were stored at 3 °C until used in FTIR analysis.

The 500 μm mesh used to filter larger undigested sample material was backwashed with Milli-Q water into a filtration vessel and filtered onto a 10 μm mesh, then rinsed and processed as above. Filters were then stored at 3 °C until visually inspected for any microplastic debris.

2.2.3. Filtration of water samples

The filters used to collect the water samples were gently rinsed with Milli-Q water and a few drops of ethanol (30%) and the filtrate was collected in a filtration vessel. Filters were visually inspected using an Olympus (SZX16) microscope to ensure the sample had been rinsed off. They were then filtered and processed using the same methodology as the above zooplankton samples. The filter was then placed into a sterile Petri dish and left to dry. Filters were stored at 3 °C until FTIR analysis.

2.3. Microplastic identification

FTIR spectroscopy to identify microplastic particles was performed on a PerkinElmer Spotlight 400 (PerkinElmer, UK) in reflectance mode. Spectral imaging was carried out at a resolution of 16 cm^{-1} using 4 accumulations (4 scans per spectrum) at a pixel resolution of 6.25 μm and an interferometer speed of 1 cm s^{-1}. Scans were carried out from 4000 to 750 cm^{-1}. All spectra were corrected for light reflectance penetration and baseline displacement using a clean silver filter (5 μm pore size, Sterlitech) as a background sample. Each sample, on a 13 mm silver filter, required 16 h to be scanned entirely.

The free software programme, SIMPLE (https://simple-plastics.eu/), was used to quantify and identify particles by comparing spectra to a polymer database with reference spectra of known plastic polymers (Primpke et al., 2020). Sample spectra were matched against the database using a Pearson's correlation coefficient threshold of 0.65 against the first and second derivative. This threshold was used as a compromise between allowing for spectral modifications that may occur due to weathering in the marine environment and having a reasonable confidence in the spectral match

(Johnson et al., 2020). The second and third thresholds that were used for particle building (the pixels adjoining the particle already identified as a polymer) were set using the Pearson's correlation coefficient thresholds of 0.4 and 0.3, respectively. Anthropogenic cellulosic fibres (e.g. rayon) return the same spectra as cellulose within the SIMPLE software, and due to their structure, not all of the fibre is often in the correct plane of focus for scanning. Therefore, the images generated using the FTIR prior to scanning, were visually checked for the presence of anthropogenic fibres using colour and structure as markers of anthropogenic origin. The lengths of the fibres were measured using the Olympus cellSens software on an Olympus (SZX16) microscope.

2.4. Contamination and prevention of microplastic loss

2.4.1. On-board quality assurance/quality control (QA/QC)

QA/QC procedures were designed and implemented at all stages to reduce sample contamination. Metal and glass equipment were used as much as possible; all equipment was thoroughly cleaned with ethanol (70%) and triple rinsed with Milli-Q water prior to use. The same personal protective equipment was worn for the duration of the sampling and stored separately. Sample fibres were taken from all clothing, along with any potential contaminants such as ropes, pipes etc. to be analysed alongside zooplankton samples. Blank control vials containing only homogenising buffer (5 mL) were prepared alongside zooplankton samples at each station. Blank controls were also taken in parallel with the water samples using the filtration rig and mesh covered filtration vessel with no water sample.

2.4.2. Laboratory QA/QC

Samples were prepared and analysed in an ultra-clean laboratory (positive pressure system with HEPA filters, cotton lab coats, key card entry and tact mats) at Plymouth Marine Laboratory in a positively pressured laminar flow hood. All surfaces were thoroughly cleaned with ethanol (70%) before use. Glass and metal equipment were used where possible, and consumables were used directly from sterile packaging. All equipment was triple rinsed with Milli-Q water before use. When not in use, samples were kept covered. Natural fibre clothing was worn underneath a clean 100% cotton laboratory coat, stored within the laboratory to avoid contact with synthetic fibres.

Background laboratory contamination was assessed by exposing a damp filter paper (47 mm, Whatman, UK) in a clean Petri dish for the duration of the experimental work to catch airborne microplastics. These were sealed and labelled for further analysis. Samples of potential contaminants such as sterile packaging, natural clothing fibres etc. were taken and added to the FTIR reference library to be analysed alongside zooplankton samples.

Positive controls, in triplicate, of known spiked microplastic quantities were conducted to assess the capture efficiency of our filtration methodology. Ten fluorescent 20 μm spherical polystyrene beads (Spherotech, USA) and ten 19 × 250 μm Nile Red stained nylon fibres (Goodfellow Cambridge Ltd., prepared following the method by Cole (2016)) were added to each positive control vial. These controls were processed and filtered using the same methodology as for the zooplankton samples (see Section 2.2.2). Using a microscope (Olympus SZ X16) with fluorescence, silver filters were visually inspected and microplastics of both types counted. Using this methodology with these sized microplastics, our capture efficiency was 97%.

Negative controls, in triplicate, for sample filtration were taken by filtering Milli-Q water and a few drops of ethanol (30%) to take into consideration any contamination from the filtration process. These negative controls were also processed using the same methodology as for the zooplankton samples (see Section 2.2.2). Silver filters were left to dry in sterile Petri dishes and then stored in a fridge at 3 °C until used in FTIR analysis.

2.5. Statistical and mapping analyses

All data were analysed using Microsoft Excel (Microsoft Corporation, 2018) and the statistical software R (version 3.4.1, R Development Core Team, 2017). Data were tested for normality using a Shapiro–Wilk test, and homogeneity of variance was assessed by using the Fligner-Killeen test (Thomas et al., 2013). Microplastic ingestion was calculated as the total number of microplastic particles ingested/No. of organisms. Whilst the amphipod species were collected individually, they were pooled together to match that of the copepods to keep both sets of data constant. A Kruskal Wallis test, with following pairwise comparisons using Dunn's test, was used to compare the ingestion of microplastics between species, and also the relationship between species and the size of the microplastics ingested (Thomas et al., 2013). Size differences between the ingested microplastics in the 2019 samples and those found within the water samples were analysed using a Mann-Whitney *U* test. It was also used to investigate the differences in the mean number of microplastics ingested by copepods between 2018 and 2019 and mean microplastic size between 2018 and 2019. A Spearman's rank coefficient was used to compare the correlation between ingestion of microplastics per organism and distance to land, sea ice, latitude and longitude. Identical tests were carried out for microplastics found in water samples. A Spearman's rank coefficient was also used to compare the correlation between microplastic size and latitude for both biota and water samples. A Fisher's exact test (Thomas et al., 2013) was conducted to assess differences in the polymer compositions of water and zooplankton samples. The significance level for all tests was set at $\alpha = 0.05$.

Land and coastline data were sourced from Natural Earth (http://www.naturalearthdata.com) and imported into ArcGIS 10.2.2 (ESRI, 2011). Geographic locations of sampling stations were provided as longitude and latitude (WGS1984). An assessment of the sea ice condition in Fram Strait at the time of sampling was made using standard satellite products. The applied sea ice concentration product is provided by the Center for Satellite Exploitation and Research (CERSAT) and based on 85 GHz SSM/I brightness temperatures, using the ARTIST Sea Ice (ASI) algorithm. The product is available on a 12.5 × 12.5 km grid (Ezraty et al., 2007). This data set was then also used to calculate the distance between sampling locations and the ice edge. For this, we first smoothed the sea ice concentration data set by convolution with a 2 × 2 grid cell kernel. Next, the shortest distance between the sampling location and an area with more than 15% ice cover is calculated.

3. Results

3.1. Number of microplastics found

We assessed microplastic ingestion in several species of zooplankton including the copepods *Calanus hyperboreus* ($n = 177$) and *Calanus glacialis/finmarchicus* ($n = 1229$), and the amphipods *Themisto libellula* ($n = 5$), *Themisto abyssorum* ($n = 5$) and *Apherusa glacialis* ($n = 1$). All species were found to have ingested microplastics, all of which were fragments ($n = 64$). Ingestion of microplastic (total number of microplastic particles ingested/no. of individuals) varied between species with relatively more amphipods found to have ingested microplastics than copepods. The mean number (±SE) of microplastics ingested per zooplankton individual was 1.8 ± 0.2 in *T. libellula* (frequency of occurrence ((no. of microplastics found/no. of individuals analysed)*100): 180%), 1 in *T. abyssorum* (100%), 1 in *A. glacialis* (100%), 0.21 ± 0.03 in *C. hyperboreus* (21%), and 0.01 ± 0.003 in *C. glacialis/finmarchicus* (1%) (Table 1). There was a significant difference in the ingestion of microplastics (per individual) between species (Kruskal-Wallis test, $H = 407.76$, d.f. = 4, $P = 2.2 \times 10^{-16}$). Pairwise comparisons using Dunn's test indicate that *T. libellula*, *T. abyssorum*, and *A. glacialis* had ingested significantly more than *C. hyperboreus* ($P < 0.001$ for all tests) and *C. glacialis/finmarchicus* ($P < 0.001$ for all tests). *C. hyperboreus* had ingested significantly more microplastics than *C. glacialis/finmarchicus* ($P < 0.001$). There was no significant correlation between the ingestion of microplastics per individual and distance to land (Spearman's rank $r_s = -0.05$, $P = 0.86$), sea ice ($r_s = 0.19$, $P = 0.49$), latitude ($r_s = -0.49$, $P = 0.15$) or longitude ($r_s = -0.17$, $P = 0.62$). Comparing the difference in microplastic ingestion per individual in copepods

Table 1
Ingestion of microplastics by zooplankton from the Fram Strait.

Species	No. of individuals analysed	No. of microplastics found	Incidence of ingestion for amphipods that were processed individually (no. of ZP that ingested MP/total no. ZP digested)	Ingestion of microplastics (total no. of microplastic particles ingested/no. ZP digested) mean ± SE	Frequency of occurrence (N microplastics in N individuals)
Calanus finmarchicus/glacialis	1229	12	n/a[1]	0.01 ± 0.003	1 in 102 (1%)
Calanus hyperboreus	177	37	n/a[1]	0.21 ± 0.03	1 in 5 (21%)
Apherusa glacialis	1	1	1	1	1 in 1 (100%)
Themisto abyssorum	5	5	0.6	1	1 in 1 (100%)
Themisto libellula	5	9	0.6	1.8 ± 0.2	2 in 1 (180%)

[1] Cannot be calculated due to individuals pooled in samples.

for each year, *C. hyperboreus* ingested substantially more microplastics in 2019 (Mann-Whitney U = 638.5, $P = 0.056$), and *C. finmarchicus/glacialis* ingested significantly more microplastics in 2018 (Mann-Whitney U = 284, $P = 2.2 \times 10^{-16}$) (**Supplementary materials Table S3**).

Six water samples (2 L) were collected at the same stations as the zooplankton samples during JR18007. In total, we found 32 microplastic particles and 52 fibres in these samples. The average microplastic concentration was 7 MP L^{-1} (range: 0–18.5 MP L^{-1}) (7000 MP m^{-3}; range 0–18,500 MP m^{-3}) (**Supplementary material Table S2**). There was no correlation between the number of microplastics found at each station with distance to land (Spearman's rank $r_s = 0.07$, $P = 1$), distance to ice ($r_s = 0.58$, $P = 0.14$) or latitude ($r_s = -0.2$, $P = 0.72$).

3.2. Size of microplastics found

The microplastic fragment sizes found in the zooplankton samples ranged from 8 to 286 μm with a mean size ± SE of 41 ± 6 μm. The majority (75%) of the microplastics were below 50 μm in size (Fig. 2a). Further breakdown of this size category showed that microplastics between 11 and 20 μm were the most common (Fig. 2b). Analysis of the size difference showed that there was a significant difference between species (Kruskal-Wallis, $H = 21.82$, d.f. = 4, $P = 0.0002$). Pairwise comparisons using Dunn's test indicated that *C. hyperboreus* had ingested significantly smaller microplastics than *T. abyssorum* ($P = 0.03$), *T. libellula* ($P = 0.01$), and *C. finmarchicus/*glacialis ($P = 0.02$) (Table 2). Comparing the size of the ingested microplastics in each year showed that *C. hyperboreus* ingested significantly smaller microplastics in 2019 (Mann-Whitney U = 93, $P = 0.02$). There was no significant difference in the microplastics size for *C. finmarchicus/glacialis* between 2018 and 2019 (Mann-Whitney U = 18, $P = 0.8$) (**Supplementary materials Table S3**). There was a significant positive correlation between the size of the ingested microplastics and latitude (Spearman's rank $r_s = 0.61$, $P = 7 \times 10^{-8}$) with the size of the microplastics increasing as latitude increases.

The mean size of the microplastics fragments found in water samples was 69 (±9) μm (range: 6.3–271 μm). The microplastic particles ingested by zooplankton (JR18007) were significantly smaller than those found in the water samples (Mann-Whitney U = 3290, $P = 2.7 \times 10^{-15}$). The average length of the fibres was 577 (±77) μm (range: 45–2552 μm)

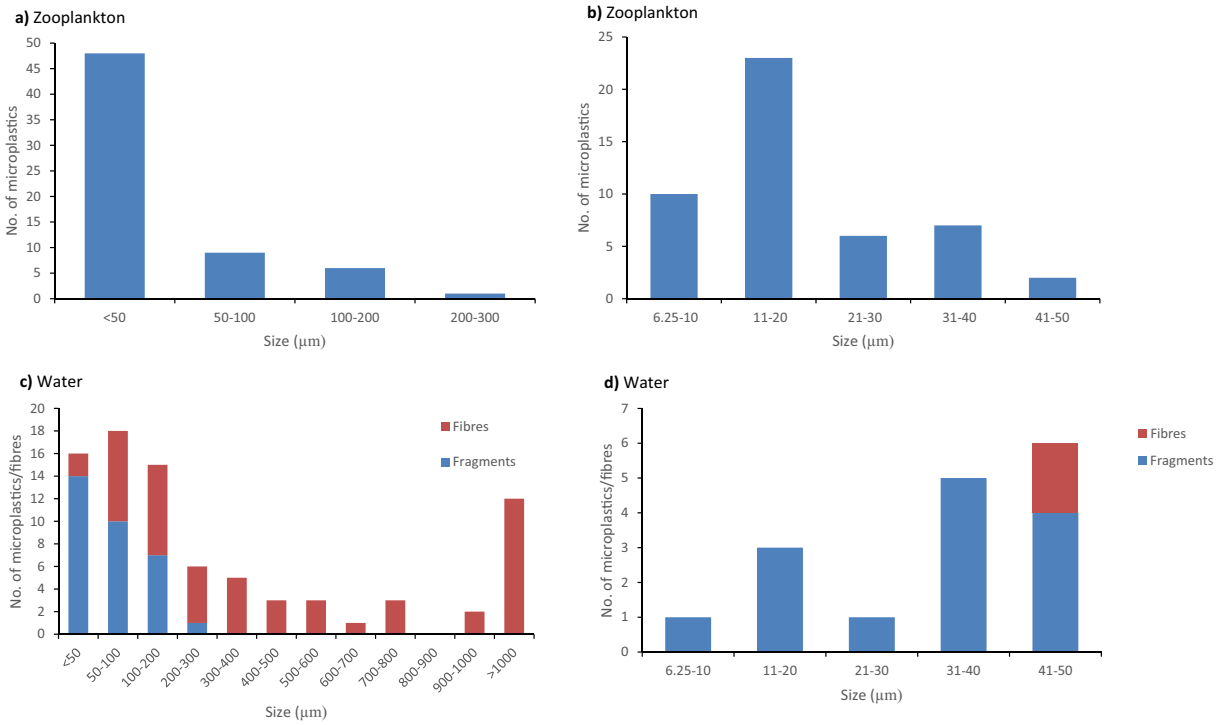

Fig. 2. a) Size distribution of microplastic fragments ($n = 64$) found within the copepod and amphipod samples, b) Size distribution of microplastic fragments smaller than 50 μm ($n = 48$) in zooplankton, c) Size distribution of the microplastic fragments ($n = 32$) and fibres ($n = 52$) found in the water samples, d) Size distribution of fragments ($n = 14$) and fibres ($n = 2$) smaller than 50 μm in the water samples.

Table 2
Mean microplastic size and polymer types ingested by zooplankton species (PE: polyethylene; PS: polystyrene; PU: polyurethane; PVDC: polyvinylidene chloride).

Species	Mean microplastic size ± SE (range) (μm)	Polymer types identified
Calanus finmarchicus/glacialis	58.2 ± 13.4 (8–158)	Acrylic, PS, PU
Calanus hyperboreus	26.2 ± 7.5 (8–286)	PS, PU
Apherusa glacialis	31	Polyester
Themisto abyssorum	64 ± 14.8 (33 − 113)	Acrylic, PE, Polyester
Themisto libellula	65.4 ± 16.8 (15–146)	Acrylic, PE, PS, PU, PVDC

(Fig. 2d, **Supplementary material Table S2**). There was no correlation between the size of microplastics and latitude (Spearman's rank $r_s = -0.15$, $P = 0.16$).

3.3. Microplastic polymers found

Six plastic polymers were identified in the zooplankton samples (Fig. 4). Polyurethane (PU) was the most prevalent (66%, $n = 42$), followed by acrylic and polystyrene (PS) (both 11%, $n = 7$), polyethylene (PE) (6%, n = 4), polyester and polyvinylidene chloride (PVDC) (both 3%, $n = 2$). Polymer types varied between the different size categories, with the greatest diversity found in the smallest size category (Fig. 3). Those species that had a higher ingested particle mean size also had the highest diversity of microplastic polymers, i.e. the amphipods *T. abyssorum* and *T. libellula* (Table 2).

In the water samples, anthropogenic cellulose fibres were most common ($n = 54$, 63.5%), followed by six polymer types; PS ($n = 21$, 25%), PVDC (n = 4, 4.8%), PU ($n = 3$, 3.6%), PE (n = 2, 2.4%), PVC and PA (both $n = 1$, 1.2%) (Fig. 4). The stations 114-4, NT11 and F7 had the greatest diversity of polymer types. While zooplankton samples contained exclusively polymer fragments, anthropogenic cellulose fibres dominated the water samples. Many of the polymers found ingested are also present in the water samples, however they varied in number and between stations (Fig. 4). There was a significant difference between the polymer types present in the water and zooplankton samples at all of the stations, which had both zooplankton and water samples (NT11 - Fisher's exact test, $P = 2.2 \times 10^{-16}$, F7 - $P = 0.007$ and D3 - $P = 0.009$).

3.4. Blank results

No contaminating particles from the polymers PU, PVDC and acrylic were found in any of the blanks taken and there were no particles found on any of the laboratory air contamination filters. There was limited contamination from PE, polyester, anthropogenic cellulose fibres, PA and PS. Despite considerable efforts to limit contamination of samples, there was still persistent PP contamination in our sample blanks and negative controls. To take this contamination into account, the number of each microplastic polymer found in the samples were adjusted by subtracting the average number of each polymer found in the procedural blanks from sample values. Average number of particles found on the blanks (where this was less than 1, it was rounded up): PE = 1, polyester = 1, anthropogenic cellulose fibres = 1, PA = 1, PS = 1 and PP = 28.

A blank control was taken alongside each water sample. There were no contaminating particles from the polymers PU and PVDC found, but there was limited contamination from PE, anthropogenic cellulose fibres, polyester, PA, PS and PVC. Similar to the zooplankton sample blanks, persistent PP contamination was present in all our water sample blanks. Using the same method as above, microplastics counts in samples were adjusted by subtracting the average number of each polymer from the six procedural blanks. Average number of particles found on the blanks (where this was less than 1, it was rounded up): PE = 2, polyester = 1, anthropogenic cellulose fibres = 1, PA = 1, PS = 5, PVC = 1 and PP = 8.

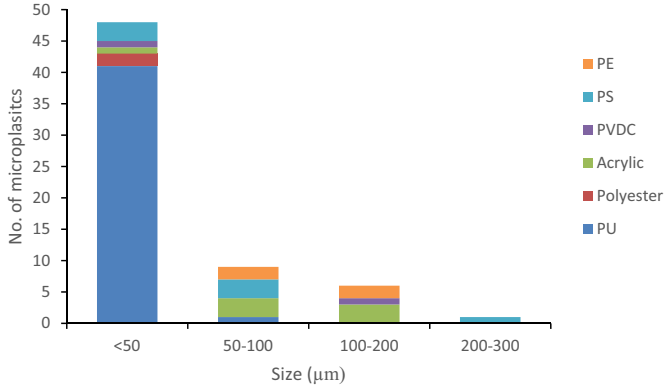

Fig. 3. Polymer types within different size ranges found in zooplankton samples (PE: polyethylene; PS: polystyrene; PU: polyurethane; PVDC: polyvinylidene chloride).

4. Discussion

This is the first study to use FTIR scanning spectroscopy, in combination with automated polymer identification software, to investigate microplastic ingestion by zooplankton. Using this novel technique, we were able to identify that the majority of microplastics found ingested were below 50 μm in size and therefore could have previously been missed by other standard techniques. All five species of Arctic zooplankton investigated had ingested microplastics. Amphipod species i.e., *Themisto* spp. and *Apherusa glacialis*, which are mainly found in surface waters or closely associated with ice, had ingested significantly more microplastics than copepod species (*Calanus finmarchicus/glacialis*, *Calanus hyperboreus*). We also showed that, contrary to our hypothesis, microplastics found ingested by zooplankton were not representative of those found in water samples. In comparison with the water samples, those microplastics found ingested were all fragments, had significantly different polymer composition, and were also significantly smaller (mean size 41 μm in zooplankton, 383 μm in water samples), indicating that the smaller sized microplastics were being selected for by the zooplankton. Recent research on water samples taken from the Fram Strait and the North Atlantic Ocean has shown that the smallest microplastics are often found at the highest concentrations (Lindeque et al., 2020; Tekman et al., 2020). If the smaller microplastics also have a higher bioavailability to zooplankton, these organisms could be at particular risk of ingesting high concentrations of microplastics. This could have negative impacts not only for the zooplankton themselves but for the species that depend on them as a food source promoting bioaccumulation.

Whilst the impacts of microplastic ingestion in the field are currently unknown, several laboratory studies have shown that if the zooplankton are ingesting microplastics it is likely that they are ingesting less natural prey (Cole et al., 2013, 2019; Coppock et al., 2019). This could lead to an energy deficit that could result in impacts on growth, development, reproduction, and life span (Lee et al., 2013; Cole et al., 2015; Lo and Chan, 2018). Microplastic ingestion by *Calanus helgolandicus* also led to 1.7–3-fold decreased in metabolic rates and time spent swimming, similar to that of starving copepods (Isinibilir et al., 2020). In addition, chemical additives, either present on the surface of the plastic or incorporated within, have been shown to potentially be a contributing factor causing premature moulting in the copepod *Calanus finmarchicus* (Cole et al., 2019).

4.1. Microplastic ingestion by Arctic zooplankton species

Some of the highest marine microplastics concentrations to date have been reported from Arctic surface waters (Tekman et al., 2020) and sea ice (Peeken et al., 2018), indicating that zooplankton found in the Arctic

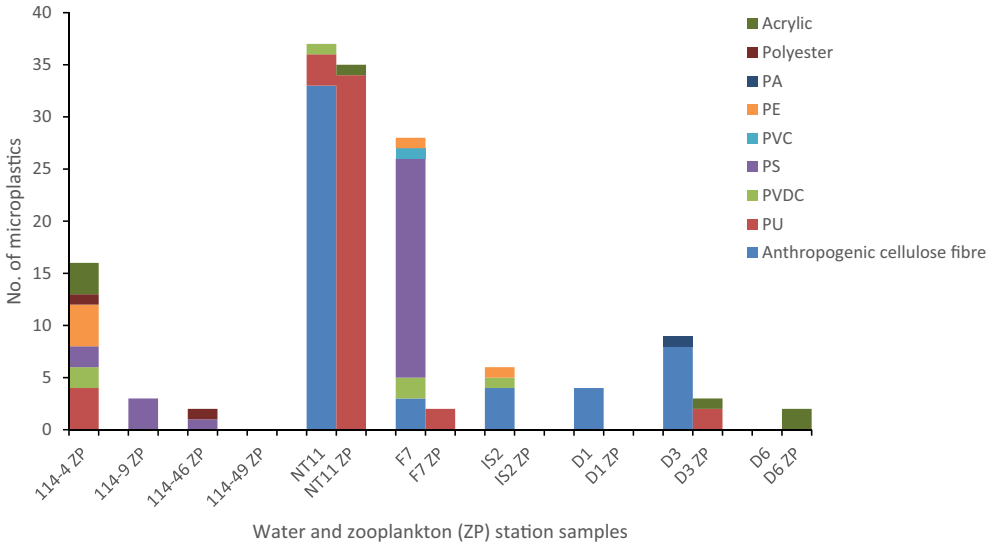

Fig. 4. Comparison of microplastic polymers found in water and zooplankton (ZP) samples at each station (PA: polyamide; PE: polyethylene; PS: polystyrene; PU: polyurethane; PVC: polyvinyl chloride; PVDC: polyvinylidene chloride). During PS114, no water samples were taken.

may have a greater chance of encountering microplastics in the environment. Of the five species of Arctic zooplankton investigated, the amphipod *T. libellula* had the highest frequency of occurrence (180%), followed by *T. abyssorum* (100%) and *A. glacialis* (100%). These species typically inhabit the highly productive surface waters (epipelagic: *Themisto*) or are associated with sea ice (sympagic: *A. glacialis*) in the Arctic where algal blooms ensure that there is a sufficiently high abundance of prey (Kraft et al., 2013; Kunisch et al., 2020). They are an important food source for many species, especially polar cod (McNicholl et al., 2016) and seabirds (Dalpadado et al., 2001, 2008), and have been identified as possible indicator species for biomonitoring in the Arctic (Collard and Ask, 2021). *Apherusa glacialis* is a herbivorous-detritivorous suspension-feeding species (Poltermann, 2001), whereas *T. abyssorum* and *T. libellula* are considered omnivorous visual feeders (Kraft et al., 2013). These species may have directly ingested microplastics from the water, having mistaken them for prey, or may accumulate microplastics via consumption of other zooplankton species, which may have ingested microplastics themselves (Setälä et al., 2014). If so, the accumulation from prey items may explain the higher microplastic ingestion, however further research into the retention times of both predator and prey species would be essential. By contrast, the copepod species investigated had a much lower frequency of occurrence (21% in *C. hyperboreus*; 1% in *C. glacialis/finmarchicus*). Copepods were sampled in both years of sampling and showed a high interannual variation in microplastic ingestion. In 2018, *C. finmarchicus/glacialis* ingested significantly more microplastics per individual than in 2019. However, the opposite was true for *C. hyperboreus*, which ingested substantially more microplastics per individual in 2019. These species are pelagic suspension feeders primarily consuming phytoplankton. Since copepods use chemosensory cues to locate their prey (Breckels et al., 2013), the presence of infochemicals from algal blooms or biofilms could influence microplastic uptake as they mimic the scent of prey (Botterell et al., 2020). Indeed, Tekman et al. (2020) reported a correlation between microplastics quantities and particulate organic carbon indicating interactions with biological processes in the water. The short time period of food available to Arctic zooplankton during the spring/summer bloom means that any negative effects due to microplastic ingestion could affects individuals at a critical life stage in terms of energy budgets, development, and lipid storage. Recent research by Rodríguez-Torres et al., 2020 has shown that exposure to microplastics (200 and 20,000 MP L^{-1}) in Arctic copepods caused stress-induced spawning, which could impact population dynamics due to a temporal mismatch in nauplii development and maximum food availability.

Research from the Canadian Arctic reported microplastic presence in 90% of the zooplankton samples with a mean particle concentration of 3.51 particles g^{-1} (range: 0–16 particles g^{-1}) (Huntington et al., 2020). However, a direct comparison of their findings with our data is limited as samples were processed in batches of unknown number of different species. Elsewhere, 1 in 125 (0.8%) amphipods from Antarctica have also been shown to ingest microplastics, yet the lower concentrations of microplastics reported in water samples may mean that there is a lower chance of encountering microplastics in the environment (Jones-Williams et al., 2020). Microplastic ingestion in zooplankton from regions, which have reported high microplastic concentrations in surface waters (i.e. South China Sea), report frequencies of microplastic occurrence of up to 8% in copepods, and up to 143% in predatory species such as fish larvae (Sun et al., 2017). Whereas in the northeast Pacific Ocean, 1 in 34 (3%) copepods were found to have ingested microplastics (Desforges et al., 2015), and in the Black Sea, 2.1% in *Calanus euxinus* and 0.8% in *Acartia clausi* (Aytan et al., 2022). These studies highlight the variation in microplastic ingestion in similar zooplankton species in different regions. In all these studies, however, microplastics were manually picked out from samples by hand, which may mean that not all microplastics, especially from the smallest size fraction, will have been captured. This may explain in part the differences in reported ingestion between these studies and our values reported here.

Of the five species investigated, *C. glacialis/finmarchicus* were the smallest (2–4 mm (Leinaas et al., 2016)), followed by *C. hyperboreus* (6–7 mm (Leinaas et al., 2016)), *A. glacialis* (7–16 mm (Kunisch et al., 2020) and Themisto spp. (5–18 mm (Koszteyn et al., 1995)). Ultimately, the size of any microplastic ingested is going to be constrained by the gape size of the species' mouthparts. Those species with a larger gape will have a wider range of microplastics sizes bioavailable to them (Botterell et al., 2019). As seen in our results, whereby the larger *Themisto* amphipods had the highest mean size of ingested microplastics. Additionally, predatory species (i.e. amphipods) may show a wider range of ingested microplastic sizes due to accumulation of smaller particles ingested by their prey (Setälä et al., 2014). These factors highlight that the life history and ecology of certain species could put them at increased risk of microplastic ingestion.

The majority of the microplastics found ingested by the zooplankton were below 50 μm in size and were all fragments. In comparison to the microplastics found in the water samples, they were significantly smaller, indicating that the smaller-sized microplastics were potentially being selected for by the zooplankton. Fragments were also primarily found in

pelagic copepods from the Black Sea (Aytan et al., 2022). In addition, recent experiments on the closely related *Calanus helgolandicus* showed a significant preference for the smallest particles when offered microplastics of 6, 12, and 26 μm diameter (Isinibilir et al., 2020). However, polymer analysis indicated that some of these small fragments found are polymer types that are typically fibres (e.g. polyester). This could be due to fibres fragmenting into very small pieces that no longer resemble classic fibres. Recent research has shown that in only a matter of months, UV degradation (under laboratory conditions) causes fragmentation of fibres, which could easily occur over the Arctic summer timescale with potentially 24 h of sunlight (Sørensen et al., 2021) or while entrained in sea ice, whose cold temperature may affect the crystalline structure of plastic (Peeken et al., 2018). In addition, fragmentation through ingestion by the organism could also be a contributing factor to the smaller sizes found. The freshwater amphipod *Gammarus duebeni*, for example, fragmented polyethylene spheres into a variety of different shapes and sized fragments, including nanoplastics (Mateos-Cárdenas et al., 2020) as did Antarctic krill (Dawson et al., 2018).

The greatest diversity of polymers was found at the smallest size range, consistent with fragmentation of larger plastics that have been in the environment for a long time. Whilst a wide range of polymer types were found within the zooplankton, PU was the most commonly ingested. Nearly all (98%) of these fragments are below 50 μm in size, which may have increased the bioavailability. However, to show these particles were actively selected, a selectively index (e.g. Ivlev's selectivity index) would need to be calculated (Lechowicz, 1982; Moreno-Rueda et al., 2018). This is complicated for field data, however, as the total number of potential prey items (including microplastics in this case) is required. Unless this is explicitly measured, with large sample/volume sizes, this is not possible to calculate. Different polymers will break down at different rates because of differences in polymer structure. In PU, the ester bond in the mainchain causes the polymer to break down more easily via hydrolysis, photo-oxidation, and biodegradation (Gewert et al., 2015). As a result, PU is more likely to fragment than other polymers, which may explain the small sizes found in our samples.

While our zooplankton ingestion data are a snapshot in time, with egestion rates of 2–168 h reported for *Calanus finmarchicus* and *Calanus helgolandicus* (Cole et al., 2013; Vroom et al., 2017) and 16 h for the freshwater amphipod *Gammarus fossarum* (Blarer and Burkhardt-Holm, 2016), they do provide a vital glimpse of the present microplastic burden in these important species in a remote and fragile ecosystem, which is strongly exposed to the effects of climate change. Any alteration to energy budgets in Arctic zooplankton species could be critical, as there is only a short period of time when food becomes available to the organisms for lipid storage, which is vital for over-wintering. Current research from the Barents Sea shows that in areas where sea ice extent has decreased substantially, the timing of the peak phytoplankton spring bloom has already advanced by over a month (Dalpadado et al., 2020). As this region warms the abundance of Arctic zooplankton species such as *C. glacialis* and *T. libelula* has already decreased (Dalpadado et al., 2012; Aarflot et al., 2018).

4.2. Microplastics in Arctic water samples

We found that the average concentration of microplastics in the water samples varied between stations; from 0 MP m^{-3} at D6 to 18,500 MP m^{-3} at NT11. A similar variation in concentrations and high microplastic concentrations (range: 0–1287 particles m^{-3}) have previously been reported for the Fram Strait (Tekman et al., 2020). However, due to methodological constraints, the Tekman study did not include fibres. As fibres are a prevalent anthropogenic contaminant, found in other studies examining Arctic water and ice, it is likely that these concentrations are an underestimate (Lusher et al., 2015; Rist et al., 2020). Still, even without fibres, microplastic concentrations from sea ice in the Fram Strait are some of the highest currently reported microplastic concentrations (1.2×10^7 m^{-3}) worldwide (Peeken et al., 2018). This sea ice is exported south with the Transpolar Drift, through the Fram Strait, eventually melting, representing an important transport vector, sink and source of microplastics in the Fram Strait and the North Atlantic (Krumpen et al., 2016; Peeken et al., 2018). The water masses at those stations toward the west of the Fram Strait are likely characterised by the East Greenland Current/Transpolar Drift, whereas the other stations are probably influenced by the Atlantic-West Spitsbergen Current (Fahrbach et al., 2001; Beszczynska-Möller et al., 2012). However, in this study, there was no correlation between either the number of microplastics in water or zooplankton samples and with distance to ice, distance to land, latitude, or longitude. Except for the size of microplastics in zooplankton samples, which showed a positive correlation with latitude, indicating that as latitude increased so did microplastic size. Yet this could be linked to species, as *C. hyperboreus* was shown to ingest significantly smaller microplastics, which when compared to the station information, predominately occurred at the lowest latitude station.

In comparison to the microplastics found within the zooplankton samples, fibres were present as well as fragments in the water samples. In addition, there was a much larger range of microplastic sizes present (6.3–2552 μm), but there was a similarly high diversity of polymer types. The most common size class was 100–200 μm followed closely by <50 μm, with the majority of those microplastics under 50 μm being fragments and all particles over 300 μm being fibres. This agrees with other studies in the same region using similar methodology where the majority of the microplastics identified were below 50 μm in size (Peeken et al., 2018; Bergmann et al., 2019; Tekman et al., 2020). This highlights the importance of using scanning FTIR and an automated polymer identification approach (e.g. SIMPLE software) to include smaller particles that otherwise escape detection. But it also crucially indicates that the majority of the microplastics found have a high bioavailability to zooplankton (Botterell et al., 2019). Whilst this method provides vital information to understand ingestion in the field, it is a time consuming and labour-intensive technique requiring very clean samples (i.e., little other organic or inorganic material remaining) which may not be appropriate for all field-based studies. We need a combination of monitoring studies, that take less time and provide a broad estimate of risk (i.e., the encounter rate), but also more detailed studies such as ours to better understand the fundamental mechanism of the interactions between zooplankton and microplastics, which is essential to understand the complex relationship and ultimately to better interpret the risks.

Whilst the water samples taken at each station were of a small volume (2 L), they do provide an important indication of microplastic concentrations, size distribution and polymer types present in a remote and difficult area to access. Moreover, it enables comparisons with microplastics found within zooplankton, which inform on whether this is representative of the microplastics available in the marine environment or indicates selectivity. It should be noted that while the zooplankton samples were taken in vertical tows from 0 to 200 m, water samples originated from a discrete water depth of 6.5 m, which may have caused some bias. However, the data can be compared since zooplankton migrate vertically throughout the water column (Turner, 2015). Vertical water sampling, on the other hand, would have resulted in excess organic matter that would have rendered analysis via FTIR virtually impossible even after additional sample purification steps. Previous research has shown that, in comparison to net tows, sampling with grab bottles (1 L bottles used in Green et al. (2018) or using plastic-free pumps (Rist et al., 2020) is the most effective way of capturing smaller microplastics. However, further research has shown that there can be high within-site variation and using larger grab water volumes (10*L*) combined with multiple replicates is recommended to provide a more accurate estimate of microplastics abundances in water samples (Ryan et al., 2020). Sampling from the ship is complicated, as there are many sources of contamination, which require rigorous control measures and protocols, such as multiple blanks and recording of any clothing, equipment, and potential sources in the sampling area to be compared to samples in the FTIR library, as conducted in this study. An additional problem of operating on ships in cold environments is the prevalence of fleecy material that is commonly used in clothing (Jones-Williams et al., 2020). Limiting the use of fleece and ensuring any is covered during sampling is

recommended. Lastly, as a ship passes through an area it can release microplastic pollution through paint, equipment such as rope, and grey water including washing machine effluent, creating a contamination 'footprint' as it travels (Leistenschneider et al., 2021). To minimise this contamination in our samples, water samples were taken as soon as we arrived on station as washing machine usage was prohibited. Samples of paint and equipment from the sampling area were taken, scanned into the FTIR, and compared to those found in samples.

5. Conclusion

In our study, we highlight which microplastics (size, shape) have the highest bioavailability to Arctic amphipods and copepods, which species have the highest risk of microplastic ingestion and report, in accordance with other studies, high microplastic concentrations in surface waters. Understanding the factors behind microplastic ingestion is an important step to understanding the risk threshold to a species, population, and an ecosystem. As the Arctic continues to warm due to climate change, increasing quantities of ice-bound microplastics will be released and ice-free areas will be open to known polluting anthropogenic activities such as fishing, shipping, and resource exploitation. At the same time, global plastic production continues to grow and rising production leads to increasing plastic leakage to the environment (Borrelle et al., 2020; Lau et al., 2020) beyond safe planetary boundaries (Persson et al., 2022), with more microplastics occurring due to increasing fragmentation of legacy plastic that is already in the environment (Barnes et al., 2009; Hohn et al., 2020). This subsequently leads to the inevitable increase of plastic pollution in the Arctic, exacerbating the pressure from climate change on an already vulnerable ecosystem (Lannuzel et al., 2020). Global action is needed to reduce the leakage of plastic pollution to the environment.

CRediT authorship contribution statement

All authors have agreed to be listed and have approved the submitted version of our manuscript. ZLRB conducted the research and led the writing with contributions from MB, NH, MS, RCT, and PKL (conceptualization: ZLRB, PKL, MB; data collection: ZLRB, NH; TK; methodology, data analysis and writing-original draft: ZLRB, supervision: PKL, MS, RCT; writing-review and editing: all authors).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.154886.

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