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Summer sea ice melt and wastewater are important local sources of microlitter to Svalbard waters

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ABSTRACT

Human activities leave traces of marine litter around the globe. The Arctic is, despite its remoteness, emerging as an area of no exception to this environmental issue. Arctic sea ice has previously been found to constitute a temporal sink of microplastics, but the potential release and subsequent fate of microplastics in the marine environment are yet unknown. Furthermore, the relative importance of local sources of microplastics in the Arctic marine environment is under discussion. In this study, the concentration and distribution of anthropogenic microparticles (AMPs, < 5 mm, including microplastics) have been investigated in marine waters and sea ice of Svalbard. Seawater samples throughout the water column and floating sea ice samples were collected along a transect originating in Rijpfjorden, reaching northward to the sea ice-edge. Seawater samples were also collected along a transect extending westwards from head to mouth of Kongsfjorden. Samples were collected throughout the water column with stations positioned to enable detection of potential AMP emissions from the wastewater outlet in Ny-Ålesund. Along both transects, environmental parameters were measured to explore potential correlations with AMP distribution. High concentrations of AMPs were detected in sea ice (158 ± 155 AMPs L⁻¹). Based on both AMP concentrations and characteristics, AMPs identified in seawater of the marginal ice zone are to a large extent likely released during the melting of sea ice. The release of AMPs during summer melting of sea ice was concomitantly taking place with the ice-edge bloom, suggesting increased bioavailability to Arctic marine biota. Concentrations of AMPs were up to an order of magnitude higher in Kongsfjorden (up to 48.0 AMPs L⁻¹) than in Rijpfjorden (up to 7.4 AMPs L⁻¹). The distribution and composition of AMPs in Kongsfjorden suggest the wastewater outlet in Ny-Ålesund to be a likely source. Our results emphasize the importance of local point- and diffuse sources of AMPs in the Arctic and stress the urgency of considering their associated environmental impact. Implementation of regulatory policy is of importance, particularly since human activities and environmental pressures are increasing in the Arctic.

1. Introduction

Anthropogenic marine litter is found all around the globe (Li et al., 2016) and despite its remoteness, the Arctic is no exception (Bergmann et al., 2017a). Studies have shown that the quantity of marine litter found on beaches around Svalbard is similar to amounts found in more densely populated areas of the world, and that over 80% of the litter consists of plastic (Bergmann et al., 2017b). Tekman et al. (2017) found a marked increase in plastic litter density km⁻² in 2014 compared to 2002 at 2500 m depth in the Fram strait, particularly in the smaller fractions below 10 cm. Furthermore, deep-sea sediments collected at the scientific sampling station “Hausgarten” in the Fram strait were found to be highly polluted by plastic, holding between 42 and 6595 plastic particles (≥11 µm) kg⁻¹ dry weight (Bergmann et al., 2017c). Improved knowledge on sources, distributions, temporal sinks and areas of elevated bioavailability (in time and space) of microplastics in the Arctic marine environment are needed to identify risks and mitigation strategies through science-based management.

Abbreviations: AMP, anthropogenic microparticle (human produced- or modified materials, including microplastics); WWO, wastewater outlet; PCC, procedural contamination control

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Although microplastics in the environment have been widely studied during the recent decade, non-synthetic human-modified materials such as wool and cotton have been largely overlooked (Ladewig et al., 2015). Non-synthetic materials have, however, recently been identified as being of emerging concern due to their high detected abundance when measured and reported (Barrows et al., 2018; Stanton et al., 2019). Non-synthetic materials such as cotton and wool are, similarly to synthetic materials, often industrially treated with several chemicals and pigments. Due to differences in surface properties and persistence they are likely to have different fate in the marine environment, and the relevance of also including non-synthetic particles in studies of anthropogenic microlitter has been put forward (Barrows et al., 2018; Ladewig et al., 2015). There is an ongoing discussion about appropriate size definitions, with arguments both for keeping the 5 mm upper limit established in monitoring programs and for moving to a more SI consistent 1 mm upper limit (Frias and Nash, 2019; Hartmann et al., 2019; Kershaw et al., 2019). Here, a definition up to 5 mm was used to enable comparison to the majority of existing studies. The collective term used for all synthetic and non-synthetic anthropogenic particles is from now on anthropogenic microparticles (AMPs) in the current study.

The few existing studies on microplastics in sea ice identify the ice as a temporal sink for AMPs. Sea ice samples from across the high Arctic were first found to contain a concentration of micropolystics several orders of magnitude higher than reported in surface waters from the North Pacific subtropical gyre (Obbard et al., 2014). It was further verified by Peeken et al. (2018) who found even higher microplastic concentrations in pack ice from the Fram strait and north of Svalbard, as well as in land-fast ice from east Greenland. It has therefore been suggested that a pulse of micropolystics could be released during seasonal and/or climate change induced sea ice melting, where older multi-year ice could release micropolystics sequestered under longer time-periods (Obbard et al., 2014; Peeken et al., 2018). So far, this process has neither been modelled nor studied in greater detail.

The relative importance of local point sources versus diffuse transport of AMPs from lower latitudes is currently largely unknown in the Arctic (Hallanger and Gabrielsen, 2018; PAME, 2019). Large amounts of floating fragmented plastic litter have been observed in the northern and eastern parts of the Greenland and Barents Seas, and the hypothesis of a “plastic conveyor belt”, following the patterns of the global thermohaline circulation, has been put forward (Cózar et al., 2017). Results from studies on dispersal routes of floating buoys released around the globe suggest that there may be a sixth garbage patch in Barents Sea, in addition to the five previously identified in other oceans around the world (van Sebille et al., 2012). Ocean currents thus seem likely to transport litter to the Arctic from neighboring seas. Transportation from more densely populated areas around the North Atlantic has been further supported as a route by Lusher et al. (2015), who found that coastal surface waters of Svalbard, diluted by freshwater, contain less micropolystics than warmer offshore Atlantic water. However, the study by Lusher et al. (2015) did not include the actual fjord systems. The North Pacific has also been suggested to contribute with micropolystics into the Arctic Ocean via the inflow through Bering strait (Obbard et al., 2014; Peeken et al., 2018).

Local sources of AMPs are vastly unexplored in Svalbard (Granberg et al., 2019; PAME, 2019). Potential point sources of pollution have been proposed to include wastewater outlets, dumping sites, shipping and fishing activity as well as land- and ocean-based industries (Granberg et al., 2017). Wastewater discharge has previously been indicated to constitute a point source of micropolystics to the environment, also in polar regions (Magnusson et al., 2016a, 2016b; Murphy et al., 2016; Reed et al., 2018). Lusher et al. (2015) reported high concentrations of microfibres as well as polymers heavier than seawater outside the west coast of Svalbard, which were suggested to at least partially possibly originating from wastewater outlets or other local sources of Svalbard. Wastewater treatment is generally lacking in the Arctic. However, since 2015, a small-scale wastewater treatment system has been installed in Ny-Ålesund, Kongsfjorden (Granberg et al., 2017). The general retention efficiency of AMPs in wastewater treatment plants is largely varying, from negligible removal to > 99% (Leslie et al., 2017; Magnusson et al., 2016b; Magnusson and Wahlberg, 2014). Wastewater from Ny-Ålesund, a remote settlement facilitating a year-round research community (~ 50 persons in winter, ~ 170 in summer), could therefore represent a point source of AMPs in this otherwise largely uninhabited area. Granberg et al. (2019) performed a pilot study where AMPs in incoming and outgoing wastewater from the wastewater treatment plant in Ny-Ålesund were quantified, and the treatment plant was found to retain > 99% of the incoming AMPs. Yet, the outgoing wastewater contained a concentration of 83 AMPs L⁻¹, thus constituting a point source to Kongsfjorden. This was further supported by higher concentrations of AMPs measured close to the wastewater outlet compared to reference sites (Granberg et al., 2019). Considering the rapidly increasing industrial development and tourism activities in the Arctic, infrastructure is highly under-dimensional and local contamination sources cannot be considered negligible.

Consensus regarding the relative importance of different environmental matrices as sinks for microplastics is increasing, and the very surface water seems less important than previously thought (Cozar et al., 2014; Sherrington, 2016). Evidence that AMPs are in fact distributed throughout the water column is emerging (e.g. Bagaev et al., 2017; Kanhai et al., 2018; Railo et al., 2018). This calls for research on mechanistic explanations of particle behavior in terms of distribution and transport dynamics towards the sediment, which is believed to hold most sequestered micropolystics (Sherrington, 2016). Since AMPs consist of a range of materials of diverse morphologies and sizes, and with different intrinsic properties such as density and surface charge, these characteristics will influence dynamics and fate in the marine environment (Bagaev et al., 2017; Kooi et al., 2016). The distribution of AMPs throughout the water column can also be affected by biotic parameters such as biofilm formation (Lobelle and Cunliffe, 2011; Rummel et al., 2017), interaction with biota (Lusher, 2015) and marine snow (Porter et al., 2018). Abiotic parameters affecting the vertical distribution of AMPs are e.g. salinity, temperature, currents, mixing and the prevailing sedimentation and advection regimes (Crichtell and Lambrecchts, 2016; Welden and Lusher, 2017). Micropolystics have indeed been identified throughout the water column in the Arctic central basin with the highest concentrations in the polar mixed layer (0–50 m depth) (Kanhai et al., 2018). The vertical distribution of AMPs should further be addressed in Svalbard waters, and also investigated in coastal environments in order to understand fate processes related to local pollution sources.

The objectives of this study were to quantify, characterize and describe the occurrence and spatial (vertical and horizontal) pattern of AMPs throughout the water column in two fjords in Svalbard; Kongsfjorden and Rijpfjorden. Sampling was performed in transects to assess the relative importance of sea ice as a temporal sink - and potential diffuse source of AMPs, and to evaluate a local wastewater outlet as a point source of AMPs to the Arctic marine environment. Higher concentrations of AMPs were expected in seawater of both the marginal ice zone and in the vicinity of the wastewater outlet of Ny-Ålesund compared to more distant regions. Hydrographic parameters were further assessed to explore potential mechanistic support for AMP distribution and fate processes.

2. Methods

2.1. Study site

Sampling stations were arranged along two transects following a longitudinal gradient through Kongsfjorden located at the west coast of Spitsbergen and a latitudinal gradient from Rijpfjorden located at the northern coast of Nordaustlandet (Fig. 1). These two fjords are oceano-graphically different, where Rijpfjorden is dominated by Arctic
surface water and glacial meltwater, and Kongsfjorden is characterized by an inflow of Atlantic and Arctic waters as well as an input of glacial meltwater. The transects covered a bathymeric gradient including both shallow shelf areas and the deeper continental slope towards the Nansen basin, with sampling depths ranging from surface water to 1000 m (Table 1, Table A-1). Transects and sampling stations were located to enable an assessment of input of anthropogenic microparticles (AMPs) to seawater from sea ice north of Rijpfjorden and from the wastewater outlet (WWO) in Ny-Ålesund (abbreviated as MP1-MP4 in the figure).

2.2. Sample collection and processing

The field study was conducted between the 29th of July and 8th of August 2017 on-board RV *Lance*. In total, 10 stations (MP1, MP2, MP4, KB3, KB1, R1, R3, R5, R7, R7-b) were visited for sampling of AMPs at defined depths throughout the water column (Table 1). Sea ice was sampled at three of these stations (R1, R3, R7). Water samples were collected in Niskin bottles (8 L) using a rossette sampler mounted around a central conductivity, temperature and depth (CTD) package (Sea-Bird SBE9; depth, temperature, salinity, density, fluorescence, oxygen, 1 m averaged for each sampling depth of AMPs). The sampler descended to the bottom and based on the obtained profile, three to five sampling depths were chosen during ascent. Depths were determined to be at least one above (A), one below (B) and one at the pycnocline (P). When the pycnocline was very wide or vague, the chlorophyll a maximum was targeted since it usually coincided with the pycnocline. When possible, samples were also collected at greater depths (D, > 100 m). To avoid resuspension and sediment contamination, the deepest sample of each station was never collected deeper than 10 m above the seafloor. Water was subsampled from Niskin bottles via silicone tubing. Collecting containers (white plastic canisters, 4 L), lids and tubing were before-hand rinsed with filtered seawater (GF/F, 0.7 μm, Whatman) followed by rinsing three times with the sample water itself. Collected water was vacuum filtered through 10 μm filters (Nucleopore, Track-Eth Membrane, Polycarbonate, Whatman) supported by a glass microfiber filter (GF/F, 0.7 μm, Whatman) underneath. To avoid salt crystal formation, filters were rinsed with 50 ml Milli-Q water (18.2 MΩ cm TC, 0.22 μm, Millipore) before dark storage in individual sealed petri-slides (47 mm gamma-sterilized polystyrene, Millipore). Filtration took place in the onboard laboratory. During filtration, the exposed water surface in the used metal funnel, as well as all other equipment used, was covered with clean (new) aluminum foil at all times possible. The filtered water volume ranged from 1930 ml (KB3, 10 m) to 4400 ml (MP4, 81 m). When using Niskin bottles for sampling of water, there is a drawback in terms of the volume available for investigation, as particle counts may be too low to conduct statistical analyses, or even below the detection limit. On the other hand, advantages of the sampling method include accessibility to targeted sampling depths and that it may have a lower contamination risk compared to other methods where air exposure can be harder to control (however, note that there can be a risk of old Niskin bottles starting to shed particles). These are trade-offs that should be considered upon choosing sampling method. CTD casts providing standard hydrographical information throughout the water column were performed at all stations shown in Fig. 1.

Pieces of free-floating sea ice (approximately 40 × 20 × 20 cm) were sampled directly from the deck at RV *Lance* at stations R1, R3 and R7 using a metal boat hook and a green plastic basket connected to a white rope. The pieces of sea ice were immediately placed in pre-rinsed (three times with filtered seawater, GF/F, 0.7 μm, Whatman) plastic buckets (white or dark blue) and left to thaw covered in clean aluminum foil in the onboard laboratory. The samples were then filtered according to the same protocol as the seawater samples, excluding addition of Milli-Q water. The filtered volumes of melted sea ice were 3130 ml (R3), 3690 ml (R1) and 4020 ml (R7).

Table 1

<table>
<thead>
<tr>
<th>Area</th>
<th>Included stations</th>
<th>Sampled depths (m)</th>
<th>n</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>WWO</td>
<td>MP1, MP2, MP4</td>
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<td>10</td>
<td>Seawater: wastewater outlet</td>
</tr>
<tr>
<td>KB3</td>
<td>KB3</td>
<td>5, 10, 100, 300</td>
<td>4</td>
<td>Seawater: central Kongsfjorden</td>
</tr>
<tr>
<td>KB1</td>
<td>KB1</td>
<td>0, 7, 100, 300</td>
<td>4</td>
<td>Seawater: outer Kongsfjorden</td>
</tr>
<tr>
<td>R</td>
<td>R1, R3, R5</td>
<td>5, 10, 11, 25, 100</td>
<td>9</td>
<td>Seawater: Rijpfjorden</td>
</tr>
<tr>
<td>MIZ</td>
<td>R7, R7-b</td>
<td>5, 11, 15, 50, 100, 951, 1000</td>
<td>3</td>
<td>Seawater: marginal ice zone</td>
</tr>
<tr>
<td>ICE</td>
<td>R1, R3, R7</td>
<td></td>
<td>5</td>
<td>Sea ice</td>
</tr>
<tr>
<td>PCC</td>
<td></td>
<td></td>
<td></td>
<td>Procedural contamination control</td>
</tr>
</tbody>
</table>
2.3. Procedural contamination controls

Along with the substantial contamination precautions mentioned above during sample collection and processing, procedural contamination controls (PCC) were performed. Five replicates of filtered seawater (GF/F, 0.7 µm, Whatman) were filled (via silicone tubing) and exposed in identical canisters as the actual seawater samples next to the CTD during a sample collection from the Niskin bottles and processed in the same way as the actual seawater samples. The volume ranged from 4000 to 4130 ml. To simulate possible sources of contamination of sea ice samples, scraping tests in a plastic bucket and off the hull of RV Lance were performed to examine similarities in particle color and morphology.

2.4. Visual analysis of anthropogenic microparticles

The filters were examined under a stereomicroscope (Leica M205C, magnification 7.8–160 ×) where potential AMPs were classified based on morphology (evenness, roundness), color (homogeneity, shininess) and texture (stiffness, brittleness). The limit of detection in terms of particle size was estimated to > 50 µm, as it below this size becomes very challenging to visually investigate the texture of a particle. All suspected AMPs were photographed (Leica DFC420C), and length and width determined as the two largest measurements (Leica Application Suite 4.8.0). No analysis based on length and width of identified particles in sea ice was performed. Identified AMPs were visually sorted into two categories; fibers and fragments.

2.5. Spectroscopic analysis of anthropogenic microparticles

A subsample of the particles visually identified as AMPs were analyzed with Fourier Transform infrared spectroscopy (FTIR) for validation of the visual classification along with polymer specific identification. The FTIR (Thermo Scientific, NicoletiN10) was run in reflection of the visual classification along with polymer specific identification and was run in reflection of the visual classification along with polymer specific identification. The FTIR (Thermo Scientific, Nicolet iN10) was run in reflection mode with 256 scans (resolution 4 cm⁻¹, spectral range 4000–675 cm⁻¹). All particles larger than 100 × 30 µm from the seawater samples were analyzed (n = 34/584, 6%). From the ice samples, a random subset of 12 particles from each sample (n = 36/1579, 2%) were analyzed with FTIR. Library search (OMNIC Picta) to reference polymer spectra libraries was utilized and correlative match rates with similarities over 70% were accepted, below 60% rejected and between 60 and 70% carefully examined to ensure concordant key peaks as previously done in studies of AMPs in the Arctic (Kanhai et al., 2018; Obbard et al., 2014).

Lately, semi-synthetic cellulose fibers such as rayon/viscose have been reported as a major part of marine microfibers also in the Arctic (Lusher et al., 2015; Obbard et al., 2014). However, the possibility to distinguish between natural and man-made cellulose fibers by comparing FTIR spectra from available search libraries to environmental particles is under discussion (Comnea-Stancu et al., 2017). Comnea-Stancu et al (2017) found that the application of library search as an aid in identification of polymers might not be reliable unless the library spectra are generated with the same acquisition mode as the investigated samples. Therefore, to avoid overestimation of semi-synthetic fibers, a library of 100% cotton fibers (white lab coat, n = 3) was created and added to the collection of search libraries. Similarly, a library of 100% wool fibers (white clothing: n = 3, grey raw untreated: n = 1, brown raw carded: n = 1) was created due to its spectral similarity to polyamide. Additionally, a library of pre-degraded commonly reported polymers in the marine environment was created and included (polyethylene (PE), polypropylene (PP), polyester (PS) and polyethylene terephthalate (PET)). After FTIR analysis, the visual categories fibers and fragments were further divided into subcategories; synthetic, semi-synthetic and non-synthetic fibers and fragments respectively. Two categories of particles that were visually determined to be anthropogenic but did not produce any known FTIR spectra was included in the present study and referred to as “unknown-pigment” and “paint” due to a suspicion of it originating from synthetic pigments and paints. Particles classified as “acryl blend” comprise polyacrylonitrile, polystyrene:acrylonitrile:methacrylate and polymethylmethacrylate.

2.6. Identification of water masses

To evaluate potential correlations between AMP concentration and water mass, major hydrographical features were analyzed and water masses defined in accordance to Svendsen et al. (2002) and Cottier et al. (2005). Based on combinations of salinity and temperature features, water masses were categorized into Atlantic water (AW: transported from lower latitudes with the West Spitsbergen current; warm and high salinity), transformed Atlantic water (TAW: cooled down AW; cooler and high salinity), surface water (SW: influenced by sea ice melt, freshwater and glacial runoff; cold and low salinity), intermediate water (IW: formed through mixing between SW and underlying AW or TAW; medium warm and medium salinity), and Arctic water (ArW: deep water of the Arctic ocean, flowing into the East Spitsbergen current; cold and high salinity but not as high as AW).

2.7. Data analysis and statistics

The concentration of AMPs is in the present study measured as count data, where low counts result in a Poisson distribution rather than a normal distribution of the data. When particle counts are low (≤ 5) the statistical power becomes too low to accurately compare concentrations between stations and/or matrices (Karlsdottir et al., 2018). When AMP-counts were high enough (i.e. > 5), abundance-based differences in AMP spatial distribution as well as characteristics (color: 13 different; morphology: fiber or fragment; size: 5 size classes 10–100, > 100–300, > 300–1000, > 1000–5000, > 5000 µm) were evaluated between and/or within each matrix (seawater, sea ice and PCC). To test for geographical differences, stations were pooled into “areas”, thus increasing the count of AMPs as well as the number of replicates within that area (Table 1). All calculations on abundance of AMPs in water and sea ice were performed based on the full particle count from stereomicroscopy, i.e. not only the FTIR subsamples. Evaluation of the difference in polymer assemblage at different areas was based on the presence or absence of specific polymers, i.e. not abundance based.

Multivariate analyses were performed in PRIMER-E (6.1.13) with the PERMANOVA + (1.0.3) extension (Clarke and Gorley, 2006) using Bray-Curtis similarity on square root transformed abundance (AMPs L⁻¹) or presence/absence data (1/0). If non-metric multi-dimensional scaling (nMDS) plots showed tendencies of clustering, one-way analysis of similarities (ANOSIM) was performed. Here, a global R-statistic based on 4999 permutations was created with a significance level of 0.05, followed by pairwise tests between groups generating local R-statistics. Groups were tested against each other in different set-ups (matrices, stations, areas and depths) to investigate patterns of AMP concentrations and characteristics. Comparisons between matrices included all samples belonging to seawater, sea ice and PCC respectively. In comparisons between stations, each sampled depth at a station constituted one data point. Comparisons between areas included several stations pooled together (Table 1). The comparison between different depth categories was based on all samples belonging to a certain depth category grouped within areas and/or full transects (i.e. Rijp jorden and Kongsfjorden separated). Between areas, analysis of similarity percentages (SIMPER) subsequently revealed the relative contribution of primary particle characteristics (i.e. polymer, size & morphology, color) to the dissimilarity between groups. If necessary, values were log-transformed to obtain homogenous variances. The
3. Results

3.1. Abundance and distribution of anthropogenic microparticles

The concentration of anthropogenic microparticles (> 50 µm, AMPs L⁻¹) in seawater ranged between 0.7 in deep water collected in outer Kongsfjorden (KB1) and 48.0 in surface water from central Kongsfjorden (KB3), corresponding to a particle count between 3 and 95 per sample. The number of AMPs were in general considered adequately high (i.e. > 5) to further explore differences between areas and depths. Procedural contamination controls (PCC) contained 1–6 AMPs per sample (> 50 µm, 3 ± 2) corresponding to an average of 0.7 ± 0.5 AMPs L⁻¹ which thus constitutes the detection limits in terms of particle count and concentration in this study. In relation to the detection limits, 32/36 samples were above the particle count detection limit and 35/36 above the particle concentration detection limit. The results of the PCCs was used for comparison to environmental samples in terms of AMP concentration (L⁻¹) and characteristics (morphology, size, color, polymer), i.e. no subtraction or correction of environmental data (sea ice and seawater) was made based on the PCC results. The average number of AMPs L⁻¹ (> 50 µm) in sea ice was 158 ± 155 with a particle count per sample between 221 (R7) and 1054 (R3). Thus, the number of AMPs was considered sufficiently high to further explore differences in abundance and particle characteristics between sea ice and seawater. The scraping test of the plastic bucket used for thawing sea ice did not produce particles resembling any of those identified in the sea ice samples. The scraping test of the ship hull demonstrated that the antifouling paint (in water contact) was red, and the above-water hull paint was blue. Due to their specific features, particles originating from such paints are easy to distinguish from other types of AMPs. AMPs with antifouling paint morphology were found in sea ice samples and occasionally also in sea water samples. These particles are included in the data set. Characteristics of all detected AMPs described in more detail (morphology, color and size distribution as well as identified polymers) can be found in supplemental material B.

Concentrations of AMPs were compared among different areas (each station with all its depths as individual data points within that area: for the pooling of stations into areas see Table 1), of where sea ice constitutes one area of itself. The highest concentration of AMPs L⁻¹ was identified in sea ice followed by seawater in the central part of Kongsfjorden, the marginal ice zone (MIZ) and the wastewater outlet (WWO), whereas the lowest concentrations were identified in seawater in the outer part of Kongsfjorden (KB1) and in Rijpfjorden (R) (Fig. 2) (One-way ANOVA, F₃,14 = 31.669, p < 0.0005).

No differences in the concentration of AMPs were identified between the four depth categories (A: above pycnocline, P: at pycnocline, B: below pycnocline, D: deep > 100 m) in Kongsfjorden (Kruskal Wallis, p = 0.814) or Rijpfjorden (One-way ANOVA, F₃,14 = 0.082, p = 0.969).

3.2. Spatial- and matrix related differences in AMP characteristics

3.2.1. Color

The color distribution of identified AMPs differed significantly between the matrices seawater, sea ice and PCC (ANOSIM, Global R = 0.618, p < 0.0005) (Fig. 3A). The main contributor to dissimilarity was blue in all comparisons, being most abundant in sea ice followed by seawater and least abundant in the PCC (Table C-1). The color blue was well represented in many different size classes as both fibers and fragments. Particle colors also differed between areas (ANOSIM, global R = 0.419, p < 0.0005) except between KB1-R, KB1-MIZ and WWO-MIZ (Fig. 3B, Table C-1). See Table C-1 for local R- and p-values of all pairwise comparisons. There was a higher abundance of blue AMPs in the marginal ice zone (MIZ) compared to inside Rijpfjorden (R). There was a higher abundance of blue and black AMPs in the central part of Kongsfjorden (KB3) compared to other areas (Table C-1).

3.2.2. Morphology and size

There was a significant difference in the “morphology and size” distribution of identified AMPs between the matrices seawater and the
The main contributor to dissimilarity was the category fibers >300–1000 µm, being more abundant in seawater than in the PCC (Table C-2). The morphology and size distribution of identified AMPs also differed between areas (ANOSIM, global R = 0.239, p < 0.0005), except between KB1-R, KB1-MIZ and WWO-MIZ (Fig. 3D, Table C-2). See Table C-2 for local R- and p-values of all pairwise comparisons. There was a higher abundance of fragments 10–100 µm in the MIZ compared to inside Rijpfjorden (R). There was a higher abundance of large fibers >1000–5000 µm at the WWO compared to outer Kongsfjorden (KB1) (Table C-2). No general pattern of size and morphology-dependent depth category distribution was identified across all stations (ANOSIM, global R = -0.056, p = 0.872) (Table C-2).

### 3.2.3. Polymer assemblage

Based on the dataset produced through FTIR analysis (subsampled AMPs from each matrix; n = 34 from seawater, n = 36 from sea ice, n = 3 from PCC), polymer assemblage was evaluated as presence/absence (1/0) of polymers in each sample, see section 2.7. The only difference in polymer assemblage identified between areas was attributed to cotton, which was more present at the WWO than in Rijpfjorden (R) (ANOSIM, local R = 0.119, p = 0.011). See Table C-3 for local R- and p-values of all pairwise comparisons. There was no difference in polymer assemblage between depth categories (ANOSIM, global R = 0.02, p = 0.193) (Table C-3). The total polymer assemblage of AMPs in the matrix seawater was different from that in the PCC (ANOSIM, global R = 0.053, p = 0.01). The main contributor to dissimilarity was PET, being more often present in the PCC (Table C-3). In fact, PET was the only identified polymer in the PCC. The polymers identified in seawater were paint, unknown-pigment (see Section 2.5), acrylic, PS, polyetherurethane, polyurethane (PU), cotton and wool (supplemental material B). The polymers identified in sea ice were unknown-pigment (see Section 2.5), PET, paint, polyamide (PA), low-density PE, urethane alkyd, acrylic, polyetherurethane, cellophane, cotton and wool (supplemental material B).

### 3.3. Correlation between environmental parameters, water masses and anthropogenic microparticles

Several of the measured environmental parameters were found to correlate with AMP-concentration in the water column of some areas (Table 2), but no overarching correlation was identified between AMPs L⁻¹ and environmental parameters across the two full transects (BEST, sample statistic = 0.057, p = 0.780).

#### 3.3.1. Rijpfjorden

A positive correlation was identified between fluorescence and AMP concentration in the marginal ice zone (MIZ) (R² = 0.629, p = 0.011) as well as along the Rijpfjorden transect as a whole (R + MIZ) (R² = 0.377, p = 0.007) (Table 2).

Along the transect in Rijpfjorden, the following water masses were identified: surface water, Atlantic water, transformed Atlantic water and Arctic water, displaying a typical hydrographic structure across the continental slope (Fig. 4). The inner part of Rijpfjorden was characterized by cold water (< 0°C) and a more pronounced low salinity surface layer due to input of glacial- and sea ice meltwater. Towards the marginal ice zone, the core of Atlantic water (high temperature, high salinity) was identified at a depth of 128 m between R6 and R7-b, but with a pronounced colder water mass with low salinity in the upper 50 m due to summer sea ice melt. Identification of water masses in
terms of sampling location demonstrated that AMPs were sampled from all present water masses except from transformed Atlantic water (Fig. 4). The northernmost sampling stations (R7 and R7-b) included Atlantic water and Arctic deep water in contrast to the other AMP sampling locations (R1, R3 and R5) where all depths were located in the surface water layer. Patterns of vertical AMP-distribution were graphically evaluated for individual stations and some potential trends may be seen (Fig. 4). The highest concentrations of AMPs (7.4 and 4.9 AMPs L$^{-1}$) at the stations in the marginal ice zone (R7 and R7-b) were detected in sub-surface layers, whereas lower concentrations of AMPs (1.2 and 3.7 AMPs L$^{-1}$) were found in the deeper layers of Atlantic- and deep Arctic water (Fig. 4). The highest concentrations of AMPs at stations R1 and R5 (2.0 and 1.5 AMPs L$^{-1}$) were identified in the very surface water. This was in contrast to R3 where the minimum

<table>
<thead>
<tr>
<th>Area</th>
<th>Environmental parameter (best fit)</th>
<th>Sample statistic (correlation)</th>
<th>p-Value</th>
<th>Linear regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R²</td>
</tr>
<tr>
<td>All stations</td>
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<td>0.550**</td>
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<tr>
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<td>0.109</td>
</tr>
<tr>
<td>(KB3 + WWO + KB1)</td>
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<td>0.379**</td>
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</tr>
<tr>
<td>R</td>
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</tr>
<tr>
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<td>Fluorescence &amp; Temperature</td>
<td>0.303</td>
<td>0.120**</td>
<td>0.377</td>
</tr>
</tbody>
</table>

Table 2: Correlations between environmental parameters and abundance of anthropogenic microparticles (AMPs, >50 µm) in seawater of different areas and transects. Evaluated by BEST-analysis and linear regression. BEST-analysis generates a selection of environmental parameters best describing the AMP abundance. These environmental parameters were then individually analyzed with linear regressions. N.s. refers to non-significant, * to p < 0.05 and ** to p < 0.01. KB3: central Kongsfjorden, WWO: wastewater outlet (MP1, MP2 & MP4), KB1: outer Kongsfjorden, R: Rijpfjorden (R1, R3 & R5), MIZ: marginal ice zone (R7 & R7-b).

Fig. 4. Vertical distribution of anthropogenic microparticles (AMPs, > 50 µm) in Rijpfjorden and identified water masses. (A) Vertical distribution of AMPs L$^{-1}$ at the different stations along the Rijpfjorden transect. MIZ: marginal ice zone, R: Rijpfjorden. Created with Ocean Data View (Schlitzer, 2018), (B) CTD surfer plot presenting fluorescence (chlorophyll a, mg m$^{-3}$) along the Rijpfjorden transect. Black dots represent the sampling locations of AMPs. Created with kriging gridding in Surfer (Golden Software). Note that the plot demonstrates the upper 300 m as in contrast to graph A, (C) temperature-salinity plot of all CTD data points along Rijpfjorden transect, where AMP concentrations are marked with symbols from figure A in different size classes, representing the relative concentration of AMPs within each station. Boxes represent the identified water mass written as acronyms within/next to the respective box; SW: surface water, AW: Atlantic water, TAW: transformed Atlantic water, ArW: Arctic water. Black lines represent the isopycnals.
concentration of AMPs (1.0 AMPs L⁻¹) was found in the very surface water, rather showing a tendency to increase with water depth and density (Fig. 4).

3.3.2. Kongsfjorden

A positive correlation was identified between temperature and AMP concentration in outer Kongsfjorden (R² = 0.744, p = 0.028) (Table 2). Identified water masses in Kongsfjorden were surface water, intermediate water, Atlantic water and transformed Atlantic water. Major hydrographical features were high temperature water (> 6.5 °C) in the outer part due to spreading of Atlantic water, and low salinity water (< 31.5 PSU) in the surface of the inner part of the fjord due to input of glacial meltwater. Well pronounced frontal zones were discovered across the whole depth between KB7 and KB4 and extending to the central part (KB3) in the very surface water (< 10 m), separating oceanic high temperature and high salinity water from low temperature and low salinity water from inner Kongsfjorden. The highest concentration of AMPs (48.0 AMPs L⁻¹) in Kongsfjorden was identified at the detected frontal zone in the very surface layer of central Kongsfjorden (KB3) (Fig. 5). The lowest concentrations of AMPs (0.7–1.7 AMPs L⁻¹) were measured throughout the water column in the outer part of Kongsfjorden (KB1). Of the three stations at the wastewater outlet (MP1, MP2, MP4), MP1 demonstrated the highest concentration of AMPs (11.4 AMPs L⁻¹) in the very surface water. When graphically evaluating the concentration of AMPs in the different water masses, a trend of higher concentration in the very surface water was observed across all stations except at MP2, where the concentration instead was higher in the deeper Atlantic water (Fig. 5).

4. Discussion

4.1. Rijpfjorden: Sea ice as a temporal sink and diffuse local source of anthropogenic microparticles

Sea ice contained up to two orders of magnitude higher concentration of anthropogenic microparticles (AMPs, > 50 µm) compared to seawater along the Rijpfjorden transect. The highest concentration of AMPs in seawater of this transect was detected in the subsurface layers of the marginal ice zone (MIZ) at the northernmost two stations R7 and R7-b. Concentrations of AMPs were positively correlated with fluorescence. High fluorescence values were found in surface waters of station R7 and R7-b which indicates that this area was the position of the ice-edge bloom at the time of sampling. Arctic sea ice displays seasonal dynamics of withdrawing each summer, releasing nutrients and allowing light to reach the water column with a subsequent bloom following along the melting edge zone (Sakshaug et al., 2009). The observed higher concentrations of AMPs in seawater of the MIZ compared to more open waters may therefore be a result of the melting of sea ice releasing sequestered AMPs. Since sampling was not conducted during several time periods, AMP concentration alone cannot provide a clear answer to whether sea ice is the source. However, the theory was further supported by identified patterns in particle characteristics of sea
ice and seawater. The particle morphology “fragment” dominated in sea ice, whereas “fibers” in general dominated in seawater (supplemental material B). The main difference in AMP morphology in seawater along Rijpfjorden transect was indeed fragments, being more abundant in the area of melting sea ice (MIZ) than within the fjord itself (R). Furthermore, the AMP color distribution along the transect also suggests sea ice as a point of AMP release since 65% of the AMPs identified in sea ice were blue (supplemental material B) and the average abundance of blue particles in seawater increased with proximity to the sea ice-edge.

High concentrations of AMPs were identified in all three sea ice samples along the Rijpfjorden transect, whereas relatively high concentrations of AMPs in seawater were only identified in the MIZ. This confirms that sea ice is a temporal sink of AMPs, becoming a diffuse source of AMPs in the active melting zone. Although there was sea ice to be found to some extent along the whole Rijpfjorden transect, it was only in the MIZ that a solid ice cover was found. Therefore, the amount of melt water will here be a lot higher than further south along the transect. Upon release, AMPs do not seem to stagnate in the surface of the water column but rather to re-distribute either by sedimentation, advection or interaction with biota and/or marine snows. In comparison with findings in seawater from the Arctic central basin by Kanhai et al (2018), the concentrations of AMPs recorded in Rijpfjorden in the current study were generally an order of magnitude higher than the maximum recorded abundance from the Arctic central basin (polar mixed layer: 8–51 m, largely influenced by sea ice melt). This difference between the studies may be due to the geographical differences and/or the inclusion of non-synthetic AMPs in the particle count in our study. Here, non-synthetic AMPs represented overall 57% of the particles analyzed and identified with FTIR (i.e. particles > 100 × 30 µm). Furthermore, a larger filter mesh size was used by Kanhai et al (2018) (250 µm) compared with the present study (10 µm).

Several observations in the current study point to the fact that sequestered AMPs are released from melting sea ice at the time of the ice-edge bloom, creating an AMP “hotspot” where increased bioavailability of AMPs to the sympagic and pelagic ecosystems could be the consequence. The ice-edge bloom is a constrained period in time and space of very high biological activity across trophic levels (Bluhm et al., 2018; Sakshaug et al., 2009). There is reason for concern that these Arctic organisms may be subjected to elevated concentrations and increased bioavailability of AMPs and associated chemicals during this event, while aiming to stock up necessary resources for the coming winter. Exposure to microplastics has experimentally been associated with reduced intake of prey in e.g. copepods, crabs, bivalves and worms which can cause alterations in the energy budget and the scope for growth and reproduction (Cole et al., 2015; Rist et al., 2016; Watts et al., 2015; Wright et al., 2013), however it should be noted that such experimental exposure studies have often been conducted with non-environmentally realistic microplastic concentrations. Alterations in the energy budget is a process that could be even more critical in the high Arctic, if one considers the short window of biological production that organisms’ phenology are heavily governed by (Sakshaug et al., 2009). Further investigations of effects on both the internal sea ice community and on the pelagic ice-edge community are now urgently needed, based on environmentally realistic concentrations of AMPs.

The occurrence of microplastics in Arctic sea ice has been reported twice before, presenting high (i.e. higher than most reported concentrations in seawater, see e.g. Rezania et al., 2018) abundances of 38–234 particles L−1 (Obbard et al., 2014) and 1100–12000 particles L−1 (Peeken et al., 2018). Peeken et al. (2018) suggested that the main contributing reason to the difference in particle concentrations between the two studies was the difference of applied analytical method. The study of Obbard et al (2014) relied on a first visual identification to select suspected particles for subsequent spectroscopic analysis, whereas Peeken et al (2018) applied direct FTIR-imaging, identifying that 67% of all particles found were in the smallest size class (11 µm). This size fraction would be largely, if not completely, overlooked during a visual analysis. The analytical methodology of the present study is similar to that of Obbard et al (2014) and presents an abundance of AMPs in the same order of magnitude as they found (including non-synthetic fibers: 158 ± 155 AMPs L−1, excluding non-synthetic fibers: 123 AMPs L−1). The high concentration of AMPs in sea ice could be a result from the scavenging behavior of ice crystals upon formation of frazil ice (Garrison et al., 1983, 1989; Geilfus et al., 2019). It could also be incorporated during the formation of anchor ice, which is formed at the bottom in shallow areas and may therefore enclose sediment related particles (Reimnitz et al., 1987). By the two previous studies of microplastics in Arctic sea ice, the main, although not only, suggested input pathway is long-distance transport with Pacific or Atlantic water rather than input from local sources (Obbard et al., 2014; Peeken et al., 2018). Atmospheric deposition has further been suggested as a potentially important pathway (Bergmann et al., 2016). Additionally, high volumes of Arctic sea ice are formed in the Laptvei sea where several large Russian rivers discharge water. Rivers are from other geographical areas known to be main transport routes of litter to the ocean (Lebreton et al., 2017). River-discharged litter could thus become incorporated during sea ice formation in the Laptvei sea region, subsequently being transported towards Svalbard via the transpolar drift, a process well-known for other pollutants (e.g. Rigor and Colony, 1997). The processes of AMP incorporation, sequestration, transportation and release from sea ice are all important and may further affect fate upon melting, and dynamics of other pollutants being released from the ice. No extensive conclusions regarding potential origin of AMPs in sea ice can be drawn from the here presented data, but a pattern corroborating a previous observation (Peeken et al., 2018) is the presence of shipping-related particles such as suspected paint particles. Peeken et al (2018) discovered large amounts of varnish, especially in the ice core collected north of Svalbard in the Nansen basin. In the present study, paint particles where found in sea ice samples and occasionally in seawater samples. AMPs with this typical paint morphology have previously been widely identified in e.g. sediment (Karlsson et al., 2019). There is a possibility that some observed paint particles in sea ice in the present study originate from the hull of the research vessel, as sea ice samples were obtained next to the ship. However, it is not considered likely as these AMPs were found in all three sea ice samples and active ice breaking only took place at the northermost station (R7). During ice breaking, blue particles could be spread from the research vessel to the ice, whereas during regular sailing in more or less open waters (i.e. at stations R1-R5) with occasional bumping into smaller sea ice pieces would generate, if any, red paint particles. No red paint particles were identified in any samples.

Lusher et al (2015) found that coastal Arctic surface water contained less microplastics than offshore Atlantic water, proposing Atlantic water as a vehicle for microplastic transport from more polluted areas around the North Atlantic, whereas coastal Arctic surface water was proposed to be rather unpolluted and/or diluted by less polluted freshwater. This would be in contrast to Obbard et al (2014), who, based on their findings of microplastics in sea ice, expected high microplastic input to Arctic surface water from the melting of sea ice. This would assume that particles released from melting sea ice stay afloat. Data from the present study further supports hypotheses that AMPs sequestered in sea ice will be released when the Arctic sea ice is melting as initially suggested by Obbard et al (2014). By the data provided here, additional steps are taken towards understanding the dynamics of such release events. An elevated AMP concentration in the immediate melting zone is detected, but with increasing distance to the melting zone the concentrations are decreasing thus suggesting sedimentation, advection and/or biological interaction. The release of AMPs from sea ice provides an example of solvent depletion where the carrying matrix disappears and the contaminant is released, a mechanism by which climate change may influence the distribution of environmental pollutants between matrices in the environment (Noyes et al., 2009).
4.2. Kongsfjorden: The wastewater outlet of Ny-Ålesund as a local point source of anthropogenic microparticles

The concentrations of AMPs (> 50 μm) measured along the Kongsfjorden transect were up to an order of magnitude higher than those measured along the Rijpfjorden transect. Higher concentrations of AMPs were found in central Kongsfjorden and at the wastewater outlet compared to the outer part of Kongsfjorden, especially in the shallow frontal zone between Atlantic and local waters in central Kongsfjorden (KB3: 48 AMPs L⁻¹ at 5 m depth). The wastewater outlet is positioned closest to sampling station MP1 at a depth of ~ 4–6 m. Here, high concentrations (> 10 AMPs L⁻¹) were found in surface water, suggesting that the wastewater outlet acts as a point source of AMPs. Furthermore, a particle characteristic supporting this theory was the high presence of larger fibers (> 1000–5000 μm) close to the wastewater outlet when compared to other areas in Kongsfjorden. This is in line with observations of high fiber concentrations in wastewater, originating from sources such as laundry wash water (Magnusson et al., 2016b; Salvador Cesa et al., 2017). Indeed, a study of anthropogenic microlitter in wastewater from Ny-Ålesund quantified a high concentration of 83 AMPs L⁻¹ in effluent wastewater to Kongsfjorden, consisting of 93% synthetic and non-synthetic fibers (Granberg et al., 2019).

In terms of polymer composition of identified AMPs, a significant difference was identified between the wastewater outlet (WWO) and Rijpfjorden (R) with cotton being present at the wastewater outlet and absent in Rijpfjorden. This furthermore indicates a potential particle source from Ny-Ålesund, probably largely consisting of clothing/textile fibers. The highest polymer richness was identified in the water column above the pycnocline right outside the wastewater outlet (MP1) with five different polymers present. This suggests that wastewater discharge contributes with an AMP-mixture into Kongsfjorden. In the study by Granberg et al (2019) the highest polymer richness values were detected in effluent wastewater and receiving seawater as compared to reference sites. Several of the polymers corresponded to observations from the present study, e.g. PU, PA, wool and cotton.

It has previously been suggested that a transport of AMPs away from the immediate vicinity of wastewater outlets is taking place, with AMPs mainly staying buoyant rather than becoming deposited in the sediment as found in a study from Longyearbyen, Svalbard (Sundet et al., 2016). A similar fate process could explain the high concentrations identified in central Kongsfjorden (KB3), 2–3 km from the wastewater outlet in the present study. Here, we further propose the importance of water mass characteristics and frontal zones for particle accumulation patterns. Further investigation of this phenomenon will need to cover spatial and temporal scales with a higher resolution.

The results of the present investigation, where AMPs (> 50 μm) were identified in seawater at all locations and depths at concentrations of 0.7–48.0 AMPs L⁻¹, are contradictory to previous studies where no, or noticeably lower, levels of AMPs were found in Kongsfjorden (Granberg et al., 2019, Sundet et al., 2017). Discrepancies may be due to differences in hydrodynamics at the point of sampling, specific sampling sites, sampling methodologies and size fractions (as imposed by filter mesh size) analyzed (present study: > 10 μm, Sundet et al., 2017: > 250 μm, Granberg et al., 2019: > 50 μm). It should further be noted that there may be other important sources contributing to AMP pollution in Kongsfjorden, e.g. discharges from cruise ships and other boating activities, atmospheric deposition and meltwater from glaciers. Ship-based tourism, along with commercial shipping, is rapidly increasing in Svalbard and will likely continue to do so as climate change progresses. The number of cruise passengers disembarking outside the main settlements of Svalbard increased from around 24,000 in 1999 to 83,500 in 2017 (MOSJ, 2019). Compared to the peak number of inhabitants of Ny-Ålesund (~ 170 in summer), even occasional discharges of wastewater from cruise ships could cause significant pollution in remote polar regions such as Svalbard.

Land-based sources along the coast of Svalbard have traditionally been considered less important for Arctic pollution than long-range transport. When it comes to AMPs, this has been attributed to the low number of inhabitants, however, studies suggesting this like Lusher et al (2015) and Peeken et al (2018) did not include coastal fjord systems in their investigations. Our findings from Kongsfjorden highlight that local point sources are not negligible within individual fjords. However, a rapid decrease towards the mouth of the fjord seems to take place, potentially explaining the low concentrations previously detected by Lusher et al (2015) in more offshore coastal waters. Decreasing abundance of microplastics with increased distance from land-based sources have previously been observed in Antarctica (Munari et al., 2017; Reed et al., 2018).

Previous studies indicate that temperature, salinity and wind can affect the distribution of microplastics in Arctic waters (Kanhai et al., 2018; Lusher et al., 2015). In the present analysis, temperature did show a positive correlation with AMPs L⁻¹ in the outer part of Kongsfjorden. However, due to AMP concentrations being very low in that area (0.7–1.7 AMPs L⁻¹) and not statistically different from the PCGs, the correlation to temperature should be interpreted with caution. None of the measured environmental parameters were found to correlate with AMP concentrations in the wastewater discharge area. Assuming that the wastewater outlet constitutes a continuous point source of AMPs, the subsequent distribution of AMPs in the absolute vicinity of the discharge point is expected to be randomly mixed with the surrounding seawater. Further out in the fjord the distribution of AMPs is more likely governed by hydrographical conditions such as stratification and frontal zones between water masses.

5. Conclusions

In this study we present results demonstrating that high levels of anthropogenic microparticles (AMPs, > 50 μm) are sequestered in Arctic sea ice north of Svalbard. Based on both AMP concentrations and characteristics (morphology, size and color), we present data supporting the hypothesis of that AMPs are released to the seawater column during summer sea ice melting. This coincides with the ice-edge bloom which raises concern about potential ecological impacts due to the significance of this biological event during an Arctic year. The results suggest that the ice-edge bloom, a constrained event in time and space, could be particularly exposed to elevated levels of AMPs released from sea ice. To gain further insight into the ecological consequences of this synchronization of litter release and biological activities, studies with a higher temporal and spatial resolution are proposed along with experimental exposure studies on population- and ecosystem levels.

We further present data suggesting that a wastewater outlet in Kongsfjorden, Svalbard, constitutes a point source of AMPs with the subsequent distribution suggested to be mainly local within the fjord. The importance of hydrographical parameters and frontal zones between water masses for the distribution and fate of AMPs is emphasized.

Local sources (e.g. point sources as wastewater outlets) and temporal sinks (e.g. diffuse sources as sea ice) of AMPs are thus not negligible in the Arctic and may be as, or even more important than long-range transport of microlitter from other ocean basins. The data presented here contributes with essential steps towards understanding the fate and importance of AMPs from local sources in the Arctic marine environment.

CRediT authorship contribution statement

Lisa W. von Friesen: Data curation, Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing - original draft, Writing - review & editing. Maria E. Granberg: Conceptualization, Formal analysis, Funding acquisition, Methodology, Resources, Supervision, Validation, Writing - review & editing. Olga
Pavlova: Software, Validation, Visualization, Writing - review & editing. Kerstin Magnusson: Methodology, Supervision, Validation, Writing - review & editing. Martin Hasselöv: Methodology, Supervision, Validation, Writing - review & editing. Geir W. Gabrielsen: Funding acquisition, Project administration, Resources, Supervision, Writing - review & editing.

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 material

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References
