Microlitter in arctic marine benthic food chains and potential effects on sediment dwelling fauna

Nordic Council of Ministers

Contents

Preface

This report summarizes results from the two-year project 'Microplastics in benthic fauna and sediments in Arctic waters funded by the Marine Group (HAV) under the Nordic Council of Ministers in 2017 -2018. A small part of the total analyses (μ-FTIR analyses) were performed with support from the DANCEA funded SUMAG2-project and from Danish Centre for Environment and Energy (DCE) at Aarhus University.

The experimental work in Ny Ålesund, Svalbard was in part funded by Svalbard Science Forum-Arctic Field Grant (RIS ID 11024), the JPI Oceans project 'PLASTOX' (Grant No EC-696324) and Miljøringen (MSc field support issued to Amalie Ask).

The overall aim of the project was to determine abundance of microlitter pollution in sediments and benthic food chains in Norwegian (Svalbard) and Greenlandic Arctic coastal marine ecosystems and to evaluate it in relation to potential local sources and background levels. Further, the aim was to investigate potential effects of microplastic pollution on benthic organisms by conducting laboratory studies using an arctic amphipod as a model organism.

This report describes findings of microlitter particles in sediment and biota samples in gradients from outlets of untreated wastewater in Sisimiut, West Greenland and Longyearbyen, Svalbard, as well as from an outlet of treated wastewater in Ny-Ålesund, Svalbard. Further, the experimental studies on effects of microplastic particles (including both fibres and fragments), are described with respect to experimental aims, setups and results. The aim of the experimental studies was to investigate whether environmentally relevant and future predicted concentrations of microplastics could impact feeding rate, microplastic ingestion, respiration and locomotion activity in an Arctic sediment dwelling amphipod. The project thus provides quantitative- and impact data related to microlitter pollution specifically focusing on the arctic marine environment.

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Summary

Marine litter pollution affects oceans globally and has today also made its way to the pristine arctic environment adding to the microlitter from local pollution sources. Marine litter pollution is recognized as a serious threat to the marine environment at all levels, from the UN to regional (EU, OSPAR, HELCOM) and national authorities. The risk posed by microlitter to marine biota is related to their documented ubiquity and long residence time in marine ecosystems. Risks are also mediated by intrinsic toxicity of added chemicals and potential adsorbance of other pollutants. When released into the marine environment, a major part of microlitter likely accumulate in beach sand and marine sediments either immediately or after acquiring a biofilm. It is therefore expected that benthic food chains will be key to understanding fate and effects (i.e. concentrations, potential trophic transfer and biological impact) of microlitter in the marine environment. The overall aim of the project was to determine abundance of microlitter pollution in marine sediments and benthic food chains in the Arctic, and to evaluate the abundance in relation to potential local sources and background levels. The aim was also to investigate potential effects of microplastic pollution on benthic organisms through laboratory studies using an arctic amphipod as a model organism.

The field investigations in Svalbard, Norway and Greenland focused on determining microlitter particle concentrations and characteristics in marine sediments and biota collected close to and far from potential local pollution sources and pathways, i.e. outlets of untreated wastewater and effluents from a dumping site in Sisimiut, West Greenland and outlets of treated (Ny-Ålesund) and untreated (Longyearbyen) wastewater in Svalbard. Overall, higher concentrations and a higher diversity of microlitter types and polymers were found in sediments and organisms (blue mussels and cod) closer to human settlements (wastewater outlets and dumping sites) and in places where lost and/or dumped fishing gear accumulate. Thus, we can confidently conclude that local pollution sources for anthropogenic microlitter do exist in the Arctic.

The experimental studies investigated whether environmentally relevant and future predicted concentrations of microplastics could impact feeding rate, microplastic ingestion, respiration and locomotion activity in an arctic sediment dwelling amphipod. The experimental results confirm previous microplastics studies on marine invertebrates showing effect only at very high concentrations not yet relevant in the arctic environment. The shape of the plastic particles was found to affect the particle fate. While microplastic fragments were ingested, short microplastic fibres attached to the carapace of the amphipods and likely obstructed normal ventilation behaviour. Furthermore, biofilm cover was found to affect the behaviour and effects of the particles. Microlitter naturally become covered by biofilms in the environment and our results stress the importance of effect experiments being carried out using naturally fouled plastics for ecological relevance.

This report provides both environmental- and impact data related to microlitter pollution in the arctic marine environment. Although the levels of microplastics required to cause effects in experimental organisms in this study were much higher

than what was detected in the field, there may be other species that are more sensitive than the one tested in nature. The currently relatively low microlitter concentrations detected in the field should be considered as a "window of opportunity" to act to at least reduce local pollution. Consequently, introduction of sustainable waste management and wastewater treatment should be an important focus of local management initiatives.

1. Introduction

1.1 Purpose, aims and design of project

The purpose of the project was to quantify the occurrence and effects of microlitter (ML) including microplastic in coastal marine benthic food chains of the Norwegian and Greenlandic Arctic. Here, microlitter is defined as manmade or modified materials <5 mm, e.g. plastics, paints, rubber and textile fibres. Specific aims of the study were;

1. To quantify and characterize the composition of ML along short benthic food chains, i.e. in sediments, benthic invertebrates (i.e. blue mussels and amphipods) and fish (e.g. Arctic sculpin and/or Greenland cod) at sites expected to be pristine and polluted in Greenland and Svalbard. Study regions included Sisimiut in Greenland and Longyearbyen and Ny-Ålesund in Svalbard.
2. To measure uptake rates, accumulation and effects of two types of polyethylene terephthalate (PET) microparticles, i.e. fragments and fibres with and without natural microbial biofilms, in selected arctic sediment dwelling amphipods.

The project was conducted over a two-year period, 2017-2018. The first year was devoted to aim number one and focused on field sampling in Svalbard and Greenland. Year two was focused on investigating aim number two and involved experimental studies. This report compiles data obtained from both field and experimental studies with the aim to synthesize our findings.

1.2 Background: microlitter pollution

Pollution by marine litter is affecting the oceans globally and has today also made its way to the pristine arctic marine environment adding to local litter pollution sources. Marine litter pollution is recognized by the UN as a serious threat to the marine environment (UNEP 2009, 2016) and by the EU through the Marine Strategy Framework Directive (2008/56/EC). Regional organization and authorities like the Nordic Council (Nordisk Miljøhandlingsprogram 2013–2018), the Arctic Council through AMAP (Arctic Monitoring and Assessment Programme), CAFF (Conservation of Arctic Flora and Fauna) and PAME (Protection of the Arctic Marine Environment), and local arctic national governments are also recognizing the threat. The risk posed by microlitter to marine biota is related to their documented ubiquity and long residence times in marine ecosystems, potential intrinsic toxicity and potential adsorbance of other pollutants as well as their propensity to be ingested by biota (Anderson et al. 2016).

Microlitter (ML) originate from a multitude of sources (Anderson et al., 2016; Magnusson et al 2016). Some are produced and emitted as microplarticles, e.g. industrial plastic pellets, microplastics from personal care products or plastic granulates from artificial turfs. Others are formed when larger objects are fragmented during wear and tear or in the environment into smaller pieces as a

result of weathering (Cole et al. 2011, Anderson et al. 2016). All marine litter share the characteristic of being potential carriers of contaminants from production, processing or adsorption during their environmental journey (Mato et al. 2001, Bakir et al. 2014, Yu et al. 2019).

When released into the marine environment, investigations show that the major part of total ML likely accumulate in beach sand and marine sediments either immediately or after acquiring a biofilm (Lusher 2015, Rummel et al. 2017). Due to various biological processes, such as the aggregation of organic material and/or biofouling, the density of ML particles that settle in the sediments of aquatic ecosystems may be several orders of magnitudes higher than that of the surrounding waters (Haegerbaeumer et al. 2019). It is therefore expected that uptake in benthic food chains will be key to understanding fate and effects (i.e. concentrations, potential trophic transfer and biological impact) of ML in the marine environment. Many sediment dwelling organisms, e.g. amphipods, echinoderms and polychaetes, ingest their bodyweight of sediment several times per day and will thus be exposed to ML particles through their normal feeding behaviour. Selective feeding may also augment the uptake rate and exposure in species belonging to particular functional groups. In addition, sediment dwelling organisms serve as food for many benthic fish species including those of commercial value, e.g. flat fish and cod, and ML particles may thus be transferred along benthic food chains with humans as top consumers. Possible negative effects on individual organisms, populations and ecosystems involve both direct physical effects of the ML particles themselves and possible exposure to various associated hazardous substances (Teuten et al. 2009, Engler 2012, Herzke et al. 2016). The potential toxicity of ML is likely to be mediated by: 1) digestive stress e.g. congestion and energy expenditure for egestion, 2) leakage of plastic additives or production chemicals, and 3) exposure to contaminants (e.g. persistent organic pollutants- POPs) adsorbed to the ML particles (Anderson et al. 2016). The effects of ML particles depend on size, polymer type, shape, associated chemicals, biofouling state and abundance in water, sediment, or biota. Currently, many of these factors are not well understood, and need to be better characterized in various environments (Anderson et al. 2016).

The impacts of ML ingestion are not well known, especially not in lower trophic organisms despite the growing interest in that topic. When focusing on crustaceans, the exposure to high (and environmentally irrelevant) concentrations of polyethylene microspheres led to an altered feeding selectivity of *Calanus helgolandicus* and subsequent modifications in faecal sinking speeds (Coppock et al. 2019). Also, the amphipod *Orchestoidea tuberculata* showed changes in its consumption rates and preferences when microplastics were incorporated into food (Carrasco et al. 2019). To our knowledge, the effects of ML particle ingestion on arctic amphipods are unknown.

1.3 Marine litter pollution in the Arctic

The number of studies on macro- and microlitter in arctic marine waters, sea ice, sediments and biota is increasing and the published studies do confirm the spreading of ML particles with oceans currents (Zarfl & Matthies 2010, Van Sebille et al. 2012) and air (Bergmann et al. 2019) to the Arctic. Macro- and/or microlitter pollution has been documented in all arctic marine habitats; on beaches (Sundet et al. 2016, Bergmann et al. 2017a, Granberg et al. 2019), in surface and in subsurface water layers (Lusher et al. 2014, Bergmann et al. 2016, Tekman et al. 2017, von Friesen et al. 2020), in shallow sediment and down to 5 500 m depth (Sundet et al. 2016, Bergmann et al. 2017b, Granberg et al. 2019), frozen into sea ice of the Arctic Ocean (Obbard et al. 2014, Peeken et al. 2018, von Friesen et al. 2020) and in the guts of organisms, e.g. intertidal amphipods *(Gammarus setosus)* (Iannilli et al. 2019), polar cod, northern fulmar (*Fulmarus glacialis*) (Trevail et al. 2015) and little auk (*Alle alle*) (Amelineau et al. 2016).

Marine macro- and microlitter found in the arctic may originate from global, regional (Cozar et al. 2017) and local sources (von Friesen et al. 2020). Local sources include emission related to shipping, fishing, tourism (Grøsvik et al. 2018, Halsband & Herzke 2019) and runoff from land-based industries, dumping sites and wastewater outlets (Granberg et al. 2019). Wastewater outlets are identified as important sources of microlitter to the marine environment in temperate areas (Gatidou et al. 2019). An investigation comparing municipal wastewater treatment systems in Sweden, Finland and Iceland showed that multi-step wastewater treatment plants (WWTPs) retained up to 99% of inflowing ML particles ≥300 µm, while mechanical separation retained 0% of the inflowing ML particles, i.e. leading to 100% emissions (Magnusson et al. 2016). Similar high retention was found in a pilot study from the WWTP in Ny-Ålesund, Svalbard (Granberg et al. 2019).

Wastewater treatment is generally lacking in the Arctic, and in smaller settlements sewage and garbage disposal is comparable to conditions observed in developing countries. The reasons are incapacities and high costs related to maintenance and warming of treatment ponds and plants. Consequently, municipal, industrial and hospital wastewater is discharged directly into the sea while garbage is piled on dumping sites sometimes located close to the shore. In the wake of climate change, industrial development and tourism is expected to increase in the Arctic leading to temporal population increases in these ecologically sensitive areas, with a highly insufficient municipal infrastructure. Baseline- and source related investigations of ML and other pollutants are vital to support decision making in this rapidly changing region. Indeed, because of global change and its consequences for the arctic environment, an intensification of human activities is expected, likely leading to an increase of macro- and microlitter pollution in this area. Macrolitter and waste will fragment into micro- and nanolitter particles over time and become available to coastal marine ecosystems possibly affecting marine organisms. The relative importance of global, regional and local sources for ML pollution is currently unknown, as well as the impact of ML on coastal marine organisms, ecosystems and resources in the Arctic.

2. Field measurements

2.1 Aim

The main aim of the first part of the project was to determine concentrations and characteristics of ML pollution in coastal marine areas without direct human impact and around sites with suspected local ML pollution sources such as wastewater outlets and vicinity to arctic towns and dumping sites. The matrices investigated were beach sand, sediments, marine invertebrates, *i.e.* blue mussels (*Mytilus edulis*) and amphipods (*Gammarus setosus*), and Greenland cod (*Gadus ogac*) representing different trophic levels along a benthic food chain.

2.2 Sites

Sites for sampling ML in both Greenland and Svalbard were selected to target both background concentrations at expected pristine reference sites (R) and expected polluted sites (P) close to local land based sources (Table 1). All samples were collected during the summer of 2017.

Table 1: Geographical positions of sampling stations in Greenland (GL) and Svalbard (SV) indicating area, site and sampled matrices with number of replicates (n) in brackets.

Station	Region	Area	Site	Position	Sampled matrix (n)
GL R1	Greenland	Amerdloq fjord	Manitsoq Island	66°52.861'N, 53°33.474'W	C (1), M (8)
GL R2	Greenland	Amerdloq fjord	Sarfanguaq land	66°51.771'N, 53°23.905'W	C (3), M (8), S (2)
GL R3	Greenland	Sisimiut SW	Møllers Island	66°55.573'N, 53°45.214'W	S (2)
GL P1	Greenland	Ulkebugt bay	Ulkebugt WWO	66°56.624'N, 53°39.191'W	C (2), M (8), S (2)
GL P2	Greenland	Sisimiut S	Dumping site WWO	66°55.654'N, 53°40.364'W	C (2), S (2)
SV R1	Svalbard	Krossfjord	Ebeltofthamna	79°09.347'N, 11°36.004'E	A (10), S (2)
SV R2	Svalbard	Kongsfjord	Krykkjefjellet	78°53.857'N, 12°12.131'E	S (2), B (1)
SV R3	Svalbard	Krossfjord	Signehamna	79°16.245'N, 11°32.036'E	S (3)
SV R4	Svalbard	Kongsfjord	Hukbogen	78°58.771'N, 11°23.337'E	B (2)
SV P1	Svalbard	Kongsfjord	Thiisbukta	78°55.639'N, 11°54.119'E	A (10), S (2)
SV P2	Svalbard	Kongsfjord	Ny Ålesund WWO	78°55.720'N, 11°56.975'E	S (2)
SV P3	Svalbard	Adventfjord	Longyear WWO2	78°14.125'N, 15°40.716'E	S (2)
SV P4	Svalbard	Adventfjord	Longyear WWO1	78°14.063'N, 15°40.856'E	S (2)

Note: *For stations, R refers to reference site and P to polluted site. WWO refers to wastewater outlet. C: Cod (*Gadhus ogac*), M: Mussel (*Mytilus edulis*), S: Sediment, A: Amphipod (*Gammarus setosus*), B: Beach sand.*

Source: *Own data.*

2.2.1 Greenland

The island and country of Greenland has a population of approximately 56,000 where almost 18,000 live in the capital Nuuk in the south. In Greenland, samples were collected around the town of Sisimiut (Fig. 1). With its 5,524 (2018) inhabitants, Sisimiut is the second largest town in Greenland with local entrepreneurs, several schools, a hospital and a shrimp factory. People in Sisimiut engage in hunting and fishing both professionally and for recreation and small summer huts are sparsely scattered on some small islands and along fjords in the vast wilderness surrounding the town.

Figure 1: Sisimiut, Greenland.

Note: *Sisimiut town situated on the west coast of Greenland surrounded by vast wilderness. Photo taken from the north. Right: map showing Greenland with the polar circle (dotted line) and the location of Sisimiut.*

Photo and map: *Maria Granberg.*

Sisimiut has no wastewater treatment facility. Wastewater drainage from the municipal housing, schools and hospital connects to pipes (Sisimiut has seven) draining directly into the sea close to the shore (Fig. 2 top) During low tide the pipes visibly release wastewater above sea level (tidal range ~4 m). Not all homes are connected to the wastewater system. Greywater is then released directly onto the ground a few meters from the individual houses or into ditches. This greywater forms ice falls on the hills when it freezes during winter. Toilets in these houses are often not water-flushed but instead lined with plastic bags. Full toilet bags are collected, and the contents released directly into the sea by the dumping site.

The main dumping site holds all types of waste and is located near Sisimiut on the coast facing the open ocean (Fig. 2 bottom). The waste is sorted into categories and waste piles are situated uncovered directly on the frozen ground. A large incinerator works to burn combustible waste. However, as in most other places in Greenland, Sisimiut is impacted by rough arctic weather with strong winds, which contributes to spreading waste, such as different types of plastic materials, to the surroundings. Leakage from the dumping site is collected in ditches draining directly into the ocean.

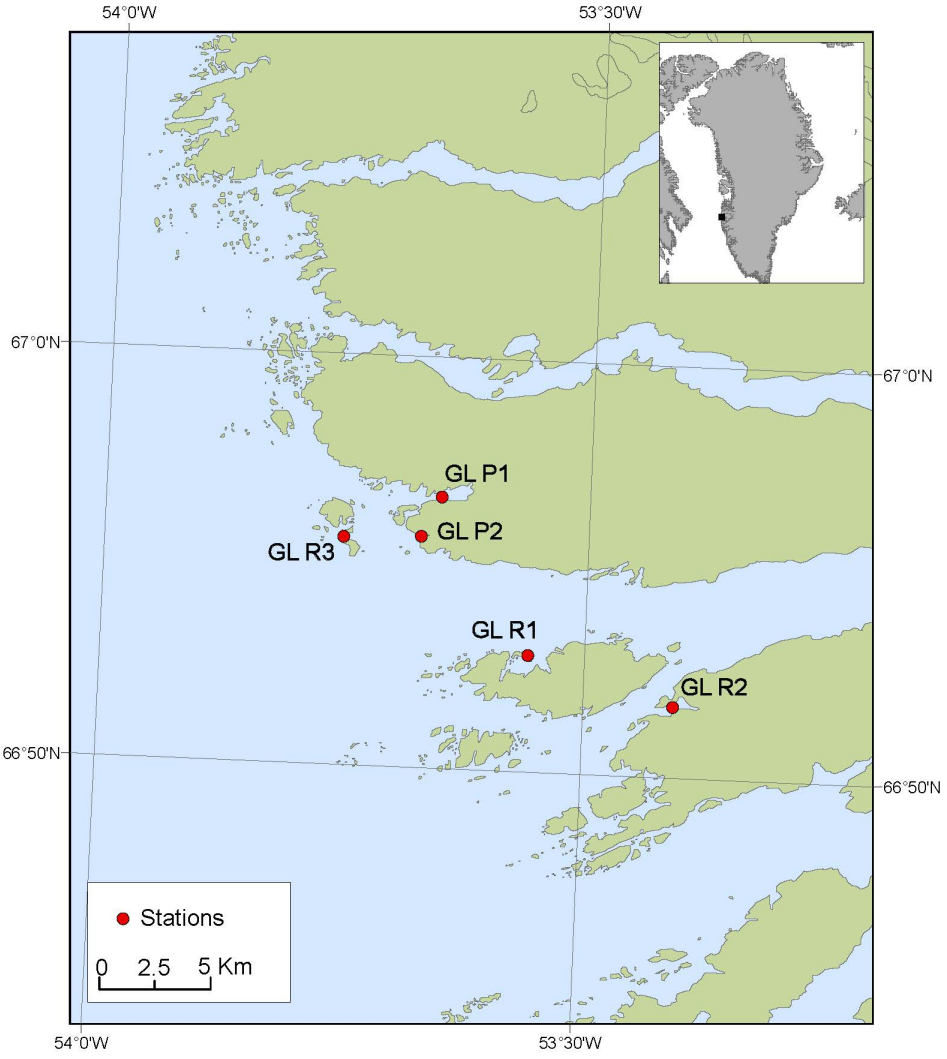
Figure 2: Wastewater outlet (sewage pipe) in Ulkebugt bay (top) and by the main dumping site with drainage pipe releasing waste leachate into the sea, Sisimiut (bottom).

Note: *Expected local plastic and contaminant pollution sources to coastal waters in Greenland.*

Photos: *Maria Granberg.*

Polluted sampling sites (P) were located in direct vicinity to two local ML sources, i.e. the main wastewater outlet in the Ulkebugt bay (GL P1) and the Sisimiut dumping site (GL P2) (Fig. 3, Table 1). Samples were also collected at expected clean reference sites (R) away from Sisimiut into the Amerdloq fjord, far from permanent settlements (GL R1–3) (Fig. 3, Table 1).

Figure 3: Sampling sites in Sisimiut, Greenland.



Note: *Map showing the area around Sisimiut town with polluted (P) sampling sites GL P1 by the wastewater outlet and GL P2 by the dumping site and reference (R) sampling sites (GL R1-R3) in uninhabited areas.*

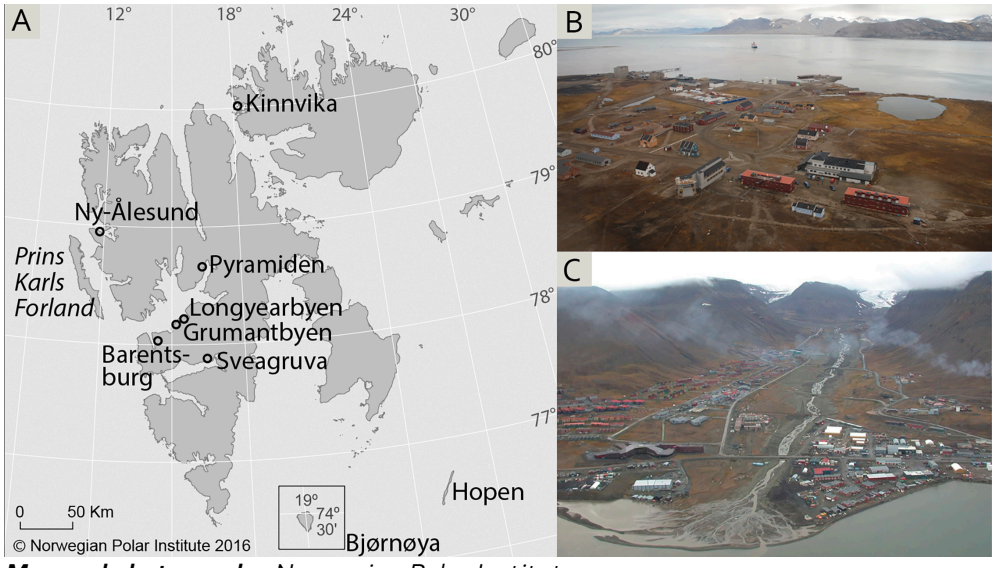
Source: *Maps created with ESRI ArcMap 10.5.1.*

2.2.2 Svalbard

The Norwegian island group Svalbard has a resident population of 2667 (2016), which increases dramatically during tourist seasons, reaching over 130,000 "guest nights" in 2015. Longyearbyen is the largest settlement and the administrative centre of Svalbard (Fig. 4A & C). This is where most permanent residents live and where all tourism is based. The town has a large harbour regularly frequented by cruise ships, an airport, a hospital, primary and secondary schools, a university centre and a sports centre with a swimming pool. There is no wastewater treatment in Longyearbyen and untreated wastewater is thus released directly into Adventfjorden.

The settlement of Ny-Ålesund is situated in Kongsfjorden (Fig. 4A & B). It was founded as a mining town by Kings Bay AS in 1917 and terminated as such in 1963. It is now run exclusively as an international research facility, hosting ~50 persons in winter and ~170 in the summer. The community of Ny-Ålesund, including the research facilities and infrastructure, is operated by Kings Bay AS under the Norwegian Ministry of Climate and Environment. As one of the first settlements in Svalbard, Kings Bay AS installed a wastewater treatment plant in Ny-Ålesund summer 2015. The treatment plant collects all wastewater from the settlement, which passes through a sedimentation step followed by chemical and biological treatment steps. The outgoing wastewater is released into Kongsfjorden.

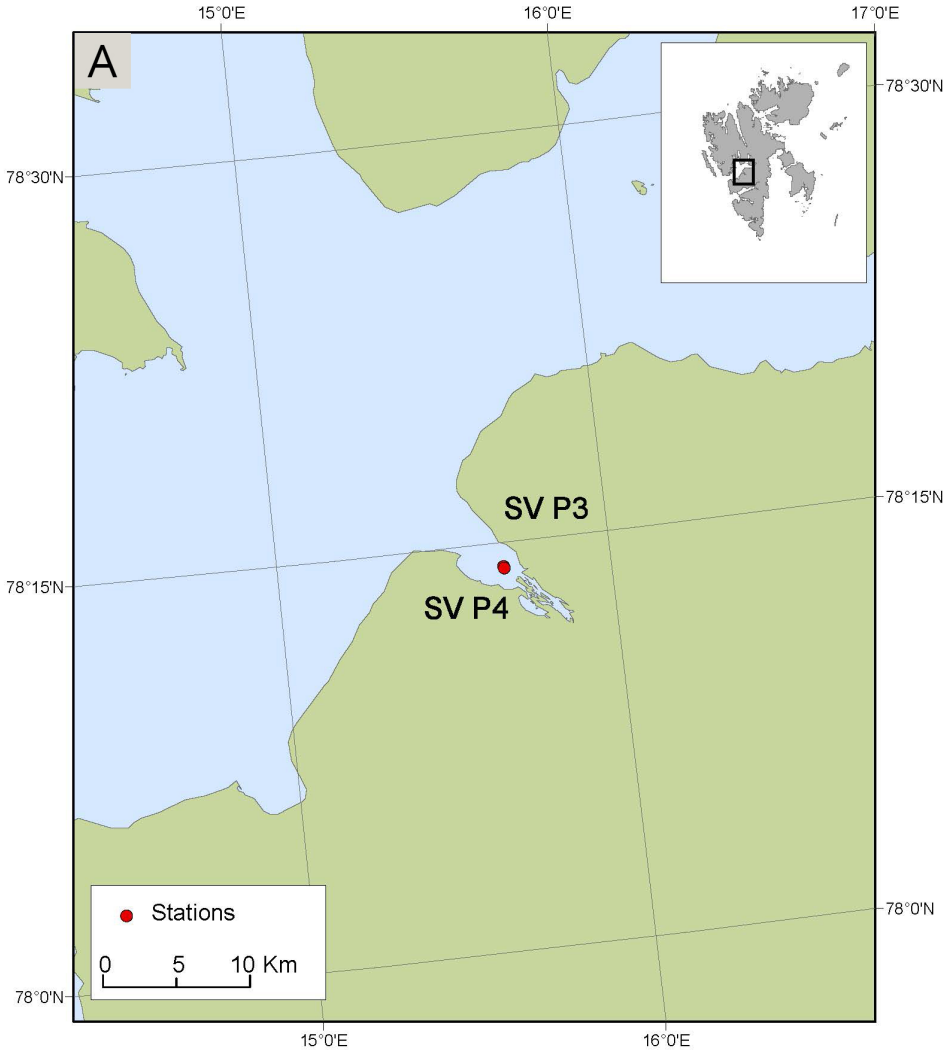
Figure 4: Map of Svalbard with main settlements (A) and aerial photographs of B) Ny-Ålesund by Kongsfjorden and C) Longyearbyen by Adventfjorden.

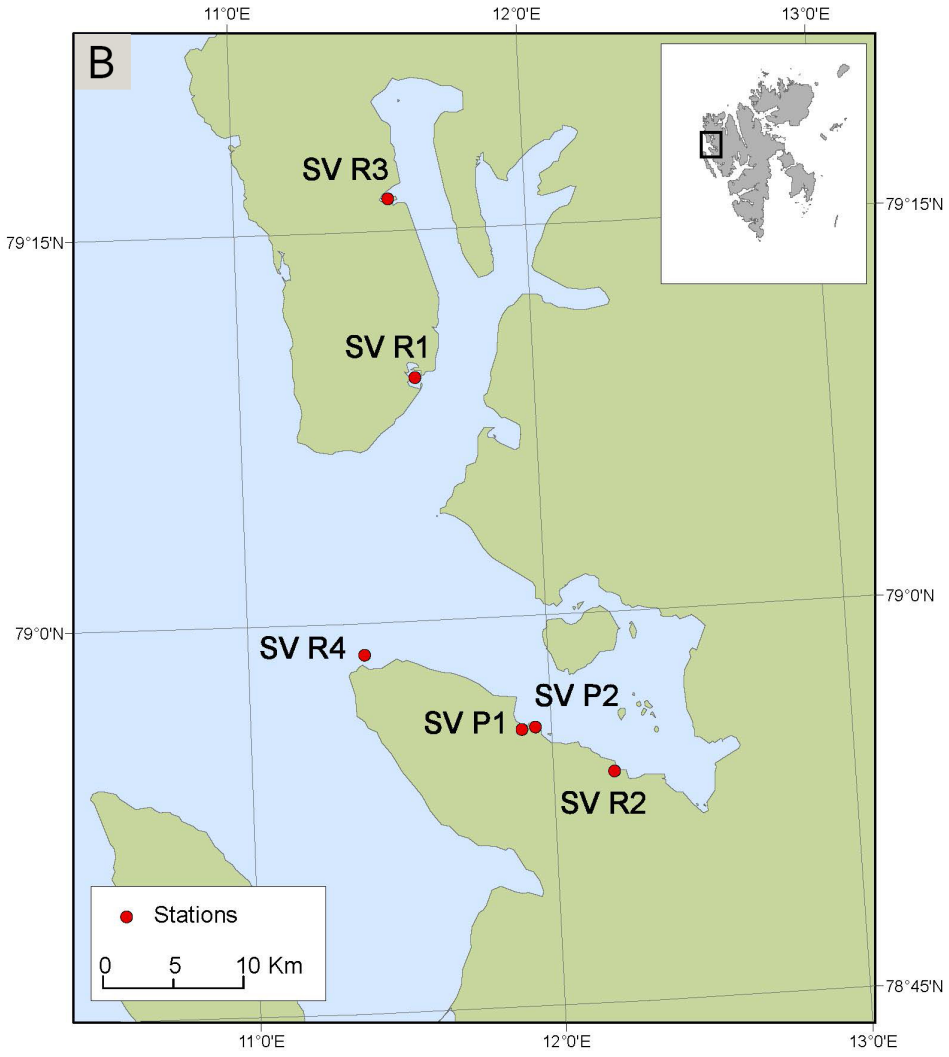


Map and photographs: *Norwegian Polar Institute.*

Sampling sites in Svalbard were located close to the wastewater outlet in Adventfjorden, Longyearbyen (SV P3 & SV P4) (Fig. 5A, Table 1) and in Kongsfjorden along the south shore from Krykkjefjellet (SV R2) close to the Kongsbreen glacier, by the wastewater outlet and harbor of Ny-Ålesund (SV P1 & SV P2) and at Hukbogen (SV R4) in the mouth of the fjord (Fig. 5B, Table 1). Samples were also collected in Krossfjorden in the bays Ebeltofthamna (outer part, SV R1) and Signehamna (SV R3) close to the Liljehöökbreen glacier (Fig. 5B, Table 1).

Figure 5: Sampling sites in Longyearbyen and Ny-Ålesund, Svalbard.





***Note: A**: Map showing Longyearbyen with sampling sites in Adventfjorden close to the wastewater outlet. **B**: Map showing Ny-Ålesund with sampling sites in Kongsfjorden and Krossfjorden including Lilliehöökfjorden. R refers to expected reference/unpolluted site far from local sources and P refers to expected polluted site close to local pollution sources.*

***Source:** Maps created with ESRI ArcMap 10.5.1.*

2.3 Sampling of sediment, beach sand and biota

2.3.1 Sampling of sediments and beach sand

Sediment sampling in deeper waters was performed from a small boat using a hand operated Van Veen grab sampler (Fig. 6 left). The grab sample was expelled in a seawater washed plastic box. The grab sample was considered valid when a clear structure could be observed, and the layering was intact. All equipment was rinsed with o.2 µm filtered Milli-Q (MQ) before the sampling and with seawater and MQ between each sample. After collection, the uppermost 2–3 cm of the sediment was scraped off using a metal spoon and transferred into rinsed glass jars sealed with aluminium foil and lids. Beach sand or sediment was sampled by collecting the uppermost 2–3 cm of the surface using a metal spoon. Sand/sediment was transferred into glass jars. The aim was to collect three sand/sediment samples from each site. Sediment samples were stored frozen and dark until extraction.

Figure 6: Sediment sampling in deeper waters using a manually operated Van Veen grab sampler (left) and by hand on beaches (right).

Photos: *Ingrid Gabrielsen (left) and Maria Granberg (right).*

2.3.2 Sampling of biota

Sediment dwelling invertebrates and fish were sampled at each site in Svalbard and Greenland to represent different trophic levels of benthic food chains. Invertebrates were represented by blue mussels (*Mytilus edulis*) in Greenland and amphipods (various species including *Gammarus setosus*) in Svalbard. Amphipods were collected by hand at low tide and using a Van Veen grab sampler followed by sediment sieving. In Greenland blue mussels were easily collected by hand from ca. 0.5 m depth (Fig. 7) while this species is not found in most of Svalbard.

Figure 7: Sampling of blue mussels (*Mytilus edulis*) by hand in Greenland.

Note: *Lis Bach successfully collects blue mussels in shallow waters in the Amerdloq fjord, Greenland.*

Photos: *Maria Granberg.*

In Svalbard the amphipod *Gammarus setosus* (Fig. 8) is a common species of the shallow intertidal. Directly after collection, invertebrates were rinsed first with seawater and then with filtered (0.2 µm) MQ water to remove debris, and subsequently stored individually in aluminium foil covered and lidded pre-rinsed (3 times with 0.2 µm filtered MQ water) glass jars. All invertebrate samples were stored frozen (-20°C) and dark until ML extraction and analysis. The swift handling of individuals after collection prevented loss of ML through organisms expelling material from their guts or ingesting ML particles.

Figure 8: The amphipod *Gammarus setosus.*

Note: *These amphipods are between 2 and 5 cm long.*

Photo: *Maria Granberg.*

Greenland cod (*Gadus ogac*) were caught using a fishing rod with a shiny lure. This method was chosen to make sure that ML was neither ingested nor expelled during catchment as can be the case in trawls or nets (Lusher et al. 2017b). Fish were immediately killed, and the gastrointestinal tract dissected out and stored in individual aluminium foil covered and lidded pre-rinsed (3 times with 0.2 µm filtered MQ water) glass jars (Fig. 9). All samples were stored frozen (-20°C) and dark until ML extraction and analysis. In Svalbard fishing was not successful.

Figure 9: Field sampling of the gastrointestinal tract and contents from line caught Greenland cod in Sisimiut, Greenland.

Photo: *Maria Granberg.*

2.4 Pre-treatment of samples

2.4.1 Sediment and beach sand samples

The extraction of MLs from sediment was performed by density separation using a down-scaled Munich Plastic Sediment Separator (MPSS, Imhof et al. 2012). The setup consists of three main parts; I) an electric engine driving a rotating propeller in the bottom of a sediment container, II) a high conically shaped standpipe, and III) a device with a ball valve which enables the division of the sample and sequential filtering. A detailed description of the stepwise procedure can be found in Appendix 1. Sediment samples from each station and replicate were homogenised, subsampled (450–650 g wet weight) and added to the density separator. Saturated sodium chloride (dissolved in MQ and 20 µm filtered, ρ = 1.2 g cm^{-3}) was used as separation liquid. When density separation was completed, the top part of the liquid, now containing only particles lighter than the saturated saline solution, which theoretically included microparticles of most plastic polymers, was vacuum filtered through nylon filters (Sefar Nitex) with the smallest mesh size of 20 µm. Filters were then stored individually in closed petri dishes. To determine the water content and establish the wet weight to dry weight ratio, the sediment was thoroughly mixed, and a subsample moved to a pre-weighed aluminium container in 105°C until constant weight was reached (n=3 per sample). ML particle abundance in sediment is here reported as numbers per unit mass (dry weight)

2.4.2 Biota samples

Preparation of biota for ML extraction took place in a clean air cabinet (Clean Air Techniek B.V.) and the tissue to be treated was weighed and transferred into new individual glass jars beforehand rinsed twice with o.2 µm filtered MQ water. Aluminium foil was placed over the mouth of the jars prior to lidding to avoid potential contamination. The following biota was processed for ML extraction: blue mussels from station GL R2 (n=8), GL P1 (n=8) and GL R1 (n=8), amphipods from station SV R1 (n=10) and SV P1 (n=10), gastrointestinal tract of Greenland cod from station GL R2 (n=4), GL R1 (n=1), GL P1 (n=2) and GL P2 (n=2). The blue mussels were beforehand measured (maximum shell length), thawed and carefully separated from the shell, and the inner part of the shell rinsed with o.2 µm filtered MQ water.

A gentle and effective digestion protocol using pancreatic enzymes (Creon 40,000, Abbott Laboratories GmbH, Germany, Mylan) was applied for the extraction of ML particles from biota (Piarulli et al. 2019, von Friesen et al. 2019). The pancreatic enzyme originated from swine pancreas and contained lipase (40,000 Ph.Eur), amylase (25,000 Ph.Eur) and protease (1,600 Ph.Eur) as active substances. The enzymes were added together with tris hydrochloride solution (Trizma, pH 8.0, 1 M, 0.2 µm filtered, Sigma-Aldrich, T3038, USA). The dosage of pancreatic enzymes was 0.05 g per g wet weight tissue. Tris was added until the pH reached the optimal performing range of pancreatic enzymes (8±0.1) (Berdutina et al. 2000) (pH-Fix 7.0–14.0, Macherey-Nagel), but with a minimum of 10 ml. Samples were incubated in 37.5°C on 145 rpm overnight (Innova 40, Incubator Shaker Series, New Brunswick Scientific). After digestion, the solution was vacuum filtered through nylon filters (Sefar Nitex) with the smallest mesh size of 20 µm, and filters were then stored individually in closed petri dishes until analysis.

2.5 Analyses of anthropogenic microlitter

2.5.1 Visual analysis

The filters were examined under a stereomicroscope (Leica M205C) with a maximal magnification of 160x where potential MLs were classified based on shape (evenness, roundness), colour (homogeneity, shininess, unnatural) and texture (stiffness). All suspected MLs were photographed with a camera (Leica DFC420C) mounted on the stereomicroscope and processed in Leica Application Suite (Version 4.8.0) for measurements of size. ML particles were assorted in four main visual categories; synthetic fibres, non-synthetic fibres, synthetic filaments or synthetic fragments. Combustion particles were excluded due to the uncertainty of their nature. White/transparent non-synthetic fibres were not quantified due to their ubiquitous presence also in procedural contamination controls, possibly originating from the use of cotton lab coats. Before opening the individual petri dishes, a swift visual scan for larger particles (i.e. fibres that can easily contaminate via air) was performed. Then a few drops of MQ were added, and filters were visually analysed.

2.5.2 FTIR analysis

Subsamples of particles visually identified as suspected ML were further analysed with Fourier-Transform Infrared spectroscopy (FTIR) for validation of the visual classification along with polymer specific identification. The percentage of analysed particles ranged from 21.5% in sediment to 31% in amphipods (*Gammarus setosus*) (Table 2). Subsampling of particles was performed to optimize the relative distribution, i.e. to include particles from all replicates within a station and species, to represent all four visual categories (synthetic fibres, non-synthetic fibres, synthetic filaments and synthetic fragments) and to represent both frequently occurring as well as rare particle types. Particles classified as rubber were only subjected to visual analysis due to the limited possibility to receive reliable FTIR spectra of black particles

FTIR is irradiating the particle with infrared light with subsequent measurements of how specific vibrations in different chemical bonds of polymers absorb the light, creating a fingerprint-absorbance spectrum (Ismail et al., 1997). The FTIR technique used in the present study was focal plane array (FPA, 128 x 128) transmission μFTIR (Agilent Technologies, Cary 600 Series FTIR Microscope, Cary 620/670 FTIR) run with a liquid Nitrogen cooled detector, resolution of 8 cm^{-1} and a scan range of 3800–850 cm^{-1}. 120 background scans were collected before 30 sample scans to adjust for background noise. Suspected ML particles were moved onto a ZnSe disc (Zinc Selenide, Ø 13 mm, thickness: 2 mm), of which an initial photograph was taken in order to correctly set the area for assembly of a mosaic scan with an IR pixel size of 5.5 x 5.5 μm. Obtained spectra were matched (MineIt, KnowItAll Informatics System, vibrational spectroscopy edition) to both licensed commercial libraries of polymers (ATR-IR Polymers Bio-Rad Sadtler and IR- Polymers Hummel-BioRad Sadtler) as well as locally produced libraries at Aarhus University, Department of Bioscience additionally containing both weathered and natural materials. Additionally, wool fibres were added into the library in order to minimize the risk of incorrect identification of polyamide, due to their similarity in the spectra they generate. However, this may similarly have led to the underestimation of polyamide fibres and therefore they are grouped together in the present study.

Correlative matching rates to library reference spectra were generated with in-program optimized corrections, including baseline corrections. The results generated by library search were carefully observed to ensure concordant key peaks. An unknown particle category is included in the present study that was visually classified as anthropogenic but did not produce identifiable spectra, and a category called 'synthetic undefined' for clearly synthetic spectra but lacking polymer specific identification. Non-synthetic fibres with an uncertain visual appearance in combination with the FTIR match of cellulose were discarded as natural organic material, but when showing clear visual ML particle characteristics (e.g. unnatural colour) retained as cotton fibres.

2.5.3 QA/QC

Precaution was taken during all steps to mitigate contamination. All tools, jars and equipment that were used in contact with samples, were rinsed in MQ and kept covered in aluminium foil. White 100% cotton lab coats were always used when samples were handled. When the clean air cabinet (Laminar Air Flow – LAF bench) could not be used, the positioning in laboratories was placed away from ventilation and doors, and surfaces were carefully cleaned beforehand, and movement minimized in the room. Prior to choosing working location, an estimation of background contamination levels in four potential working environments was performed. Dampened 20 µm nylon filters were air exposed in the different working environment for two hours followed by visual analysis at 25 x magnification. The lowest contamination was 0.7 ± 0.6 fibres per filter and consequently this place was chosen for work outside of the clean air cabinet. Filters were beforehand rinsed thoroughly in tap water and visually analysed under a stereomicroscope with the same magnification that analysis was later performed, and any contaminating particles removed. Prior to filtration, filters were mounted in sequence to minimize air exposure and handling, thus lowering the contamination risk. Each filter was stored separately in pristine sealed petri dishes (polystyrene) until further analysis. Blank samples were performed for sediment and beach sand as well as for biota as procedural contamination controls (PCC), i.e. these blank samples were exposed to identical handling and analysis as the actual sediment and biota samples.

2.6 Results of field measurements

2.6.1 Evaluation of analysis

A subsample of the particles visually categorized as ML particles were further analysed with FTIR to verify polymeric identity. The percentage of FTIR-analysed particles ranged from 21.5% in sediment and beach to 31.0% in amphipods (Table 2). The percentage of particles visually identified as ML particles, which subsequently were verified as such by FTIR was relatively high, varying between 54% for amphipods to 82% for Greenland cod (Table 2). This indicates the accuracy level in the visual examination procedure.

Table 2: Percentage of visually identified particles analysed with FTIR and subsequently identified as microlitter (ML) particles.

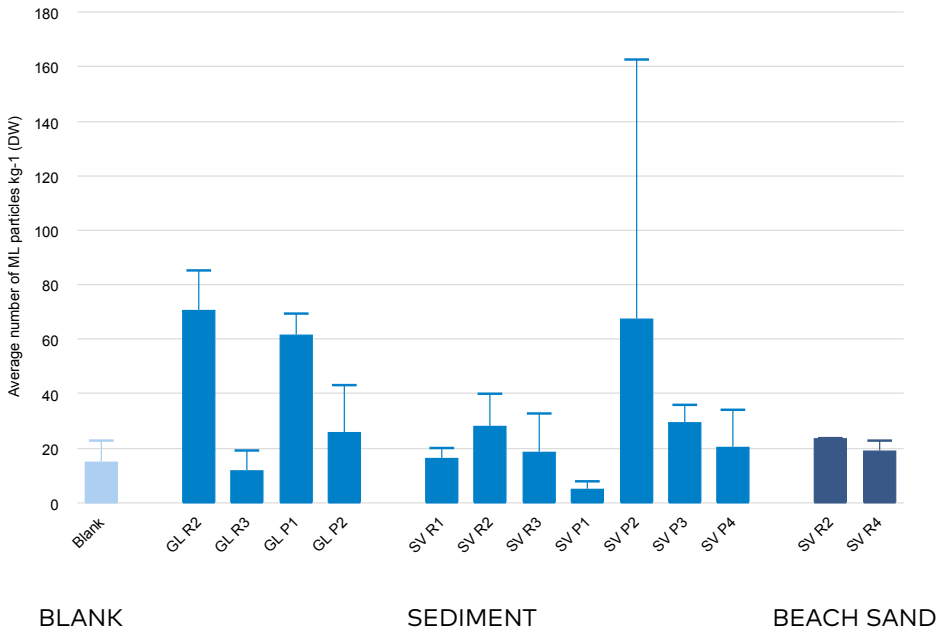
Matrix	% particles analysed with FTIR	% identified as ML particles
Cod	22.0	82.0
Blue mussel	25.0	61.0
Amphipod	31.0	54.0
Sediment & beach	21.5	77.0

Source: *Own data.*

2.6.2 Sediment and beach sand

The average concentration of ML particles kg^{-1} (DW) >20 µm in sediment varied between 70 ± 15 (measured at station GL R2 in the Amerdloq fjord, Greenland) and 5 ± 3 (measured at station SV P1 in Thiisbukta, Svalbard) (Fig. 10). When solely looking at the concentration of ML, a limit of detection was determined by the amount present in the blank (control) samples (15 ± 8 ML particles $sample^{-1}$). Thereby, stations demonstrating lower average concentrations than the detection limit, i.e. GL R3: Møllers Island, Greenland; SV P1: Thiisbukta, Svalbard, could not confidently be interpreted as true environmental concentrations (Fig. 10). When considering the colour distribution of ML particles at these two stations, there were also clear similarities to the contamination control, which further support the uncertainty of data from these two stations (Fig. 11). Three stations were characterised by their high average number of ML particles kg^{-1} DW; GL R2 (Sarfanguaq land, reference site), GL P1 (Ulkebugt wastewater outlet, WWO) and SV P2 (Ny-Ålesund WWO) (Figs. 10, 12 & 15). A large variation between replicates was identified, especially at SV P2 (Fig 10).

Figure 10: Average number of ML particles kg^{-1} (DW) ± SD > 20 µm in sediment, beach sand and the contamination control (blank).

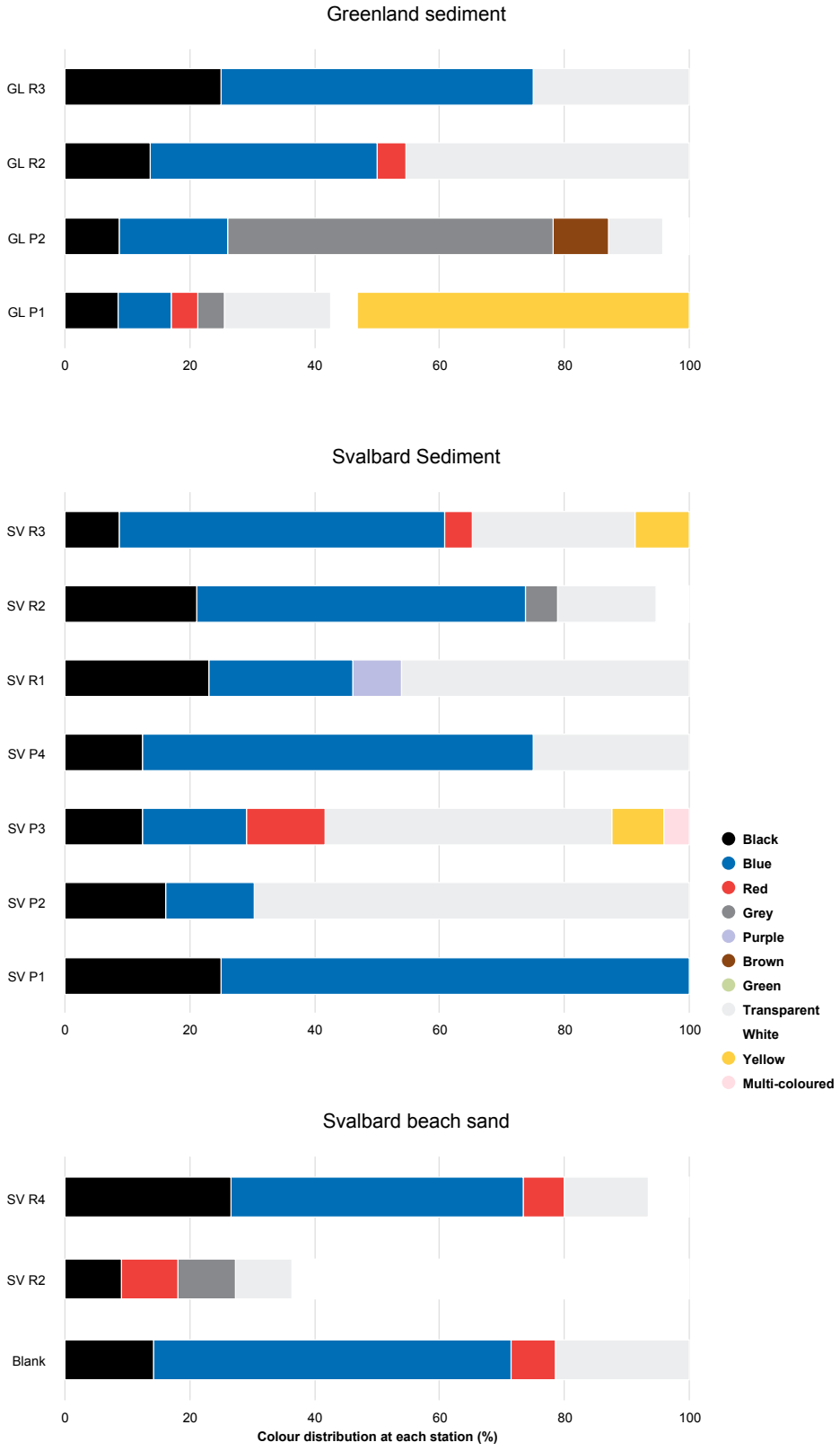


Note: *For site IDs see* ***Table 1****. GL: Greenland, SV: Svalbard, R: reference, P: polluted.*

Source: *Data generated in this study.*

The dominating colours of ML particles in sediment and beach sand were generally blue, black and transparent (Fig. 11). However, some differences were identified with a higher number of different colours present in sediment at GL P1 (Ulkebugt WWO, 7), GL P2 (dumping site Sisimiut, 6) and SV P3 (Longyearbyen WWO, 6) (Fig. 11 A & B). The contamination control demonstrated a similar colour set up as the stations GL R2, SV P4, SV P2 and SV P1 (Fig. 11). However, the polymeric composition was different between the ML particles identified in the blank samples compared to ML particles identified in the actual sediment samples, except cotton fibres which were detected in all sediments (Table3). In contrast to the high diversity of ML colours identified outside the point sources, the colour diversities outside the WWO in Ny-Ålesund (SV P2) and in Thiisbukta (SV P1) were lower and dominated by black, blue and transparent ML particles (Fig. 11). In contrast to SV P3 (Longyearbyen WWO2), SV P4 (Longyearbyen WWO1) demonstrated a lower colour diversity (Fig. 11).

Figure 11: Colour distribution (%) of identified ML particles in sediment and beach sand at the different stations.

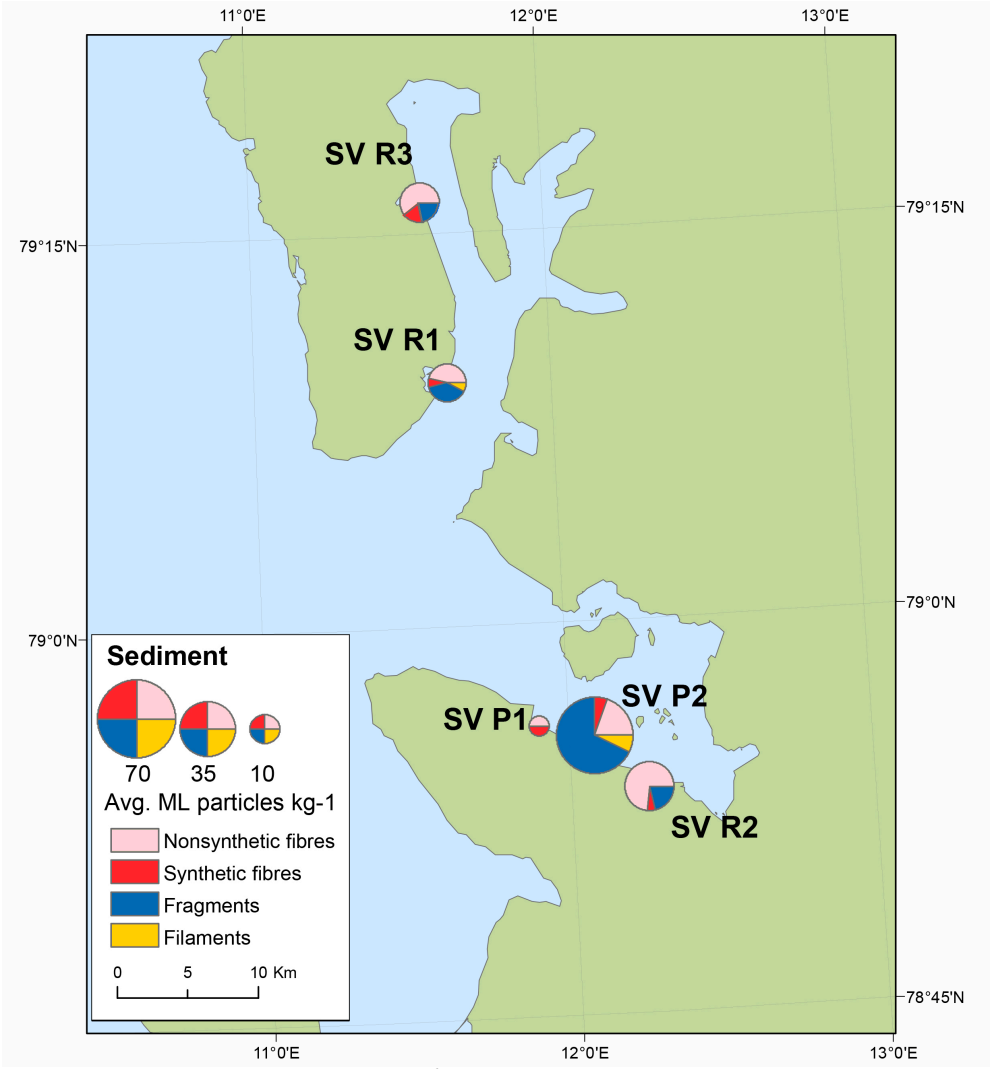


Note: *The blank sample in Svalbard beach sand is the contamination control for all samples and thus also represents the contamination in Greenland sediment & Svalbard sediment. For site IDs see* ***Table 1****.*
Source: *Data generated in this study.*

Close to suspected local point sources (WWOs and the dumping site), the fraction of fragments was higher in both Svalbard North (Ny-Ålesund) and Greenland (Sisimiut) compared to the reference sites where non-synthetic fibres were more commonly dominating (Fig. 12, 15 and 16 B–E). Exceptions to this pattern were GL R2 (Sarfanguaq land) (Fig. 15 & 16 A), SV P3 & P4 (Longyearbyen WWO) (Fig. 14). The only stations where the ML shape category of filaments was identified in sediment were SV P2, SV R1 and GL P2 (Fig. 12 & 15). The shape distribution of the contamination control (blank) was 29% non-synthetic fibres, 43% fragments and 29% synthetic fibres.

In the Svalbard samples, the concentrations of ML particles were similar between the two beach sand reference locations Krykkjefjellet (SV R2) and Hukbogen (SV R4) (Fig. 13), but the shape distribution differed markedly with fragments dominating (82%) at Krykkjefjellet (SV R2) and non-synthetic fibres dominating (67%) at Hukbogen (SV R4) (Fig. 13 & 16 F). The colour distribution was clearly different between the two beach sand samples as well, with Hukbogen (SV R4) showing an almost identical distribution to the blank whereas Krykkjefjellet (SV R2) was dominated by white ML particles (64%) (Fig. 16 F). Polymers identified at SV R2 were polyurethane (PU) and an undefined synthetic polymer (Table 3). No FTIR analysis was performed on ML particles from SV R4.

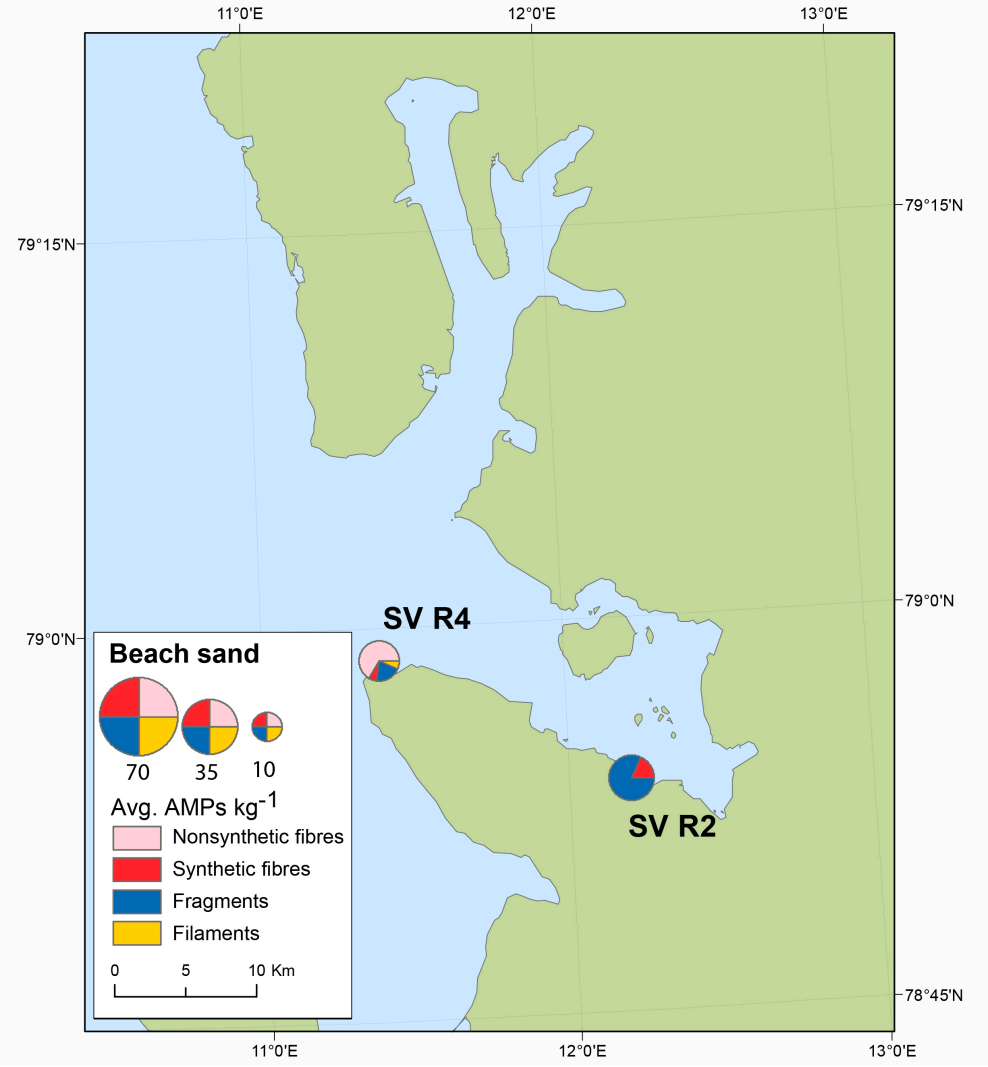
Figure 12: Concentration, shape distribution (non-synthetic fibres, synthetic fibres, fragments, filaments) of ML particles identified in sediment in Ny-Ålesund, Svalbard.



Note: *Svalbard North sediment (Ny-Ålesund and reference sites).*

Source: *Maps created with ESRI ArcMap 10.5.1.*

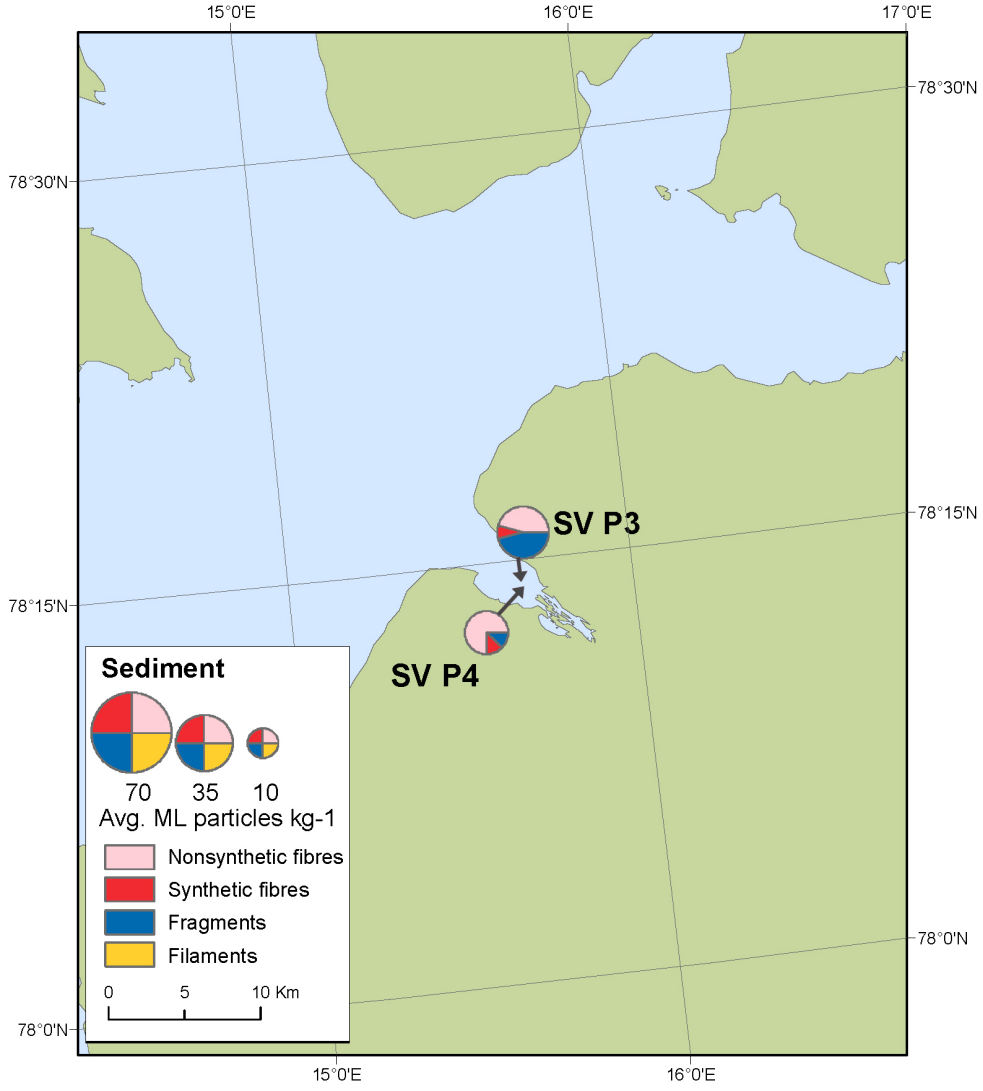
Figure 13: Concentration, shape distribution (non-synthetic fibres, synthetic fibres, fragments, filaments) of ML particles identified in beach sand in Ny-Ålesund, Svalbard.



Note: *Svalbard North beach sand (reference sites).*

Source: *Maps created with ESRI ArcMap 10.5.1.*

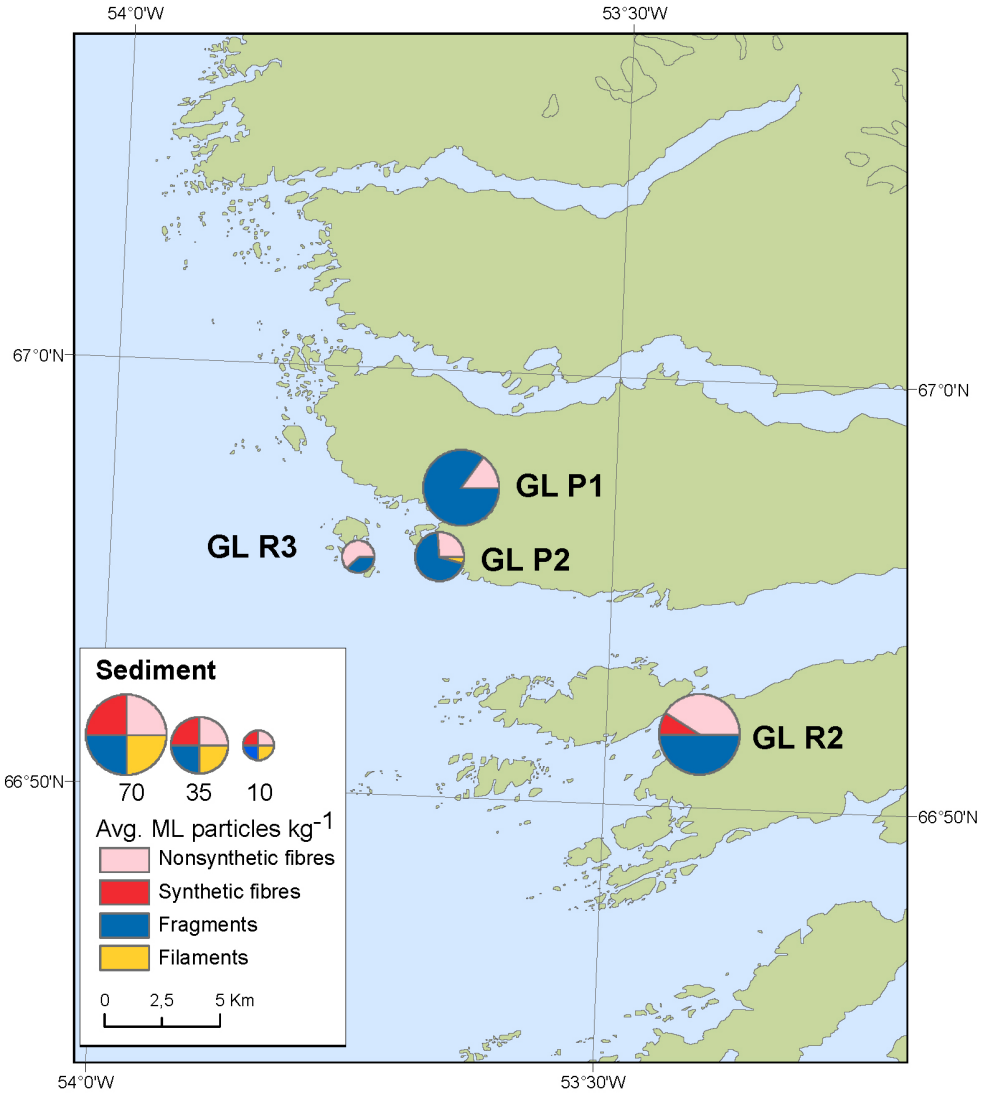
Figure 14: Concentration, shape distribution (non-synthetic fibres, synthetic fibres, fragments, filaments) of ML particles identified in sediment in Longyearbyen, Svalbard.



Note: *Svalbard South sediment (Longyearbyen).*

Source: *Maps created with ESRI ArcMap 10.5.1.*

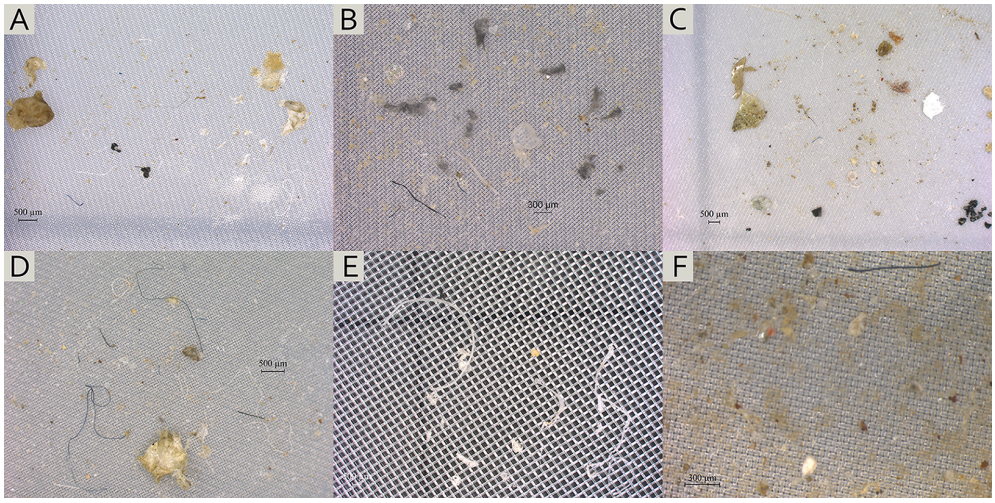
Figure 15: Concentration, shape distribution (non-synthetic fibres, synthetic fibres, fragments, filaments) of ML particles identified in sediment in Greenland.



Note: *Greenland sediment (Sisimiut and reference sites).*

Source: *Maps created with ESRI ArcMap 10.5.1.*

Figure 16: Photographs of ML particles identified in sediments and beach sand in Greenland and Svalbard.



***Note: A**: ML particles at GL R2 (sediment, Sarfanguaq land), **B**: ML particles at GL P2 (sediment, dumping site Sisimiut), **C**: ML particles at GL P1 (sediment, Ulkebugt wastewater outlet), **D**: ML particles at SV R2 (sediment, Krykkjefjellet), **E**: ML particles at SV P2 (sediment, Ny-Ålesund wastewater outlet), **F**: ML particles at SV R2 (beach sand, Krykkjefjellet). All photographs of ML particles (A–F) are presented on a filter with the mesh size of 20 µm, except I (SV P2, sediment) that is presented on a 300 µm filter.*

***Photos:** Lisa von Friesen.*

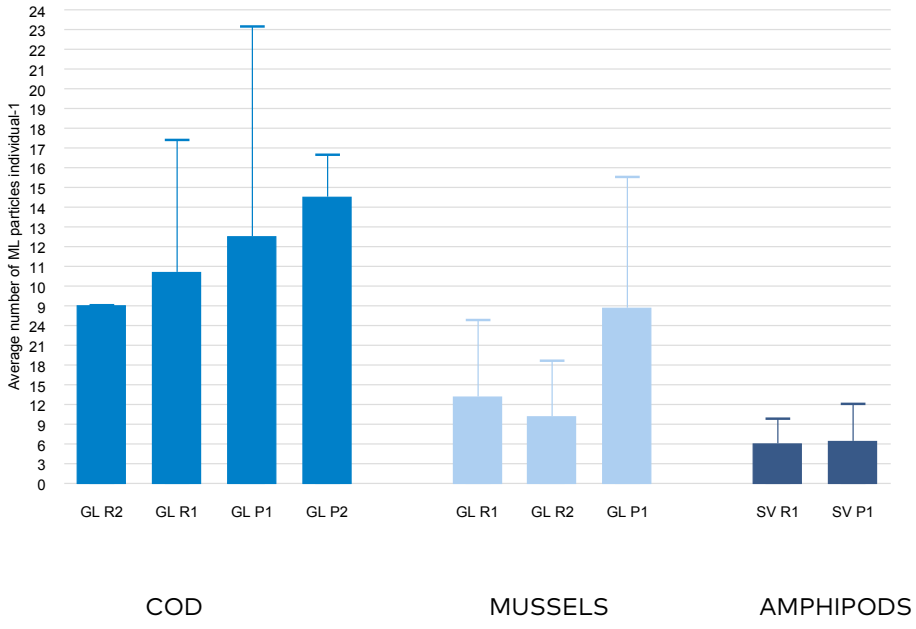
In terms of the identified materials, a clear difference in polymer composition was found between the polluted and the reference sites with only cotton, low density polyethylene (LDPE) and polyamide (PA)/wool overlapping (Table 3). The stations where the highest polymer richness was identified at GL R2 (4), GL P1 (5), SV R3 (4) and SV P2 (4) (Table 3). Materials identified at polluted stations included cotton, the product chewing gum, LDPE, polylactic acid (PLA), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), rubber, synthetic undefined and PA/wool. Materials identified at reference stations were cellophane, cotton, epoxy resin, ester gum, LDPE, polyethylene terephthalate (PET), unknown and PA/wool. Materials identified in blank samples were only cotton (thought to originate from the lab coats worn), rubber (from equipment seals) and styrene copolymer resin (unknown origin, not identified in any other sample) (Table 3).

2.6.3 Biota samples

A higher average number of ML particles individual^{-1} was identified in the gastrointestinal tracts of Greenland cod (12 ± 6) (100% containing ML particles, n=9) compared to blue mussels (6 ± 5) (100% containing ML particles, n=24) and amphipods (varia) (2 ± 2) (80% containing ML particles, n=20) across all stations. However, it should be noted that the variance between replicates within single stations was high and results should be interpreted with caution (Fig. 17). The average number of ML particles individual^{-1} of Greenland cod was higher at the

polluted sites GL P1 (Ulkebugt WWO) and GL P2 (dumping site Sisimiut) than at the two reference sites GL R1 (Manitsoq island) and GL R2 (Sarfanguaq land) (Fig. 17). Correspondingly, in blue mussels the highest average number of ML particles individual^{-1} was identified at a polluted site, GL P1, and lower at the two reference sites GL R1 and GL R2. Very low, and similar, numbers of ML particles were identified in amphipods at SV R1 (Ebeltofthamna) and SV P1 (Thiisbukta) (Fig. 17).

Figure 17: Average number of ML particles individual^{-1} ± SD > 20 µm in Greenland cod, blue mussels and amphipods.

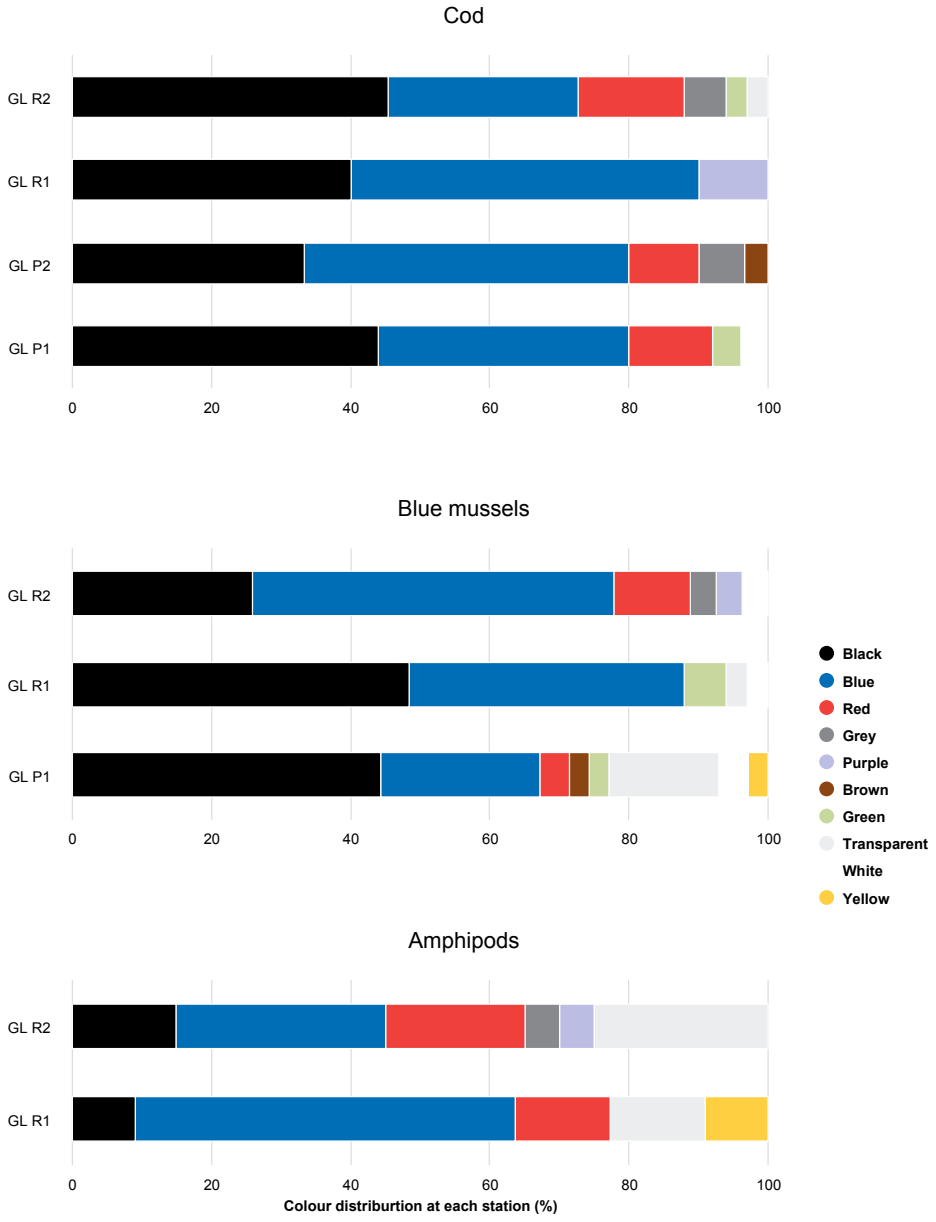


Note: *For site IDs see* ***Table 1****.*

Source: *Data generated in this study.*

In general, higher colour diversities were identified in biota than in sediment and beach sand, but both matrices were dominated by black and blue ML particles (Figs. 18 & 11). In biota, red and grey were additionally commonly identified colours. The pattern found in sediments and beach sand with a higher colour- and particle diversity observed at more polluted sites, was not observed in biota. The exception was blue mussels from GL P1 (Ulkebugt WWO), where the highest colour diversity (8 different kinds) was found (Fig. 18 Mussels).

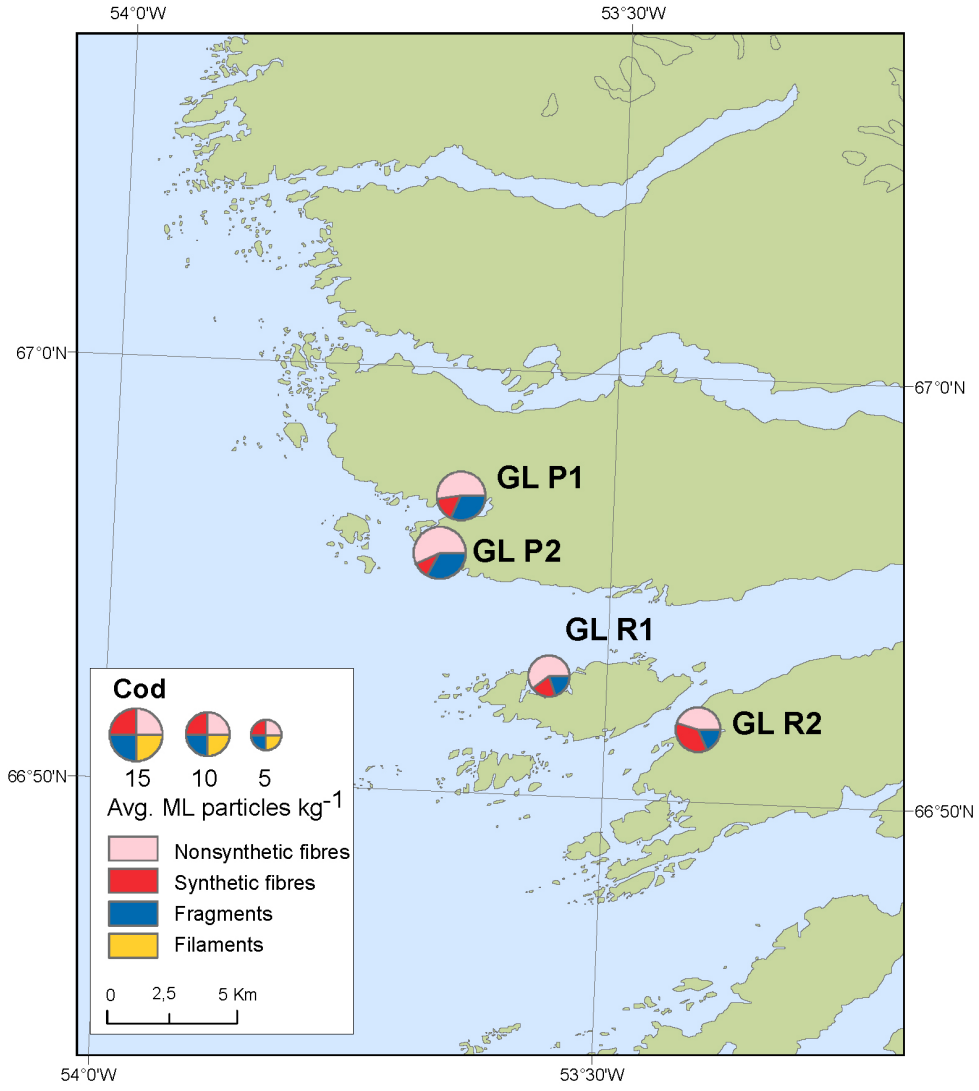
Figure 18: Colour distribution (%) of identified ML particles in Greenland cod, blue mussels and amphipods at the different stations.



Note: *For site IDs see* ***Table 1****.*
Source: *Data was generated in this study.*

The shape distribution of non-synthetic fibres, synthetic fibres and fragments was very similar between the sampling stations in Greenland (GL P1, GL P2 and GL R1), both for Greenland cod and blue mussels. However, for both species, the shape distribution at GL R2 differed from the other stations by containing a larger fraction of synthetic fibres (Figs. 19, 20 & 22 C–F). A larger fraction of fibrous ML particles (both non-synthetic and synthetic) was also identified in sediment at this station (GL R2 –Sarfanguaq land) (Fig. 15). In amphipods from Svalbard, non-synthetic fibres and fragments were dominating at both stations (Fig. 21 & 22 A & B). No filaments were identified in biota.

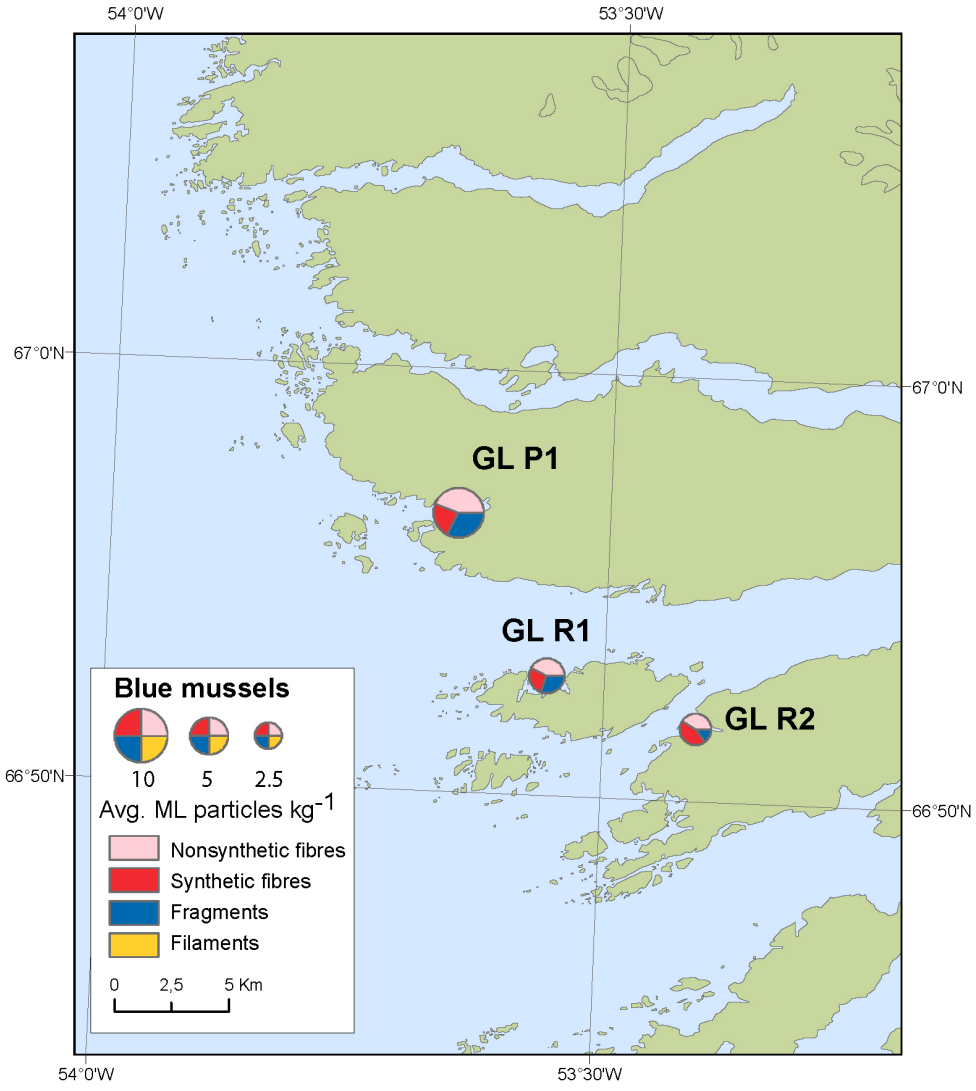
Figure 19: Concentration, shape distribution (non-synthetic fibres, synthetic fibres, fragments, filaments) and photographs of ML particles identified in Greenland cod in Greenland.



Note: *Greenland cod (Sisimiut and reference sites).*

Source: *Maps are created with ESRI ArcMap 10.5.1.*

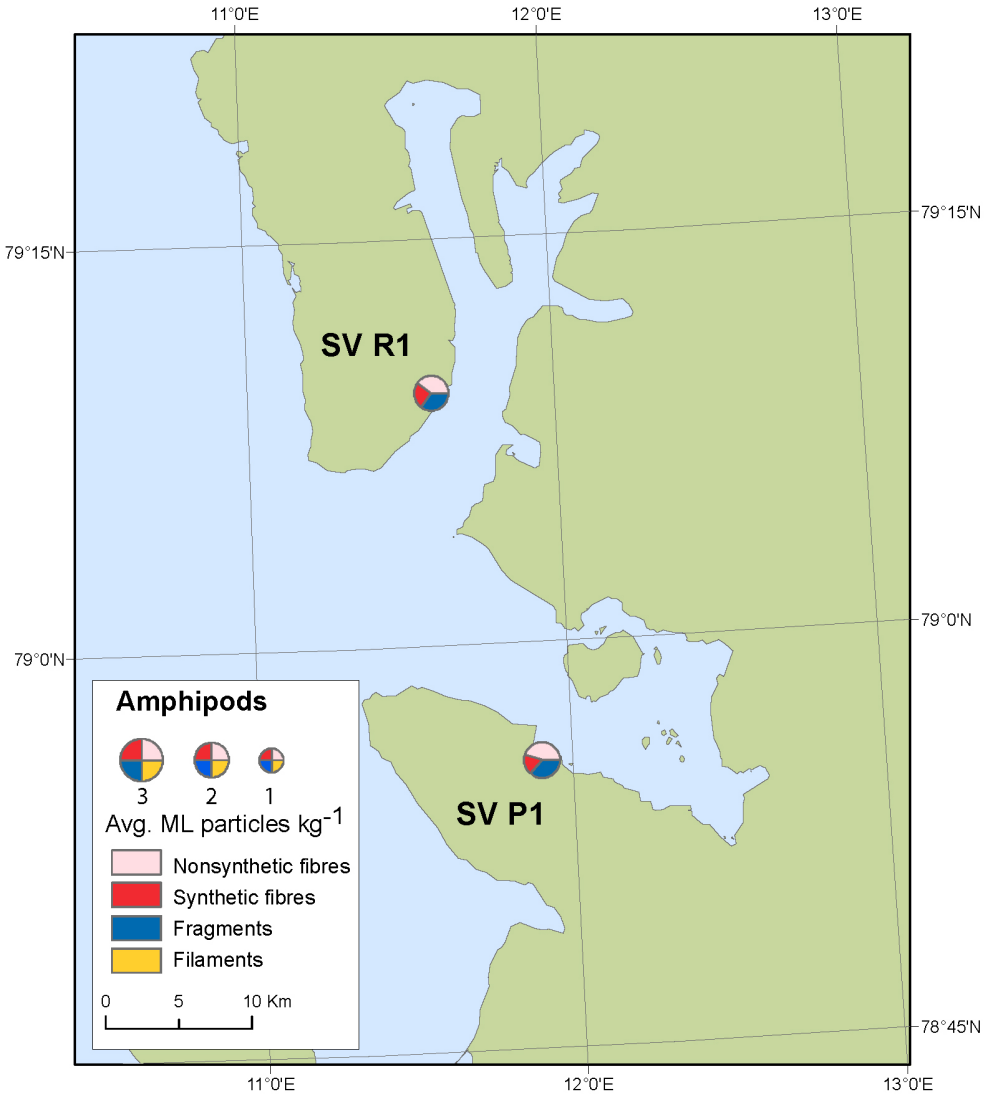
Figure 20: Concentration, shape distribution (non-synthetic fibres, synthetic fibres, fragments, filaments) and photographs of ML particles identified in blue mussels in Greenland.



Note: *Greenland, blue mussels (Sisimiut and reference sites).*

Source: *Maps are created with ESRI ArcMap 10.5.1.*

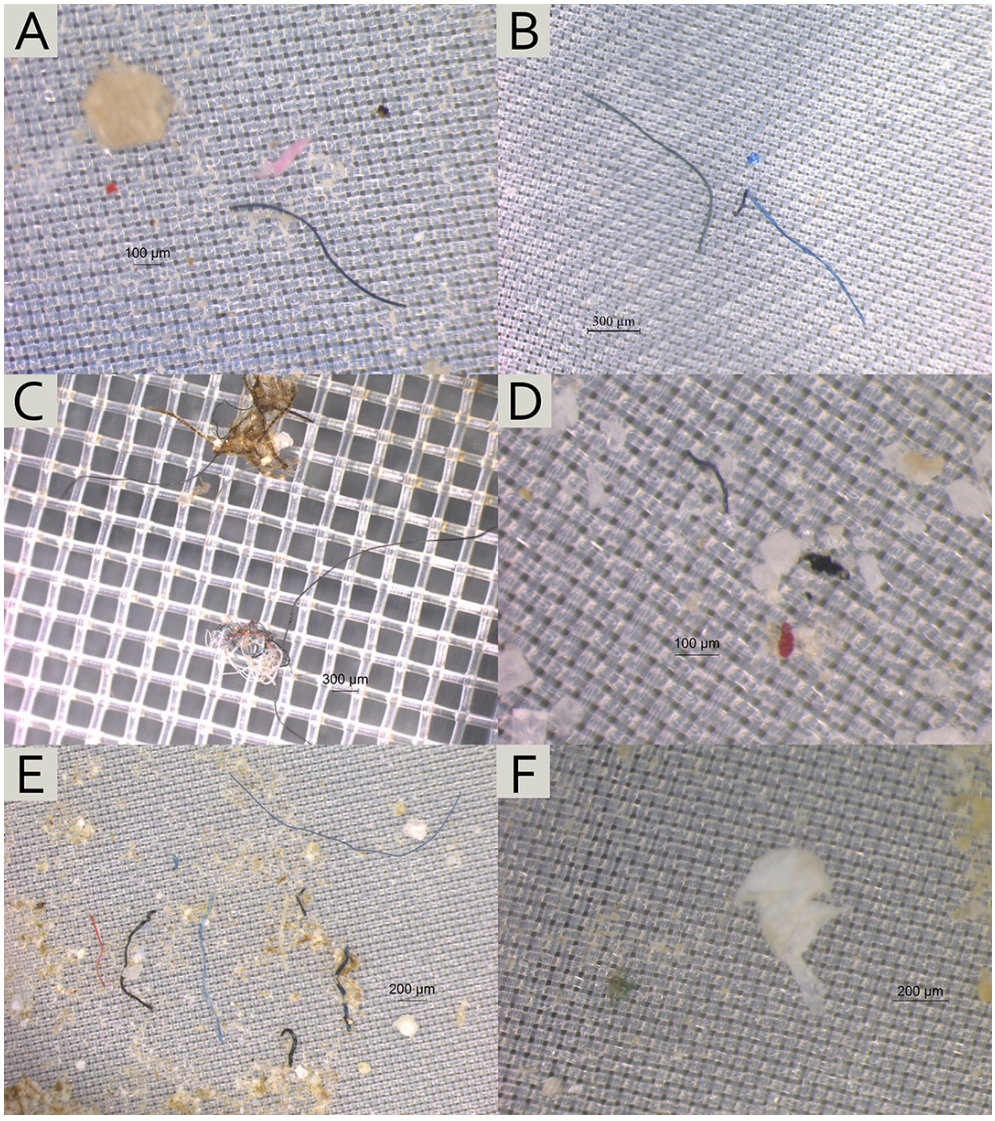
Figure 21: Concentration, shape distribution (non-synthetic fibres, synthetic fibres, fragments, filaments) ML particles identified in amphipods in Ny-Ålesund area, Svalbard.



Note: *A: Svalbard North, Amphipods (Ny-Ålesund and reference site).*

Source: *Maps are created with ESRI ArcMap 10.5.1.*

Figure 22: Photographs of ML particles identified in amphipods, Greenland cod and blue mussels at the different stations.



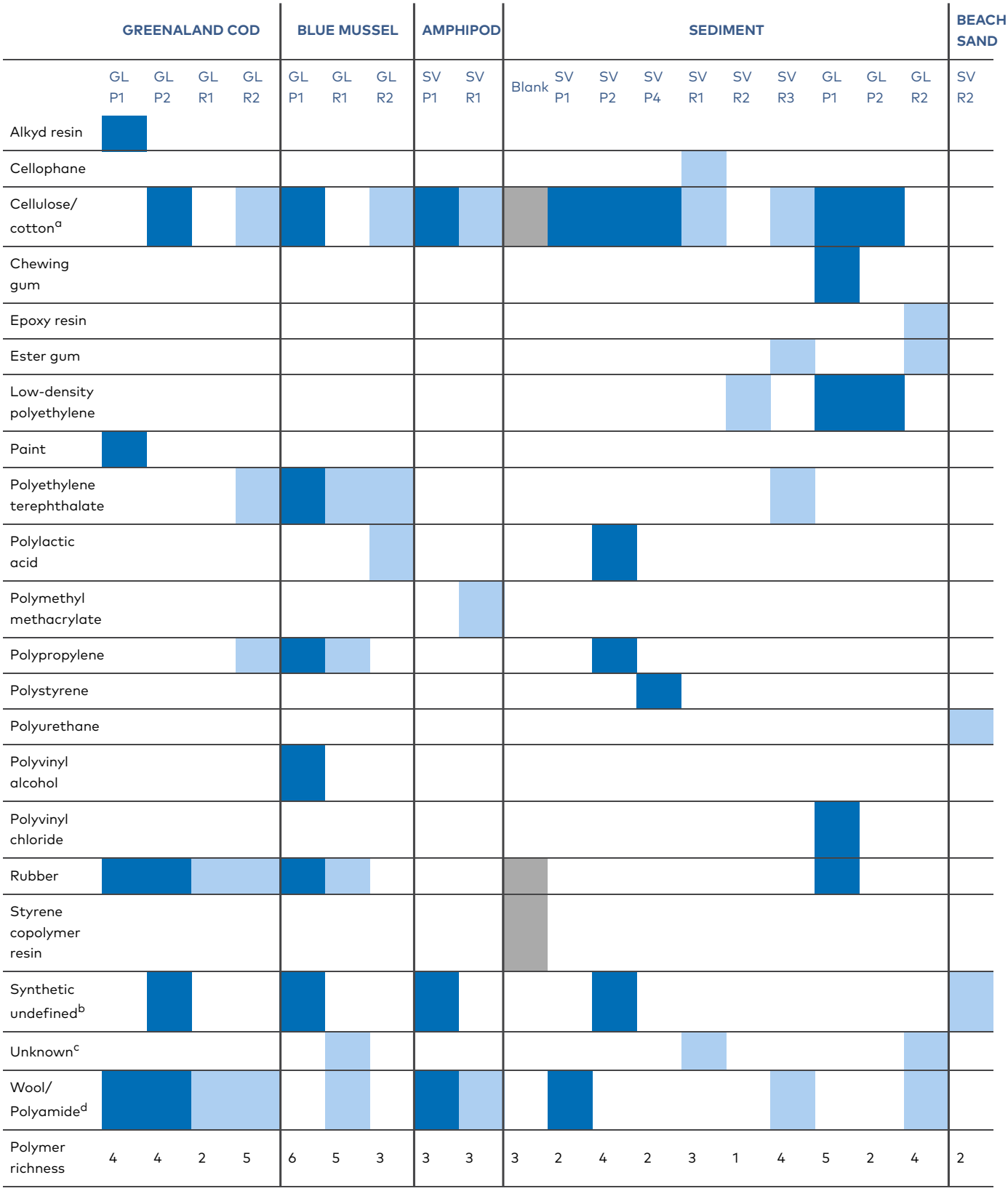
***Note: A**: ML particles at SV R1 (amphipods, Ebeltofthamna), **B**: ML particles at SV P1 (amphipods, Thiisbukta), **C**: ML particles at GL R2 (Greenland cod, Sarfanguaq land), **D**: ML particles at GL P1 (Greenland cod, Ulkebugt wastewater outlet), **E**: ML particles at GL R2 (blue mussels, Sarfanguaq land), **F**: ML particles at GL P1 (blue mussels, Ulkebugt wastewater outlet). All photographs of ML particles (A-F) are presented on a filter with the mesh size of 20 µm, except F (GL R2, cod) that is presented on a 300 µm filter.*

***Photos:** Lisa von Friesen.*

GL P1 is furthermore the station where particles with the greatest span of different plastic polymers were identified (in blue mussels), thus having the highest polymeric richness (Table 3). As mentioned above, GL R2 was different from other stations by having a larger fraction of synthetic fibres. Synthetic materials identified at this station in Greenland cod and blue mussels were PET, PP, rubber and PLA (Table 3). Polymers identified in blue mussels at the reference sites (GL R1 and GL R2) were very similar to the ones identified in cod at the same reference sites (cotton, PET, PP, rubber, PA/wool), with an additional PLA particle in mussels at GL R2 and a particle of an unknown polymer at GL R1 (Table 3). Polymers identified in blue mussels, solely at the polluted site Ulkebugt WWO (GL P1), were PVA and an undefined synthetic polymer (Table 3). Polymers identified in Greenland cod, solely at the polluted sites, were alkyd resin, paint and a synthetic undefined material (Table 3). The only synthetic materials identified in amphipod samples were polymethyl methacrylate (PMMA) at SV R1 and an unknown polymer at SV P1 (Table 3).

Rubber was recurrently identified in biota (Greenland cod and blue mussels), but only once in sediment (GL P1). Since rubber was also identified in the blank sample of sediment, rubber in GL P1 (sediment) is assumed to be a false positive possibly originating from seals of the density separation unit itself (see Appendix 1). Furthermore, the density of the separation solution (saturated NaCl, ~1.2 g cm^{-3}) applied for ML extraction from sediment is not expected to retrieve the heavier rubber particles, whose density can be higher than 1.2 g cm^{-3}. However, during extraction of biota, all ML particles present in the body (or targeted body part) of the organisms are retrieved since no density separation is applied (von Friesen et al. 2019). In total, for all matrices in Greenland (sediment, Greenland cod, blue mussels), the polymer richness was 12 at polluted sites and 8 at reference sites. In Svalbard, the total polymer richness for all matrices (sediment, beach sand, amphipods) was 10 at polluted sites and 11 at reference sites.

Table 3: Polymers and materials identified in the different matrices at the different stations. Dark blue represents locations close to wastewater outlets or dumping sites (P=polluted), lighter blue represents the reference sites (R=reference) and grey represents the blank samples for contamination control in sediment and beach sand. Polymer richness is the total number of different materials identified at the respective station and sample type. GL: Greenland, SV: Svalbard.



Note: [a]A match to cellulose/cotton was only accepted for ML particles exhibiting clear visual anthropogenic properties, e.g. unnatural color. [b]Synthetic undefined were a group of particles where no confident polymer specific identification could be made based on the spectra, but a synthetic origin was clear. [c]The category unknown were particles that could neither confidently be rejected as natural material nor accepted as synthetic. [d]Wool and polyamide were grouped together due to their similarity in FTIR spectral appearance.

Source: Data obtained in this study.

2.7 Discussion

The current investigation identified several patterns of microlitter (ML) particle pollution in arctic coastal environments. In general, higher abundances, higher diversity in terms of polymers and colours as well as specific shape distributions of ML particles were identified closer to land-based human activities. This observation supports and emphasizes the expected importance of local point sources and pathways to ML pollution even in small remote arctic towns, as well as providing an indication of the levels of background concentrations potentially originating from global diffuse pathways. The concentration of ML particles measured in sediment in this study (5–67 ML particles kg^{-1} DW > 20 µm) was within the same range as previously reported findings of microlitter in sediments (Van Cauwenberghe et al., 2015). However, much higher microlitter concentrations (but also high variance) have been reported from arctic deep-sea sediments collected in the Fram Strait (42–6595 ML particles kg^{-1} DW, > 11 µm) (Bergmann et al. 2017b). Regarding marine biota, reported concentrations of microlitter varies vastly (Rezania et al., 2018) and our reported concentrations do not stand out in either direction.

2.7.1 Wastewater and dumping sites

In Ny-Ålesund there is a small-scale wastewater treatment plant (WWTP) installed as opposed to Sisimiut where no wastewater treatment is taking place. A recent pilot investigation of microlitter retention in the WWTP of Ny-Ålesund showed that the treatment resulted in a >99% decrease of ML particles (>20 µm) in outgoing as compared to incoming wastewater (Granberg et al. 2019). Despite this drastic reduction, effluent wastewater was measured to contain 83 ML particles L^{-1} at the time of sampling, and the wastewater was thus concluded to constitute an important source and pathway of ML particles to the recipient Kongsfjorden. Granberg et al. (2019) also detected higher concentrations of ML particles in both seawater and sediments close to Ny-Ålesund as compared to reference sites. Similarly, increasing concentrations of microlitter closer to a point source have been identified in a remote polar settlement in Antarctica (Reed et al. 2018). The higher concentrations of ML particles identified in the vicinity of Sisimiut and Ny-Ålesund (particularly in sediment) in our investigation confirm this pattern.

In addition to the higher abundance of ML particles found around wastewater outlets, a trend of higher ML diversity in terms of colour and polymeric composition was also observed closer to a point source. Higher colour diversities of ML particles were identified in marine sediments collected by the Ulkebugt WWO, the dumping site in Sisimiut (Fig. 23) and the Longyearbyen WWO as compared to other sites. In biota, a similar pattern was only found in blue mussels collected near the Ulkebugt WWO. In general, biota demonstrated higher colour diversity than sediment, potentially resulting from the concentrating effect created by active feeding on particles of different density. In terms of polymeric composition, similar materials were found in blue mussels and Greenland cod at both reference and polluted sites. In sediments, completely different materials were identified at the reference sites as compared to the polluted sites. This suggests that different "streams" of microlitter exist in the arctic coastal marine environment, probably originating from vastly different sources such as local points sources versus ML particles becoming

transported to this region from diffuse global sources. The large variation in numbers of ML particles kg^{-1} DW between replicates in sediment outside Ny-Ålesund WWO indicates that there are elevated ML concentrations within this area, but with large variation on small spatial scales. This scenario calls for a larger sample size (i.e. number of replicates and sampling sites) in order to understand the acting forcers and further enable statistical analyses.

Figure 23: The dumping site in Sisimiut, Greenland extending to the border of the ocean.

Note: *The pictures show uncovered waste and garbage piles waste and the incineration plant with a modest smokestack.*

Photos: *Lis Bach.*

The concentrations of ML particles identified in sediments outside Longyearbyen were not particularly high, as was found at the other point sources, even though this was expected due to the lack of wastewater treatment and larger human population than Ny-Ålesund. ML particles have been suggested to be transported with currents away from the immediate vicinity of this WWO, with ML particles possibly staying buoyant rather than becoming deposited in the sediment close to the WWO (Sundet et al. 2016). This complicates the use of sediment analysis as a

way to detect and quantify actual impact from the point source in the recipient. It also stresses the importance of upstream sampling, i.e. measuring ML contents in wastewater before it enters the sea. In order to link ML pollution to a source by measuring field concentrations, increased spatial and temporal resolution is required (Magnusson et al. 2016, von Friesen et al. 2020). Hydrodynamic processes and ML particle characteristics could also explain the deviating results found in sediment from Thiisbukta (SV P1) close to Ny-Ålesund, where a very low number of ML particles were detected. Since ML particles released with wastewater may be rapidly transported with prevailing currents, accumulation zones could exist far from the actual WWO discharge point. A slightly higher percentage of fibrous ML particles was observed in sediments collected near one of the WWO in Sisimiut, i.e. GL P2 (26%) compared to GL P1 (15%). A relatively large laundry facility connects its discharge water to the wastewater released by the dumping site by GL P2 (Dam et al., 2017), which likely contributes to the higher prevalence of fibrous ML particles at this site. Laundry is known to produce secondary microlitter in the shape of textile fibres (Salvador Cesa et al., 2017) and a link between the recipient and the local source can likely be established.

ML particles released with wastewater will have different intrinsic properties such as density and shape, which consequently affect their environmental journey, e.g. whether they will float or sink to the bottom (Bagaev et al. 2018). Such particle characteristics will influence the fate of ML particles in the marine environment already at the point of release, acting in combination with abiotic factors such as temperature, salinity and currents (Critchell and Lambrechts 2016) as well as with biotic factors such as biofouling (Lobelle and Cunliffe 2011). The pattern observed in this study with more fragments in sediments close to local point sources in both Greenland and Svalbard may be a result of such acting forcers. Although fibres are known to be the dominant category from WWOs (Browne et al. 2011, Mintenig et al. 2017, Granberg et al. 2019), they may be more readily transported with currents due to their complex sinking behaviour (Bagaev et al. 2017), hence prolonging the temporal importance over other factors influencing their environmental journey. The fact that polyurethane (PU) was solely identified in beach sand, may be due to the low density of some forms of PU, making it stay afloat and eventually becoming washed up on the beach rather than sinking out of the water column to the sediment. PU could also have been transported to beaches with sea birds after being ingested at sea. No other clear patterns related to polymer density were observed in this study, suggesting that the fate of ML particles is driven by a combination of factors influencing and determining their distribution in the marine environment.

In addition to sites close to expected point sources (WWOs, dumping site), two reference sites were identified as different from other reference sites due to their deviating patterns of ML abundances and/or shape distributions, i.e. GL R2 (Sarfanguaq land) and SV R2 (Krykkjefjellet).

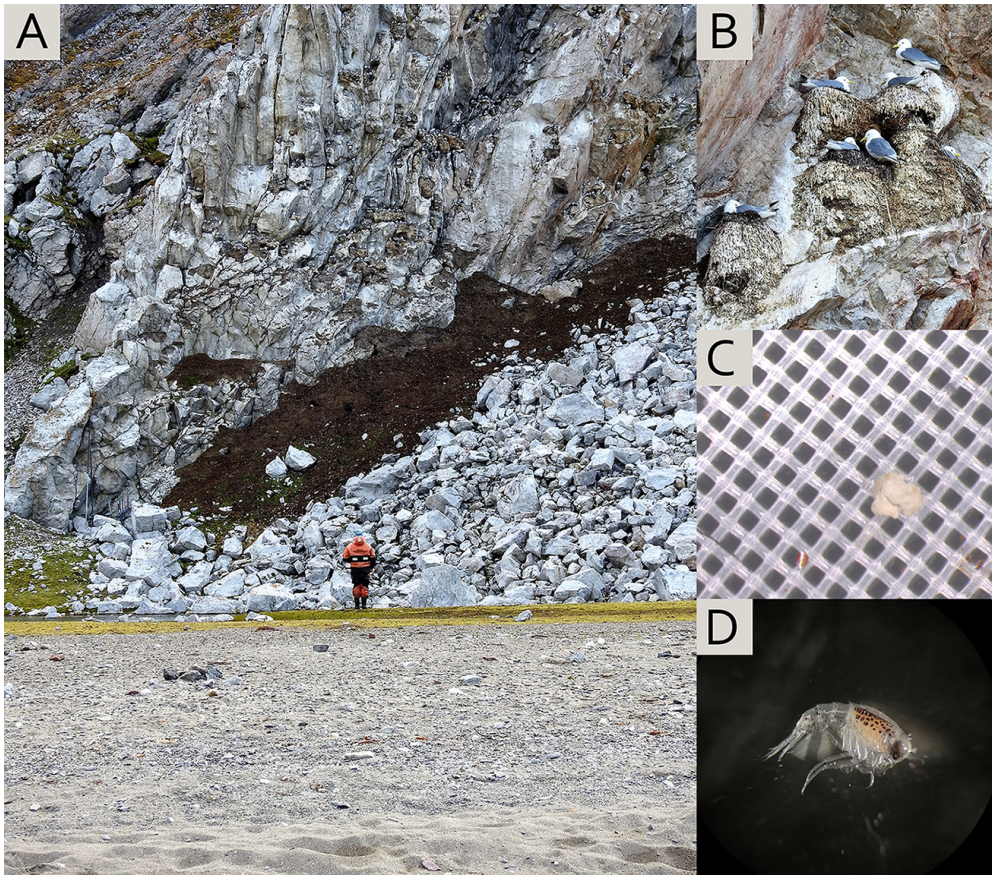
2.7.2 Beached fishing gear – a potential microlitter source off Sisimiut?

The remote uninhabited Sarfanguaq land (GL R2) was chosen as reference site in Greenland. However, a high average number of ML particles was identified here, particularly in sediment samples. The station demonstrated a pattern with a higher fraction of synthetic fibres in all sampled matrices (sediment, Greenland cod and blue mussels) as compared to other sampling sites in Greenland. Observations of large amounts of lost and/or dumped fishing gear at nearby beaches were made during sampling, which may explain the larger relative abundance of synthetic fibres in samples from this area. Several polymers identified in the sampled matrices at Sarfanguaq land (PET, PP, PA) are commonly used in fishing nets. This observation emphasises the lack of knowledge about where marine litter accumulates in the Arctic and the subsequent fragmentation and spreading of microlitter to nearby marine environments. In addition to the fishing gear that might be lost/dumped at the shores in the sampling area, it is likely that ocean currents bring ML particles to the rather closed bay area where the sampling at Sarfanguaq was conducted. Our results suggest that actions such as beach clean-ups can be useful to avoid further spread of litter fragments to local ecosystems. Beaches around Sarfanguaq land are here identified as potential hot spots. A high incidence of lost and/or dumped fishing gear has been reported in the arctic marine environment around Svalbard (e.g. Bergmann et al., 2017), and has also been observed around the areas of Sisimiut (Beach litter workshop Sisimiut 2019 – pers. comm. L. Bach). The issue needs to be further addressed at the source.

2.7.3 Kittiwake colony – a potential pathway for microlitter accumulation in Svalbard

The Krykkjefjellet (direct translation is Kittiwake mountain) beach site (SV R2) is located in Kongsfjorden, Svalbard below a bird cliff where 450–600 black-legged kittiwakes (*Rissa tridactyla*) nest during the arctic summer season (Fig. 24 A & B). Arctic seabirds are known to ingest plastic pieces when foraging at sea (e.g. Trevail et al. 2015) and may cover vast areas during their feeding journeys. Plastic particles from extensive areas may thus be concentrated to nesting sites, used as building material or expelled as parts of guano or regurgitates after ingestion (Hartwig et al., 2007). At Krykkjefjellet, the ML particles found in beach sand mainly consisted of fragments (82%) with the majority (64%) being white in colour (Fig. 24 C). A common prey of Kittiwakes is a pelagic amphipod of the genus *Themisto* (Mehlum and Gabrielsen 1993, Vihtakari et al. 2018). These amphipods are small and white (Fig. 24 D), and floating white ML pieces could easily be mistaken for amphipods. *Themisto* sp. are found in seawater adjacent to glaciers where freshwater output causes osmotic paralysis of pelagic invertebrates making them easy prey (Lydersen et al. 2014). Kittiwakes are often found foraging in front of glaciers and Krykkjefjellet is situated next to one of the major glaciers of Kongsfjorden, Kongsbreen. Whether microlitter is found in seawater close to the Kongsbreen glacier is yet unexplored. It cannot be excluded that plastic pollution at Krykkjefjellet also may originate from scientists studying these bird colonies during summertime.

Figure 24: White microplastic fragments dominated the sand samples collected below the kittiwake (*Rissa tridactyla*) colony at Krykkjefjellet in Kongsfjorden, Svalbard.



***Note: A:** Sampling below the Krykkjefjellet bird mountain, Svalbard (SV R2), **B:** Kittiwakes (*Rissa tridactyla*), **C:** White plastic particle collected on a 100 µm nylon filter, **D:** Arctic pelagic amphipod Themisto sp. varying in size from 0.2 to 60 mm.*

***Photos: A** & **B**) Lis Bach, **C**) Lisa Winberg von Friesen, **D**) D. Sneppova.*

Little auks (*Alle alle*) were recently suggested to feed selectively on lighter coloured microplastic particles in arctic waters (Amélineau et al., 2016). Lighter coloured plastics are more easily detected from above than darker ones (Thayer 1896) and are thus more frequently found in gut contents of seabirds foraging from the sea surface than in species feeding from below (Santos et al. 2016). Our observations support the theory that areas around sea bird colonies may function as important biological accumulation sites for microlitter. Reports of sea birds ingesting plastics are not a new phenomenon (e.g. Baltz and Morejohn 1976, van Franeker 1985, van Franeker and Law 2015) and one of the most severely affected species is the northern fulmar (*Fulmarus glacialis*). Individuals of this species have been found with large numbers of plastic fragments (> 1 mm) in their stomachs, constituting up to 80% of their gut contents (Trevail et al., 2015a). Northern fulmars are now in focus of arctic monitoring programs for microlitter pollution and impacts on biota (AMAP monitoring guidelines, in prep.).

2.7.4 Microlitter particles in marine biota

Fibres dominated the ML particles extracted from biota. This is in accordance with Sundet et al. (2016) who observed a dominance of fibrous ML particles in blue mussels from the waters outside Longyearbyen, Svalbard. Granberg et al. (2019) reported a larger fraction of fibrous ML particles found in the water column compared to the more fragment dominated sediments in the coastal environment of Svalbard. The observed fibre dominance in biota may indicate that certain ML particles are more bioavailable to free-living and filter feeding species than others, or that selective feeding is occurring (Shaw and Day, 1994). Another theory is that fibres have a longer retention time in organisms than fragments do and thus accumulate in the guts. Dominance of fibres in biota is frequently reported also from other waters (Lusher et al. 2017a, Bråte et al. 2018, Rezania et al. 2018). However, in a recent large-scale study of microplastics in bivalves collected in Nordic waters, Bråte et al. (2020) reported that fragments constituted 87% of the overall count while fibres only accounted for the remaining 13%. The authors attribute this "reverse" shape relationship mainly to improved analytical methodologies involving better organic matter degradation steps.

In the present study both synthetic and non-synthetic fibres are included under the term microlitter (ML). The reason for not only considering microplastics are 1) that non-synthetic microfibres in part may consist of synthetics, e.g. in technical textiles, 2) that non-synthetic fibres may carry hazardous chemicals from production or adsorbed during their lifetime, and 3) that non-synthetic fibres are ingested by biota and mechanical blocking by these fibres may be just as harmful as that of synthetic fibres. Fibres of all kinds dominate wastewater effluents (Magnusson et al. 2016, Granberg et al. 2019) and wastewater treatment is generally lacking in the Arctic (Granberg et al. 2017). Wastewater thus constitutes an important coastal ML point source or pathway (Granberg et al. 2019, von Friesen et al. 2020). When excluding or methodologically destroying non-synthetic fibres or particles in marine microlitter studies, the environmental risk may be underestimated or misinterpreted. Our recommendation for future investigations and monitoring is thus to focus on marine microlitter instead of only considering microplastics.

In terms of polymeric diversity in biota, the composition was almost identical in blue mussels and in Greenland cod at the reference sites. This may indicate that microlitter at the reference sites indeed originates from similar sources and/or represent a background microlitter composition in this region. At the polluted sites, no such similarity was identified between the two species, which further suggests that a stream of a wide range of ML particles is introduced to the environment through these point sources. The concentrations of ML particles measured in organisms in this field study represent a snapshot in time and space. In order to fully understand microlitter fate and effects, it is important to increase the spatial-temporal resolution and investigate the complex mechanisms of ML ingestion, retention times and egestion rates.

Blue mussels have been suggested as an indicator species for marine microlitter monitoring (e.g. Bråte et al. 2018, Kazour and Amara 2020). It is, however, important to recognise that ML particles do not accumulate as, for example, organic contaminants do, but are particles subjected to selective feeding and varying

ingestion- and egestion rates that will enter and leave the organism at various rates. Thus, in order to detect changes in microlitter pollution in the environment through blue mussels, these changes must likely be very large, and the processes better understood. Due to individual variation, ingested ML particle numbers may also vary greatly within a species as was observed in this study. Many or pooled samples are therefore required.

To compare concentrations of ML particles measured here with other studies is complicated due to the lack of standardisation of sampling and analytical procedures as well as of units of reporting (Hartmann et al., 2019; Lusher et al., 2017). We further argue that valuable information is not solely in the concentration of ML particles, but also in other particle properties as addressed in the present study. When studying particle concentrations, the data obtained is count data, which hampers data interpretation when counts are low (< 5) (Karlsson et al., 2018). The number of counts also influences the probabilities for detecting a certain polymer, shape or colour. Thus, the patterns reported here should be interpreted thereafter and larger sample sizes with increased temporal and spatial resolution should be aimed for in future studies.

3. Experimental studies

3.1 Aims and objectives

The main aim of the experiments was to measure ingestion and effects of microplastics in an arctic benthic lysianassid amphipod. The objectives were 1) to determine potential different effects of particle shape (fragment or fibre) of the same plastic polymer, and 2) to determine effects of plastic particle biofilm-cover. Effects were measured as change in feeding, respiration and locomotory movement.

3.2 Background

3.2.1 Measuring effects of microplastics

Microplastic particles vary in size, shape, composition and origin. Plastic materials may also contain different types of toxic chemical additives such as metal-holding dyes, flame retardants or plasticizers like phthalates (Cole et al. 2011). In the marine environment, all surfaces are subject to microbial biofilm growth and microplastic particles have been found to constitute suitable substrates (Rummel et al. 2017). The formation of biofilms often start with settling bacteria and successively develop into diverse microbial communities consisting of microalgae, bacteria and even multicellular organisms (Costerton et al. 1995, Lobelle and Cunliffe 2011). With or without biofilms, microplastics will also attract hydrophobic pollutants already present in the environment. The complexity of microplastic particles and how they subsequently affect living organisms in nature is not evident. When it comes to microplastics it is thus not obvious in what way or ways they will affect living organisms in their natural habitats, and it is not trivial to determine which effect parameters to measure with environmental relevance.

Typical effect endpoints that have been used in microplastic studies are mortality, growth, development, reproduction, energy allocation, respiration, cellular responses (e.g. enzyme activity, oxidative stress and genotoxicity) and behaviour (mainly feeding and locomotion) (Haegerbaeumer et al. 2019 and references therein). These different effect endpoints act on different levels of biological organisation from cells to individuals. The goal is to be able to determine the concentration at which microplastics have an impact, not only on cells or individuals, but on populations and ecosystems. One relevant endpoint to measure is therefore behaviour since it links physiological function with ecological processes (Scott and Sloman 2004). Behavioural change can be used to assess ecologically important effects also when the modes of action of a stressor is unclear or has multifaceted effects (Dell'Omo 2002). Behavioural change also predates mortality and thus serves as an early warning signal of stress. In this study, we measure feeding behaviour, movement and ventilation as well as respiration as effect endpoints.

Because the microplastics research field is young, standard practices for field and

laboratory investigations are not yet developed. For instance, many laboratory studies use unnaturally high concentrations of microplastic particles in artificial exposure systems (e.g. Cole et al. 2013, Setälä et al. 2014, Cole et al. 2016, Phuong et al. 2016). These studies do function as "proof of concept", indicating that marine organisms can ingest plastic particles, but the studies do not provide knowledge on the fate and effects of microplastics in nature. When aiming to perform ecologically relevant effect studies, scientists are faced with challenges and studies inevitably become exploratory.

Fibres are the most commonly occurring microplastic shape in marine benthic habitats (Woodall et al. 2014). The most commonly used plastic shape in experimental studies is, however, uniform, spherical pellets made of polyethylene (PE) or polystyrene (PS) (Lusher 2015). While PE and PS are among the most abundant polymers in the environment (Wagner et al. 2014), the impacts of other plastic types remain unstudied. In this study we thus use irregular polyethylene terephthalate (PET) particles, i.e. fragments and fibres. PET is predominantly used as packaging material and makes up to 7.1% of the total European plastic consumption (Plastics Europe 2019). PET was chosen in this experimental study in part because denser polymers ($\rho > 1$ g cm^{-3}) sink in the water column and become available to benthic species.

3.3 Amphipod biology

Natural populations of the amphipod *Orchomene* sp. were used as study organisms. The amphipod is a small (5–10 mm length) benthic crustacean belonging to the order Amphipoda. Like most other amphipods, it has a shrimp-like body, flattened from side to side and is characterized by its rather stocky body compared to other amphipod species. It has large coxal plates, light orange coloured body and large red compound eyes (Fig. 25). This species is ubiquitous within arctic shallow waters (Nygård et al. 2009). By brooding their off-spring, amphipods are generally successful and can outcompete larger and competitively superior species with planktonic larval dispersal, by preying upon their settling larvae and newly settled juveniles. The brooding strategies of amphipods and their limited dispersal also confer population stability once they are established (Conlan 1994).

Figure 25: The *Orchomene* sp. used for microplastic toxicity testing

Note: *The photo shows a group of amphipods scavenging on the piece of fish (cod) used for collecting the amphipods.*

Photo: *Lis Bach.*

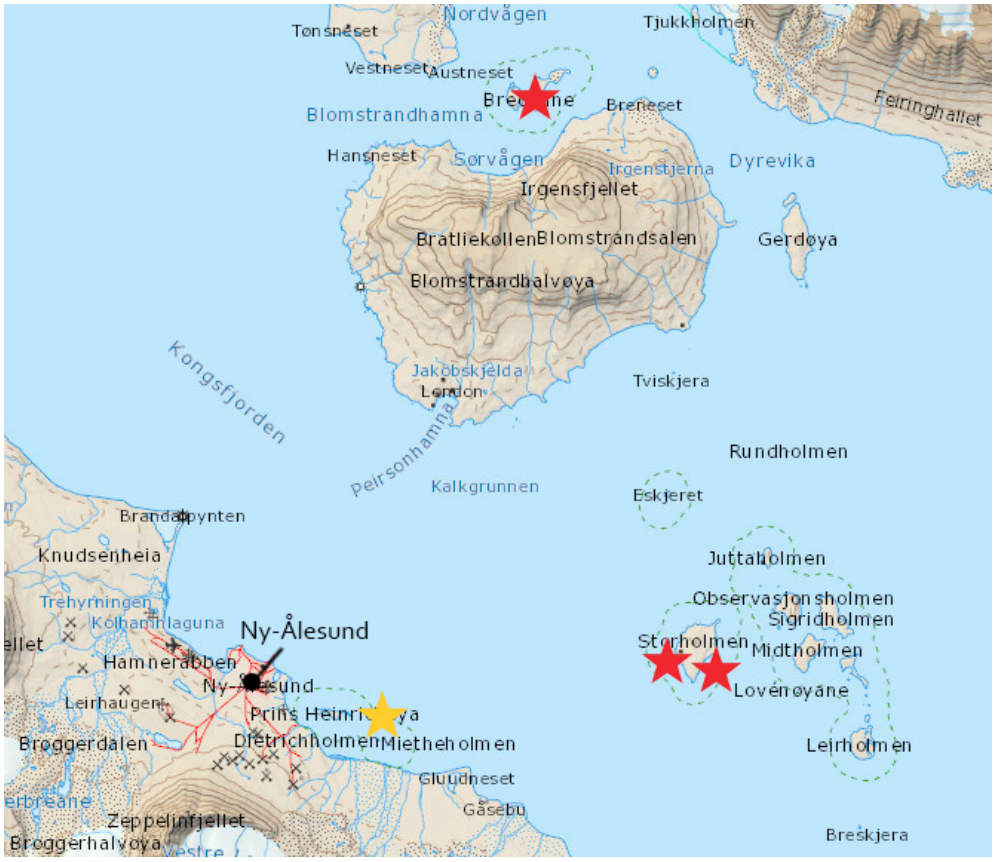
Orchomene species are scavengers, feeding on detritus. In the arctic food web, the species forms an important link between the benthic and pelagic systems as prey for fish, birds and benthic-feeding mammals such as seals. The frequent occurrence of sediment grains in the guts of this amphipod suggests a scavenging behaviour on the bottom rather than an active pelagic predation (Nygård et al. 2009). Laboratory observations also revealed that these species feed on dead zooplankton, while live ones are taken only when they were stranded near the bottom and non-active. These feeding strategies makes this amphipod an ideal model organism for studying microplastic uptake and effects. Due to the high abundance of this amphipod also in shallow coastal waters (>5 m), it is likely to be among the first organisms to be affected by sediment associated contaminants originating from land.

3.4 Experimental setup – Methods and materials

3.4.1 Collection of sediment and amphipods

Sediment was collected at two beaches (78.9678°N, 12.1347°E; 78.9869°N, 12.2258°E) on Blomstrandhalvøya in Kongsfjorden, Svalbard (Fig. 26). The sampling of amphipods was carried out in shallow waters (2–4 m) in Kongsfjorden, Svalbard. After an initial screening of various sites (Fig. 26), amphipods were successfully collected using fish (frozen cod for human consumption) baited traps in the vicinity of Prins Heinrich Island (78.9195°N, 11.9851°E) (Fig. 26 & 27).

Figure 26: Sampling sites for amphipods in Kongsfjorden, Svalbard

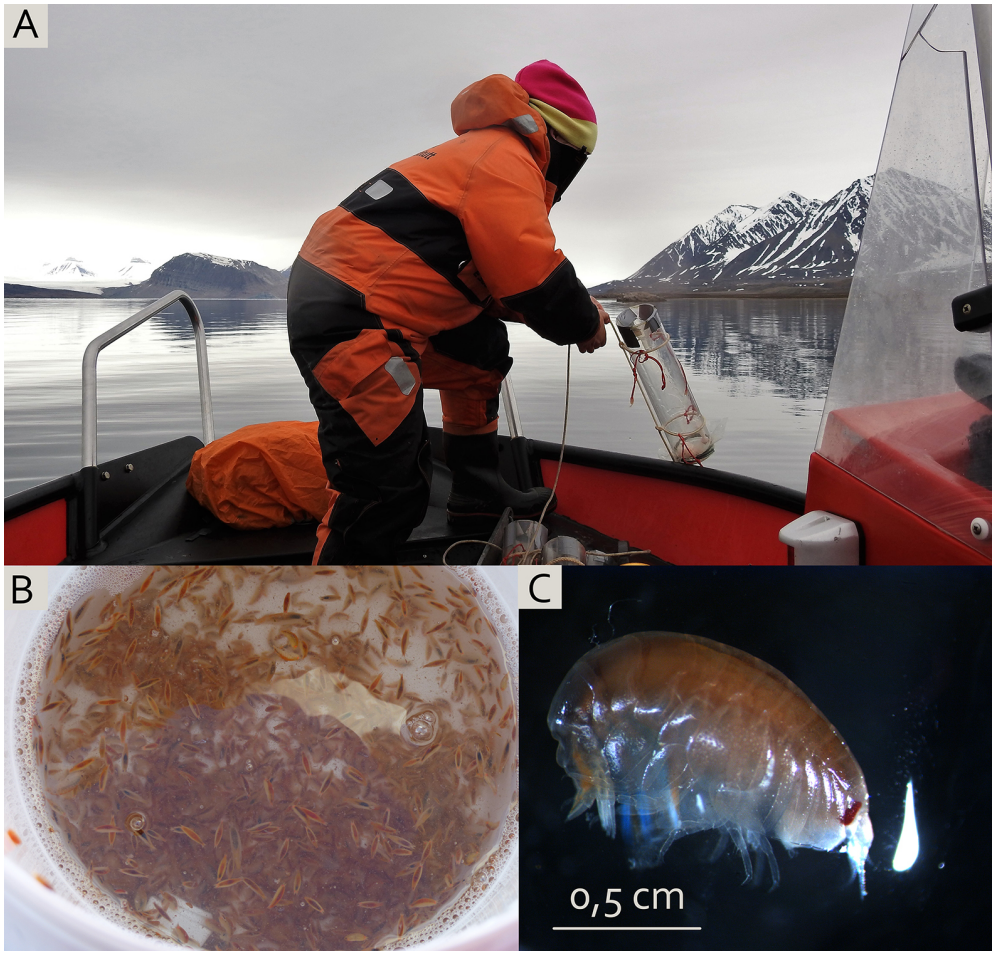


Note: *Map of sampling sites in Kongsfjorden, Svalbard with the island Blomstrandhalvøya where sediments were collected. Stars indicate sites screened for amphipods using baited traps. Yellow star indicates site for collecting amphipods for experimental exposures by Prins Heinrich Island.*

Source: *https://toposvalbard.npolar.no/*

Traps were deployed overnight and after collection, the amphipods were immediately transferred to 10 L thermoses filled with fresh seawater and transported to the laboratory in Ny-Ålesund (Marine lab) (Fig. 27). At the laboratory the amphipods were kept in large glass aquaria filled with a 3 cm layer of sieved (500 µm) and oven dried (150°C) sediment, organically enriched with *Thalassiosira weissflogii* microalgal paste (Instant Algae®, Reed Mariculture Inc.) corresponding to a 1% increase in sediment total organic carbon (TOC). The aquaria were filled with filtered and UV-treated seawater and maintained at +3°C in darkness with continuous aeration.

Figure 27: Amphipod sampling



Note: *Collection of amphipods using baited traps.* ***A)*** *deployment of trap from boat,* ***B)*** *amphipods retrieved from a trap and* ***C)*** *the amphipod Orchomene sp. in magnification.*

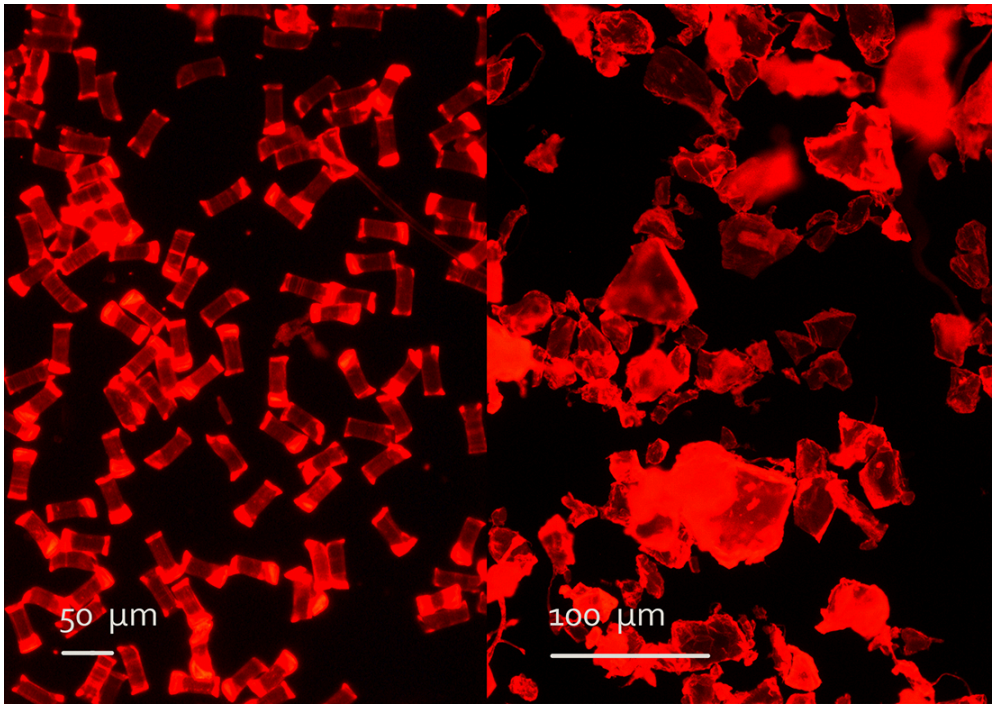
Photos: *Lis Bach, Maria Granberg and France Collard.*

3.4.2 Preparation and staining of microplastic particles

For the microplastic exposure experiment, two shapes, fibres and fragments, of polyethylene terephthalate (PET) microparticles were used to spike experimental sediments. The microfibres (l: 50 µm, Ø: 5 µm) were manually prepared from a long plastic thread according to Cole (2016). The PET fragments consisted of microparticles with a wide size range (10–100 µm). The PET fragments were ground from pellets (Goodfellow) using a pin-mill (alpine C160, IKTS, Germany). In order to detect the microplastic particles with a fluorescence microscopy, they were stained using Nile Red dye (Sigma-Aldrich) according to a protocol modified after Erni-Cassola et al. (2017) (Figs. 28 & 29 B). After the staining procedure the microparticles were either stored in MQ water or filtered onto membrane filters (pore size 0.2 µm, track etched, hydrophilic black, Millipore) and mixed with 10 ml unfiltered seawater to allow biofilms to grow on the plastic particles. All prepared plastic microparticles were stored in darkness at room temperature until use, ca. two days. In order to determine the concentration of plastic, 1 ml of each plastic

solution was added to a Bürker chamber. The stained plastic microparticles were then counted under a fluorescence microscope (DM 2000, Leica microsystems) equipped with a mercury lamp (EBQ 100). The final concentration was calculated to estimate the total amount of plastics in each solution.

Figure 28: Nile red dyed PET microplastics in a fluorescence microscope



Note: *Fluorescence microscopic images of polyethylene terephthalate (PET) fibres (left) and fragments (right) dyed with Nile red staining as used in the experimental exposures.*

Photos: *France Collard.*

3.4.3 Preparation of sediment exposures

The collected sediment was sieved below 250 µm and heated at 150°C to dryness. The sediment was then stored at room temperature in glass jars until use. Batches of exposure sediment were prepared by mixing the dried sediment with seawater (256 µl g^{-1} DW sediment) and enriching the slurry with 1% TOC by adding 1.7 µl concentrated *Thalassiosira weissflogii* microalgal paste (-0.32*10^9 cells ml^{-1}, TW 1200, Reed Mariculture Inc.) g^{-1} DW sediment. The slurry was thoroughly mixed. Subsets of the organically enriched sediment slurry were transferred to individual glass jars and microplastic particles were added to achieve an exposure gradient with the following final sediment concentrations; 0; 5,000; 50,000; 500,000 and 5,000,000 particles kg^{-1} DW sediment. From these sediments a fraction was used for each experimental exposure. The concentrations were chosen to reflect a gradient from a representative ML pollution scenario to a future worst-case scenario. An extremely high exposure concentration was included to determine "proof of concept". The lowest exposure concentration (5,000 microparticles (MPs)

kg^{-1} DW sediment) had to be in the higher end of what is found in nature for the amphipods to actually encounter any microplastic particles. 5,000 MPs kg^{-1} DW translates into 25 MP particles per glass jar. For reference, a range of 42–6,595 MPs kg DW-1 (≥ 11 µm) was measured at different stations located at the arctic deep sea sampling site HAUSGARTEN in the Fram Strait (Bergmann et al. 2017b).

All experiments were performed with four different treatments; 1) biofilm-coated PET fragments, 2) uncoated or naïve PET fragments, 3) biofilm coated PET microfibres and 4) naïve PET microfibres. Fitness-based effect endpoints were measured, i.e. microplastic ingestion, respiration and swimming/ventilation behaviour.

3.4.4 Ingestion, feeding and distribution

Experiments were carried out to determine ingestion, feeding rate and distribution of microplastics in and on the amphipods. Amphipods in each treatment were exposed together to the different exposure sediments in larger glass aquaria. After 12 h of feeding, 6 amphipods per treatment were removed, rinsed with distilled water and immediately frozen in individual Eppendorf tubes to determine gut contents. Also, in order to determine feeding rate 6 amphipods per treatment were removed after 12 h from each treatment and placed in individual scintillation vials (20 ml) containing clean, oxygenated FSW only. Vials were placed cold (+3°C) and dark and faecal pellet production was monitored and removed from each vial with a glass pipette at 2, 4, 8, and 12 hours. Faecal pellets were stored frozen (-20°C) in individual Eppendorf tubes. All samples were sent for analysis to IVL at Kristineberg Marine Research Station, Sweden.

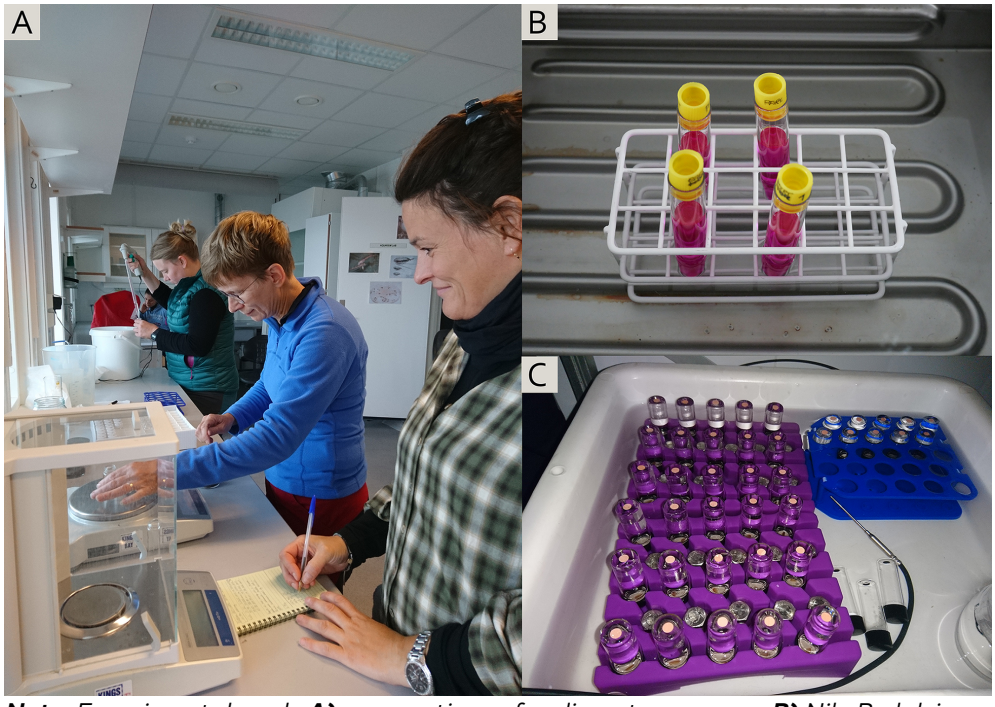
Microplastic content was analysed in the individuals and in the faecal pellets. Organisms were analysed after enzymatic digestion (von Friesen et al. 2019). The digested material and the faecal pellets were filtered onto 0.2 µm track etched, hydrophilic black membrane filters (Frisenette A/S, Denmark) and the microplastic particles still fluorescent with Nile Red dye were quantified microscopically under epifluorescence. Only results from the faecal pellets are considered here. Egestion of MPs was followed until no further MPs could be detected. The total number of egested MPs were plotted against the sediment MP concentration and statistical differences among treatments were tested using ANOVA.

3.4.5 Respiration and metabolic rates

Respiration was measured after sediment exposures. Exposure aquaria consisted of scintillation vials (20 ml) filled with ~5 g DW of exposure sediment and 18 ml of oxygenated filtered seawater (FSW). Amphipods were exposed individually for 24 h at +3°C in darkness. During measurements, the amphipods (n=6) were placed in individual respiration vials (4 ml) filled with oxygen saturated FSW at +3°C in darkness. The vials were fitted with optical oxygen sensor spots (PreSens GmbH) glued to the inner wall at the bottom of each vial (Fig. 29 C). Oxygen consumption was measured as photoluminescence of the sensor spot using a fiber optic cable placed directly above the spot on the outside wall of each respiration vial. The fiber optic supplied excitation light (505 nm) and transported the emitted fluorescence

signal (600 nm) back to the oxygen meter (Fibox 3; PreSens GmbH). Data was recorded using OxyView 3.51 software (PreSens GmbH). Measurements were performed with two-hour intervals over a period of eight hours. Metabolic rates were calculated as weight specific oxygen consumption per day and plotted against MP exposure concentration. Linear regression analysis was used to explore statistical significance.

Figure 29: Experimental preparations and exposures.



Note: *Experimental work.* ***A)*** *preparations of sediment exposures,* ***B)*** *Nile Red dying of PET microparticles and* ***C)*** *respiration incubations with amphipods in temperature-controlled bath.*

Photos: *Maria Granberg and France Collard.*

3.4.6 Movement and ventilation

Effects of microplastic exposure on movement and ventilation capacity were quantified using a Multispecies Freshwater Biomonitor (MFB, LimCo International GmbH). The MFB test chambers were partially (2 cm) embedded in sieved (250 µm) and oven dried (150°C) sediment and completely submerged in seawater (+3°C) (Fig. 30). Sediment was included in the test chamber to ensure that these burrowing amphipods maintained their natural behavior during measurements. After 24 hours of exposure in microplastic amended sediments, 4 amphipods from the control group and 4 from the second-highest concentration (500,000 particles kg DW-1 sediment) were carefully placed in the chambers. The chambers were then left to run for 24 hours in darkness at a room temperature of +12–13°C to record organism behavior. For each individual, the percentage of activity was recorded at a range of signal frequencies over a 12-h test period. Kruskal Wallis tests were performed to determine differences in percent activity at each signal frequency. Individual size

differences were tested on ln-transformed data applying ANOVA followed by a Tukey post hoc test.

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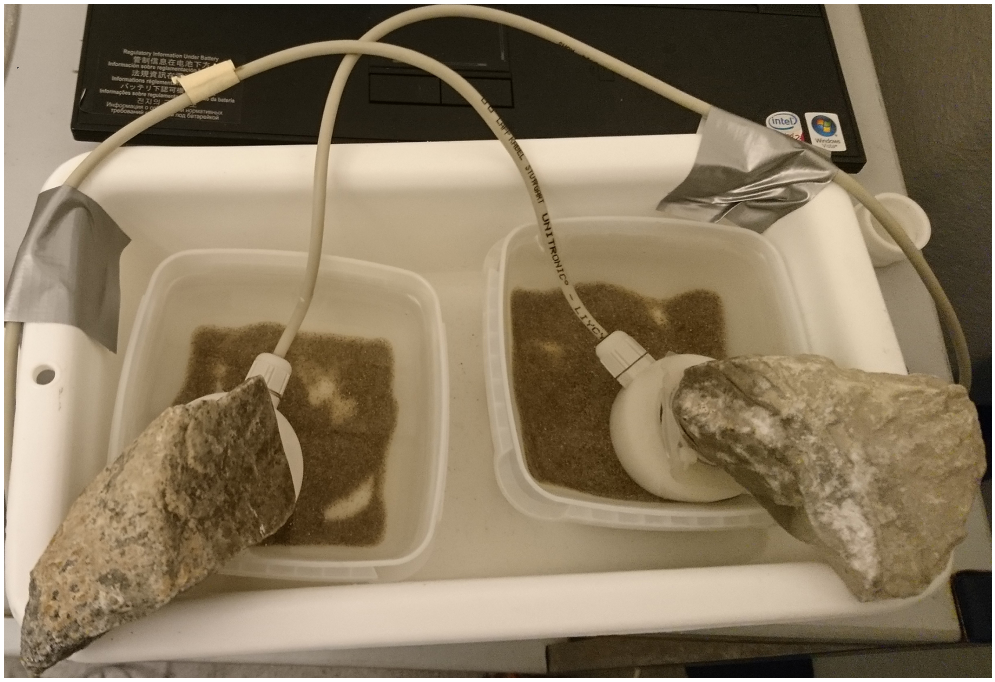
Figure 30: Experimental MFB exposure chambers submerged and resting in sediments.



Note: *Amphipods were placed in individual Multispecies Freshwater Biomonitor (MFB) chambers where electric currents generated by all types of movements were registered at a range of frequencies. The rocks were used as weights to keep the chambers in place.*

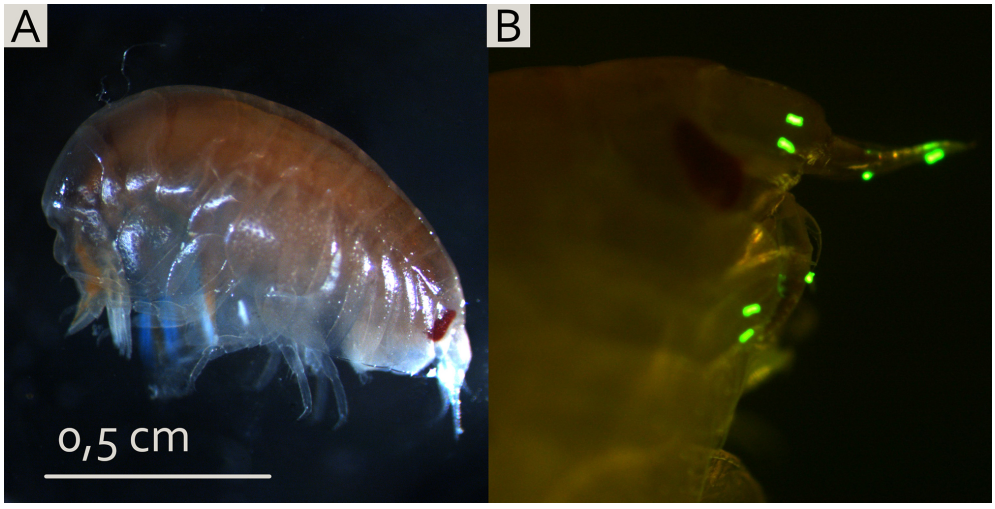
Photo: *France Collard.*

3.5 Results of experimental studies

3.5.1 Ingestion and feeding

No PET fibres were detected in the faecal material in any of the treatments or at any concentration, indicating a complete lack of ingestion of the fibre-shaped PET microplastics. Pieces of PET fibres were, however, observed to stick to the carapace of the amphipods as if the fibres were charged with static electricity (Fig. 31 B). The adhesion of PET fibres to the carapace of the amphipods can possibly have a physical impact on organism movement and/or respiration.

Figure 31: Stereomicroscopic images of the amphipod under natural and fluorescent light.



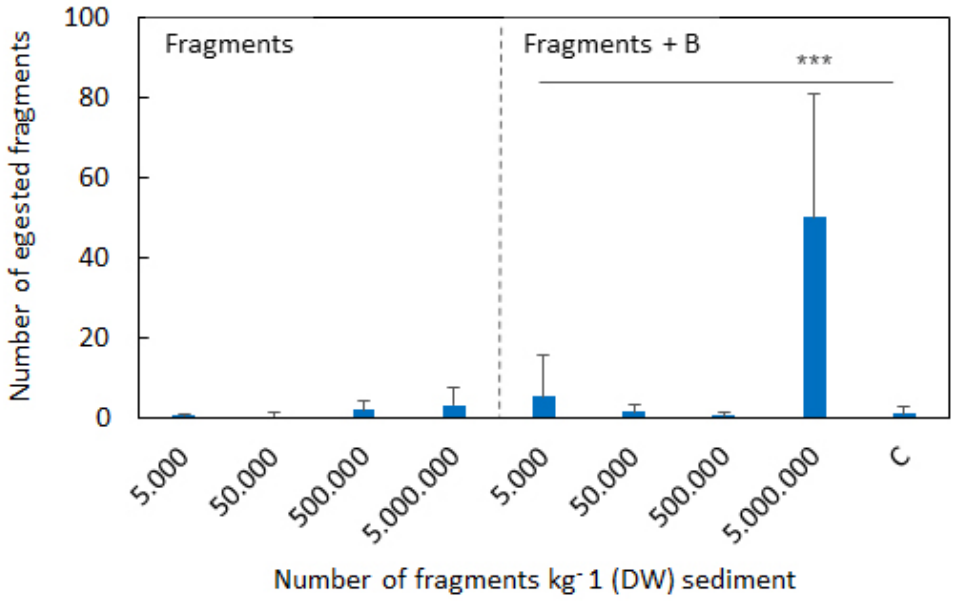
Note: *Stereomicroscopic image of the amphipod under natural light* ***A)*** *and in higher magnification under epifluorescent lighting displaying microplastic PET fibres attached to the carapace and antennae of the organism.*

Photos: *France Collard and Lis Bach.*

There was a significantly higher number of egested biofilm coated PET fragments (50 ± 31 pieces, n=6) in the highest exposure concentration, 5,000,000 fragments kg^{-1} DW compared to all other exposure concentrations including the control (1.2 ± 1.6 pieces) (One-way ANOVA; $F_{4,26}$=12.097, p<0.001) (Fig. 31). The egestion of fragments was negligible for the other exposure concentrations and did not differ statistically from the control.

The number of egested fragments from sediment exposures with naïve PET fragments did not differ from the control in any of the tested concentrations (Kruskal-Wallis One-way ANOVA on the Ranks; H4 = 2.468, p=0.650) (Fig. 25) indicating that biofilm-coating stimulates ingestion of sediment associated plastic fragments. These results indicate that the fragments are not ingested randomly, but rather selected for when covered by a biofilm.

Figure 32: Egestion of plastic fragments from feeding experiments.



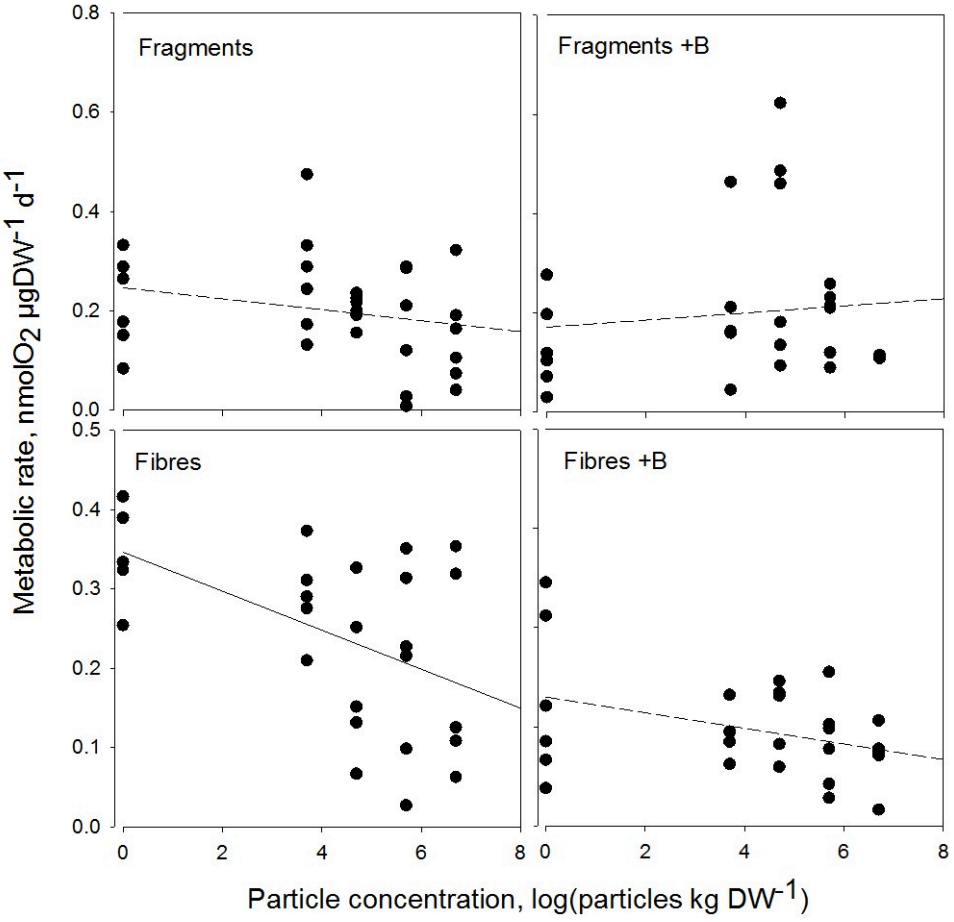
Note: *Number of fragments egested by amphipods exposed for 24 hours to sediment containing either 5,000; 50,000; 500,000 or 5,000,000 PET fragments kg* DW^{-1} *either with (Fragments +B) or without (Fragments) natural biofilms. There was a significant increase in the number of egested fragments in amphipods exposed to the highest concentration of biofilm-coated fragments (ANOVA;* $F_{4,26}$*=12.097, p<0.001 as indicated by ***).*

Source: *Own measurements.*

3.5.2 Respiration and metabolic rates

Amphipod respiration and metabolic rates were negatively affected by PET fibres. This effect was significant for naïve fibres (fibres) (Linear regression: r^2=0.256, p=0.008) and almost significant for fibres with biofilm (fibre +B) (Linear regression: r^2=0.137, p=0.057) (Fig. 33). The negative effect of fibres may be mediated by their observed tendency to adhere to the carapace of the amphipods (Fig 31 B) and thus possibly obstruct, e.g. respiratory movements. Plastic fragments had no effect on respiration or metabolic rates of the amphipod (Fig. 33).

Figure 33: Weight specific metabolic rates in amphipods exposed to the different treatments.



Note: *Weight specific metabolic rates ($nmolO_2$ µg DW^{-1} d^{-1}) calculated from respiration measurements and plotted against log transformed particle concentrations (number of particles (5,000; 50,000; 500,000; 5,000,000) kg DW^{-1} sediment). Treatments constituted sediment exposures with fragments or fibres, naïve (without biofilm) or coated with a natural marine biofilm (+B). Dashed lines indicate nonsignificant ($p > 0.05$) linear regressions while unbroken lines indicate significant ($p < 0.05$) linear regression.*

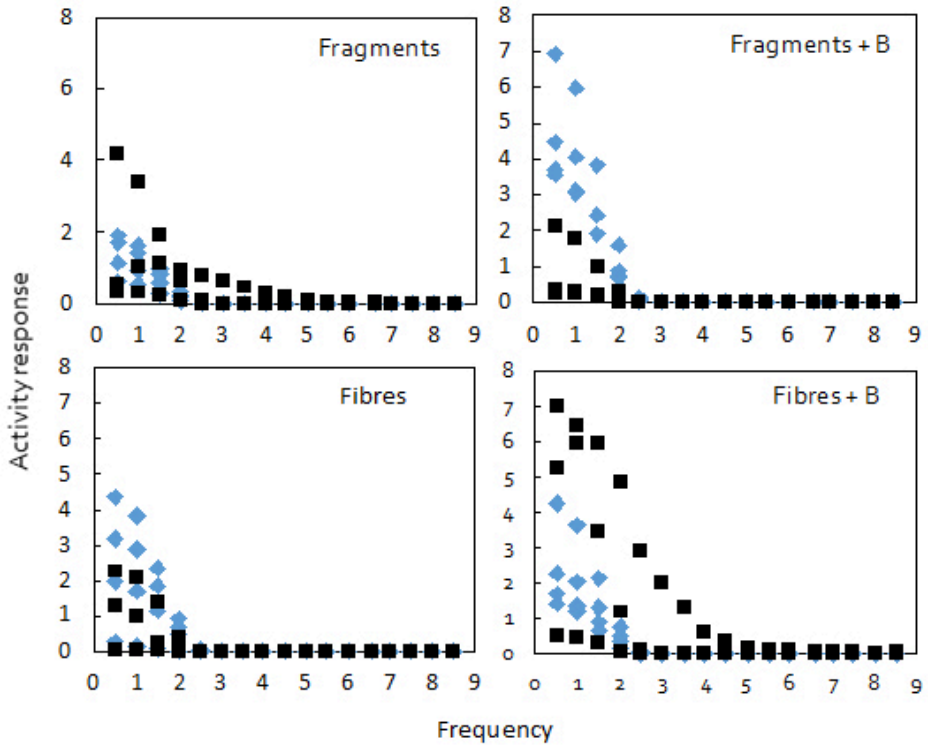
Source: *Own measurements.*

3.5.3 Movement and ventilation

The behavioural activity detected at the different frequencies of each individual in each treatment is displayed in Fig. 34. Amphipods were randomly allocated to the different treatments of the experimental setups and no significant size difference was observed between the control and treatment group within the same experimental day or between control groups or exposed groups across experimental days (ANOVA; Tukey Post hoc test; all p's >0.05). One of the exposure chambers displayed an error and was omitted from further measurements, thus n= 4 for control individuals and n= 3 for exposed individuals. All MFB measurements were

conducted during night-time when the amphipods usually leave the sediment to roam the sediment surface and possibly swim into the water column searching for food. Amphipod movement frequencies were observed at 0.5, 1.0, 1.5, and 2.0 Hz and only a few individuals generated movements at frequencies higher than 2.0 Hz (Fig. 34). There was a significantly lower response from amphipods exposed to fragments coated with biofilm up to 2.5 Hz (all p's < 0.05), while no other significant effects were observed in any of the other test conditions. There was a trend towards higher activity levels in amphipods exposed to biofilm covered fibres compared to those exposed to naïve fibres.

Figure 34: Multispecies Freshwater Biomonitor (MFB) measurements of amphipods.



Note: *MFB measurements of amphipods during a 12-hour measurement period after a 24-hour exposure period to either clean sediment (blue symbol, controls) or to microplastic contaminated sediments (black symbol, fragments and fibres with and without biofilm). Data presents the activity for each individual at each frequency at 1–9 Hz. No activity was observed at frequency 10–17.*

Source: *Own measurements.*

3.6 Discussion

The aim of this set of experiments was to determine whether shape and biofilm cover of polyethylene terephthalate (PET) microplastic particles had an impact on the ingestion of the PET particles themselves as well as on respiration and locomotory behaviour in the arctic coastal amphipod *Orchomene sp.* Although not conclusive, our results show that both particle shape (fragments or fibres) and biofilm cover affected all measured endpoints. The microplastic fibres had specific dimensions (l: 50 µm, Ø: 5 µm) while the fragments varied in sizes from 10–100 µm.

Fibres were observed to adhere to the carapace of the amphipods as though the fibres were electrostatic (Fig. 31 B). Respiration and weight specific metabolic rates decreased with increasing fibre concentration, indicating that the microfibres may have been physically obstructing the respiratory apparatus located under the carapace of the amphipods. A decreasing metabolic rate with increasing microparticle concentration was statistically significant for naïve microfibres and showed a clear trend also for biofilm covered microfibres (Fig. 33). Fibres have been observed to obstruct the digestive tract of Norwegian lobsters (*Nephrops norvegicus*) (Welden and Cowie 2016), and low concentrations of polypropylene rope fibres have been shown to hamper food uptake and cause starvation by forming fibrous balls with high gut residence times in shore crabs (*Carcinus maenas*) (Watts et al. 2015). Horn et al. (2020) observed negative effects on both reproductive success and survival in Pacific mole crabs (*Emerita analoga*) exposed to very low and environmentally relevant concentrations of polypropylene rope fibres. In the present study, fibres were not detected in faecal pellets indicating that they were not ingested. In all previous reported cases where fibres have been found to have an effect on biota they were ingested, and to our knowledge this is the first observation of plastic fibres adhering to the carapace and appendages of amphipods possibly affecting respiration and metabolic rates negatively. Furthermore, the PET fibres in the present study were relatively short and are not likely to form entangled balls.

Low levels of PET fragments were egested in exposures to particles both with and without biofilms and at all exposure concentrations. However, the number of egested fragments increased significantly for biofilm covered fragments at the highest exposure concentration, i.e. 5,000,000 MPs kg DW^{-1} (Fig. 32). These results are proof of concept that sediment associated PET fragments are ingested by *Orchomene* sp and show that biofilm cover stimulates ingestion. The smell of biofilms has been shown to attract surface feeding birds to forage on floating plastics (Savoca et al. 2016). Hodgson et al. (2018) further observed a substantial increase in ingestion and shredding of stranded plastic bags by the intertidal amphipod *Orchestia gammarellus* when the plastic bags were covered with a biofilm.

Exposure to PET fragments had no effect on respiration or metabolic rates in the amphipods. This is in line with findings by Weber et al. (2018) who tested irregular PET fragments of various size classes and concentrations on a subset of physiological endpoints in the freshwater amphipod *Gammarus pulex* without finding any effects in adults or juveniles. However, amphipod locomotory activity was in the current study significantly affected in treatments with biofilm-covered fragments. This may be a result of lacking stimuli from biofilm covered fragments

present in the pre-exposure but absent in the MFB chamber sediment. Due to instrumental failure, locomotory activity was only measured in organisms pre-exposed to a microplastic concentration of 0 and 500,000 MPs kg DW^{-1}, and the sediment in the MFB chambers did not contain any plastic. If measurements had been performed at all concentrations and the MFB sediment had been spiked with plastic, changes in locomotory activity or behaviour may have been detected. Results from this part of the study are inconclusive.

Behavioural patterns like locomotion are characteristic features for organisms and can be important for evaluating their physiological health. Changes in an organism's movement can therefore be used as a suitable early warning in ecotoxicological risk assessment (Tahedl and Häder 2001). This is particularly true for organisms like amphipods that ventilate their gills by moving their legs, distortions of this type of movement may cause severe organism impact. The activities observed at different signal frequencies could not be related to a specific behaviour for this amphipod species, as we were not able to observe the individuals during the recordings. Previous studies on *Daphnia magna* have shown that recordings at 0–0.5 Hz were related to swimming movements, while at 1–1.5 Hz the recordings were related to ventilation behaviour (Gerhardt 2006). Another study on a palaemonid shrimp (*Macrobrachium nipponense*) related locomotory behaviour to recordings at 0.5–2.5 Hz and ventilation behavior to 3–5.5 Hz (Gerhardt et al. 2002). The amphipod *Crangonyx pseudogracilis* showed swimming activity within the frequency range of 0.1–1.0 Hz, while ventilation was found to be at higher signal frequency (> 2 Hz) (Kirkpatrick et al. 2006). A study on the amphipod *Gammarus pulex* showed feeding behaviour within the frequency range of 3.0–4.5 Hz (Alonso et al. 2009). Consequently, the behaviour at lower signal frequencies are related to slower movements such as swimming, while ventilation movements are reported at higher frequencies. In the present study, only a few individuals showed movements above 2 Hz, but as mentioned above we did not observe the amphipod behaviour and specific movement patterns. Therefore, activities cannot be appointed to specific signal frequencies. Consequently, the method needs to be further adapted to each study organism and is most likely more suitable for pelagic than for sediment dwelling species.

4. Conclusion and recommendation

We found higher concentrations and a higher diversity of microlitter types and polymers in sediments and organisms closer to human settlements (wastewater outlets and dumping sites) and in places with lost and/or dumped fishing gear accumulation. We can confidently conclude that local pollution sources and pathways for microlitter do exist in the Arctic. Microlitter pollution is thus not solely transported to the Arctic via global pathways (ocean currents, atmospheric transport). The positive aspect of this is that emissions from these identified land-based sources can be reduced. Actions to introduce sustainable waste- and wastewater treatment in the Arctic should be the focus of management actions to reduce arctic plastic pollution. Treatment of both waste and wastewater would also lead to a reduction in the spread of other pollutants in the arctic environment. Additionally, based on our results, actions such as beach clean-ups can prove to be an effective measure against uncontrolled fragmentation of macrolitter and thus limit the spread of microlitter in the marine environment.

Blue mussels contained low and variable concentrations of microlitter, likely related to a general high throughput of particles in these organisms as well as the comparably low pollution levels in this region. We thus recommend that microlitter monitoring using blue mussels should be done with caution or avoided in the Arctic until more scientific data is obtained. The main reason for this is the generally lower environmental contamination levels and low retention times of microplastics in mussels. It is further costly to obtain and analyze enough samples to detect differences between stations and sites in this region.

Greenland cod contained relatively high numbers of microlitter. This finding will be important to consider in relation to marine food chain transfer. Since only the gastrointestinal tract was analysed in this study, our results have no implications for human consumption or health. For monitoring purposes our recommendation is to investigate the more stationary species with a clearer benthic feeding preference than cod, e.g. the Arctic sculpin (*Myoxocephalus scorpioides*) as a biomonitoring organism for ML particles. Although cod is attracted by wastewater and will forage there, it will also visit other areas, which will obscure the interpretation of data. Catching fish using a rod and lure is recommended. Even using this non-invasive collection method, it was apparent that the fish when stressed during catchment often expelled their gut contents. Fish caught with trawls, nets or collected at food markets are likely not providing accurate data regarding microlitter ingestion.

Our experimental results confirm previous microplastics studies on marine invertebrates showing effect only at very high concentrations not yet relevant in the arctic environment. Our results demonstrate that biofilm cover affects the behaviour of the particles and influences their effects. Microlitter rapidly become covered by a microfilm in nature and future effect studies should be carried out using naturally biofouled plastics. The shape of the plastic particles also affected the particle fate. While fragments were ingested, short fibres attached to the carapace

of the amphipods and likely obstructed normal ventilation behaviour. The effects of a particle thus also depend on other factors than the polymer composition, which should be accounted for in experimental work and monitoring programmes.

This report provides both quantitative- and impact data related to microlitter pollution in the arctic marine environment. Overall, taking together findings from the field- and laboratory investigations, the levels of microplastics required to cause effects in experimental organisms in this study were much higher than what was detected in the field (5–67 ML particles kg^{-1} DW, > 20 µm), there may be other species that are more sensitive than the one tested. The currently relatively low microlitter concentrations detected in the field should be considered as a "window of opportunity" to act to at least reduce local pollution. Consequently, introduction of sustainable waste management and wastewater treatment should be the focus of local management initiatives.

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Appendix 1: Detailed description of the extraction procedure for ML particles from sediment

See figure A1.

1. All parts are beforehand carefully rinsed with MQ and kept closed/covered with aluminum foil at all times
2. Part I and II are mounted together and filled to the upper part of the glass cylinder with NaCl (ρ=1.2 g cm^{-3}, approximately 4 L), leaving space for the sample addition
3. Around 550 g (481–653 g) of wet sediment or beach sand is wet sieved with saturated NaCl through a 2 mm metal sieve to remove larger stones and gravel that could prevent thorough rotation of the propeller
4. The propeller is started, and the sediment sample is slowly poured in from the top of part II
5. The level of NaCl is filled up to the top of part II and covered in aluminum foil
6. The propeller is kept rotating by 14 rpm for 3 h
7. The sample is left to sediment overnight (>12 h)
8. Part III is mounted up until before the 300 mm filter, with the ball valve open and the air valve closed
9. NaCl is added through the NaCl inlet/outlet until the surface reaches up to the 300 mm position
10. Ball valve is closed, the targeted sample is now separated in the top part
11. Air valve and NaCl inlet/outlet are opened to retrieve the level of NaCl below the top of part II
12. All filters (pre-washed and contamination controlled: 300, 100 and 20 mm) are being mounted in sequence (i.e. full part III)
13. Part III is being separated from part II, turned around and put into a vacuum flask, upon the ball valve is opened and the sample is vacuum filtered through the three filters
14. Part III is stepwise dissembled while filters and equipment are being rinsed by plenty of MQ water to avoid the formation of salt crystals and flush down any potential particles adhering to the walls of the equipment
15. Filters are carefully removed and stored in individual petri-dishes (polystyrene)
16. All parts are being emptied and washed thoroughly, finishing with MQ rinsing and covering by aluminium foil

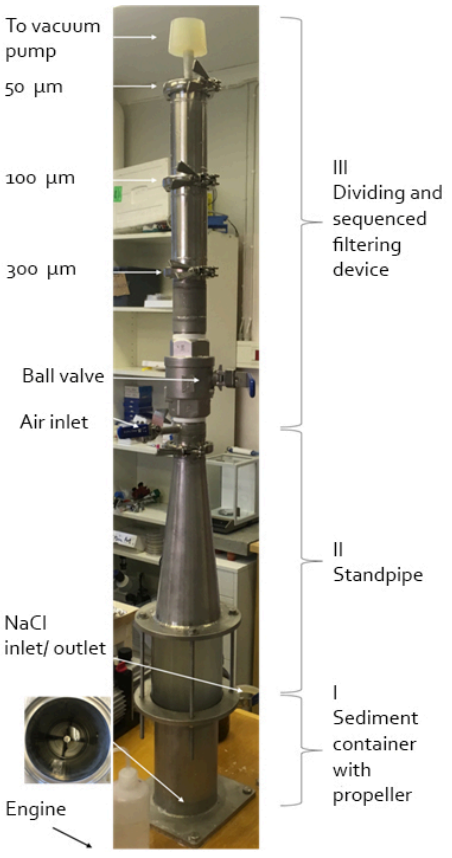

Figure A1: Schematic presentation of the density separation module for the extraction of anthropogenic micro particles from sediment. See text for detailed description on usage.

About this publication

Microlitter in arctic marine benthic food chains and potential effects on sediment dwelling fauna

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