Copyright Statement

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MICROPLASTIC ABUNDANCE, DISTRIBUTION AND COMPOSITION IN THE ATLANTIC AND ARCTIC OCEANS

by

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Author's Declaration

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award without prior agreement of the Doctoral College Quality Sub-Committee.

Work submitted for this research degree at the University of Plymouth has not formed part of any other degree at the University of Plymouth.

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Microplastic abundance, distribution and composition in the Atlantic and Arctic oceans

La Daana Kada Kanhai

Abstract

Microplastics are ubiquitous, persistent particles that are capable of posing a threat to organisms that inhabit or depend upon marine ecosystems. Understanding the origin, transport pathways and fate of these particles in the ocean is fundamentally important when evaluating the risks associated with such particles to marine organisms. In the Atlantic Ocean, the Canary and Benguela Upwelling Ecosystems are regions of high primary productivity which sustain large commercial fisheries. The influence of oceanic phenomena such as upwellings on microplastic abundance, distribution and composition remains unknown. Any microplastics that are present within such upwelling ecosystems can potentially interact with the associated biota of such systems. The Arctic Ocean, one of the most remote oceanic basins in the world, is particularly distinct due to its abiotic features and the highly specialised ecosystem that it supports. Thus far, a few studies have reported the presence of microplastics in various environmental compartments of this polar ecosystem. To date, however, there has been a particular paucity of information regarding the Arctic Central Basin (ACB).

The overall goal of the research presented in this dissertation was to target specific knowledge gaps regarding microplastics in the Atlantic and Arctic Ocean. This dissertation is divided into 6 chapters which include an introductory chapter, four core chapters which detail specific components of the research and a

discussion chapter that contextualizes the research findings and indicates prospects for future research.

The first core chapter (Chapter 2) of the present dissertation details the sampling of sub-surface waters at a single depth (11 m) between the Bay of Biscay, France and Cape Town, South Africa. This component of the research presented information regarding microplastic abundance, distribution and composition in the Benguela Upwelling Ecosystem (BUE). This specific sampling technique (sub-surface waters at a single depth, 11 m) revealed that there were no significant differences between microplastic abundance at upwelled and non-upwelled sites in the Atlantic Ocean. The provision of information about the environmentally relevant concentrations and composition of microplastics at the BUE is particularly important for laboratory experiments which seek to assess the potential threats posed by microplastics to organisms that inhabit or depend upon such productive regions.

The second core chapter (Chapter 3) of the present dissertation details the sampling of sub-surface waters in the ACB by two independent methods i.e. bow water sampling at a single depth (8.5 m) and sampling using a CTD rosette sampler at multiple depths (8 – 4400 m). Both methods provided data on microplastic abundance, distribution and composition within the ACB and emphasised the pervasiveness of these particles throughout the water column in this oceanic basin. Such findings suggested that there were mechanisms operating within this oceanic basin that were responsible for the vertical transport of these particles through the water column.

The third core chapter (Chapter 4) of the present dissertation presented preliminary information regarding the presence of microplastics in surficial sediments in the ACB. Opportunistic sampling that involved the retrieval of surficial sediments from 11 sampling locations in the ACB, indicated that microplastics were potentially making their way to the deep-sea realm of this oceanic basin and that the sediment environmental compartment was potentially functioning as a sink.

The fourth and final core chapter (Chapter 5) of the present dissertation detailed the sampling of surface waters underlying ice floes as well as sea ice at 25 ice stations in the ACB. Microplastic concentrations in sea ice from the ACB were several orders of magnitude higher than those recorded in surface waters underlying the ice floes. Backward drift trajectories for the sampled sea ice indicated that they possibly originated from the Siberian shelves, the western Arctic and the central Arctic basin. The present study found that there was no apparent pattern in the vertical distribution of microplastics in the sampled ice cores. These findings suggest that sea ice in the Arctic Ocean is functioning as a temporary sink, transport medium and a secondary source of microplastics.

While the research presented in this dissertation does provide some headway in addressing some of the knowledge gaps regarding microplastics in the Atlantic and Arctic Ocean, there is still much that remains unknown and thus there is much scope for future research.

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Chapter 1

Introduction

1.1. Microplastics in the ocean

Marine litter is broadly defined as, "*any persistent, manufactured or processed solid material discarded, disposed of or abandoned in the marine and coastal environment*", with plastics comprising over 60% of all marine litter in the ocean (UNEP 2009; AWI Litterbase 2018). Within the past few decades, plastic debris in the ocean has emerged as an issue of global concern due to the potential threat that plastics pose to organisms that inhabit or depend upon the marine environment (UNEP 2014). Plastics comprise of a diverse array of synthetic polymers that are often sub-divided based on size classifications into macro-, meso- and microplastics (Ryan 2015). Microplastics can be defined as plastic particles < 5 mm in diameter that can enter the environment from a combination of terrestrial and marine anthropogenic activities (Arthur et al. 2009). They may be of primary origin i.e. industrially created for use as exfoliants in cosmetics, as abrasives in synthetic 'sandblasting' media and resin pellets, or secondary origin i.e. whereby they are formed as a result of the fragmentation of macro or mesoplastics (Andrady 2017). Concern regarding the presence of microplastics in the marine environment stems from the fact that they are (i) ubiquitous, (ii) persistent and, (iii) a potential threat to marine biota.

The ubiquitous nature of microplastics is such that they have been recorded in every environmental compartment of the world's oceans (Lusher 2015). Some of the highest microplastic abundances have been recorded in (i) oceanic waters of the North East Pacific (279 ± 178 particles m^{-3}), (ii) deep sea sediments of the Fram Strait (42 – 6595 microplastics kg^{-1} dry sediment) and, (iii) sea ice of the Arctic Ocean (1.1×10^6 – 1.2×10^7 particles m^{-3}), (Desforges et al. 2014;

Bergmann et al. 2017; Peeken et al. 2018). Within the ocean, surface waters are not the ultimate repository for plastic debris (Cózar et al. 2014; Eriksen et al. 2014). In fact, it has been suggested that deep sea sediments and sea ice act as sinks for microplastics in the ocean (Woodall et al. 2014; Obbard et al. 2014). Furthermore, there are several factors which may potentially influence the vertical flux and overall fate of microplastics in the ocean. Some of these include (i) the ingestion and subsequent egestion of microplastics by marine organisms in faecal pellets (Cole et al. 2016), (ii) the attachment of microplastics to mucus 'houses' of larvaceans (Katija et al. 2017), (iii) the incorporation of microplastics in aggregates of various algal species (Long et al. 2015), (iv) biofouling of microplastics by microorganisms (Fazey and Ryan 2016) and, (v) other abiotic factors such as oceanic currents, wind stress, etc (Kukulka et al. 2012; van Sebille et al. 2012).

Concern about microplastics in the world's oceans is in part driven by their discovery in several phyla of marine organisms (Lusher 2015). Since the presence of a contaminant does not automatically imply impact, laboratory studies have sought to investigate impact by conducting exposure experiments. Some of these have shown that microplastics can negatively affect (i) algae (*Scenedesmus obliquus*) by hindering their photosynthesis/growth (Bhattacharya et al. 2010; Besseling et al. 2014), (ii) lugworms (*Arenicola marina*) by reducing their feeding and energy reserves (Besseling et al. 2013; Wright et al. 2013), (iii) mussels (*Mytilus edulis*) by reducing their filtering activity and decreasing lysosomal membrane stability (Von Moos et al. 2012; Wegner et al. 2012), (iv) copepods (*Calanus helgolandicus*) by reducing their feeding and reproductive output (Cole et al. 2015) and, (v) fish (*Pomatoschistus microps*; *Oryzias latipes*)

by causing liver stress, negatively impacting upon cholinergic neurotransmission and leading to endocrine disruption (Oliveira et al. 2013; Rochman et al. 2013; Rochman et al. 2014). However, since the majority of these experiments exposed marine organisms to microplastic concentrations that were not environmentally relevant i.e. not recorded in the natural environment, great uncertainty remains regarding this issue and thus it is important that risk should not be overstated (Phuong et al. 2016; Burton 2017; Paul-Pont et al. 2018).

1.2. Microplastics in the Atlantic Ocean

The Atlantic Ocean, the second largest ocean in the world, is bound by continents (Europe, Africa, North and South America) on its eastern and western edges. This ocean has the largest meridional extent since it stretches to the Arctic in the north and the Antarctic in the south (Stramma 2001). Within the Atlantic basin, some of the oceanic phenomena which are of particular interest when discussing plastic pollution are its (i) sub-tropical gyres and, (ii) coastal upwelling ecosystems. Both environmental data (Law et al. 2010; Cózar et al. 2014) and ocean models (van Sebille et al. 2012; Eriksen et al. 2014) have indicated that sub-tropical gyres of the world's oceans (including those of the Atlantic Ocean) are accumulation zones for plastic debris (Figure 1.1). With respect to microplastics in the Atlantic Ocean, some of the highest abundances (0 – 300 particles m^{-3}) have been recorded in near-surface waters (depth ~ 3 m) of the North Atlantic sub-tropical gyre (Enders et al. 2015). Although microplastic concentrations in these regions are very high in comparison to other areas in the world's oceans, gyres have been regarded as biological deserts due to their low levels of marine biodiversity (Rochman et al. 2014; Polovina et al. 2008), (Figure 1.2).

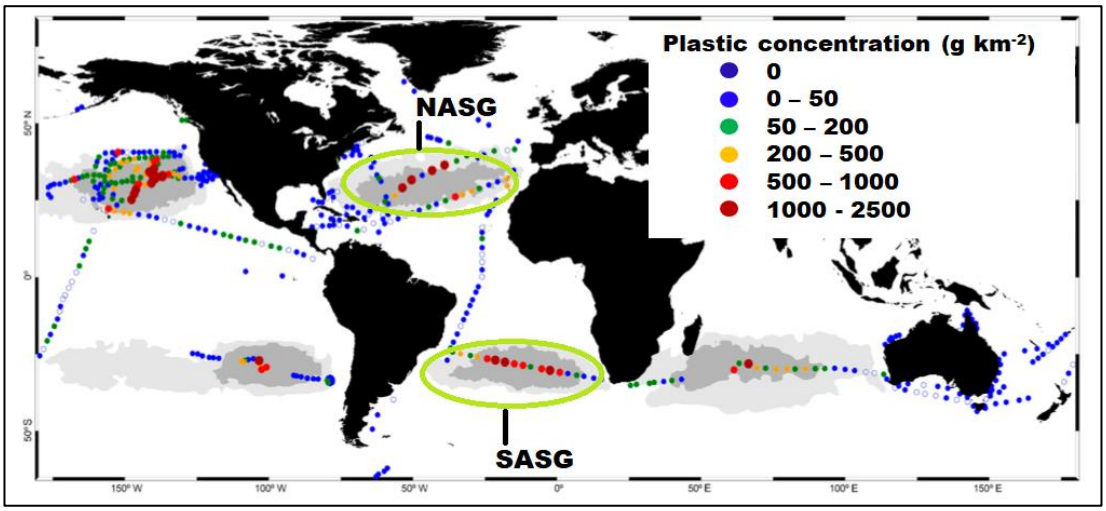

Figure 1.1: Plastic concentration in oceanic surface waters, highlighted are those of north (NASG) and south (SASG) Atlantic sub-tropical gyres [Adapted from Cózar et al. 2014]

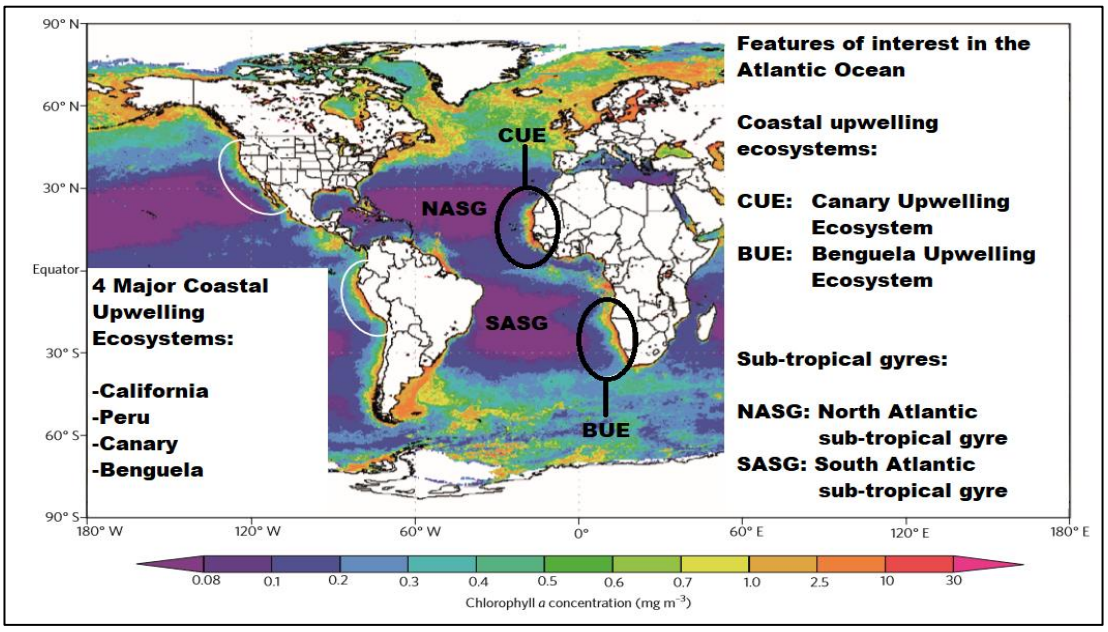

Figure 1.2: Upwelling regions and sub-tropical gyres in the Atlantic Ocean [Adapted from Capone and Hutchins 2013]

In the Atlantic Ocean, coastal upwelling is responsible for high biological productivity in the (i) Canary Upwelling Ecosystem (CUE), and (ii) the Benguela Upwelling Ecosystem (BUE), (Figure 1.2). For the most part, however, oceanic

waters considered to be ‘biota-rich’ have been understudied. An understanding of microplastic pollution in such waters is particularly important due to the potential that exists for enhanced interactions between microplastics and marine organisms that inhabit or depend upon such waters (Cole et al. 2015). Furthermore, the influence of oceanic phenomena such as coastal upwellings and their influence on microplastics remains an area that is to date not well understood. Microplastic sampling along a latitudinal gradient in the Atlantic Ocean therefore presented an opportunity to study microplastics in ‘biota-rich’ waters, i.e. at the Canary and Benguela Upwelling Ecosystems, and to investigate whether oceanic phenomena such as upwellings were capable of influencing microplastic abundance, distribution and composition. In the present dissertation, Chapter 2 reports on ‘Microplastic, abundance, distribution and composition along a latitudinal gradient in the Atlantic Ocean’.

1.3. Microplastics in the Arctic Ocean

The Arctic Ocean, the world’s smallest ocean, is comprised of a deep central basin surrounded by extensive continental shelves (CAFF 2013). This oceanic basin is particularly distinct due to (i) its abiotic features, some of which include a central area of perennial pack ice, seasonally extreme environmental conditions and an upper layer of lower salinity water and, (ii) the highly specialised ecosystem it supports, with some examples of its inhabitants including marine organisms which are endemic, commercially important and central to the functioning of the ecosystem (CAFF 2013, CAFF 2017). Despite its remote location away from major population centres, it has been suggested that plastic debris may enter this ecosystem as a result of (i) riverine input from Siberian and Canadian rivers, (ii) the influx of contaminated Atlantic (via the Fram Strait) and

Pacific waters (via the Bering Strait), (iii) local anthropogenic activities such as shipping, (iv) biotransport and, (v) atmospheric deposition (Mallory 2008; Zarfl and Matthies 2010; Provencher et al. 2012; Bergmann and Klages 2012; Trevail et al. 2015; Tekman et al. 2017; Cózar et al. 2017). The presence of plastic debris, specifically microplastics, in the Arctic Ocean is an issue that warrants attention due to the potential threats that these contaminants may pose to inhabitants of this ecosystem. Within this oceanic basin, only a few studies have focused on the issue of microplastics (Figure 1.3).

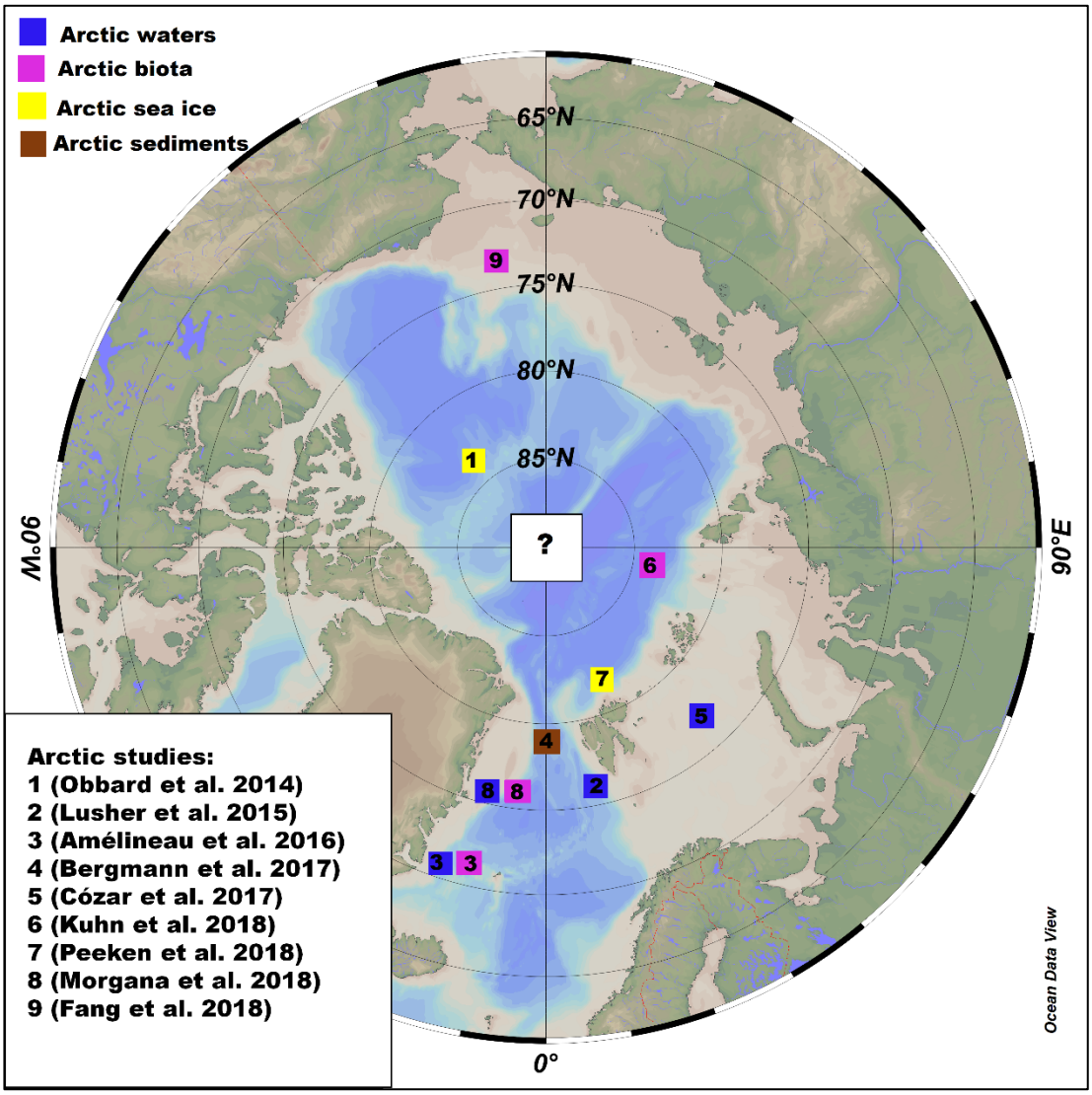

Figure 1.3: Previous studies that reported on microplastics in the Arctic Ocean

Prior to the research presented in this dissertation, only 9 studies reported on microplastics in Arctic (i) waters, i.e. south and south-west of Svalbard (Lusher et al. 2015), east of Greenland (Amélineau et al. 2016; Morgana et al. 2018) and along a circumpolar track (Cózar et al. 2017), (ii) deep-sea sediments, i.e. from the Fram Strait, the Atlantic gateway to the central Arctic (Bergmann et al. 2017), (iii) biota, i.e. from east of Greenland, the central Arctic and the Bering-Chukchi Sea (Amélineau et al. 2016; Kuhn et al. 2018; Fang et al. 2018), and (iv) sea ice (Obbard et al. 2014; Peeken et al. 2018), (Figure 1.3). Of these, only 3 analysed samples (biota and sea ice) from the central Arctic Ocean for microplastics. The research presented in Chapters 3, 4 and 5 of this dissertation focuses specifically on the Arctic Central Basin and thus addresses this knowledge gap.

Microplastic sampling of oceanic waters has in the past focused primarily on surface and near-surface waters due to the presumption that the majority of particles would be found in that region of the water column given the inherent densities of individual synthetic polymers. However, when estimated plastic production and projected inputs to the oceans were considered, it was evident that a mismatch existed between observed and expected plastic concentrations in surface oceanic waters (Cózar et al. 2014; Eriksen et al. 2014). Despite this realization that surface waters were not the ultimate repository for microplastics, few studies ventured deeper than near-surface microplastic monitoring to investigate their vertical distribution in the water column. Monitoring microplastics in sub-surface waters is particularly relevant as it can also provide some insight into the whereabouts of the ‘missing plastic’ from surface waters. Chapter 3

therefore details the research that was conducted on ‘Microplastics in sub-surface waters of the Arctic Central Basin’.

Elucidating the fate of microplastics in the marine environment is important as it can lead to the identification of environmental compartments which may be acting as sinks. Subsequently, such information can be used to identify the marine organisms that are most likely to be threatened by microplastics in an ecosystem. Within the past 5 years, deep sea sediments have been identified as a potential sink for microplastics with only four studies having reported on microplastics in deep sea sediments in various oceanic basins (Van Cauwenberghe et al. 2013; Woodall et al. 2014; Fischer et al. 2015; Bergmann et al. 2017). In the present dissertation, Chapter 4 details the research on ‘Deep sea sediments of the Arctic Central Basin: A potential sink for microplastics’.

Sea ice is an integral abiotic component of the Arctic Ocean’s ecosystem. In this oceanic basin, the presence of sea ice is closely linked to the survival of numerous species of marine organisms which either use the ice as a habitat (Søreide et al. 2010; Hardge et al. 2017; Bluhm et al. 2010) or use the ice as a key structural feature in their overall habitat (Tynan et al. 2010; Kovacs et al. 2011). Within recent times, sea ice was reported to contain orders of magnitude more microplastics than highly contaminated surface waters (Obbard et al. 2014). Sea ice in the Arctic Ocean was therefore identified as a sink for microplastics and it was suggested that upon melting it could also function as a source of these contaminants (Obbard et al. 2014). Most recently, Peeken et al. (2018) highlighted that sea ice can also function as a means of transport for microplastics in the Arctic Ocean. To date, all findings regarding microplastics in sea ice from

the Arctic Ocean were based on the analysis of either 4 sea ice cores (Obbard et al. 2014) or 5 sea ice cores (Peeken et al. 2018). In the present dissertation, Chapter 5, which is based on 25 sea cores, details the research that was conducted on 'Sea ice in the Arctic Central Basin: A temporary sink, transport medium and secondary source of microplastics'.

1.4. Overview of dissertation

The aim of this research was to address some key knowledge gaps that existed regarding microplastics in the Atlantic and Arctic Oceans. The dissertation is comprised of a total of 6 Chapters (Figure 1.4). Aside from Chapters 1 (introduction) and 6 (discussion), all other chapters target specific knowledge gaps in either the Atlantic Ocean (Chapter 2) or the Arctic Ocean (Chapters 3, 4 and 5). Below is an overview of the core chapters of the dissertation with their specific aims:

Chapter 2- Microplastic abundance, distribution and composition along a latitudinal gradient in the Atlantic Ocean

(i) To determine whether microplastic abundance in upwelled areas was significantly different from non-upwelled areas

Chapter 3- Microplastics in sub-surface waters of the Arctic Central Basin

(i) To provide a spatial overview of microplastic abundance, distribution and composition in the Polar Mixed Layer (PML) of the Arctic Central Basin (ACB)

(ii) To determine whether microplastics in the ACB were being transported out of surface waters by assessing their vertical distribution in the water column

Chapter 4- Deep sea sediments of the Arctic Central Basin: A potential sink for microplastics

(i) To provide a preliminary assessment of microplastic presence in surficial sediments of the Arctic Central Basin (ACB)

(ii) To establish whether the deep sea in this oceanic basin is possibly acting as a sink for microplastics

Chapter 5- Sea ice in the Arctic Ocean: A temporary sink, transport medium and secondary source of microplastics

(i) provide a more spatially comprehensive assessment of microplastic concentration and composition in sea ice cores in the Arctic Ocean,

(ii) assess the vertical distribution of microplastics in sea ice cores

(iii) estimate backward drift trajectories and source areas of sampled sea ice cores

(iv) assess microplastic abundance, distribution and composition in surface waters (beneath ice floes) in the Arctic Ocean

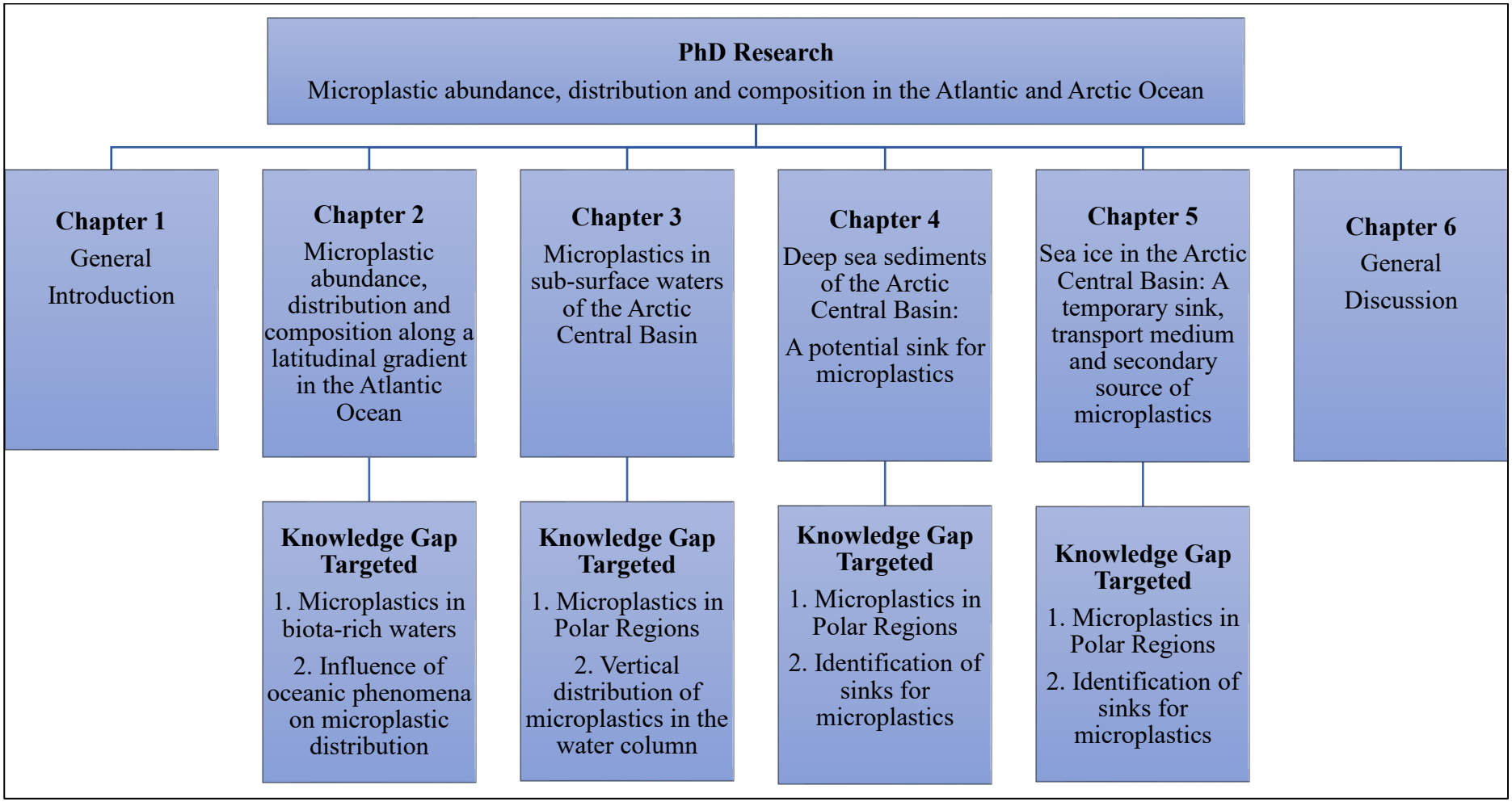

Figure 1.4: Overview of dissertation

1.5. References

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Chapter 2

Microplastic abundance, distribution and composition along a latitudinal gradient in the Atlantic Ocean

Chapter presented as a poster (see Appendix I):

Kanhai L.K, Officer R, O'Connor I, Thompson RC. (2016) Investigation of the abundance, distribution and composition of microplastics at coastal upwelling sites in the Atlantic Ocean. P*eerJ PrePrints* 4:e1629v1. Mares Conference on Marine Ecosystems Health and Conservation (Olhão, Portugal, February 1st to 5th 2016)

This chapter is a verbatim reproduction from the following published paper (published version found in Appendix II, supplementary tables in Appendix III):

Kanhai, L.K., Officer, R., Lyashevska, O., Thompson, R.C., O'Connor, I., 2017. Microplastic abundance, distribution and composition along a latitudinal gradient in the Atlantic Ocean. Marine Pollution Bulletin 115, 307-314. https://doi.org/10.1016/j.marpolbul.2016.12.025

2.1. Abstract

Microplastics in the world's oceans are a global concern due to the potential threat they pose to marine organisms. This study investigated microplastic abundance, distribution and composition in the Atlantic Ocean on a transect from the Bay of Biscay to Cape Town, South Africa. Microplastics were sampled from sub-surface waters using the underway system of the RV *Polarstern*. Potential microplastics were isolated from samples and FT-IR spectroscopy was used to identify polymer types. Of the particles analysed, 63% were rayon and 37% were synthetic polymers. The majority of microplastics were identified as polyesters (49%) and blends of polyamide or acrylic/polyester (43%). Overall, fibres (94%) were predominant. Average microplastic abundance in the Atlantic Ocean was 1.15 ± 1.45 particles m^{-3}. Of the 76 samples, 14 were from the Benguela upwelling and there was no statistically significant difference in microplastic abundance between upwelled and non-upwelled sites.

2.1.1. Keywords

Microplastic, Sub-surface waters, Upwelling, Atlantic Ocean, Marine Debris

2.2. Introduction

Within the past decade, microplastics in the world's oceans have emerged as an issue of global importance (UNEP 2011). Concern regarding these particles stems from their ubiquity, persistence and the potential threat they pose to marine organisms. The gravity of the situation is compounded by the fact that even if the introduction of plastic debris to the marine environment were to be halted, microplastic abundances are projected to increase as a result of the fragmentation of plastics that are already in the world's oceans (Thompson 2015).

Global concern about microplastics, i.e. plastic particles < 5 mm in diameter (Arthur et al. 2009), has prompted numerous investigations regarding this type of marine debris. Microplastics have been discovered in oceanic waters, deep sea sediments, sea ice and marine organisms (Lusher 2015). Studies that investigated microplastics in surface and sub-surface waters of the world's oceans found that microplastic abundance was highest in the convergence zones of the five sub-tropical gyres which are regarded as biological deserts due to their low levels of marine biodiversity (Cozar et al. 2014; Polovina et al. 2008).

Even though information exists regarding microplastics in the world's oceans, a greater understanding of microplastic abundances in biota rich waters is particularly important due to the enhanced possibilities for interactions between microplastics and organisms (Cole et al. 2015). Areas which experience coastal upwelling sustain high primary productivity and it is this enhanced productivity which supports more complex food webs comprising biota from a range of trophic levels. Coastal upwelling in the Atlantic Ocean occurs primarily at the (i) Canary Upwelling Ecosystem (CUE) which is comprised of three zones (12–19ºN, 21–26ºN, 26–35ºN) and, (ii) Benguela Upwelling Ecosystem (BUE) which stretches from the southern tip of Africa to approximately 15ºS where it is bounded by the Angola front (Santos et al. 2012; Cropper et al. 2014).

Effectively addressing the issue of microplastics in the marine environment requires information on the abundance, distribution and composition of microplastics in the world's oceans. Information from the natural environment is particularly important as it (i) provides an indication of the extent of the problem

and, (ii) informs laboratory studies by providing data on the environmentally relevant concentrations of microplastics that biota are exposed to in the natural environment. More specifically, information about microplastics at coastal upwelling sites in the Atlantic Ocean is particularly important as it could provide (i) an indication of the probability of encounter between organisms and microplastics at such sites and, (ii) insight into the potential effect of oceanographic phenomena such as upwelling on microplastics in the world's oceans. The present study investigated microplastic abundance, distribution and composition along a latitudinal gradient in the Atlantic Ocean. The specific aim was to determine whether microplastic abundance in upwelled areas were significantly different from non-upwelled areas.

2.3. Materials and Method

2.3.1. Sample Collection

This study was conducted onboard the RV *Polarstern* during Expedition PS95 and covered 7345 nautical miles (13,603 km) between Bremerhaven, Germany and Cape Town, South Africa. Sub-surface oceanic waters pumped onboard the vessel via the underway system were sampled for microplastics using the method described by Lusher et al. (2014). Sampling was conducted during November 2015 (1st to 28th) at vessel speeds of between 8 to 13 knots. Since each sample constituted the filtration of 2,000 L of water (Lusher et al. 2014), the survey effort for this study was 152,000 L of water (76 samples).

Seawater from a continuous intake located at the keel of the ship (depth 11 m) was pumped onboard the vessel using a Klaus Union Sealex Centrifugal Pump

(Bochum, Germany) at a flow rate of 25 m^3/hr and transported to the laboratory via stainless steel pipes. Prior to reaching the laboratory, the seawater passed through a primary filter (pore size 2 mm) to remove large debris items. The inclusion of this primary filter was standard operating procedure onboard the vessel and thus was beyond the control of the investigator. Potential contamination of the seawater intake by waste water generated onboard did not occur since grey water from the vessel was stored onboard for subsequent treatment. In the laboratory, seawater from the vessel's underway system was allowed to flow through a covered stainless steel sieve (250 µm) by means of a connection hose fitted into a wooden sieve cover. For the duration of the sampling, the stainless steel sieve was supported in a wooden stand. For each sample, 2,000 L of water was filtered. The length of time taken for the filtration of the specified volume of water was determined by calculation of the flow rate of the seawater. Once the specified volume of water was filtered, the sieve was removed and distilled water used to wash retained material from the sieve into a clean container. The collected material was then filtered under vacuum onto glass microfiber paper (GF/C); Whatman: 47 mm, pore size: 1.2 µm, using a Buchner funnel and a vacuum flask (Lusher et al. 2014). Each filter paper was then placed into a clean petri dish, covered and stored in a freezer (-20 ºC) until returned to the laboratory. At the start and at the end of each sample, positioning data were collected. Data for various environmental variables were obtained from the vessel's (i) thermosalinometer-keel (water temperature, salinity, conductivity), (ii) ferrybox (chlorophyll a and pH), and (iii) weather station (wind speed, wind direction).

2.3.2. Method Validation and Contamination Prevention

Method blanks and controls were used to determine whether there was any contamination during sample processing. Clean petri dishes and filter paper were left exposed to the air during vacuum filtration to determine if there was any airborne contamination. To determine whether there was any additional contamination during vacuum filtering, distilled water was passed through clean GF/C filter paper under vacuum. During visual identification of potential microplastics in samples, checks were also made for airborne contamination by exposing a clean petri dish and filter paper to the air. In order to prevent contamination in the laboratory, the following measures were taken (i) lab coats, cotton clothing and gloves were worn during sample processing, (ii) a wooden cover was placed over the stainless steel sieve to prevent airborne contamination, and (iii) all containers used during sample processing were covered and cleaned using distilled water before reuse (Lusher et al. 2014).

2.3.3. Laboratory Analyses

Samples were removed from the freezer and left to dry. Individual filter papers were then visually examined under a dissecting microscope (Olympus SZX10) equipped with a polariser and camera (Q Imaging Retiga 2000R). Potential microplastics were identified based on characteristic features such as (i) colour-homogenous colour, shininess, unnatural colours, (ii) thickness-fibres homogenous in thickness and, (iii) bending-fibres demonstrated three dimensional bending. Potential microplastics from each sample were photographed and length measurements were taken prior to transferring to a clean filter paper. Filter papers with potential microplastics from each sample were stored in clean, labelled petri dishes. Potential microplastics were assigned

to two broad categories (fibres, fragments) and to five length categories: 0.25 – 0.5 mm, 0.5 – 0.75 mm, 0.75 – 1.0 mm, 1.0 – 2.0 mm, 2.0 – 5.0 mm.

All potential microplastics as well as a subset of particles not considered to be microplastics (n = 499) were analysed by Fourier transform infrared (FT-IR) spectroscopy on a Bruker Vertex 70 Infrared Spectrometer coupled to a Hyperion 1000 microscope. The instrument was equipped with a potassium bromide (KBr) beamsplitter and an internal mercury cadmium telluride (MCT) detector. Microscope-transmission sampling was performed using a Specac DC-2 Diamond Compression cell. Spectra were recorded as the average of 32 scans in the spectral wave number range of 4000 - 600 cm^{-1} at a resolution of 4 cm^{-1} (Blackman-Harris 3-term apodisation). Bruker's Opus 7.5 spectroscopy software was used for processing and evaluating all spectra. Prior to analysing each sample, background scans were performed and sample spectra were automatically corrected. Each sample spectrum was compared with those of known standard polymers in the (i) Bruker Optics Attenuated Total Reflectance (ATR) Polymer and (ii) Synthetic Fibres ATR libraries. An initial hit quality with a score ranging between 0 and 1000 was produced for each match between sample and reference spectra, with the highest score representing the closest match. Following this preliminary matching, the top ten matches for each sample spectrum were then further evaluated using the Quick Identity Test / Euclidean Distance (ED) option. A hit quality ranging between 0 and 2 was produced for each match between the sample spectrum and the reference spectra, with the lowest number representing the closest match. Overall, matches with >70% similarity were accepted while those with 60-70% similarity were individually examined to ensure that there was clear evidence of peaks from the sample

corresponding to known peaks of standard polymers. Samples which produced spectra with a match <60% were automatically rejected.

2.3.4. Statistical Analyses

All statistical analyses were performed using R version 3.2.3 (R Core Team 2015). Descriptive statistics, histograms and box plots were generated and tests of normality (Supplementary Table 2.1) were conducted on all data sets to determine whether parametric or non-parametric statistical analyses were appropriate. Univariate (Kruskal Wallis test) and multivariate (Principal Component Analysis) analyses were conducted to determine whether sampling occurred in the Benguela and Canary Upwelling Ecosystems. Correlation analyses were performed to determine whether there were any correlations between individual environmental variables and microplastic abundance. A Generalised Additive Model (GAM) was also developed to determine which environmental variables had an effect on microplastic abundance.

2.4. Results

2.4.1. Quality Control

Microplastics were not found in the (i) air contamination controls set up during sample collection (n = 4), (ii) method blanks set up during vacuum filtration of distilled water (n = 8), and (iii) air contamination controls set up during visual identification (n = 76). This indicates that microplastics were not introduced into the samples either as a result of airborne contamination or as a result of contamination during the vacuum filtration process. Airborne contamination by

microplastics during the filtration of each sample was prevented by the use of a wooden cover over the stainless steel sieve.

2.4.2. Confirmation of sampling in upwelling ecosystems

Kruskall Wallis tests indicated that there were statistically significant differences in both water temperature (Kruskal-Wallis chi-squared = 16.599, df = 2, p-value = 0.0002) and chlorophyll a concentrations (Kruskal-Wallis chi-squared = 28.086, df = 2, p-value = 7.967e-07) amongst non-upwelled sites, Canary upwelling sites and Benguela upwelling sites. Post hoc tests indicated that there were statistically significant differences in water temperature and chlorophyll a concentrations between (i) non-upwelled sites and Benguela upwelling sites (water temperature: Nemenyi test-p value = 0.0026, Dunn's test-p value = 0.0011; chlorophyll: Nemenyi test-p value = 0.0003, Dunn's test-p value = 4.1e-07) and, (ii) between Canary upwelling sites and Benguela upwelling sites (water temperature: Nemenyi test-p value = 0.0005, Dunn's test-p value = 0.0003; chlorophyll: Nemenyi test-p value = 0.0005, Dunn's test-p value = 9.3e-07). The fact that the Benguela upwelling sites exhibited water temperatures that were significantly lower than those of all other sites and chlorophyll a concentrations that were significantly higher than those of all other sites suggests that sampling in this study occurred within the Benguela upwelling ecosystem. However, the same cannot be said for the Canary upwelling ecosystem.

Multivariate analyses were also utilised to confirm whether sampling occurred within upwelling ecosystems in the Atlantic Ocean. PCA conducted on available data (n = 76) revealed that principal components 1 (PC1), 2 (PC2) and 3 (PC3) accounted for 84.48% of the variation. Eigenvectors indicated that PC1 was

governed by increasing temperature (0.601), decreasing chlorophyll (-0.595) and decreasing wind speed (-0.519), PC2 was governed by decreasing salinity (-0.719) and PC3 was governed by increasing pH (0.803). The biplot (Figure 2.1) revealed that while the majority of sites were located towards the middle of the plot, there were a few distinct groups of sites. Of importance is the group of sites located in the upper left quadrant of the biplot characterised by low water temperatures, high chlorophyll a concentrations, high wind speeds and low salinities. Since the majority of these sites were located within the region where the Benguela upwelling was expected to occur (i.e. from the southern tip of Africa to 15°S) and certain features (low water temperatures, high chlorophyll concentrations) could be attributed to the phenomenon of upwelling, these sites were henceforth referred to as 'upwelling sites'.

2.4.3. Overview of findings

Of the 499 particles analysed by FT-IR spectroscopy, 37% were confirmed as synthetic polymers (n = 183) and 63% as Rayon (n = 316). The majority (96%) of synthetic polymers were < 5 mm in length (Figure 2.2) and thus were considered as microplastics (n = 175), with only a small percentage (4%) of synthetic polymers >5 mm in length; all fragments were less than 1 mm in length. The majority of the microplastics were fibres (n = 165) with only a few fragments (n = 10) while the Rayon particles were solely fibres. Seventy-two percent of the microplastics were blue, 9% were transparent, 8% were pink and 11% were comprised of other colours such as purple, brown, red, green, grey, black, yellow and white (Figure 2.3). Microplastic polymer types included polyester (n = 86), blends (n = 76), polyamide (n = 4), polypropylene (n = 3), acrylic (n = 2), polyvinyl chloride (n = 2), polystyrene (n = 1) and polyurethane (n = 1).

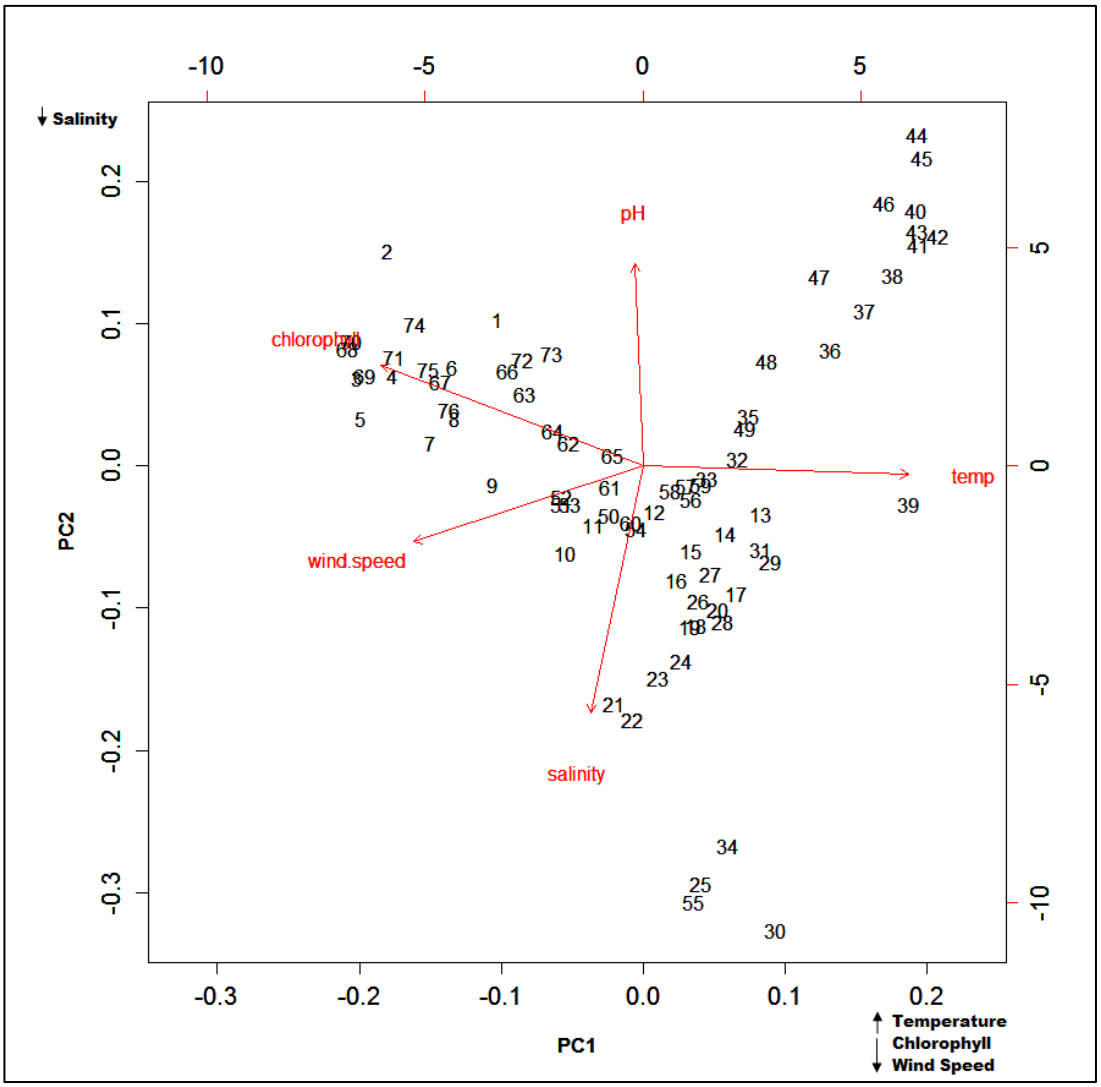

Figure 2.1: Biplot showing sampling sites based on environmental variables

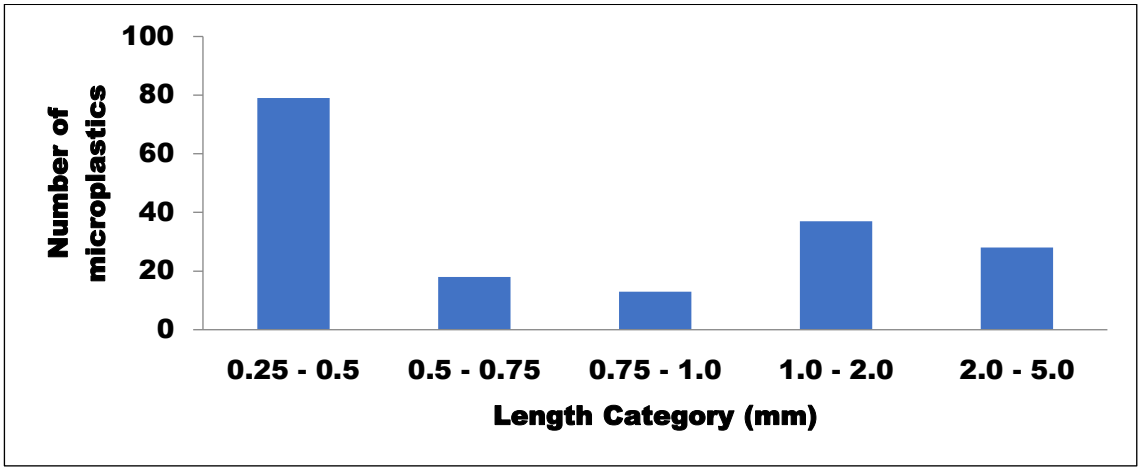

Figure 2.2: Lengths of confirmed microplastics

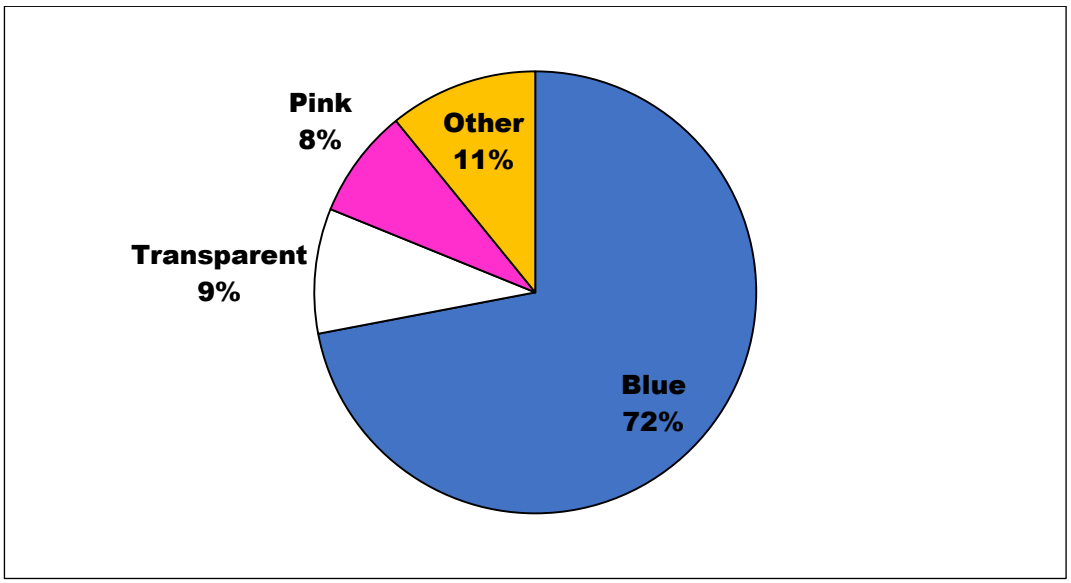

Figure 2.3: Colours of confirmed microplastics

The overall category of polyester also included particles identified as polyethylene terephthalate (PET), (n = 18). Particles identified as blends were either polyamide blends or acrylic/polyester blends and the polyamides were comprised of specific polymers such as nylon and Kevlar.

2.4.4. Microplastic abundance and distribution in the Atlantic Ocean

Microplastic abundance along the North/South latitudinal gradient in the Atlantic Ocean ranged from 0 – 8.5 particles m^{-3} (Figure 2.4). For the majority of sampling sites, microplastic abundance ranged between 0 – 2.5 particles m^{-3}. However, the areas where this range was exceeded included (i) offshore of Namibia (8.5 particles m^{-3}), (ii) off the west coast of Morocco (6 – 6.5 particles m^{-3}), (iii) the Bay of Biscay (3.5 particles m^{-3}), and (iv) off the western coast of Portugal (3.5 particles m^{-3}). A Mann-Whitney Wilcoxon test indicated that there was no statistically significant difference (Wilcoxon rank sum test p-value = 0.7111) in microplastic abundance between the Benguela upwelling sites and all other sites considered as non-upwelled sites (Figure 2.5).

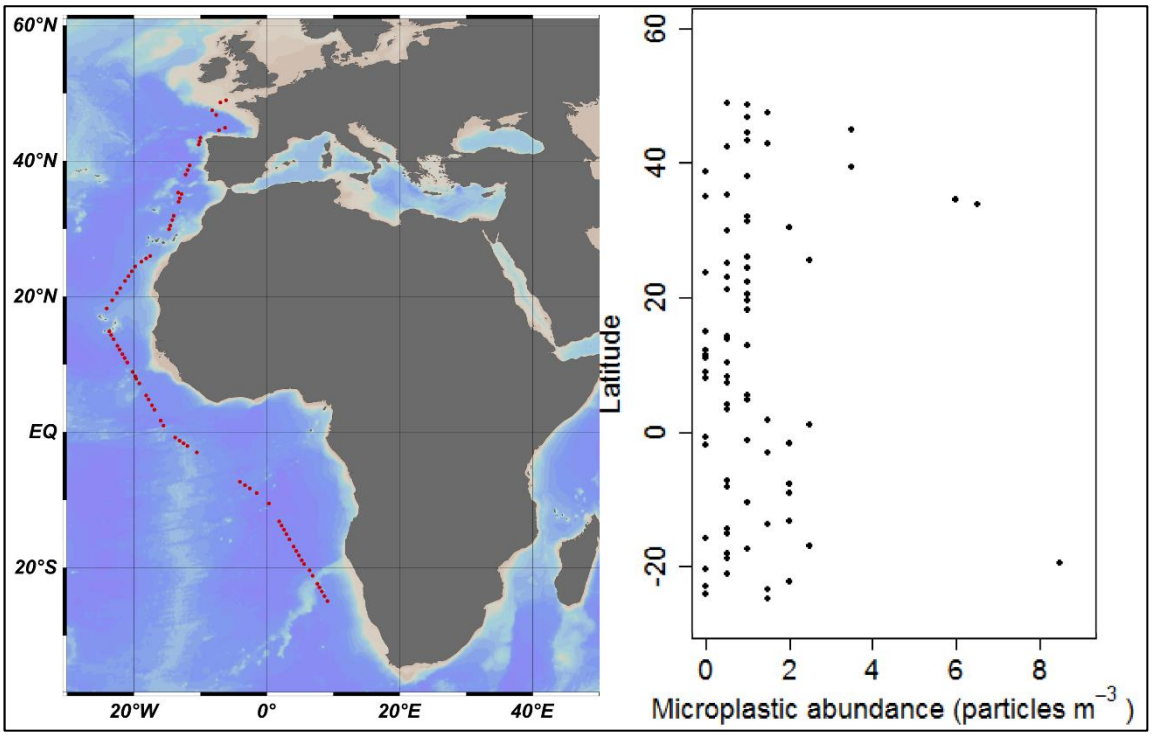

Figure 2.4: Map of sampling locations and microplastic abundance along the north/south transect in the Atlantic Ocean

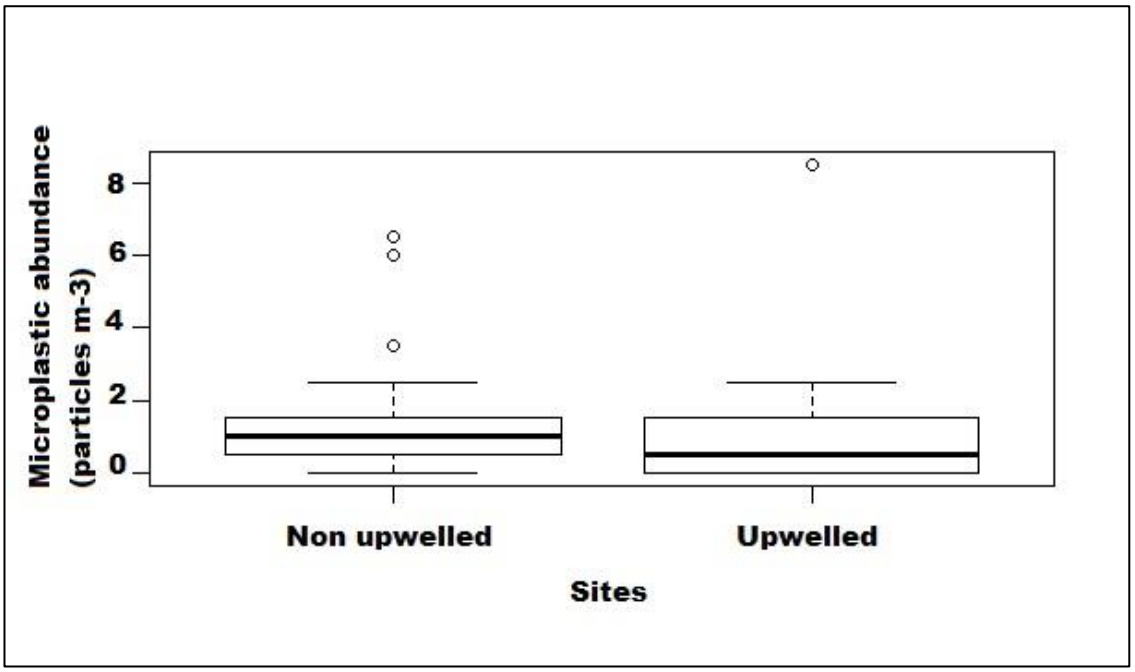

Figure 2.5: Comparison of microplastic abundances at upwelled and non-upwelled sites in the Atlantic Ocean

2.4.5. Influence of environmental variables on microplastic abundance

Correlation analyses were conducted to determine whether environmental variables influenced microplastic abundance. Overall, there were no statistically significant correlations between microplastic abundance and: chlorophyll, pH, salinity and wind speed (Supplementary Table 2.1). However, there was a statistically significant weak negative correlation between microplastic abundance and: sub-surface water temperature (Spearman's rank correlation, rho = -0.25, p-value = 0.03); and conductivity (Spearman's rank correlation, rho = -0.27, p-value = 0.02). A Generalized Additive Model (GAM) was developed to further determine the influence of environmental variables on microplastic abundance. In this model, the response variable was microplastic count (number of microplastics per sample) and initial explanatory variables included location (latitude, longitude), physico-chemical properties associated with sub-surface waters (temperature, pH, salinity), chlorophyll a concentration, weather data (wind direction, wind speed), presence of upwelling and duration of filtration. In the model, (i) the poisson family distribution of error terms was specified with a log link function due to the fact that microplastic abundance data was count data, and (ii) the explanatory variable water temperature was included as the difference between the highest and lowest water temperature recorded during sample collection (Δ water temperature). The output of the initial model was examined and based on this non-parametric smoothers (s) were applied to all explanatory variables except latitude, temperature, wind speed and upwelling. Non-significant explanatory variables (as evidenced by their p-values) were eliminated in a stepwise manner until a GAM with the lowest Akaike Information Criterion (AIC) score (283.334) and the fewest explanatory variables was obtained. The final GAM (R-sq = 0.548) was as shown below:

$$\text{Microplastic count} \sim \mathbf{latitude} + s(\mathbf{longitude}) + \mathbf{\Delta water\ temperature} + \\ + s(\mathbf{wind\ direction}) + \mathbf{wind\ speed} + \text{upwelling} + s(\mathbf{salinity})$$

Of the explanatory variables that were present in the final model, latitude, longitude, water temperature, wind direction, wind speed and salinity were the six variables found to have a significant effect on the abundance of microplastics in the Atlantic Ocean (Table 2.1).

Table 2.1: Explanatory variables included in the final best fit GAM

Explanatory variables	**p value**
Wind Direction	9.12e-09
Δ Temperature	9.25 e-05
Latitude	5.23e-05
Wind Velocity	0.000333
Longitude	0.008280
Salinity	0.014380
Upwelling	0.050666

2.5. Discussion

Interactions between microplastics and marine organisms are of particular interest due to the potential negative effects that this category of anthropogenic debris may have on marine organisms. The assessment of microplastic abundance in 'biota rich' waters is therefore particularly important due to the potential that exists for enhanced interactions between these particles and

abundant biota at such sites. Along the western coast of Africa, there were two areas considered ‘biota rich’ of specific interest: Canary Upwelling Ecosystem (CUE) and the Benguela Upwelling Ecosystem (BUE). The present study availed of a platform of opportunity aboard a research vessel transit; consequently the investigators had no influence over the vessel’s track. Both univariate and multivariate analyses indicated that although the Benguela upwelling was definitely sampled, the same could not be said for the Canary upwelling. This was possibly due to the fact that in the region where the Canary upwelling was expected to occur, the research vessel was too far offshore from the African continent. The present study found that there were no statistically significant differences between microplastic abundance in upwelled and non-upwelled areas in the Atlantic Ocean. Previous studies had suggested that upwelling may (i) provide a source of deepwater with relatively low levels of microplastics and, (ii) lead to a dilution of plastics in surface waters thus resulting in lower plastic abundances at sites within close proximity to such oceanic phenomena (Desforges et al. 2014; de Lucia et al. 2014). The findings of the present study must be taken in the context that only 14 of the 76 samples for microplastics were taken in the Benguela upwelling. More definitive statements about the microplastic abundance at upwelling regions in the Atlantic Ocean can only be made if more intensive sampling is conducted in such regions in the future.

In certain respects, the composition of microplastics along the North/South Atlantic transect was comparable to that found in other marine environments across the world. The predominance of fibrous microplastics noted in this study was consistent with similar previous findings in both surface and sub-surface waters (Cole et al. 2014; Desforges et al. 2014; Lusher et al. 2014; Zhao et al.

2014; Enders et al. 2015; Lusher et al. 2015). It has been suggested that an important source of microplastic fibres in the environment may be from the washing of clothes, with laboratory experiments demonstrating that a single garment may potentially produce > 1900 fibres per wash (Browne et al. 2011) and an average 6 kg load of acrylic fabric could release over 700,000 fibres (Napper and Thompson 2016). While fibres may in fact be more dominant in the natural environment, it is important to note that as a category of microplastics, they are generally more discernible than other categories of microplastics. Fragments, for example, have a higher chance of being disregarded due to their similarity in appearance to natural materials. Cole et al. (2014) suggests the presence of an 'operator selection bias' towards fibrous microplastics.

Analytical techniques such as FT-IR spectroscopy are immensely useful in microplastic studies as they confirm whether particles from environmental samples are indeed synthetic and, if so, identify the polymer type. In this present study, the major polymer types included polyester (50%) and blends that were either polyamide or acrylic/polyester (42%) with a minority (8%) of acrylic, polyamide, polypropylene, polyvinyl chloride, polystyrene and polyurethane. While previous studies have reported the presence of similar polymer types in their samples, low density polymers such as polyethylene and polypropylene were not as abundant in this study when compared to other studies which sampled microplastics in surface waters or even from shallower sub-surface depths (Hidalgo-Ruz et al. 2012; Cole et al. 2014; Obbard et al. 2014; Frias et al. 2014; Enders et al. 2015; Lusher et al. 2015; Kang et al. 2015a; Woodall et al. 2015). This raises the question as to whether sampling depth within the water column influences microplastic composition due to differences in the densities

and buoyancies of particular polymer types (Cole et al. 2013; Desforges et al. 2014; Woodall et al. 2015). Although techniques such as FT-IR spectroscopy can identify polymers, this information does not allow the investigator to pinpoint the exact origin of the polymers in the environment but instead reduces the possibilities (Claessens et al. 2011; Desforges et al. 2014). The synthetic polymers that were found in this study may have been derived from clothing, ropes, fishing gear (nets, lines, etc), plastic beverage bottles, as well as packaging materials (Smith 1999; Andrady 2011; Claessens et al. 2011; Napper and Thompson 2016).

In the quest to assess microplastic abundance and composition in the marine environment, one of the issues that has emerged is the prevalence of rayon fibres in the environment. Rayon is essentially regenerated cellulosic material, it is man-made and is therefore considered as semi-synthetic (Mishra 2010). In addition to being used in textiles, rayon has also been used in cigarette filters and personal hygiene products (Woodall et al. 2015). This study found that 63% of the particles analysed by FT-IR spectroscopy were rayon fibres. Previous studies have also reported that rayon fibres were the most prevalent synthetic microparticle in (i) fish from the English Channel (58%), (Lusher et al. 2013), (ii) surface and sub-surface waters in the Arctic Ocean (30 %), (Lusher et al. 2015), (iii) sea ice cores from the Arctic Ocean (54%), (Obbard et al. 2014), (iv) deep sea sediments (57%), (Woodall et al. 2015), and (v) coastal sediments from Portuguese shelf waters (81%), (Frias et al. 2016). The prevalence of rayon fibres in the marine environment suggests heightened propensity for the potential impact of this material upon biota. Laedwig et al. (2015) suggested that although natural fibres may exhibit different degradability and chemical sorption behaviours when

compared to synthetic fibres, natural fibres may still warrant environmental concerns, for example, in chemical pollution dispersion. Remy et al. (2015) further suggested that while the natural material of cellulose may not be an issue, the associated dyes or additives in the semi-synthetic fibres may pose a threat to biota.

Beyond the provision of data about microplastic abundance in the world's oceans, it is important that there is an understanding of the environmental variables that may potentially influence this issue. In this study, a generalized additive model (GAM) was developed to gain a preliminary insight into the environmental variables which had an effect on microplastic abundance in the Atlantic Ocean. A GAM model was chosen in lieu of the more common general linear model (GLM) in order to better capture the relationship between the response variable and the explanatory variables without assuming a parametric form (Crawley 2013). The best fitting GAM generated in this study indicated that location (latitude, longitude), certain physico-chemical parameters of oceanic waters (water temperature, salinity) and atmospheric variables (wind direction, wind speed) had a significant effect on microplastic abundance. These findings must be taken in the context that the model in this study was based on data from 76 samples in the Atlantic Ocean. Notwithstanding this, GLMs based on datasets from the Northeast Atlantic and Arctic Ocean also indicated that sea surface temperature and wind affected microplastic abundance (Lusher et al. 2014; Lusher et al. 2015). Based on the combination of field data and a theoretical model, Kukulka et al. (2012) indicated that that wind stress results in vertical mixing of buoyant microplastics in the surface mixed layer of the ocean. Overall then, it appears that microplastic abundance is influenced by a combination of

factors, some of which include location, atmospheric parameters and oceanographic conditions.

While comparison of microplastic abundances between studies is possible, one must be cognisant of the differences between sampling, processing and analytical techniques for microplastic identification. These differences may account for some of the variation in the findings between studies. Bearing that in mind, average microplastic abundance (1.15 ± 1.45 particles m^{-3}) in sub-surface waters along the North/South Atlantic transect in this study was lower than that reported for sub-surface waters in the north eastern Pacific Ocean (279 ± 178 particles m^{-3}), Arctic Ocean (2.68 ± 2.95 particles m^{-3}) and north eastern Atlantic Ocean (2.46 ± 2.43 particles m^{-3}), (Table 2.2). The lower microplastic abundances that were reported in this study were possibly due to the fact that the vessel (i) did not traverse waters where microplastics have been known to accumulate (i.e. either the North Atlantic or the South Atlantic Sub-Tropical Gyre), (ii) was too far offshore the African continent to sample nearshore sites which usually have higher microplastic abundances than open oceanic sites, or (iii) was sampling from a different vertical fraction in the water column. Although microplastic abundance in the present study was comparable to abundances reported for surface waters in the Atlantic Ocean, Pacific Ocean and the Mediterranean and European Seas, considerably higher microplastic abundances in surface waters were reported for nearshore sites in the US, Korea, South Africa, UK and Sweden (Table 2.2). The comparisons of microplastic abundance in sub-surface waters must be taken in the context of variations in the depth (3 – 11 m) at which seawater was sampled and mesh sizes (62.5 -300 µm) of the sieves that were used amongst the studies. For surface water samples,

there were also variations in the mesh sizes (50 – 505 µm) of the nets that were used. These factors may influence microplastic abundance as (i) there may be vertical stratification of microplastics in the water column and, (ii) smaller mesh sizes would increase the quantity of microplastics collected during sampling. Standardisation and intercalibration protocols for sampling microplastics in surface and sub-surface waters are key issues to be addressed by the scientific community if greater comparability between studies is to be achieved.

2.6. Conclusion

This study provided an assessment of microplastics in sub-surface waters along a North/South latitudinal gradient in the Atlantic Ocean. Overall, average microplastic abundance as reported by this study for the Atlantic Ocean (1.15 ± 1.45 particles m^{-3}) was lower than was reported for sub-surface waters across the world. Additionally, there were no statistically significant differences between microplastic abundance at Benguela upwelling sites (n = 14) and all other non-upwelled sites (n = 62). Rayon (63%) was the predominant polymer of the particles that were analysed. Of the confirmed microplastics, the most abundant polymer types were polyester (49%) and blends of polyamide or acrylic/polyester (43%). Fibres (94%) were also the predominant type of microplastics. The information provided by this study is important as it provides an indication of the environmentally realistic concentrations and types of microplastics that biota are exposed to in the natural environment.

Table 2.2: Microplastic abundances reported for surface and sub-surface oceanic waters across the world

Location	**Microplastic abundance (particles per m^3)**	**Method for surface waters (unless otherwise indicated)**	**Study**
Arctic Ocean			
Svalbard, Norway	0.34 ± 0.31; 0 – 1.31 (mean, range)	Manta trawl (333 µm)	Lusher et al. (2015)[1]
	2.68 ± 2.95; 0 -11.5 (mean, range)	Underway system (250 µm)*	
Pacific Ocean			
Southern California, USA	7.25 (mean)	Manta trawl (333 µm)	Moore et al. (2002)
Santa Monica Bay, USA	3.92 (mean)	Manta net (333 µm)	Lattin et al. (2004)
South Californian current	0 – 3.141	Manta net (505 µm)	Gilfillan et al. (2009)
Southeast Bering Sea	0.004 – 0.19	Sameoto neuston/manta net (505 µm)	Doyle et al. (2011)
NP Subtropical Gyre	0.425 (median)	Manta net (333 µm)	Goldstein et al. (2012)

*Sub-surface waters sampled at the following depths ([1]6 m, [2]4.5 m)

Table 2.2: Microplastic abundances reported for surface and sub-surface oceanic waters across the world

Location	**Microplastic abundance (particles per m^3)**	**Method for surface waters (unless otherwise indicated)**	**Study**
Pacific Ocean			
North eastern Pacific Ocean	279 ± 178 (mean)	Underway system (62.5 – 250 µm)*	Desforges et al. (2014)[2]
Geoje Island, South Korea	0.4 – 54	Manta trawl (330 µm)	Song et al. (2014)
East China Sea	0.167 ± 0.138 (mean)	Neuston net (333 µm)	Zhao et al. (2014)
Southern Sea of Korea	1.92 – 5.51; 2.3 – 38.77 (2012)	Manta trawl (330 µm)	Kang et al. (2015a)
	582 – 924; 10 – 375 (2013)	Hand Net (50 µm)	
Atlantic Ocean			
Bristol Channel, UK	0 – 100	Lowestoft plankton sampler (270 µm)	Morris & Hamilton (1974)

*Sub-surface waters sampled at the following depths ([1]6 m, [2]4.5 m)

Table 2.2: Microplastic abundances reported for surface and sub-surface oceanic waters across the world

Location	**Microplastic abundance (particles per m^3)**	**Method for surface waters (unless otherwise indicated)**	**Study**
Atlantic Ocean			
Offshore Ireland	2.46 ± 2.43; 0 – 22.5 (mean, range)	Underway system (250 µm)*	Lusher et al. (2014)[3]
Western English Channel	0.27	Plankton nets (200, 500 µm)	Cole et al. (2014)
Portuguese coastal waters	0.002 – 0.036	WP2 (180 µm), Neuston (280 µm), LH Plankton Recorder (335 µm)	Frias et al. (2014)
St. Peter/St. Paul Archipelago, Brazil	0.01	Plankton net (300 µm)	Ivar do Sul et al. (2013)
Western Tropical Atlantic Ocean	0.015 – 0.04	Manta trawl (300 µm)	Ivar do Sul et al. (2014)
North Atlantic Ocean	13 – 501	Underway system (10, 300 µm)*	Enders et al. (2015)[4]

*Sub-surface waters sampled at the following depths ([3]3 m, [4]3 m, [5]11 m)

Table 2.2: Microplastic abundances reported for surface and sub-surface oceanic waters across the world (continued)

Location	**Microplastic abundance (particles per m^3)**	**Method for surface waters (unless otherwise indicated)**	**Study**
Atlantic Ocean			
Atlantic Ocean	**1.15 ± 1.45; 0 – 8.5 (mean, range)**	**Underway system (250 µm)***	**This study[5]**
Mediterranean and European Seas			
West Coast, Sweden	167 – 2400	Plankton net (80 µm)	Noren (2007)
	72 – 141	Zooplankton net (450 µm)	
West Sardinian Coast	0.15	Manta trawl (500 µm)	de Lucia et al. (2014)
Southwest Finland	0 – 0.74	Manta trawl (333 µm)	Magnusson et al. (2014)
Baltic Sea	10^2 - 10^4	WP2 net (90 µm mesh)	Gorokhova (2015)

*Sub-surface waters sampled at the following depths ([3]3 m, [4]3 m, [5]11 m)

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Chapter 3

Microplastics in sub-surface waters of the Arctic Central Basin

This chapter is a verbatim reproduction from the following published paper (published version found in Appendix IV, Supplementary Tables in Appendix V):

Kanhai, L.K., Gårdfeldt, K., Lyashevska, O., Hassellöv, M., Thompson, R.C., O'Connor, I., 2018. Microplastics in sub-surface waters of the Arctic Central Basin. Marine Pollution Bulletin 130, 8-18. https://doi.org/10.1016/j.marpolbul.2018.03.011

3.1. Abstract

Polar oceans, though remote in location, are not immune to the accumulation of plastic debris. The present study, investigated for the first time, the abundance, distribution and composition of microplastics in sub-surface waters of the Arctic Central Basin. Microplastic sampling was carried out using the bow water system of icebreaker Oden (single depth: 8.5 m) and CTD rosette sampler (multiple depths: 8 – 4369 m). Potential microplastics were isolated and analyzed using Fourier Transform Infrared Spectroscopy (FT-IR). Bow water sampling revealed that the median microplastic abundance in near surface waters of the Polar Mixed Layer (PML) was 0.7 particles m^{-3}. Regarding the vertical distribution of microplastics in the ACB, microplastic abundance (particles m^{-3}) in the different water masses was as follows: Polar Mixed Layer (0 - 375) > Deep and bottom waters (0 – 104) > Atlantic water (0 – 95) > Halocline i.e. Atlantic or Pacific (0 – 83).

3.1.1. Keywords

Microplastic, Marine debris, Arctic Ocean, Sub-surface waters, Pollution, Water column

3.2. Introduction

The Arctic Ocean, though the smallest in the world, is unique due to its distinct abiotic features and the highly specialised ecosystem it supports. Key anthropogenic drivers which may put pressure on this ecosystem include (i) climate change, (ii) harvest and fisheries, (iii) persistent, bio-accumulative and toxic contaminants, (iv) industrial development, (v) shipping, and (vi) invasive

alien species (CAFF 2017). Plastic contaminants in the world's oceans have emerged as an issue of global importance due to their ubiquitous distribution, long-range transport potential, persistence and perhaps most importantly the potential threat they pose to marine organisms (UNEP 2011). Remote polar oceans such as the Arctic Ocean have not been immune to the entry of plastics as a combination of long-range transport processes and local anthropogenic activities have contributed to the plastic debris in these areas.

Characteristic abiotic features which set the Arctic Ocean apart from other oceanic basins include (i) a central area of perennial pack ice, (ii) seasonal extremes in solar irradiance, ice and snow cover, temperature and riverine inflow, and (iii) an upper layer of lower salinity water due to freshwater input from rivers and seasonal sea-ice melt (CAFF 2013). This unique ecosystem is a habitat for a vast array of marine organisms, some of which are (i) endemic to the region, (ii) commercially important, (iii) apex predators, (iv) central to the functioning of the ecosystem, and (v) threatened as evidenced by their inclusion in the IUCN Red List of Threatened Species (CAFF 2013, CAFF 2017).

Despite its remote location away from major population centres and the low coastal population in its surrounding shelf areas, both macro and microplastics were detected in the various environmental compartments of the Arctic Ocean. Between 2002 and 2014, macroplastics were detected on the seafloor (2500 m depth) of the eastern Fram Strait at the HAUSGARTEN observatory (Bergmann and Klages 2012; Tekman et al. 2017). Sightings of buoyant macroplastics were also made during ship and helicopter observation surveys in the Barents Sea and Fram Strait (Bergmann et al. 2016). A citizen-science study also recently reported

the presence of macroplastics on six beaches of the Svalbard Archipelago (Bergmann et al. 2017a). Arctic sea ice was reported by Obbard et al. (2014) as having microplastic concentrations (38 – 234 particles m^3 of ice) several orders of magnitude greater than highly contaminated oceanic waters. Lusher et al. (2015) first reported on microplastic abundances in surface and sub-surface waters south and southwest of Svalbard. Amélineau et al. (2016) later reported on microplastic abundance in surface waters east of Greenland. Regarding Arctic species, microplastics have been detected in the gular pouches of Little Auks (*Alle Alle*), (Amélineau et al. 2016), as well as in the stomachs of juvenile Polar Cod (*Boreogadus saida*), (Kuhn et al. 2018). Microplastics were also detected in sediments (collection depths 2340 – 5570 m) from the Fram Strait (Bergmann et al. 2017b). Recently, results from a circumpolar expedition of the Arctic indicated that concentrations of floating plastic ranged between 0 – 320,000 items km^{-2} in the Greenland and Barents Sea and 0 – 27,000 items km^{-2} in the rest of the Arctic Ocean (Cózar et al. 2017).

Plastic contaminants are introduced to the Arctic Ocean due to a combination of (i) long-range transport processes, e.g. via oceanic currents, biotransport and riverine input, and (ii) local anthropogenic activities, e.g. shipping. The three oceanic currents which supply the greatest water volumes to the Arctic Ocean are the (i) West Spitsbergen Current i.e. the polar limb of the North Atlantic circulation which carries warm water from the North Atlantic Current (9.5 Sverdrup, Sv = $10^6 m^3 s^{-1}$), (ii) a cold ocean current that enters from the Pacific Ocean via the Bering Strait (1.5 Sv) and, (iii) a branch of the North Atlantic Current, which flows along the Siberian coastline (1.0 Sv), (Zarfl and Matthies 2010). These oceanic currents may also transport plastics to the Arctic Ocean

with the estimated plastic flux to this region ranging between 62,000 to 105,000 tons per year (Zarfl and Matthies 2010). Models based on a particle-trajectory approach for studying the fate of marine debris in the open ocean highlighted the northward transport of marine debris to polar regions and the formation of a sixth so-called garbage patch in the Barents Sea (van Sebille et al. 2012). Bio-transport is another long-range transport process via which plastics may enter polar regions. Plastic ingestion was reported in Northern Fulmars (*Fulmaris glacialis*) and Thick-billed Murres (*Uria lomvia*) in the Arctic (Mallory 2008; Provencher et al. 2012; Trevail et al. 2015). Some studies suggested that the seabirds had ingested plastics during their wintering in the North Atlantic Ocean and had then transported the contaminants to the Arctic upon migration (Mallory 2008; Provencher et al. 2012). Riverine discharge from Siberian (Ob, Yenisei and Lena) and Canadian (Mackenzie) rivers are other potential sources of plastics to the Arctic. Obbard et al. (2014), however, point out that the contribution of riverine discharge to plastic input in the Arctic is projected to be low due to the fact that these rivers flow through sparsely populated watersheds. Local anthropogenic activities are another source of plastics to the Arctic. Increased ship traffic due to shipping and tourism was found to be positively correlated with increased litter densities in the Fram Strait (Bergmann and Klages 2012; Tekman et al. 2017).

The intense focus by scientists on the near-surface layer of the ocean for microplastics has been due in part to the presumption that the majority of particles would be found in this region of the water column given the inherent densities of individual synthetic polymers. Such a theorization led to traditional techniques that involved nets, manta trawls as well as the seawater intake of vessels that sampled only the upper few metres of the water column for microplastics. Yet,

several studies indicated that a mismatch existed between observed and expected plastic concentrations in surface oceanic waters when estimated plastic production and projected inputs to the oceans were considered (Cózar et al. 2014; Eriksen et al. 2014). It was therefore proposed that several mechanisms potentially influenced the vertical distribution of microplastics within the water column and led to their transport out of surface waters. Some of these mechanisms included (i) incorporation into marine aggregates (Long et al. 2015), (ii) biofouling (Fazey and Ryan 2016), (iii) incorporation into faecal matter (Cole et al. 2016) and, (iv) hydrodynamic factors such as wind (Kukulka et al. 2012). Despite the theorization that surface waters are not the ultimate repository for plastic debris in the marine environment (Cózar et al. 2014), few studies ventured beyond traditional near-surface microplastic monitoring to investigate their vertical distribution in the water column.

Microplastic pollution in the Arctic Ocean is an issue that warrants attention due to the potential threats that these contaminants may pose to the inhabitants of this unique ecosystem. A practical step towards addressing this issue and evaluating the extent of the problem involves assessing the abundance, distribution and composition of microplastics in Arctic waters. Whilst microplastic monitoring in the marine environment has traditionally focused on surface waters, the reality is that the vast majority of marine organisms inhabit sub-surface waters. Monitoring microplastics in sub-surface waters is particularly relevant as it can also provide some insight into the whereabouts of the ‘missing plastic’ from surface waters. To our knowledge, the present study sought for the first time (i) to provide a spatial overview of microplastic abundance, distribution and composition in the Polar Mixed Layer (PML) of the Arctic Central Basin (ACB)

and, (ii) to determine whether microplastics in the ACB were being transported out of surface waters by assessing their vertical distribution in the water column.

3.3. Materials and Method

3.3.1. Study Area

The Arctic Ocean is comprised of a deep central basin surrounded by extensive continental shelves (CAFF 2013). The bathymetry of the Arctic Ocean is such that the Lomonosov Ridge separates the central basin into the Canadian (Amerasian) and Eurasian basins with the basins being further sub-divided by the (i) Gakkel Ridge, into the Amudsen and Nansen basins and, (ii) Alpha Ridge, into the Makarov and Canada basins (Jakobssen et al. 2004; Rudels 2015, Figure 3.1).

A major structuring element of the Arctic marine ecosystem is sea ice which floats on the surface layer impeding surface mixing and influencing freshwater and heat fluxes (CAFF 2013). In the Arctic Ocean, there is a distinct vertical stratification of the water column giving rise to three major water layers (i) Polar Surface Water (PSW) which includes the Polar Mixed Layer (PML) and the halocline, (ii) Atlantic Water and, (iii) deep and bottom waters (Rudels 2015, Figure 3.1). The PML (approximately 50 m deep) is the uppermost surface layer of low salinity water formed as a result of sea ice melt and the influx of freshwater from riverine sources (Rudels et al. 1991; CAFF 2013; Jakobsson et al. 2014).

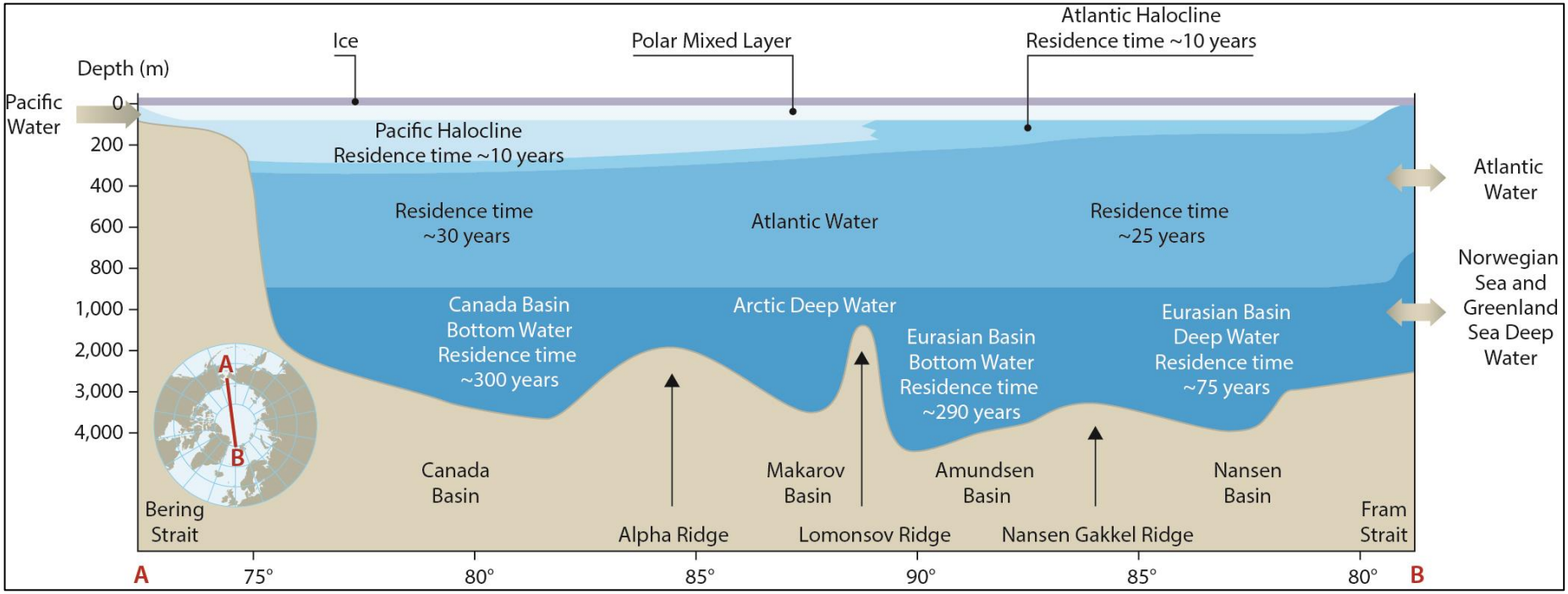

Figure 3.1: General overview of the bathymetry and water masses of the Arctic Central Basin

[reprinted here with permission from CAFF], (CAFF 2013)

Beneath the PML is a halocline (50 – 250 m), characterised by a strong salinity increase with depth and comprised of either Pacific waters or Atlantic waters with the Pacific halocline being deeper than the Atlantic halocline (Rudels et al. 1991; Jakobsson et al. 2004). Below the halocline lies an intermediate water layer comprising of dense saline Atlantic water. The deep and bottom waters also referred to as Arctic deep water ranges from a depth of approximately 900 m and extend to the seafloor (Rudels et al. 1991; CAFF 2013).

3.3.2. Sample Collection

Underway samples

This study was conducted onboard the Swedish icebreaker Oden during the Arctic Ocean 2016 expedition. The vessel departed Longyearbyen, Svalbard on August 8th 2016 and traversed approximately 4943 nautical miles in the Arctic Ocean until its return on September 19th 2016 (Figure 3.2). Sub-surface oceanic water pumped onboard the vessel via the bow water system was sampled for microplastics according to Lusher et al. (2014). Sampling was conducted for a period of approximately 6 weeks (9th August to 16th September 2016). Since each sample constituted the filtration of approximately 2,000 L of water, the total survey effort for this study was approximately 116,000 L of water (58 samples).

Seawater from a continuous intake located at the keel of the ship (depth 8.5 m) was pumped onboard the vessel using a rotary positive displacement pump (Universal II Series Pump, Waukesha Cherry-Burrell) at a flow rate of 85 L/min (at optimal capacity) and transported to the laboratory via stainless steel pipes.

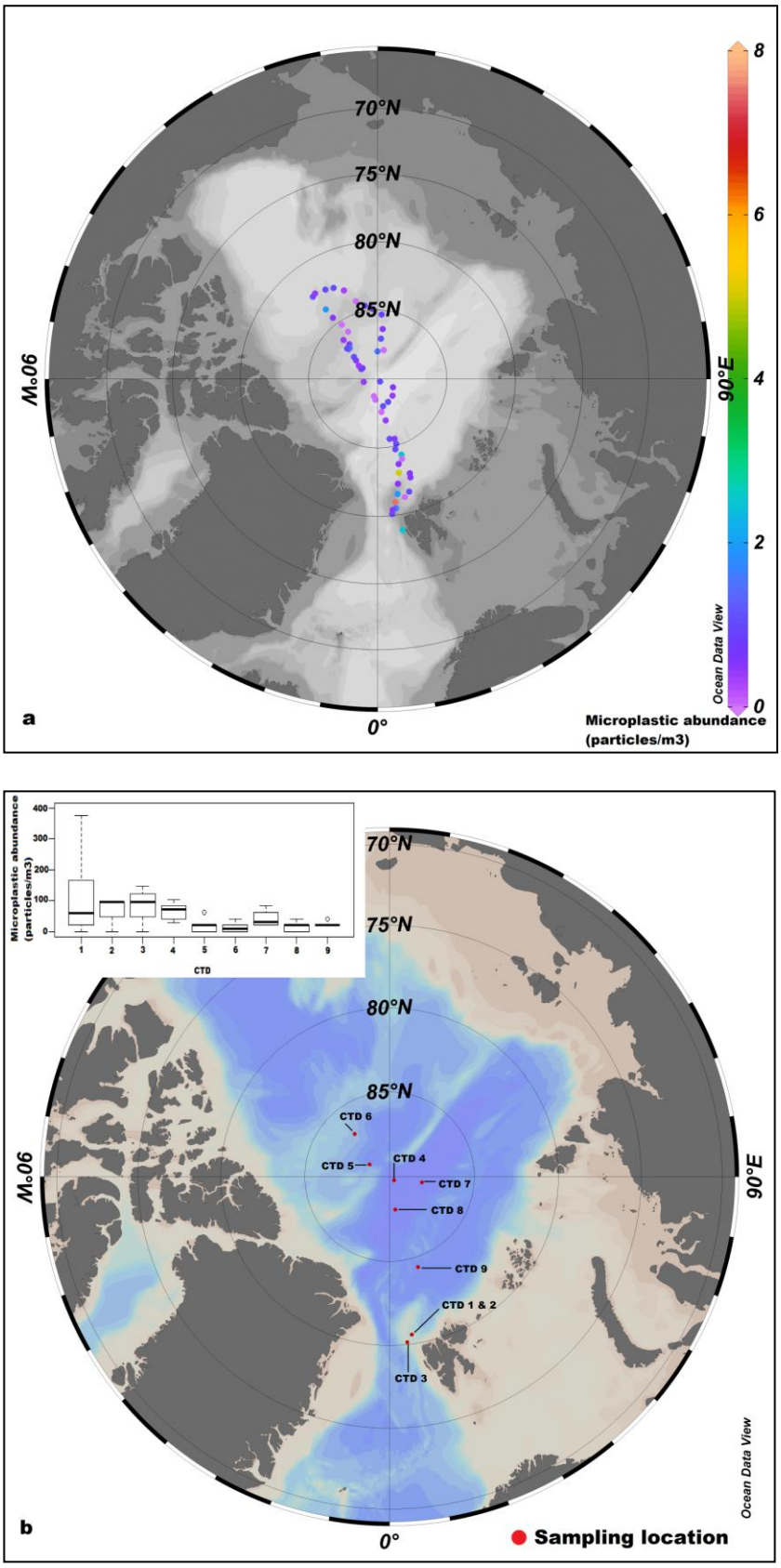

Figure 3.2: Microplastic abundance in the Arctic Central Basin (a) based on bow water sampling at a single depth of 8.5 m, (b) based on CTD sampling at multiple depths (8 – 4369 m)

Prior to reaching the laboratory, the seawater passed through a stainless steel primary filter (pore size 2.5 mm) which was beyond the control of the investigator. The discharge of grey water in relation to the seawater intake was not an issue since the seawater intake was located towards the front of the vessel whilst grey water was discharged mid-vessel. In the laboratory, seawater from the vessel's bow water system was allowed to flow through a covered stainless steel sieve (250 µm) by means of a connection hose fitted into the wooden sieve cover. For the duration of the sampling, the stainless steel sieve was supported in a wooden stand. Based on Lusher et al. (2014), approximately 2,000 L of water was filtered for each sample. The length of time taken for the filtration of the specified volume of water was determined by calculation of the flow rate of the seawater. A flow meter, attached at a point prior to the entry of the water into the sieve, was also used to verify the volume of water filtered. Once the specified volume of water was filtered, the sieve was removed and Milli-Q water was used to wash retained material from the sieve into a clean container. The collected material was then filtered under vacuum onto glass microfiber paper (GF/C); Whatman: 47 mm, pore size: 1.2 µm, using a Buchner funnel and a vacuum flask (Lusher et al. 2014). Each filter paper was then placed into a clean plastic petri dish, covered and stored in a freezer (-20 ºC) until returned to the laboratory. At the start and at the end of each sample, positioning data were collected. Data for various environmental variables were obtained from the vessel's (i) thermosalinometer (water temperature, salinity) and (ii) weather station (wind speed and direction).

CTD samples

A rosette water sampler containing 24 Niskin bottles coupled to a Sea-Bird SBE 911 conductivity-temperature-depth (CTD) sensor suite (hereafter referred to as

CTD) was used to collect sub-surface water samples and hydrographic data at 9 sampling locations in the Arctic Ocean. Upon deployment from the vessel, the CTD entered the water and was allowed to descend to the bottom layer. During the descent, Niskin bottles were open with water flowing through them. It was during the up-cast that multiple Niskin bottles were closed at specific depths in order to facilitate the collection of a specified volume of water. A total of 48 water samples were retrieved during the 9 CTD casts to sample for microplastics. At 7 of the CTD casts, 6 water depths were sampled with 48 L of water collected at each depth i.e. 4 Niskin bottles (12 L) per depth. At 2 CTD casts, 3 water depths were sampled with 21 L of water collected at each depth i.e. 3 Niskin bottles (7 L) per depth. At a particular sampling location, the overall goal was to collect samples in the near-surface, mid-water and bottom layers within the water column thereby reflecting the main water masses. As such, exact sampling depths were determined by the information provided by the salinity and temperature sensors on each downcast. The deepest samples at each CTD cast were collected at least 10 m above the seafloor at a given location. Following each CTD cast, Niskin bottle taps were rinsed with Milli-Q water and a clean hose was attached. Water from bottles closed at the same depth was passed through the same stainless steel sieve (250 µm) held in a covered wooden stand. Once water from all bottles at a specific depth had been filtered, the sieve was removed and Milli-Q water was used to wash retained material from the sieve into a clean container. The collected material was filtered under vacuum onto glass microfiber paper (GF/C); Whatman: 47 mm, pore size: 1.2 µm, using a Buchner funnel and a vacuum flask. Each filter paper was then folded and placed into an aluminium foil packet and stored in a freezer (-20 ºC) until returned to the laboratory.

3.3.3. Method Validation and Contamination Prevention

For the underway samples, potential contamination during sample processing was evaluated by (i) leaving clean plastic petri dishes with filter paper exposed to the air during vacuum filtration, and (ii) passing an aliquot (250 mL) of Milli-Q water through clean GF/C filter paper under vacuum. For the CTD samples, potential contamination was assessed by filling a clean Niskin bottle with Milli-Q water and subjecting it to the exact process a sample underwent. Measures taken to prevent contamination in the laboratory included (i) wearing lab coats (cotton/polyester blend), cotton clothing and gloves (nitrile) during sample processing, (ii) placing a wooden cover over the stainless steel sieve during filtration to prevent airborne contamination, and (iii) washing all containers used during sample processing with Milli-Q water before reuse.

3.3.4. Laboratory analyses

Filter papers were removed from the freezer, left to dry and then visually examined under a dissecting microscope (Olympus SZX10) equipped with a polariser and camera (Q Imaging Retiga 2000R). Potential microplastics were isolated and processed (photographed and length measurements taken) prior to transferring to a clean filter paper in a labelled petri dish (Kanhai et al. 2017). All potential microplastics were analysed by Fourier transform infrared (FT-IR) spectroscopy on a Thermo Scientific Nicolet iN10 FT-IR spectrometer. The instrument was equipped with a potassium bromide (KBr) beamsplitter and an internal mercury cadmium telluride (MCT) detector which was cooled with liquid nitrogen. Microscope-reflectance sampling was performed and spectra were recorded as the average of 256 scans in the spectral wave number range of 4,000 - 675 cm^{-1} at a resolution of 4 cm^{-1}. Thermo Scientific's OMNIC Picta Version 9

spectroscopy software was used for processing and evaluating all spectra. Prior to analysing each sample, background scans were performed and sample spectra were automatically corrected. Each sample spectrum was compared with those of known standard polymers in the (i) Hummel Polymer Sample library, (ii) Polymer Laminate Films library, and (iii) Wizard library. Values of between 0 and 100% were produced for each match between sample and reference spectra with the highest percentage representing the closest match. Particles for which there was uncertainty regarding the identity of the polymer (specifically fragments and some fibres) were subjected to further FT-IR spectroscopy on a Bruker Vertex 70 Infrared Spectrometer coupled to a Hyperion 1000 microscope (Kanhai et al. 2017). Samples which produced spectra with a match < 60% were automatically rejected while those with a match of > 70% were automatically accepted. All spectra with matches > 60% were individually examined to ensure that there was clear evidence of peaks from the sample corresponding to known peaks of standard polymers and that instances of the misidentification of natural and semi-synthetic polymers was reduced (Comneau-Stancu et al. 2017).

3.3.5. Statistical analyses

All statistical analyses were performed using R version 3.2.3 (R Core Team 2015). Descriptive statistics, histograms and box plots were generated and tests of normality (Supplementary Tables 3.1 and 3.2) were conducted on all data to determine whether parametric or non-parametric statistical analyses were appropriate. Correlation analyses were performed between individual environmental variables and microplastic abundance for both underway and CTD samples. A generalized additive model (GAM) was developed using the underway data and a generalized linear mixed model (GLMM) was developed

using the CTD data to determine which environmental variables had an effect on microplastic abundance.

3.4. Results

3.4.1. Quality Control

In conjunction with the collection of samples via the underway system of the vessel, a total of 24 blanks (air contamination-12, method-12) were run (Supplementary Table 3.3). No synthetic polymers were found in the method blanks. However, a single synthetic fibre (blue, polyethylene terephthalate, 0.438 mm) was found in the last air contamination blank. For 6 of the 9 CTD casts, at least one method blank was run (Supplementary Table 3.4). Between 0 and 3 synthetic fibres were found in each of the method blanks. The synthetic fibres that were found included polyethylene terephthalate (n = 8), polyacrylonitrile (n = 1) and polyvinyl chloride (n = 1).

3.4.2. Overview of findings

Underway samples

A total of 303 particles were isolated from the underway samples and analysed by FT-IR spectroscopy. Of these, 46 particles were excluded because of uncertainty regarding their identity (< 60% match to reference spectra) and in the minority of cases (n = 6) due to their length (< 250 µm). Of the remaining particles (n = 257), 14 were macro-particles (i.e. > 5 mm in length) and 243 were micro-particles (< 5 mm in length). Of the macro-particles, 11 were confirmed as macroplastics having the following polymer types: polyethylene terephthalate (4), polyamide blend (4) and polyacrylonitrile (3). Of the 243 micro-particles, 110 were

natural (cellulosic), 16 were semi-synthetic (cellulose-based e.g. rayon) and 117 were synthetic. All further analyses and discussions focus on the 117 confirmed microplastics. The majority (94%) of microplastics were fibres and 6% were fragments. In terms of colour, the most prevalent were blue (49%) and transparent (25%) (Figure 3.3a). Approximately 62% of the microplastics occurred in the larger size classes of 1.0 – 2.0 mm and 2.0 – 5.0 mm (Figure 3.4a).

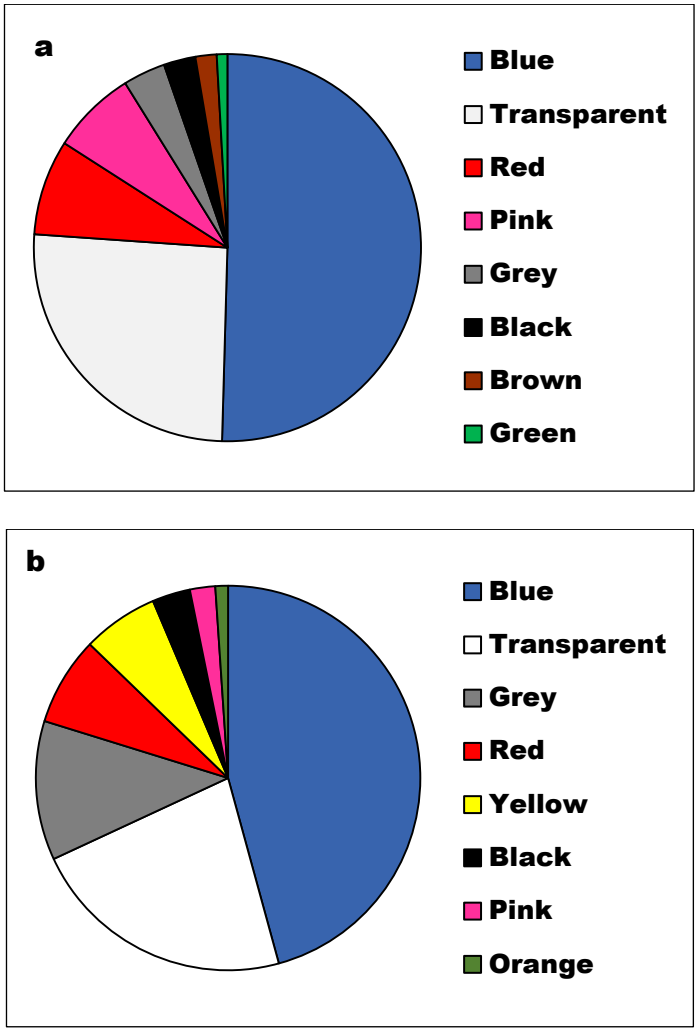

Figure 3.3: Colours of microplastics found in (a) underway and (b) CTD samples at depths 8 – 4369 m

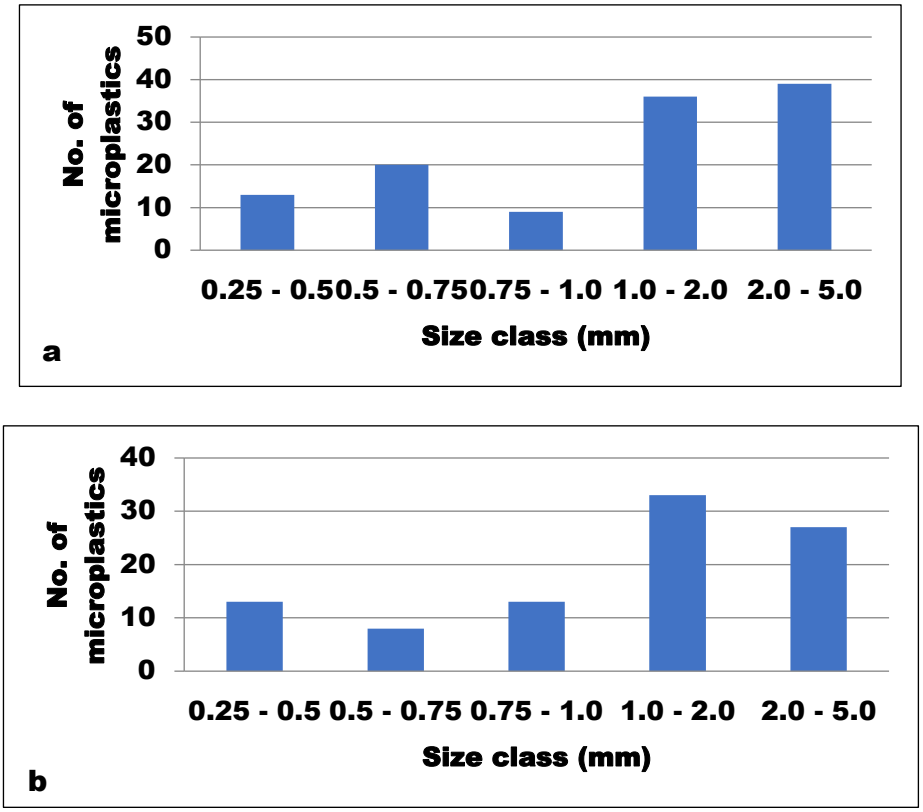

Figure 3.4: Size classes of microplastics found in (a) underway and (b) CTD samples at depths 8 – 4369 m

Microplastic polymer types included polyester (n = 88), blends (n = 11), polyacrylonitrile (n = 8), polyamide (n = 5) and polyvinyl chloride (n = 5), (Figure 3.5a). The overall category of 'polyester' included both polyethylene terephthalate (PET) and other polyesters while blends included either polyamide blends or polyester blends.

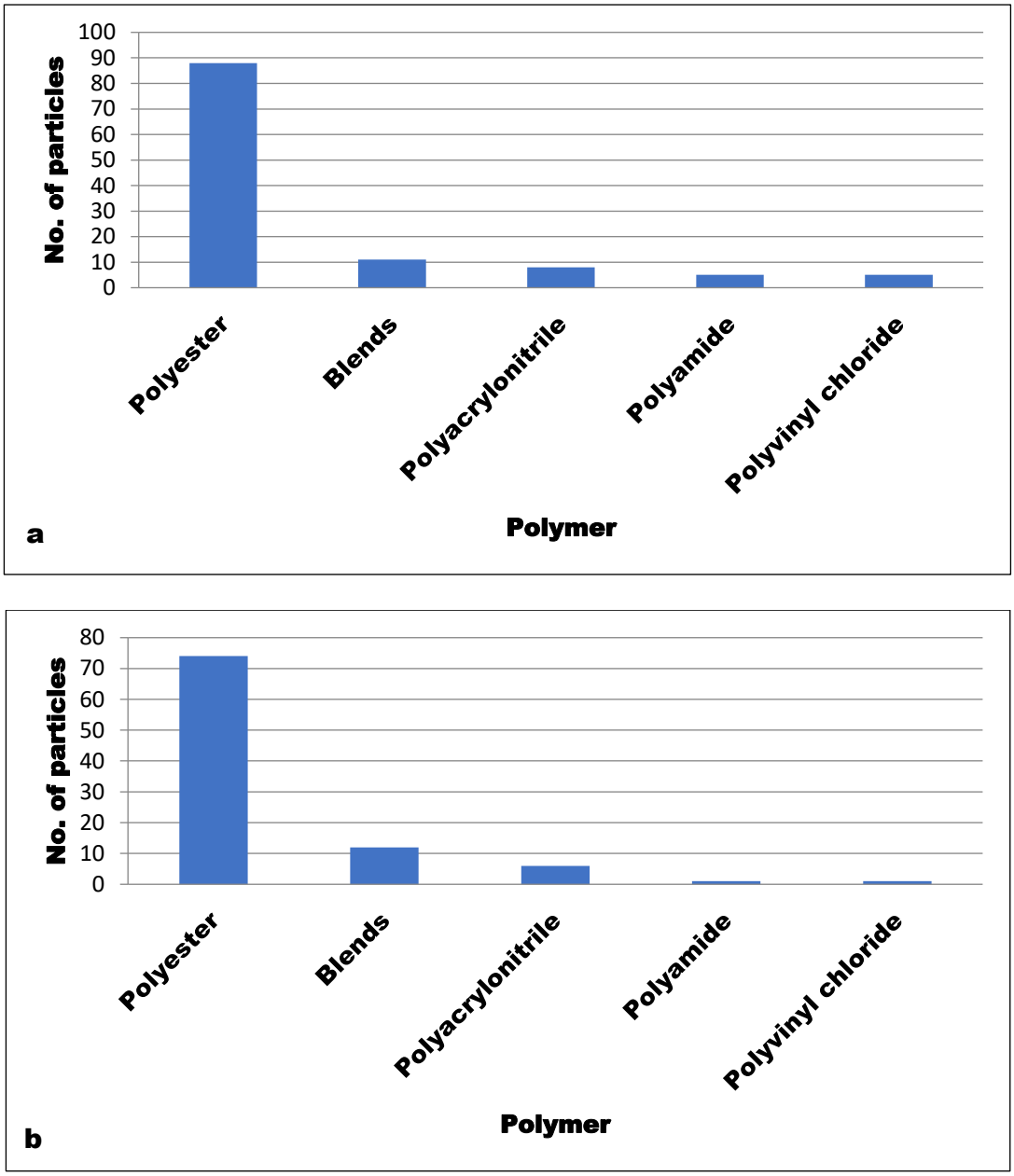

Figure 3.5: Synthetic polymers found in (a) underway and (b) CTD samples at depths 8 – 4369 m

CTD samples

A total of 157 particles were isolated from the CTD samples and analysed by FT-IR spectroscopy. Of these, 14 were excluded for the reasons mentioned above for the underway samples. Of the remaining particles, 2 were categorised as macroplastics (> 5 mm) and included PET and polyacrylonitrile. Of the 141 micro-

particles (< 5 mm), 39 were natural (cellulosic), 8 were semi-synthetic (cellulose-based e.g. rayon) and 94 were synthetic. All further analyses and discussions focus on the 94 confirmed microplastics.

Overall, the characteristics of the microplastics isolated from CTD samples were similar to those from the underway samples in that (i) the majority (96%) of microplastics were fibres and 4 % were fragments, (ii) the most prevalent colours were blue (46%) and transparent (22%) (Figure 3.3b), (iii) the majority (64%) of microplastics were in the larger size classes of 1.0 – 2.0 mm and 2.0 – 5.0 mm (Figure 3.4b), and (iv) microplastic polymer types included polyester (n = 74), blends (n = 12), polyacrylonitrile (n = 6), polyamide (n = 1) and polyvinyl chloride (n=1), (Figure 3.5b). The overall category of polyester included both polyethylene terephthalate (PET) and polyester while blends included only polyamide blends.

3.4.3. Microplastic abundance and distribution in the Arctic Ocean

Based on the underway samples (collection depth 8.5 m), microplastic abundance in sub-surface waters in the Arctic Ocean ranged between 0 – 7.5 particles m^{-3} with a median of 0.7 particles m^{-3} (interquartile range 0.4 – 1.0), (Figure 3.2, Supplementary Table 3.5). For the majority of the sampling sites, microplastic abundance ranged between 0 – 1.0 particles m^{-3}. However, at a few sites, microplastic abundances were between 2 – 2.5 particles m^{-3} and at two sites it was at 5 and 7.5 particles m^{-3} respectively. Based on the CTD samples (collection depths between 8 – 4369 m), microplastic abundance in sub-surface waters in the Arctic Ocean ranged between 0 – 375 particles m^{-3} with a median of 20.8 particles m^{-3} (interquartile range 20.8 – 62.5) (Figures 3.2 and 3.5, Supplementary Table 3.6). With the exception of CTD cast 4, the CTD casts (1 –

3) nearer the periphery of the Arctic Central Basin (ACB), i.e. in the Nansen Basin (Yermak Plateau), reflected a comparatively higher abundance of microplastics in the water column than other CTD casts within the ACB (Figures 3.2, 3.6).

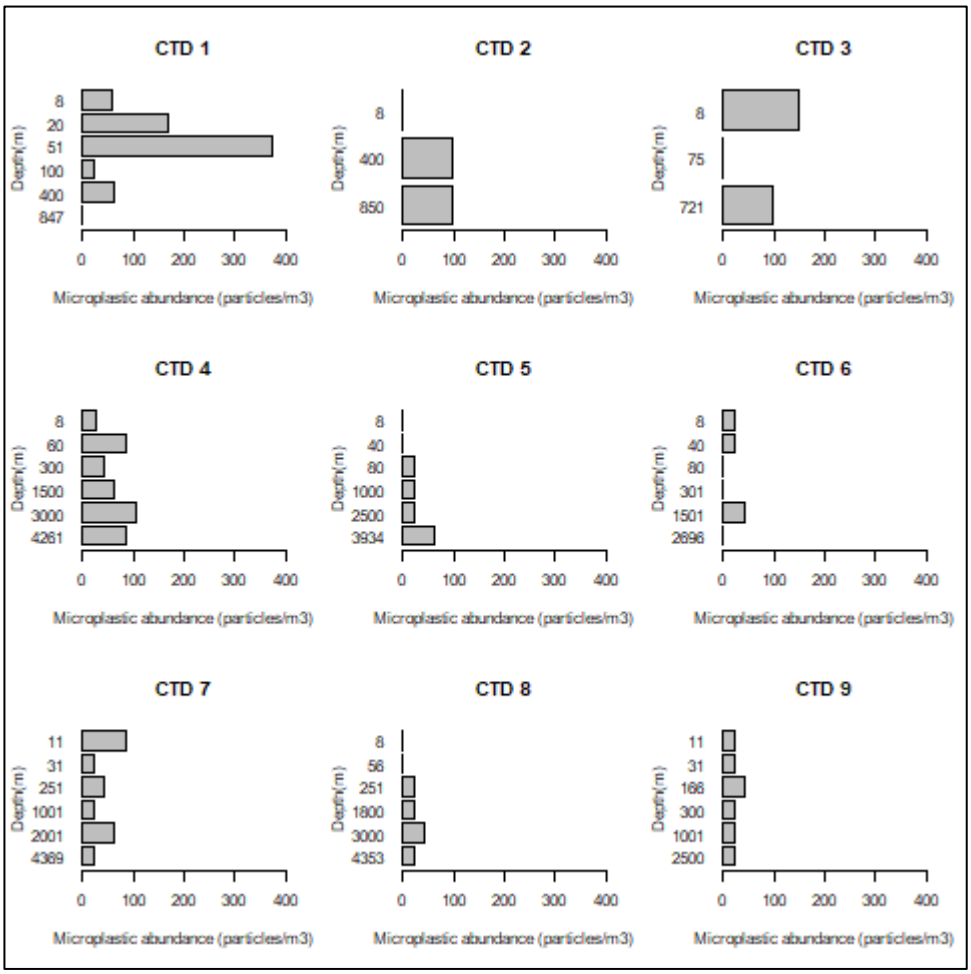

Figure 3.6: Microplastic abundance from the various CTD casts in the Arctic Ocean

It must be noted however that CTD casts 1 – 3 sampled the upper 850 m of the water column and as such would have sampled particles from the Polar Mixed Layer (PML), Atlantic halocline and Atlantic water (Figures 3.1, 3.6). This is in contrast to the other CTD casts which sampled a much more extensive vertical range throughout the water column by including deep bottom water in excess of

1,000 m depth. Overall, there was no statistically significant correlation between microplastic abundance and depth (Spearman's rank correlation, rho = 0.06, p-value = 0.7). However, upon examination of individual CTD casts, it is apparent that microplastic abundance was not uniform at various depths in the water column and that there were certain depths that reflected higher microplastic abundances (Figure 3.6). Additionally, microplastic abundance (particles m^{-3}) in the different water masses of the ACB was as follows: Polar Mixed Layer (0 - 375) > Deep and bottom waters (0 – 104) > Atlantic water (0 – 95) > Halocline i.e. Atlantic or Pacific (0 – 83), (Table 3.1).

Table 3.1: Microplastic abundance in the various water layers of the Arctic Central Basin (ACB)

Water Mass	**Depths Sampled (m)**	**Number of depths sampled**	**Microplastic abundance (particles m^{-3})**
Polar Mixed Layer (PML)	8 – 51	15	0 – 375
Halocline (Atlantic or Pacific)	56 – 166	7	0 – 83
Atlantic Water	251 – 850	10	0 – 95
Deep & Bottom Waters	1001 – 4369	16	0 – 104

3.4.4. Association between environmental variables and microplastic abundance in samples

Correlation analyses were conducted to determine whether there was any association between environmental variables and microplastic abundance in the

samples. For both the underway and CTD samples, there was no statistically significant correlation between microplastic abundance and any of the ancillary environmental variables of temperature, salinity, wind direction, wind speed, depth and density (Supplementary Tables 3.1 and 3.2). Specifically, there was no statistically significant correlation between microplastic abundance at depth (Supplementary Table 3.2). However, for the underway samples, there was a statistically significant weak negative correlation between microplastic abundance and latitude (Spearman's rank correlation, rho = -0.286, p-value = 0.03).

Using the underway data, a Generalized Additive Model (GAM) was developed to further determine whether environmental variables influenced microplastic count in the underway samples. In this model, the response variable was microplastic count (number of microplastics per sample) and initial explanatory variables included location (latitude, longitude), physico-chemical properties associated with sub-surface waters (temperature, salinity) and weather data (wind direction, wind speed). In the model, the Poisson family distribution of error terms was specified with a log link function since microplastic abundance data were count data. The output of the initial model was examined and based on this non-parametric smoothers were accordingly applied to the explanatory variables. A scale invariant tensor product smooth (te) was applied to latitude and longitude while a cubic regression spline (bs = cr) was applied to all other variables except wind direction to which no smoother was applied (based on initial model plots). Non-significant explanatory variables (as evidenced by their p-values) were eliminated in a stepwise manner until a GAM with the lowest Akaike Information

Criterion (AIC) score and the fewest explanatory variables was obtained. The final GAM (R-sq = 0.396) was as shown below:

Microplastic count ~ te(**latitude, longitude**) + s(**temperature**, br = “cr”) + s(**wind speed,** bs = “cr”)

All of the explanatory variables that were present in the final model (shown in bold) were found to have a significant influence on microplastic count in water samples from the Arctic Ocean (wind speed, p-value = 0.0006, latitude, longitude, p-value = 0.0007, temperature, p-value = 0.0483).

A generalized linear mixed model (GLMM) was developed using the CTD data to determine the influence of environmental variables on microplastic count in the CTD samples. In this model, the response variable was microplastic count (number of microplastics per sample) and initial explanatory variables included location (latitude, longitude), physicochemical properties associated with sub-surface waters (temperature, salinity), depth at which water was sampled and CTD number. All the explanatory variables were included in the model as fixed effects, with the exception of CTD number which was included as a random effect. In the model, the Poisson family distribution of error terms was specified with a log link function since microplastic abundance data were count data. Based on the preliminary finding that there was a statistically significant correlation between depth and salinity (Spearman’s rank correlation, rho = 0.852, p-value = 8.156e-13), temperature and salinity (Spearman’s rank correlation, rho = 0.506, p-value = 0.00074) and depth and density (Spearman’s rank correlation, rho = 0.973, p-value = < 2.2 e-16), interactions between these variables (denoted by

':') were included in the initial model. Non-significant explanatory variables (as evidenced by their p-values) were eliminated in a stepwise manner until a model with the lowest Akaike Information Criterion (AIC) score and the fewest explanatory variables was obtained. The significance of the random effect (CTD number) in the final model was verified by using analysis of variance (ANOVA) to compare the full final model to a reduced model (random effect deleted). The model with the random effect was shown to be significantly different (ANOVA, p-value = 0.0008, ΔAIC = 9.19) from the model without the random effect. The model with the lower AIC score (AIC = 190.59) was retained as the final mixed effects model as shown below:

Microplastic count ~ **latitude** + **temperature**:**salinity** + (1|ctd)

Latitude (p-value = 0.0198) and the physicochemical parameters of temperature and salinity (p-value = 7.46 e-05), as shown in bold, were the explanatory variables that were found to have a significant influence on microplastic count in the CTD samples.

3.5. Discussion

The discovery of microplastics in virtually every environmental phase (sea ice, water, sediments, biota) of the Arctic and Southern Oceans has revealed that polar oceans, though remote, are not immune to the entry of plastic contaminants to their ecosystems (Bergmann and Klages 2012; Obbard et al. 2014; Lusher et al. 2015; Amélineau et al. 2016; Bergmann et al. 2016; Bergmann et al. 2017a; Bergmann et al. 2017b; Cincinelli et al. 2017; Cózar et al. 2017; Isobe et al. 2017; Tekman et al. 2017; Waller et al. 2017). The present study expands the

knowledge base about plastics in the Arctic by providing evidence for the existence of microplastics in the Polar Mixed Layer (PML) as well as some insight into the vertical distribution of microplastics in the Arctic Central Basin (ACB). This region of the Arctic, though of low productivity, has been recognised as an Ecologically/Biologically Significant Marine Area (EBSA) due to its uniqueness/rarity, provision of a critical habitat and ability to support specialised biota (CAFF 2017). There is cause for concern about microplastics in Arctic waters since laboratory studies have shown that these contaminants may (i) hinder algal photosynthesis/growth (Bhattacharya et al. 2010; Besseling et al. 2014), (ii) reduce feeding and energy reserves of lugworms (Besseling et al. 2013; Wright et al. 2013), (iii) reduce filtering activity and decrease lysosomal membrane stability in mussels (Von Moos et al. 2012; Wegner et al. 2012), (iv) reduce feeding and reproductive output in copepods (Cole et al. 2015) and, (v) cause liver stress, negatively impact upon cholinergic neurotransmission and lead to endocrine disruption in fish (Oliveira et al. 2013; Rochman et al. 2013; Rochman et al. 2014). It must be pointed out, however, that some laboratory experiments which reported negative effects of microplastics on marine organisms used microplastic concentrations of 42 to 10,000 particles/mL or 42 million to 10 billion particles m^{-3} (Phuong et al. 2016). In context, microplastic abundance in the ACB as reported by the present study ranged from 0 – 7.5 particles m^{-3} (based on underway sampling) and 0 – 375 particles m^{-3} (based on CTD sampling). Although the ecological impact of microplastics upon the Arctic ecosystem presently remains unknown, it is plausible that these contaminants could pose a threat to its inhabitants.

The present study showed for the first time the pervasiveness of microplastics throughout the water column of the Arctic Central Basin. Between depths of 8 – 4400 m, microplastic abundance in the ACB ranged between 0 – 375 particles m^{-3} (based on CTD sampling). Such findings provide evidence that in natural conditions microplastics are being vertically transported out of surface waters. These findings also give some indication as to the whereabouts of the 'missing plastic' from oceanic surface waters (Cózar et al. 2014; Eriksen et al. 2014). Recently, Courtene-Jones et al. (2017) also reported on microplastic abundance (70.8 particles m^{-3}) in deep oceanic waters (2227 m at the Rockall Trough, North East Atlantic Ocean) and similarly suggested the possibility of vertical re-distribution of microplastics within the water column. Although it remains unclear as to which mechanisms are specifically operating in the ACB to influence the vertical transport of particles, previous studies have provided several possibilities. Specifically, some laboratory experiments showed that aggregates of algae species (*Chaetoceros neogracile*, *Rhodomonas salina*) were capable of incorporating and concentrating microplastics and that the microplastics impacted the sinking rates of the aggregates (Long et al. 2015). In the Arctic Ocean, it is certainly plausible that marine aggregates may be playing a role in the vertical transport of microplastics due to the existence of phytoplankton in the ACB (CAFF 2017) and the fact that transparent exopolymer particles (TEPs); which are excreted by algae and are important components of marine aggregates, have been reported in sub-surface waters as far north as the Fram Strait (Engel et al. 2017). Biofouling is another possibility as field studies have shown that plastic particles exposed to natural conditions became sufficiently fouled, had their average material density affected leading them to sink (Fazey and Ryan 2016). The incorporation of microplastics into faecal matter is another

means by which microplastics may be vertically transported out of surface waters given that laboratory experiments have shown that zooplankton may egest microplastics within densely packed faecal pellets which in natural conditions would sink or in some cases be eaten by other biota (Cole et al. 2016).

Sea ice is an integral component of the Arctic Ocean's ecosystem and as such possibly exerts an influence on microplastic abundance in sub-surface waters. Sea ice floating on the surface of the water column in the Arctic Ocean can potentially act as (i) a source of microplastics upon melting, (ii) a physical barrier to wind and as such reduce vertical mixing of surface waters and, (iii) a physical barrier to influx of polluted surface waters. Based on the analysis of sub-sections of four ice cores, sea ice in the Arctic Ocean was reported to contain orders of magnitude more microplastic than contaminated oceanic waters suggesting that sea ice potentially acts as both a sink and a source of microplastics (Obbard et al. 2014). Apart from Obbard et al. (2014) no data exists in the published literature regarding either the spatial or vertical distribution of microplastics in sea ice from the Arctic Ocean. In the upper water column, the absence of sea ice cover means that wind stress can generate turbulence and lead to vertical mixing of buoyant plastic debris (Kukulka et al. 2012). More recently, Cózar et al. (2017) suggested that sea ice can also act as a physical barrier preventing the surface advance of polluted Atlantic water into the Arctic Ocean. The present study highlighted that the Polar Mixed Layer (PML) of the ACB reflected the highest overall microplastic abundance (particles m^{-3}): Polar Mixed Layer (0 - 375) > Deep and bottom waters (0 – 104) > Atlantic water (0 – 95) > Halocline i.e. Atlantic or Pacific (0 – 83). As previously mentioned, the PML is the uppermost surface layer of low salinity water (approximately 50 m deep) formed as a result of sea ice melt and the influx

of freshwater from riverine sources (Rudels et al. 1991; CAFF 2013). It is possible that one of the reasons that the highest microplastic abundances were recorded in this layer is due to its proximity to microplastic sources such as melting sea ice as well as sea-going vessels (especially if they are releasing wastewater to the environment). Furthermore, in the present study, the highest microplastic abundances were reported nearer to the periphery of the Arctic Central Basin (ACB), i.e. in waters north of Svalbard. It is possible that the lack of permanent sea ice cover in this region of the Arctic allows incoming Atlantic water to have a greater influence on near surface waters thereby resulting in higher microplastic abundances. A recent circumpolar expedition of the Arctic Ocean similarly reported that the north eastern Atlantic sector of the Arctic was a hotspot of plastic debris due to the influence of incoming Atlantic water (Cózar et al. 2017).

The present study showed that there was a predominance of fibrous microplastics (> 90%) in sub-surface waters of the ACB. This dominance of fibres in sub-surface waters was similarly reported in (i) the north east Pacific Ocean (75%), (ii) the north east Atlantic Ocean (96%), (iii) south/southwest of Svalbard (95%), and (iv) the Atlantic Ocean (96%) (Desforges et al. 2014; Lusher et al. 2014; Lusher et al. 2015; Kanhai et al. 2017). Fibrous microplastics in the marine environment most likely originate from textile materials and fishing gear (Andrady 2017). Studies have indicated that washing clothes may lead to the release of fibrous materials in the order of > 1900 fibres per wash or as much as 700,000 fibres per 6 kg load of acrylic fabric (Browne et al. 2011; Napper and Thompson 2016). A recent study in the Ross Sea revealed that the highest concentration of fibrous microplastics (54%) was found close to the effluent of a sewage treatment plant at the scientific Mario Zucchelli Station, Antarctica (Cincinelli et al. 2017).

Fibrous microplastics may enter the Arctic Ocean through a combination of long range transport processes (e.g. via oceanic currents, riverine input) or more in-situ activities such as the release of wastewater from vessels operating in the region. Another mechanism which was recently suggested as being responsible for plastic fibres in Arctic sea ice was atmospheric transport (Cózar et al. 2017). This is certainly plausible as there have been reports of the atmospheric fallout of synthetic polymers in both urban and sub-urban environments in France (Dris et al. 2016). It must be highlighted that while fibrous microplastics seem to be dominant in certain sub-surface waters, other studies conducted in surface Arctic waters reported a predominance of filaments (97%) and fragments (73%), (Amélineau et al. 2016; Cózar et al. 2017).

Analytical techniques such as FT-IR and Raman spectroscopy are a central component of microplastic studies which aim to provide unambiguous identification of synthetic polymers in environmental samples. Omission of this critical step is likely to be a key contributor to an overestimation of microplastic abundances due to the inclusion of non-synthetic polymers in microplastic counts. In the present study, for example, only a percentage (underway - 48%, CTD - 67%) of the particles were confirmed as synthetic polymers with the remainder being a combination of natural and semi-synthetic polymers. Of the synthetic polymers in the present study, the most abundant (underway – 74%, CTD – 78%) was polyethylene terephthalate (PET). A member of the polyester family, PET is one of the five major types of commodity plastics commonly found in the marine environment (Andrady 2011; Andrady 2017). As a thermoplastic, PET is often used in manufacturing beverage containers and packaging materials and its fibres are used in clothing. Overall, this finding of a high prevalence of

polyesters in sub-surface waters of the Arctic Central Basin was also corroborated by those of other Arctic studies which reported that polyester was the most prevalent synthetic polymer in waters south/southwest of Svalbard (15%), in waters of the east Greenland Sea (53%), and in sea ice (21%), (Obbard et al. 2014; Lusher et al. 2015; Amélineau et al. 2016). Other polymers in sub-surface Arctic waters in this study included polyacrylonitrile, polyamide and polyvinyl chloride. Of note is the fact that the majority of synthetic polymers in the present study had densities greater than that of seawater (Andrady 2017). Even though investigators are able to generate information regarding the identity of polymers in environmental samples, definitive statements cannot be made about the origin of the plastics. Based on the identity and type of synthetic polymers found in sub-surface Arctic waters, it is likely that they originated from textiles, fishing gear, beverage containers and packaging materials (Andrady 2011; Andrady 2017).

Within the water column, the distribution of microplastics is in a state of flux due to the influence of multiple factors. The development of models based on simultaneously acquired environmental and microplastic data is immensely useful in this respect as they can provide some discernment regarding the variables influencing measures of microplastic abundances in the marine environment. In the present study, the utilisation of a generalized additive model (GAM) and a generalized linear mixed effects model (GLMM) was particularly relevant due to the ability of both models to handle non-normal data and in the case of the GLMM to differentiate between fixed and random effects. Visual inspection of microplastic abundances in sub-surface waters (Figure 3.2) revealed that the highest microplastic abundances were located to some extent

towards the periphery of the Arctic Central Basin (ACB). Bearing this in mind, it was presumed that the 'location' of sampling could have influenced the number of microplastics that were found in the samples. Cózar et al. (2017) also noted this spatial heterogeneity in the distribution of plastics in the Arctic. For these reasons, latitude and longitude were included as a proxy of location in the models. In the GAM, both variables were included using a smoother and therefore it was not assumed that latitude and longitude had a linear effect on microplastic count. Furthermore, during the period of sampling (August/September 2016), the Arctic Central Basin (ACB) was not completely covered by sea ice. Since the vessel traversed areas of open water, wind was included in the model as it could have influenced microplastic abundance at specific sampling sites. Both models suggested that location, oceanographic (temperature, salinity) and atmospheric variables (wind) had a significant influence on microplastic counts in samples of water from the Arctic Ocean. The findings of the present study must be taken in the context of the number of samples used in the generation of the GAM (n = 58). Models that are based on a low number of samples are weak e.g. standard errors are inflated, etc. However, such models allow us to delve a bit deeper into the factors influencing measurements of microplastic abundance in the Arctic Ocean. Findings of the present study were also corroborated by previous studies which indicated that water temperature, salinity and wind also had a significant effect on microplastic abundance (Lusher et al. 2014; Lusher et al. 2015; Kanhai et al. 2017).

Comparative assessments between oceanic basins are critical in providing an indication of the extent of microplastic pollution in the marine environment. A major challenge, which demands caution when drawing conclusions from such

comparisons, is the lack of standardization of microplastic sampling methods (depth of collection, mesh size of net/sieve, etc). Microplastic abundances in the present study were not normally distributed and therefore the median was reported as it is the most relevant measure of central tendency for such data. However, in order to enable comparability with other published studies, which generally did not report median microplastic abundances nor made statements about the normality of their data, the mean was also reported in Supplementary Table 3.7. In the present study, sub-surface waters (depth 8.5 m) in the Arctic Central Basin (ACB), sampled via the bow water system, had a mean microplastic abundance of 0.97 ± 1.20 particles m^{-3}. In comparison to other studies that employed similar methods (i.e. the underway system of vessels) to sample sub-surface waters, microplastic abundance in the ACB was lower than values reported (i) in the north eastern Pacific Ocean (279 ± 178 particles m^{-3}), (ii) in the North Atlantic Ocean (13 – 501 particles m^{-3}), (iii) off Svalbard (2.68 ± 2.95 particles m^{-3}), (iv) in the north east Atlantic Ocean (2.46 ± 2.43 particles m^{-3}), and (v) in the Atlantic Ocean (1.15 ± 1.45 particles m^{-3}), (Desforges et al. 2014; Lusher et al. 2014; Enders et al. 2015; Lusher et al. 2015; Kanhai et al. 2017, Supplementary Table 3.7). The only oceanic basin for which there were reports of lower microplastic abundances in sub-surface waters was the Ross Sea (0.17 ± 0.34 particles m^{-3}), (Cincinelli et al. 2017; Supplementary Table 3.7). Although the methods used to sample sub-surface waters for microplastics in the above mentioned studies were similar, the fact remains that the variation of several factors e.g. mesh size of sieve (1 – 300 µm), sampling depth (3 – 11 m), etc., amongst the studies could have impacted the reported microplastic abundances. In terms of assessing the vertical distribution of microplastics in the marine environment, Bagaev et al. (2017) was the only other published study which

utilised a similar sampling method (Niskin bottles) in the Baltic Sea. Being cognisant of the fact that no confirmatory analytical techniques or blanks were used by Bagaev et al. (2017), microplastic abundance at multiple depths in sub-surface waters of the Arctic Ocean (mean: 46 ± 62 particles m^{-3}; range: 0 – 375 particles m^{-3}; depths sampled: 8 – 4400 m) was lower than reported for the Baltic Sea (mean: 310 ± 520 particles m^{-3}; 70 – 2600 particles m^{-3}; depths sampled: 1 - 218 m). Similar to the findings of the present study whereby the highest microplastic abundances were found in the uppermost water layer i.e. the PML, Bagaev et al. (2017) reported that near- surface and near-bottom water layers in the Baltic Sea had higher fibre concentrations than intermediate layers and that this was possibly due to greater turbulence and density stratification in those layers. Of note is the fact that microplastic abundance in deep waters of the ACB (0 – 104 particles m^{-3}, depths 1,000 – 4,400 m, sieve 250 µm) was similar to those reported for deep waters at the Rockall Trough, North East Atlantic Ocean (70.8 particles m^{-3}, depth 2227 m, sieve 80 µm), (Courtene-Jones et al. 2017). Overall, it must be acknowledged that an underestimation of microplastic abundance in the Arctic Central Basin (ACB) could have occurred in the present study as the mesh size of the sieve was only 250 µm leading to an exclusion of smaller sized particles. Nevertheless, the Arctic's remote geographic location away from major population centres, its low population in its surrounding continental shelves and relatively low in-situ anthropogenic activities (e.g. shipping) are all factors which may explain the lower microplastic abundances in sub-surface waters within the Arctic Central Basin (ACB). From an oceanographic perspective, the reduced contribution of Atlantic water in its upper water layers due to the dominance of the polar mixed layer is another possible explanation for lower microplastic abundances in the Arctic in comparison to other oceanic basins. Presumably

more polluted, Atlantic water which originates from the more densely populated southern latitudes has its surface advance into the Arctic Ocean hindered due to freshwater released from melting ice and other physical barriers such as the sea ice itself and the Novaya Zemlya islands (Cózar et al. 2017). However, a plausible future scenario for the Arctic in the context of a changing climate is that microplastic abundance in near-surface layers of the Arctic Ocean may increase upon melting of contaminated sea ice and opening up of shipping lanes due to a decrease in sea ice extent (Obbard et al. 2014; Cózar et al. 2017).

Of interest is the fact that the present study managed to sample microplastics in sub-surface waters at approximately 8.5 m depth by two independent methods i.e. by the bow water system of the vessel (underway sampling) and the rosette water sampler (CTD sampling). CTD samples (n = 9) retrieved from an average depth of 8.5 m indicated that microplastic abundance in the ACB ranged between 0 – 148 particles m^{-3}, with a median of 20.8 particles m^3. By comparison, samples collected via the underway system (n = 58) at 8.5 m indicated that microplastic abundance in the ACB ranged between 0 – 7.5 particles m^3, with a median of 0.7 particles m^3. Although both methods sampled water at an average depth of 8.5 m, calculated microplastic abundances from both methods are not directly comparable due to the differences associated with the methods. Whereas underway sampling involved filtration of a greater volume of water (approximately 2,000 L) over a longer distance and a longer sampling time (> 2 hours), the CTD sampling involved the collection and subsequent filtration of a smaller volume of water (21 L or 48 L) at a single location in a shorter period of time (minutes). The advantage of using the underway system is that microplastic abundances over a larger spatial area can be quantified whilst the vessel is in transit. By comparison,

CTD sampling facilitates the quantification of microplastic abundance at specific locations making it less likely to mask contamination hotspots. However, some of the major limitations associated with CTD microplastic sampling are (i) the vessel must stop at sampling stations to collect samples, (ii) deployment and retrieval of the rosette water sampler is time consuming and, (iii) only small volumes of water can be collected in comparison to the underway sampling. The limitation of filtering smaller volumes of water is twofold in that there can be (i) false negatives whereby microplastics are not sampled despite being present in the environment or, (ii) microplastics are found in the samples but scaling up to relevant units (particles m^{-3}) has a greater effect on microplastic abundances.

One of the major challenges that investigators face when quantifying marine microplastic abundance is sample contamination. In addition to employing strict measures to control contamination during sampling and processing, it is important that checks are carried out to quantify potential contamination of samples. For underway samples in the present study, although method blanks were free of contamination by synthetic particles, a single synthetic fibre was found in one air contamination check. In context, there were between 0 – 15 synthetic particles in each underway sample, with an average of 2 synthetic particles per sample. For CTD samples in the present study, between 0 – 3 synthetic fibres were found in the method blanks. In context, between 0 – 18 synthetic particles were found per CTD sample (21 L or 48 L), with an average of 2 particles per sample. In both cases, if contamination were an issue, its contribution to the reported microplastic abundances in the present study would be substantial. However, the possibility of airborne contamination in the actual underway samples is projected to be low since (i) 92% of the air contamination

checks (11 of the 12 petri dishes) were free of synthetic particles and, (ii) air contamination checks had maximum exposure to the atmosphere while actual samples had minimal exposure. With respect to the CTD samples, it is proposed that synthetic fibres in the method blanks may have been introduced into the Niskin bottle during the transfer of Milli-Q water or could have been present from the previous CTD cast and remained in the bottle due to insufficient rinsing with Milli-Q water prior to the blank. This should not have been an issue for the actual samples since Niskin bottles were rinsed during the downcast and were closed within the water column thus preventing the possibility of airborne contamination.

3.6. Conclusion

The present study demonstrated the pervasiveness of microplastics in sub-surface waters of the Arctic Central Basin (ACB). Two independent sampling techniques led to the discovery of microplastics in near surface waters of the Polar Mixed Layer (PML) i.e. at a single depth of 8.5 m as well as throughout the water column i.e. at multiple depths (8 – 4369 m) of the ACB. Such findings confirm that microplastics are entering the central Arctic Ocean, that they are being vertically transported out of surface waters and that the water column is one of the reservoirs of microplastics in this region. Presently, however, uncertainty exists regarding the actual mechanisms responsible for the vertical transport of microplastics in the Arctic Ocean. Although there was a predominance of fibrous microplastics, the majority of which were polyester, the exact sources of microplastics to the Arctic Ocean remain unknown as they could have been introduced to the ecosystem via long range transport processes or originated from more local sources. The fact that the highest microplastic abundances were recorded in the PML nearer to the periphery of the ACB

suggests the influence of location-specific factors e.g. absence of sea ice, proximity to microplastic sources, wind, etc. Knowledge about microplastic abundance, distribution and composition in the Arctic Ocean is vital as it provides (i) quantitative data on the concentrations and types of microplastics that polar organisms are exposed to, (ii) a sound starting point for investigating the potential threat that microplastics pose to the Arctic ecosystem and, (iii) insight into the whereabouts of the 'missing plastic' from oceanic surface waters.

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Chapter 4

Deep sea sediments of the Arctic Central Basin:

A potential sink for microplastics

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4.1. Abstract

Deep sea sediments have in the past decade emerged as a potential sink for microplastics in the marine environment. The discovery of microplastics in various environmental compartments (sea ice, biota, water column) of the Arctic Central Basin (ACB) suggested that these contaminants were potentially being transported to the deep-sea realm of this oceanic basin. For the first time, the present study conducted a preliminary assessment to determine whether microplastics were present in surficial sediments from the ACB. Gravity and piston corers were used to retrieve sediments from depths of 855 – 4353 m at 11 sites in the ACB during the Arctic Ocean 2016 (AO16) expedition. Surficial sediments from the various cores were subjected to density flotation with sodium tungstate dihydrate solution ($Na_2WO_4.2H_2O$, density 1.4 g cm^{-3}). Potential microplastics were isolated and analysed by Fourier Transform Infrared (FT-IR) spectroscopy. Of the surficial samples, 7 of the 11 samples contained synthetic polymers which included polyester (n = 3), polystyrene (n = 2), polyacrylonitrile (n = 1), polypropylene (n = 1), polyvinyl chloride (n = 1) and polyamide (n = 1). Fibres (n = 5) and fragments (n = 4) were recorded in the samples. In order to avoid mis-interpretation, these findings must be taken in the context that (i) sampling equipment did not guarantee retrieval of undisturbed surficial sediments, (ii) low sample volumes were analysed (~ 10 g per site), (iii) replicate sediment samples per site was not possible, (iv) no air contamination checks were included during sampling and, (v) particles <100 µm were automatically excluded from analysis. While the present study provides a preliminary indication that microplastics may be accumulating in the deep-sea realm of the ACB, further work is necessary to assess microplastic abundance, distribution and composition in surficial sediments of the ACB.

4.1.1. Keywords

Microplastic, Marine debris, Arctic Ocean, Sediment, Deep Sea

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4.2. Introduction

Microplastics are pervasive, persistent contaminants in the world's oceans that warrant concern due to the potential threat they pose to marine organisms. Traditionally, microplastic sampling has been conducted in surface and near-surface waters due to the presumption that the majority of microplastics would be present in that layer of the water column. However, when plastic production and projected plastic input to the ocean was considered, there was an evident mismatch between reported and expected plastic concentrations in surface oceanic waters (Cózar et al. 2014; Eriksen et al. 2014). It was therefore apparent that apart from surface waters, microplastics were present in various environmental compartments in the world's oceans (water column, sea ice, sediments, biota) and that some of these potentially functioned as sinks (Obbard et al. 2014; Woodall et al. 2014).

Deep sea sediments have recently been identified as a potential sink for microplastics (Woodall et al. 2014; Bergmann et al. 2017). To date, only four studies have reported on microplastics in deep sea sediments in various oceanic basins (Van Cauwenberghe et al. 2013; Woodall et al. 2014; Fischer et al. 2015; Bergmann et al. 2017). Despite the fact that each of these studies employed

different sampling equipment, extraction techniques and reported microplastic abundance in different units, the consensus was that microplastics have made it to the deep-sea and that they are pervasive in its sediments. Presently, uncertainty still exists regarding the exact mechanisms that are responsible for the vertical transport of microplastics out of surface oceanic waters and into deep sea sediments.

The Arctic Ocean, though one of the most remote oceanic basins in the world, has been subject to the entry of plastic debris into its ecosystem. It has been suggested that this plastic debris, in particular microplastics, could have entered the Arctic ecosystem via a combination of (i) long-range transport processes, e.g. via oceanic currents (Zarfl and Matthies 2010; van Sebille et al. 2012), biotransport (Mallory 2008; Provencher et al. 2012) and riverine input (Obbard et al. 2014) and, (ii) local anthropogenic activities, e.g. shipping (Tekmann et al. 2017). At the Atlantic gateway to the Arctic Ocean, i.e. the Fram Strait, macroplastics were reported in surface waters, on the seafloor and even on beaches of Svalbard (Bergmann & Klages 2012; Bergmann et al. 2016; Bergmann et al. 2017a; Cózar et al. 2017; Tekman et al. 2017) while microplastics were discovered in the surface/sub-surface waters and sediments (Lusher et al. 2015; Bergmann et al. 2017b; Cózar et al. 2017). Further north, in the Arctic Central Basin (ACB), microplastics were recorded in sea ice, biota such as juvenile polar cod (*Boreogadus saida*) and benthic organisms and sub-surface waters (Obbard et al. 2014; Kanhai et al. 2018; Kuhn et al. 2018; Peeken et al. 2018; Fang et al. 2018).

The fact that microplastics have been reported in the various water layers of the ACB, in particular its deep waters, suggests that these particles are pervasive in the water column and that they are being transported out of its surface waters (Kanhai et al. 2018). It was therefore hypothesized that microplastics would be present in deep sea sediments in the ACB. To our knowledge, the present study sought for the first time to determine whether microplastics were present in surficial sediments of the Arctic Central Basin (ACB) and to establish whether the deep sea in this oceanic basin is possibly acting as a sink for microplastics.

4.3. Material and methods

4.3.1. Study area

The Arctic Ocean, the world's smallest ocean, is comprised of a deep central basin surrounded by extensive continental shelves (CAFF 2013). The bathymetry of the Arctic Ocean is such that the Lomonosov Ridge divides the central basin into the Canadian (Amerasian) and Eurasian sub-basins (Jakobsson et al. 2004). Within each of the sub-basins, there are further divisions as well as the existence of Abyssal Plains (APs) which are deep water areas of low relief. In the Amerasian basin, the Alpha-Mendeleev Ridge separates the Canada Basin (with its Canadian AP) and the Makarov Basin (with its Fletcher AP) while in the Eurasian basin, the Gakkel Ridge separates the Amundsen Basin (with its Pole AP) and the Nansen Basin (with its Barents AP), (Jakobsson et al. 2004).

4.3.2 Sample collection

This study was conducted onboard the Swedish icebreaker Oden between August 8th to September 19th 2016 during the Arctic Ocean 2016 expedition.

During the transit of 4943 nautical miles, sediments were retrieved using a gravity corer or a piston corer with an associated trigger weight corer. Cores from 11 stations were sampled for microplastics (Table 4.1, Figure 4.1). Cores were split, wrapped in plastic film (polyethylene) and transported to the laboratory where they were processed prior to sampling for microplastics. Processing usually involved (i) scraping the core half (with a polypropylene scraper) to remove liner fragments and sediment disturbed during core splitting and, (ii) visually describing the lithostratigraphic properties. Sheer strength measurements, sediment pH and samples for paleomagnetic measurements were taken prior to microplastic sampling for four of the eleven cores (gravity and piston cores). Approximately 10 cm^3 of sediment was sampled from the top 2 cm of the working half of each core using a scoop (polypropylene). Sediment samples were placed into clean, labelled plastic bags (polyethylene) and stored in a freezer (-20°C). Although it is acknowledged that such a low volume of sediment may not be representative of sediments at individual sampling stations, the sampling technique used in the present study, i.e. coring, limited the volume of sediment that was available per site. Precautions taken onboard the ship to limit cross-contamination included (i) minimal exposure of the sediment samples to the atmosphere, (ii) samples collected/stored in new materials (scoops, bags) and, (iii) sampling conducted by one individual. A record was also kept of all plastic materials that came into contact with the sample during collection and processing. One limitation was that no air contamination check was included during sediment sampling onboard the vessel.

Table 4.1: Site-specific information for sediment cores sampled for microplastics in the Arctic Central Basin

Sample No.[a]	Core ID	Latitude	Longitude	Location in the ACB	Water Depth (m)	Core Type[b]
1	AO16-1-GC1	80.5532	8.0520	Yermak Plateau	855	GC
2	AO16-2-PC1	88.5022	-6.6195	Amundsen Basin	4353	PC
3	AO16-3-TWC1	89.2530	-66.6097	Foot of Lomonosov Ridge	3777	TWC
4	AO16-4-TWC1	88.5290	-128.5048	Marvin Spur	3936	TWC
5	AO16-5-TWC1	89.0780	-130.5470	Crest of Lomonosov Ridge	1253	TWC
6	AO16-7-PC1	88.6332	-121.4477	Marvin Spur	3941	PC
7	AO16-8-GC1	86.7795	-140.6433	Alpha Ridge	2620	GC
8	AO16-9-TWC1	85.9557	-148.3258	Alpha Ridge	2212	TWC
9	AO16-10-TWC1	82.3980	-141.2450	Nautilus Basin	2872	TWC
10	AO16-11-TWC1	86.0993	173.1877	Makarov Basin	3066	TWC
11	AO16-12-TWC1	87.8577	136.9875	Crest of Lomonosov Ridge	1269	TWC

[a]Sample numbers correspond to those on Figure 4.1a; [b]GC (Gravity core); PC (Piston core); TWC (Trigger weight core)

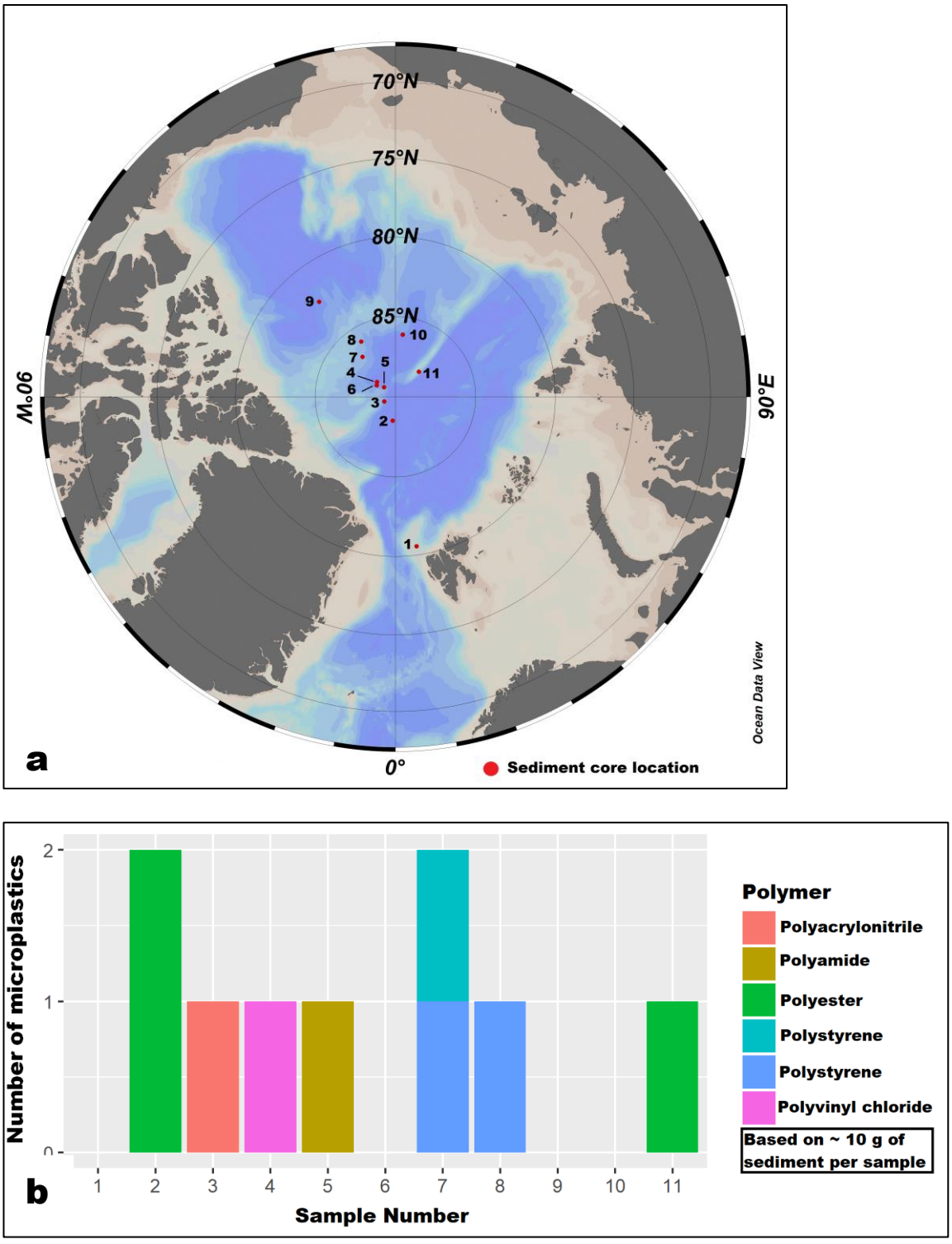

Figure 4.1: Locations at which sediment cores were collected in the Arctic Central Basin (a) and synthetic polymer composition in surficial sediments from the various cores (b)

4.3.3. Laboratory analyses (microplastics)

In the laboratory, sediments were defrosted, transferred into clean covered aluminium foil trays and oven dried at 60°C for approximately 96 hours. Approximately 10 g of oven-dried sediment was weighed and placed into a pre-cleaned glass jar. 105 mL of sodium tungstate dihydrate ($Na_2WO_4.2H_2O$, 40% w/v, density 1.4 g cm^{-3}), as recommended by Frias et al. (2018) and Pagter et al. (2018), was added to each glass jar, the mixture was shaken for approximately 1 minute and the sediments were allowed to settle. From each sample jar, the overlying sodium tungstate dihydrate solution was removed using a pipette and filtered under vacuum onto glass microfiber paper (GF/C), Whatman 47 mm, pore size 1.2 µm, using a Buchner funnel and an Erlenmeyer flask. Minimal volumes of ultrapure water (< 2 mL) were used to wash down the sides of the glass jars with the sediments. Introducing water into the remaining extraction solution can lead to a change in the density of the solution and thus this was minimised. Ultrapure water was also used to wash the pipette and sides of the Buchner funnel. Filter papers for the samples were placed into clean petri dishes and stored until analysis. Potential contamination was evaluated by using (i) air contamination checks-clean petri dishes with filter paper (n = 2) were exposed to the air during sample processing and, (ii) method blanks-jars devoid of sediment (n = 2) were processed in the same manner as actual samples. Measures taken to prevent contamination in the laboratory included (i) wearing lab coats and gloves during sample processing and, (ii) washing all glass jars used during sample processing with a 6% nitric acid solution and Ultra-pure water.

Filter papers were visually examined under a dissecting microscope (Olympus SZX10) equipped with a polariser and camera (Q Imaging Retiga 2000R).

Potential microplastics were isolated and processed (photographed and length measurements taken) prior to transferring to a clean filter paper in a labelled petri dish (Kanhai et al. 2017). Due to the difficulties that arise when handling particles < 100 µm, such particles were automatically excluded for any analysis. All potential microplastics and any plastic material that was in direct contact with the samples either during sampling or laboratory processing were analysed by Fourier transform infrared (FT-IR) spectroscopy on a Bruker Vertex 70 Infrared Spectrometer coupled to a Hyperion 1000 microscope (Kanhai et al. 2017). Samples which produced spectra with a match < 60% were automatically rejected while those which produced a match of > 70% were accepted. All spectra with matches > 60% were individually examined to ensure that there was clear evidence of peaks from the sample corresponding to known peaks of standard polymers and that instances of misidentification of natural and semi-synthetic polymers were reduced (Comnea-Stancu et al. 2017).

4.3.4. Particle size and organic carbon content analysis

For particle size analysis, approximately 0.1 g of wet sediment from each sediment core was transferred to a test tube. To this, 3 mL of sodium metaphosphate solution (10%) was added and the total volume brought to 10 mL using de-ionized water. The contents of the tube were stirred, ultrasonicated for approximately 30 seconds to facilitate disaggregation and then transferred to the Hydro LV wet dispersion unit of a Mastersizer 3000 laser diffraction particle size analyser (Malvern Instruments, UK). De-ionized water was used to bring the final volume in the wet dispersion unit to 600 mL prior to analysis. The particle size of surficial sediments from each core was based on the analysis of three sub-samples. Following Pagter et al. (2018), approximately 3 g of dried sediment (105

°C, 4 h) from each sample was placed into a furnace at 450 °C for 6 hours in order to estimate the organic content of the sediment samples based on loss on ignition.

4.3.5. Data Analyses

The software Ocean Data View (ODV) Version 4.7.10 (Schlitzer 2017) was used to generate a map of sediment sampling locations (Figure 4.1a). Grain size composition of the surficial sediment data was conducted using Gradistat Version 8 (Blott 2010). Figure 4.1b was generated using R version 3.4.4 (R Core Team 2018).

4.4. Results

4.4.1. Quality Control

In the present study, the following plastic materials made direct contact with the sediment samples either during collection or processing (i) plastic film – low density polyethylene (LDPE), (ii) core liner – polyvinyl chloride (PVC) or polycarbonate (PC), (iii) scraper - polypropylene (PP), (iv) sediment collection scoop – polypropylene (PP) and, (v) sample bag – low density polyethylene (LDPE). In the surficial sediment samples, no polyethylene particles were recovered. However, in two instances, synthetic polymers from surficial sediments matched plastic materials used during sample processing but were not eliminated since they were of different colours than the materials used. This was so for 2 particles in sediment core 4 (single PVC fragment, different colour from liner) and sediment core 7 (polypropylene fragment, different colour from collection scoop/scraper). To assess whether synthetic polymers were introduced

during laboratory processing of the samples, air contamination checks (ACs), (n = 2), and method blanks (MBs), (n = 2), were included. No synthetic polymers were found in the air contamination blanks. However, a total of three fibres were found in the two method blanks (MB1 – blue polyester fibre, 1.28 mm; MB2 – blue polyester fibre, 0.49 mm and red polyester fibre, 0.53 mm). All samples were blank corrected such that if any blue or red polyester fibres were found in the sediment samples, they were removed from the final results.

4.4.2. Microplastic composition in surficial sediments

Based on visual identification, fifteen particles from the sediment samples were isolated and subjected to FTIR spectroscopy. Of these, four were natural cellulosic fibres. The remaining eleven were identified as synthetic polymers with one macroplastic (> 5 mm) and ten microplastics (< 5 mm). After blank correction of the samples, there were a total of nine microplastics (<5 mm). Synthetic polymers detected in the sediments included polyester (n = 3), polystyrene (n = 2), polyacrylonitrile (n = 1), polyamide (n = 1), polypropylene (n = 1) and polyvinyl chloride (n = 1). Both fibres (n = 5) and fragments (n = 4) were present in the samples. In terms of colour, most of the synthetic polymers were transparent (n = 5) with the remainder black (n = 1), brown (n = 1), white (n = 1) and blue (n = 1). With respect to length, most (n = 5) were < 1 mm, 3 were between 1 – 2 mm and 1 was > 2 mm. Of the surficial sediment samples analysed from the Arctic Central Basin (ACB), 7 of the 11 samples contained between 1 – 2 synthetic polymers (Figure 4.1b). When the number of microplastics and the dry weight of the sediment samples were taken into account, it was estimated that microplastic abundance in surficial sediments in the ACB possibly ranged from 0 – 200 particles kg^{-1} dry sediment (Supplementary Table 4.1). Excluding the four (4) sites

at which no microplastics were recorded, microplastic abundance at most of the sites was estimated at approximately 100 particles kg^{-1} dry sediment.

Regarding the particle size composition of the sediments, all sampled sites had a predominance of fine-grained sediments where the percentage of silt (59 – 87%) > clay (10 - 24%) > sand (1 – 23%) > gravel (0 – 0.2%), (Supplementary Table 4.1). Organic content of the surficial sediment samples ranged between 2.3 – 4.6% (Supplementary Table 4.1).

4.5. Discussion

Elucidation of the transport and fate of microplastics in the marine environment is a critical step towards assessing the threat that these contaminants potentially pose to organisms inhabiting different compartments of an ecosystem. In the Arctic Central Basin (ACB), only a few studies have reported on microplastic presence in the sea ice, biota and water column (Obbard et al. 2014; Kuhn et al. 2018; Kanhai et al. 2018; Peeken et al. 2018). Based on these studies, the key suggestions regarding microplastics in this oceanic basin are that (i) sea ice acts as a sink and means of transport for microplastics, and (ii) the pervasiveness of microplastics in the various water layers of the ACB indicates that there is vertical transport of microplastics out of surface waters into deeper waters (Obbard et al. 2014; Kanhai et al. 2018; Peeken et al. 2018). In context, the findings of the present study expand the knowledge base about microplastics in the Arctic Ocean by providing preliminary information that suggests microplastics are present in surficial sediments of the Arctic Central Basin and that within this oceanic basin the sediment compartment is potentially acting as one of the sinks for microplastics. Microplastic presence on the seafloor of the ACB lends

credence to the suggestion that there is vertical transport of microplastics within the water column. Laboratory and field studies have shown that marine organisms, such as zooplankton, larvaceans and other pelagic filter feeders, which are capable of ingesting microplastics and egesting them in their faecal pellets and discarded houses (as in the case of the larvaceans), could contribute to the vertical flux of microplastics in the water column when their waste products sink (Cole et al. 2016; Katija et al. 2017). Various algal species, capable of incorporating and concentrating microplastics in their aggregates, may also contribute to this process in the oceans due to the sinking of their aggregates (Long et al., 2015). Additionally, the biofouling of microplastics by microorganisms is another process which potentially influences the vertical transport of these particles in the marine environment (Fazey and Ryan 2016).

The presence of microplastics in sediments of the Arctic Central Basin implies that interactions between these particles and deep-sea organisms that inhabit or depend upon this environmental phase is plausible. Although the Arctic Ocean has generally been regarded as oligotrophic, the fact remains that marine organisms do inhabit its' deep-water environment with the most speciose groups being arthropods, foraminiferans, annelids and nematodes (Bodil et al. 2011). Depending on the foraging behaviours and feeding habits of deep-sea benthos in the ACB, the possibility exists that some of them may be interacting with microplastics in the sediment phase. Recently, Fang et al. (2018) reported that microplastics were discovered in 11 different benthic species that were recovered from depths of 35 – 151 m in the Bering-Chukchi Sea shelves. Fibres were the predominant type of microplastics found in the organisms with synthetic polymers including polyamide, polyethylene, polyester and cellophane (Fang et al. 2018).

Microplastics were also discovered in 3 different phyla (Echinodermata, Arthropoda, Cnidaria) of deep sea organisms recovered from depths of 334 – 1783 m in the equatorial mid-Atlantic and SW Indian Ocean (Taylor et al. 2016). Although the presence of a contaminant in the marine environment does not directly imply harm, laboratory experiments have indicated that benthic organisms exposed to microplastics in sediments may be negatively impacted. For example, Wright et al. (2013) reported that exposure of the deposit-feeding marine polychaete worm (*Arenicola marina*) to unplasticised polyvinyl chloride (UPVC) led to a depletion in energy reserves of the worms which could have been caused by reduced feeding, longer gut residence time of ingested matter and inflammation.

Identification of the synthetic polymer composition of particles recovered from the natural environment is fundamentally important if investigators are to provide unambiguous evidence regarding the identity of particles from their samples. The present study confirmed via FT-IR spectroscopy that polyesters were recovered from surficial sediments of the ACB. Such findings are corroborated by previous studies which investigated other environmental phases in this oceanic basin. Obbard et al. (2014) reported that of the synthetic polymers found in sea ice, the majority were polyester (21%). Kanhai et al. (2018) similarly found that of the synthetic particles present in the sub-surface waters, polyesters (74 – 78%) were also predominant. Upon melting, sea ice can act as a local source of microplastics to the water column (Obbard et al. 2014; Peeken et al. 2018). Synthetic polymers that are present in surface waters of this oceanic basin could then be subject to vertical transport, persist in the water column as evidenced by Kanhai et al. (2018) and at some stage a fraction of these particles could end up in the

sediment phase. Of interest is the fact that the present study found low density polymers such as polypropylene and polystyrene fragments in the sediments of the ACB. Based on the inherent densities of the virgin resins, such particles are unlikely candidates for the sediment phase in that they are positively buoyant and are expected to float. However, this suggests that there are mechanisms operating within the ACB that are affecting the density of these particles and in effect causing them to end up in the sediment phase. Long et al. (2015) showed that under laboratory conditions marine aggregates of various algal species (*Chaetoceros neogracile*, *Rhodomonas salina*) were capable of incorporating and concentrating polystyrene microbeads which in turn led to an increase in their sinking rates. Such mechanisms can potentially explain the presence of low-density polymers in surficial sediments of the ACB. Of note is the fact that the present study is not the first to report the presence of low density polymers in deep sea sediments since polyethylene and polypropylene particles were found in surficial sediments from the Fram Strait (Bergmann et al. 2017).

Within the last decade, deep-sea sediments were for the first time identified as a potential sink for microplastics with four studies reporting on the issue in various oceanic basins (Van Cauwenberghe et al. 2013; Woodall et al. 2014; Fischer et al. 2015; Bergmann et al. 2017), (Table 4.2). Comparison between these studies is particularly challenging and not straightforward due to the fact that each used different sampling equipment, extraction techniques and reported microplastic abundance/concentration in different units (Table 4.2). Microplastic abundance in surficial sediments of the ACB was estimated to range between 0 – 200 microplastics kg^{-1} dry sediment based on the findings of the present study. However, it is unlikely that these estimates are reflective of the situation in the

ACB since (i) the equipment used for sample retrieval (gravity and piston corers) may have led to the collection of disturbed surficial sediment samples, (ii) low sample volumes (~ 10 g per site) were used to assess microplastic abundance, (iii) the density of the extraction solution was only 1.4 g cm^{-3} and thus could have excluded high density polymers, (iv) replicate sediment samples per site was not possible, (v) particles < 100 µm were excluded by virtue of the procedure used to identify/isolate potential microplastics.

Table 4.2: Microplastics in deep sea sediments of various oceanic basins

Location	**Depth (m)**	**Sampling Equipment**	**Extraction Method**	**Microplastic abundance**	**Synthetic polymers**	**Study**
Porcupine Abyssal Plain Nile Deep Sea Fan Atlantic Sector of the Southern Ocean	1176 – 4843	Multicorer	Density flotation, NaI (1.6 g cm^{-3})	0.5 particles cm^{-3} (average, n = 11) 1 particle cm^{-3} (max)	No data	Van Cauwenberghe et al. (2013)
Subpolar North Atlantic Ocean NE Atlantic Ocean Mediterranean Sea SW Indian Ocean	300 – 3500	Megacorers Boxcorers	Density flotation, NaCl, Ludox-TM 40 extraction	1.4 - 40 pieces per 50 ml (mean ± s.e., 13.4 ± 3.5)	PA, PES, Acrylic Rayon	Woodall et al. (2014)
Kuril-Kamchatka Trench, NW Pacific	4869 - 5768	Box corer	Sieve-washing of sediments	60 - 2020 pieces m^{-2}	No data	Fischer et al. (2015)

PA-Polyamide, PAN-Polyacrylonitrile, PES-Polyester, PP-Polypropylene, PS-Polystyrene, PVC-Polyvinyl chloride

Table 4.2: Microplastics in deep sea sediments of various oceanic basins

Location	**Depth (m)**	**Sampling Equipment**	**Extraction Method**	**Microplastic abundance**	**Synthetic polymer**	**Study**
HAUSGARTEN observatory, Fram Strait	2340 - 5570	Multiple corer	Density separation, $ZnCl_2$ (1.8 g cm^{-3})	42 – 6595 microplastics kg^{-1} dry sediment	18 polymer types detected. Majority: PE, PA, PP	Bergmann et al. (2017b)
Arctic Central Basin	855 - 4353	Gravity and piston corer	Density separation, $Na_2WO_4.2H_2O$ (1.4 g cm^{-3})	0 - 200 microplastics kg^{-1} dry sediment	PA, PAN, PES, PP, PS, PVC	This study

PA-Polyamide, PAN-Polyacrylonitrile, PES-Polyester, PP-Polypropylene, PS-Polystyrene, PVC-Polyvinyl chloride

During the AO16 expedition, gravity and piston corers were used to retrieve sediments from several metres in depth from the ACB. Among the corers, gravity and piston corers are not guaranteed to retrieve undisturbed surficial sediment samples due to the shock wave that they generate during descent (Gallmetzer et al. 2016). It is therefore likely that any shock waves generated by the corers used in the present study may have triggered a resuspension of surficial material (sediments and microplastics) into the water column leading to an overall reduction and subsequent underestimation of microplastic abundance in the samples. When sampling surficial marine sediments, equipment such as box and multi-corers are more suitable for the recovery of undisturbed surficial sediment samples (Georgiopoulou 2018). Box corers were recommended by Frias et al. (2018) due to (i) the minimal impact they have on surface deformation of sediments and, (ii) their ability to maintain sediment integrity during sampling.

Furthermore, due to the heterogenous nature of sediments, it is unlikely that the low sample volumes (~ 10 g of sediment) used in the study were reflective of the situation at the respective sites. Future studies should ensure that replicate samples are collected per site. Multi-corers may be particularly useful since they can facilitate the collection of replicate samples in a single deployment. When density separation is used to extract microplastics from sediment samples, the density of the extraction solution is important in determining which synthetic polymers are extracted from the samples. Although sodium tungstate dihydrate (density 1.4 g cm^{-3}) was used in the present study based on a safety-price index assessment (i.e. cost and health hazard), the density of the solution could have led to the exclusion of some high-density polymers (Frias et al. 2018). Recent studies have recommended the use of zinc chloride based on its cost

effectiveness and its higher density of 1.8 g cm^{-3} (Loder and Gerdts 2015; Coppock et al. 2017). However, the health hazard is high for this particular extraction solution (Frias et al. 2018). Finally, the methods employed by investigators for the isolation and identification of microplastics influences the final reported microplastic abundance. In the present study, particles < 100 µm were automatically excluded from analysis. However, it must be noted that at the Atlantic gateway to the Arctic Ocean i.e. the Fram Strait, Bergmann et al. (2017) reported that the majority (80%) of microplastics in surficial sediments from that area were < 25 µm. Bergmann et al. (2017) used a combination of ATR-FTIR spectroscopy as well as a µFTIR microscope equipped with a focal plane array detector to detect microplastics. It is therefore likely that if similar analytical techniques were employed to analyse the surficial sediment samples of the present study, higher microplastic abundances may have been reported, especially in the cases where zero microplastics were reported in certain samples.

Another limitation of the present study is the non-inclusion of an air contamination check during sampling onboard the vessel. Such a check would have been necessary to rule out airborne contamination during sampling. Since this was not done, the possibility exists that one or more of the particles reported as present in the surficial sediments of the ACB could have been introduced into the samples as a result of airborne contamination. The findings of the present study should therefore be regarded as preliminary and be used as a justification for future studies which can provide more comprehensive assessments of microplastics in deep-sea sediments of the Arctic Central Basin. Grain size and total organic carbon content of sediments are important ancillary parameters in sediment

studies as they are capable of providing information about the dynamics of the natural environment especially related to particle deposition. For example, sediments with smaller particle sizes are generally indicative of zones where sediment deposition is occurring. A recent study by Maes et al. (2017) in the North-East Atlantic indicated that sediments with smaller grain sizes and higher total organic carbon contents contained higher amounts of microplastics. While the findings of the present study cannot be used to make such statistical interpretations, the consultation of ancillary parameters such as grain size and organic carbon content of sediments may be particularly useful in the selection of sampling sites in future microplastic studies.

4.6. Conclusion

To our knowledge, this is the first study to present preliminary information regarding microplastics in surficial sediments of the Arctic Central Basin (ACB). The potential discovery of these particles in the sediment phase of this seemingly remote oceanic basin emphasizes the pervasiveness of microplastics in the marine environment. The possible presence of microplastics, specifically low-density polymers such as polypropylene (PP) and polystyrene (PS), in the sediment phase of the ACB suggests that there are mechanisms operating within this oceanic basin that are potentially affecting the density of microplastics and that are potentially driving the vertical transport of these particles through the water column. Microplastics that are present in sediments of the ACB are likely to interact with organisms inhabiting or depending upon this environmental phase. At present, whether those interactions are occurring with benthic organisms within the ACB and the consequences of those interactions to individual organisms and the ecosystem services that they perform remains

uncertain. Due to the numerous limitations of the present study, the findings should not be taken as conclusive regarding the status of microplastics in the surficial sediments of the ACB but instead be used as a foundation for future work seeking to quantify microplastic abundance, distribution and composition in surficial sediments of the Arctic Ocean.

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Chapter 5

Sea ice in the Arctic Central Basin:
A temporary sink, transport medium and secondary source of microplastics

Portion of chapter presented in oral platform presentation:

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5.1. Abstract

Understanding the sources, transport pathways and fate of microplastics in the Arctic Ocean is a fundamental step in assessing the potential threat that such particles may pose to polar organisms that inhabit or depend upon various environmental compartments of the ecosystem. In the Arctic Ocean, sea ice is an integral abiotic structural feature that has the potential to influence the distribution of microplastics. The present study assessed microplastic abundance, distribution and composition in surface waters underlying ice floes and in sea ice from the Arctic Central Basin (ACB). At various ice stations in the ACB, sea ice cores (n = 25) were retrieved and seawater underlying ice floes (n = 22) were filtered for microplastics. Following sample processing, potential microplastics were isolated and analysed using Fourier Transform Infrared (FT-IR) Spectroscopy. Microplastic abundance in surface waters underlying ice floes ranged between 0 – 18 particles m^{-3}. Ice cores whose melt water volumes ranged between 3 – 12 L contained between 8 - 117 microplastics. Microplastic concentration in sea ice cores ranged between 2 – 17 particles L^{-1}. In both environmental compartments, there was an overall predominance of fibrous polyesters and polyamides. While it is not possible to conclude with any certainty, backward drift trajectories suggested that the sampled cores possibly originated from Siberian shelves in the Eurasian Basin, western Arctic and the central Arctic. Microplastics may have been introduced to the Arctic marine ecosystem via (i) riverine input, (ii) the influx of contaminated Pacific and Atlantic waters, (iii) atmospheric deposition and, (iv) grey water discharge from vessels. Given the dynamics of sea ice formation and transport, it is quite likely that microplastic entrainment, transport and release from sea ice has an associated (i) temporal component i.e. related to periods of increased melting such as spring and

summer and, (ii) spatial component i.e. relevant to regions subjected to greatest melting ice such as nearshore areas, central Arctic, marginal ice zone.

5.1.1. Keywords

Microplastic, Arctic Ocean, Sea Ice, Surface Waters, debris

5.2. Introduction

Sea ice is an integral abiotic component of the Arctic Ocean's marine ecosystem that is capable of exerting an influence on numerous processes in that oceanic basin. Sea ice functions as a habitat for numerous marine microbes and meiofauna (Søreide et al. 2010; Hardge et al. 2017; Bluhm et al. 2010) and is a key structural feature in the habitat of ice-associated mammals such as polar bears, pinnipeds and cetaceans (Tynan et al. 2010; Kovacs et al. 2011). When sea ice is present, it presents a physical barrier to the (i) influence of wind stress upon oceanic waters, (ii) influx of surface waters and, (iii) availability of light. One of the characteristic features of sea ice in the Arctic Ocean is its occurrence as both multi-year pack ice over the central basin and as younger seasonal ice over its peripheral shelf areas (CAFF 2017). In the Arctic Ocean, sea ice formation generally occurs when seawater becomes supercooled to temperatures below the freezing point (< 1.8 °C), ice crystals called 'frazils' form in the water column, frazils rise to the surface and aggregate to form a layer of 'grease ice' and if freezing continues there is 'congelation' with vertical growth ensuing on the underside of the ice (Pfirman et al. 1990). Ice floes in the Arctic Ocean are complex and dynamic structures that can endure seasonal freeze-thaw cycles and be influenced by rafting/ridging, suspension freezing and the incorporation of anchor-ice from the peripheral shelves (Pfirman et al. 1990; Reimnitz et al. 1992;

Reimnitz et al. 1993; Pfirman et al. 1995). Important sources of sea ice in the Arctic Ocean are the shallow Siberian shelves in the Eurasian Basin and the Beaufort Sea in the Amerasian Basin (Pfirman et al. 1990). Some of the sea ice which forms over shelves of the Laptev, Kara and East Siberian Seas is advected off the coast, joins the Transpolar Drift with some eventually exiting through the Fram Strait (Pfirman et al. 1990; Pfirmann et al. 1995). In the western Arctic, ice from the Beaufort, Chukchi and East Siberian seas may become incorporated into the Beaufort gyre with some of the sea ice eventually escaping this gyre (Pfirmann et al. 1995).

For many years, it has been acknowledged that sea ice played an important role in influencing the fate of sediments in the Arctic Ocean. Observational records of 'dirty ice', laboratory-based experiments and modelling studies all highlighted that sea ice functioned as a (i) temporary sink, (ii) transport medium and, (iii) source of sediments in the Arctic Ocean (Pfirman et al. 1990; Reimnitz et al. 1992; Reimnitz et al. 1993). Following an extensive body of work on sediments, it was subsequently suggested that sea ice potentially played a similar role in influencing the fate of contaminants in the Arctic Ocean (Pfirman et al. 1995; Rigor and Colony 1997; Korsnes et al. 2002; Pavlov et al. 2004). As was described for sediments, contaminants could potentially be entrained in sea ice during formation creating a scenario whereby sea ice acted as a temporary sink for contaminants (Reimnitz et al. 1993; Pfirmann et al. 1995; Rigor and Colony 1997). To date, a few studies have confirmed contaminant entrainment of metals, organochlorines and organophosphates in sea ice from the Arctic Ocean (Hargrave et al. 1988; Muir et al. 1992; Chernyak et al. 1996; Melnikov et al. 2003; Pućko et al. 2010a; Pućko et al. 2010b). Sea ice is neither a stationary nor

permanent structural feature of the Arctic Ocean. Once advected off shallow shelf areas, sea ice is mobile and based on its drift patterns can play a role in the re-distribution of contaminants due to the eventual release of contaminants upon melting of the ice (Pfirmann et al. 1995; Rigor and Colony 1997). Areas which may be particularly vulnerable to the release of contaminants from sea ice are areas where melting occurs and include the nearshore areas, central basin and marginal ice zones (Pfirman et al. 1995; Rigor and Colony 1997; Pavlov et al. 2004; Newton et al. 2017).

Almost two decades after metal and organic contaminants were first reported in Arctic sea ice (Hargrave et al. 1988; Muir et al. 1992; Chernyak et al. 1996; Melnikov et al. 2003; Pućko et al. 2010a; Pućko et al. 2010b), microplastics, i.e. plastic particles < 5 mm in diameter, were discovered in sub-sections of 4 ice cores retrieved from various locations in the Arctic Ocean (Obbard et al. 2014). This initial study reported a total of 6 synthetic polymers (polystyrene, acrylic, polyethylene, polypropylene, nylon, polyester) in the ice cores and estimated that microplastic concentrations in Arctic sea ice were between (1.3 – 9.6) × 10^4 particles m^{-3} (where N refers to the number of microplastics, range reported excludes rayon), (Obbard et al. 2014). Rayon, a semi-synthetic polymer was also reported in the ice cores and comprised of 54% of the particles (Obbard et al. 2014). Of note was the fact that the estimated microplastic concentrations in Arctic sea ice were several orders of magnitude higher than highly contaminated surface waters which suggested that sea ice was functioning as a sink for microplastics and that upon melting it could act as a source (Obbard et al. 2014). Four years later, a further 5 sea ice cores were retrieved from the Arctic Ocean and the application of improved analytical technologies led to the discovery of a

more diverse array of smaller (< 100 μm in diameter) synthetic polymers (n = 17), (Peeken et al. 2018). Microplastic concentrations in Arctic sea ice as reported by Peeken et al. (2018), ($1.1 \times 10^6 – 1.2 \times 10^7$ particles m^{-3}), were higher than those initially reported by Obbard et al. (2014). Building on the work of Obbard et al. (2014), Peeken et al. (2018) went a step further, estimated the backward drift trajectories of the sea ice, reported the potential source areas of the cores of both studies and thus confirmed that sea ice was also functioning as a transport medium for microplastics in the Arctic Ocean.

Climate change and pollution are key anthropogenically driven stressors of the Arctic marine ecosystem (CAFF 2013; CAFF 2017). Changes in the global climate have been shown to have a direct impact on Arctic sea ice with reports of decreasing sea ice extent, reductions in sea ice thickness (less multi-year ice) and alterations in the rate of sea ice drift (Serreze and Meier 2018). In the Arctic Ocean, a changing climate is also likely to influence contaminant fate and transport (Pućko et al. 2015), especially if the contaminants of interest are capable of being entrained within, transported and subsequently released by sea ice. Microplastics have been reported in surface and sub-surface waters (Lusher et al. 2015; Amélineau et al. 2016; Cózar et al. 2017; Kanhai et al. 2018; Morgana et al. 2018), sediments (Bergman et al. 2017; Chapter 4) and biota (Amélineau et al. 2016; Fang et al. 2018; Kuhn et al. 2018; Morgana et al. 2018) of the Arctic Ocean. Previous studies also highlighted that microplastics are capable of being entrained within sea ice such that the sea ice is capable of functioning as a temporary sink, transport medium and a secondary source of these particles (Obbard et al. 2014; Peeken et al. 2018). Given that the 18 Arctic Large Marine Ecosystems (LMEs) support an extensive and diverse array of marine life

(AMAP/CAFF/SDWG 2013), an understanding of microplastic contamination in the various environmental phases of the Arctic Ocean is vitally important. Some of the key areas for which knowledge gaps exist and which require greater understanding are (i) the spatial extent of the issue of microplastics in Arctic sea ice given the limited data provided by previous studies, (ii) the vertical distribution of microplastics within sea ice cores given that Obbard et al. (2014) analysed isolated sub-sections of ice cores and Peeken et al. (2018) provide data from only 5 ice cores, (iii) the potential threat posed by microplastics to polar organisms, (iv) the dynamics of microplastic fate and transport in Arctic waters and sea ice and, (v) regions at risk from microplastic contamination in the Arctic Ocean. In order to address some of these issues, the specific objectives of the present study were to (i) provide a more spatially comprehensive assessment of microplastic concentration and composition in sea ice cores in the Arctic Ocean, (ii) assess the vertical distribution of microplastics in sea ice cores, (iii) estimate backward drift trajectories and source areas of sampled sea ice cores and, (iv) assess microplastic abundance, distribution and composition in surface waters (beneath ice floes) in the Arctic Ocean.

5.3. Materials and Methods

5.3.1 Sample Collection

This study was conducted onboard the Swedish icebreaker Oden during the Arctic Ocean 2016 expedition. The vessel departed Longyearbyen, Svalbard on August 8th 2016 and traversed approximately 4943 nautical miles in the Arctic Ocean until its return on September 19th 2016. During the expedition, there were 25 ice stations at which sea ice cores were retrieved and water was pumped from

beneath the ice. Upon arrival at an ice station, the area was surveyed, wind direction was noted and an appropriate sampling site chosen. All microplastic sampling was conducted at a location upwind of all other activities on the ice station. At each site, an area of approximately 0.5 m^2 was shovelled to remove any overlying snow. A Nordic ice drill with an attached Husqvarna X-series 326A125 motor and a stainless-steel core barrel of 12.5 cm diameter was used to drill the sea ice. Once the core barrel was filled with ice, drilling ceased until ice in the core barrel was retrieved and placed on an undisturbed area adjacent to the site. In cases where the drill had not completely penetrated the ice, extensions were added to the core barrel and further drilling ensued until underlying water beneath the ice was reached. The individual sub-sections of the retrieved ice core were placed into a clean bag (polyethylene) and tie straps used to cordon off individual sub-sections. Sea ice cores were then transported to the laboratory until further processing.

Once the sea ice core was retrieved at a site, water was then pumped from under the ice floe. A plastic hose (approximately 2 m in length) was inserted into the drilled hole. This hose was then connected to the inlet of a manual JABSCO Amazon Warrior pump (Model Number 29280-0000). Another hose of approximately 1 m in length was then connected to the outlet of the pump. Prior to any further connections, water was pumped from beneath the ice to flush the system of any contaminants. At this point, the flow rate of water through the system was manually checked in order to estimate the length of time that was needed for pumping the relevant volume of water. Following this, the hose from the outlet of the pump was positioned into the cover of a wooden stand containing a stainless steel sieve (250 µm). This wooden stand with the sieve was positioned

over a bucket into which filtered water flowed. The water that entered the bucket exited the site via a hose that was approximately 3 m in length. At each ice station, water was manually pumped for at least 40 minutes thus ensuring that 1200 L of water was pumped at all sites, with the exception of two sites at which lower volumes (780 L, 1036 L) were pumped. Once pumping of water from beneath the ice was completed, the sieve was covered with aluminium foil, secured in the wooden stand and transported to the lab for further processing.

5.3.2 Laboratory Processing

Once in the lab, each subsection of an ice core was removed from the plastic bag and placed unto a clean wooden surface. A boomerang scraper was used to scrape off the outer surface of the ice and a stainless steel hand saw was used to cut each ice core into 10 cm vertical subsections. Subsections were then placed into individual clean Ziploc bags (polyethylene) and allowed to melt for 24 – 48 hours. Once melted, the water from each subsection was transferred to a graduated cylinder and its volume measured. Each Ziploc bag was rinsed with Milli-Q water to ensure that all particles were transferred out of the bag. Water from each sub-section was filtered under vacuum onto glass microfiber paper (GF/C); Whatman: 47 mm, pore size: 1.2 µm, using a Buchner funnel and a vacuum flask. Each filter paper was then folded, placed into an aluminium foil packet and stored in a freezer (-20 ºC) until returned further processing. The sieve that was used for filtering water from beneath the ice was also processed in the same manner.

5.3.3 Method Validation and Contamination Prevention

Several measures were taken to minimise contamination of samples. In the field, (i) microplastic sampling was conducted upwind of all other activities, (ii) nitrile gloves were used when handling ice cores, (iii) the manual pump used at the ice stations was flushed with water prior to the commencement of a sample, and (iv) the stainless steel sieve that was used at the ice stations had a wooden cover affixed to it during filtration. In the lab, (i) ice processing was conducted on a wooden surface, (ii) the wooden work area was washed down with Milli-Q water in between processing of individual ice core subsections, (iii) all equipment (scraper, saw) was washed with MilliQ water, (iv) lab coats, cotton clothing and gloves were worn during sample processing, and (v) all containers used during sample processing were covered and cleaned using Milli-Q water before reuse. Checks were conducted to determine whether there was any contamination during sample processing. Clean petri dishes with filter paper were left exposed to the air during ice core processing to determine if there was any airborne contamination. To determine whether there was any additional contamination during the processing of the melted sea ice, method blanks were conducted. For each method blank, 750 mL of Milli-Q water was placed into clean Ziploc bags and left for 24 – 48 hours. This water then underwent the exact processing as would have occurred for water from an ice core sub-section (transfer to graduated cylinder and vacuum filtration).

5.3.4. Laboratory Analyses

Filter papers were removed from the freezer, left to dry and then visually examined under a dissecting microscope (Olympus SZX10) equipped with a polariser and camera (Q Imaging Retiga 2000R). Potential microplastics were

identified based on characteristic features such as (i) colour- homogenous colour, shininess, unnatural colours, (ii) thickness-fibres homogenous in thickness and, (iii) bending-fibres demonstrated three-dimensional bending. Potential microplastics from each sample were photographed and length measurements were taken prior to transferring to a clean filter paper. Filter papers with potential microplastics from each sample were stored in clean, labelled petri dishes. Potential microplastics were assigned to two broad categories (fibres, fragments) and to six length categories: <0.5 mm, 0.5 – 1.0 mm, 1.0 – 2.0 mm, 2.0 – 3.0 mm, 3.0 – 4.0 mm, 4.0 – 5.0 mm. During the visual identification process, clean filter papers were also left in petri dishes exposed to the air to determine whether there was any airborne contamination during this procedure.

All potential microplastics were analysed by Fourier transform infrared (FT-IR) spectroscopy on a Bruker Vertex 70 Infrared Spectrometer coupled to a Hyperion 1000 microscope. The instrument was equipped with a potassium bromide (KBr) beamsplitter and an internal mercury cadmium telluride (MCT) detector. Microscope-transmission sampling was performed using a Specac DC-2 Diamond Compression cell. Spectra were recorded as the average of 32 scans in the spectral wave number range of 4,000 - 600 cm^{-1} at a resolution of 4 cm^{-1} (Blackman-Harris 3-term apodisation). Bruker's Opus 7.5 spectroscopy software was used for processing and evaluating all spectra. Prior to analysing each sample, background scans were performed and sample spectra were automatically corrected. Each sample spectrum was compared with those of known standard polymers in the (i) Bruker Optics Attenuated Total Reflectance (ATR) Polymer and (ii) Synthetic Fibres ATR libraries. An initial hit quality with a score ranging between 0 and 1,000 was produced for each match between

sample and reference spectra, with the highest score representing the closest match. Following this preliminary matching, the top ten matches for each sample spectrum were then further evaluated using the Quick Identity Test / Euclidean Distance (ED) option. A hit quality ranging between 0 and 2 was produced for each match between the sample spectrum and the reference spectra, with the lowest number representing the closest match. Samples which produced spectra with a match < 60% were automatically rejected. All remaining spectra (> 60%) were individually examined to ensure that there was clear evidence of peaks from the sample corresponding to known peaks of standard polymers. Overall, matches with > 70% similarity were accepted while some between 60 - 70% similarity were accepted.

5.3.5. Sea ice trajectories

Source areas and backward drift trajectories for the sea ice cores were estimated using the AWI Ice Track application (Krumpen 2018). For each ice core, input to the application included sampling date and location (latitude, longitude) while output included a plot of the estimated pathway as well as ancillary data associated with the pathway. Model predicted and measured sea ice thickness was compared in order to assess the validity of the findings for the various sea ice cores. Percentage matches that were < 75% were viewed with caution.

5.4. Results

5.4.1. Quality Control

Of the shipboard air contamination checks (n = 13) that were carried out, a single fibre (blue polyester fibre, 0.47 mm) was found. This indicates a low likelihood

that microplastics were introduced to the samples as a result of airborne contamination. Of the method blanks (n = 15), 7 were free of contamination while the remainder contained either a single fibre (n = 6) or 2 fibres (n = 2). Fibres in the method blanks were polyester (n = 6), polyamide (n = 1) or a polyamide blend (n = 3). In order to account for any contamination that might have been introduced during ice core processing, a blank correction was applied whereby a single fibre was removed from each sub-section total. Additionally, if there were any matches between synthetic polymers found in the samples and those that came into contact with the samples (either during sampling or laboratory processing), these particles were excluded from the results. Although the application of these measures may lead to an underestimation of microplastic concentrations in sea ice, they are important in order to correct for potential contamination.

5.4.2. Microplastics in sea ice cores

A total of 2031 particles were isolated from the sea ice cores and analysed using FT-IR spectroscopy. Of these, 501 particles were rejected due to (i) poor spectral matches, (ii) matches with polymers used during sample collection or processing and, (iii) identification as being natural or semi-synthetic polymers. A further 117 synthetic polymers were excluded from further analyses since they were categorized as being macroplastics (> 5mm). Of the 1413 confirmed synthetic polymers, 223 were removed during the blank correction process. Subsequent analyses are therefore based on 1190 synthetic polymers < 5mm from the sea ice cores.

Microplastic concentration in sea ice cores (n = 25) from the Arctic Central Basin ranged between 2 – 17 particles L^{-1} (Figure 5.1, Table 5.1). Of the sampled cores,

the majority had estimated microplastic concentrations < 8 particles L^{-1} (Figure 5.1). The two cores with the highest microplastic concentrations were cores 1 (16.5 particles L^{-1}) and 3 (15 particles L^{-1}), (Figure 5.1). Backward trajectories of the sampled ice cores indicated that they possibly originated from the (i) Siberian shelves in the Eurasian basin i.e. Laptev Sea, East Siberian Sea, (ii) western Arctic i.e. Beaufort Sea, Chukchi Sea and, (iii) Central Arctic Ocean (Figure 5.2, Table 5.1). It must be highlighted that for only 12 of the sea ice cores there was a > 75% match between measured sea ice thickness and model-predicted sea ice thickness (Table 5.1). For these cores, there is therefore a measure of certainty that the backward trajectories and source areas obtained by the model were reliable. Of the sampled cores, those which had the highest estimated microplastic concentrations i.e. > 8 particles L^{-1} possibly originated in the Laptev, East Siberian and Chukchi Seas (Figure 5.2).

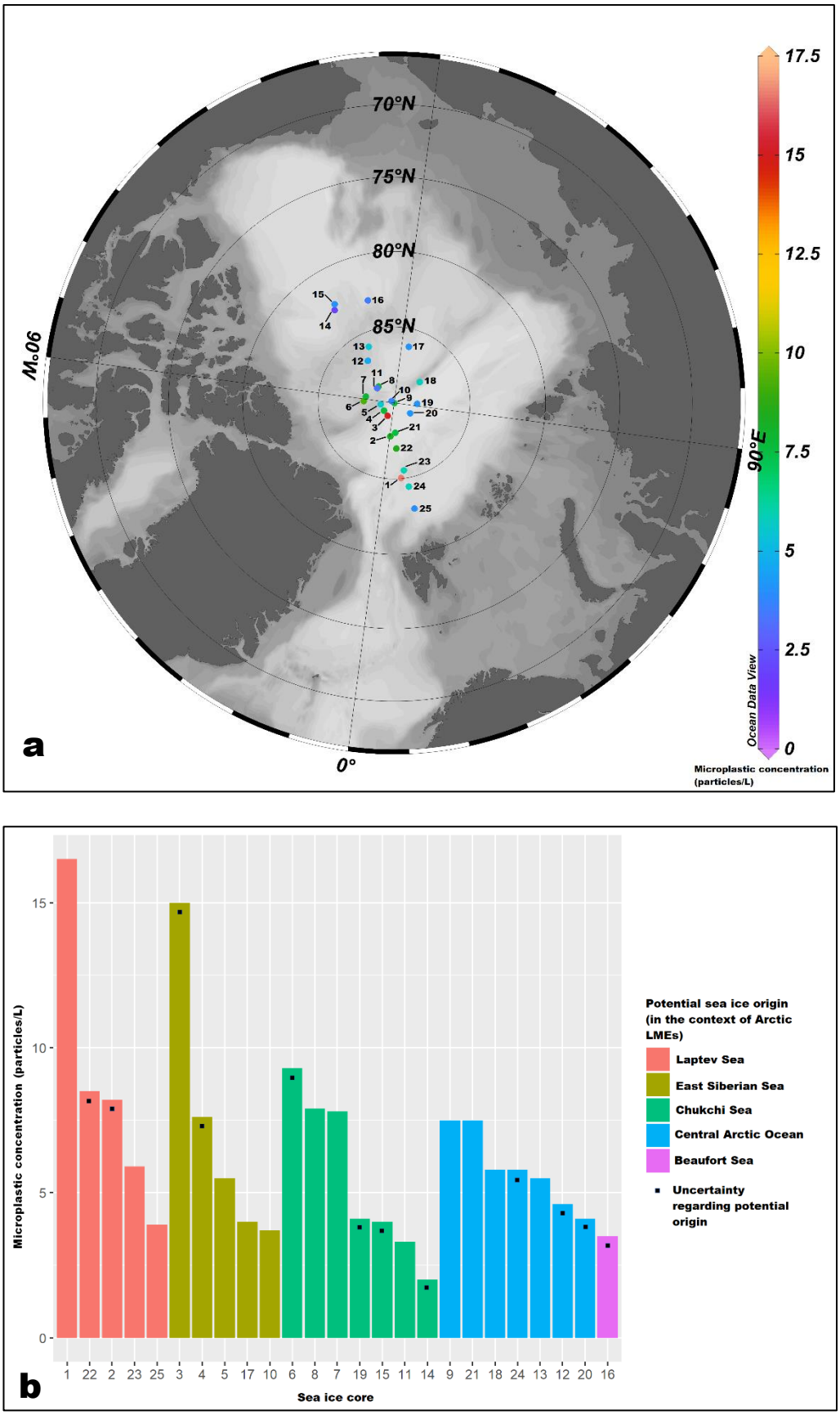

Figure 5.1: Microplastic concentration in sea ice cores from the Arctic Central Basin by sampling location (a) and potential origin (b).

Table 5.1: Model-predicted data (age, ice type, ice thickness, formation zone) for the sea ice cores based on retrieval date and location

Core	Latitude	Longitude	Sampling Date	No. of MPs	Melt water volume (L)	Microplastic concentration (number of particles/L)	SI age (days)	Type of Ice	CL (cm)	MPT (cm)	Difference [CL & MPT] (%)	FZ
1	85.0414	12.9208	13/08/2016	117	7.1	16.5	581	SYI	118	113	4	LS
2	87.7889	0.9455	14/08/2016	32	3.9	8.2	641	SYI	48	116	NA	LS
3	87.7889	0.9455	15/08/2016	108	7.2	15.0	692	SYI	85	124	46	ESS
4	89.0468	-19.3978	15/08/2016	30	3.9	7.6	694	SYI	48	125	160	ESS
5	89.1639	-45.5045	17/08/2016	47	8.6	5.5	684	SYI	113	116	3	ESS
6	89.1245	-76.5614	18/08/2016	38	4.1	9.3	703	SYI	56	123	120	CS

CL-Core length (measured in the field); MPT-Model predicted sea-ice thickness (estimated by AWI Ice Track); NA-CL recorded not representative of ice thickness as ice was not penetrated by drilling; FZ-Formation zone; BS-Beaufort Sea; CAO-Central Arctic Ocean; CS-Chukchi Sea; ESS-East Siberian Sea; LS-Laptev Sea.

Table 5.1: Model-predicted data (age, ice type, ice thickness, formation zone) for the sea ice cores based on retrieval date and location

Core	Latitude	Longitude	Sampling Date	No. of MPs	Melt water volume (L)	Microplastic concentration (number of particles/L)	SI age (days)	Type of Ice	CL (cm)	MPT (cm)	Difference [CL & MPT] (%)	FZ
7	88.0025	-85.9708	19/08/2016	76	9.7	7.8	697	SYI	120	118	2	CS
8	88.0952	-94.9308	20/08/2016	98	12.3	7.9	674	SYI	147	114	22	CS
9	88.5268	-128.7235	21/08/2016	89	11.9	7.5	348	FY1	135	105	22	CAO
10	89.9885	48.4153	22/08/2016	41	11.1	3.7	479	SY1	125	103	18	ESS
11	89.7980	-120.0168	24/08/2016	37	11.1	3.3	678	SYI	137	114	17	CS
12	88.5182	-123.2861	26/08/2016	31	6.7	4.6	1422	MYI	83	144	73	CAO
13	86.7316	-140.4501	29/08/2016	31	5.6	5.5	341	FYI	75	84	12	CAO

CL-Core length (measured in the field); MPT-Model predicted sea-ice thickness (estimated by AWI Ice Track); NA-CL recorded not representative of ice thickness as ice was not penetrated by drilling; FZ-Formation zone; BS-Beaufort Sea; CAO-Central Arctic Ocean; CS-Chukchi Sea; ESS-East Siberian Sea; LS-Laptev Sea.

Table 5.1: Model-predicted data (age, ice type, ice thickness, formation zone) for the sea ice cores based on retrieval date and location

Core	Latitude	Longitude	Sampling Date	No. of MPs	Melt water volume (L)	Microplastic concentration (number of particles/L)	SI age (days)	Type of Ice	CL (cm)	MPT (cm)	Difference [CL & MPT] (%)	FZ
14	85.9559	-148.1900	30/08/2016	8	4.0	2.0	343	FYI	53	96	81	CS
15	82.7416	-139.8267	02/09/2016	18	4.5	4.0	355	FYI	51	92	80	CS
16	82.4130	-141.3739	03/09/2016	11	3.1	3.5	334	FYI	38	89	134	BS
17	83.0300	-158.1485	05/09/2016	31	7.7	4.0	336	FYI	87	83	5	ESS
18	86.1915	172.7474	07/09/2016	51	8.8	5.8	1440	MYI	109	141	29	CAO
19	87.8334	136.6409	08/09/2016	20	4.9	4.1	697	SYI	59	104	76	CS
20	88.4850	95.3654	10/09/2016	20	4.8	4.1	368	SYI	64	97	NA	CAO

CL-Core length (measured in the field); MPT-Model predicted sea-ice thickness (estimated by AWI Ice Track); NA-CL recorded not representative of ice thickness as ice was not penetrated by drilling; FZ-Formation zone; BS-Beaufort Sea; CAO-Central Arctic Ocean; CS-Chukchi Sea; ESS-East Siberian Sea; LS-Laptev Sea.

Table 5.1: Model-predicted data (age, ice type, ice thickness, formation zone) for the sea ice cores based on retrieval date and location

Core	Latitude	Longitude	Sampling Date	No. of MPs	Melt water volume (L)	Microplastic concentration (number of particles/L)	SI age (days)	Type of Ice	CL (cm)	MPT (cm)	Difference [CL & MPT] (%)	FZ
21	88.7405	64.7840	11/09/2016	62	8.3	7.5	376	SYI	104	102	2	CAO
22	88.0378	9.9060	12/09/2016	44	5.2	8.5	578	SYI	59	102	73	LS
23	86.9902	10.3032	13/09/2016	49	8.3	5.9	717	SYI	111	116	5	LS
24	85.5214	15.6777	14/09/2016	67	11.6	5.8	14	FYI	150	12	92	CAO
25	84.4055	17.4587	15/09/2016	34	8.8	3.9	669	SYI	113	112	1	LS

CL-Core length (measured in the field); MPT-Model predicted sea-ice thickness (estimated by AWI Ice Track); NA-CL recorded not representative of ice thickness as ice was not penetrated by drilling; FZ-Formation zone; BS-Beaufort Sea; CAO-Central Arctic Ocean; CS-Chukchi Sea; ESS-East Siberian Sea; LS-Laptev Sea.

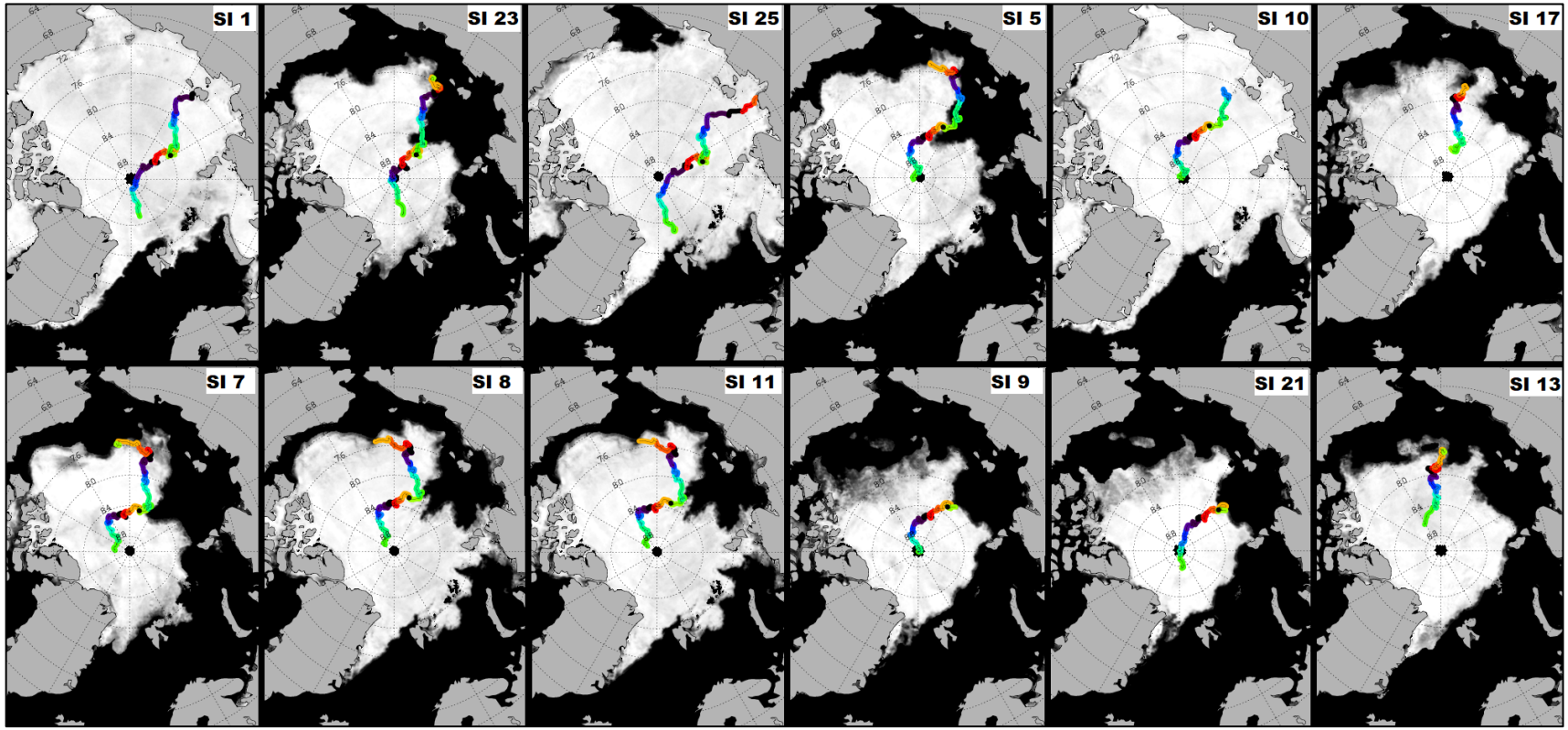

Figure 5.2: Backward trajectories derived using the AWI Ice Track application indicate formation zones for sampled sea ice in the (i) Laptev Sea (cores 1, 23, 25), (ii) East Siberian Sea (cores 5, 10, 17), (iii) Chukchi Sea (cores 7, 8, 11), (iv) Central Arctic Ocean (cores 9, 21, 13). [Trajectories shown here were for cores with a > 75% match between model-predicted and field-recorded sea ice thickness, colours rep. months]

Based on the backward trajectories of the sampled sea ice, age estimations indicate that the majority were at least second year ice (SYI, n = 16) and first year ice (FYI, n = 7) with only two cores being classified as multi-year ice or greater than 3 years old (MYI, n = 2), (Table 5.1).

5.4.3. Vertical distribution of microplastics in sea ice

Overall, there appears to be no consistent pattern in the vertical distribution of microplastics within sea ice cores as well as no relation between sub-section depth of the ice core and microplastic concentration (Figure 5.3). Notwithstanding this, for some ice cores (i.e. core 3, 6, 7, 8, 23) it is apparent that microplastic concentration is comparatively higher in the upper sub-sections of the core (Figure 5.3). The only exception to this was core 25 where microplastic concentration in the sub-section that was in contact with the underlying seawater had the highest microplastic concentration (Figure 5.3). Microplastics were also shown to be pervasive throughout the majority of the ice cores with only a few cores (8, 10, 14, 15, 17, 19, 23) having sub-sections in which no microplastics were found (Figure 5.3). In terms of polymer composition, a total of 9 types of synthetic polymers were found in the ice cores with an overall predominance of polyesters (57%) and polyamides (19%) followed by polyurethane (6%), styrene/acrylates (6%), polyacrylonitrile (6%), polyvinyl chloride (5%) and other polymers (1.3%) which included polypropylene and polyethylene (Figure 5.4a). The majority of the microplastics that were found in the ice cores were fibres (79%) with the remainder (21%) being fragments. Size class distributions of microplastics in the ice cores were as follows: <0.5 mm (32%) > 1 – 2 mm (23%) > 0.5 – 1 mm (20%) > 2 – 3 mm (13%) > 3 – 4 mm (7%) > 4 – 5 mm (4%), (Figure 5.4b).

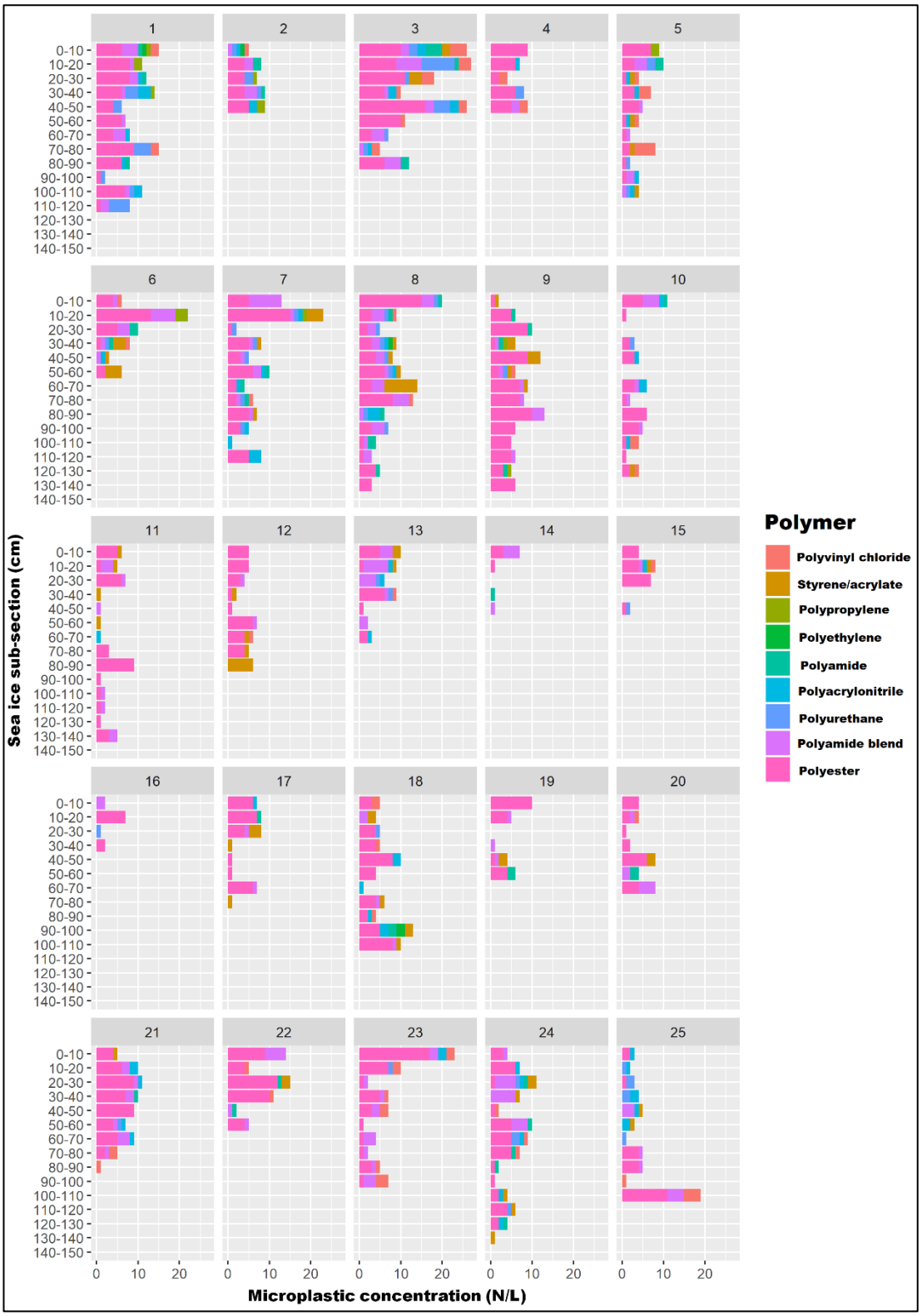

Figure 5.3: Vertical distribution of microplastics in sea ice cores from the Arctic Central Basin [In all cases (except cores 2, 8, 17, 20, 23), last bar indicative of sub-section of ice core in contact with the underlying seawater]

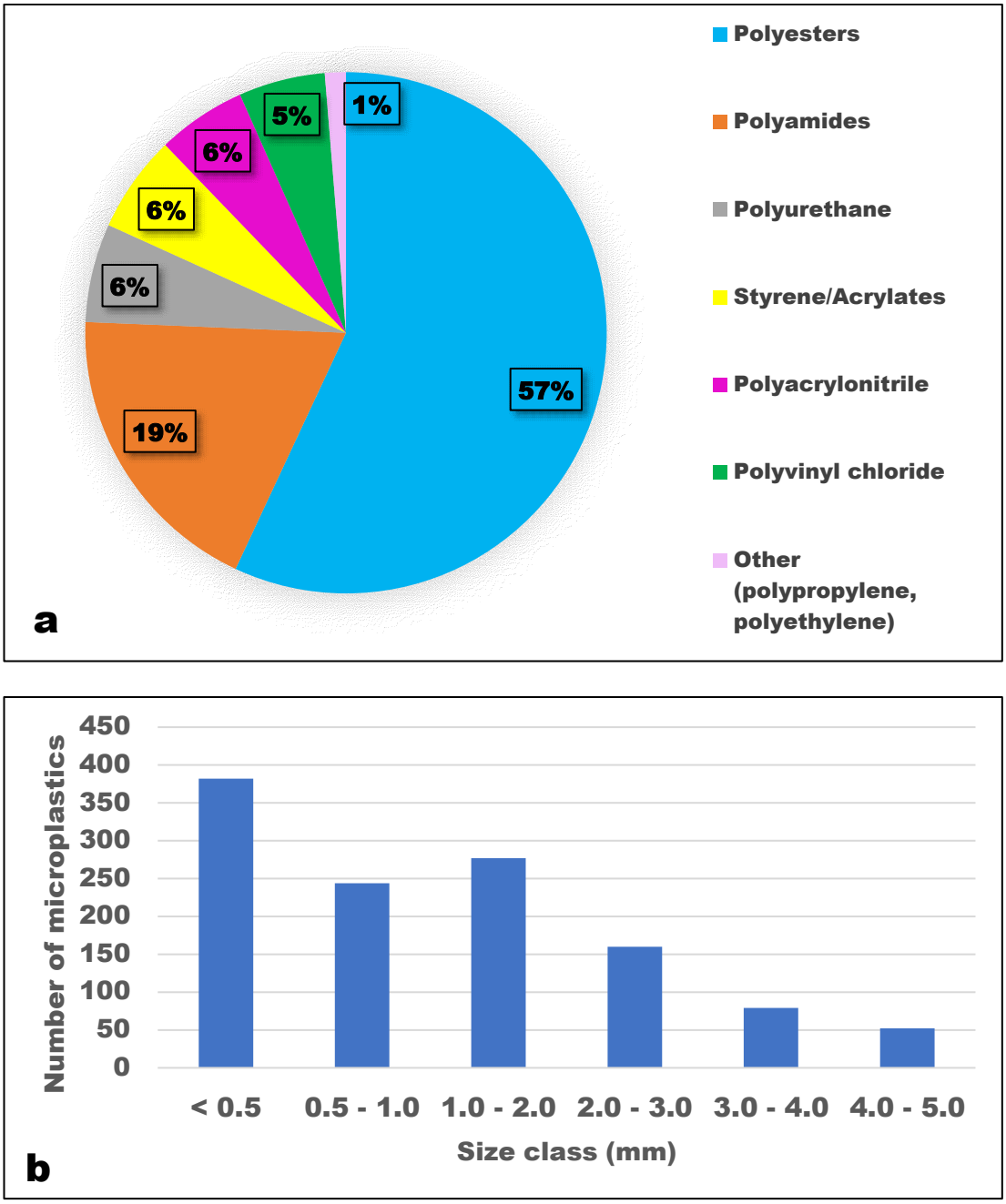

Figure 5.4: Polymer composition (a) and size class distribution (b) in sea ice cores from the Arctic Central Basin.

In terms of colour, the majority of microplastics recorded in the sea ice cores were blue (53%) followed by red (10%), pink (9%), yellow (7%), black (5%), green (3.5%), transparent (3.5%), white (3%), grey (3%) and orange, purple and brown (3%).

5.4.4. Microplastics in surface waters beneath ice floes

A total of 189 particles were isolated from surface waters beneath ice floes in the ACB. Of these, 47 were rejected for reasons mentioned in section 5.4.3. A further 17 synthetic polymers were excluded since they were categorized as macroplastics (> 5mm). Subsequent analyses were based on 125 synthetic polymers < 5 mm. Microplastic abundance in seawater beneath the ice floes ranged from 0 – 18 particles m^{-3} (Figure 5.5). Of the 22 sites where sampling was conducted, at only one site was microplastics not detected in its surface waters. The present study therefore shows that in the Arctic Central Basin (ACB), sea ice contains microplastic concentrations several orders of magnitude higher than those recorded in its surface waters. In terms of polymer composition, the majority of polymers detected in surface waters of the ACB were polyesters (70%) and polyamides (23%) with a minority of polyvinyl chloride (7 %). Microplastic size class distributions were as follows: <0.5 mm (16%), 0.5 – 1 mm (18%), 1 - 2 mm (34%), 2 – 3 mm (14%), 3 – 4 mm (11%), 4 – 5 mm (6%), with the majority of microplastics (68%) being <2 mm. Overall, fibrous microplastics predominated (89%). In terms of colour, the majority of microplastics were blue (58%) and red (18%), with other colours such as transparent (5%), pink (4%), yellow (4%), grey (4%), purple (3%), green (2%), black (2%) and brown (1%) also being recorded.

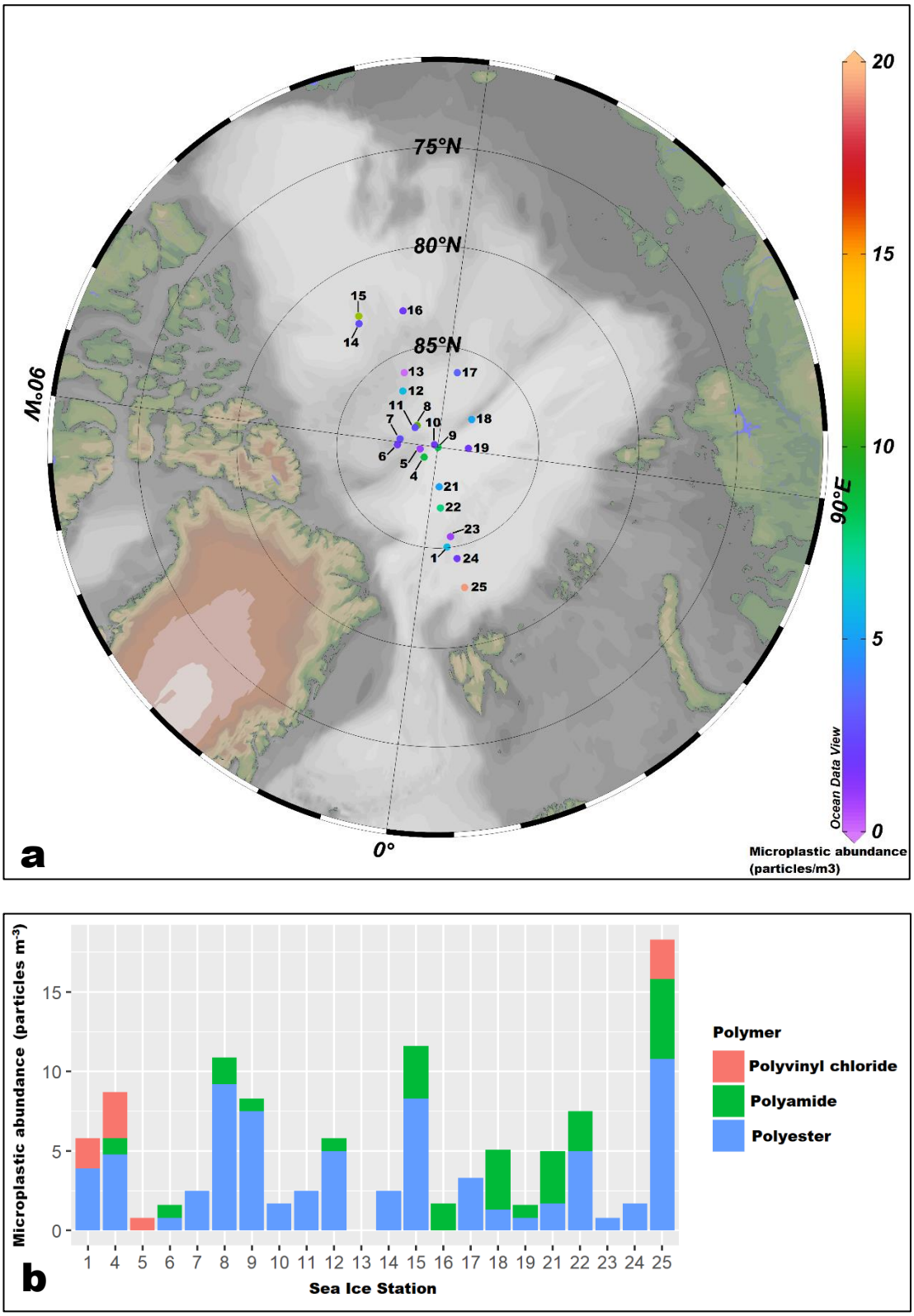

Figure 5.5: Microplastic abundance in surface waters beneath ice floes in the Arctic Central Basin

5.5. Discussion

Elucidating the sources, transport pathways and the fate of microplastics in the Arctic Ocean is critical to understanding the potential threats posed by such particles to marine organisms that inhabit or depend upon different environmental compartments in this ecosystem. Building on the work of previous studies by Obbard et al. (2014) and Peeken et al. (2018), the present study highlighted that microplastics were present in surface waters underlying ice floes in the Arctic Central Basin (ACB) and based on a greater spatial coverage indicated that sea ice cores retrieved from the ACB had microplastic concentrations that were orders of magnitude higher than recorded in the underlying surface waters. Understanding where these microplastics may be originating from and where they might be released is ultimately linked to the dynamics of sea ice formation and transport in the Arctic Ocean. During the life cycle of an ice floe, there are several instances during which microplastics may either be incorporated within sea ice or lost from it (Figure 5.6).

When sea ice formation occurs over the shallow marginal shelves of the Arctic Ocean, it is possible that similar to the situation described for sediments, microplastics that are in the water column or underlying sediments may be entrained in the sea ice during frazil ice formation, suspension freezing or anchor-ice formation (Reimnitz et al. 1993; Pfirman et al. 1995). To date, however, there are no published studies regarding microplastic concentrations in sea ice at the marginal shelves of the Arctic Ocean.

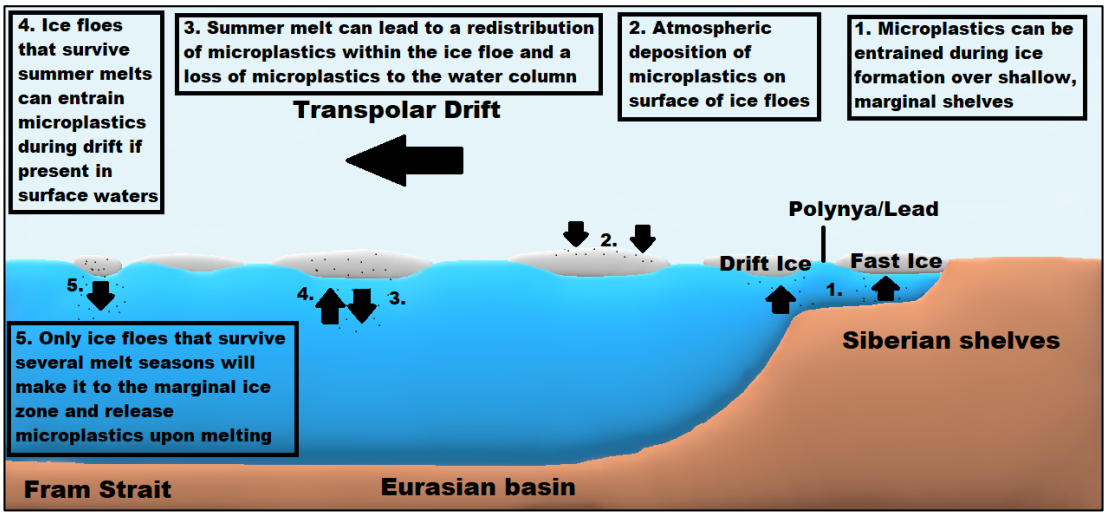

Figure 5.6: Conceptual framework showing instances where microplastic entrainment, re-distribution, release and transport may occur within sea ice in the Arctic Ocean

However, by estimating backward drift trajectories, both Peeken et al. (2018) and the present study indicated that the majority of the sea ice cores that were retrieved from the Arctic Ocean originated from the shallow, marginal shelf areas. Specifically, the present study indicated that sampled sea ice possibly originated from the (i) Siberian shelves in the Eurasian basin i.e. Laptev Sea, East Siberian Sea, (ii) western Arctic i.e. Beaufort Sea, Chukchi Sea and, (iii) Central Arctic Ocean. If microplastics were entrained during sea ice formation in these areas, it is possible that microplastic composition in sampled sea ice cores is reflective of the microplastic composition in surface waters and possibly even sediments from these areas. At the marginal shelves of the Arctic, the freshwater discharge of Siberian and Canadian rivers could be potential contributors to the microplastic load in the Arctic Ocean. Of particular relevance are the Dvina and Pechora (Barents Sea), Ob and Yenisei (Kara Sea), Lena (Laptev Sea) and the Kolyma rivers (East Siberian Sea) which discharge freshwater over the Siberian shelves and the Mackenzie river which discharges into the Beaufort Sea in the western

Arctic (AMAP/CAFF/SDWG 2013; CAFF 2017), (Figure 5.7). Since there are numerous anthropogenic activities (domestic, agricultural, industrial e.g. mining, oil and gas exploitation) occurring within the catchment areas of these rivers, the influx of freshwater into the Arctic basin is a potential pathway via which contaminants could enter this marine ecosystem (Harms et al. 2000; Pavlov 2007). Of interest is the fact that Cózar et al. (2017) reported on plastic concentrations in surface waters following a circumpolar navigation of the Arctic Ocean and with the exception of the Greenland and Barents Seas reported no hotspots of contamination at the marginal shelves. Presently, the contribution of riverine discharge to the microplastic load in Arctic sea ice remains uncertain. Apart from freshwater sources, there is also a notable influx of Pacific waters into the Arctic Ocean through the Bering Strait (Figure 5.7). Pacific waters represent a potential source of contamination in that they are influenced by anthropogenic activities that occur in northern America and eastern Russia. The Chukchi, Beaufort and East Siberian Seas which border the central Arctic are influenced by Pacific waters and thus any sea ice formed in these seas could potentially reflect microplastics that are present in the surface waters in these areas.

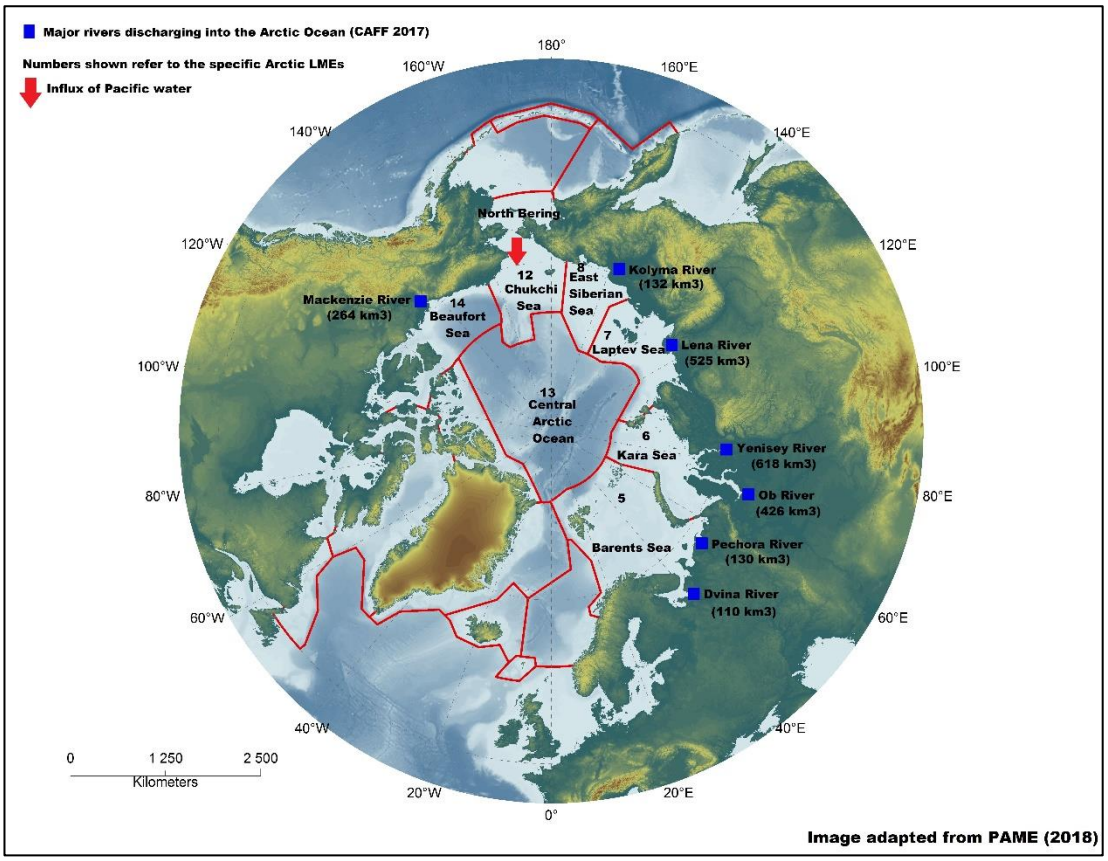

Figure 5.7: Location of riverine freshwater inputs in the Arctic Ocean

Although the marginal shelves are areas of substantial ice production in the Arctic Ocean, sea ice is not a stationary structural feature of the Arctic Ocean but is in fact mobile. A portion of the ice which forms over these marginal shelves in winter will not survive summer and thus will melt in nearshore areas (Rigor and Colony 1997). However, any sea ice that is advected off the coast will eventually enter the Transpolar Drift (particularly relevant to ice floes originating in the Eurasian Basin) or the Beaufort Gyre (relevant to ice floes originate in the western Arctic). If ice floes survive at least one summer melt and at least one period of freezing, microplastics may also be incorporated into ice floes during drift over the central Arctic. The backward drift trajectories produced for sea ice sampled in the present study indicate that 24 of the 25 sea ice cores survived at least one summer melt and endured at least one winter period and that the sea ice drifted across the

Arctic Central Basin (ACB) after formation was initiated. The backward drift trajectories also highlighted the fact that some of the sampled sea ice originated over the central Arctic. Together, these findings are indicative that the microplastics that were found in the sampled sea ice cores were potentially reflective of microplastics present in surface waters of the ACB. In fact, the present study reported that microplastics were present in surface waters beneath ice floes in the central Arctic and that polymer compositions were similar to those reported in the ice cores. The point is that once microplastics are present in the Arctic Central Basin, periods of freezing will facilitate the entrainment of these particles in newly formed ice or on the underside of existing ice floes. Furthermore, at any point during transport, atmospheric deposition of microplastics unto the surface of an ice floe may occur. Unlike other organic contaminants whose presence have been previously reported in the atmospheric environmental compartment of the Arctic (Hargrave et al. 1988; Chernyak et al. 1996; Pućko et al. 2010a), to date, there are no published studies on microplastics in this environmental phase. At lower latitudes, atmospheric fallout of microplastics was reported with the subsequent suggestion that the atmospheric compartment is a potential source of microplastics (Dris et al. 2016). In the Arctic Ocean, it is probable that microplastics are being transported into the region by winds and that these particles are then being deposited either unto ice floes during transport or directly unto surface waters. Both Cózar et al. (2017) and Kanhai et al. (2018) previously postulated that atmospheric transport of microplastics into the Arctic is a possibility. During transport, it is important to note that seasonal environmental conditions can exert an influence on microplastic distribution within an ice floe. During summer, snow and ice melt off the upper portion of the ice floe with meltwater either running off the ice floe, percolating

into the surface of the floe, accumulating in melt ponds or refreezing on the underside (Pfirman et al. 1995). Summer melt periods can therefore facilitate the redistribution of microplastics within an ice floe in that particles that were present in the lower layers of an ice floe may eventually make their way to the top layer of an ice floe. In the present study, some of the sea ice cores that were examined did reflect comparatively higher concentrations of microplastics in the upper sub-sections and it is therefore postulated that re-distribution processes may have resulted in this pattern. Melt seasons are also critical periods during which microplastics may potentially be lost to the underlying seawater.

Identifying the polymer type of individual microplastics is a vitally important component of studies which aim to provide unambiguous proof of the presence of synthetic polymers in their samples. Incorporation of this step in the analysis limits particle misidentification by ensuring that natural and semi-synthetic particles are excluded from microplastic abundance estimates. Additionally, information regarding polymer type can narrow potential sources of microplastics. In the present study, 9 different types of synthetic polymers were reported in the sea ice cores while only 3 were reported in surface waters underlying the ice floes. In both environmental compartments, the majority of microplastics were comprised of fibrous polyesters (57%- sea ice cores, 70%- surface waters) and polyamides (19%- sea ice cores, 23%- surface waters). Of interest is the fact that both polyesters and polyamides have a higher density than seawater (Andrady 2017), thus raising the issue as to why these particles were so prevalent in both the sea ice cores and surface waters of the Arctic Central Basin. Overall, however, these findings were corroborated by those of Obbard et al. (2014) who also utilised similar analytical techniques and reported that the most common

synthetic polymers (exclusive of rayon) in 4 ice cores retrieved from the Arctic Ocean were polyesters (21%) and polyamides (16%). By contrast, although Peeken et al. (2018) did report polyester and polyamide presence in 5 sea ice cores from the Arctic Ocean, the most dominant synthetic polymer in the examined cores was polyethylene (48%) with the difference in findings possibly occurring due to the exclusion of fibres. In the present study, the majority of microplastics in sea ice cores (79%) and surface waters (89%) were fibrous. Andrady (2017) reported that fibres in the marine environment may be originating from fishing gear, textiles due to laundering fabric and cigarette filters. Polyesters and polyamides are two of the most important man-made fibres in the world (Oerlikon 2010). The input of textile fibres into the marine environment can occur following the discharge of wastewater from domestic washings (Browne et al. 2011; Hernandez et al. 2017). Some laboratory experiments have estimated that (i) a single polyester (PET) fleece can release 110,000 fibres per wash, (ii) washing a 6 kg load of polyester fabric can release approximately 496,030 fibres and, (iii) washing a 5 kg load of polyester fabrics can release up to 6 million fibres (Napper and Thompson 2016; Almroth et al. 2018; De Falco et al. 2018). For the Arctic Ocean, it is difficult to pinpoint the exact origin of fibrous microplastics. Although there have been no published studies on this issue, the discharge of grey water from vessels operating in Arctic waters can be a potential source of fibrous microplastics in this remote oceanic basin.

Since sea ice is neither a permanent nor stationary structural feature of this ecosystem, it is likely that any risk posed by microplastics in sea ice has both a spatial and temporal component associated with it. From a spatial perspective, regions which could be most at risk from an influx of contaminants (e.g.

microplastics) from melting sea ice include (i) nearshore areas over the marginal shelves, (ii) the central Arctic basin and, (iii) the marginal ice zone e.g. Fram Strait, Barents Sea (Pfirman et al. 1995; Rigor and Colony 1997; Pavlov et al. 2004; Newton et al. 2017). From a temporal perspective, seasonal environmental conditions influence sea ice formation and melting in the Arctic Ocean and thus by extension can influence microplastic entrainment or release from sea ice. During periods of freezing (autumn and winter), it is assumed that if microplastics are present in surface waters, particle entrainment can occur in newly forming sea ice. However, during spring/summer when melting occurs, microplastics that are entrained in sea ice can possibly be released to surface waters underlying the ice floes. Of note is the fact that sea ice melting in the Arctic Ocean usually coincides with bursts of biological activity, i.e. ice algal blooms in spring and phytoplankton blooms in summer. Therefore, any potential risks associated with microplastic release from sea ice may also have a temporal component. Sea ice in the Arctic Ocean functions as a key habitat for numerous species of marine organisms (Søreide et al. 2010; Hardge et al. 2017; Bluhm et al. 2010). Presently, no information exists regarding the location of entrainment of microplastics within sea ice i.e. whether this is occurring within the ice crystal structure or brine channels. Therefore, it is uncertain whether microplastics may pose a threat to sea ice meiofauna or in-ice fauna which inhabit specific environmental compartments within sea ice. Marine organisms which live in close association with sea ice, specifically under-ice or sea ice macrofauna and sub-ice fauna, are likely to interact with microplastics once these particles are released into the underlying water column from melting ice. In the Arctic Ocean, the dominant under-ice fauna are the gammarid amphipods while sub-ice fauna include various species of copepods and fish such as the polar cod (*Boreogadus saida*) and

Arctic cod (*Arctogadus glacialis*), (Bluhm et al. 2010). Recently, Kuhn et al. (2018) reported microplastic fragments in the stomachs of polar cod (*Boreogadus saida*) sampled from waters in the Eurasian Basin of the Arctic Ocean and north of Svalbard. Morgana et al. (2018) also reported microplastics in the digestive tracts of polar cod (*Boreogadus saida*) sampled in Arctic waters east of Greenland. Based on the fact that interactions can potentially occur between marine organisms and microplastics in the Arctic Ocean, there is an urgent need for laboratory experiments to elucidate the impact of those interactions with polar organisms.

The present study was able to demonstrate for the first time that microplastics are indeed present in surface waters underlying ice floes in the Arctic Central Basin (ACB) with microplastic abundances ranging between 0 – 18 particles m^{-3}. Outside the central Arctic, previous studies reported that microplastic abundance in surface Arctic waters south and southwest of Svalbard ranged between 0 – 1.3 particles m^{-3} (Lusher et al. 2015) and east of Greenland it ranged between 0.2 – 2.6 particles m^{-3} in 2005 and 0.8 – 4.5 particles m^{-3} in 2014 (Amélineau et al. 2016). Microplastics were also recorded in surface Arctic waters following a circumpolar expedition of the Arctic Ocean but the grouping of macro- and microplastics in that study resulted in the reporting of overall plastic concentration (Cózar et al. 2017). Being cognizant of the fact that there were several differences in the sampling techniques (e.g. use of nets, mesh sizes of nets, etc.) between Lusher et al. (2015), Amélineau et al. (2016) and the present study, it must be noted that the present study reported higher microplastic abundances in surface waters beneath the ice floes than were reported in surface Arctic waters that were ice-free. At this point, it is uncertain whether this difference in microplastic

abundance is due to sampling method or whether the sea ice is acting as a secondary source of microplastics to the underlying water column. Regardless, microplastic presence in surface waters of the ACB provides evidence of the availability of these particles for entrainment in newly forming sea ice, whether that is first year ice forming over the central basin or vertical ice growth on the underside of the floes.

In order to facilitate comparisons between microplastic studies, it is important that investigators report their findings using comparable units. Of the two studies (Obbard et al. 2014; Peeken et al. 2018) that reported on microplastic concentrations in sea ice in the Arctic Ocean, both reported in units of particles m^{-3}. While the use of such units facilitates comparison with microplastic abundances in surface/near-surface waters, the issue arises as to whether such units are relevant for sea ice given the low volumes of meltwater that were analysed per ice core. In the present study, for example, approximately 1200 L of surface waters were filtered for microplastics at an individual site. Reporting microplastic concentrations in surface waters of the ACB in units of particles m^{-3} was therefore relevant. By comparison, meltwater volumes from ice cores in the present study were considerably lower as they ranged between 3 – 12 L. It is therefore more relevant to report microplastic concentrations in the ice cores as particles L^{-1} due to the low meltwater volumes on which these concentrations are based. In the present study microplastic concentration in sea ice cores were found to range between 2 – 17 particles L^{-1}. When these values are extrapolated to particles m^{-3}, it is estimated that microplastic concentrations in sea ice in the Arctic Ocean ranges between 2000 – 17,000 particles m^{-3}. When investigators extrapolate microplastic concentrations from < 20 L of meltwater from a single ice

core and estimate microplastic concentrations in 1,000 L or 1 m^{-3} of meltwater, such estimations are based on limited datasets and thus can be overestimations. Obbard et al. (2014) reported that meltwater volumes from isolated sub-sections of 4 ice cores were between 50 – 100 mL and yet these low meltwater volumes were extrapolated and microplastic concentrations in sea ice cores were reported in particles m^{-3}. Peeken et al. (2018) similarly analysed 5 ice cores and used this limited dataset to extrapolate and report microplastic concentrations in sea ice in particles m^{-3}. Based on the low meltwater volumes that are expected from individual ice cores, it is suggested that microplastic concentration in sea ice is reported as particles L^{-1}. In cases where extrapolations are made to facilitate comparisons with surface/near-surface waters, investigators should clearly indicate the number of particles that such estimations were based on such that the uncertainties associated with such extrapolations are clear.

Comparative assessments of reported microplastic concentrations in sea ice cores from the Arctic Ocean is not a straightforward issue primarily due to methodological differences that exist between studies. In the present study, for example, microplastic concentrations were lower than those reported by Obbard et al. (2014) and Peeken et al. (2018), (Table 5.2). Interpreting these findings in the context of the methods used (e.g. number of cores sampled, portion of core analysed, techniques used to identify polymer type, etc) shows that there are numerous factors which could have contributed to the reported differences (Table 5.2). For example, Obbard et al. (2014) estimated microplastic concentrations in sea ice based on the analysis of isolated sub-sections (50 – 100 cm^3) of 4 ice cores. By contrast, estimated microplastic concentrations in the present study

were based on analysis of the entire sea ice core with meltwater volumes ranging from 3 – 12 L.

Table 5.2: Details of the methods used and reported findings from studies on microplastics in sea ice from the Arctic Ocean

Study	**Microplastic concentration (particles m^{-3})**	**Synthetic polymers**	**Cores analysed**	**Entire core analysed**	**Method employed for polymer ID**
Obbard et al. (2014)	$1.3 – 9.6 \times 10^4$	6	4	No	Visual ID, FT-IR spectrometry
Peeken et al. (2018)	$(1.1 \times 10^6) – (1.2 \times 10^7)$	17	5	Yes	Imaging FT-IR spectrometry
Present study	$(2.0 \times 10^3) – (1.7 \times 10^4)$	9	25	Yes	Visual ID, FT-IR spectrometry

Since the present study highlighted that the vertical distribution of microplastics within sea ice cores was not uniform, it is quite likely that extrapolations based on isolated sea ice sub-sections may either underestimate or overestimate microplastic concentrations. Furthermore, analytical techniques employed in the present study led to the exclusion of particles <100 µm from analysis and thus may have resulted in an underestimation of microplastic abundance in sea ice cores. This is particularly relevant due to the fact that the majority of microplastics

in the 5 sea ice cores analysed by Peeken et al. (2018) were <50 µm. It is therefore evident that the inclusion of smaller size fractions will lead to the reporting of higher microplastic concentrations. Of note is the fact that although Peeken et al. (2018) reported the highest microplastic concentrations in sea ice, fibres were excluded from the analysis. The present study, however, reported that there was a pre-dominance of fibrous microplastics (79%) in sea ice from the Arctic Ocean. It is therefore very likely that if either fibrous particles or particles < 100 µm are excluded from analysis, microplastic concentrations in sea ice will be underestimated.

5.6. Conclusion

The present study demonstrated that microplastics were present in surface waters underlying ice floes (0 – 18 particles m^{-3}) in the Arctic Central Basin (ACB) and that these particles were also present in the overlying sea ice floes at concentrations that were several orders of magnitude higher (2×10^3) to (1.7×10^4) particles m^{-3} (latter statement substantiated by 3 independent studies). In both environmental compartments, there was an overall predominance of fibrous polyesters and polyamides. Backward drift trajectories estimated that the sampled cores possibly originated from (i) Siberian shelves in the Eurasian Basin, (ii) western Arctic and, (iii) the central Arctic. Of note was the fact that all drift trajectories indicated that the sampled sea ice drifted through the central Arctic. Although definitive statements cannot be made about the origin of microplastics in surface waters or sea ice of the ACB, potential sources of these contaminants may include (i) riverine discharge from the Siberian and Canadian rivers, (ii) influx of contaminated Pacific and Atlantic waters, (iii) grey water discharge from vessels operating in the Arctic and, (iv) atmospheric deposition. Given the

dynamics of sea ice formation and transport, it is quite likely that microplastic entrainment, transport and release from sea ice has a spatial and temporal component associated with it. From a temporal perspective, periods of sea ice melting (spring/summer) coincide with bursts of biological activity (ice algal blooms in spring and phytoplankton blooms in summer), thus increasing the possibility of interactions between microplastics released from sea ice and marine organisms. From a spatial perspective, the regions in the Arctic Ocean that are most likely at risk from microplastic release from sea ice are those where melting occurs and thus include (i) nearshore areas, (ii) central Arctic and, (iii) marginal ice zone. Under-ice fauna, e.g. gammarid amphipods, and other organisms which have a close association with sea ice, e.g. polar and Arctic cod, are likely to be the ones which have the greatest interactions with microplastics in surface waters underlying ice floes and those released from sea ice. Knowledge about microplastics in the various environmental compartments of the Arctic Ocean is a fundamentally important step in assessing the potential threats that these particles pose to polar organisms.

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Chapter 6

Discussion

6.1. Overview

The main goal of the research presented in this dissertation was to address some key knowledge gaps regarding microplastics in the Atlantic and Arctic Oceans. Since the core chapters of this dissertation (Chapters 2, 3, 4, 5) already presented individual components of the research in detail, the primary objective of this final chapter is to contextualize the findings of this study and highlight prospects for future research.

6.2. Atlantic Ocean

Eastern Boundary Upwelling Ecosystems (EBUEs) have amongst the highest primary production rates in the world which in turn sustain large commercially important fisheries (Pauly and Christensen 1995). Within the Atlantic Ocean, upwelling ecosystems off the west coast of the African continent i.e. the Canary (CUE) and Benguela Upwelling Ecosystem (BUE), are regions of high primary production that support commercially important sardine, mackerel and anchovy fisheries (Schwartzlose et al. 1999; Crawford et al. 2007; Aŕistegui et al. 2009). Microplastics in oceanic waters of upwelling ecosystems are capable of interacting with organisms that inhabit or depend upon these waters. Furthermore, oceanic processes such as coastal upwellings may exert an influence on microplastic abundance, distribution and composition. Previous studies have postulated that the upwelling process provides deep water, that has relatively low levels of microplastics, to the surface and that such a process can lead to the dilution of plastic concentrations in surface waters (Desforges et al. 2014; de Lucia et al. 2014).

Prior to the research presented in Chapter 2 (Kanhai et al. 2017), there were no published studies that reported on microplastics in either of the coastal upwelling ecosystems in the Atlantic Ocean. Kanhai et al. (2017) was able to (i) provide evidence for the existence of microplastics in sub-surface waters of the Benguela Upwelling Ecosystem (BUE) with information provided on microplastic abundance, distribution and composition and, (ii) demonstrate based on sampling sub-surface waters at a single depth (11 m) that there were no statistically significant differences between microplastic abundance in 'upwelled areas' i.e. Benguela sampling sites and 'non-upwelled' areas. It is unlikely that sampling sub-surface waters at a single depth using the underway system of a vessel will unravel the dynamics of microplastic abundance, distribution and composition at upwelling ecosystems in the Atlantic Ocean. While opportunistic sampling does play an important role in providing baseline data about microplastics in the marine environment, such sampling opportunities are often associated with limitations (e.g. vessel track, sampling frequency and site, etc) which are beyond the control of the investigator and may impose restrictions on a research project.

Given the inherent value of upwelling ecosystems in the Atlantic Ocean, it is proposed that further research should be carried out regarding microplastics in these regions. Future microplastic sampling efforts should be focused on understanding the sources, transport pathways and fate of microplastics in such systems. Such research efforts should specifically attempt to (i) assess the vertical distribution of microplastics within the water column, (ii) assess whether microplastics are present in commercially important fish species that depend upon such ecosystems and, (iii) identify potential sources of microplastics in these ecosystems. Since some of regions of the CUE are either seasonal or

permanent, microplastic sampling programs ought to incorporate a seasonal factor in their sampling program as this may give some insight into the potential impact of upwellings on microplastic abundance, distribution and composition. Apart from using the underway system of the vessel at a single depth to sample sub-surface waters, it is suggested that future research projects use a CTD rosette sampler as was done by Bagaev et al. (2017) and Kanhai et al. (2018) in order to assess the vertical distribution of microplastics in the water column. The perceived benefits of this are twofold in that investigators may be able to determine whether an upwelling event is occurring based on the collection of physicochemical data and it may facilitate a greater understanding of the influence of the upwelling process on microplastic abundance. Investigating the potential impact of microplastics on organisms, in particular fish, that depend upon upwelling ecosystems is also important given that large commercial fisheries are associated with upwelling ecosystems. Therefore, in addition to finding out whether the fish associated with upwelling ecosystems are ingesting microplastics, it is important that laboratory exposure experiments should also be carried out to elucidate the potential impact of environmentally relevant concentrations of microplastics on commercially important species e.g. sardine (*Sardinops sagax*), horse mackerel (*Trachurus trachurus*), Atlantic chub mackerel (*Scomba colias*), anchovy (*Engraulis encrasicolus*), (Schwartzlose et al. 1999; Crawford et al. 2007; Ańistegui et al. 2009).

6.3. Arctic Ocean

Polar regions, such as the Arctic Ocean, are amongst those where there is an evident paucity of information regarding marine plastic debris (Gall and Thomspson 2015). It has been postulated that plastic debris can potentially enter

the remote Arctic Ocean as a result of (i) oceanic currents, (ii) biotransport, (iii) riverine input from Siberian and Canadian rivers, (iv) atmospheric deposition and, (v) local anthropogenic activities such as shipping (Mallory 2008; Zarfl and Matthies 2010; Bergman and Klages 2012; Provencher et al. 2012; Obbard et al. 2014; Trevail et al. 2015; Cózar et al. 2017; Tekman et al. 2017). Yet, it was only within the past 5 years that published studies starting emerging on microplastics in the various environmental compartments of the Arctic Ocean. Apart from the research presented in this dissertation, microplastics have been reported in (i) Arctic waters south and southwest of Svalbard (Lusher et al. 2015) and east of Greenland (Amélineau et al. 2016; Morgana et al. 2018), (ii) sediments from the Fram Strait (Bergmann et 2017), (iii) biota such as Little Auks (*Alle Alle*), (Amélineau et al. 2016) and polar cod (*Boreogadus saida*) and, (iv) sea ice (Obbard et al. 2014; Peeken et al. 2018). Of note is the fact that of these studies only three (Obbard et al. 2014; Kuhn et al. 2018; Peeken et al. 2018) reported on microplastics in the central Arctic Ocean. The research presented in Chapters 3, 4 and 5 of this dissertation sought to fill that knowledge gap by assessing microplastics in the various environmental compartments (surface and sub-surface waters, sediments, sea ice) of the Arctic Central Basin (ACB).

One of the key contributions of the present thesis is the provision of data about microplastics in the water column of the ACB. Based on two independent sampling methods, microplastic presence was reported in sub-surface waters of the ACB (Chapter 2, Kanhai et al. 2018). Bow water sampling at a single depth (8.5 m) indicated that microplastic abundance in the Polar Mixed Layer ranged between 0 – 8 particles m^{-3} while CTD rosette sampling at multiple depths (8 – 4400 m) indicated that microplastic abundance ranged between 0 – 375 particles

m^{-3} (Kanhai et al. 2018). For the first time within this oceanic basin, information was presented about the vertical distribution of microplastics in the water column. To our knowledge, the only other published study to have reported on the vertical distribution of microplastics in the water column was conducted in the Baltic Sea (Bagaev et al. 2007). Apart from sub-surface waters, for the first time microplastics were recorded in surface waters underlying ice floes in the ACB with microplastic abundance ranging between 0 – 18 particles m^{-3} (Chapter 4). Overall, these findings indicated that microplastics were pervasive in the water column and furthermore suggested that vertical transport of microplastics out of surface waters was occurring within the ACB. Another contribution of the present thesis was the presentation of data which highlighted that microplastics were making their way to the deep-sea realm of the Arctic Central Basin (ACB). Firstly, the discovery of microplastics in deep/bottom waters, i.e. at depths between 1,000 – 4,000 m, of the ACB was reported in Chapter 3 (Kanhai et al. 2018). Following that, Chapter 4 presented some preliminary insight into the presence of microplastics in deep sea sediments of the ACB. While it is acknowledged that the sediment sampling presented in Chapter 4 was associated with numerous limitations, that component of the research provides proof of concept that there are mechanisms operating within the ACB that are possibly responsible for the vertical transport of microplastics through the water column and that the sediment phase of that oceanic basin is possibly functioning as a sink for microplastics. The final contribution of the present thesis was the provision of information regarding microplastics in sea ice from the Arctic Central Basin (ACB). Acknowledging the fact that the present study was not the first to do so, Chapter 5 of the present dissertation was able to provide a more spatially comprehensive overview of microplastics in sea ice cores as it was based on the analysis of 25

sea ice cores collected within the ACB. Microplastic concentrations in these cores ranged from 2 to 17 particles L^{-1}. By assessing the vertical distribution of microplastics within the sea ice cores, it was shown that these particles were pervasive within the sea ice sub-sections. Backward drift trajectories on the sampled sea ice cores also showed that the sampled ice cores had diverse origins that included the Siberian shelves of the Eurasian basin, western Arctic and the central Arctic Ocean. Figure 6.1 therefore provides a summary of the findings of the present dissertation as it relates to microplastics in the Arctic Central Basin (ACB). As highlighted on figure 6.1, various processes may be responsible for the transport of microplastics between the different environmental compartments of the ACB.

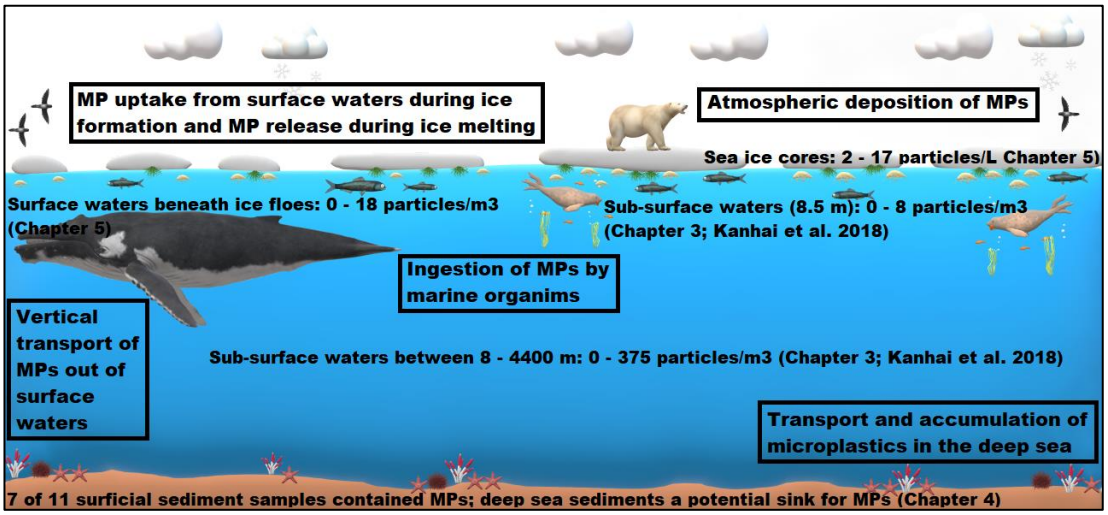

Figure 6.1: Findings of present dissertation pertaining to microplastics in the Arctic Central Basin [information in boxes indicate processes that may result in the transfer of microplastics between different environmental compartments]

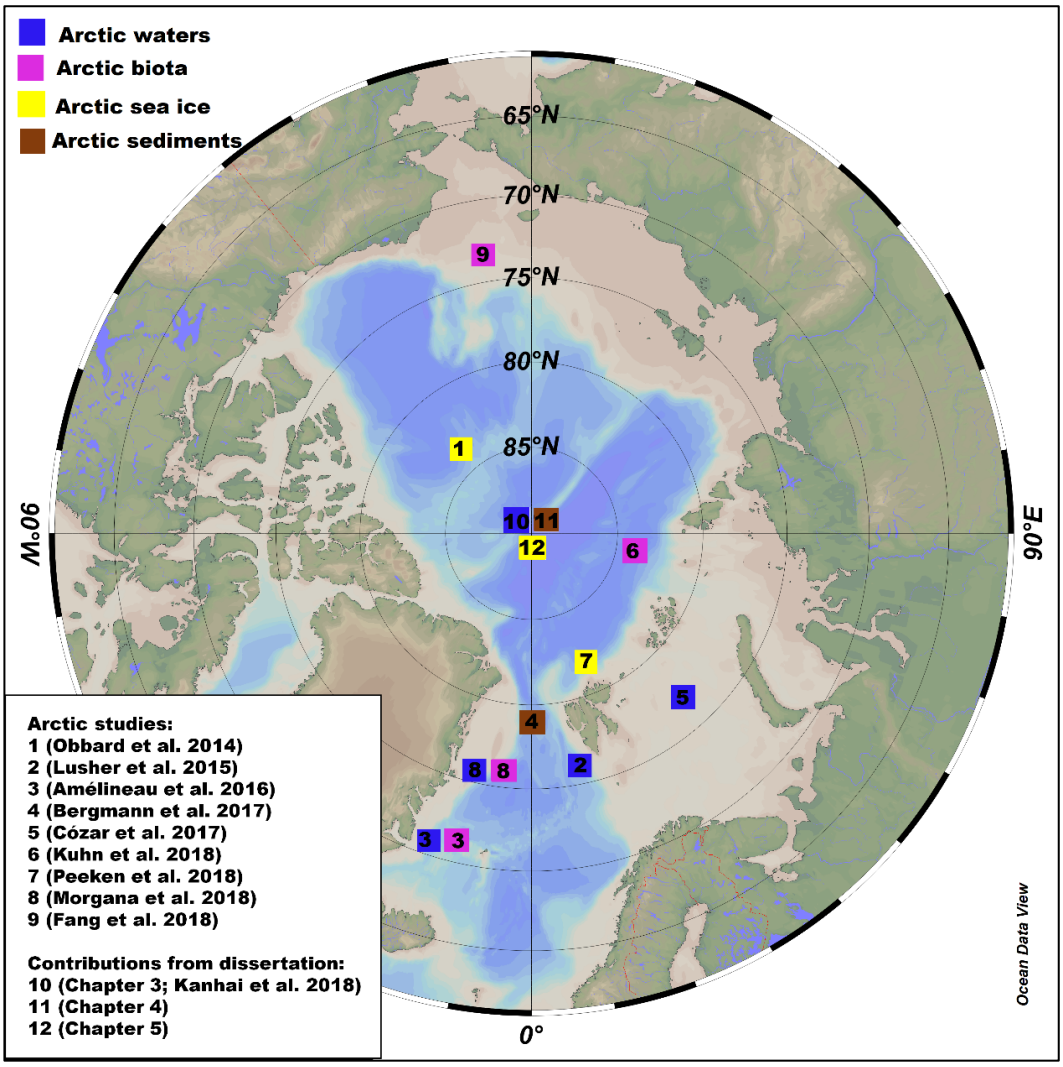

Figure 6.2: Overview of studies that reported on microplastics in Arctic Ocean

Collectively, the scientific community has made progress investigating microplastics in the various environmental compartments of the Arctic Ocean (Figure 6.2). To date, there is still much that remains unknown about microplastics in the Arctic Ocean and thus there is scope for future research. Specific areas which should be targeted include (i) determining the potential impacts that microplastics may have on polar organisms, (ii) assessing microplastic abundance, distribution and composition in sediments of the Arctic Central Basin, (iii) assessing whether atmospheric transport is an important route for microplastics in the Arctic Ocean, (iv) monitoring vessels that are operating in

the Arctic Ocean to determine whether their wastewater effluents are a potential source of microplastics, (v) identifying whether the main Siberian and Canadian rivers that are discharging into the Arctic Ocean are contributing to the microplastic load in the Arctic Ocean. Specific polar organisms that should be targeted for laboratory exposure experiments include gammarid amphipods, copepods and fish such as polar cod (*Boreogadus saida*) and Arctic cod (*Arctogadus glacialis*) due to the fact that they are amongst the most dominant under-ice and sub-ice fauna in the Arctic Ocean and thus are likely to interact with microplastics in the water column (Bluhm et al. 2010). Any future research that is focused on sampling surficial sediments in the ACB for microplastics should utilise sampling equipment that is capable of retrieving undisturbed sediment samples such as a multi-corer or box corer (Georgiopoulou 2018) and incorporate the collection of replicate sediment samples per site in their sampling plan. Research vessels operating within the Arctic Ocean may be a relevant platform for monitoring microplastics in the atmospheric environmental compartment of the Arctic Ocean.

6.4. Overarching issues relevant to microplastic studies

The present study utilized two independent methods to assess microplastic abundance in sub-surface waters in the Atlantic and Arctic Ocean. Seawater taken in via the underway system of vessels such as the RV Polarstern (depth of intake 11 m) and icebreaker Oden (depth of intake 8.5 m) was sampled for microplastics. The method utilized in both of these studies (Kanhai et al. 2017; Kanhai et al. 2018) followed the method of Lusher et al. (2014) where the filtration of approximately 2000 L of water constituted a single sample. In order to achieve comparability between these and other microplastic studies, microplastic

abundances were reported in particles m^{-3}. In this case, the use of such a reporting unit was relevant due to the large volume of water that was filtered per sample. The CTD rosette sampler was also used to sample sub-surface waters at multiple depths in the Arctic Ocean. While this method facilitated an assessment of the vertical distribution of microplastics at multiple depths in the Arctic Central Basin (ACB), lower seawater volumes (either 21 L or 48 L) were sampled at a single depth. In order to facilitate comparison with the findings from the bow water system of icebreaker Oden, Kanhai et al. (2018) extrapolated findings based on low seawater volumes (21 L or 48 L) from the CTD sampling and reported microplastic abundances in particles m^{-3}. The issue that arises is whether that extrapolation from <50 L of seawater to 1,000 L of seawater was relevant or whether such extrapolations can lead to an overestimation of microplastic abundances. One of the ways in which this issue may be addressed is by utilizing different equipment which will lead to either the collection of larger volumes of water at individual depths in the water column or by the development of new systems which facilitate the in-situ filtration of larger volumes of water at different depths in the water column. A similar issue arises when assessing microplastic concentrations in sea ice cores. Previous studies by Obbard et al. (2014) and Peeken et al. (2018) reported microplastic concentrations in particles m^{-3}. While Obbard et al. (2014) analysed between 50 – 100 mL of meltwater from isolated sub-sections of 4 individual ice cores, Peeken et al. (2018) analysed meltwater from 5 ice cores but did not indicate the meltwater volumes per core. In the present study, sea ice cores contained between 3 – 12 L of meltwater. Due to the fact that meltwater volumes were < 20 L, Chapter 5 of the present study reported microplastic concentrations as particles L^{-1}. Extrapolations of microplastic concentrations in sea ice cores to particles m^{-3} based on low

meltwater volumes (< 20 L per core) are likely to lead to an overestimation of microplastics in sea ice. Such extrapolations are also making assumptions about the homogeneity of microplastics within sea ice from individual ice floes in the Arctic Ocean. To date, however, there is no data about variability in microplastic concentrations in individual ice floes in the Arctic Ocean. Future studies which use low volumes of water samples, i.e. either seawater or meltwater from sea ice, should therefore consider reporting microplastic abundances/concentrations in particles L^{-1} and if extrapolating to particles m^{-3} should highlight the uncertainties that are associated with such extrapolations.

Apart from collecting information about the size and polymer composition of microplastics, investigators often record the colour of these particles. Although colour may be affected by weathering (Duis and Coors 2016), microplastics from environmental samples usually have very distinct colours. In the present study, there was a pre-dominance of blue microplastics in both the Atlantic and Arctic Oceans. Kanhai et al. 2017 reported that 72% of the microplastics found in sub-surface waters at a single depth of 11 m in the Atlantic Ocean were blue. In the Arctic Ocean, blue microplastics were also predominant in sub-surface waters at a single depth of 8.5 m in the Polar Mixed Layer (PML), (49%), as well as at various water depths in the ACB (46%), (Kanhai et al. 2018). Blue microplastics were also predominant in sea ice (53%) as well as in surface waters underlying ice floes (58%) in the ACB (Chapter 5). In the Arctic Ocean, this predominance of blue and dark coloured microplastics was also reported in (i) waters south and south-west of Svalbard (black- 45%, blue- 29%), (Lusher et al. 2015), (ii) surface waters east of Greenland (blue- 83% in 2005 and 53% in 2014) and in the gular pouches of Little Auks (*Alle alle*), (19 – 25 %), (Amélineau et al. 2016), (iii) surface

waters east of Greenland (blue- 29%) and in Bigeye Sculpin (*Triglops nybelini*), (blue- 58%) and Polar Cod (*Boreogadus saida*), (35%), (Morgana et al. 2018) and, (iv) sea ice from the Arctic Ocean (blue, black, green, red-most common), (Obbard et al. 2014). It is uncertain why there is this predominance of blue and darker coloured microplastics in the various environmental compartments of the Arctic Ocean. Shaw and Daly (1994) suggest that this could be as a result of a human bias during sample processing in that there are difficulties associated with the identification of lighter coloured microplastics. Another suggestion is that there is a selective removal of lighter coloured particles in surface waters by biota (Shaw and Daly 1994). Many marine organisms such as fish and squid are visual predators and rely on colour for prey selection and it was therefore suggested that these organisms may be selectively ingesting microplastics that resemble their prey (Shaw and Daly 1994; Wright et al. 2013). In the North Pacific Central Gyre (NPCG), the most prevalent microplastics (white, clear and blue) detected in planktivorous fish were similar to the prey (plankton) of the fish which suggests that there could have been a case of mistaken identity of microplastics for food (Boerger et al. 2010). In the South Pacific Subtropical Gyre (SPSG), Amberstripe Scad (*Decapterus muroadsi*) were reported to have ingested blue microplastics similar to blue copepods which were their natural food source (Ory et al. 2017). Although uncertainty exists regarding the reasons for the predominance of darker coloured particles, especially blue, in the various environmental compartments of the Arctic Ocean, information on particle colour ought to be collected during microplastic investigations.

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Appendices

Appendix I

Investigation of microplastics at coastal upwelling sites in the Atlantic Ocean

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Microplastics are an emerging contaminant

Microplastics are one category of marine debris which may potentially pose threats to marine biota. While previous studies have reported on microplastics in various environmental phases of the marine environment, there has been a recent call for the investigation of microplastics in biota-rich waters[1].

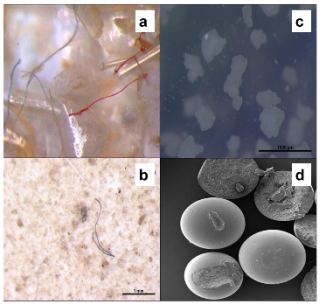

Figure 1: Examples of microplastics (a, b-fibres found in environmental samples; c,d[2]-fragments and microbeads from cosmetics)

Coastal upwelling sites are biota-rich

Within the world's oceans, areas which experience coastal upwelling are associated with high primary productivity which in turn supports key biological resources. In the Atlantic Ocean, coastal upwelling is responsible for high biological productivity in the (i) Canary Upwelling Ecosystem (CUE), and (ii) Benguela Upwelling Ecosystem (BUE), (Fig. 2).

Objective of this study:

To investigate microplastic abundance, distribution and composition in areas of coastal upwelling in the Atlantic Ocean.

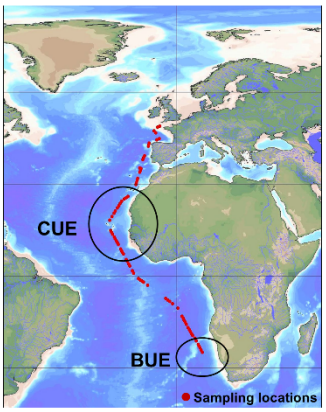

Figure 2: Map showing sampling locations in the Atlantic Ocean

Sub-surface oceanic waters sampled

Based on an opportunistic voyage aboard the RV Polarstern, microplastics were sampled from sub-surface waters in November 2015 along the North/South Atlantic Transect i.e. between Bremerhaven, Germany and Cape Town, South Africa (Fig. 2). Sampling was based on the method shown in Fig. 3 (Lusher et al. 2014[3]). Each sample constituted the filtration of 2000 L of water with a total survey effort of 152 000 L (76 samples).

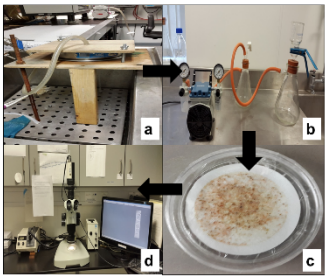

Figure 3: Flow diagram of method used for microplastic sampling of sub-surface waters

Work in Progress

1. Identification of microplastics:
   - Visual identification
   - Confirmation of microplastic type by Fourier Transform Infrared Spectroscopy

2. Statistical Analyses
   - Differences between upwelled and non-upwelled areas
   - Influence of environmental factors on microplastics

Overall Significance

1. Insight into the potential effect of oceanographic phenomena such as coastal upwelling on microplastics in the world's oceans.
2. Field data can be used to inform laboratory experiments that investigate the impact of microplastics on marine biota.

Acknowledgments

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Appendix II

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Microplastic abundance, distribution and composition along a latitudinal gradient in the Atlantic Ocean

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ABSTRACT

Microplastics in the world's oceans are a global concern due to the potential threat they pose to marine organisms. This study investigated microplastic abundance, distribution and composition in the Atlantic Ocean on a transect from the Bay of Biscay to Cape Town, South Africa. Microplastics were sampled from sub-surface waters using the underway system of the RV *Polarstern*. Potential microplastics were isolated from samples and FT-IR spectroscopy was used to identify polymer types. Of the particles analysed, 63% were rayon and 37% were synthetic polymers. The majority of microplastics were identified as polyesters (49%) and blends of polyamide or acrylic/polyester (43%). Overall, fibres (94%) were predominant. Average microplastic abundance in the Atlantic Ocean was 1.15 ± 1.45 particles m^{-3}. Of the 76 samples, 14 were from the Benguela upwelling and there was no statistically significant difference in microplastic abundance between upwelled and non-upwelled sites.

1. Introduction

Within the past decade, microplastics in the world's oceans have emerged as an issue of global importance (UNEP, 2011). Concern regarding these particles stems from their ubiquity, persistence and the potential threat they pose to marine organisms. The gravity of the situation is compounded by the fact that even if the introduction of plastic debris to the marine environment were to be halted, microplastic abundances are projected to increase as a result of the fragmentation of plastics that are already in the world's oceans (Thompson, 2015).

Global concern about microplastics, i.e. plastic particles < 5 mm in diameter (Arthur et al., 2009), has prompted numerous investigations regarding this type of marine debris. Microplastics have been discovered in oceanic waters, deep sea sediments, sea ice and marine organisms (Lusher, 2015). Studies that investigated microplastics in surface and sub-surface waters of the world's oceans found that microplastic abundance was highest in the convergence zones of the five sub-tropical gyres which are regarded as biological deserts due to their low levels of marine biodiversity (Cozar et al., 2014; Polovina et al., 2008).

Even though information exists regarding microplastics in the world's oceans, a greater understanding of microplastic abundances in biota rich waters is particularly important due to the enhanced possibilities for interactions between microplastics and organisms (Cole et al., 2015). Areas which experience coastal upwelling sustain high primary productivity and it is this enhanced productivity which supports more complex food webs comprising biota from a range of trophic levels. Coastal upwelling in the Atlantic Ocean occurs primarily at the (i) Canary Upwelling Ecosystem (CUE) which is comprised of three zones (12–19°N, 21–26°N, 26–35°N) and, (ii) Benguela Upwelling Ecosystem (BUE) which stretches from the southern tip of Africa to approximately 15°S where it is bounded by the Angola front (Santos et al., 2012; Cropper et al., 2014).

Effectively addressing the issue of microplastics in the marine environment requires information on the abundance, distribution and composition of microplastics in the world's oceans. Information from the natural environment is particularly important as it (i) provides an indication of the extent of the problem and, (ii) informs laboratory studies by providing data on the environmentally relevant concentrations of microplastics that biota are exposed to in the natural environment. More specifically, information about microplastics at coastal upwelling sites in the Atlantic Ocean is particularly important as it could provide (i) an indication of the probability of encounter between organisms and microplastics at such sites and, (ii) insight into the potential effect of oceanographic phenomena such as upwelling on microplastics in the world's oceans. The present study investigated microplastic abundance, distribution and composition along a latitudinal gradient in the Atlantic Ocean. The specific aim was to determine whether microplastic abundance in upwelled areas were significantly different from non-upwelled areas.

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0025-326X/

2. Materials and method

2.1. Sample collection

This study was conducted onboard the RV *Polarstern* during Expedition PS95 and covered 7345 nautical miles (13,603 km) between Bremerhaven, Germany and Cape Town, South Africa. Sub-surface oceanic waters pumped onboard the vessel via the underway system were sampled for microplastics using the method described by Lusher et al. (2014). Sampling was conducted during November 2015 (1st to 28th) at vessel speeds of between 8 and 13 knots. Since each sample constituted the filtration of 2000 L of water (Lusher et al., 2014), the survey effort for this study was 152,000 L of water (76 samples).

Seawater from a continuous intake located at the keel of the ship (depth 11 m) was pumped onboard the vessel using a Klaus Union Sealex Centrifugal Pump (Bochum, Germany) at a flow rate of 25 m^3/h and transported to the laboratory via stainless steel pipes. Prior to reaching the laboratory, the seawater passed through a primary filter (pore size 2 mm) to remove large debris items. The inclusion of this primary filter was standard operating procedure onboard the vessel and thus was beyond the control of the investigator. Potential contamination of the seawater intake by waste water generated onboard did not occur since grey water from the vessel was stored onboard for subsequent treatment. In the laboratory, seawater from the vessel's underway system was allowed to flow through a covered stainless steel sieve (250 μm) by means of a connection hose fitted into a wooden sieve cover. For the duration of the sampling, the stainless steel sieve was supported in a wooden stand. For each sample, 2000 L of water was filtered. The length of time taken for the filtration of the specified volume of water was determined by calculation of the flow rate of the seawater. Once the specified volume of water was filtered, the sieve was removed and distilled water used to wash retained material from the sieve into a clean container. The collected material was then filtered under vacuum onto glass microfiber paper (GF/C); Whatman: 47 mm, pore size: 1.2 μm, using a Buchner funnel and a vacuum flask (Lusher et al., 2014). Each filter paper was then placed into a clean petri dish, covered and stored in a freezer (−20 °C) until returned to the laboratory. At the start and at the end of each sample, positioning data were collected. Data for various environmental variables were obtained from the vessel's (i) thermosalinometer-keel (water temperature, salinity, conductivity), (ii) ferrybox (chlorophyll *a* and pH), and (iii) weather station (wind speed, wind direction).

2.2. Method validation and contamination prevention

Method blanks and controls were used to determine whether there was any contamination during sample processing. Clean petri dishes and filter paper were left exposed to the air during vacuum filtration to determine if there was any airborne contamination. To determine whether there was any additional contamination during vacuum filtering, distilled water was passed through clean GF/C filter paper under vacuum. During visual identification of potential microplastics in samples, checks were also made for airborne contamination by exposing a clean petri dish and filter paper to the air. In order to prevent contamination in the laboratory, the following measures were taken (i) lab coats, cotton clothing and gloves were worn during sample processing, (ii) a wooden cover was placed over the stainless steel sieve to prevent airborne contamination, and (iii) all containers used during sample processing were covered and cleaned using distilled water before reuse (Lusher et al., 2014).

2.3. Laboratory analyses

Samples were removed from the freezer and left to dry. Individual filter papers were then visually examined under a dissecting microscope (Olympus SZX10) equipped with a polariser and camera (Q Imaging Retiga 2000R). Potential microplastics were identified based on characteristic features such as (i) colour- homogenous colour, shininess, unnatural colours, (ii) thickness-fibres homogenous in thickness and, (iii) bending-fibres demonstrated three dimensional bending. Potential microplastics from each sample were photographed and length measurements were taken prior to transferring to a clean filter paper. Filter papers with potential microplastics from each sample were stored in clean, labelled petri dishes. Potential microplastics were assigned to two broad categories (fibres, fragments) and to five length categories: 0.25–0.5 mm, 0.5–0.75 mm, 0.75–1.0 mm, 1.0–2.0 mm, 2.0–5.0 mm.

All potential microplastics as well as a subset of particles not considered to be microplastics ($n = 499$) were analysed by Fourier transform infrared (FT-IR) spectroscopy on a Bruker Vertex 70 Infrared Spectrometer coupled to a Hyperion 1000 microscope. The instrument was equipped with a potassium bromide (KBr) beamsplitter and an internal mercury cadmium telluride (MCT) detector. Microscope-transmission sampling was performed using a Specac DC-2 Diamond Compression cell. Spectra were recorded as the average of 32 scans in the spectral wave number range of 4000–600 cm^{-1} at a resolution of 4 cm^{-1} (Blackman-Harris 3-term apodisation). Bruker's Opus 7.5 spectroscopy software was used for processing and evaluating all spectra. Prior to analysing each sample, background scans were performed and sample spectra were automatically corrected. Each sample spectrum was compared with those of known standard polymers in the (i) Bruker Optics Attenuated Total Reflectance (ATR) Polymer and (ii) Synthetic Fibres ATR libraries. An initial hit quality with a score ranging between 0 and 1000 was produced for each match between sample and reference spectra, with the highest score representing the closest match. Following this preliminary matching, the top ten matches for each sample spectrum were then further evaluated using the Quick Identity Test/Euclidean Distance (ED) option. A hit quality ranging between 0 and 2 was produced for each match between the sample spectrum and the reference spectra, with the lowest number representing the closest match. Overall, matches with >70% similarity were accepted while those with 60–70% similarity were individually examined to ensure that there was clear evidence of peaks from the sample corresponding to known peaks of standard polymers. Samples which produced spectra with a match <60% were automatically rejected.

2.4. Statistical analyses

All statistical analyses were performed using R version 3.2.3 (R Core Team, 2015). Descriptive statistics, histograms and box plots were generated and tests of normality (Supplementary Table 1) were conducted on all data sets to determine whether parametric or non-parametric statistical analyses were appropriate. Univariate (Kruskal Wallis test) and multivariate (Principal Component Analysis) analyses were conducted to determine whether sampling occurred in the Benguela and Canary Upwelling Ecosystems. Correlation analyses were performed to determine whether there were any correlations between individual environmental variables and microplastic abundance. A generalized additive model (GAM) was also developed to determine which environmental variables had an effect on microplastic abundance.

3. Results

3.1. Quality control

Microplastics were not found in the (i) air contamination controls set up during sample collection ($n = 4$), (ii) method blanks set up during vacuum filtration of distilled water ($n = 8$), and (iii) air contamination controls set up during visual identification ($n = 76$). This indicates that microplastics were not introduced into the samples either as a result of airborne contamination or as a result of contamination during the vacuum filtration process. Airborne contamination by microplastics

during the filtration of each sample was prevented by the use of a wooden cover over the stainless steel sieve.

3.2. Confirmation of sampling in upwelling ecosystems

Kruskall Wallis tests indicated that there were statistically significant differences in both water temperature (Kruskal-Wallis chi-squared = 16.599, df = 2, p-value = 0.0002) and chlorophyll a concentrations (Kruskal-Wallis chi-squared = 28.086, df = 2, p-value = 7.967e−07) amongst non-upwelled sites, Canary upwelling sites and Benguela upwelling sites. Post hoc tests indicated that there were statistically significant differences in water temperature and chlorophyll a concentrations between (i) non-upwelled sites and Benguela upwelling sites (water temperature: Nemenyi test-p value = 0.0026, Dunn's test-p value = 0.0011; chlorophyll: Nemenyi test-p value = 0.0003, Dunn's test-p value = 4.1e−07) and, (ii) between Canary upwelling sites and Benguela upwelling sites (water temperature: Nemenyi test-p value = 0.0005, Dunn's test-p value = 0.0003; chlorophyll: Nemenyi test-p value = 0.0005, Dunn's test-p value = 9.3e−07). The fact that the Benguela upwelling sites exhibited water temperatures that were significantly lower than those of all other sites and chlorophyll a concentrations that were significantly higher than those of all other sites suggests that sampling in this study occurred within the Benguela upwelling ecosystem. However, the same cannot be said for the Canary upwelling ecosystem.

Multivariate analyses were also utilised to confirm whether sampling occurred within upwelling ecosystems in the Atlantic Ocean. PCA conducted on available data (n = 76) revealed that principal components 1 (PC1), 2 (PC2) and 3 (PC3) accounted for 84.48% of the variation. Eigenvectors indicated that PC1 was governed by increasing temperature (0.601), decreasing chlorophyll (−0.595) and decreasing wind speed (−0.519), PC2 was governed by decreasing salinity (−0.719) and PC3 was governed by increasing pH (0.803). The biplot (Fig. 1) revealed that while the majority of sites were located towards the middle of the plot, there were a few distinct groups of sites. Of importance is the group of sites located in the upper left quadrant of the biplot characterised by low water temperatures, high chlorophyll a concentrations, high wind speeds and low salinities. Since the majority of these sites were located within the region where the Benguela upwelling was expected to occur (i.e. from the southern tip of Africa to 15°S) and certain features (low water temperatures, high chlorophyll concentrations) could be attributed to the phenomenon of upwelling, these sites were henceforth referred to as 'upwelling sites'.

3.3. Overview of findings

Of the 499 particles analysed by FT-IR spectroscopy, 37% were confirmed as synthetic polymers (n = 183) and 63% as Rayon (n = 316). The majority (96%) of synthetic polymers were <5 mm in length (Fig. 2) and thus were considered as microplastics (n = 175), with only a small percentage (4%) of synthetic polymers >5 mm in length; all fragments were <1 mm in length. The majority of the microplastics were fibres (n = 165) with only a few fragments (n = 10) while the Rayon particles were solely fibres. Seventy-two percent of the microplastics were blue, 9% were transparent, 8% were pink and 11% were comprised of other colours such as purple, brown, red, green, grey, black, yellow and white (Fig. 3). Microplastic polymer types included polyester (n = 86), blends (n = 76), polyamide (n = 4), polypropylene (n = 3), acrylic (n = 2), polyvinyl chloride (n = 2), polystyrene (n = 1) and polyurethane (n = 1). The overall category

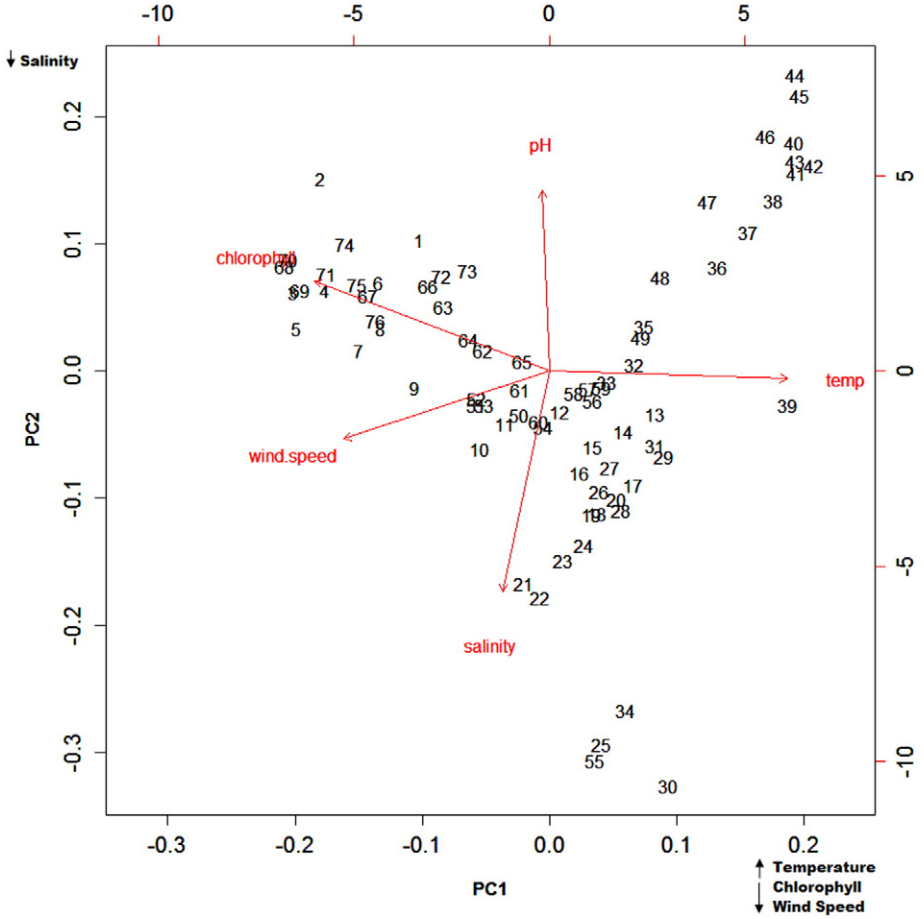

Fig. 1. Biplot showing sampling sites based on environmental variables.

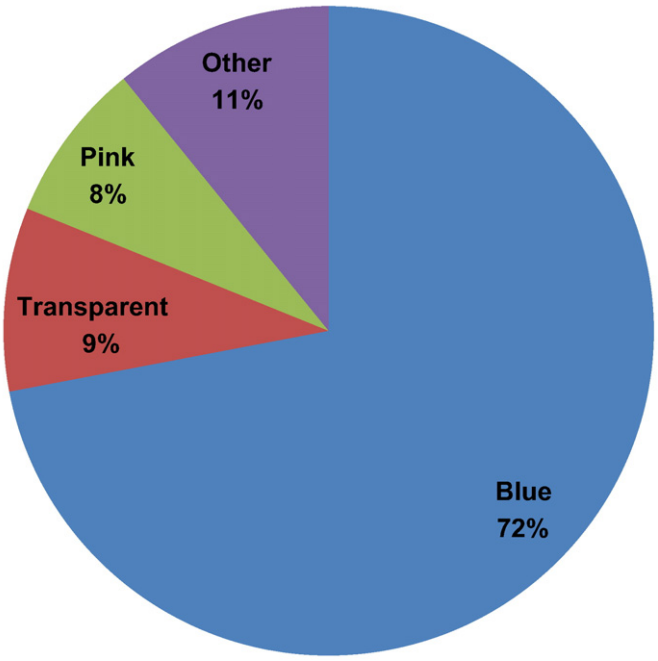

Fig. 2. Colours of confirmed microplastics.

of polyester also included particles identified as polyethylene terephthalate (PET), ($n = 18$). Particles identified as blends were either polyamide blends or acrylic/polyester blends and the polyamides were comprised of specific polymers such as nylon and Kevlar.

3.4. Microplastic abundance and distribution in the Atlantic Ocean

Microplastic abundance along the North/South latitudinal gradient in the Atlantic Ocean ranged from 0 to 8.5 particles m^{-3} (Fig. 4). For the majority of sampling sites, microplastic abundance ranged between 0 and 2.5 particles m^{-3}. However, the areas where this range was exceeded included (i) offshore of Namibia (8.5 particles m^{-3}), (ii) off the west coast of Morocco (6–6.5 particles m^{-3}), (iii) the Bay of Biscay (3.5 particles m^{-3}), and (iv) off the western coast of Portugal (3.5 particles m^{-3}). A Mann-Whitney Wilcoxon test indicated that there was no statistically significant difference (Wilcoxon rank sum test p-value $= 0.7111$) in microplastic abundance between the Benguela upwelling sites and all other sites considered as non-upwelled sites (Fig. 5).

3.5. Influence of environmental variables on microplastic abundance

Correlation analyses were conducted to determine whether environmental variables influenced microplastic abundance. Overall, there were no statistically significant correlations between microplastic abundance and: chlorophyll, pH, salinity and wind speed (Supplementary Table 1). However, there was a statistically significant weak negative correlation between microplastic abundance and: sub-surface water temperature (Spearman's rank correlation, rho $= -0.25$, p-value $= 0.03$); and conductivity (Spearman's rank correlation, rho $= -0.27$, p-value $= 0.02$).

A generalized additive model (GAM) was developed to further determine the influence of environmental variables on microplastic abundance. In this model, the response variable was microplastic count (number of microplastics per sample) and initial explanatory variables included location (latitude, longitude), physico-chemical properties associated with sub-surface waters (temperature, pH, salinity), chlorophyll *a* concentration, weather data (wind direction, wind speed), presence of upwelling and duration of filtration. In the model, (i) the Poisson family distribution of error terms was specified with a log link function due to the fact that microplastic abundance data was count data, and (ii) the explanatory variable water temperature was included as the difference between the highest and lowest water temperature recorded during sample collection (Δ water temperature). The output of the initial model was examined and based on this non-parametric smoothers (s) were applied to all explanatory variables except latitude, temperature, wind speed and upwelling. Non-significant explanatory variables (as evidenced by their p-values) were eliminated in a stepwise manner until a GAM with the lowest Akaike Information Criterion (AIC) score (283.334) and the fewest explanatory variables was obtained. The final GAM (R-sq $= 0.548$) was as shown below:

$$\text{Microplastic count} \sim \textbf{latitude} + s(\textbf{longitude}) + \textbf{Δwater temperature} + +s(\textbf{wind direction}) + \textbf{wind speed} + \text{upwelling} + s(\textbf{salinity})$$

Of the explanatory variables that were present in the final model, latitude, longitude, water temperature, wind direction, wind speed and salinity were the six variables found to have a significant effect on the abundance of microplastics in the Atlantic Ocean (Table 1).

4. Discussion

Interactions between microplastics and marine organisms are of particular interest due to the potential negative effects that this category of anthropogenic debris may have on marine organisms. The assessment of microplastic abundance in 'biota rich' waters is therefore

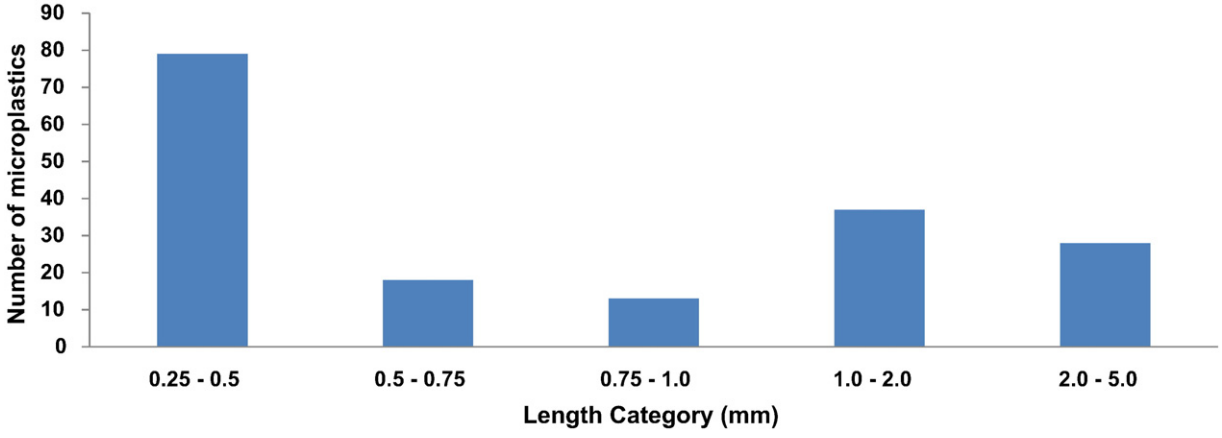

Fig. 3. Lengths of confirmed microplastics.

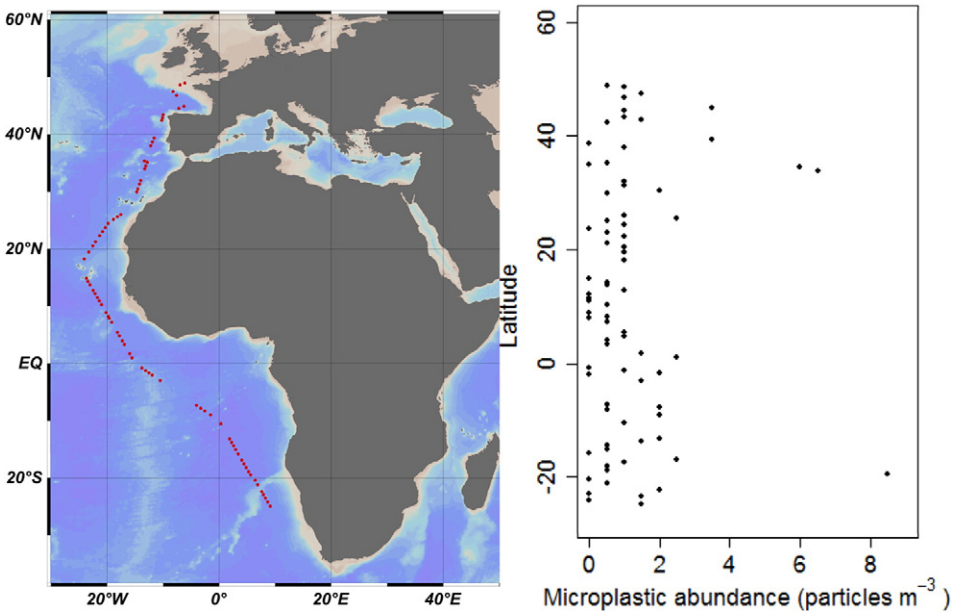

Fig. 4. Map of sampling locations and microplastic abundance along the north/south transect in the Atlantic Ocean.

particularly important due to the potential that exists for enhanced interactions between these particles and abundant biota at such sites. Along the western coast of Africa, there were two areas considered 'biota rich' of specific interest: Canary Upwelling Ecosystem (CUE) and the Benguela Upwelling Ecosystem (BUE). The present study availed of a platform of opportunity aboard a research vessel transit; consequently the investigators had no influence over the vessel's track. Both univariate and multivariate analyses indicated that although the Benguela upwelling was definitely sampled, the same could not be said for the Canary upwelling. This was possibly due to the fact that in the region where the Canary upwelling was expected to occur, the research vessel was too far offshore from the African continent. The present study found that there were no statistically significant differences between microplastic abundance in upwelled and non-upwelled areas in the Atlantic Ocean. Previous studies had suggested that upwelling may (i) provide a source of deepwater with relatively low levels of microplastics and, (ii) lead to a dilution of plastics in surface waters thus resulting in lower plastic abundances at sites within close proximity to such oceanic phenomena (Desforges et al., 2014; de Lucia et al., 2014). The findings of the present study must be taken in the context that only 14 of the 76 samples for microplastics were taken in the Benguela upwelling. More definitive statements about the microplastic abundance at upwelling regions in the Atlantic Ocean can only be made if more intensive sampling is conducted in such regions in the future.

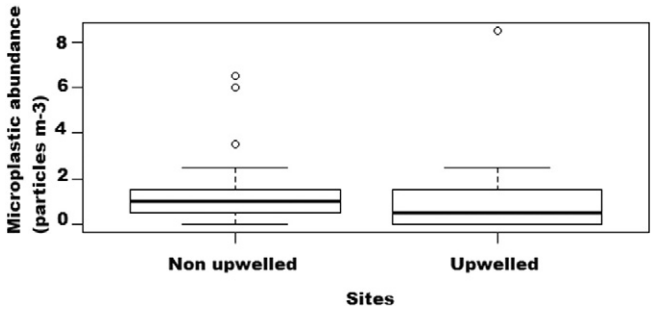

Fig. 5. Comparison of microplastic abundances at upwelled and non-upwelled sites in the Atlantic Ocean.

In certain respects, the composition of microplastics along the North/South Atlantic transect was comparable to that found in other marine environments across the world. The predominance of fibrous microplastics noted in this study was consistent with similar previous findings in both surface and sub-surface waters (Cole et al., 2014; Desforges et al., 2014; Lusher et al., 2014; Zhao et al., 2014; Enders et al., 2015; Lusher et al., 2015). It has been suggested that an important source of microplastic fibres in the environment may be from the washing of clothes, with laboratory experiments demonstrating that a single garment may potentially produce >1900 fibres per wash (Browne et al., 2011) and an average 6 kg load of acrylic fabric could release over 700,000 fibres (Napper and Thompson, 2016). While fibres may in fact be more dominant in the natural environment, it is important to note that as a category of microplastics, they are generally more discernible than other categories of microplastics. Fragments, for example, have a higher chance of being disregarded due to their similarity in appearance to natural materials. Cole et al. (2014) suggests the presence of an 'operator selection bias' towards fibrous microplastics.

Analytical techniques such as FT-IR spectroscopy are immensely useful in microplastic studies as they confirm whether particles from environmental samples are indeed synthetic and, if so, identify the polymer type. In this present study, the major polymer types included polyester (50%) and blends that were either polyamide or acrylic/polyester (42%) with a minority (8%) of acrylic, polyamide, polypropylene, polyvinyl chloride, polystyrene and polyurethane. While previous studies have reported the presence of similar polymer types in their samples, low density polymers such as polyethylene and polypropylene were not as abundant in this study when compared to other studies which sampled microplastics in surface waters or even from shallower sub-

Table 1
Explanatory variables included in the final best fit GAM.

Explanatory variables	*p* value
Wind Direction	9.12e−09
Δ Temperature	9.25e−05
Latitude	5.23e−05
Wind Velocity	0.000333
Longitude	0.008280
Salinity	0.014380
Upwelling	0.050666

surface depths (Hidalgo-Ruz et al., 2012; Cole et al., 2014; Obbard et al., 2014; Frias et al., 2014; Enders et al., 2015; Lusher et al., 2015; Kang et al., 2015a; Woodall et al., 2015). This raises the question as to whether sampling depth within the water column influences microplastic composition due to differences in the densities and buoyancies of particular polymer types (Cole et al., 2013; Desforges et al., 2014; Woodall et al., 2015). Although techniques such as FT-IR spectroscopy can identify polymers, this information does not allow the investigator to pinpoint the exact origin of the polymers in the environment but instead reduces the possibilities (Claessens et al., 2011; Desforges et al., 2014). The synthetic polymers that were found in this study may have been derived from clothing, ropes, fishing gear (nets, lines, etc), plastic beverage bottles, as well as packaging materials (Smith, 1999; Andrady, 2011; Claessens et al., 2011; Napper and Thompson, 2016).

In the quest to assess microplastic abundance and composition in the marine environment, one of the issues that has emerged is the prevalence of rayon fibres in the environment. Rayon is essentially regenerated cellulosic material, it is man-made and is therefore considered as semi-synthetic (Mishra, 2010). In addition to being used in textiles, rayon has also been used in cigarette filters and personal hygiene products (Woodall et al., 2015). This study found that 63% of the particles analysed by FT-IR spectroscopy were rayon fibres. Previous studies have also reported that rayon fibres were the most prevalent synthetic microparticle in (i) fish from the English Channel (58%), (Lusher et al., 2013), (ii) surface and sub-surface waters in the Arctic Ocean (30%), (Lusher et al., 2015), (iii) sea ice cores from the Arctic Ocean (54%), (Obbard et al., 2014), (iv) deep sea sediments (57%), (Woodall et al., 2015), and (v) coastal sediments from Portuguese shelf waters (81%), (Frias et al., 2016). The prevalence of rayon fibres in the marine environment suggests heightened propensity for the potential impact of this material upon biota. Ladewig et al. (2015) suggested that although natural fibres may exhibit different degradability and chemical sorption behaviours when compared to synthetic fibres, natural fibres may still warrant environmental concerns, for example, in chemical pollution dispersion. Remy et al. (2015) further suggested that while the natural material of cellulose may not be an issue, the associated dyes or additives in the semi-synthetic fibres may pose a threat to biota.

Beyond the provision of data about microplastic abundance in the world's oceans, it is important that there is an understanding of the environmental variables that may potentially influence this issue. In this study, a generalized additive model (GAM) was developed to gain a preliminary insight into the environmental variables which had an effect on microplastic abundance in the Atlantic Ocean. A GAM model was chosen in lieu of the more common general linear model (GLM) in order to better capture the relationship between the response variable and the explanatory variables without assuming a parametric form (Crawley, 2013). The best fitting GAM generated in this study indicated that location (latitude, longitude), certain physico-chemical parameters of oceanic waters (water temperature, salinity) and atmospheric variables (wind direction, wind speed) had a significant effect on

Table 2
Microplastic abundances reported for surface and sub-surface oceanic waters across the world.

Location	Microplastic abundance (particles per m^3)	Method for surface waters (unless otherwise indicated)	Study
Arctic Ocean			
Svalbard, Norway	0.34 ± 0.31; 0–1.31 (mean, range) 2.68 ± 2.95; 0–11.5 (mean, range)	Manta trawl (333 μm) Underway system (250 μm)[a]	Lusher et al. (2015)[1]
Pacific Ocean			
Southern California, USA	7.25 (mean)	Manta trawl (333 μm)	Moore et al. (2002)
Santa Monica Bay, USA	3.92 (mean)	Manta net (333 μm)	Lattin et al. (2004)
South Californian current	0–3.141	Manta net (505 μm)	Gilfillan et al. (2009)
Southeast Bering Sea	0.004–0.19	Sameoto neuston/manta net (505 μm)	Doyle et al. (2011)
NP Subtropical Gyre	0.425 (median)	Manta net (333 μm)	Goldstein et al. (2012)
North eastern Pacific Ocean	279 ± 178 (mean)	Underway system (62.5–250 μm)[a]	Desforges et al. (2014)[2]
Geoje Island, South Korea	0.4–54	Manta trawl (330 μm)	Song et al. (2014)
East China Sea	0.167 ± 0.138 (mean)	Neuston net (333 μm)	Zhao et al. (2014)
Southern Sea of Korea	1.92–5.51; 2.3–38.77 (2012) 582–924; 10–375 (2013)	Manta trawl (330 μm) Hand Net (50 μm)	Kang et al. (2015a)
Geoje and Jinhae Bays, Korea	1.92 ± 1.84; 5.51 ± 11.2 (2012) 1.68 ± 0.81; 1.07 ± 0.34 (2013)	Manta Trawl (330 μm)	Kang et al. (2015b)
East Asian Sea	3.7 ± 10.4; 0.03–491 (mean, range)	Neuston net (350 μm)	Isobe et al. (2015)
Indian Ocean			
Southeast South Africa	257.9–1215	WP-2 type net (80 μm)	Nel and Froneman (2015)
Atlantic Ocean			
Bristol Channel, UK	0–100	Lowestoft plankton sampler (270 μm)	Morris and Hamilton (1974)
Offshore Ireland	2.46 ± 2.43; 0–22.5 (mean, range)	Underway system (250 μm)[b]	Lusher et al. (2014)[3]
Western English Channel	0.27	Plankton nets (200, 500 μm)	Cole et al. (2014)
Portuguese coastal waters	0.002–0.036	WP2 (180 μm), Neuston (280 μm), LH Plankton Recorder (335 μm)	Frias et al. (2014)
St. Peter/St. Paul Archipelago, Brazil	0.01	Plankton net (300 μm)	Ivar do Sul et al. (2013)
Western Tropical Atlantic Ocean	0.015–0.04	Manta trawl (300 μm)	Ivar do Sul et al. (2014)
North Atlantic Ocean	13–501	Underway system (10, 300 μm)[b]	Enders et al. (2015)[4]
Atlantic Ocean	1.15 ± 1.45; 0–8.5 (mean, range)	Underway system (250 μm)[b]	This study[5]
Mediterranean and European Seas			
West Coast, Sweden	167–2400 72–141	Plankton net (80 μm) Zooplankton net (450 μm)	Noren (2007)
West Sardinian Coast	0.15	Manta trawl (500 μm)	de Lucia et al. (2014)
Southwest Finland	0–0.74	Manta trawl (333 μm)	Magnusson (2014)
Baltic Sea	10^2–10^4	WP2 net (90 μm mesh)	Gorokhova (2015)

[a] Sub-surface waters sampled at the following depths ([1]6 m, [2]4.5 m).
[b] Sub-surface waters sampled at the following depths ([3]3 m, [4]3 m, [5]11 m).

microplastic abundance. These findings must be taken in the context that the model in this study was based on data from 76 samples in the Atlantic Ocean. Notwithstanding this, GLMs based on datasets from the Northeast Atlantic and Arctic Ocean also indicated that sea surface temperature and wind affected microplastic abundance (Lusher et al., 2014; Lusher et al., 2015). Based on the combination of field data and a theoretical model, Kukulka et al. (2012) indicated that that wind stress results in vertical mixing of buoyant microplastics in the surface mixed layer of the ocean. Overall then, it appears that microplastic abundance is influenced by a combination of factors, some of which include location, atmospheric parameters and oceanographic conditions.

While comparison of microplastic abundances between studies is possible, one must be cognisant of the differences between sampling, processing and analytical techniques for microplastic identification. These differences may account for some of the variation in the findings between studies. Bearing that in mind, average microplastic abundance (1.15 ± 1.45 particles m^{-3}) in sub-surface waters along the North/South Atlantic transect in this study was lower than that reported for sub-surface waters in the north eastern Pacific Ocean (279 ± 178 particles m^{-3}), Arctic Ocean (2.68 ± 2.95 particles m^{-3}) and north eastern Atlantic Ocean (2.46 ± 2.43 particles m^{-3}), (Table 2). The lower microplastic abundances that were reported in this study were possibly due to the fact that the vessel (i) did not traverse waters where microplastics have been known to accumulate (i.e. either the North Atlantic or the South Atlantic Sub-Tropical Gyre), (ii) was too far offshore the African continent to sample nearshore sites which usually have higher microplastic abundances than open oceanic sites, or (iii) was sampling from a different vertical fraction in the water column. Although microplastic abundance in the present study was comparable to abundances reported for surface waters in the Atlantic Ocean, Pacific Ocean and the Mediterranean and European Seas, considerably higher microplastic abundances in surface waters were reported for nearshore sites in the US, Korea, South Africa, UK and Sweden (Table 2). The comparisons of microplastic abundance in sub-surface waters must be taken in the context of variations in the depth (3–11 m) at which seawater was sampled and mesh sizes (62.5–300 μm) of the sieves that were used amongst the studies. For surface water samples, there were also variations in the mesh sizes (50–505 μm) of the nets that were used. These factors may influence microplastic abundance as (i) there may be vertical stratification of microplastics in the water column and, (ii) smaller mesh sizes would increase the quantity of microplastics collected during sampling. Standardisation and intercalibration protocols for sampling microplastics in surface and sub-surface waters are key issues to be addressed by the scientific community if greater comparability between studies is to be achieved.

5. Conclusion

This study provided an assessment of microplastics in sub-surface waters along a North/South latitudinal gradient in the Atlantic Ocean. Overall, average microplastic abundance as reported by this study for the Atlantic Ocean (1.15 ± 1.45 particles m^{-3}) was lower than was reported for sub-surface waters across the world. Additionally, there were no statistically significant differences between microplastic abundance at Benguela upwelling sites ($n = 14$) and all other non-upwelled sites ($n = 62$). Rayon (63%) was the predominant polymer of the particles that were analysed. Of the confirmed microplastics, the most abundant polymer types were polyester (49%) and blends of polyamide or acrylic/polyester (43%). Fibres (94%) were also the predominant type of microplastics. The information provided by this study is important as it provides an indication of the environmentally realistic concentrations and types of microplastics that biota are exposed to in the natural environment.

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Appendix III

Published Manuscript I Supplementary Tables

Kanhai, L.K., Officer, R., Lyashevska, O., Thompson, R.C., O'Connor, I., 2017. Microplastic abundance, distribution and composition along a latitudinal gradient in the Atlantic Ocean. Marine Pollution Bulletin 115, 307-314. https://doi.org/10.1016/j.marpolbul.2016.12.025

Supplementary Table 1: Descriptive statistics, tests of normality and correlation findings for environmental variables

Descriptive Statistics	**Chlorophyll a**	**pH**	**Conductivity**	**Salinity**	**Temperature**	**Wind Speed**
Min	0.12140000	7.67550000	4.281900e+01	3.439620e+01	14.3396000	2.0417000
Max	1.36460000	8.25950000	5.755580e+01	3.700730e+01	29.1567000	12.8200000
Range	1.24320000	0.58400000	1.473680e+01	2.611100e+00	14.8171000	10.7783000
Median	0.20565000	8.20175000	5.322960e+01	3.595205e+01	23.2692000	7.7576000
Mean	0.40497500	8.17863289	5.212323e+01	3.589371e+01	22.9075079	7.6621461
SE.mean	0.03914829	0.01325830	5.027554e-01	6.838279e-02	0.4898121	0.2986146
CI.mean	0.07798740	0.02641189	1.001540e+00	1.362255e-01	0.9757557	0.5948707
Var	0.11647675	0.01335947	1.920999e+01	3.553917e-01	18.2336076	6.7769695
Std.dev	0.34128691	0.11558317	4.382920e+00	5.961474e-01	4.2700829	2.6032613
Coef.var	0.84273575	0.01413233	8.408765e-02	1.660868e-02	0.1864054	0.3397562
Skewness	1.139311	-3.539398	-0.4683603	-0.2530947	-0.1625543	-0.3214113
Kurtosis	3.145469	14.98953	1.936009	2.510892	1.916949	2.314437
Shapiro Wilk Normality Test (p value)	4.033e-09	5.951e-15	5.31e-05	0.3192	0.003877	0.08189
Anderson-Darling Test (p value)	3.372e-16	< 2.2e-16	7.044e-06	0.4458	0.008971	0.1098
*Spearman's rank correlation						
rho	0.31	-0.11	0.21	0.22	-0.25	-0.27
p value	0.12	0.34	0.07	0.06	0.03	0.02

*All correlation analyses were between microplastic abundance and the specified parameter

APPENDIX IV

Published Manuscript II

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Microplastics in sub-surface waters of the Arctic Central Basin

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ABSTRACT

Polar oceans, though remote in location, are not immune to the accumulation of plastic debris. The present study, investigated for the first time, the abundance, distribution and composition of microplastics in sub-surface waters of the Arctic Central Basin. Microplastic sampling was carried out using the bow water system of ice-breaker Oden (single depth: 8.5 m) and CTD rosette sampler (multiple depths: 8–4369 m). Potential microplastics were isolated and analysed using Fourier Transform Infrared Spectroscopy (FT-IR). Bow water sampling revealed that the median microplastic abundance in near surface waters of the Polar Mixed Layer (PML) was 0.7 particles m^{-3}. Regarding the vertical distribution of microplastics in the ACB, microplastic abundance (particles m^{-3}) in the different water masses was as follows: Polar Mixed Layer (0–375) > Deep and bottom waters (0–104) > Atlantic water (0–95) > Halocline i.e. Atlantic or Pacific (0–83).

1. Introduction

The Arctic Ocean, though the smallest in the world, is unique due to its distinct abiotic features and the highly specialised ecosystem it supports. Key anthropogenic drivers which may put pressure on this ecosystem include (i) climate change, (ii) harvest and fisheries, (iii) persistent, bio-accumulative and toxic contaminants, (iv) industrial development, (v) shipping, and (vi) invasive alien species (CAFF, 2017). Plastic contaminants in the world's oceans have emerged as an issue of global importance due to their ubiquitous distribution, long-range transport potential, persistence and perhaps most importantly the potential threat they pose to marine organisms (UNEP, 2011). Remote polar oceans such as the Arctic Ocean have not been immune to the entry of plastics as a combination of long-range transport processes and local anthropogenic activities have contributed to the plastic debris in these areas.

Characteristic abiotic features which set the Arctic Ocean apart from other oceanic basins include (i) a central area of perennial pack ice, (ii) seasonal extremes in solar irradiance, ice and snow cover, temperature and riverine inflow, and (iii) an upper layer of lower salinity water due to freshwater input from rivers and seasonal sea-ice melt (CAFF, 2013). This unique ecosystem is a habitat for a vast array of marine organisms, some of which are (i) endemic to the region, (ii) commercially important, (iii) apex predators, (iv) central to the functioning of the ecosystem, and (v) threatened as evidenced by their inclusion in the IUCN Red List of Threatened Species (CAFF, 2013; CAFF, 2017).

Despite its remote location away from major population centres and the low coastal population in its surrounding shelf areas, both macro and microplastics were detected in the various environmental compartments of the Arctic Ocean. Between 2002 and 2014, macroplastics were detected on the seafloor (2500 m depth) of the eastern Fram Strait at the HAUSGARTEN observatory (Bergmann and Klages, 2012; Tekman et al., 2017). Sightings of buoyant macroplastics were also made during ship and helicopter observation surveys in the Barents Sea and Fram Strait (Bergmann et al., 2016). A citizen-science study also recently reported the presence of macroplastics on six beaches of the Svalbard Archipelago (Bergmann et al., 2017a). Arctic sea ice was reported by Obbard et al. (2014) as having microplastic concentrations (38–234 particles m^3 of ice) several orders of magnitude greater than highly contaminated oceanic waters. Lusher et al. (2015) first reported on microplastic abundances in surface and sub-surface waters south and southwest of Svalbard. Amélineau et al. (2016) later reported on microplastic abundance in surface waters east of Greenland. Regarding Arctic species, microplastics have been detected in the gular pouches of Little Aulks (*Alle alle*), (Amélineau et al., 2016), as well as in the stomachs of juvenile polar cod (*Boreogadus saida*), (Kuhn et al., 2018). Microplastics were also detected in sediments (collection depths 2340–5570 m) from the Fram Strait (Bergmann et al., 2017b). Recently,

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results from a circumpolar expedition of the Arctic indicated that concentrations of floating plastic ranged between 0 and 320,000 items km^{-2} in the Greenland and Barents Sea and 0–27,000 items km^{-2} in the rest of the Arctic Ocean (Cózar et al., 2017).

Plastic contaminants are introduced to the Arctic Ocean due to a combination of (i) long-range transport processes, e.g. via oceanic currents, biotransport and riverine input, and (ii) local anthropogenic activities, e.g. shipping. The three oceanic currents which supply the greatest water volumes to the Arctic Ocean are the (i) West Spitsbergen Current i.e. the polar limb of the North Atlantic circulation which carries warm water from the North Atlantic Current (9.5 Sverdrup, Sv = 10^6 m^3 s^{-1}), (ii) a cold ocean current that enters from the Pacific Ocean via the Bering Strait (1.5 Sv) and, (iii) a branch of the North Atlantic Current, which flows along the Siberian coastline (1.0 Sv), (Zarfl and Matthies, 2010). These oceanic currents may also transport plastics to the Arctic Ocean with the estimated plastic flux to this region ranging between 62,000 to 105,000 tons per year (Zarfl and Matthies, 2010). Models based on a particle-trajectory approach for studying the fate of marine debris in the open ocean highlighted the northward transport of marine debris to polar regions and the formation of a sixth so-called garbage patch in the Barents Sea (van Sebille et al., 2012). Bio-transport is another long-range transport process via which plastics may enter polar regions. Plastic ingestion was reported in Northern Fulmars (*Fulmaris glacialis*) and Thick-billed Murres (*Uria lomvia*) in the Arctic (Mallory, 2008; Provencher et al., 2012; Trevail et al., 2015). Some studies suggested that the seabirds had ingested plastics during their wintering in the North Atlantic Ocean and had then transported the contaminants to the Arctic upon migration (Mallory, 2008; Provencher et al., 2012). Riverine discharge from Siberian (Ob, Yenisei and Lena) and Canadian (Mackenzie) rivers are other potential sources of plastics to the Arctic. Obbard et al. (2014), however, point out that the contribution of riverine discharge to plastic input in the Arctic is projected to be low due to the fact that these rivers flow through sparsely populated watersheds. Local anthropogenic activities are another source of plastics to the Arctic. Increased ship traffic due to shipping and tourism was found to be positively correlated with increased litter densities in the Fram Strait (Bergmann and Klages, 2012; Tekman et al., 2017).

The intense focus by scientists on the near-surface layer of the ocean for microplastics has been due in part to the presumption that the majority of particles would be found in this region of the water column given the inherent densities of individual synthetic polymers. Such a theorization led to traditional techniques that involved nets, manta trawls as well as the seawater intake of vessels that sampled only the upper few meters of the water column for microplastics. Yet, several studies indicated that a mismatch existed between observed and expected plastic concentrations in surface oceanic waters when estimated plastic production and projected inputs to the oceans were considered (Cózar et al., 2014; Eriksen et al., 2014). It was therefore proposed that several mechanisms potentially influenced the vertical distribution of microplastics within the water column and led to their transport out of surface waters. Some of these mechanisms included (i) incorporation into marine aggregates (Long et al., 2015), (ii) biofouling (Fazey and Ryan, 2016), (iii) incorporation into faecal matter (Cole et al., 2016) and, (iv) hydrodynamic factors such as wind (Kukulka et al., 2012). Despite the theorization that surface waters are not the ultimate repository for plastic debris in the marine environment (Cózar et al., 2014), few studies ventured beyond traditional near-surface microplastic monitoring to investigate their vertical distribution in the water column.

Microplastic pollution in the Arctic Ocean is an issue that warrants attention due to the potential threats that these contaminants may pose to the inhabitants of this unique ecosystem. A practical step towards addressing this issue and evaluating the extent of the problem involves assessing the abundance, distribution and composition of microplastics in Arctic waters. While microplastic monitoring in the marine environment has traditionally focused on surface waters, the reality is that the vast majority of marine organisms inhabit sub-surface waters. Monitoring microplastics in sub-surface waters is particularly relevant as it can also provide some insight into the whereabouts of the 'missing plastic' from surface waters. To our knowledge, the present study sought for the first time (i) to provide a spatial overview of microplastic abundance, distribution and composition in the Polar Mixed Layer (PML) of the Arctic Central Basin (ACB) and, (ii) to determine whether microplastics in the ACB were being transported out of surface waters by assessing their vertical distribution in the water column.

2. Materials and method

2.1. Study area

The Arctic Ocean is comprised of a deep central basin surrounded by extensive continental shelves (CAFF, 2013). The bathymetry of the Arctic Ocean is such that the Lomonosov Ridge separates the central basin into the Canadian (Amerasian) and Eurasian basins with the basins being further sub-divided by the (i) Gakkel Ridge, into the Amudsen and Nansen basins and, (ii) Alpha Ridge, into the Makarov

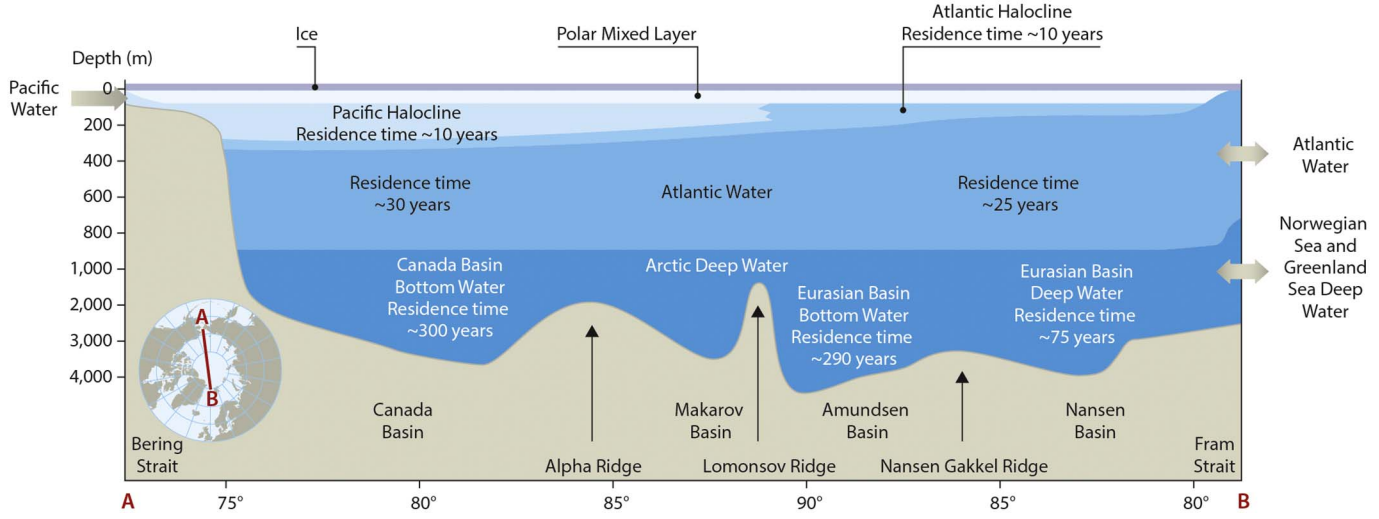

Fig. 1. General overview of the bathymetry and water masses of the Arctic Central Basin [reprinted here with permission from CAFF], (CAFF, 2013).

and Canada basins (Jakobsson et al., 2004; Rudels, 2015, Fig. 1). A major structuring element of the Arctic marine ecosystem is sea ice which floats on the surface layer impeding surface mixing and influencing freshwater and heat fluxes (CAFF, 2013). In the Arctic Ocean, there is a distinct vertical stratification of the water column giving rise to three major water layers (i) Polar Surface Water (PSW) which includes the Polar Mixed Layer (PML) and the halocline, (ii) Atlantic water and, (iii) deep and bottom waters (Rudels, 2015, Fig. 1). The PML (approximately 50 m deep) is the uppermost surface layer of low salinity water formed as a result of sea ice melt and the influx of freshwater from riverine sources (Rudels et al., 1991; CAFF, 2013; Jakobsson et al., 2004). Beneath the PML is a halocline (50–250 m), characterised by a strong salinity increase with depth and comprised of either Pacific waters or Atlantic waters with the Pacific halocline being deeper than the Atlantic halocline (Rudels et al., 1991; Jakobsson et al., 2004). Below the halocline lies an intermediate water layer comprising of dense saline Atlantic water. The deep and bottom waters also referred to as Arctic deep water ranges from a depth of approximately 900 m and extend to the seafloor (Rudels et al., 1991; CAFF, 2013).

2.2. Sample collection

2.2.1. Underway samples

This study was conducted onboard the Swedish icebreaker Oden during the Arctic Ocean 2016 expedition. The vessel departed Longyearbyen, Svalbard on August 8th 2016 and traversed approximately 4943 nautical miles in the Arctic Ocean until its return on September 19th 2016 (Fig. 2). Sub-surface oceanic water pumped onboard the vessel via the bow water system was sampled for microplastics according to Lusher et al. (2014). Sampling was conducted for a period of approximately 6 weeks (9 August to 16 September 2016). Since each sample constituted the filtration of approximately 2000 L of water, the total survey effort for this study was approximately 116,000 L of water (58 samples).

Seawater from a continuous intake located at the keel of the ship (depth 8.5 m) was pumped onboard the vessel using a rotary positive displacement pump (Universal II Series Pump, Waukesha Cherry-Burrell) at a flow rate of 85 L/min (at optimal capacity) and transported to the laboratory via stainless steel pipes. Prior to reaching the laboratory, the seawater passed through a stainless steel primary filter (pore size 2.5 mm) which was beyond the control of the investigator. The discharge of grey water in relation to the seawater intake was not an issue since the seawater intake was located towards the front of the vessel while grey water was discharged mid-vessel. In the laboratory, seawater from the vessel's bow water system was allowed to flow through a covered stainless steel sieve (250 µm) by means of a connection hose fitted into the wooden sieve cover. For the duration of the sampling, the stainless steel sieve was supported in a wooden stand. Based on Lusher et al. (2014), approximately 2000 L of water was filtered for each sample. The length of time taken for the filtration of the specified volume of water was determined by calculation of the flow rate of the seawater. A flow meter, attached at a point prior to the entry of the water into the sieve, was also used to verify the volume of water filtered. Once the specified volume of water was filtered, the sieve was removed and Milli-Q water was used to wash retained material from the sieve into a clean container. The collected material was then filtered under vacuum onto glass microfibre paper (GF/C); Whatman: 47 mm, pore size: 1.2 µm, using a Buchner funnel and a vacuum flask (Lusher et al., 2014). Each filter paper was then placed into a clean plastic petri dish, covered and stored in a freezer (−20 °C) until returned to the laboratory. At the start and at the end of each sample, positioning data were collected. Data for various environmental variables were obtained from the vessel's (i) thermosalinometer (water temperature, salinity) and (ii) weather station (wind speed and direction).

2.2.2. CTD samples

A rosette water sampler containing 24 Niskin bottles coupled to a Sea-Bird SBE 911 conductivity-temperature-depth (CTD) sensor suite (hereafter referred to as CTD) was used to collect sub-surface water samples and hydrographic data at 9 sampling locations in the Arctic Ocean. Upon deployment from the vessel, the CTD entered the water and was allowed to descend to the bottom layer. During the descent, Niskin bottles were open with water flowing through them. It was during the up-cast that multiple Niskin bottles were closed at specific depths in order to facilitate the collection of a specified volume of water. A total of 48 water samples were retrieved during the 9 CTD casts to sample for microplastics. At 7 of the CTD casts, 6 water depths were sampled with 48 L of water collected at each depth i.e. 4 Niskin

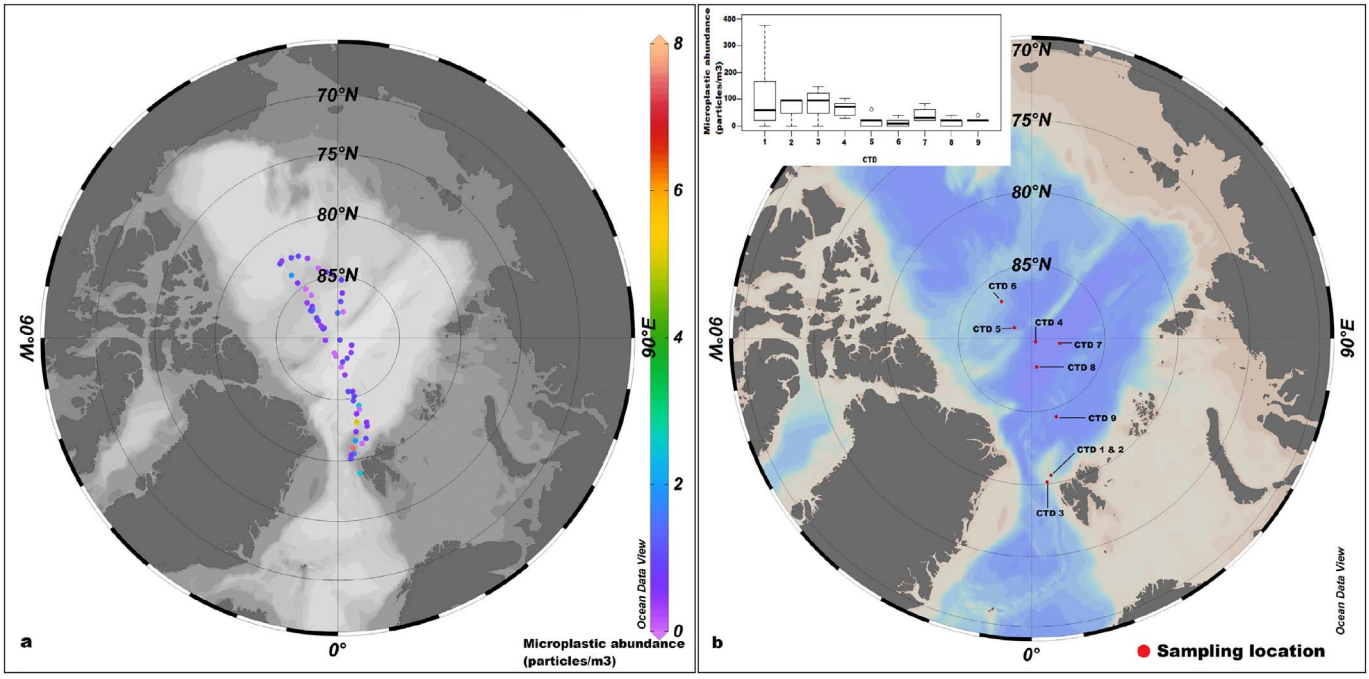

Fig. 2. Microplastic abundance in the Arctic Central Basin (a) based on bow water sampling at a single depth of 8.5 m, (b) based on CTD sampling at multiple depths (8–4369 m).

bottles (12 L) per depth. At 2 CTD casts, 3 water depths were sampled with 21 L of water collected at each depth i.e. 3 Niskin bottles (7 L) per depth. At a particular sampling location, the overall goal was to collect samples in the near-surface, mid-water and bottom layers within the water column thereby reflecting the main water masses. As such, exact sampling depths were determined by the information provided by the salinity and temperature sensors on each downcast. The deepest samples at each CTD cast were collected at least 10 m above the seafloor at a given location. Following each CTD cast, Niskin bottle taps were rinsed with Milli-Q water and a clean hose was attached. Water from bottles closed at the same depth was passed through the same stainless steel sieve (250 μm) held in a covered wooden stand. Once water from all bottles at a specific depth had been filtered, the sieve was removed and Milli-Q water was used to wash retained material from the sieve into a clean container. The collected material was filtered under vacuum onto glass microfibre paper (GF/C); Whatman: 47 mm, pore size: 1.2 μm, using a Buchner funnel and a vacuum flask. Each filter paper was then folded and placed into an aluminium foil packet and stored in a freezer (−20 °C) until returned to the laboratory.

2.3. Method validation and contamination prevention

For the underway samples, potential contamination during sample processing was evaluated by (i) leaving clean plastic petri dishes with filter paper exposed to the air during vacuum filtration, and (ii) passing an aliquot (250 mL) of Milli-Q water through clean GF/C filter paper under vacuum. For the CTD samples, potential contamination was assessed by filling a clean Niskin bottle with Milli-Q water and subjecting it to the exact process a sample underwent. Measures taken to prevent contamination in the laboratory included (i) wearing lab coats (cotton/polyester blend), cotton clothing and gloves (nitrile) during sample processing, (ii) placing a wooden cover over the stainless steel sieve during filtration to prevent airborne contamination, and (iii) washing all containers used during sample processing with Milli-Q water before reuse.

2.4. Laboratory analyses

Filter papers were removed from the freezer, left to dry and then visually examined under a dissecting microscope (Olympus SZX10) equipped with a polariser and camera (Q Imaging Retiga 2000R). Potential microplastics were isolated and processed (photographed and length measurements taken) prior to transferring to a clean filter paper in a labelled petri dish (Kanhai et al., 2017). All potential microplastics were analysed by Fourier transform infrared (FT-IR) spectroscopy on a Thermo Scientific Nicolet iN10 FT-IR spectrometer. The instrument was equipped with a potassium bromide (KBr) beamsplitter and an internal mercury cadmium telluride (MCT) detector which was cooled with liquid nitrogen. Microscope-reflectance sampling was performed and spectra were recorded as the average of 256 scans in the spectral wave number range of 4000–675 cm^{-1} at a resolution of 4 cm^{-1}. Thermo Scientific's OMNIC Picta Version 9 spectroscopy software was used for processing and evaluating all spectra. Prior to analysing each sample, background scans were performed and sample spectra were automatically corrected. Each sample spectrum was compared with those of known standard polymers in the (i) Hummel Polymer Sample library, (ii) Polymer Laminate Films library, and (iii) Wizard library. Values of between 0 and 100% were produced for each match between sample and reference spectra with the highest percentage representing the closest match. Particles for which there was uncertainty regarding the identity of the polymer (specifically fragments and some fibres) were subjected to further FT-IR spectroscopy on a Bruker Vertex 70 Infrared Spectrometer coupled to a Hyperion 1000 microscope (Kanhai et al., 2017). Samples which produced spectra with a match < 60% were automatically rejected while those with a match of > 70% were automatically accepted. All spectra with matches > 60% were individually examined to ensure that there was clear evidence of peaks from the sample corresponding to known peaks of standard polymers and that instances of the misidentification of natural and semi-synthetic polymers was reduced (Comnea-Stancu et al., 2017).

2.5. Statistical analyses

All statistical analyses were performed using R version 3.2.3 (R Core Team, 2015). Descriptive statistics, histograms and box plots were generated and tests of normality (Supplementary Tables 1 and 2) were conducted on all data to determine whether parametric or non-parametric statistical analyses were appropriate. Correlation analyses were performed between individual environmental variables and microplastic abundance for both underway and CTD samples. A generalized additive model (GAM) was developed using the underway data and a generalized linear mixed model (GLMM) was developed using the CTD data to determine which environmental variables had an effect on microplastic abundance.

3. Results

3.1. Quality control

In conjunction with the collection of samples via the underway system of the vessel, a total of 24 blanks (air contamination-12, method-12) were run (Supplementary Table 3). No synthetic polymers were found in the method blanks. However, a single synthetic fibre (blue, polyethylene terephthalate, 0.438 mm) was found in the last air contamination blank. For 6 of the 9 CTD casts, at least one method blank was run (Supplementary Table 4). Between 0 and 3 synthetic fibres were found in each of the method blanks. The synthetic fibres that were found included polyethylene terephthalate (n = 8), polyacrylonitrile (n = 1) and polyvinyl chloride (n = 1).

3.2. Overview of findings

3.2.1. Underway samples

A total of 303 particles were isolated from the underway samples and analysed by FT-IR spectroscopy. Of these, 46 particles were excluded because of uncertainty regarding their identity (< 60% match to reference spectra) and in the minority of cases (n = 6) due to their length (< 250 μm). Of the remaining particles (n = 257), 14 were macro-particles (i.e. > 5 mm in length) and 243 were micro-particles (< 5 mm in length). Of the macro-particles, 11 were confirmed as macroplastics having the following polymer types: polyethylene terephthalate (4), polyamide blend (4) and polyacrylonitrile (3). Of the 243 micro-particles, 110 were natural (cellulosic), 16 were semi-synthetic (cellulose-based e.g. rayon) and 117 were synthetic. All further analyses and discussions focus on the 117 confirmed microplastics.

The majority (94%) of microplastics were fibres and 6% were fragments. In terms of colour, the most prevalent were blue (49%) and transparent (25%) (Fig. 3a). Approximately 62% of the microplastics occurred in the larger size classes of 1.0–2.0 mm and 2.0–5.0 mm (Fig. 4a). Microplastic polymer types included polyester (n = 88), blends (n = 11), polyacrylonitrile (n = 8), polyamide (n = 5) and polyvinyl chloride (n = 5), (Fig. 5a). The overall category of 'polyester' included both polyethylene terephthalate (PET) and other polyesters while blends included either polyamide blends or polyester blends.

3.2.2. CTD samples

A total of 157 particles were isolated from the CTD samples and analysed by FT-IR spectroscopy. Of these, 14 were excluded for the reasons mentioned above for the underway samples. Of the remaining particles, 2 were categorised as macroplastics (> 5 mm) and included PET and polyacrylonitrile. Of the 141 micro-particles (< 5 mm), 39 were natural (cellulosic), 8 were semi-synthetic (cellulose-based e.g.

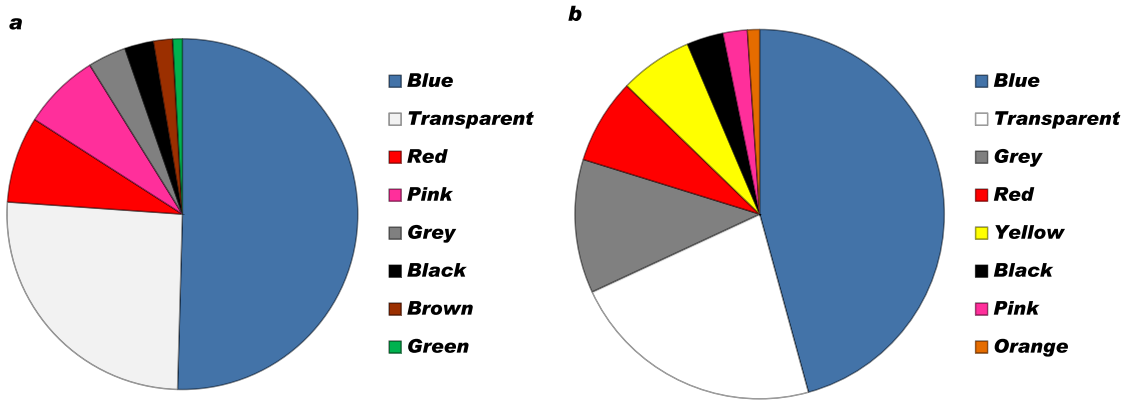

Fig. 3. Colours of microplastics found in (a) underway and (b) CTD samples at depths 8–4369 m. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

rayon) and 94 were synthetic. All further analyses and discussions focus on the 94 confirmed microplastics.

Overall, the characteristics of the microplastics isolated from CTD samples were similar to those from the underway samples in that (i) the majority (96%) of microplastics were fibres and 4% were fragments, (ii) the most prevalent colours were blue (46%) and transparent (22%) (Fig. 3b), (iii) the majority (64%) of microplastics were in the larger size classes of 1.0–2.0 mm and 2.0–5.0 mm (Fig. 4b), and (iv) microplastic polymer types included polyester (n = 74), blends (n = 12), polyacrylonitrile (n = 6), polyamide (n = 1) and polyvinyl chloride (n = 1), (Fig. 5b). The overall category of polyester included both polyethylene terephthalate (PET) and polyester while blends included only polyamide blends.

3.3. Microplastic abundance and distribution in the Arctic Ocean

Based on the underway samples (collection depth 8.5 m), microplastic abundance in sub-surface waters in the Arctic Ocean ranged between 0 and 7.5 particles m^{-3} with a median of 0.7 particles m^{-3} (interquartile range 0.4–1.0), (Fig. 2, Supplementary Table 5). For the majority of the sampling sites, microplastic abundance ranged between 0 and 1.0 particles m^{-3}. However, at a few sites, microplastic abundances were between 2 and 2.5 particles m^{-3} and at two sites it was at 5 and 7.5 particles m^{-3} respectively. Based on the CTD samples (collection depths between 8 and 4369 m), microplastic abundance in sub-surface waters in the Arctic Ocean ranged between 0 and 375 particles m^{-3} with a median of 20.8 particles m^{-3} (interquartile range 20.8–62.5) (Figs. 2 and 5, Supplementary Table 6). With the exception of CTD cast 4, the CTD casts (1–3) nearer the periphery of the Arctic Central Basin (ACB), i.e. in the Nansen Basin (Yermak Plateau), reflected a comparatively higher abundance of microplastics in the water column than other CTD casts within the ACB (Figs. 2, 6). It must be noted however that CTD casts 1–3 sampled the upper 850 m of the water column and as such would have sampled particles from the Polar Mixed Layer (PML), Atlantic halocline and Atlantic water (Figs. 1, 6). This is in contrast to the other CTD casts which sampled a much more extensive vertical range throughout the water column by including deep bottom water in excess of 1000 m depth. Overall, there was no statistically significant correlation between microplastic abundance and depth (Spearman's rank correlation, rho = 0.06, p-value = 0.7). However, upon examination of individual CTD casts, it is apparent that microplastic abundance was not uniform at various depths in the water column and that there were certain depths that reflected higher microplastic abundances (Fig. 6). Additionally, microplastic abundance (particles m^{-3}) in the different water masses of the ACB was as follows: Polar Mixed Layer (0–375) > Deep and bottom waters (0–104) > Atlantic water (0–95) > Halocline i.e. Atlantic or Pacific (0–83), (Table 1).

3.4. Association between environmental variables and microplastic abundance in samples

Correlation analyses were conducted to determine whether there was any association between environmental variables and microplastic abundance in the samples. For both the underway and CTD samples, there was no statistically significant correlation between microplastic abundance and any of the ancillary environmental variables of temperature, salinity, wind direction, wind speed, depth and density

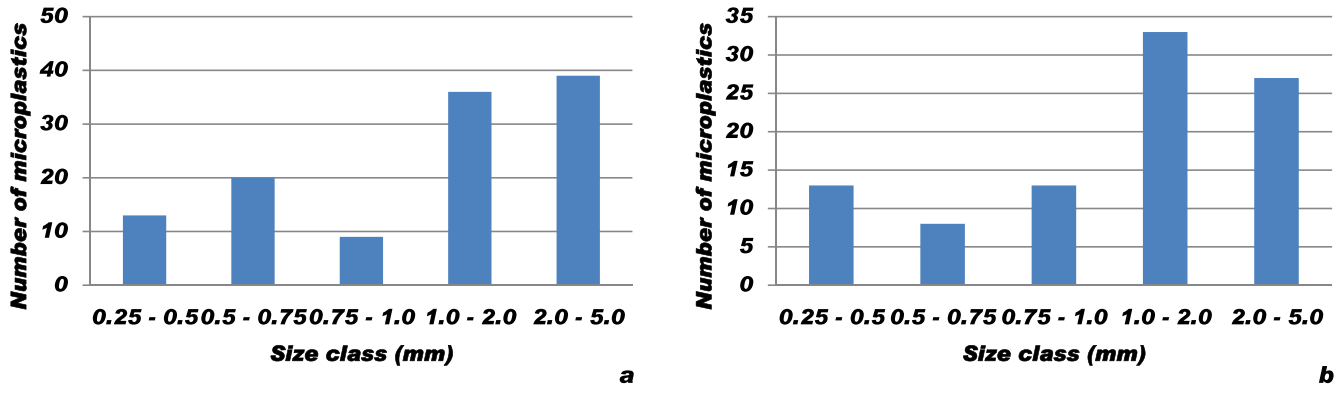

Fig. 4. Size classes of microplastics found in (a) underway and (b) CTD samples at depths 8–4369 m.

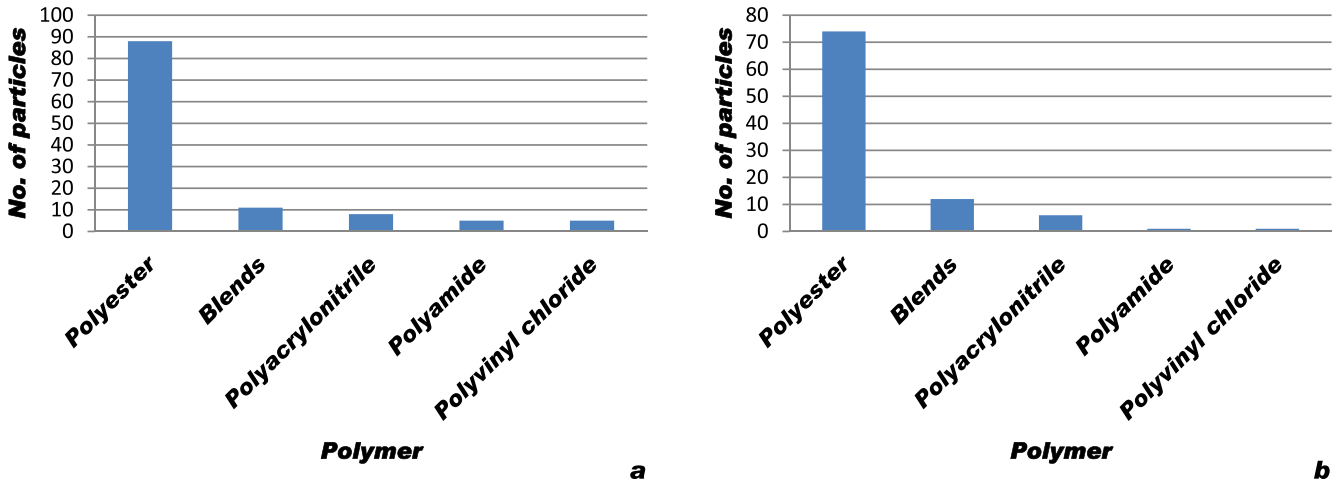

Fig. 5. Synthetic polymers found in (a) underway and (b) CTD samples at depths 8–4369 m.

(Supplementary Tables 1 and 2). Specifically, there was no statistically significant correlation between microplastic abundance at depth (Supplementary Table 2). However, for the underway samples, there was a statistically significant weak negative correlation between microplastic abundance and latitude (Spearman's rank correlation, rho = −0.286, p-value = 0.03).

Using the underway data, a Generalized Additive Model (GAM) was developed to further determine whether environmental variables influenced microplastic count in the underway samples. In this model, the response variable was microplastic count (number of microplastics per sample) and initial explanatory variables included location (latitude, longitude), physico-chemical properties associated with sub-surface waters (temperature, salinity) and weather data (wind direction, wind speed). In the model, the Poisson family distribution of error terms was specified with a log link function since microplastic abundance data were count data. The output of the initial model was examined and based on this non-parametric smoothers were accordingly applied to the explanatory variables. A scale invariant tensor product smooth (te) was applied to latitude and longitude while a cubic regression spline (bs = cr) was applied to all other variables except wind direction to which no smoother was applied (based on initial model plots). Non-significant explanatory variables (as evidenced by their p-values) were eliminated in a stepwise manner until a GAM with the lowest Akaike Information Criterion (AIC) score and the fewest explanatory variables was obtained. The final GAM (R-sq = 0.396) was as shown below:

$$\text{Microplastic count} \sim \text{te}(\mathbf{latitude}, \mathbf{longitude}) + \text{s}(\mathbf{temperature}, \text{br} = \text{“cr”}) + \text{s}(\mathbf{wind\ speed}, \text{bs} = \text{“cr”})$$

All of the explanatory variables that were present in the final model (shown in bold) were found to have a significant influence on microplastic count in water samples from the Arctic Ocean (wind speed, p-value = 0.0006, latitude, longitude, p-value = 0.0007, temperature, p-value = 0.0483).

A generalized linear mixed model (GLMM) was developed using the CTD data to determine the influence of environmental variables on microplastic count in the CTD samples. In this model, the response variable was microplastic count (number of microplastics per sample) and initial explanatory variables included location (latitude, longitude), physicochemical properties associated with sub-surface waters (temperature, salinity), depth at which water was sampled and ctd number. All the explanatory variables were included in the model as fixed effects, with the exception of ctd number which was included as a random effect. In the model, the Poisson family distribution of error terms was specified with a log link function since microplastic abundance data were count data. Based on the preliminary finding that there was a statistically significant correlation between depth and salinity (Spearman's rank correlation, rho = 0.852, p-value = 8.156e−13), temperature and salinity (Spearman's rank correlation, rho = 0.506, p-value = 0.00074) and depth and density (Spearman's rank correlation, rho = 0.973, p-value ≤ 2.2e−16), interactions between these variables (denoted by ‘:’) were included in the initial model. Non-significant explanatory variables (as evidenced by their p-values) were eliminated in a stepwise manner until a model with the lowest Akaike Information Criterion (AIC) score and the fewest explanatory variables was obtained. The significance of the random effect (ctd number) in the final model was verified by using analysis of variance (ANOVA) to compare the full final model to a reduced model (random effect deleted). The model with the random effect was shown to be significantly different (ANOVA, p-value = 0.0008, ΔAIC = 9.19) from the model without the random effect. The model with the lower AIC score (AIC = 190.59) was retained as the final mixed effects model as shown below:

$$\text{Microplastic count} \sim \mathbf{latitude} + \mathbf{temperature}{:}\ \mathbf{salinity} + (1 \mid \text{ctd})$$

Latitude (p-value = 0.0198) and the physicochemical parameters of temperature and salinity (p-value = 7.46e−05), as shown in bold, were the explanatory variables that were found to have a significant influence on microplastic count in the CTD samples.

4. Discussion

The discovery of microplastics in virtually every environmental phase (sea ice, water, sediments, biota) of the Arctic and Southern Oceans has revealed that polar oceans, though remote, are not immune to the entry of plastic contaminants to their ecosystems (Bergmann and Klages, 2012; Obbard et al., 2014; Lusher et al., 2015; Amélineau et al., 2016; Bergmann et al., 2016; Bergmann et al., 2017a; Bergmann et al., 2017b; Cincinelli et al., 2017; Cózar et al., 2017; Isobe et al., 2017; Tekman et al., 2017; Waller et al., 2017). The present study expands the knowledge base about plastics in the Arctic by providing evidence for the existence of microplastics in the Polar Mixed Layer (PML) as well as some insight into the vertical distribution of microplastics in the Arctic Central Basin (ACB). This region of the Arctic, though of low productivity, has been recognised as an Ecologically/Biologically Significant Marine Area (EBSA) due to its uniqueness/rarity, provision of a critical habitat and ability to support specialised biota (CAFF, 2017). There is cause for concern about microplastics in Arctic waters since laboratory studies have shown that these contaminants may (i) hinder algal photosynthesis/growth (Bhattacharya et al., 2010; Besseling et al.,

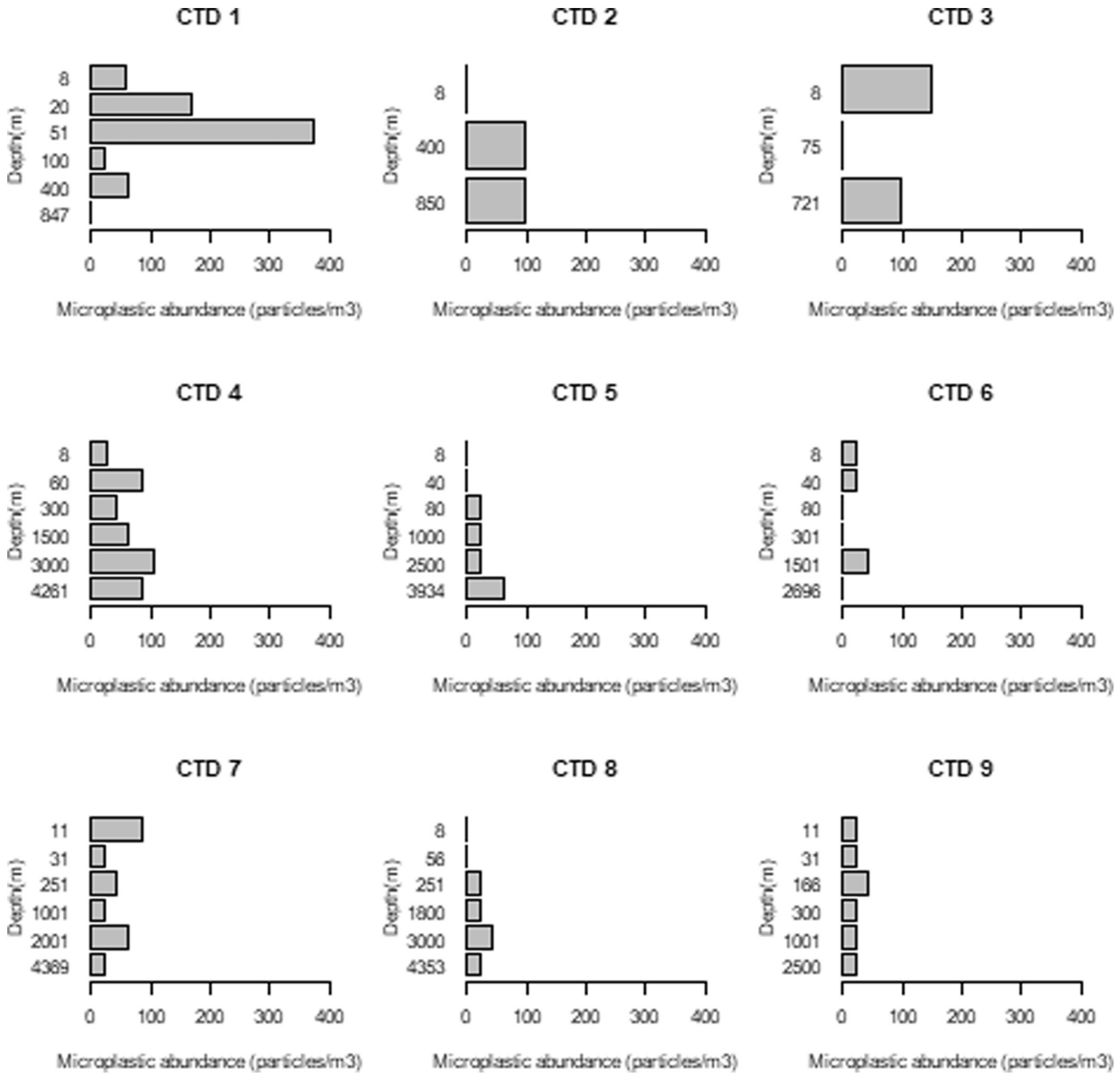

Fig. 6. Microplastic abundance from the various CTD casts in the Arctic Ocean.

Table 1
Microplastic abundance in the various water layers of the Arctic Central Basin (ACB).

Water mass	Depths sampled (m)	Number of depths sampled	Microplastic abundance (particles m^{-3})
Polar Mixed Layer (PML)	8–51	15	0–375
Halocline (Atlantic or Pacific)	56–166	7	0–83
Atlantic water	251–850	10	0–95
Deep & bottom waters	1001–4369	16	0–104

2014), (ii) reduce feeding and energy reserves of lugworms (Besseling et al., 2013; Wright et al., 2013), (iii) reduce filtering activity and decrease lysosomal membrane stability in mussels (von Moos et al., 2012; Wegner et al., 2012), (iv) reduce feeding and reproductive output in copepods (Cole et al., 2015) and, (v) cause liver stress, negatively impact upon cholinergic neurotransmission and lead to endocrine disruption in fish (Oliveira et al., 2013; Rochman et al., 2013; Rochman et al., 2014). It must be pointed out, however, that some laboratory experiments which reported negative effects of microplastics on marine organisms used microplastic concentrations of 42 to 10,000 particles/mL or 42 million to 10 billion particles m^{-3} (Phuong et al., 2016). In context, microplastic abundance in the ACB as reported by the present study ranged from 0 to 7.5 particles m^{-3} (based on underway sampling) and 0–375 particles m^{-3} (based on CTD sampling). Although the ecological impact of microplastics upon the Arctic ecosystem presently remains unknown, it is plausible that these contaminants could pose a threat to its inhabitants.

The present study showed for the first time the pervasiveness of

microplastics throughout the water column of the Arctic Central Basin. Between depths of 8–4400 m, microplastic abundance in the ACB ranged between 0 and 375 particles m^{-3} (based on CTD sampling). Such findings provide evidence that in natural conditions microplastics are being vertically transported out of surface waters. These findings also give some indication as to the whereabouts of the 'missing plastic' from oceanic surface waters (Cózar et al., 2014; Eriksen et al., 2014). Recently, Courtene-Jones et al. (2017) also reported on microplastic abundance (70.8 particles m^{-3}) in deep oceanic waters (2227 m at the Rockall Trough, North East Atlantic Ocean) and similarly suggested the possibility of vertical re-distribution of microplastics within the water column. Although it remains unclear as to which mechanisms are specifically operating in the ACB to influence the vertical transport of particles, previous studies have provided several possibilities. Specifically, some laboratory experiments showed that aggregates of algae species (*Chaetoceros neogracile*, *Rhodomonas salina*) were capable of incorporating and concentrating microplastics and that the microplastics impacted the sinking rates of the aggregates (Long et al., 2015). In the Arctic Ocean, it is certainly plausible that marine aggregates may be playing a role in the vertical transport of microplastics due to the existence of phytoplankton in the ACB (CAFF, 2017) and the fact that transparent exopolymer particles (TEPs); which are excreted by algae and are important components of marine aggregates, have been reported in sub-surface waters as far north as the Fram Strait (Engel et al., 2017). Biofouling is another possibility as field studies have shown that plastic particles exposed to natural conditions became sufficiently fouled, had their average material density affected leading them to sink (Fazey and Ryan, 2016). The incorporation of microplastics into faecal matter is another means by which microplastics may be vertically transported out of surface waters given that laboratory experiments have shown that zooplankton may egest microplastics within densely packed faecal pellets which in natural conditions would sink or in some cases be eaten by other biota (Cole et al., 2016).

Sea ice is an integral component of the Arctic Ocean's ecosystem and as such possibly exerts an influence on microplastic abundance in sub-surface waters. Sea ice floating on the surface of the water column in the Arctic Ocean can potentially act as (i) a source of microplastics upon melting, (ii) a physical barrier to wind and as such reduce vertical mixing of surface waters and, (iii) a physical barrier to influx of polluted surface waters. Based on the analysis of sub-sections of four ice cores, sea ice in the Arctic Ocean was reported to contain orders of magnitude more microplastic than contaminated oceanic waters suggesting that sea ice potentially acts as both a sink and a source of microplastics (Obbard et al., 2014). Apart from Obbard et al. (2014) no data exists in the published literature regarding either the spatial or vertical distribution of microplastics in sea ice from the Arctic Ocean. In the upper water column, the absence of sea ice cover means that wind stress can generate turbulence and lead to vertical mixing of buoyant plastic debris (Kukulka et al., 2012). More recently, Cózar et al. (2017) suggested that sea ice can also act as a physical barrier preventing the surface advance of polluted Atlantic water into the Arctic Ocean. The present study highlighted that the Polar Mixed Layer (PML) of the ACB reflected the highest overall microplastic abundance (particles m^{-3}): Polar Mixed Layer (0–375) > Deep and bottom waters (0–104) > Atlantic water (0–95) > Halocline i.e. Atlantic or Pacific (0–83). As previously mentioned, the PML is the uppermost surface layer of low salinity water (approximately 50 m deep) formed as a result of sea ice melt and the influx of freshwater from riverine sources (Rudels et al., 1991; CAFF, 2013). It is possible that one of the reasons that the highest microplastic abundances were recorded in this layer is due to its proximity to microplastic sources such as melting sea ice as well as sea-going vessels (especially if they are releasing wastewater to the environment). Furthermore, in the present study, the highest microplastic abundances were reported nearer to the periphery of the Arctic Central Basin (ACB), i.e. in waters north of Svalbard. It is possible that the lack of permanent sea ice cover in this region of the Arctic allows incoming Atlantic water to have a greater influence on near surface waters thereby resulting in higher microplastic abundances. A recent circumpolar expedition of the Arctic Ocean similarly reported that the north eastern Atlantic sector of the Arctic was a hotspot of plastic debris due to the influence of incoming Atlantic water (Cózar et al., 2017).

The present study showed that there was a predominance of fibrous microplastics (> 90%) in sub-surface waters of the ACB. This dominance of fibres in sub-surface waters was similarly reported in (i) the north east Pacific Ocean (75%), (ii) the north east Atlantic Ocean (96%), (iii) south/southwest of Svalbard (95%), and (iv) the Atlantic Ocean (96%) (Desforges et al., 2014; Lusher et al., 2014; Lusher et al., 2015; Kanhai et al., 2017). Fibrous microplastics in the marine environment most likely originate from textile materials and fishing gear (Andrady, 2017). Studies have indicated that washing clothes may lead to the release of fibrous materials in the order of > 1900 fibres per wash or as much as 700,000 fibres per 6 kg load of acrylic fabric (Browne et al., 2011; Napper and Thompson, 2016). A recent study in the Ross Sea revealed that the highest concentration of fibrous microplastics (54%) was found close to the effluent of a sewage treatment plant at the scientific Mario Zucchelli Station, Antarctica (Cincinelli et al., 2017). Fibrous microplastics may enter the Arctic Ocean through a combination of long range transport processes (e.g. via oceanic currents, riverine input) or more in-situ activities such as the release of wastewater from vessels operating in the region. Another mechanism which was recently suggested as being responsible for plastic fibres in Arctic sea ice was atmospheric transport (Cózar et al., 2017). This is certainly plausible as there have been reports of the atmospheric fallout of synthetic polymers in both urban and sub-urban environments in France (Dris et al., 2016). It must be highlighted that while fibrous microplastics seem to be dominant in certain sub-surface waters, other studies conducted in surface Arctic waters reported a predominance of filaments (97%) and fragments (73%), (Amélineau et al., 2016; Cózar et al., 2017).

Analytical techniques such as FT-IR and Raman spectroscopy are a central component of microplastic studies which aim to provide unambiguous identification of synthetic polymers in environmental samples. Omission of this critical step is likely to be a key contributor to an overestimation of microplastic abundances due to the inclusion of non-synthetic polymers in microplastic counts. In the present study, for example, only a percentage (underway - 48%, CTD - 67%) of the particles were confirmed as synthetic polymers with the remainder being a combination of natural and semi-synthetic polymers. Of the synthetic polymers in the present study, the most abundant (underway – 74%, CTD – 78%) was polyethylene terephthalate (PET). A member of the polyester family, PET is one of the five major types of commodity plastics commonly found in the marine environment (Andrady, 2011; Andrady, 2017). As a thermoplastic, PET is often used in manufacturing beverage containers and packaging materials and its fibres are used in clothing. Overall, this finding of a high prevalence of polyesters in sub-surface waters of the Arctic Central Basin was also corroborated by those of other Arctic studies which reported that polyester was the most prevalent synthetic polymer in waters south/southwest of Svalbard (15%), in waters of the east Greenland Sea (53%), and in sea ice (21%), (Obbard et al., 2014; Lusher et al., 2015; Amélineau et al., 2016). Other polymers in sub-surface Arctic waters in this study included polyacrylonitrile, polyamide and polyvinyl chloride. Of note is the fact that the majority of synthetic polymers in the present study had densities greater than that of seawater (Andrady, 2017). Even though investigators are able to generate information regarding the identity of polymers in environmental samples, definitive statements cannot be made about the origin of the plastics. Based on the identity and type of synthetic polymers found in sub-surface Arctic waters, it is likely that they originated from textiles, fishing gear, beverage containers and packaging materials (Andrady, 2011; Andrady, 2017).

Within the water column, the distribution of microplastics is in a

state of flux due to the influence of multiple factors. The development of models based on simultaneously acquired environmental and microplastic data is immensely useful in this respect as they can provide some discernment regarding the variables influencing measures of microplastic abundances in the marine environment. In the present study, the utilisation of a generalized additive model (GAM) and a generalized linear mixed effects model (GLMM) was particularly relevant due to the ability of both models to handle non-normal data and in the case of the GLMM to differentiate between fixed and random effects. Visual inspection of microplastic abundances in sub-surface waters (Fig. 2) revealed that the highest microplastic abundances were located to some extent towards the periphery of the Arctic Central Basin (ACB). Bearing this in mind, it was presumed that the 'location' of sampling could have influenced the number of microplastics that were found in the samples. Cózar et al. (2017) also noted this spatial heterogeneity in the distribution of plastics in the Arctic. For these reasons, latitude and longitude were included as a proxy of location in the models. In the GAM, both variables were included using a smoother and therefore it was not assumed that latitude and longitude had a linear effect on microplastic count. Furthermore, during the period of sampling (August/September 2016), the Arctic Central Basin (ACB) was not completely covered by sea ice. Since the vessel traversed areas of open water, wind was included in the model as it could have influenced microplastic abundance at specific sampling sites. Both models suggested that location, oceanographic (temperature, salinity) and atmospheric variables (wind) had a significant influence on microplastic counts in samples of water from the Arctic Ocean. The findings of the present study must be taken in the context of the number of samples used in the generation of the GAM (n = 58). Models that are based on a low number of samples are weak e.g. standard errors are inflated, etc. However, such models allow us to delve a bit deeper into the factors influencing measurements of microplastic abundance in the Arctic Ocean. Findings of the present study were also corroborated by previous studies which indicated that water temperature, salinity and wind also had a significant effect on microplastic abundance (Lusher et al., 2014; Lusher et al., 2015; Kanhai et al., 2017).

Comparative assessments between oceanic basins are critical in providing an indication of the extent of microplastic pollution in the marine environment. A major challenge, which demands caution when drawing conclusions from such comparisons, is the lack of standardization of microplastic sampling methods (depth of collection, mesh size of net/sieve, etc.). Microplastic abundances in the present study were not normally distributed and therefore the median was reported as it is the most relevant measure of central tendency for such data. However, in order to enable comparability with other published studies, which generally did not report median microplastic abundances nor made statements about the normality of their data, the mean was also reported in Supplementary Table 7. In the present study, sub-surface waters (depth 8.5 m) in the Arctic Central Basin (ACB), sampled via the bow water system, had a mean microplastic abundance of 0.97 ± 1.20 particles m^{-3}. In comparison to other studies that employed similar methods (i.e. the underway system of vessels) to sample sub-surface waters, microplastic abundance in the ACB was lower than values reported (i) in the north eastern Pacific Ocean (279 ± 178 particles m^{-3}), (ii) in the North Atlantic Ocean (13–501 particles m^{-3}), (iii) off Svalbard (2.68 ± 2.95 particles m^{-3}), (iv) in the north east Atlantic Ocean (2.46 ± 2.43 particles m^{-3}), and (v) in the Atlantic Ocean (1.15 ± 1.45 particles m^{-3}), (Desforges et al., 2014; Lusher et al., 2014; Enders et al., 2015; Lusher et al., 2015; Kanhai et al., 2017, Supplementary Table 7). The only oceanic basin for which there were reports of lower microplastic abundances in sub-surface waters was the Ross Sea (0.17 ± 0.34 particles m^{-3}), (Cincinelli et al., 2017; Supplementary Table 7). Although the methods used to sample sub-surface waters for microplastics in the above mentioned studies were similar, the fact remains that the variation of several factors e.g. mesh size of sieve (1–300 μm), sampling depth (3–11 m), etc., amongst the studies could have impacted the reported microplastic abundances. In terms of assessing the vertical distribution of microplastics in the marine environment, Bagaev et al. (2017) was the only other published study which utilised a similar sampling method (Niskin bottles) in the Baltic Sea. Being cognisant of the fact that no confirmatory analytical techniques or blanks were used by Bagaev et al. (2017), microplastic abundance at multiple depths in sub-surface waters of the Arctic Ocean (mean: 46 ± 62 particles m^{-3}; range: 0–375 particles m^{-3}; depths sampled: 8–4400 m) was lower than reported for the Baltic Sea (mean: 310 ± 520 particles m^{-3}; 70–2600 particles m^{-3}; depths sampled: 1–218 m). Similar to the findings of the present study whereby the highest microplastic abundances were found in the uppermost water layer i.e. the PML, Bagaev et al. (2017) reported that near- surface and near-bottom water layers in the Baltic Sea had higher fibre concentrations than intermediate layers and that this was possibly due to greater turbulence and density stratification in those layers. Of note is the fact that microplastic abundance in deep waters of the ACB (0–104 particles m^{-3}, depths 1000–4400 m, sieve 250 μm) was similar to those reported for deep waters at the Rockall Trough, North East Atlantic Ocean (70.8 particles m^{-3}, depth 2227 m, sieve 80 μm), (Courtene-Jones et al., 2017). Overall, it must be acknowledged that an underestimation of microplastic abundance in the Arctic Central Basin (ACB) could have occurred in the present study as the mesh size of the sieve was only 250 μm leading to an exclusion of smaller sized particles. Nevertheless, the Arctic's remote geographic location away from major population centres, its low population in its surrounding continental shelves and relatively low in-situ anthropogenic activities (e.g. shipping) are all factors which may explain the lower microplastic abundances in sub-surface waters within the Arctic Central Basin (ACB). From an oceanographic perspective, the reduced contribution of Atlantic water in its upper water layers due to the dominance of the polar mixed layer is another possible explanation for lower microplastic abundances in the Arctic in comparison to other oceanic basins. Presumably more polluted, Atlantic water which originates from the more densely populated southern latitudes has its surface advance into the Arctic Ocean hindered due to freshwater released from melting ice and other physical barriers such as the sea ice itself and the Novaya Zemlya islands (Cózar et al., 2017). However, a plausible future scenario for the Arctic in the context of a changing climate is that microplastic abundance in near-surface layers of the Arctic Ocean may increase upon melting of contaminated sea ice and opening up of shipping lanes due to a decrease in sea ice extent (Obbard et al., 2014; Cózar et al., 2017).

Of interest is the fact that the present study managed to sample microplastics in sub-surface waters at approximately 8.5 m depth by two independent methods i.e. by the bow water system of the vessel (underway sampling) and the rosette water sampler (CTD sampling). CTD samples (n = 9) retrieved from an average depth of 8.5 m indicated that microplastic abundance in the ACB ranged between 0 and 148 particles m^{-3}, with a median of 20.8 particles m^3. By comparison, samples collected via the underway system (n = 58) at 8.5 m indicated that microplastic abundance in the ACB ranged between 0 and 7.5 particles m^3, with a median of 0.7 particles m^3. Although both methods sampled water at an average depth of 8.5 m, calculated microplastic abundances from both methods are not directly comparable due to the differences associated with the methods. Whereas underway sampling involved filtration of a greater volume of water (approximately 2000 L) over a longer distance and a longer sampling time (> 2 h), the CTD sampling involved the collection and subsequent filtration of a smaller volume of water (21 L or 48 L) at a single location in a shorter period of time (minutes). The advantage of using the underway system is that microplastic abundances over a larger spatial area can be quantified while the vessel is in transit. By comparison, CTD sampling facilitates the quantification of microplastic abundance at specific locations making it less likely to mask contamination hotspots. However, some of the major limitations associated with CTD

microplastic sampling are (i) the vessel must stop at sampling stations to collect samples, (ii) deployment and retrieval of the rosette water sampler is time consuming and, (iii) only small volumes of water can be collected in comparison to the underway sampling. The limitation of filtering smaller volumes of water is twofold in that there can be (i) false negatives whereby microplastics are not sampled despite being present in the environment or, (ii) microplastics are found in the samples but scaling up to relevant units (particles m^{-3}) has a greater effect on microplastic abundances.

One of the major challenges that investigators face when quantifying marine microplastic abundance is sample contamination. In addition to employing strict measures to control contamination during sampling and processing, it is important that checks are carried out to quantify potential contamination of samples. For underway samples in the present study, although method blanks were free of contamination by synthetic particles, a single synthetic fibre was found in one air contamination check. In context, there were between 0 and 15 synthetic particles in each underway sample, with an average of 2 synthetic particles per sample. For CTD samples in the present study, between 0 and 3 synthetic fibres were found in the method blanks. In context, between 0 and 18 synthetic particles were found per CTD sample (21 L or 48 L), with an average of 2 particles per sample. In both cases, if contamination were an issue, its contribution to the reported microplastic abundances in the present study would be substantial. However, the possibility of airborne contamination in the actual underway samples is projected to be low since (i) 92% of the air contamination checks (11 of the 12 petri dishes) were free of synthetic particles and, (ii) air contamination checks had maximum exposure to the atmosphere while actual samples had minimal exposure. With respect to the CTD samples, it is proposed that synthetic fibres in the method blanks may have been introduced into the Niskin bottle during the transfer of Milli-Q water or could have been present from the previous CTD cast and remained in the bottle due to insufficient rinsing with Milli-Q water prior to the blank. This should not have been an issue for the actual samples since Niskin bottles were rinsed during the downcast and were closed within the water column thus preventing the possibility of airborne contamination.

5. Conclusion

The present study demonstrated the pervasiveness of microplastics in sub-surface waters of the Arctic Central Basin (ACB). Two independent sampling techniques led to the discovery of microplastics in near surface waters of the Polar Mixed Layer (PML) i.e. at a single depth of 8.5 m as well as throughout the water column i.e. at multiple depths (8–4369 m) of the ACB. Such findings confirm that microplastics are entering the central Arctic Ocean, that they are being vertically transported out of surface waters and that the water column is one of the reservoirs of microplastics in this region. Presently, however, uncertainty exists regarding the actual mechanisms responsible for the vertical transport of microplastics in the Arctic Ocean. Although there was a predominance of fibrous microplastics, the majority of which were polyester, the exact sources of microplastics to the Arctic Ocean remain unknown as they could have been introduced to the ecosystem via long range transport processes or originated from more local sources. The fact that the highest microplastic abundances were recorded in the PML nearer to the periphery of the ACB suggests the influence of location-specific factors e.g. absence of sea ice, proximity to microplastic sources, wind, etc. Knowledge about microplastic abundance, distribution and composition in the Arctic Ocean is vital as it provides (i) quantitative data on the concentrations and types of microplastics that polar organisms are exposed to, (ii) a sound starting point for investigating the potential threat that microplastics pose to the Arctic ecosystem and, (iii) insight into the whereabouts of the 'missing plastic' from oceanic surface waters.

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Appendix A. Supplementary data

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APPENDIX V

Published Manuscript II Supplementary Tables

Kanhai, L.K., Gårdfeldt, K., Lyashevska, O., Hassellöv, M., Thompson, R.C., O'Connor, I., 2018. Microplastics in sub-surface waters of the Arctic Central Basin. Marine Pollution Bulletin 130, 8-18.

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Microplastics in sub-surface waters of the Arctic Central Basin

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Supplementary Table 1: Descriptive statistics, tests of normality and correlation findings for environmental variables (underway data)

Descriptive Statistics	**Temperature**	**Salinity**	**Wind Speed**	**Wind Direction**
Min	-1.799942	6.6622757	1.62352	6.74E+01
Max	6.5476454	34.00517	20.5199	3.34E+02
Range	8.3475874	27.342894	18.8964	2.67E+02
Median	-1.495973	29.147106	7.52099	2.39E+02
Mean	-1.241312	28.184244	8.50391	2.20E+02
SE.mean	0.1628863	0.6942569	0.51361	9.36E+00
CI.mean	0.3261742	1.3902254	1.02848	1.87E+01
Var	1.5388528	27.955572	15.2999	5.08E+03
Std.dev	1.2405051	5.2873029	3.91151	7.13E+01
Coef.var	-0.9993499	0.1875978	0.45997	3.24E-01
Skewness	5.083195	-2.446492	0.81825	-0.329744
Kurtosis	29.90303	9.672563	3.53424	2.005591
Shapiro Wilk Normality Test				
(p value)	7.11E-15	3.56E-09	0.02707	0.02597
Anderson-Darling Test				
(p value)	< 2.2e-16	2.94E-12	0.03416	0.01846
*Spearman's rank correlation				
rho	0.038	0.17369	-0.0943	-0.1113
p value	0.7769	0.1922	0.4811	0.4056

*All correlation analyses were between microplastic abundance and the specified parameter

Supplementary Table 2: Descriptive statistics, tests of normality and correlation findings for environmental variables (CTD data)

Descriptive Statistics	**Depth**	**Temperature**	**Salinity**	**Density**
Min	8.00	-1.80	28.90	1023.3
Max	4369.00	3.96	35.00	1048.4
Range	4361.00	5.75	6.14	25.16
Median	275.50	-0.49	34.90	1029.22
Mean	913.90	-0.19	33.80	1031.56
SE.mean	200.35	0.22	0.28	1.02
CI.mean	404.62	0.44	0.56	2.06
Var	1.69E+06	1.99	3.22	50.28
Std.dev	1.29E+03	1.41	1.80	7.09
Coef.var	1.42	-7.25	0.0531	-0.0069
Skewness	1.53	1.14	-1.53	1.04
Kurtosis	4.19	3.79	4.01	3.09
Shapiro Wilk Normality Test (p value)	1.49e-07	5.0e-04	3.321e-08	9.688e-05
Anderson-Darling Test (p value)	1.976e-11	4.0e-04	2.859e-14	2.063e-05
*Spearman's rank correlation				
rho	0.06	0.21	0.09	0.21
p value	0.70	0.19	0.56	0.15

*All correlation analyses were between microplastic abundance and the specified parameter

Supplementary Table 3: Quality control checks associated with the underway sampling

Method blank	Number of microplastics	Air contamination check	Number of microplastics
1	0	1	0
2	0	2	0
3	0	3	0
4	0	4	0
5	0	5	0
6	0	6	0
7	0	7	0
8	0	8	0
9	0	9	0
10	0	10	0
11	0	11	0
12	0	12	1

Supplementary Table 4: Quality control checks associated with CTD sampling

	Number of synthetic polymers found in method blanks			
CTD Cast	**Polyethylene terephthalate (PET)**	**Polyvinyl chloride (PVC)**	**Polyacrylonitrile**	**Total number**
1	1	0	0	1
3	1	1	0	2
4	3	0	0	3
5	3	0	0	3
6	0	0	1	1
8	0	0	0	0

Supplementary Table 5: Number of microplastics and calculated microplastic abundances for the underway samples

Date	Sample	Latitude	Longitude	Number of microplastics	Microplastic abundance (particles m^{-3})
09-08-16	1	78.8495	9.39896	5	2.5
10-08-16	2	80.5442	8.03564	4	2.0
11-08-16	3	80.5066	8.03402	3	1.5
11-08-16	4	80.1287	6.08353	2	1.0
11-08-16	5	80.4314	6.55955	1	0.5
12-08-16	6	80.9713	8.02501	15	7.5
12-08-16	7	81.5383	9.7702	4	2.0
12-08-16	8	82.2486	10.9693	1	0.5
12-08-16	9	83.0332	12.6703	10	5.0
13-08-16	10	83.6607	13.752	1	0.5
13-08-16	11	84.7648	14.101	2	1.0
13-08-16	12	85.575	11.2663	2	1.0
15-08-16	13	88.5031	-6.6458	0	0.0
15-08-16	14	88.7435	-12.546	0	0.0
17-08-16	15	88.9613	-78.621	1	0.4
20-08-16	16	88.6493	-125.86	1	0.4
22-08-16	17	89.7614	43.7088	3	1.0
23-08-16	18	88.5118	-123.88	2	1.0
23-08-16	19	88.6097	-121.8	2	0.9
24-08-16	20	88.3172	-125.31	1	0.4
25-08-16	21	87.9254	-130.91	1	0.5
25-08-16	22	87.6501	-133.3	2	1.0
26-08-16	23	86.7524	-140.86	1	0.5
27-08-16	24	86.9682	-138.16	4	1.7
27-08-16	25	86.9868	-135.84	3	1.5
28-08-16	26	86.8767	-135.06	2	1.0
28-08-16	27	86.2319	-138.81	1	0.4

Supplementary Table 5: Number of microplastics and calculated microplastic abundances for the underway samples

Date	Sample	Latitude	Longitude	Number of microplastics	Microplastic abundance (particles m^{-3})
29-08-16	28	85.9511	-148.01	0	0.0
29-08-16	29	85.279	-146.58	0	0.0
30-08-16	30	84.4969	-143.82	1	0.5
30-08-16	31	83.6959	-143.37	4	2.0
02-09-16	32	82.394	-141.82	2	1.0
02-09-16	33	82.293	-143.86	1	0.4
03-09-16	34	82.4763	-149.69	2	1.0
03-09-16	35	82.6463	-154.18	2	1.0
03-09-16	36	83.1181	-159.13	1	0.3
04-09-16	37	84.1428	-164.44	0	0.0
04-09-16	38	84.6056	-169.94	2	0.9
04-09-16	39	84.9017	-175.91	1	0.5
04-09-16	40	85.3211	176.568	2	0.9
05-09-16	41	86.3528	174.321	1	0.5
05-09-16	42	87.0606	175.479	2	1.0
06-09-16	43	87.9905	-179.75	3	1.5
06-09-16	44	87.8512	168.207	0	0.0
10-09-16	45	88.7471	61.6513	1	0.5
10-09-16	46	88.3891	42.1193	1	0.4
11-09-16	47	88.1952	24.3452	2	1.0
11-09-18	48	88.0011	11.3847	2	1.0
12-09-16	49	87.6096	6.37678	0	0.0
12-09-16	50	86.959	10.9778	1	0.4
13-09-16	51	85.4929	15.6535	1	0.5
13-09-16	52	85.1201	16.0128	2	1.0
14-09-16	53	84.2656	17.3778	5	2.5
14-09-16	54	83.9565	17.018	0	0.0

Supplementary Table 5: Number of microplastics and calculated microplastic abundances for the underway samples

Date	Sample	Latitude	Longitude	Number of microplastics	Microplastic abundance (particles m^{-3})
15-09-16	55	82.7728	18.8161	1	0.5
15-09-16	56	82.4838	18.381	1	0.5
16-09-16	57	81.5187	15.6088	2	1.0
16-09-16	58	81.1909	12.8995	0	0.0

Supplementary Table 6: Number of microplastics and calculated microplastic abundances for each CTD cast

Date	CTD Cast	Depth (m)	Latitude	Longitude	# of microplastics	Microplastic abundance (particles m^{-3})
10-08-16	1	847	80.56276	8.03511	0	0
10-08-16	1	400	80.56276	8.03511	3	63
10-08-16	1	100	80.56276	8.03511	1	21
10-08-16	1	51	80.56276	8.03511	18	375
10-08-16	1	20	80.56276	8.03511	8	167
10-08-16	1	8	80.56276	8.03511	3	56
10-08-16	2	850	80.56208	8.00078	5	95
10-08-16	2	400	80.56208	8.00078	5	95
10-08-16	2	8	80.56208	8.00078	0	0
11-08-16	3	721	80.1349	6.14062	5	95
11-08-16	3	75	80.1349	6.14062	0	0
11-08-16	3	8	80.1349	6.14062	7	148
22-08-16	4	4261	89.66148	52.0587	4	83
22-08-16	4	3000	89.66148	52.0587	5	104
22-08-16	4	1500	89.66148	52.0587	3	63
22-08-16	4	300	89.66148	52.0587	2	42
22-08-16	4	60	89.66148	52.0587	4	83
22-08-16	4	8	89.66148	52.0587	1	28
23-08-16	5	3934	88.6113	-121.77868	3	63
23-08-16	5	2500	88.6113	-121.77868	1	21
23-08-16	5	1001	88.6113	-121.77868	1	21
23-08-16	5	80	88.6113	-121.77868	1	21
23-08-16	5	40	88.6113	-121.77868	0	0
23-08-16	5	8	88.6113	-121.77868	0	0
26-08-16	6	2696	86.75014	-140.88221	0	0
26-08-16	6	1501	86.75014	-140.88221	2	42
26-08-16	6	301	86.75014	-140.88221	0	0

Supplementary Table 6: Number of microplastics and calculated microplastic abundances for each CTD cast

Date	CTD Cast	Depth (m)	Latitude	Longitude	# of microplastics	Microplastic abundance (particles m^{-3})
26-08-16	6	80	86.75014	-140.88221	0	0
26-08-16	6	40	86.75014	-140.88221	1	21
26-08-16	6	8	86.75014	-140.88221	1	21
10-09-16	7	4369	88.06206	80.27812	1	21
10-09-16	7	2001	88.06206	80.27812	3	63
10-09-16	7	1001	88.06206	80.27812	1	21
10-09-16	7	251	88.06206	80.27812	2	42
10-09-16	7	31	88.06206	80.27812	1	21
10-09-16	7	11	88.06206	80.27812	4	84
11-09-16	8	4353	88.04016	10.04138	1	21
11-09-16	8	3000	88.04016	10.04138	2	42
11-09-16	8	1800	88.04016	10.04138	1	21
11-09-16	8	251	88.04016	10.04138	1	21
11-09-16	8	56	88.04016	10.04138	0	0
11-09-16	8	8	88.04016	10.04138	0	0
14-09-16	9	2500	84.40488	17.45691	1	21
14-09-16	9	1001	84.40488	17.45691	1	21
14-09-16	9	300	84.40488	17.45691	1	21
14-09-16	9	166	84.40488	17.45691	2	42
14-09-16	9	31	84.40488	17.45691	1	21
14-09-16	9	11	84.40488	17.45691	1	21

Supplementary Table 7: Microplastic abundances reported for surface and sub-surface waters across the world

Location	**Microplastic abundance (particles per m^3)**	**Method for surface waters (unless otherwise indicated)**	**Study**
Arctic Ocean			
Svalbard, Norway	0.34 ± 0.31; 0 – 1.31 (mean, range)	Manta trawl (333 µm)	Lusher et al. (2015)
	2.68 ± 2.95; 0 -11.5 (mean, range)	Underway system (250 µm)[a]	
Greenland Sea	0.99 ± 0.62; 0.15 – 2.64 (mean, range)	WP-2 net (500 µm) in 2005	Amélineau et al. (2016)
	2.38 ± 1.11; 0.81 – 4.52 (mean, range)	WP-2 net (500 µm) in 2014	
Central Arctic Ocean	**0.7; 0.97 ± 1.2; 0 – 7.5**	**Underway system (250 µm)[b]**	**This study**
	20.8; 46.4 ± 62.2; 0 – 375	**CTD rosette[c]**	
	(median, mean ± SD, range)		
Antarctic/Southern Ocean			
Ross Sea	0.17 ± 0.34; 0.003 – 1.18 (mean, range)	Underway system (1 µm)[d]	Cincinelli et al. (2017)
Southern Ocean	0.0035 – 0.099 (range)	Neuston net (350 µm)	Isobe et al. (2017)
Pacific Ocean			
Southern California, USA	7.25 (mean)	Manta trawl (333 µm)	Moore et al. (2002)
Santa Monica Bay, USA	3.92 (mean)	Manta net (333 µm)	Lattin et al. (2004)
South Californian current	0 – 3.141	Manta net (505 µm)	Gilfillan et al. (2009)
Southeast Bering Sea	0.004 – 0.19	Sameoto neuston/manta (505 µm)	Doyle et al. (2011)
NP Subtropical Gyre	0.425 (median)	Manta net (333 µm)	Goldstein et al. (2012)
North eastern Pacific Ocean	279 ± 178 (mean)	Underway system (62.5 – 250 µm)[e]	Desforges et al.(2014)
Geoje Island, South Korea	0.4 – 54	Manta trawl (330 µm)	Song et al. (2014)
East China Sea	0.167 ± 0.138 (mean)	Neuston net (333 µm)	Zhao et al. (2014)
Southern Sea of Korea	1.92 – 5.51; 2.3 – 38.77 (2012)	Manta trawl (330 µm)	Kang et al. (2015a)
	582 – 924; 10 – 375 (2013)	Hand Net (50 µm)	
Geoje and Jinhae Bays, Korea	1.92 ± 1.84; 5.51 ± 11.2 (2012)	Manta Trawl (330 µm)	Kang et al. (2015b)
	1.68 ± 0.81; 1.07 ± 0.34 (2013)		
East Asian Sea	3.7 ± 10.4; 0.03 – 491 (mean, range)	Neuston net (350 µm)	Isobe et al. (2015)

*Sub-surface waters sampled at the following depths ([a]6 m, [b]8.5 m, [c]8 – 4463 m, [d]5 m, [e]4.5 m)

Supplementary Table 7: Microplastic abundances reported for surface and sub-surface waters across the world

Location	**Microplastic abundance (particles per m^3)**	**Method for surface waters (unless otherwise indicated)**	**Study**
Indian Ocean			
Southeast South Africa	257.9 - 1215	WP-2 type net (80 µm)	Nel & Froneman (2015)
Atlantic Ocean			
Bristol Channel, UK	0 - 100	Lowestoft plankton sampler (270 µm)	Morris & Hamilton (1974)
Offshore Ireland	2.46 ± 2.43; 0 – 22.5 (mean, range)	Underway system (250 µm)[f]	Lusher et al. (2014)
Western English Channel	0.27	Plankton nets (200, 500 µm)	Cole et al. (2014)
Western English Channel	0.27	Plankton nets (200, 500 µm)	Cole et al. (2014)
Portuguese coastal waters	0.002 – 0.036	WP2 (180 µm), Neuston (280 µm), LH Plankton Recorder (335 µm)	Frias et al. (2014)
St. Peter/St. Paul Archipelago, Brazil	0.01	Plankton net (300 µm)	Ivar do Sul et al. (2013)
Western Tropical Atlantic Ocean	0.015 – 0.04	Manta trawl (300 µm)	Ivar do Sul et al. (2014)
North Atlantic Ocean	13 - 501	Underway system (10, 300 µm)[g]	Enders et al. (2015)
Atlantic Ocean	1.15 ± 1.45; 0 – 8.5 (mean, range)	Underway system (250 µm)[h]	Kanhai et al. (2017)
Mediterranean and European Seas			
West Coast, Sweden	167 – 2400 72 - 141	Plankton net (80 µm) Zooplankton net (450 µm)	Noren (2007)
West Sardinian Coast	0.15	Manta trawl (500 µm)	de Lucia et al. (2014)
Southwest Finland	0 – 0.74	Manta trawl (333 µm)	Magnusson et al. (2014)
Baltic Sea	10^2 - 10^4	WP2 net (90 µm mesh)	Gorokhova (2015)

*Sub-surface waters sampled at the following depths ([f]3 m, [g]3 m, [h]11 m)

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APPENDIX VI

Published Manuscript III Supplementary Tables

Kanhai, L.K., Johansson, C., Frias, J.P.G.L., Gardfeldt, K., Thompson, R.C., O'Connor, I., 2019. Deep sea sediments of the Arctic Central Basin: A potential sink for microplastics. Deep Sea Research Part I: Oceanographic Research Papers. https://doi.org/10.1016/j.dsr.2019.03.003

Supplementary Table 1: Site-specific information for sediment cores sampled for microplastics in the Arctic Central Basin

Sample No.[a]	Core ID	Latitude	Longitude	Location in the ACB	Water Depth (m)	Core Type[b]
1	AO16-1-GC1	80.5532	8.0520	Yermak Plateau	855	GC
2	AO16-2-PC1	88.5022	-6.6195	Amundsen Basin	4353	PC
3	AO16-3-TWC1	89.2530	-66.6097	Foot of Lomonosov Ridge	3777	TWC
4	AO16-4-TWC1	88.5290	-128.5048	Marvin Spur	3936	TWC
5	AO16-5-TWC1	89.0780	-130.5470	Crest of Lomonosov Ridge	1253	TWC
6	AO16-7-PC1	88.6332	-121.4477	Marvin Spur	3941	PC
7	AO16-8-GC1	86.7795	-140.6433	Alpha Ridge	2620	GC
8	AO16-9-TWC1	85.9557	-148.3258	Alpha Ridge	2212	TWC
9	AO16-10-TWC1	82.3980	-141.2450	Nautilus Basin	2872	TWC
10	AO16-11-TWC1	86.0993	173.1877	Makarov Basin	3066	TWC
11	AO16-12-TWC1	87.8577	136.9875	Crest of Lomonosov Ridge	1269	TWC

[a]Sample numbers correspond to those on Figure 1a; [b]GC (Gravity core); PC (Piston core); TWC (Trigger weight core)

Supplementary Information related to particle size and organic carbon content analysis

Method: For particle size analysis, approximately 0.1 g of wet sediment from each sediment core was transferred to a test tube. To this, 3 mL of sodium metaphosphate solution (10%) was added and the total volume brought to 10 mL using de-ionized water. The contents of the tube were stirred, ultrasonicated for approximately 30 seconds to facilitate disaggregation and then transferred to the Hydro LV wet dispersion unit of a Mastersizer 3000 laser diffraction particle size analyser (Malvern Instruments, UK). De-ionized water was used to bring the final volume in the wet dispersion unit to 600 mL prior to analysis. The particle size of surficial sediments from each core was based on the analysis of three sub-samples. Following Pagter et al. (2018), approximately 3 g of dried sediment (105 °C, 4 h) from each sample was placed into a furnace at 450 °C for 6 hours in order to estimate the organic content of the sediment samples based on loss on ignition. Grain size composition of the surficial sediment data was conducted using Gradistat Version 8 (Blott 2010).

Results: Regarding the particle size composition of the sediments, all sampled sites had a predominance of fine-grained sediments where the percentage of silt (59 – 87 %) > clay (10 - 24 %) > sand (1 – 23 %) > gravel (0 – 0.2 %), (Supplementary Table 2). Organic content of the surficial sediment samples ranged between 2.3 – 4.6 % (Supplementary Table 2).

Reference: Blott, S., 2010. Gradistat Version 8: A grain size distribution and statistics package for the analysis of unconsolidated sediments by sieving or laser granulometer. Kenneth Pye Associates Limited, Berkshire, UK.

Supplementary Table 2: Synthetic polymer composition and grain sizes of surficial sediments in the Arctic Central Basin

	Sample Number										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Grain size composition (%)											
Total Gravel (%)	0.0	0.0	0.0	0.1	0.0	0.1	0.2	0.0	0.0	0.1	0.0
Total Sand (%)	3.3	5.2	1.2	7.5	12.4	15.2	17.5	16.4	22.6	10.2	7.5
Total Silt (%)	86.9	73.6	76.4	68.4	70.5	65.6	63.4	63.0	59.3	69.8	73.7
Total Clay (%)	9.8	21.2	22.5	24.1	17.1	19.2	18.9	20.5	18.0	19.8	18.7
Organic content (%)	4.6	4.0	4.4	3.5	3.3	2.6	3.4	3.6	2.6	2.3	2.8
Polymer Type (n)											
Polyacrylonitrile (PAN)			1								
Polyamide (PA)					1						
Polyester (PES)		2									1
Polypropylene (PP)							1				
Polystyrene (PS)							1	1			
Polyvinyl chloride (PVC)				1							
Total number of synthetic polymers	0	2	1	1	1	0	2	1	0	0	1
Size (mm); Type (Fi-fibre; Fr-fragment) of microplastics		1.84 (Fi) 1.38 (Fi)	0.47 (Fi)	0.91 (Fr)	3.58 (Fi)		0.88 (Fr) 0.54 (Fr)	0.45 (Fr)			1.03 (Fr)
Microplastic concentration											
Mass of dry sediment used (g)	10.02	10.12	7.86	9.83	10.20	10.89	9.31	10.09	10.07	10.36	4.54
Microplastic concentration (items kg^{-1})	0	198	127	102	98	0	215	99	99	97	220

Supplementary Table 3: Microplastics in deep sea sediments of various oceanic basins

Location	**Depth (m)**	**Sampling Equipment**	**Extraction Method**	**Microplastic abundance**	**Synthetic polymers**	**Study**
Porcupine Abyssal Plain Nile Deep Sea Fan Atlantic Sector of the Southern Ocean	1176 – 4843	Multicorer	Density flotation, NaI (1.6 g cm^{-3})	0.5 particles cm^{-3} (average, n = 11) 1 particle cm^{-3} (max)	No data	Van Cauwenberghe et al. (2013)
Subpolar North Atlantic Ocean NE Atlantic Ocean Mediterranean Sea SW Indian Ocean	300 – 3500	Megacorers Boxcorers	Density flotation, NaCl, Ludox-TM 40 extraction	1.4 - 40 pieces per 50 ml (mean ± s.e., 13.4 ± 3.5)	PA, PES, Acrylic Rayon	Woodall et al. (2014)
Kuril-Kamchatka Trench, NW Pacific	4869 - 5768	Box corer	Sieve-washing of sediments	60 - 2020 pieces m^{-2}	No data	Fischer et al. (2015)
HAUSGARTEN observatory, Fram Strait	2340 - 5570	Multiple corer	Density separation, zinc chloride (1.8 g cm^{-3})	42 – 6595 microplastics kg^{-1} dry sediment	18 polymer types detected. Majority: PE, PA, PP	Bergmann et al. (2017)
Arctic Central Basin	855 - 4353	Gravity and piston corer	Density separation, sodium tungstate dihydrate (1.4 g cm^{-3})	0 - 200 microplastics kg^{-1} dry sediment	PA, PAN, PES, PP, PS, PVC	This study

PA-Polyamide, PAN-Polyacrylonitrile, PES-Polyester, PP-Polypropylene, PS-Polystyrene, PVC-Polyvinyl chloride

APPENDIX VII

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Deep sea sediments of the Arctic Central Basin: A potential sink for microplastics

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ABSTRACT

Deep sea sediments have emerged as a potential sink for microplastics in the marine environment. The discovery of microplastics in various environmental compartments of the Arctic Central Basin (ACB) suggested that these contaminants were potentially being transported to the deep-sea realm of this oceanic basin. For the first time, the present study conducted a preliminary assessment to determine whether microplastics were present in surficial sediments from the ACB. Gravity and piston corers were used to retrieve sediments from depths of 855–4353 m at 11 sites in the ACB during the Arctic Ocean 2016 (AO16) expedition. Surficial sediments from the various cores were subjected to density flotation with sodium tungstate dihydrate solution ($Na_2WO_4{\cdot}2H_2O$, density 1.4 g cm^{-3}). Potential microplastics were isolated and analysed by Fourier Transform Infrared (FT-IR) spectroscopy. Of the surficial samples, 7 of the 11 samples contained synthetic polymers which included polyester (n = 3), polystyrene (n = 2), polyacrylonitrile (n = 1), polypropylene (n = 1), polyvinyl chloride (n = 1) and polyamide (n = 1). Fibres (n = 5) and fragments (n = 4) were recorded in the samples. In order to avoid mis-interpretation, these findings must be taken in the context that (i) sampling equipment did not guarantee retrieval of undisturbed surficial sediments, (ii) low sample volumes were analysed (~10 g per site), (iii) replicate sediment samples per site was not possible, (iv) no air contamination checks were included during sampling and, (v) particles < 100 μm were automatically excluded from analysis. While the present study provides preliminary indication that microplastics may be accumulating in the deep-sea realm of the ACB, further work is necessary to assess microplastic abundance, distribution and composition in surficial sediments of the ACB.

1. Introduction

Microplastics are pervasive, persistent contaminants in the world's oceans that warrant concern due to the potential threat they pose to marine organisms. Traditionally, microplastic sampling has been conducted in surface and near-surface waters due to the presumption that the majority of microplastics would be present in that layer of the water column. However, when plastic production and projected plastic input to the ocean was considered, there was an evident mismatch between reported and expected plastic concentrations in surface oceanic waters (Cózar et al., 2014; Eriksen et al., 2014). It was therefore apparent that apart from surface waters, microplastics were present in various environmental compartments in the world's oceans (water column, sea ice, sediments, biota) and that some of these potentially functioned as sinks (Obbard et al., 2014; Woodall et al., 2014). Deep sea sediments have recently been identified as a potential sink for microplastics (Woodall et al., 2014; Bergmann et al., 2017). To date, only a few studies have reported on microplastics in deep sea sediments in various oceanic basins (Van Cauwenberghe et al., 2013; Woodall et al., 2014; Fischer et al., 2015; Bergmann et al., 2017). Despite the fact that each of these studies employed different sampling equipment, extraction techniques and reported microplastic abundance in different units, the consensus was that microplastics have made it to the deep-sea and that they are pervasive in its sediments. Presently, uncertainty still exists

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regarding the exact mechanisms that are responsible for the vertical transport of microplastics out of surface oceanic waters and into deep sea sediments.

The Arctic Ocean, though one of the most remote oceanic basins in the world, has been subject to the entry of plastic debris into its ecosystem. It has been suggested that this plastic debris, in particular microplastics, could have entered the Arctic ecosystem via a combination of (i) long-range transport processes, e.g. via oceanic currents (Zarfl and Matthies, 2010; Van Sebille et al., 2012), biotransport (Mallory, 2008; Provencher et al., 2012) and riverine input (Obbard et al., 2014) and, (ii) local anthropogenic activities, e.g. shipping (Tekman et al., 2017). Specifically, microplastics were discovered in the surface/sub-surface waters and sediments (Lusher et al., 2015; Bergmann et al., 2017; Cózar et al., 2017; Mu et al., 2019) of the Arctic. Further north, in the Arctic Central Basin (ACB), microplastics were recorded in sea ice, biota, such as juvenile polar cod (*Boreogadus saida*) and benthic organisms, and sub-surface waters (Obbard et al., 2014; Kanhai et al., 2018; Kuhn et al., 2018; Peeken et al., 2018; Fang et al., 2018). The fact that microplastics have been reported in the various water layers of the ACB, in particular its deep waters, suggests that these particles are pervasive in the water column and that they are being transported out of its surface waters (Kanhai et al., 2018). It was therefore hypothesized that microplastics would be present in deep sea sediments in the ACB. To our knowledge, the present study sought for the first time to determine whether microplastics were present in surficial sediments of the Arctic Central Basin (ACB) and to establish whether the deep sea in this oceanic basin is possibly acting as a sink for microplastics.

2. Material and methods

The Arctic Ocean, the world's smallest ocean, is comprised of a deep central basin surrounded by extensive continental shelves. The bathymetry of the Arctic Ocean is such that the Lomonosov Ridge divides the central basin into the Canadian (Amerasian) and Eurasian sub-basins (Jakobsson et al., 2004). Within each of the sub-basins, there are further divisions as well as the existence of Abyssal Plains (APs) which are deep water areas of low relief. In the Amerasian basin, the Alpha-Mendeleev Ridge separates the Canada Basin (with its Canadian AP) and the Makarov Basin (with its Fletcher AP) while in the Eurasian basin, the Gakkel Ridge separates the Amundsen Basin (with its Pole AP) and the Nansen Basin (with its Barents AP), (Jakobsson et al., 2004).

This study was conducted aboard the Swedish icebreaker Oden between August 8th to September 19th, 2016 during the Arctic Ocean 2016 expedition. During the transit of 4943 nautical miles, sediments were retrieved using a gravity corer or a piston corer with an associated trigger weight corer. Cores from 11 stations were sampled for microplastics (Supplementary Table 1, Fig. 1).

Cores were split longitudinally, wrapped in plastic film (polyethylene) and transported to the laboratory where they were processed prior to sampling for microplastics. Processing usually involved (i) scraping the core half (with a polypropylene scraper) to remove liner fragments and sediment disturbed during core splitting and, (ii) visually describing the lithostratigraphic properties. Sheer strength measurements, sediment pH and samples for paleomagnetic measurements were taken prior to microplastic sampling for four of the eleven cores (gravity and piston cores). Approximately 10 cm^3 of sediment was sampled from the top 2 cm of the working half of each core using a scoop (polypropylene). Sediment samples were placed into clean, labelled plastic bags (polyethylene) and stored in a freezer (−20 °C).

Although it is acknowledged that such a low volume of sediment may not be representative of sediments at individual sampling stations, the sampling technique used in the present study, i.e. coring, limited the volume of sediment that was available per site. Precautions taken onboard the ship to limit cross-contamination included (i) minimal exposure of the sediment samples to the atmosphere, (ii) samples collected/stored in new materials (scoops, bags) and, (iii) sampling conducted by one individual. A record was also kept of all plastic materials that came into contact with the sample during collection and processing. One limitation was that no air contamination check was included during sediment sampling onboard the vessel.

In the laboratory, sediments were defrosted, transferred into clean covered aluminium foil trays and oven dried at 60 °C for approximately 96 h. Approximately 10 g of oven-dried sediment was weighed and placed into a pre-cleaned glass jar. 105 mL of sodium tungstate dihydrate ($Na_2WO_4{\cdot}2H_2O$, 40% w/v, density 1.4 g cm^{-3}), as recommended by Frias et al. (2018) and Pagter et al. (2018), was added to each glass jar, the mixture was shaken for approximately 1 min and the sediments were allowed to settle. From each sample jar, the overlying sodium tungstate dihydrate solution was removed using a pipette and filtered under vacuum onto glass microfiber filter paper (GF/C), Whatman 47 mm, pore size 1.2 μm, using a Buchner funnel and an Erlenmeyer flask. Minimal volumes of ultrapure water (< 2 mL) were used to wash down the sides of the glass jars with the sediments. Introducing water into the remaining extraction solution can lead to a change in the density of the solution and thus this was minimised. Ultrapure water was also used to wash the pipette and sides of the Buchner funnel. Filter papers for the samples were placed into clean petri dishes and stored until analysis. Potential contamination was evaluated by using (i) air contamination checks-clean petri dishes with filter paper (n = 2) were exposed to the air during sample processing and, (ii) method blanks-jars devoid of sediment (n = 2) were processed in the same manner as actual samples. Measures taken to prevent contamination in the laboratory included (i) wearing lab coats and gloves during sample processing and, (ii) washing all glass jars used during sample processing with a 6% nitric acid solution and Ultra-pure water.

Filter papers were visually examined under a dissecting microscope (Olympus SZX10) equipped with a polariser and camera (Q Imaging Retiga 2000R). Potential microplastics were isolated and processed (photographed and length measurements taken) prior to transferring to a clean filter paper in a labelled petri dish (Kanhai et al., 2017). Due to the difficulties that arise when handling particles < 100 μm, such particles were automatically excluded for any analysis. All potential microplastics and any plastic material that was in direct contact with the samples either during sampling or laboratory processing were analysed by Fourier transform infrared (FT-IR) spectroscopy on a Bruker Vertex 70 Infrared Spectrometer coupled to a Hyperion 1000 microscope (Kanhai et al., 2017). Samples which produced spectra with a match < 60% were automatically rejected while those which produced a match of > 70% were accepted. All spectra with matches > 60% were individually examined to ensure that there was clear evidence of peaks from the sample corresponding to known peaks of standard polymers.

3. Results

In the present study, the following plastic materials made direct contact with the sediment samples either during collection or processing (i) plastic film – low-density polyethylene (LDPE), (ii) core liner – polyvinyl chloride (PVC) or polycarbonate (PC), (iii) scraper – polypropylene (PP), (iv) sediment collection scoop – polypropylene (PP) and, (v) sample bag – low-density polyethylene (LDPE). In the surficial sediment samples, no polyethylene particles were recovered. However, in two instances, synthetic polymers from surficial sediments matched plastic materials used during sample processing but were not eliminated since they were of different colours than the materials used. This was so for 2 particles in sediment core 4 (single PVC fragment, different colour from liner) and sediment core 7 (polypropylene fragment, different colour from collection scoop/scraper). To assess whether synthetic polymers were introduced during laboratory processing of the samples, air contamination checks (ACs), (n = 2), and method blanks (MBs), (n = 2), were included. No synthetic polymers were found in the

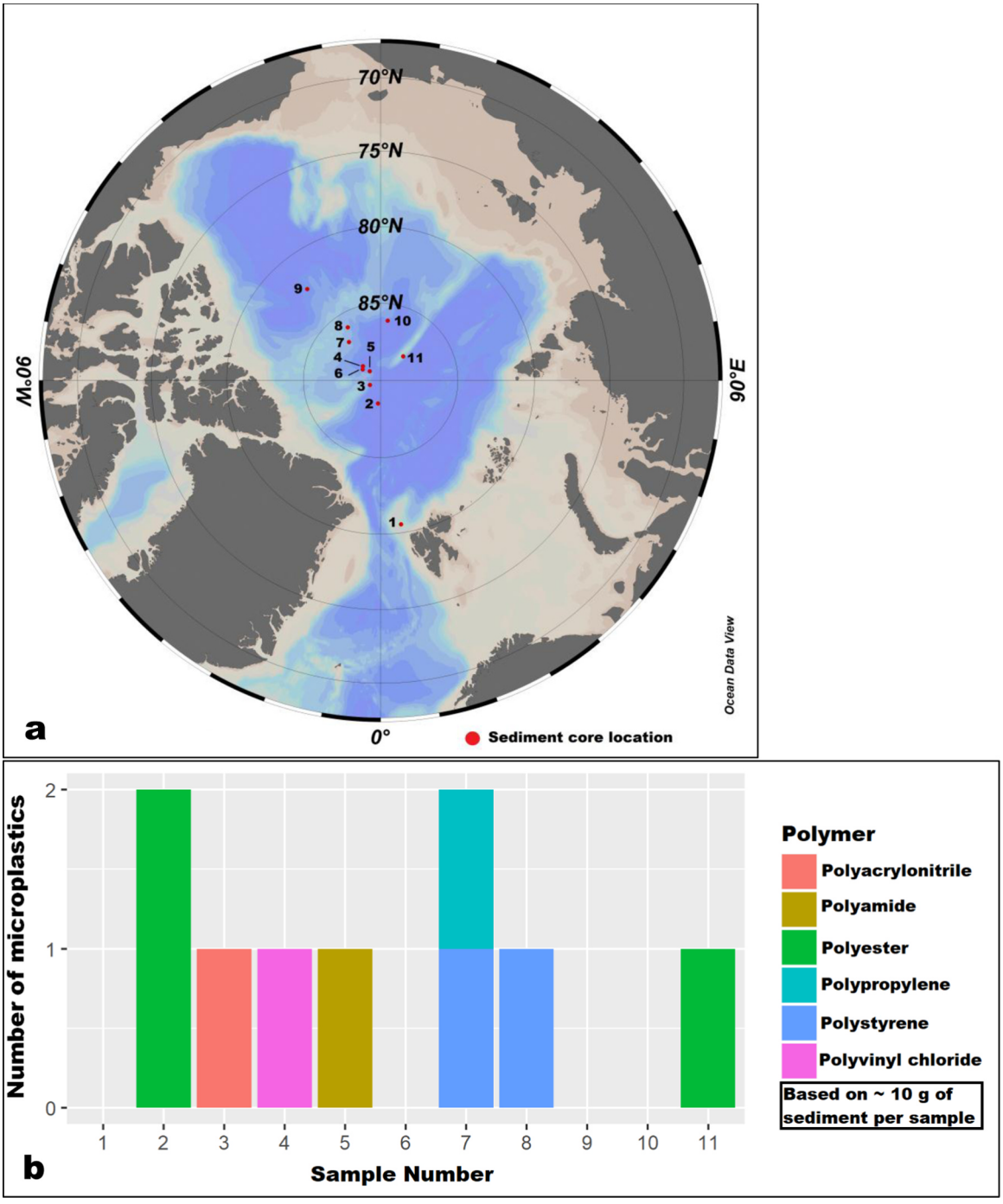

Fig. 1. Locations at which sediment cores were collected in the Arctic Central Basin (a) and synthetic polymer composition in surficial sediments from the various cores (b). [Fig. 1a generated using Ocean Data View (ODV) Version 4.7.10 (Schlitzer, 2017); Fig. 1b generated using R version 3.4.4 (R Core Team, 2018)].

air contamination blanks. However, a total of three fibres were found in the two method blanks (MB1 – blue polyester fibre, 1.28 mm; MB2 – blue polyester fibre, 0.49 mm and red polyester fibre, 0.53 mm). All samples were blank corrected such that if any blue or red polyester fibres were found in the sediment samples, they were removed from the final results.

Based on visual identification, fifteen particles from the sediment samples were isolated and subjected to FTIR spectroscopy. Of these, four were natural cellulosic fibres. The remaining eleven were identified as synthetic polymers with one macroplastic (> 5 mm) and ten microplastics (< 5 mm). After blank correction of the samples, there were a total of nine microplastics (< 5 mm), (Supplementary Table 2). Synthetic polymers detected in the sediments included polyester (n = 3), polystyrene (n = 2), polyacrylonitrile (n = 1), polyamide (n = 1), polypropylene (n = 1) and polyvinyl chloride (n = 1). Both fibres (n = 5) and fragments (n = 4) were present in the samples. In terms of colour, most of the synthetic polymers were transparent (n = 5) with the remainder black (n = 1), brown (n = 1), white (n = 1) and blue (n = 1). With respect to length, most (n = 5) were < 1 mm, 3 were between 1 and 2 mm and 1 was > 2 mm. Of the surficial sediment samples analysed from the Arctic Central Basin (ACB), 7 of the 11 samples contained between 1 and 2 synthetic polymers (Fig. 1b).

4. Discussion

Elucidation of the transport and fate of microplastics in the marine environment is a critical step towards assessing the threat that these contaminants potentially pose to organisms inhabiting different compartments of an ecosystem. In the Arctic Central Basin (ACB), only a few studies have reported on microplastic presence in the sea ice, biota and water column (Obbard et al., 2014; Kuhn et al., 2018; Kanhai et al., 2018; Peeken et al., 2018). Based on these studies, the key suggestions regarding microplastics in this oceanic basin are that (i) sea ice acts as a sink and means of transport for microplastics, and (ii) the pervasiveness of microplastics in the various water layers of the ACB indicates that there is vertical transport of microplastics out of surface waters into deeper waters (Obbard et al., 2014; Kanhai et al., 2018; Peeken et al., 2018). In context, the findings of the present study expand the knowledge base about microplastics in the Arctic Ocean by providing preliminary information that suggests microplastics are present in surficial sediments of the Arctic Central Basin and that within this oceanic basin the sediment compartment is potentially acting as one of the sinks for microplastics. Microplastic presence on the seafloor of the ACB lends credence to the suggestion that there is vertical transport of microplastics within the water column. Laboratory and field studies have shown that marine organisms, such as zooplankton, larvaceans and other pelagic filter feeders, which are capable of ingesting microplastics and egesting them in their faecal pellets and discarded houses (as in the case of the larvaceans), could contribute to the vertical flux of microplastics in the water column when their waste products sink (Cole et al., 2016; Katija et al., 2017). The incorporation of microplastics into marine aggregates and the biofouling of microplastics are other processes which may influence the vertical transport of these particles in the water column (Long et al., 2015; Fazey and Ryan, 2016).

The presence of microplastics in sediments of the Arctic Central Basin implies that interactions between these particles and deep-sea organisms that inhabit or depend upon this environmental phase is plausible. Although the Arctic Ocean has generally been regarded as oligotrophic, the fact remains that marine organisms do inhabit its' deep-water environment with the most speciose groups being arthropods, foraminiferans, annelids and nematodes (Bodil et al., 2011). Depending on the foraging behaviours and feeding habits of deep-sea benthos in the ACB, the possibility exists that some of them may be interacting with microplastics in the sediment phase. Recently, Fang et al. (2018) reported that microplastics were discovered in 11 different benthic species that were recovered from depths of 35–151 m in the Bering-Chukchi Sea shelves. Fibres were the predominant type of microplastics found in the organisms with synthetic polymers including polyamide, polyethylene, polyester and cellophane (Fang et al., 2018). Microplastics were also discovered in 3 different phyla (Echinodermata, Arthropoda, Cnidaria) of deep sea organisms recovered from depths of 334–1783 m in the equatorial mid-Atlantic and SW Indian Ocean (Taylor et al., 2016). Although the presence of a contaminant in the marine environment does not directly imply harm, laboratory experiments have indicated that benthic organisms exposed to microplastics in sediments may be negatively impacted. For example, Wright et al. (2013) reported that exposure of the deposit-feeding marine polychaete worm (*Arenicola marina*) to unplasticised polyvinyl chloride (UPVC) led to a depletion in energy reserves of the worms which could have been caused by reduced feeding, longer gut residence time of ingested matter and inflammation.

The present study confirmed via FT-IR spectroscopy that polyesters were recovered from surficial sediments of the ACB. Such findings are corroborated by previous studies which investigated other environmental phases in this oceanic basin. Obbard et al. (2014) reported that of the synthetic polymers found in sea ice, the majority were polyester (21%). Kanhai et al. (2018) similarly found that of the synthetic particles present in the sub-surface waters, polyesters (74–78%) were also predominant. Upon melting, sea ice can act as a local source of microplastics to the water column (Obbard et al., 2014; Peeken et al., 2018). Synthetic polymers that are present in surface waters of this oceanic basin could then be subject to vertical transport, persist in the water column as evidenced by Kanhai et al. (2018) and at some stage a fraction of these particles could end up in the sediment phase. Of interest is the fact that the present study found low-density polymers such as polypropylene and polystyrene fragments in the sediments of the ACB. Based on the inherent densities of the virgin resins, such particles are unlikely candidates for the sediment phase in that they are positively buoyant and are expected to float. However, this suggests that there are mechanisms operating within the ACB that could be affecting the density of these particles and in effect causing them to end up in the sediment phase. Long et al. (2015) showed that under laboratory conditions marine aggregates of various algal species (*Chaetoceros neogracile*, *Rhodomonas salina*) were capable of incorporating and concentrating polystyrene microbeads which in turn led to an increase in their sinking rates. Such mechanisms can potentially explain the presence of low-density polymers in surficial sediments of the ACB. Of note is the fact that the present study is not the first to report the presence of low-density polymers in deep sea sediments since polyethylene and polypropylene particles were found in surficial sediments from the Fram Strait (Bergmann et al., 2017).

Within the last decade, deep-sea sediments were for the first time identified as a potential sink for microplastics with four studies reporting on the issue in various oceanic basins (Supplementary Table 3). Comparison between these studies is particularly challenging and not straightforward due to the fact that each used different sampling equipment, extraction techniques and reported microplastic abundance/concentration in different units (Supplementary Table 3). Microplastic abundance in surficial sediments of the ACB was estimated to range between 0 and 200 microplastics kg^{-1} dry sediment based on the findings of the present study. However, it is unlikely that these estimates are reflective of the situation in the ACB since (i) the equipment used for sample retrieval (gravity and piston corers) may have led to the collection of disturbed surficial sediment samples, (ii) low sample volumes (~10 g per site) were used to assess microplastic abundance, (iii) the density of the extraction solution was only 1.4 g cm^{-3} and thus could have excluded high density polymers, (iv) replicate sediment samples per site was not possible, (v) particles < 100 μm were excluded by virtue of the procedure used to identify/isolate potential microplastics. During the AO16 expedition, gravity and piston corers were used to retrieve sediments from several metres in depth at specific sites in the ACB. Among the corers, gravity and piston corers are not guaranteed to retrieve undisturbed surficial sediment samples due to the shock wave that they generate during descent (Gallmetzer et al., 2016). It is therefore possible that any shock waves generated by the corers used in the present study may have triggered a resuspension of surficial material (sediments and microplastics) into the water column leading to an overall reduction and subsequent underestimation of microplastic abundance in the samples. When sampling surficial marine sediments, equipment such as box and multi-corers may be more suitable for the recovery of undisturbed surficial sediment samples (Georgiopoulou, 2018). Box corers were recommended by Frias et al. (2018) due to (i) the minimal impact they have on surface deformation of sediments and, (ii) their ability to maintain sea-floor integrity during sampling.

Furthermore, due to the heterogenous nature of sediments, it is unlikely that the low sample volumes (~10 g of sediment) used in the study were reflective of the situation at the respective sites. Future studies should ensure that replicate samples are collected per site. Multi-corers may be particularly useful since they can facilitate the collection of replicate samples in a single deployment. When density separation is used to extract microplastics from sediment samples, the density of the extraction solution is important in determining which synthetic polymers are extracted from the samples. Although sodium tungstate dihydrate (density 1.4 g cm^{-3}) was used in the present study based on a safety-price index assessment (i.e. cost and health hazard),

the density of the solution could have led to the exclusion of some high-density polymers (Frias et al., 2018). Löder and Gerdts (2015) recommended the use of zinc chloride based on its cost effectiveness and its higher density of 1.8 g cm^{-3}. However, the health hazard is high for this particular extraction solution (Frias et al., 2018). Finally, the methods employed by investigators for the isolation and identification of microplastics influences the final reported microplastic abundance. In the present study, particles < 100 µm were automatically excluded from analysis. However, it must be noted that at the Atlantic gateway to the Arctic Ocean i.e. the Fram Strait, Bergmann et al. (2017) reported that the majority (80%) of microplastics in surficial sediments from that area were < 25 µm. Bergmann et al. (2017) used a combination of ATR-FTIR spectroscopy as well as a µFTIR microscope equipped with a focal plane array detector to detect microplastics. If similar analytical techniques had been employed in the present study, the microplastic abundances reported would have likely been higher.

Another limitation of the present study is the non-inclusion of an air contamination check during sampling onboard the vessel. Such a check would have been necessary to rule out airborne contamination during sampling. Since this was not done, the possibility exists that one or more of the particles reported as present in the surficial sediments of the ACB could have been introduced into the samples as a result of airborne contamination. The findings of the present study should therefore be regarded as preliminary and be used as a justification for future studies which can provide more comprehensive assessments of microplastics in deep-sea sediments of the Arctic Central Basin.

5. Conclusion

To our knowledge, this is the first study to present preliminary information regarding microplastics in surficial sediments of the Arctic Central Basin (ACB). The potential discovery of these particles in the sediment phase of this seemingly remote oceanic basin emphasizes the pervasiveness of microplastics in the marine environment. The possible presence of microplastics, specifically low-density polymers such as polypropylene (PP) and polystyrene (PS), in the sediment phase of the ACB suggests that there are mechanisms operating within this oceanic basin that are affecting the density of microplastics and that are potentially driving the vertical transport of these particles through the water column. Microplastics that are present in sediments of the ACB are likely to interact with organisms inhabiting or depending upon this environmental phase. At present, whether those interactions are occurring with benthic organisms within the ACB and the consequences of those interactions to individual organisms and the ecosystem services that they perform remains uncertain. Due to the numerous limitations of the present study, the findings should not be taken as conclusive regarding the status of microplastics in the surficial sediments of the ACB but instead be used as a foundation for future work seeking to quantify microplastic abundance, distribution and composition in surficial sediments of the Arctic Ocean.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.dsr.2019.03.003.

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