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 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).

Keywords

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

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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, 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.

2.0 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 and Canada basins (Jakobssen et al. 2004; Rudels 2015, Figure 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 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). 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

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 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 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 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 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.

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 (Comneau-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

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 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 4a). Microplastic polymer types included polyester (n = 88), blends (n = 11), polyacrylonitrile (n = 8), polyamide (n = 5) and polyvinyl chloride (n = 5), (Figure 5a). The overall category of 'polyester' included both polyethylene terephthalate (PET) and other polyesters while blends included either polyamide blends or polyester blends.

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 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 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 5b).

297 The overall category of polyester included both polyethylene terephthalate (PET) and polyester
298 while blends included only polyamide blends.
299
300 **3.2 Microplastic abundance and distribution in the Arctic Ocean**
301 Based on the underway samples (collection depth 8.5 m), microplastic abundance in sub-surface
302 waters in the Arctic Ocean ranged between 0 – 7.5 particles m^{-3} with a median of 0.7 particles m^{-3}
303 (interquartile range 0.4 – 1.0), (Figure 2, Supplementary Table 5). For the majority of the
304 sampling sites, microplastic abundance ranged between 0 – 1.0 particles m^{-3}. However, at a few
305 sites, microplastic abundances were between 2 – 2.5 particles m^{-3} and at two sites it was at 5 and
306 7.5 particles m^{-3} respectively. Based on the CTD samples (collection depths between 8 – 4369 m),
307 microplastic abundance in sub-surface waters in the Arctic Ocean ranged between 0 – 375 particles
308 m^{-3} with a median of 20.8 particles m^{-3} (interquartile range 20.8 – 62.5) (Figures 2 and 5,
309 Supplementary Table 6). With the exception of CTD cast 4, the CTD casts (1 – 3) nearer the
310 periphery of the Arctic Central Basin (ACB), i.e. in the Nansen Basin (Yermak Plateau), reflected
311 a comparatively higher abundance of microplastics in the water column than other CTD casts
312 within the ACB (Figures 2, 6). It must be noted however that CTD casts 1 – 3 sampled the upper
313 850 m of the water column and as such would have sampled particles from the Polar Mixed Layer
314 (PML), Atlantic halocline and Atlantic water (Figures 1, 6). This is in contrast to the other CTD
315 casts which sampled a much more extensive vertical range throughout the water column by
316 including deep bottom water in excess of 1000 m depth. Overall, there was no statistically
317 significant correlation between microplastic abundance and depth (Spearman's rank correlation,
318 rho = 0.06, p-value = 0.7). However, upon examination of individual CTD casts, it is apparent that
319 microplastic abundance was not uniform at various depths in the water column and that there were

certain depths that reflected higher microplastic abundances (Figure 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).

3.3. 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 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:

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.

4.0 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

389 immune to the entry of plastic contaminants to their ecosystems (Bergmann and Klages 2012;
390 Obbard et al. 2014; Lusher et al. 2015; Amélineau et al. 2016; Bergmann et al. 2016; Bergmann
391 et al. 2017a; Bergmann et al. 2017b; Cincinelli et al. 2017; Cózar et al. 2017; Isobe et al. 2017;
392 Tekman et al. 2017; Waller et al. 2017). The present study expands the knowledge base about
393 plastics in the Arctic by providing evidence for the existence of microplastics in the Polar Mixed
394 Layer (PML) as well as some insight into the vertical distribution of microplastics in the Arctic
395 Central Basin (ACB). This region of the Arctic, though of low productivity, has been recognised
396 as an Ecologically/Biologically Significant Marine Area (EBSA) due to its uniqueness/rarity,
397 provision of a critical habitat and ability to support specialised biota (CAFF 2017). There is cause
398 for concern about microplastics in Arctic waters since laboratory studies have shown that these
399 contaminants may (i) hinder algal photosynthesis/growth (Bhattacharya et al. 2010; Besseling et
400 al. 2014), (ii) reduce feeding and energy reserves of lugworms (Besseling et al. 2013; Wright et
401 al. 2013), (iii) reduce filtering activity and decrease lysosomal membrane stability in mussels (Von
402 Moos et al. 2012; Wegner et al. 2012), (iv) reduce feeding and reproductive output in copepods
403 (Cole et al. 2015) and, (v) cause liver stress, negatively impact upon cholinergic neurotransmission
404 and lead to endocrine disruption in fish (Oliveira et al. 2013; Rochman et al. 2013; Rochman et al.
405 2014). It must be pointed out, however, that some laboratory experiments which reported negative
406 effects of microplastics on marine organisms used microplastic concentrations of 42 to 10 000
407 particles/mL or 42 million to 10 billion particles m^{-3} (Phuong et al. 2016). In context, microplastic
408 abundance in the ACB as reported by the present study ranged from 0 – 7.5 particles m^{-3} (based
409 on underway sampling) and 0 – 375 particles m^{-3} (based on CTD sampling). Although the
410 ecological impact of microplastics upon the Arctic ecosystem presently remains unknown, it is
411 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

435 that laboratory experiments have shown that zooplankton may egest microplastics within densely
436 packed faecal pellets which in natural conditions would sink or in some cases be eaten by other
437 biota (Cole et al. 2016).
438
439 Sea ice is an integral component of the Arctic Ocean's ecosystem and as such possibly exerts an
440 influence on microplastic abundance in sub-surface waters. Sea ice floating on the surface of the
441 water column in the Arctic Ocean can potentially act as (i) a source of microplastics upon melting,
442 (ii) a physical barrier to wind and as such reduce vertical mixing of surface waters and, (iii) a
443 physical barrier to influx of polluted surface waters. Based on the analysis of sub-sections of four
444 ice cores, sea ice in the Arctic Ocean was reported to contain orders of magnitude more
445 microplastic than contaminated oceanic waters suggesting that sea ice potentially acts as both a
446 sink and a source of microplastics (Obbard et al. 2014). Apart from Obbard et al. (2014) no data
447 exists in the published literature regarding either the spatial or vertical distribution of microplastics
448 in sea ice from the Arctic Ocean. In the upper water column, the absence of sea ice cover means
449 that wind stress can generate turbulence and lead to vertical mixing of buoyant plastic debris
450 (Kukulka et al. 2012). More recently, Cózar et al. (2017) suggested that sea ice can also act as a
451 physical barrier preventing the surface advance of polluted Atlantic water into the Arctic Ocean.
452 The present study highlighted that the Polar Mixed Layer (PML) of the ACB reflected the highest
453 overall microplastic abundance (particles m^{-3}): Polar Mixed Layer (0 - 375) > Deep and bottom
454 waters (0 – 104) > Atlantic water (0 – 95) > Halocline i.e. Atlantic or Pacific (0 – 83). As previously
455 mentioned, the PML is the uppermost surface layer of low salinity water (approximately 50 m
456 deep) formed as a result of sea ice melt and the influx of freshwater from riverine sources (Rudels
457 et al. 1991; CAFF 2013). It is possible that one of the reasons that the highest microplastic

458 abundances were recorded in this layer is due to its proximity to microplastic sources such as
459 melting sea ice as well as sea-going vessels (especially if they are releasing wastewater to the
460 environment). Furthermore, in the present study, the highest microplastic abundances were
461 reported nearer to the periphery of the Arctic Central Basin (ACB), i.e. in waters north of Svalbard.
462 It is possible that the lack of permanent sea ice cover in this region of the Arctic allows incoming
463 Atlantic water to have a greater influence on near surface waters thereby resulting in higher
464 microplastic abundances. A recent circumpolar expedition of the Arctic Ocean similarly reported
465 that the north eastern Atlantic sector of the Arctic was a hotspot of plastic debris due to the
466 influence of incoming Atlantic water (Cózar et al. 2017).
467
468 The present study showed that there was a predominance of fibrous microplastics (> 90%) in sub-
469 surface waters of the ACB. This dominance of fibres in sub-surface waters was similarly reported
470 in (i) the north east Pacific Ocean (75 %), (ii) the north east Atlantic Ocean (96 %), (iii)
471 south/southwest of Svalbard (95 %), and (iv) the Atlantic Ocean (96 %) (Desforges et al. 2014;
472 Lusher et al. 2014; Lusher et al. 2015; Kanhai et al. 2017). Fibrous microplastics in the marine
473 environment most likely originate from textile materials and fishing gear (Andrady 2017). Studies
474 have indicated that washing clothes may lead to the release of fibrous materials in the order of >
475 1900 fibres per wash or as much as 700 000 fibres per 6 kg load of acrylic fabric (Browne et al.
476 2011; Napper and Thompson 2016). A recent study in the Ross Sea revealed that the highest
477 concentration of fibrous microplastics (54 %) was found close to the effluent of a sewage treatment
478 plant at the scientific Mario Zucchelli Station, Antarctica (Cincinelli et al. 2017). Fibrous
479 microplastics may enter the Arctic Ocean through a combination of long range transport processes
480 (e.g. via oceanic currents, riverine input) or more in-situ activities such as the release of wastewater

481 from vessels operating in the region. Another mechanism which was recently suggested as being
482 responsible for plastic fibres in Arctic sea ice was atmospheric transport (Cózar et al. 2017). This
483 is certainly plausible as there have been reports of the atmospheric fallout of synthetic polymers
484 in both urban and sub-urban environments in France (Dris et al. 2016). It must be highlighted that
485 while fibrous microplastics seem to be dominant in certain sub-surface waters, other studies
486 conducted in surface Arctic waters reported a predominance of filaments (97 %) and fragments
487 (73 %), (Amélineau et al. 2016; Cózar et al. 2017).
488
489 Analytical techniques such as FT-IR and Raman spectroscopy are a central component of
490 microplastic studies which aim to provide unambiguous identification of synthetic polymers in
491 environmental samples. Omission of this critical step is likely to be a key contributor to an
492 overestimation of microplastic abundances due to the inclusion of non-synthetic polymers in
493 microplastic counts. In the present study, for example, only a percentage (underway - 48 %, CTD
494 - 67%) of the particles were confirmed as synthetic polymers with the remainder being a
495 combination of natural and semi-synthetic polymers. Of the synthetic polymers in the present
496 study, the most abundant (underway – 74 %, CTD – 78 %) was polyethylene terephthalate (PET).
497 A member of the polyester family, PET is one of the five major types of commodity plastics
498 commonly found in the marine environment (Andrady 2011; Andrady 2017). As a thermoplastic,
499 PET is often used in manufacturing beverage containers and packaging materials and its fibres are
500 used in clothing. Overall, this finding of a high prevalence of polyesters in sub-surface waters of
501 the Arctic Central Basin was also corroborated by those of other Arctic studies which reported that
502 polyester was the most prevalent synthetic polymer in waters south/southwest of Svalbard (15 %),
503 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 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

550 Arctic Central Basin (ACB), sampled via the bow water system, had a mean microplastic
551 abundance of 0.97 ± 1.20 particles m^{-3}. In comparison to other studies that employed similar
552 methods (i.e. the underway system of vessels) to sample sub-surface waters, microplastic
553 abundance in the ACB was lower than values reported (i) in the north eastern Pacific Ocean (279
554 ± 178 particles m^{-3}), (ii) in the North Atlantic Ocean (13 – 501 particles m^{-3}), (iii) off Svalbard
555 (2.68 ± 2.95 particles m^{-3}), (iv) in the north east Atlantic Ocean (2.46 ± 2.43 particles m^{-3}), and (v)
556 in the Atlantic Ocean (1.15 ± 1.45 particles m^{-3}), (Desforges et al. 2014; Lusher et al. 2014; Enders
557 et al. 2015; Lusher et al. 2015; Kanhai et al. 2017, Supplementary Table 7). The only oceanic basin
558 for which there were reports of lower microplastic abundances in sub-surface waters was the Ross
559 Sea (0.17 ± 0.34 particles m^{-3}), (Cincinelli et al. 2017; Supplementary Table 7). Although the
560 methods used to sample sub-surface waters for microplastics in the above mentioned studies were
561 similar, the fact remains that the variation of several factors e.g. mesh size of sieve (1 – 300 µm),
562 sampling depth (3 – 11 m), etc., amongst the studies could have impacted the reported microplastic
563 abundances. In terms of assessing the vertical distribution of microplastics in the marine
564 environment, Bagaev et al. (2017) was the only other published study which utilised a similar
565 sampling method (Niskin bottles) in the Baltic Sea. Being cognisant of the fact that no
566 confirmatory analytical techniques or blanks were used by Bagaev et al. (2017), microplastic
567 abundance at multiple depths in sub-surface waters of the Arctic Ocean (mean: 46 ± 62 particles
568 m^{-3}; range: 0 – 375 particles m^{-3}; depths sampled: 8 – 4400 m) was lower than reported for the
569 Baltic Sea (mean: 310 ± 520 particles m^{-3}; 70 – 2600 particles m^{-3}; depths sampled: 1 - 218 m).
570 Similar to the findings of the present study whereby the highest microplastic abundances were
571 found in the uppermost water layer i.e. the PML, Bagaev et al. (2017) reported that near- surface
572 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 – 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 2000 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.

5.0 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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827

828 **List of Tables**

830

831 **List of Figures**

842

List of Supplementary Tables

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

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

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

Supplementary Table 4: Quality control checks associated with CTD sampling

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

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

Supplementary Table 7: Microplastic abundances reported for oceanic basins across the world