Close encounters - microplastic availability to pelagic amphipods in sub-antarctic and antarctic surface waters

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ABSTRACT

This study investigated the distribution of plastic debris from the Atlantic portion of the Sub-Antarctic to the Antarctic Peninsula. This region is home to some of the highest concentrations of zooplankton biomass but is also threatened by increasing shipping traffic from fishing and the growing tourism market. Samples were collected using a surface-towed neuston net during the Austral summer 2018, aboard the *RRS James Clark Ross*. Using Fourier Transform Infrared Spectrometry it was found that 45.6% of the plastic particles isolated from seawater samples were sampling contamination, originating predominantly from the ship. Of the remaining particles, both low density (polyethylene, polypropylene) and high-density (phenoxy and epoxy resins) polymers were found in the surface water suggesting both long-range and local sources of origin. Whilst we found that micro and mesoplastic concentrations in seawater were significantly low ($0.013 \pm 0.005 \text{n/m}^3$) compared to global averages, they were higher along the Antarctic Peninsula than the open ocean (Sub-Antarctic) stations. The potential availability of micro and mesoplastics (MP) to pelagic amphipods was explored, using an observed encounter rate (OER) and a possible encounter rate (PER). The total OER (0.8%) was higher than the PER (0.15%), suggesting that even at low concentrations, microplastics are encountered, and potentially consumed, by amphipods. This study highlights the need to prioritise regions of high zooplankton abundance and to investigate both water and biota to build up a picture of plastic pollution and its potential interaction with the Antarctic Ecosystem.

1. Introduction

Since we began the mass production of plastics in the 1950′s, humans have generated approximately 300 million metric tonnes (Mt) of plastic waste (Geyer et al., 2017). Despite international recognition of the ubiquitous nature of this pollutant, we continue to produce plastic waste, reaching 4–12 Mt in 2010 alone (Jambeck et al., 2015). Recent projections estimate an increase of plastic litter by three orders of magnitude by 2050 and the marine environment represents the ultimate fate for this mismanaged waste (Geyer et al., 2017).

Plastics are a pervasive and complex marine contaminant, existing in many and varied forms; differing in size, shape, colour and polymer composition (Hartmann et al., 2019). The smaller size fraction of this waste, such as microplastic particles and fibers, potentially pose the greatest threat to the marine environment as their size is analogous to diatoms and microzooplankton which are fed upon by zooplankton and other suspension feeding organisms (Barnes et al., 2009; Law & Thompson, 2014). The terminology used to describe one of the smallest manifestations of plastics is evolving, however for the purposes of this paper we define microplastic (1–1000 μm) and mesoplastic (1000–10000 μm), based upon recent recommendations (Hartmann et al., 2019). This smaller plastic debris can stem from the chemical and mechanical fragmentation of larger plastics or can be manufactured purposefully small (Thompson, 2004). Synthetic microfibres, which fall within this second category, are the most common form of microplastic in surface waters (Barrows et al., 2017) originating from clothing and textiles, and are being released into the environment through machine-washing (Napper & Thompson, 2016). These are purported to be one of the major forms of microplastic contamination in surface waters of the Southern Ocean (Waller et al., 2017).

The Polar Regions have been hypothesised as a "dead-end" for plastics. This is because of the prevailing currents in the Arctic (Cózar

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et al., 2017; Obbard et al., 2014), and the Polar Front in the Southern Ocean being insufficient to safeguard Antarctic waters from plastic pollution (Barnes & Milner, 2005; Fraser et al., 2016; Waller et al., 2017). While both plastics and fibres have been reported in Antarctic waters, there is a shortage of data that can reliably determine the concentration and characteristics of plastic pollution in this region (Cincinelli et al., 2017; Isobe et al., 2017; Lacerda et al., 2019; Reed et al., 2018; Waller et al., 2017). To inform policy on the extent and associated risk of plastic pollution in the polar marine environment, the polar plastics and wider plastics research community has recommended a number of priorities. It's first recommendation has been to increase the spatial and temporal coverage of microplastic investigations (SCAR Plastics AG, 2018) and to validate concentrations of plastics found in ice and ice-influenced waters (Hallanger & Gabrielsen, 2018). To date there have been just a handful of studies which have investigated the surface and subsurface waters of the Southern Ocean (Isobe et al., 2017, Lacerda et al., 2019, Suaria et al., 2020). Both the data provided in these studies and the methods they have used are key in advancing our understanding and will be addressed in the discussion of this paper. Its second recommendation was to standardise methodologies for collecting and examining microplastics in the marine environment to facilitate comparative assessment of plastic pollution globally (GESAMP, 2015). Finally, it recommends to focus on areas where a high incidence of microplastics and zooplankton is likely, and thus where there is a higher risk of uptake by the ecosystem (GESAMP, 2015; Koelmans et al., 2017; UNEP, 2016). This latter point is pertinent when considering the Polar Regions. Zooplankton species are often keystone species, having an expedited path to top predators via short food chains (Murphy et al., 2007) and also have a reduced tolerance to environmental changes such as global warming and ocean acidification (Constable et al., 2014; Manno et al., 2016; McNeil & Matear, 2008).

The uptake of microplastics by lower trophic organisms such as zooplankton has been hypothesised as a conduit for microplastics up the food chain (Nelms et al., 2018). To date, evidence for this in Southern Ocean ecosystems has been founded on positive identification of microplastics/fibres in the scats of zooplanktivore higher predators of Sub-Antarctic and Antarctic Islands (Bessa et al., 2019; Eriksson & Burton, 2003; Le Guen et al., 2020 this issue). The Scotia Sea is home to some of the highest densities of pelagic amphipods (Atkinson et al., 2009; Watts & Tarling, 2012). These zooplankton play a key role within the Southern Ocean pelagic food web (Havermans et al., 2019); being omnivorous, parasitic, and a food source for fish, higher marine predators and migratory seabirds (Stowasser et al., 2012; Waluda et al., 2012; Xavier et al., 2018). Here we investigate the distribution of micro and meso plastics from the Sub-Antarctic Scotia Sea, to the Western Antarctic Peninsula, discussing their potential availability to pelagic amphipods. By investigating the concentrations and characteristics of plastics and fibres of the surface waters relative to the concentration of the amphipods, we can determine a "Possible Encounter Rate" (PER). In addition, we provide concentrations of microplastic found from bulk digestions of the amphipods and hence provide an "Observed Encounter Rate" (OER). In combination, these two metrics provide valuable new insight into the potential uptake of microplastics by zooplankton in this region.

2. Methodology

2.1. Sample collection

Surface plastics (both particles and fibres) and zooplankton samples were collected aboard the *RRS James Clark Ross* during the JR17002 Western Core Box cruise in January 2018. Surface samples were collected at eleven stations (Fig. 1) between the mid Scotia Sea (55.25°S, 41.23 °W) and Adelaide Island, Antarctica (67.68 °S, 69.31 °W). An additional four stations (i-iv) collected zooplankton with the same net, however were not used to investigate plastic. This was done

opportunistically in order to provide a larger dataset on zooplankton distribution in the region. Of the eleven stations sampled, five stations were in the open ocean (1–5) and six along the Antarctic Peninsula (6–11), including the South Sandwich Islands, and stations eight and nine, sampled outside and within the caldera, Deception Island. A HydroBios microplastics net (300 μm mesh) was mounted inside a larger neuston sledge frame, providing stabilisation at the sea surface and enabling sampling to be carried out in sea states up to Beaufort five. The GPS locations and time at the start and end of deployment (Table A1), were used to calculate the distance travelled as has been done recently (Lacerda et al., 2019; Suaria et al., 2020). The volume of water sampled was subsequently estimated by multiplying the distance by the net's aperture (0.28 m²), assuming a laminar flow through the cod end of the net, whilst also applying a correction factor, commonly applied for other neustonic nets, which assumes that 95% of the net aperture was submerged (Skjoldal et al., 2013). This volume was used to quantify the concentration of plastics and zooplankton. However, the authors recognise that the resistance of the net in the water is not accounted for when using the ship speed and therefore any associated increases in water volume will lead to an underestimation in the concentration of plastics and zooplankton. This caveats the absolute concentrations and highlights the importance of including the PER and OER as a measure for the relative concentration of microplastics to zooplankton.

During each sampling period of approximately 30 min, the ship transited at a speed of 2–3 knots. In order to limit ship-based contamination, the net was lowered into the water off the portside of the ship, approximately 5 m off the ship outside of the wake, which was recently recognised as a suitable method for deployment off larger vessels (Michida et al., 2019). The net was most commonly deployed below Beaufort 5 wind conditions, but on occasion at higher wind speed, as long as the ship was head to wind during deployment (Table A1) improving stabilisation of the ship. These measures were in place for each deployment and ensured the contamination due to ocean currents was minor.

Once on the deck, the soft cod end was removed and sealed with aluminium foil to prevent further airborne contamination during transport to the on-ship laboratory. A dedicated laboratory was established, which was isolated to ensure no footfall when processing the samples. Each cod end was flushed with Milli-Q water through a 200 μm brass sieve and decanted into an appropriately sized high-density polyethylene (HDPE) Nalgene bottle, preserved in 90% ethanol and sealed with tape before being stowed.

2.2. Sample preparation

A series of preparatory steps (schematic shown in Fig. 2) were required for each sample, using an adaptation of an alkaline digestion protocol (Kühn et al., 2017). Alkaline digestions have been suggested as a cheaper alternative to enzymatic digestions, with effective hydrolysis of proteins and denaturing of enzymes at temperatures that do not simultaneously cause the breakdown of polymeric particles or fibres (Cole et al., 2014; Jin et al., 2009).

Each sample was decanted from its respective ethanol preservative and rinsed through a 200 μm nylon mesh using approximately 500 ml of Milli-Q water. Firstly, zooplankton were picked, identified to genus level, counted and wet biomass recorded (Table A2) To prevent prolonged interference with the samples, sub-samples of zooplankton were created by taking weight/volume (w/v) fractions. Zooplankton not identified as belonging to the order Amphipoda, were individually investigated for microplastics using an Olympus SZX16 Stereomicroscope, made possible due to the low frequency (n < 10) per sample. The amphipods were then incubated in Duran bottles containing 20% potassium hydroxide (KOH), with volumes approximately fourfold the sample volume. The remaining fraction left on the nylon mesh comprised phytoplankton and surface flotsam and was
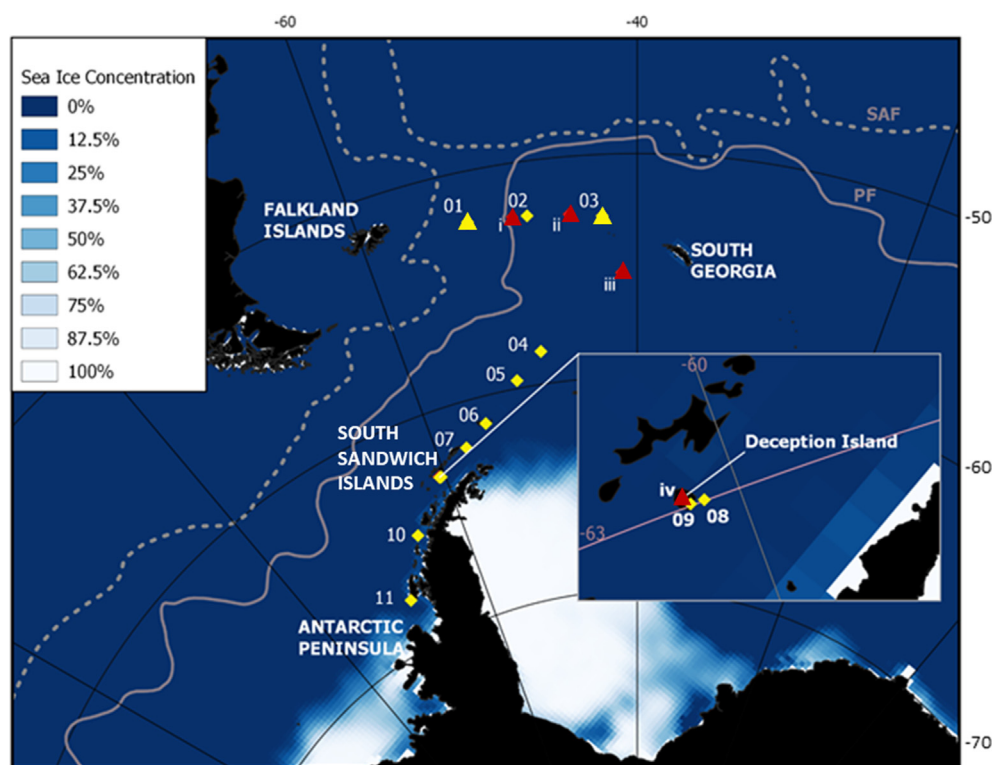
Fig. 1. A map showing the study region bounded by the Sub-Antarctic Front (SAF) and crossing the Polar Front (PF), with transect located poleward from the mid-Scotia Sea to Adelaide Island on the Antarctic Peninsula. Triangle symbols denote where only zooplankton are investigated, with diamond symbols indicating where both the surface water and zooplankton were investigated. Plastics were investigated at stations 1–11(yellow), and additional sampling locations which did not investigate microplastics, and calculated zooplankton concentrations at stations i - iv (red). The climatological position of the Polar Front and Sub-Antarctic front have been presented, as per the estimations provided by Orsi et al., (1995) and provided in Raymond, (2007).
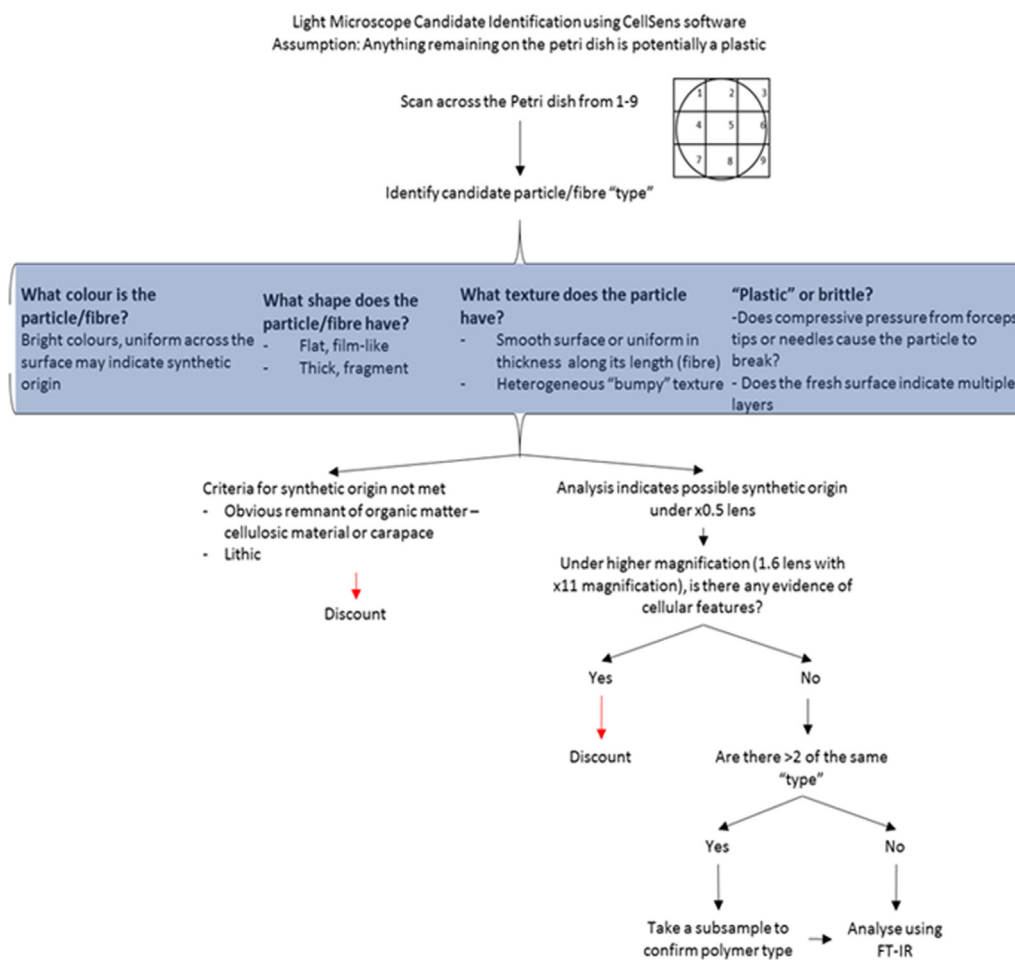
categorised as the residual "waterborne" matter. These were treated as per the amphipod samples and all bottles were shaken at 120 RPM, at 40 °C, for up to two weeks, depending on the level of organic matter present.

After the first week, those samples with little original organic matter were removed from the incubator and analysed. Applying the same methods of digestion across all samples, we were unable to isolate plastics from organic matter at stations one and three and thus only the zooplankton from these samples were isolated. Additionally, initial sub-samples before splitting into zooplankton and organic matter were taken at stations one, two and four due to its high organic matter content, correcting concentration calculation accordingly (Table A1). The samples were then removed from the alkaline solution, decanted into a Buchner flask in an ultraclean lab, under a positive pressure laminar flow hood with HEPA filter. Each sample was vacuum filtered through a 200 μm nylon mesh, flushing it with approximately 200 ml of Milli-Q water to remove any particulate or fibrous material that had potentially adhered to the sides of the bottles.
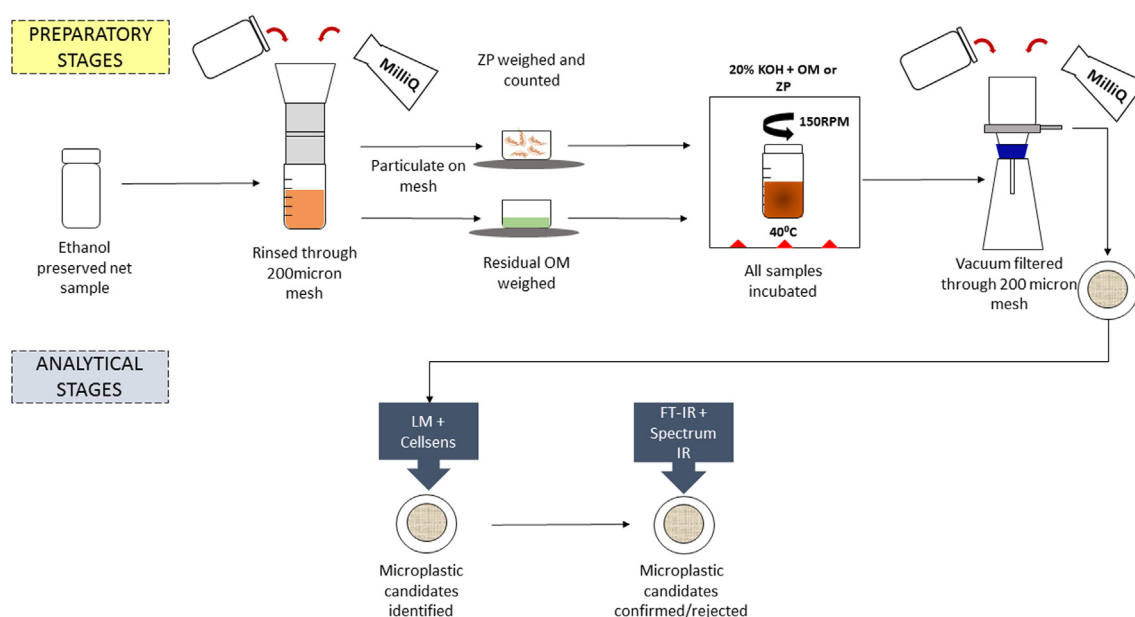
Fig. 2. A schematic illustrating both the minimum steps required to prepare each sample and the subsequent two-part analysis for characterising and quantifying the microplastics. (ZP): Zooplankton (Amphipods). (OM): Remaining organic matter once the zooplankton were removed was determined to be the waterborne fraction.

Light Microscope Candidate Identification using CellSens software
Assumption: Anything remaining on the petri dish is potentially a plastic

Scan across the Petri dish from 1-9

Identify candidate particle/fibre "type"

What colour is the particle/fibre?
Bright colours, uniform across the surface may indicate synthetic origin

What shape does the particle/fibre have?
- Flat, film-like
- Thick, fragment

What texture does the particle have?
- Smooth surface or uniform in thickness along its length (fibre)
- Heterogeneous "bumpy" texture

"Plastic" or brittle?
- Does compressive pressure from forceps tips or needles cause the particle to break?
- Does the fresh surface indicate multiple layers

Criteria for synthetic origin not met
- Obvious remnant of organic matter – cellulosic material or carapace
- Lithic

Discount

Analysis indicates possible synthetic origin under x0.5 lens

Under higher magnification (1.6 lens with x11 magnification), is there any evidence of cellular features?

Yes → Discount

No → Are there >2 of the same "type"

Yes → Take a subsample to confirm polymer type → Analyse using FT-IR

No → Analyse using FT-IR

Fig. 3. A flow diagram depicting the step-wise elimination of non– polymeric particles or fibers, and to collect "polymer candidates" for identification using spectral analysis.

2.3. Sample analysis

2.3.1. Optical sorting of polymers

Each mesh was placed in a Petri dish and examined under an Olympus SZX16 Stereomicroscope, and visualised using CellSens software (Olympus). The meshes were systematically visualised to identify candidate plastic debris. Principles laid out by Hidalgo-Ruz et al., (2012), were used as an initial set of guidelines, with these considerations incorporated into a flow diagram steering final identification (Fig. 3). Criterion of plastic debris was established using recommendations proposed in Hartmann et al., 2019, where micro = 1–1000 µm, meso = > 1000 – 10000 µm and macroplastics = > 10000 µm.

Sizes were calculated using the CellSens software to measure the maximal ferret diameter (Isobe et al., 2019). Fibres introduce additional complexities in reporting on synthetic polymer debris. Dyed natural fibres, whilst distinguishable as being manufactured, rather than naturally occurring, have previously been reported as semi-synthetic and included in microplastic counts (Hartmann et al., 2019; Barrows et al., 2018; Cincinelli et al., 2017; Kanhai et al., 2018; Taylor et al., 2016; Woodall et al., 2014). However, beyond optical identification, FTIR illustrates the cellulosic nature of these fibres. Materials such as viscose and rayon have the same chemical composition as cotton, yield very similar spectra and biodegrade in the same manner, if not more efficiently (Park et al., 2004). For this reason, concentrations of MF do not include cellulosic fibres, although the total counts are included in Table A3.

Where possible, all potential candidates were taken for analysis with

Fourier Transform Infrared Spectral analysis (henceforth, FT-IR). Where there was a recurrence of the same particle and fibre types (based on colour and shape), particularly where these particles were brittle, a subsample was analysed using FT-IR. Particles and fibres were lifted from the mesh with fine forceps, suspended in a Milli-Q water droplet and dried up to 50° on a glass slide covered with aluminium foil; proven as a cheaper alternative for reflective FTIR analysis (Cui et al., 2016). The FT-IR spectrometer was located in the same ultraclean microplastics laboratory, preventing loss of sample during transition from the optical to spectral analysis stages.

2.3.2. Polymer identification

FT-IR was used to identify the candidate microplastics using a Perkin Elmer Spotlight 400 spectrometer (MCT detector, KBr window) with the Spectrum software (PerkinElmer version 10.5.4.738). The spectrometer was operated in reflectance mode for MF and plastic particles, with a drop-down µFTIR attenuated total reflectance (ATR) needle for those particles producing a very weak signal, with 1.8 kspi maximum pressure applied. A standard resolution of 4 cm^{-1} was used for each sample, scanning between wavelengths of 600–4000 cm^{-1}. A minimum of eight scans were collected for each candidate, with visibly heterogeneous particles using 16 scans and multiple markers. A baseline correction was applied to each first derivative spectra. The spectra were investigated against reference libraries (ATRSPE ∼ 1, ATR Polymer Intro Library, Rampoym, ATRSPE ∼ 1, POLYMER) and a number of bespoke libraries developed by the microplastics research group at Plymouth Marine Laboratory. Matches of > 70% with the spectral library, in combination with analysis of key spectral peaks

informed by principles of organic chemistry, were used to positively identify polymer type.

2.3.3. Calculating concentrations of plastic debris

Concentrations of plastic debris are reported as a total of meso and microplastics (MP) and synthetic microfibres (MF). Observed concentrations in the waterborne fraction have been reported as particle or fibre frequency per volume (n/m^3). In order to compare with a recent publication carried out in a similar area (Lacerda et al., 2019), numbers have also been presented as frequency per area (n/km^2). To determine the frequency per area, the vertical component was removed by multiplying the total distance covered, by the net's width (0.4 m). These concentrations have been plotted on maps composed with QGIS Madeira 3.4 with averaged sea ice concentration for January 2018, courtesy of the National Snow and Ice data centre (Fetterer et al., 2017). The climatological position of the Polar Front and Sub-Antarctic front have been presented, as per the estimations provided by Orsi et al., (1995) and provided in Raymond, (2007).

It should be noted that the use of a microplastics net is optimal for calculating particle concentrations; however, the derivation of concentrations of fibres is caveated by the fact that fibres may be smaller than the pore size of the net. For this reason, this study reports on the concentrations of MF and MP separately and constrains discussions of availability of microplastics to pelagic amphipods through exploration of MP concentrations only. To examine the potential availability, this study has investigated the "encounter rate" of MP to amphipods, which can be subsequently divided into two separate metrics (Botterell et al., 2019). The encounter rate, which has been used in a number of other studies (Moore et al., 2001; Collignon et al., 2014; Kang et al., 2015) defines the ratio of the number of plastics and the number of pelagic amphipods in a cubic metre of water, expressed as a percentage, henceforth defined as the Possible Encounter Rate (PER). Secondly, the Observed Encounter Rate (OER) details the concentration of plastics found within the chemically digested (20% KOH) amphipods, written both as a number/individual but in the case of MP, as a percentage to compare with PER.

2.3.4. Statistical analysis

Unless otherwise stated, the standard error of the mean has been reported when $\pm$ is given. Statistical analyses were carried out using R Studio (Version 1.2.1335). Linear regressions were applied to determine any influence of environmental variables on the concentration of MP or MF. The *t*-test was used to test for significance between the open ocean and Antarctic Peninsula concentrations.

2.4. Anti-contamination protocol

2.4.1. Aboard the ship

Working aboard a marine vessel inevitably produces a number of sources of contamination. Aboard the *RRS James Clark Ross*, specific measures were put in place in order to account for this potential bias. Foot traffic in and out of the lab was limited to the scientific investigators involved and simultaneously, any work carried out was done whilst three glass petri dishes containing wetted 0.2 μm GF/F filters were placed near the basin, the door entry and at the corner of the workbench where sampling equipment was stored. These air contamination filters were investigated for fibres, with the number found during the preparation of each station, subtracted from the final station count (see section 3.1). All kit was cleaned with Milli-Q three times in between each sample and had been acid washed prior to packing. Potential sources of contamination on the deck were identified, photographed and "scrapings" taken, to be added to a specific ship deck contamination library. This included polymeric paints, polyamide rope and black plastic from the neuston sledge and other kit being operated from the aft deck.

2.4.2. Preparation and analysis of the samples

All preparatory stages were carried out in a class II, high-efficiency particulate absorbing (HEPA) filtered cabinet. During handling of each sample, a wetted 0.2 μm polycarbonate filter placed in a glass petri dish, was exposed to the air in order to collect any airborne fibres, which may have been present. Glassware was acid washed before use and all tools were rinsed with Milli-Q water three times between each sample. Nitrile gloves were worn and wetted with Milli-Q water before handling each sample in order to prevent the transfer of fibres from the surrounding environment, to the sample. Once decanted into appropriate glassware for KOH digestion, the samples were covered with aluminium foil. Prior to vacuum filtering, each new mesh or filter was rinsed and observed under the microscope for any fibres that may have settled during preparation. Any fibres were removed with forceps, before continuing. Three separate procedural blanks were carried out in the laboratory, with the average number of contaminants subtracted from all station final counts (Table A5).

3. Results

3.1. Contamination

Fibres were found in all procedural blanks, averaging five per sample. The air contamination filters in the laminar flow were clear, and those that may have been transmitted onto the mesh prior to analysis, were removed. There was evidence of air contamination, however, during the packing of samples on the ship; with a maximum of five fibres at station ten (Table A4). In order to take this contamination into account, the total MF counts, were corrected by subtracting the sum of contamination fibres found on each air contamination filter plus the average number of fibres isolated from the three procedural blanks ($n = 5 \pm 1$, min = 4, max = 7) (Table A5). In addition to those fibres observed post-collection, there were some fibres and plastic particles, which matched the contamination library, built in the FTIR, details of which have been outlined in supplementary material. Any plastics that were identified as contamination from the ship were eliminated from the final counts. Taking all of the above into consideration, it was estimated that 32% ($n = 52$) of the total fibre count ($n = 162$) in the waterborne fraction were contamination. Final estimations of fibres at each station were calculated by removing non-synthetics (see 3.2.3).

Contamination was estimated to constitute 45.6% ($n = 41$) of the total count ($n = 90$) of plastic candidate pieces (fragments = 84, films = 6). This number was derived by analysing 41 of the plastic candidate pieces using FTIR spectroscopy, with 46.3% ($n = 19$) matching the contamination library which contained samples from the ship hull and ship laboratory. This contamination fraction (46.3%) was applied to the remaining 49 plastic candidate pieces yielding an estimated additional 22 contamination particles to total 41/90.

Of the total number of plastic candidates found in amphipods ($n = 8$), after FTIR, 75% ($n = 6$) were identified as being derived from contamination sources on the deck of the ship. With the contamination factor applied individually for each station ($n - 5$), the 28 fibres which were found amongst four amphipod stations (stations, 1, 3, 5 and 7) were reduced firstly to 19 fibres, when non-synthetics were removed and to just four, when the contamination factor was applied to each station.

3.2. Waterborne plastics

3.2.1. Samples overview

Of the eleven stations investigated, all but two of the stations (1 and 3) were investigated for waterborne microplastics. The addition of KOH to the samples collected at stations one and three did not sufficiently digest the organic matter. The recalcitrant was a white, fine, filamentous material which trapped any potential particulate inside. Analysis of a sub-sample, using energy dispersive X-Ray spectroscopy

attached to a Hitachi TM300 scanning electron microscope, indicated that the nature of this substance was siliceous, and under higher magnification identified as pennate diatoms. Alkaline digestions cannot be used to remove silica, and instead requires the addition of sulphuric acid. The introduction of sulphuric acid as a digestive method in microplastics analysis has been suggested as potentially destructive to most polymers and in particular those such as synthetic polyamide fibres (Lares et al., 2019; Lusher et al., 2017). As a result, and due to the interference of the sample at point of discovery of the diatomaceous content, it was decided that analysis for microplastics within these samples would not be pursued. Alternatively, only zooplankton at these stations were identified, counted, and investigated for microplastics.

3.2.2. Micro and mesoplastics

Out of 90 candidate pieces, 41 were analysed using FTIR, based on the workflow laid out in Fig. 3. 54% of those pieces analysed with FTIR were identified as specific polymers (n = 20). Applying this 54% to the remaining candidate pieces, a further 28 pieces were estimated to be polymeric, based on physical characteristics comparable to those analysed with FTIR. Plastic debris was found in all but one of the stations (station 7), with an average concentration across all stations estimated at $0.013 \pm 0.005 n/m^3$ and a maximum concentration at station ten $(0.054 n/m^3)$ (Fig. 4). Expressed as a concentration per unit area, it was estimated that there was an average of 5056 ± 2158 items/km^2 amongst the analysed stations (2, 4–11) and a maximum of 20,462n/ km^2 (Table A6). Fragments were the most common forms of plastic particle (93%), with the remaining 7% being films. Microplastics comprised 90% of the plastic debris (n = 43), with the highest proportion identified as having a maximum ferret diameter of < 300 μm (Fig. 5). There were only four mesoplastics found at stations five, six and eight and the only macroplastic was identified at station ten. Whilst the highest concentration was found at station ten, the largest total surface area of plastic was found at station 8 due to the presence of two blue mesoplastic particles. Regression analysis gives indication that potential environmental variables (water depth, wind speed, ship speed and distance from nearest research station) show no significant correlation with the concentration of MP at each station (figure A1). Blue and black particles were the most common and made up 59% of micro- and mesoparticles (MPs) (Fig. 6). There was no correlation between the
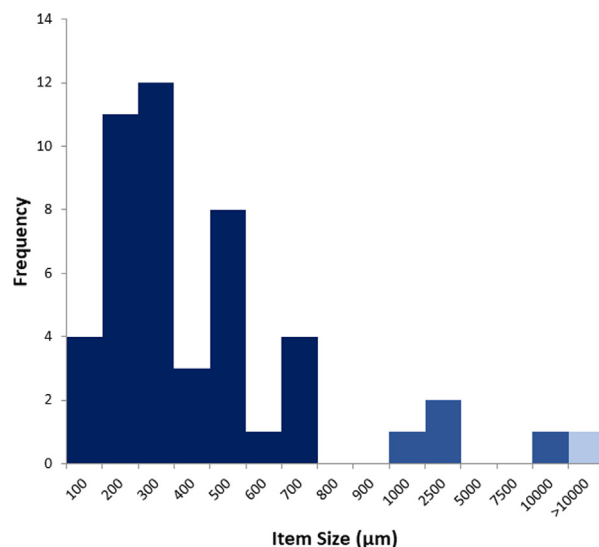

Fig. 5. Size distribution of plastic items, based on maximal ferret diameter. Dark Blue (Microplastic = 1 < x > 1000 μm), Medium Blue (Mesoplastic = 1000 ≥ x < 10000 μm) and Light Blue (Macroplastics = x > 10000 μm).

colour of plastic and the polymer type, nor any correlation between the different plastic characteristics (shapes, colours, sizes) and the environmental parameters. When comparing open ocean samples and those samples collected along the Peninsula, there was no statistically significant difference between the two concentrations (p > 0.05), however there is an evidential difference between the average concentrations of plastics found in the open ocean (2362 ± 1160) and the Antarctic Peninsula (7211 ± 3653) (Table A6).

Of those particles that underwent spectral analysis, polyethylene and phenoxy/epoxy resins were the most common, comprising 41% each. In addition, there was one polyethylene terephthalate fragment found at station six that was contained within the barbs of a feather and in one of the most remote stations (station 4), a blue microplastic was identified as polycaprolcatone, a biodegradable polyester. A red microplastic fragment at station 9 was identified as pentaerythritol, a
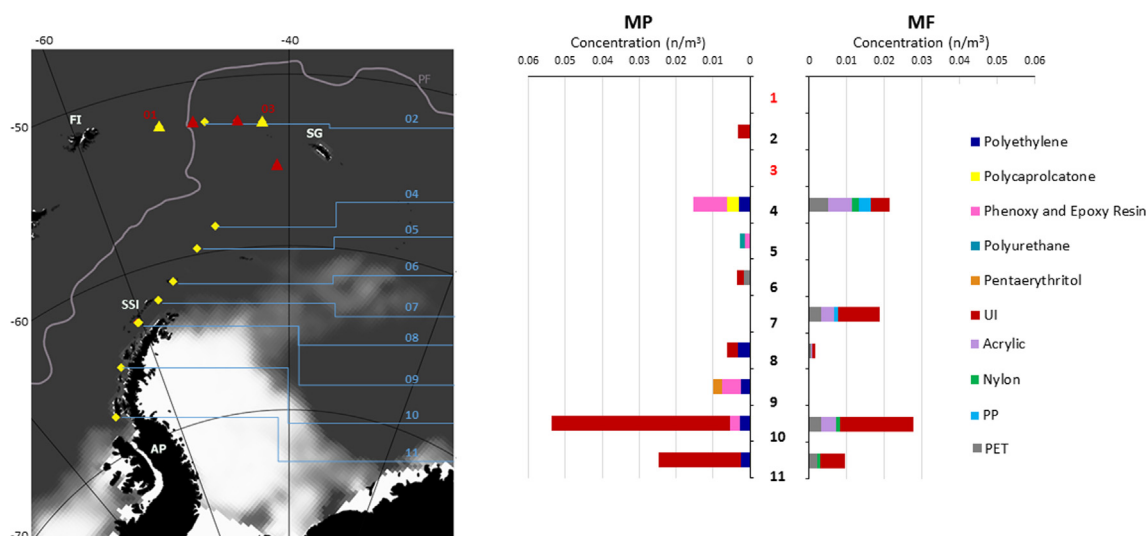

Fig. 4. FI = Falkand Islands, SG = South Georgia. DI = Deception Island. AP = Antarctic Peninsula. Sea ice concentration provide in greyscale version of Fig. 1 (white; 100% ice, dark grey; 0% ice). (Left) A map to show the location of stations investigated for micro and mesoplastics (micro and meso = MP) in water (yellow diamonds) and zooplankton (yellow triangles) and those just sampled for zooplankton (red). (Right). Two bars per station (middle values, 1–11) are plotted opposite one another, along two positive sets of × values (concentration in frequency per cubic meter). This illustrates the comparative concentrations for MP and MF at each station. The ratio of polymers is indicated within each bar, according to the legend on the right. (PP- Polypropylene, PET – Polyethylene terephthalate UI – Unidentified (Spectral signature not defined).
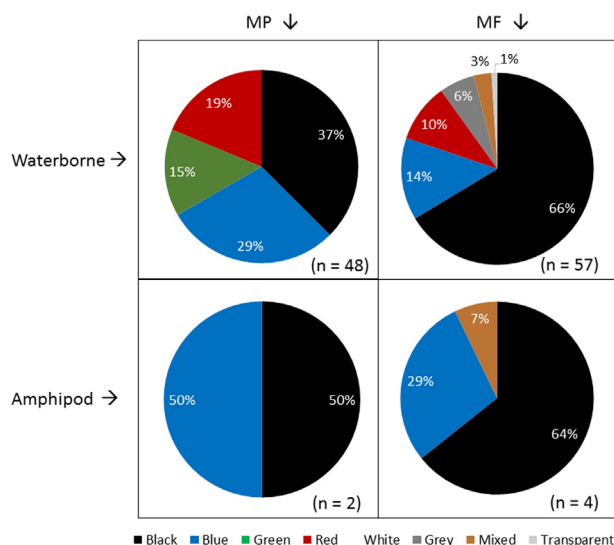
Fig. 6. Relative proportion of different colours identified amongst MP and MF within the waterborne fraction and within amphipods. MF proportions are given before correction factor applied to yield final total numbers (In parentheses).

flame retardant component found in expanded polystyrene.

3.2.3. Synthetic microfibres

Optical microscopy yielded a total fibre count of n = 135. However, this total was reduced after FTIR on a subsample (n = 102) found 34%, (n = 35) to be cellulosic. The distinction between semi-synthetics and natural cellulosic fibres, is not conclusive and these were eliminated from the final counts of MF.

Therefore, once corrected for contamination, a total of 57 fibres were identified as synthetic and were found in 5/9 stations. Polyethylene terephthalate, (Most commonly referred to as polyester in fibrous form) comprised the largest fraction (53%). The mean concentration was estimated as 0.009 ± 0.004 n/m^3, with a maximum of 0.028n/m^3 at station ten and an absence of MF at stations two, five, six and nine. Black fibres (66%) and blue fibres (14%) were the most common colours.

We acknowledge that the average thickness of fibres is 20 – 40 μm (Table A7) and consequently the fibres collected in this study, with a 300 μm mesh size net, likely represents an underestimation of the amount of fibres debris at the sea surface. However, they do serve to give indication of the levels of fibre debris that are synthetic versus those which are cellulosic.

3.3. Zooplankton distribution

Amphipoda mostly comprised of *Themisto* spp., with a greater abundance (average 5.19 individuals/m^3; max 66.35 individuals/m^3) than the other zooplankton (average 0.02 individuals/m^3 max 0.07 individuals/m^3) (Fig. 7). There was no statistically significant correlations between the environmental variables (wind speed, water depth, proximity to land, proximity to ice), and the amphipods. However, the 99.7% of the total amphipods was concentrated in the open ocean (stations 1–5) (Fig. 7).

3.4. Plastics in amphipods – Observed encounter rate (OER)

3.4.1. Micro and mesoplastics

Two microplastic fragments were found in amphipod samples isolated at stations three and five. The first, a blue polyethylene fragment measuring 200 μm, and the second a black polypropylene fragment measuring 477 μm. As a function of the number of amphipods found at

these two stations, this yields observed encounter rates (OER) of 2.7% and 1.4% at stations three and five, respectively. On average, this means that amongst the five open ocean stations (1–5), there was an OER of 0.8% or expressed as a whole number, one particle for every 125 amphipods and 0% along the Antarctic Peninsula. There were no mesoplastics found in amphipods.

3.4.2. Synthetic microfibres

In total, 28 fibres were isolated from amphipod samples. A subsample (n = 19) was analysed with FTIR and 31.6% (n = 6) were identified as cellulosic and therefore not included in the final concentration. The fibres identified as synthetic were predominantly polyester (n = 9), with the remaining fibres comprising one each of acrylic, nylon, polypropylene, poly (butylene terephthalate). Black and blue fibres dominated (Fig. 6). Fibres were only found in amphipods at station 1 and 3, 5 and 7, and once corrections had been applied (see section 3.1), yielded only a concentration of 0.0007n/individual at station one, or one fibre for every 7,000 amphipods.

3.5. Plastics and amphipods in surface waters - possible encounter rate (PER)

The total PER for MP across all stations was 0.15%, with a mean of 0.26%. Amongst the five open ocean stations (1–5), there was a PER of 0.03%, and a mean of 0.06%. The Antarctic Peninsula stations had inflated PER values due to the negligible concentration of amphipods, recording a total and mean PER of 470.26%, and 166.67% respectively. Inflated values such as this, generate a bias which can only be overcome by calculating a PER where zooplankton numbers are sufficiently high.

4. Discussion

Using a combination of optical microscopy and single spectral FTIR analysis, we estimated the mean concentration of micro- and mesoplastics to be 0.013n/m^3, with highest concentrations in Antarctic Peninsula stations (0.019n/m^3) compared to open ocean stations (0.006n/m^3). These numbers are comparable to other investigations in the Southern Ocean, which have used the same method for collecting in the surface waters (Isobe et al., 2017; Lacerda et al., 2019, Suaria et al., 2020). The concentrations in this study along the Peninsula are slightly greater than that of Lacerda et al. (2019), which found 0.008n/m^3, in the previous year around the Antarctic Peninsula. Our orders of magnitude are in agreement with Isobe et al. (2017) that found an average of 0.031n/m^3. Whilst our observations show average differences between the open ocean stations and those along the Peninsula, there was no observable trend along the Peninsula, and there was a considerable variation in polymer type and concentrations across all stations. This finding is concurrent with Lacerda et al. (2019) that similarly found no correlative relationship between microplastic concentrations and proximity to land and bases. Investigations as part of the recent Antarctic Circumnavigation Expedition (ACE) estimated an average concentration of small plastic particles to be 188n/km^2 in the Southern Ocean (Suaria et al., 2020). This is considerably lower than the average of this study (5056n/km^2), however there was only one sample taken near the Antarctic Peninsula, which yielded one of the few measurable concentrations (734.57n/km^2). Following FTIR investigations, a significant proportion (64.5%) of the small plastic pieces were paint, matching the research vessel that is similar to our investigations of 45.6% ship-based contamination. Similarly, the prevalence of paint polymers in both Lacerda et al. (2019) and Suaria et al. (2020) suggests the emergence of a new subset of polymers, which requires further investigation. Overall, our investigations of micro- and mesoplastic concentrations (5056n/km^2) are on the lower end of the global average, which has been estimated at 63,320 particles/km^2 (Eriksen et al., 2014), and is significantly smaller when compared with populated coastlines such as in East Asia, which registered 1,720,000 particles/
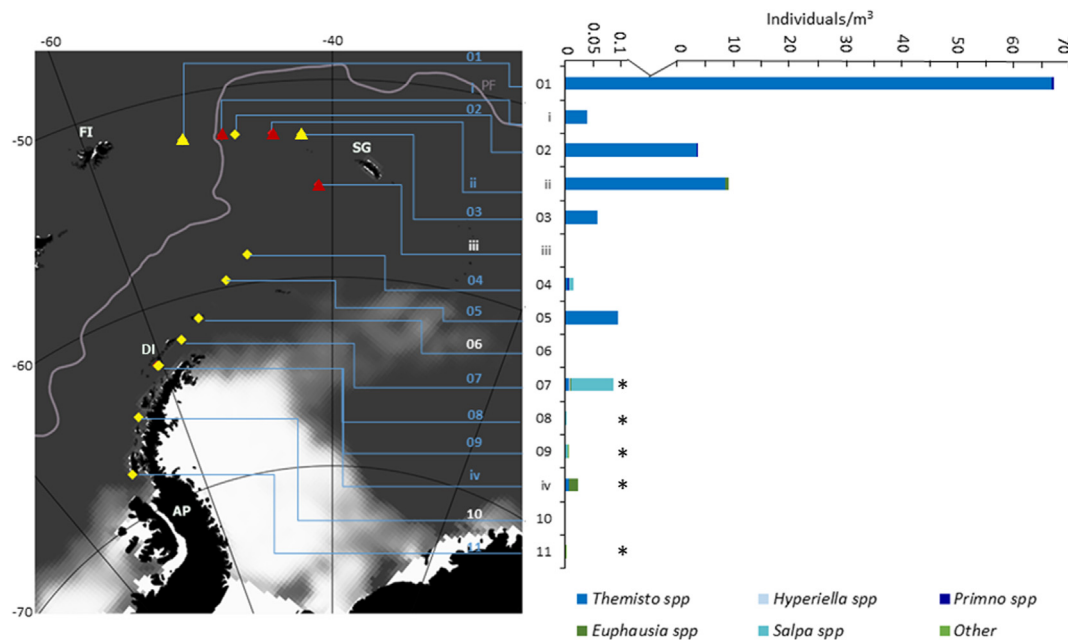
Fig. 7. Concentration and fraction of zooplankton at each station with a map to show the location of stations investigated for microplastics and zooplankton (yellow) and those just sampled for zooplankton (red). The bar chart illustrates the concentration of zooplankton at each station (individuals/m³) with a scale break to include concentrations above 0.1n/m³. White station numbers identify those where there were no zooplankton. Other = Fish larvae and pteropod (Limacina helicina). FI = Falkland Islands, AP = Antarctic Peninsula, SG = South Georgia. Ice concentration is displayed as per Fig. 1, in gray. *Scats were present in observable amounts within the waterborne fraction.

km² (Isobe et al., 2015).

Although microplastic concentrations are low in this study, the presence of polyethylene continues to be a persistent plastic pollutant in our global oceans (Cole et al., 2014) and is no exception here in the Southern Ocean. The ubiquitous nature of polyethylene can be attributed, in part, to its dominance of the global plastics market, and its long residence time within the upper water column, due to its low density (0.917–0.965 g/cm³) compared with seawater (1.025 g/cm³) (GESAMP, 2015). Oceanographic Lagrangian modelling which incorporates fluid flow mechanisms such as stokes drift, have recently demonstrated that small plastic particles may be transported long distances and as far south as the Southern Ocean (Onink et al., 2019; Wichmann et al., 2019). The presence of low-density particles may be indicative of higher residence times and a proxy for distant transport. On the contrary the presence of higher density plastics, such as acrylics, phenoxy and epoxy resins point towards a more localised source. These polymers are often incorporated in paints, and therefore may originate from marine vessels (Finnie & Williams, 2010) . With the total number of people visiting the Antarctic Peninsula by sea last season (2018/2019) approximating 55,391 (IAATO, 2019) and fishing effort concentrated around the Peninsula (McCarthy et al., 2019), this region of the Southern Ocean may be considered a "hot-spot" for potential ship-based plastic pollution. Similar conclusions have been derived in a recent study which found large concentrations of higher density polymers in regions of the Adriatic Sea which experience high shipping traffic (Suaria et al., 2016).

The numbers of synthetic microfibres (MF) in this study are low (0.009n/m³), and arguably so due to the sampling method which was designed to focus on micro and meso particle collection. Whilst the concentrations within the waterborne fraction are caveated, the fact that fibres were not found in every sample, gives indication that airborne contamination was minimised through the anti-contamination protocols in place. For both waterborne samples and amphipod samples, the highest concentrations of MF mimicked those of MP concentrations, with the highest waterborne concentrations also being found at station ten (0.028n/m³) and the presence of synthetic

microfibres in amphipods found at stations one, three and five, for which the latter two stations showed MP in amphipod samples. In the case of the waterborne fraction, discerning whether the origin of these fibres are from local or distant sources is complicated. Despite recent authors, having suggested that there is a high microfibre concentration with increasing proximity to research bases and wastewater outfalls in Antarctica (Reed et al., 2018; Waller et al., 2017), there was no correlative relationship in our study. The variance in concentrations is potentially due to the many pathways for transport of fibres, which are yet to be fully understood. For example, more research is necessary to understand the amount of fibres released from washing machine effluent on passing shipping vessels (Waller et al., 2017), combined with the prevailing currents along the Western Antarctic Peninsula transporting these fibres northward from bases, (Lacerda et al., 2019) as well as the possible airborne transmission of these fibres.

Using spectral analysis to discern any cellulose-derived fibres from naturally occurring cellulose, is not currently possible (Stark, 2019), and for this reason, this sudy eliminated all cellulosic fibres (34% of fibres) from the final MF counts. Similarly, Kuklinski et al., (2019) collected surface samples around Antarctica and proposed that fibres which appeared to be synthetic, according to optical microscopy, were of natural origin, and not plastic. Furthermore, in agreement with our study, an investigation of sediment outside Rothera Research station, (close to stations 10 and 11 of this study), found that 42% of the fibres, were cellulosic (Reed et al., 2018). Irrefutably, the use of FTIR here and the aforementioned studies has been key in being able to at least distinguish purely synthetic fibres (MF) from cellulosic, a vital step towards removing the bias of "false positives".

We calculated an average Observed Encounter Rate (OER) of 0.8%, which is low, compared to studies that have used the same metric for analysing the ratio of microplastics to pelagic zooplankton in lower latitude waters. For example, OERs for Euphausiids were higher (5.6%) than in Calanoid copepods (2.6%) in the Northeast Pacific Ocean (Desforges et al., 2015). Steer et al. (2017) recorded an OER of 3.7% in fish larvae of the English Channel. The highest OER has been recorded by Sun et al. (2017) in the South China Sea, with 120% OER amongst

five different zooplankton. In a study also in the Southern Ocean, in Terra Nova Bay of the Ross Sea, an OER of 100% (one plastic per organism) was found in benthic invertebrates (Sfriso et al., 2020). This significantly higher OER is most likely a result of sampling in close proximity to the research base and the seabed, which is the recipient of settled detritus and particulate matter. However, encounter rates were on average three to five times higher in filter feeders and grazers than predators and omnivores. This would suggest that a wider investigation of a range of feeding strategies amongst pelagic zooplankton would render a useful dataset for comparison. Whilst our study has a comparatively small absolute OER, in relative terms, it is higher than the calculated PER with a total PER of 0.15% across all stations and an average of 0.06% across open ocean stations. This suggests that even at low concentrations, microplastics are being consumed by the zooplankton in the Southern Ocean. This also demonstrates the possible underestimation of encounter rate when using the PER in isolation, without investigating zooplankton samples. Conversely, in isolation the PER can significantly overestimate the encounter rate when the concentration of zooplankton is low. For example, the PER is disproportionately higher along the Antarctic Peninsula (average 166.67%), due to the negligible amount of amphipods collected and should not be used as a measure of encounter rate in isolation.

No other studies, to date, have looked at microplastics in zooplankton from the Southern Ocean, despite microplastics having been found in higher trophic organisms in the Sub-Antarctic (Bessa et al., 2019; Le Guen et al., 2020). Bessa et al. (2019), recently found that 20% of Gentoo Penguin scats sampled from the Sub-Antarctic islands, contained microplastics. In a study investigating King Penguins foraging around South Georgia, for the presence of synthetic microfibres, it was found that 77% of the 47 faecal samples contained microfibres, however only 12% of these were determined to be synthetic (Le Guen et al., 2020). Results from Bessa et al. (2019) and Le Guen et al. (2020), together with the observations in our study emphasise that we constrain our understanding of plastic pollution in the Antarctic if we limit our investigations to surface waters without also examining the biota.

5. Recommendations and conclusions

This study has used the recommended standardised criterion in Hartmann et al. (2019), for classifying micro-, meso- and macroplastics particles; however, there is a desperate need for the same criteria to be established for fibre pollution. Here, we provide a number of additional recommendations to address the challenges faced in this study during the collection and processing of the samples, particularly with respect to fibres.

Firstly, as evidenced by this study and others aforementioned in the Polar Regions, contamination from the user and scientific equipment has a greater influence on final concentrations, compared with "high pollution" areas. This problem is twofold. Firstly, as indicated by 45.6% of our particles being shown to be sources of contamination from the ship, a fact that would not have been detectable without the use of FTIR, highlights the necessity to refrain from reporting microplastic pollution without the use of FTIR. Secondly, the prevalence of fibres on a ship operating in cold environments, where fleecy materials are commonly used, or have been traditionally used, is inherently problematic. Studies should ensure scientists and deck engineers limit the use of fleece, or as a minimum, do not expose fleece clothing during sampling. Furthermore, the same level of attention to building up a contamination library using FTIR could be achieved for fibres in the future, as a minimum analysing the fibres being worn by the samplers. The use of a laminar flow hood and a clean isolated laboratory is recommended when analysing samples (Kühn et al., 2017), however this should also be prioritised when working on a ship and as a minimum, to prohibit entry by other personnel during sample preparation. Crucially, a reduction in steps when preparing and processing the samples and limiting the length of time in which the sample is exposed to the air is important. This not only reduces contamination but also prevents loss of sample (Woodall et al., 2014). The application of correction factors is commonly used, but should be done so with caution (Kühn et al., 2017). The caveats of reporting MF concentrations are addressed in our study, and we attempted to overcome this by reporting them separately from MP concentrations. Additionally, individual air-contamination correction factors have been applied to each sample concentration, as well as the overall procedural blank correction factor.

Finally, samples collected during a phytoplankton bloom make alkaline digestions problematic due to their high concentration of siliceous biogenic material (as for this study in the areas around the Polar Front– stations 1–3). The observed concentrations of fibres in the amphipods at station one and again at station three indicate that investigations of the waterborne samples, across the frontal zone may have yielded observable concentrations, useful for comparison. For future research, it is recommended that a sub-sample of phytoplankton-rich samples are extracted and the efficacy of acid or alkali digestion is determined before further analysis.

In order to advance our understanding of plastic pollution in the Polar Regions, we recommend that "hot-spot" areas of zooplankton biomass are targeted, and that both water and biota are investigated in order to comprehend fully the potential availability of plastic pollution to the Antarctic ecosystem.

CRediT authorship contribution statement

Kirstie Jones-Williams: Conceptualization, Methodology, Formal analysis, Writing - review & editing, Supervision. **Tamara Galloway:** Writing - review & editing. **Matthew Cole:** Investigation, Writing - review & editing. **Gabriele Stowasser:** Writing - review & editing. **Claire Waluda:** Writing - review & editing. **Clara Manno:** Investigation, Resources, Writing - review & editing.

Declaration of Competing Interest

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

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.105792.

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