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Microplastics in the Southern Ocean: Findings from the Continuous Plankton Recorder in the Ross Sea and the East Antarctic Regions

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Microplastics are extremely abundant and widely distributed in the marine environment. Recently they have been found in the Southern Ocean around the continent of Antarctica. Research from around the world has begun to demonstrate that microplastics can have detrimental effects on marine organisms. There is almost no information about how microplastics might affect Antarctic marine species. The present article reviews the current state of knowledge about microplastics in the Southern Ocean. The growing alarm about the ubiquitous nature of microplastic pollution has led the Antarctic and Southern Ocean Coalition (ASOC) to recommend that the Continuous Plankton Recorder (CPR) be used as a source of information about microplastics in the Southern Ocean. The CPR analysts from the Australian Antarctic Division (AAD) and New Zealand's National Institute for Water and Atmospheric research (NIWA) have been collecting a limited amount of data about microplastics alongside their primary research on plankton since 2008. Their data is presented for the first time in this report. The findings support the growing body of evidence that demonstrates the pervasiveness of microplastics, even in remote places such as the Southern Ocean. The findings also demonstrate that while the CPR has some value, it is not the ideal tool for understanding the abundance, distribution and impacts of

microplastics on the Antarctic marine ecosystem. There needs to be a more formal protocol for the identification and reporting of microplastics from the CPR. Furthermore, a deliberate and comprehensive survey of the potential sources of microplastics needs to occur, using higher-powered techniques such as FTIR or Ramen spectroscopy for the identification and characterisation of microplastics. We also call for urgent studies that seek to understand how microplastics will affect Antarctic marine organisms, especially the key-stone species, which appear to have the greatest exposure to microplastic pollution.

Contents

List of Acronyms

AAD	Australian Antarctic Division
AWI	Alfred Wegner Institute (Germany)
ASOC	Antarctic and Southern Ocean Coalition
BAS	British Antarctic Survey
CCAMLR	The Convention on the Conservation of Antarctic Marine Living Resources
CPR	Continuous Plankton Recorder
EEZ	Exclusive Economic Zone
FTIR	Fourier transform infrared spectroscopy
GACS	Global Alliance of CPR Surveys
NIPR	Japanese National Institute of Polar Research
NIWA	National Institute of Water and Atmospheric Research (New Zealand)
PFZ	Polar-Front Zone
POOZ	Permanent Open-Ocean Zone
POP	Persistent Organic Pollutant
SAZ	Sub-Antarctic Zone
SAHFOS	Sir Alister Hardy Foundation for Ocean Sciences (UK)
SCAR	Scientific Committee on Antarctic Research
SIZ	Sea-Ice Zone
SO-CPR	SCAR Southern Ocean Continuous Plankton Recorder
SOOS	Southern Ocean Observing System
STF	Sub-Tropical Front

Introduction

Plastic pollution is well recognised as a major threat to the health of our oceans and the innumerable species that are dependent on them for survival. Much less visible to the naked eye is microplastic pollution. The problem was initially identified in 1972 (Carpenter et al. 1972), though it was only in 2004 that the term 'microplastic' was first used (Thompson et al. 2004). Microplastics are now also recognised as a significant threat to our marine ecosystems, though their abundance, distribution and impacts on marine organisms are only beginning to be understood. This issue is of increasing concern as plastic use skyrockets globally. Evidence of microplastics has now been found in all of our oceans (Thompson et al. 2004, Cole et al. 2011, Ivar do Sul and Costa 2014, GESAMP 2016, Andrady 2017, Auta et al. 2017, Avio et al. 2017, Galloway and Lewis 2017, Isobe et al. 2017). Alarmingly, this includes very recent findings from waters around the Antarctic continent (Cincinelli et al. 2017, Isobe et al. 2017, Munari et al. 2017, Waller et al. 2017), places that were previously considered the most remote and pristine (Ainley 2004, Halpern et al. 2008).

Microplastics are defined as plastic fragments that are less than 5 millimetres in diameter, and can be as small as a few microns across (Arthur et al. 2008, GESAMP 2015). They are categorized as primary or secondary microplastics. Primary microplastics are deliberately engineered for uses such as the micro-beads in personal hygiene and cosmetic products, or as the pre-production pellets to make common plastic products (Cole et al. 2011, Andrady 2017). Secondary microplastics come from the fragmentation and degradation of plastics (Andrady 2011, Cole et al. 2011). The makeup of these microplastics reflects the most common plastics that are used industrially: Polyethyelene (PE), Polypropylene (PP), Polystyrene (PS), Polyethylene terephthalate (PET), and Polyvinyl chloride (PVC)(Andrady 2011).

Microplastics enter our oceans from a range of sources including land-based litter transported via rivers and other waterways, waste water outlets and accidental or indiscriminate loss of fishing materials (Andrady 2011, Cole et al. 2011). There is a growing body of research demonstrating that microplastics are having an impact on a wide variety of marine organisms, mostly via ingestion (Cole et al. 2013, Wright et al. 2013b). One of the

principal concerns about the potential toxicity of microplastics to marine organisms is their ability to readily adsorb and concentrate persistent organic pollutants (POPs)(Teuten et al. 2009, GESAMP 2015, Gaw et al. 2016, GESAMP 2016). Thus microplastics are acting as a vector for comparatively high concentrations of POPs to be ingested by, and thereby cause harm to marine organisms (Teuten et al. 2009, Wright et al. 2013a, Wright et al. 2013b, GESAMP 2015, 2016).

Despite the rapidly growing volume of research on marine microplastics (Barboza and Gimenez 2015), it wasn't until 2017 that researchers began to consider the Southern Ocean and Antarctic regions (Isobe et al. 2017, Waller et al. 2017). Although there had been reports on macroplastics in the Southern Ocean (Barnes et al. 2010), the region was thought to be relatively unaffected by microplastic pollution (Halpern et al. 2008, Waller et al. 2017). Recent findings have now demonstrated that microplastics have indeed reached the Southern Ocean (Isobe et al. 2017, Waller et al. 2017). The majority of the data that exists about microplastics in the Southern Ocean comes from studies around the Antarctic Peninsula and the Sub-Antarctic Islands such as the South Shetland Islands and South Georgia (Waller et al. 2017). Only one study presents information about microplastics in the East Antarctic region of the Southern Ocean (Isobe et al. 2017), and two very recent Italian studies present findings from the Ross Sea (Cincinelli et al. 2017, Munari et al. 2017).

By 2017, the problem had reached the attention of key scientific bodies responsible for the monitoring and protection of Antarctic environments and marine species (ie: CCAMLR, ASOC). Consequently, microplastics have been identified as a '*serious and emerging threat'* (CCAMLR 2016, ASOC 2017). Both CCAMLR and ASOC have called for urgent research into the matter.

ASOC identified the Continuous Plankton Recorder (CPR) surveys, which have been in operation for many years in the Southern Ocean, as a potential source of critical information about the prevalence of microplastics around Antarctica (ASOC 2017). The CPR is a method of sampling plankton species by towing an instrument behind a vessel as it traverses the ocean (Thompson et al. 2004). It provides a valuable tool for monitoring plankton species composition, abundance and distribution over time. Although it was not specifically

designed for microplastic sampling, the CPR has been in use in European waters since the 1960s, and subsequent investigation has revealed rising levels of microplastics in the CPR from this region (Thompson et al. 2004, Cole et al. 2011).

In the Southern Ocean, CPR surveys began in 1991 (Hosie et al. 2003). This monitoring program is run by the SCAR Southern Ocean CPR survey (SO-CPR), a collaboration between the Australian Antarctic Division (AAD), Japanese National Institute of Polar Research (NIPR), the National Institute of Water and Atmospheric Research from New Zealand (NIWA), Alfred Wegner Institute from Germany (AWI), and the UK-based British Antarctic Survey (BAS) and Sir Alister Hardy Foundation for Ocean Science (SAHFOS). The objective of the SO-CPR survey is to monitor the species composition, abundance, and distribution of plankton in Antarctic waters over time (Hosie et al. 2003, Robinson et al. 2014). Since mid-late 2000s, some of these organisations also began recording information about microplastics identified in the CPR samples.

The present paper sets out to comprehensively report on the current information available about microplastics in the Southern Ocean: their sources, abundance, and impact on Antarctic marine biota. It will present hitherto unpublished data about microplastics in the Southern Ocean from the CPR records collected by the AAD and NIWA. This new data affords an opportunity to assess the use of the CPR as an ongoing tool for the monitoring of microplastics as suggested by ASOC and CCAMLR, and to propose an appropriate research program to address key questions and gaps in current knowledge.

Microplastics in the Southern Ocean: current knowledge

In the Southern Ocean, the local sources of microplastic pollution are the wastewater of research stations, science field camps, and research, tourist and fishing vessels (Waller et al. 2017). The breakdown of macroplastics such as fishing gear lost overboard by commercial fishing vessels, and other macroplastic pollutants lost directly to the environment from tourist-, research-, supply- and fishing-vessels also contribute (Waller et al. 2017). There is an unknown quantity of microplastics that may be transported from distant oceans and sources via ocean currents and winds (Waller et al. 2017).

In a 2017 review, Waller et al make an estimate of the relative contribution to microplastic pollution from local sources. Their estimate is based on figures on the use of personal care and cosmetics products, microfiber losses from laundering, and the data regarding the number of person-days in the Antarctic. Waller et al presented estimates of 44-500 kg of microplastic particles entering the southern ocean per decade from personal care products, and up to 25.5 billion plastic fibers per decade from laundering of synthetic fabrics (Waller et al. 2017). From these estimates, Waller et al. suggested that the microfibers from laundry that are in the wastewater will likely make a more substantial contribution when compared with the microplastics from personal care products (Waller et al. 2017). They also suggest that the local input of plastics makes up a relatively small contribution overall (Waller et al. 2017). These estimates are a useful first step in understanding the sources of microplastics in the Antarctic environment, however there has not yet been any empirical evidence reported to support these figures.

Waller's estimates assumed that wastewater treatment systems were in place, and that 90% of plastics were removed by this process. At the last review, only a little over half of the research stations on the Antarctic continent even had a wastewater treatment system (Gröndahl et al. 2009). Furthermore, the standard wastewater treatment systems that exist do not completely remove microplastics, particularly in the extreme weather conditions that they must operate at in the Antarctic (Gröndahl et al. 2009, Stark et al. 2015). At the time of writing, no information has been identified that reports on the discharge of microplastics from wastewater systems from any of these bases.

The input of microplastics coming from the breakdown of macroplastic debris is not currently possible given the paucity of reliable data about macroplastics pollution in the Southern Ocean (Barnes et al. 2009, Barnes et al. 2010).

Very recently published findings from two Italian studies identified a trend in the distribution of microplastics in the Ross Sea. This trend suggests that research stations are actually making an important contribution to the microplastic pollution in the area (Cincinelli et al. 2017, Munari et al. 2017). Both studies report higher concentrations of microplastics in samples taken from close to the Italian base (Mario Zuchelli Station). They did not report any findings taken directly from wastewater outflows, so it is not possible to be certain that these findings are related to theoretically high concentrations of microplastics in the wastewater, or whether it may be other activities occurring in the area that are having this effect.

The findings do agree in some respects with estimates by Waller et al. (2017) in that the vast majority of microplastics identified in their samples were microfibers (Cincinelli et al. 2017, Munari et al. 2017) . In the article by Cininelli et al (2017), they go as far as suggesting that the high proportion of red and blue fibers could be indicative that the most important source of plastics is from the laundering of the clothing. The Italian Antarctic uniform is mostly red and blue, though no direct comparison is made with fibers known to come from these items. Contrary to these findings, Munari et al (2017) report that the highest proportions of microplastics identified from sediment samples were of styrene-butadienestyre copolymer (SBS), which is a material commonly used in pneumatic tires, soles of shoes, and waterproofing systems. This difference may simply reflect different sampling techniques and thus the identification of differently weighted microplastics and their subsequent distribution in the environment.

The properties and toxicity of untreated wastewater at Davis Station (Aus), in East Antarctica have been investigated recently (Stark et al. 2015, Stark et al. 2016a, Stark et al. 2016b). The wastewater is similar to standard municipal wastewater, containing human waste, domestic waste (from kitchens and bathrooms), and light industrial waste (from

workshops, laboratories, and medical facilities) (Stark et al. 2016b). The latter also contains contaminants such as metals and persistent organic compounds (Stark et al. 2016a). A number of studies have investigated the distribution and dispersal of wastewater around Antarctic stations using different identifying features: microbes (Hughes, 2003; Hughes and Thompson, 2004); chemicals e.g. PBDEs (Hale et al., 2008) and hydrocarbons (Kennicutt et al., 1995); and sewage molecular markers (Martins et al., 2012). There is no data that adequately describes how microplastics distribute and disperse and whether their pattern follows that of wastewater or whether their dispersal pattern is influenced by other mechanism. There are no studies to date that specifically investigate the occurrence of microplastics in the wastewater from Antarctic stations.

New findings from the Southern Ocean Continuous Plankton Recorder (SO-CPR)

Overview

In light of the emerging evidence of microplastics in the Southern Ocean, the SO-CPR survey was identified as a potential source of information about microplastics. The scale of the CPR is enormous. For example, between 1991 and 2011 there were more than 400 separate CPR "tows" made by AAD ships, which provided sampling records for more than 36,000 nautical miles (Robinson et al. 2014). A "tow" describes the use of a single sampling filter in the CPR unit from when it was put in the water until when it was retrieved again (Robinson et al. 2014), a single filter can be used over approximately 450 nautical miles (Robinson et al. 2014).

The CPR has previously been used to investigate microplastics in the Northern Atlantic Ocean (Cole et al. 2011). At recent meetings by the Scientific Committee of CCAMLR in 2017, the CPR was identified as plausible tool for the monitoring of microplastics in the Southern Ocean (CCAMLR 2016, ASOC 2017). In fact, since the mid- late- 2000s, staff at the AAD and NIWA had already begun to record information about microplastics that were identified in the CPR samples. However, this data has not been reported until now, and it has also never made publically available on the SO-CPR database.

The AAD has been performing CPR tows and monitoring the Southern Ocean area south of Australia between 60°E and 160°E (Robinson et al. 2014). Since 2010, they began documenting findings about microplastics. NIWA has also been involved in the SO-CPR monitoring program since 2008, collecting samples from New Zealand's Exclusive Economic Zone (EEZ), the Ross Sea and the transit area between them (Robinson et al. 2014). NIWA has recorded data for microplastics since starting their program in 2008.

The principal objectives of both the AAD and NIWA's program with the SO-CPR survey is "*to map changes in the quantitative distribution of epipelagic plankton, including phytoplankton, zooplankton and euphausiid (krill) life stages*" (Robinson et al. 2014). They follow strict sampling and identification protocols determined by the SAHFOS and SO-CPR

surveys. A microplastics identification and recording protocol was developed by SAHFOS and distributed among CPR analysts at NIWA and the AAD working on the SO-CPR.

Methodology

The new data on microplastics presented in this report has been collected and analysed by specialist staff at NIWA (Robinson et al. 2014) and the AAD (Hosie 2017), who contribute to the SO-CPR database. The methodology has therefore been designed with the primary objective of characterizing the distribution and abundance of plankton species in the Southern Ocean. A secondary objective of their research has been to record observations of microplastics in the samples. The methodology reported here is a simplified version of the methods reported in (Robinson et al. 2014), focusing on the processes relevant to microplastic sampling and identification. Much of the methodology from that report relating to plankton has been excluded in this report for simplicity.

Vessels and dates

Sample collection for the AAD has been done by the multi-purpose research and re-supply vessel the *Aurora Australis*. NIWA collaborated with the commercial fishing company, Sanford Limited who provided the opportunity for the CPR tows to occur from one of their fishing vessels, the *FV San Aotea II.*

The *Aurora Australis* makes numerous trips between Hobart (Tasmania, Australia) and the four Australian research stations (Casey, Davis, Mawson and Macquarie Island), as well as other journeys. During these transits it tows a CPR instrument. Figure 1 illustrates the locations and paths of the CPR tows completed by *Aurora Australis* that have been included in the analysis for this report. These occurred between 2010 and 2015.

The ship *FV San Aotea II* travels to and from the Ross Sea to fish for Antarctic toothfish (Dissostichus mawsoni). It was used for CPR tows during its summer fishing voyages to the Ross Sea every year from 2008-2013. The *San Aotea II* undertook 10 different transits (5 southwards and 5 northwards) between New Zealand the Ross Sea in the Antarctic fishing season (December-February) in this period. For each transit, multiple separate tows of the

CPR occurred. The *FV San Aotea II* would leave the New Zealand port of Timaru around November/December and return in January/February. The CPR tows completed by the *FV San Aotea II* are illustrated in Figure 2.

The crew aboard these vessels were trained to deploy and retrieve the CPR at regular intervals. Specific training was provided to a dedicated 'CPR handler' to operate the instrument, this included (1) loading internal cassettes with new silks (2) adding buffered formaldehyde to the cassette storage tank, (3) loading the cassettes into the main body of the CPR and making sure the gears were meshed, (4) deploying and towing the CPR, (5) retrieving the sampled silks, labelling, preserving them, and recording hourly GPS time and position data. Training and support with sampling protocols were provided to the CPR analysts by SO-CPR, and SAHFOS (Robinson et al. 2014). There is only one CPR analyst working for NIWA and one working for the AAD.

Figure 1: Map indicating the location of CPR tows made by the vessel Aurora Australis between 2010 and 2015.

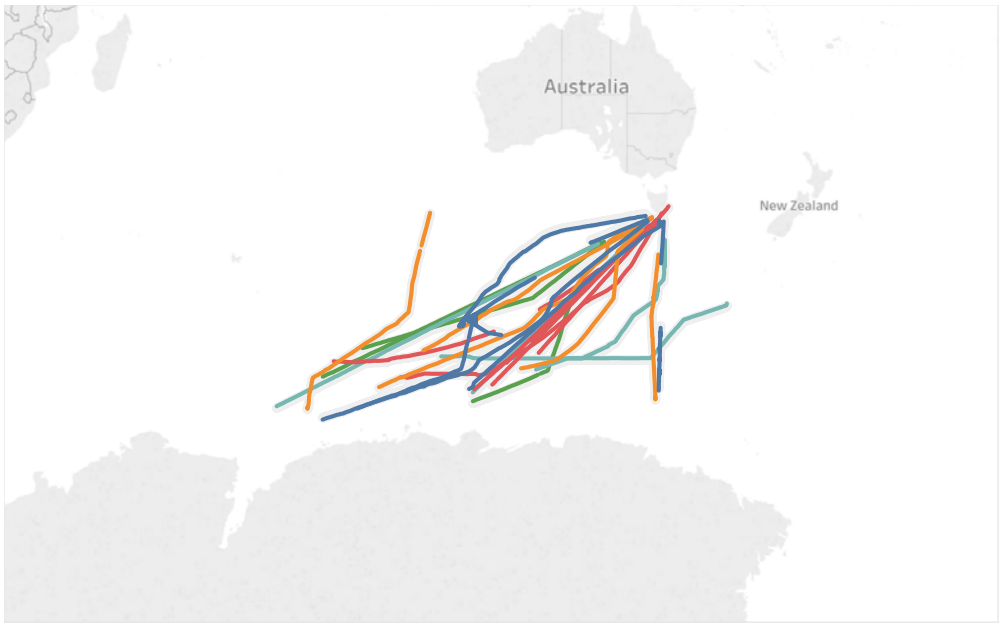
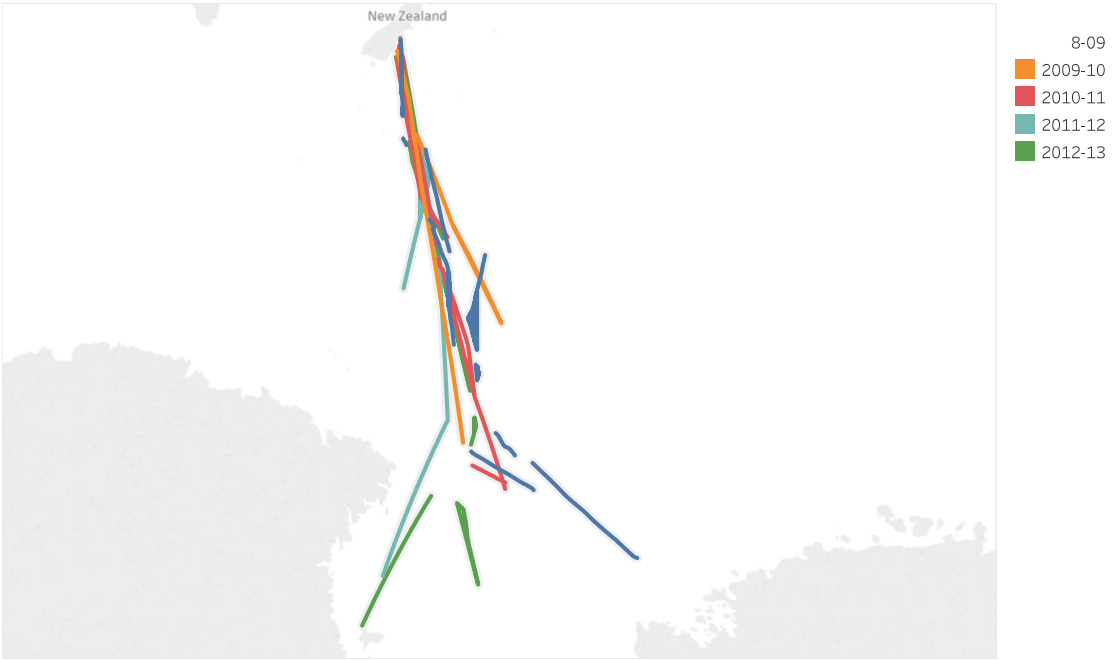


Figure 2: Map indicating the location of CPR tows made by the vessel *San Aotea II* between 2008 and 2013.



Study Area

For the purpose of this report, the Ross sea region has been defined as the area between New Zealand and the Ross Sea, which lies between 160°E and 150°W and south of 40°S. This area is within the FAO/CCAMLR statistical area 88.

The East Antarctic region defines the ocean between 60°E and 160°E, and south of the Sub-Tropical Front. This is largely contained within FAO/CCAMLR statistical areas 58 (between longitudes 80° and 150°E). This area has had a comparatively large amount of CPR sampling done since 1991.

Sample collection: The Continuous Plankton Recorder

This CPR is an instrument that is towed approximately 100m behind the ship, which can continue to travel at normal cruising speeds while it is deployed. It is towed horizontally at a depth of approximately 10 metres. While it is being towed, water enters a small opening (12.7 Å ~ 12.7 mm) at the front of the unit. The water then passes through a collecting silk, which has a mesh size that filters out particles larger than 270 µm. An external propeller advances the collecting silk at a rate of 1cm per nautical mile, irrespective of boat speed. A

second silk, the 'covering' silk, is used to cover the collecting silk and protect the particles it has collected once it is no longer being exposed to the ocean water influx. The two silks trap the plankton, microplastics and other debris between the two layers, and are then rolled up and put into a storage tank containing 40% formaldehyde for preservation of the plankton. Each silk is 6 m long, and can be used over a maximum of 450 nautical miles in a single tow. Variable ocean conditions affect the ships passage through the ocean, so the length of individual tows can be variable, for example a rough sea with large swells (larger vertical undulation) will result in a shorter horizontal tow distance. Sampling silks, once retrieved, were preserved in individual jars, labelled, and returned to the laboratory when the ship returned to New Zealand.

Sample Analysis

Once returned to the laboratory, the pairs of silks are unrolled and cut into segments that represent approximately 5 nautical miles of towing distance. A Phytoplankton Colour Index (PCI) number is assigned to each segment, and is indicative of the dominant phytoplankton group. This is a non-invasive process, thus should not impact on microplastic identification.

Each segment of silk was then rinsed off into individual sample containers to collect all zooplankton, microplastic and other debris from it. These samples, (each representing approximately a 5-nautical mile tow segment), were then viewed under a dissecting microscope using the SO-CPR Survey Method (Hosie et al. 2003). The primary purpose of the CPR analyst was to identify zooplankton to the lowest taxonomic level possible. The secondary aim of the CPR analyst, was to record the presence or absence of visualised microplastics.

The instructions to the CPR analysts were not more detailed than this, so the analyst at the AAD has followed the above methodology, as well as providing a count of the number of plastics in each segment, their size (Small ≤ 300µm, medium 300µm ≤ 2mm, large 2mm≤5mm, and x-large >5mm), their type (strand, bead or flake), and their colour (clear/transparent, blue, red, green, black, other). In samples analysed by NIWA, the presence of microplastics was reported (present = 1, absent = 0), but no formal count was

made. The NIWA analyst did make notes of the colours of the microplastics identified, and sometimes reported the type of plastic.

Specific training and support with CPR sampling protocols were provided to the CPR analysts by SO-CPR, and SAHFOS. This included training in sampling protocols, the identification of zooplankton, and the use of the Phytoplankton Colour Index. This training also required the analyst to attend international plankton identification workshops and participate with quality control check. However no formal training on microplastic identification has occurred at this stage for any analysts. Data from both the AAD-CPR samples and the NZ-CPR samples are collated and stored in the SO-CPR data base, which is managed by the AAD in Hobart. Despite information regarding plankton being freely available, the data regarding microplastics has not been made public at this stage.

Results

The important features of each of the two analysed data sets are outlined in Table 1. The microplastics data from the AAD has come from a total of 92 separate CPR tows by the vessel *Aurora Australis* between 2010 and 2015. This represents a little over 33,000 nautical miles of distance travelled by the vessel, or just under 10,000 cubic metres of sea water filtered by the CPR. Only 41 microplastic particles were identified in the 7,170 CPR segments analysed. The *San Aotea II* collected CPR data for NIWA between 2008 and 2013, performing 43 separate tows representing almost 12,000 nautical miles. Over 3,500 cubic metres of sea water were filtered for the 2,378 CPR segments collected. NIWA have identified microplastics in 547 of these samples.

Table 1: Key features of the analysed data sets from the CPR samples of the Australian Antarctic Division (AAD) and the National Institute of Water and Atmospheric research (NIWA).

	Data Source	
	AAD	**NIWA**
Ship	*Aurora Australis*	*San Aotea II*
Seasons	2010-2015	2008-2013
Number of Tows	92	43
CPR Segments	7,170	2,378
Nautical Miles	33,103	11,890^
Volume filtered (m^3)	9,888	3,552^
Microplastics (particles)	41*	547#

Average microplastic concentration (particles m^{-3})	1.24×10^{-3}	1.54×10^{-1}

^ This is an estimate based on the number of CPR segments analysed, assuming that each segment represents 5 nautical miles. Actual distances were not provided in the data-set made available to the author. Calculations for volume of sea-water filtered were also base on this assumption.
* Actual number of microplastics identified, # Number of segments with at least one microplastic particle present.

It is important to note that the number of microplastics reported by the AAD is the precise number of particles observed by the CPR analyst. Where more than one particle was identified in a single CPR segment, the number of particles in that segment was documented. The microplastic data collected by NIWA was recorded as 'presence' (1) or 'absence' (0) of microplastics for each CPR segment. Thus the number of microplastics reported represents the minimum number of particles identified in each sample. From all Southern Ocean CPR samples collected by the AAD and NIWA, the average concentration of microplastics is between 1.24×10^{-3} and 1.54×10^{-1} particles per meter[3].

The AAD also reported other characteristics of the plastic particles identified, these are summarised in Table 2. Almost half of all particles identified were red. Most of the other particles were either blue or green, and there were a very small number of particles of other colours. All particles identified were classified as 'medium' size (300µm to 2mm). The vast majority of particles were 'strands' (monofilaments), with only one bead, and one flake identified.

Table 2: Characteristics of microplastics reported in the AAD CRP

Colour		**Size**		**Type**	
Red	19	Small (≤ 300µm)	0	Strands	39
Blue	9				
Green	7	Medium (300µm to 2mm)	41	Beads	1
Yellow	3				
Clear	2	Large (2mm to 5mm)	0	Flakes	1
Black	1				
Other	0	X-Large (> 5mm)	0		
Total	41		41		41

In the NIWA dataset, CPR segments containing at least one microplastic particle were identified. Table 3 summarises the other microplastics data recorded by NIWA, though it must be examined with considerable caution as this was not in their protocol at the time of analysis, and the data is somewhat incomplete. At the time of analysis, the CPR analyst was only instructed to record 'presence' or 'absence' of microplastics for each segment, but did keep good notes on other characteristics that were identified. For each CPR segment, the colour of any and all microplastic particles were documented. Sometimes 2, 3 or even 4 colours were reported in a single CPR segment – this means that the total number of microplastics in that segment *may* reflect the actual number of colours identified. If only one colour was reported (eg: blue), there could still have been multiple blue particles in the sample. These figures should only be treated as indicative that the actual number of microplastics in the NIWA CPR samples is probably much higher than is formally documented. This data shows that red particles were probably most common, with blue, black, clear, yellow and green being successively less common. No data was recorded about particle size. The type of particle identified was inconsistently reported – when an entry in the data set was made at all, it would state that a 'thread' was present.

Table 3: Characteristics of microplastics reported in the NIWA CPR

Particle Colour^		**Particle Size**	**Particle Type**
Red	403	No data	Inconsistently reported (not part of protocol). Notes next to each sample often report 'threads', but there are also many samples with no information documented.
Blue	224		
Yellow	60		
Black	110		
Clear	94		
Green	31		
Other	6		
Not reported	4		
Total	**932**		

^ The figures reported for 'colour' represent the number of times that each colour was reported in the data set. When any microplastic particles were noted in a CPR segment, it was reported as particles present (1) or absent (0), even when there were multiple particles present. However the analyst made a note of the colour(s) of any/all plastic particles in the segment. Thus there were sometimes 2, 3 or even 4 colours reported in a single CPR segment – this means that the total number of microplastics in the CPR is likely to be much higher than has been documented. This does not represent a formal *count* of plastic particles, and is thus only discussed with extreme caution.

NIWA groups its findings about plankton from the CPR based on different oceanographic zones (Robinson et al. 2014). This means that the microplastic data can also be grouped in the same way. The relevant zones, from most northerly to most southerly, are the Sub-

Tropical Zone (STZ), the Sub-Antarctic Zone (SAZ), the Polar Frontal Zone (PFZ), the Permanent Open-Ocean Zone (POOZ) and the Sea-Ice Zone (SIZ). Table 4 summarises the following information for each zone: number of segments collected and analysed, the approximate volume of sea water filtered by the CPR, and the number of segments that had microplastics present. It also shows the concentration of microplastics in each zone. The CPR was not deployed as much in the STZ and the POOZ. This reflects the smaller amount of time spent in these zones because of their location or size. The concentration of microplastic particles was between 1.093×10^{-1} per m^3 in the STZ and 1.719×10^{-1} per m^3 in the SAZ.

Table 4: Distribution of microplastics by oceanographic zone, from NIWA data

Zone	Segments	Microplastics #	Volume (m^3)	Concentration (particles per m^3)
STZ	49	8	73.2	1.093×10^{-1}
SAZ	927	238	1,384.5	1.719×10^{-1}
PFZ	568	139	848.3	1.639×10^{-1}
POOZ	54	11	80.7	1.369×10^{-1}
SIZ	780	151	1,165.0	1.296×10^{-1}

Number of segments with at least one microplastic particle present.

Discussion

There is a striking difference between the number of microplastic particles identified in CPR samples by the AAD from waters in the East Antarctic region and in the NIWA samples from the Ross Sea and waters south of New Zealand. The AAD have identified just 41 microplastic particles in all of the CPR records analysed since introducing the basic microplastics counting protocol in 2010. This might be a reassuring finding demonstrating the relative absence of microplastics in the East Antarctic region of the Southern Ocean. However the absolute number of microplastic particles identified by NIWA (at least 547, possibly almost twice this number) is an order of magnitude greater. This is despite only processing approximately one third of the number of the number of CPR segments. Interestingly, NIWA also report a higher abundance of plankton species in the Ross Sea regions than the AAD have found in the East Antarctic regions of the Southern Ocean (Robinson et al. 2014). The Ross Sea region is considered to be a very rich ecosystem (Ainley 2010), but this should not change the concentration of microplastics. The large difference in microplastics concentrations from the AAD and NIWA is certainly an unexpected finding. Could there a particular pattern of

ocean currents and circulations that causes them to accumulate in this region? Is there a higher volume of fishing or shipping traffic? These differences also raise numerous questions about the validity and comparability of the microplastic identification protocols and techniques.

It is interesting that the microplastic particles reported by the AAD are all 'medium' (300µm to 2mm). It is possible that smaller particles were not detected as easily by the CPR analyst. Furthermore, the mesh size of the collecting silks is 270 µm, so any particles smaller than this may easily have passed through and never been collected in the CPR. The opening, or aperture, of the CPR is 12.7mm diameter. This should not exclude the 'large' (2mm-5mm) and 'x-large' (>5mm) particles, thus should not affect their collection by the CPR. The larger size particles would be at least as easy to identify as medium particles, so the fact that none were identified in the CPR tows suggest that there are indeed far fewer particles of those sizes in the sampling zone of the CPR.

The concentration of microplastics from the NIWA data was greatest in the SAZ, followed by the PFZ, the POOZ, the SIZ, and the lowest concentration of microplastics was found in the STZ. If the STZ is excluded, there seems to be a trend whereby the concentration of microplastics decreases with increasingly southerly latitudes. The comparatively low concentration of microplastics in the STZ is surprising as these are the waters closest to New Zealand where higher concentrations of microplastics might intuitively be expected.

A Japanese study reports on the abundance of microplastics in the East Antarctic region of the Southern Ocean. The average concentration of microplastics that they report is 3.1×10^{-2} particles per m^3 (Isobe et al. 2017). This is approximately 25 times greater than the average concentration reported from the SO-CPR for the same region (1.24×10^{-3}). Isobe et al. (2017) also report higher concentrations of microplastics closer to the Antarctic continent, in the waters south of 60°S, than in the open ocean between the SAF and the STF. The CPR tows by *Aurora Australis* for the AAD are contrary to these results, with only two samples South of 60°S having microplastics in them. The techniques used for sampling and identification of microplastics in the Japanese study were different to those used in the present study. Isobe et al (2017) used a Neuston net with a mesh size of 330µm. This is mesh is actually larger than the one used in the CPR, so would presumably not collect as

many small sized microplastics as the CPR. Mesh size cannot explain the difference in microplastic concentrations detected by Isobe et al (2017) and the SO-CPR. However, other features of the Neuston net and sampling technique used in the Japanese study may explain some of these differences. The net has a much wider aperture than the CPR. This was towed alongside the vessel at a depth of 1m (CPR is at 10m) for 20-40 minutes in five specific locations during the summer season of 2015-16 (Isobe et al. 2017). Any particulate matter caught in the net was returned to a laboratory for inspection and identification of microplastics under a microscope (Isobe et al. 2017). When particles were too small to determine if they were of plastic or organic matter, FTIR was used to confirm their makeup (Isobe et al. 2017). This use of FTIR possibly allowed many more small-sized particles to be detected, which may have been missed in the SO-CPR where only a dissecting microscope was used. The two studies made measurements at different depths in the water column (1m vs 10m). The density of a plastic particle will determine where it sits in the water-column. If it is positively buoyant, it will float at or near the surface, neutrally buoyant particles can be found throughout the water column – depending on the density of the water they're in, and negatively buoyant particles will sink to the seabed. Biofilms and algal growth can affect the density of a plastic particle (Andrady 2011). Without FTIR analysis of the particles identified in the SO-CPR, it is not possible to know whether the same type of plastics are detected as those found in the Japanese study. The different sampling depth may explain the differences in findings between the present study and the one by Isobe et al.

Table 5: Results of 2016 Japanese microplastic survey in the East Antarctic region of the Southern Ocean (Isobe et al. 2017). Note that GPS data was not provided

Sample number	Geographical information	Particle Count (pieces)	Seawater Volume (m^3)	Concentration (pieces m^{-3})
1	South of PFZ	20	202	9.9×10^{-2}
2	South of PFZ	18	392	4.6×10^{-2}
3	Between SAF-STF	2	566	3.5×10^{-2}
4	Between SAF-STF	2	502	4.0×10^{-2}
5	Between SAF-STF	2	417	4.8×10^{-2}
Average				**3.1×10^{-2}**

The most important criticism of these findings from both the AAD and NIWA is that microplastics identification and documentation was never the primary goal for the analysts working with the CPR samples. A very simple protocol has only recently been added at the laboratory stage that involves making a record of microplastics identified in the CPR silks while undertaking the primary task of identify plankton species and numbers. To attain robust findings on microplastics, protocols need to be designed that take all of the following steps into consideration: sample collection, storage, isolation or separation from background substrates (water, sediment, organic matter), identification, and classification. Most of these critical steps were not considered.

This has numerous implications for the validity of the present findings. The sampling process was not optimised for accurate microplastic detection – for example processes such as storage and sample preparation are only considered from the point of view of plankton data. The storage fluids used to preserve plankton and other organic matter could dissolve or degrade microplastics. Sampling and storage processes at sea, and the subsequent processing and identification processes in the laboratories might all lead to varying potential for sample contamination with microplastics from the environment, instruments used, or the analysts themselves. There has been no specific training for the analysts to help them accurately and consistently identify microplastics in their CPR samples. There has been no standardisation of microplastic identification techniques between the AAD and NIWA, and the level of inter-observer variability is unknown at this stage. It is possible that differences in the skill of the analysts and/or the technique they are using to identify microplastics accounts for the significant difference between the findings reported by the AAD and NIWA. Furthermore, the use of microscopy alone as a method for identifying microplastics has been demonstrated to have quite high levels of inaccuracy, particularly for the smaller microplastic particles (Hidalgo-Ruz et al. 2012, Miller et al. 2017, Shim et al. 2017). The inaccuracies are both positive and negative – with organic matter being falsely identified as microplastics, and true microplastics being overlooked (Shim et al. 2017). This could also contribute to the surprising differences in the findings from the AAD and NIWA. It is unclear how much the relative abundance of phytoplankton and zooplankton in a sample might impact on the ability of the analyst to identify plastics. Discussion with one of the analysts

reveals the possibility that microplastic particles are more easily missed when the abundance of plankton is higher, though this has not been formally assessed to date.

Visual sorting with the naked eye has conventionally been used for larger plastics (from 1-5mm)(Shim et al. 2017), however with the widespread presence of much smaller plastic particles requires quantification of microplastics down to the smallest possible sizes. Identification of microplastics is often done using a dissecting microscope, and provide details of the size, shape, colour and surface texture of the plastic (Shim et al. 2017). This method of identification is useful for larger microplastic, though once plastics are <100 μm, particularly when they are colourless, they become very difficult to identify (Shim et al. 2017). When there is biological material present in the sample, this becomes harder again (Shim et al. 2017). There are high rates of false identification of plastic-like particles using microscopy, with more than 20% of coloured particles, and 70% of transparent particles being falsely identified (Shim et al. 2017). Scanning electron microscopy can be used to provide very clear images, and thus is far more accurate than a simple dissecting microscope for identification of microplastics (Shim et al. 2017). This is a very expensive and slow method, thus is not a useful method for characterizing abundance of microplastics over the large scales that are being considered in most studies.

The most accurate methods available for microplastic identification are Fourier Transform InfraRed spectroscopy (FTIR) and Ramen spectroscopy (Miller et al. 2017, Shim et al. 2017). FTIR is a method for identifying microplastics that is being used quite frequently (Shim et al. 2017). This method easily discriminates between organic and inorganic materials and enables identification of specific plastic polymers (Shim et al. 2017). It also enables the identification of very small microplastic particles which cannot easily be identified with standard microsopcy (Shim et al. 2017). This method is effective when used in conjunction with standard microscopy – any plastic-like particles must first be identified with microscopy, then confirmed with FTIR. This technique reduces the likelihood of missing plastic particles, and avoid false-positives. Information about the type of plastic polymer also helps to understand the potential origin or source of the plastic (Shim et al. 2017).

Raman spectroscopy is also frequently used for identification of microplastics (Shim et al. 2017). It provides similar findings to FTIR – it identifies plastics and can determine the specific polymer composition (Shim et al. 2017). It is able to identify plastics that are even smaller than FTIR is able to do (Shim et al. 2017), and does not disrupt the sample in anyway – allowing for further analysis down the track if necessary (Shim et al. 2017). It is also useful for identifying microplastics within zooplankton samples (Shim et al. 2017).
Unlike FTIR, Raman spectroscopy is not constrained by the thickness or shape of the sample (Elert et al. 2017). It does require a process to purify the sample prior to Raman spectroscopy to decrease the amount of organic matter (Elert et al. 2017).

Importantly, the choice of microplastic identification methods depends somewhat on the overall goals of the study in question. The key characteristics in microplastic analysis are the physical characteristics (size, shape and colour), and the chemical (polymer type) properties. At present, no single method is effective at determining both of these properties, so it is recommended that a combination of analytical tools are used together. For example, the use of microscopy to identify physical characteristics followed by the use of FTIR or Ramen spectroscopy to confirm plastic identification and determine the specific polymer will be ideal (Shim et al. 2017).

To account for the potential impact of chemical separation and/or storage of samples, identification techniques such as FTIR or Raman spectroscopy should be employed both before and after any chemical separation or storage occurs for a set of control samples. This is to assess the impact of those chemicals on the structure and chemical properties of the plastics (Miller et al. 2017).

Very few studies have attempted to quantify the loss of microplastics from samples after processing them with any particular technique (Miller et al. 2017). It would be appropriate to understand the rate of loss so that accurate estimates of microplastic concentrations can be determined.

The CPR is not the most common method for collecting microplastic samples. The most frequently used method is to tow a neuston net alongside a boat (Miller et al. 2017). It is not

a dissimilar method; the neuston nets were also originally designed to collect plankton samples (Miller et al. 2017). They work on the same principal of filtering particles out of the sea-water, and use a similar mesh-size (usually 200µm to 333µm) (Miller et al. 2017). One of the important differences is that they allow a much large volume of water to pass through them,

Choice of mesh size necessarily influences the sizes of microplastics that can be collected. It is also important to be aware of the depth that samples are taken from, as the density of different plastics will affect their distribution in the water column (REF). Thus the abundance of different types of plastics may vary throughout the water column and subsequently influence the exposure of a species to different plastics. Sampling from sediments, ice or marine organisms is not discussed in the present work as the focus of this research is on seawater samples. However these types of samples must be included in a methodical plan to properly assess the state of microplastic pollution in the Southern Ocean. It needs only to be noted here that for both sediment and organic sampling, the same issues arise: the need for a reliable, standardized and efficient method to sample, separate, characterize and identify microplastics, and also to identify recovery/loss rates (Miller et al. 2017)

Since the influential work by Thompson et al. in 2004 (Thompson et al. 2004), there has been more than 10 years of research into the presence and abundance of microplastics in our oceans. Despite this rapidly growing body of research, the methods used to sample, separate, characterize and identify microplastics still highly variable (Hidalgo-Ruz et al. 2012, Miller et al. 2017) For example, the methods used to separate microplastics from the background water, sediment and biomass (Miller et al. 2017) include visual separation, density flotation, acid, alkaline, oxidative or enzyme digestion separation methods (Miller et al. 2017). At present, all these methods are time and labour intensive and is one of major issues that is holding back microplastic research (Miller et al. 2017). Furthermore, it makes a rigorous comparison of the findings from different studies almost impossible.

Other techniques for the sampling, processing and identification of microplastics do exist – however these are far more expensive and labour-intensive. Standardization of the CPR

against these more accurate techniques could significantly enhance the value of findings reported from the CPR.

Assessment of the SO-CPR as a tool for monitoring microplastics in the Southern Ocean

The CPR is a scientifically proven and cost-effective way of measuring plankton abundance, distribution and community composition (biodiversity) in the open oceans (Robinson et al. 2014). It remains to be determined whether the CPR is also an appropriate tool for monitoring microplastic pollution.

The value of the CPR for monitoring of microplastics lies the fact that it is already frequently used in the Southern Ocean for monitoring of the plankton. A microplastic monitoring protocol that can be added to the SO-CPR surveys, will require very little extra infrastructure and cost to the relevant Antarctic programs and monitoring bodies. The extra work that is needed would primarily be in the laboratory for the identifications, counting and classification of microplastics.

The strict protocols that are already in place for the SO-CPR survey, across all collaborating research parties, is also of great significance. This makes their data comparable, making the findings from different regions around the Southern Ocean far more robust than would otherwise be possible. Participating SO-CPR analysts undergo regular quality control and standardisation testing. Training for the identification of microplastics, and similar quality control procedures would make the findings of microplastics reported in the CPR highly valuable.

Furthermore, since there are some initial findings that suggest microplastics are harmful to plankton species (Cole et al. 2013, Galloway et al. 2017), and plankton dynamics are so critical to the function of the Antarctic marine ecosystem (Pinkerton et al. 2010, Pinkerton and Bradford-Grieve 2014), it could prove extremely valuable to simultaneously sample essential information on both plankton and microplastics abundance and distribution. This

may prove extremely useful to our understanding of the potential risks of rising microplastic concentrations on these populations.

The SO-CPR has many limitations that should not prevent it from being used for ongoing monitoring of microplastics, but which demand that there are parallel efforts to collect information that the CPR cannot. The major limitation is that the CPR only samples water at a depth of 10m. This will clearly exclude sampling for any plastics that do not accumulate at this depth because of their density (they may float or sink), which will create a strong sampling bias towards particular types of plastics. It also represents a very particular marine habitat, providing no information about the distribution of microplastics in the sediments, surface water, deep sea, inter-tidal zone, benthic communities or sea-ice.

The very limited microplastics identification protocol which has been used to determine the current findings from the SO-CPR calls for a major update to make the findings more reliable. Additionally, the use of FTIR or Ramen spectroscopy to provide greater accuracy as well as more details about the types of plastics being found would complement the use of the CPR, particularly to establish a baseline understanding for the sources and distribution of microplastics throughout the Antarctic marine ecosystem.

Understanding the sources and risks of microplastics in the Southern Ocean: a proposal for future research

In the first instance, the specific and deliberate collection of data to characterize the sources, abundance and distribution of microplastics in the Ross Sea, in the East Antarctic Region and around the continent is urgent. This must involve targeted sampling from greywater, wastewater treatment systems and other outflows from the research bases themselves. Given the preliminary indication from other research that there are higher concentrations of microplastics nearer to the bases (Cincinelli et al. 2017, Munari et al. 2017), it is very likely that the local sources account for a reasonable proportion of microplastic debris in coastal waters of the Southern Ocean. This is important as it presents

one of the few sources where the development of strategies to mitigate the human impact on this fragile wilderness might be possible.

This should be a very detailed study, using the most advanced techniques currently available for microplastics sampling and identification. It will be important to know what type of plastic is having the greatest contribution to the pollution, which is relevant both from the point of view of which species may be most impacted, and also in identifying the original source of those plastics (personal hygiene, laundering of clothing, kitchen, science, operations).

Further to this, a structured sampling plan to identify the habitats where microplastics are accumulating most rapidly (sediments, surface water, deep waters etc.). This will help target research on the specific collections of biota which are more likely to be impacted. The total area affected by microplastic pollution from a point-source such as a single base would also likely prove interesting. It could help to characterize the total footprint that a research station can leave on the ecosystem, improving awareness of the extent of human impact.

ASOC and CAMLR rightly identify that the impact of microplastic debris on Antarctic marine species at the individual level and at population- and ecosystem-levels is unknown (CCAMLR 2016, ASOC 2017). They recommend that CCAMLR members commence dedicated research into the onshore and ship-based sources of microplastics, their at-sea distribution and their impacts in the Southern Ocean (CCAMLR 2016, ASOC 2017).

Conclusion

The pollution of the Southern Ocean with microplastic debris is now undisputable. In 2017, new reports clearly demonstrate that even those regions such as the Ross Sea, which are considered amongst the most remote and ‘pristine’ in the world, are polluted by our universal use of plastics. It appears as if the scientific research bases are contributing in significant ways to local microplastic pollution, with higher concentrations of microplastics being identified closer to these base, probably from their wastewater outlets. Other sources of microplastics to the Southern Ocean are from the numerous ships travelling in these

waters (for fishing, support of Antarctic bases, research and tourism) the breakdown of macroplastics, and probably the global transport of microplastic particles in ocean currents.

There is evidence from other regions that demonstrates that microplastic particles are having detrimental impacts on numerous marine species. At present, we do not know to what extent Antarctic species are being affected, and whether they are more susceptible to ingestion or contamination with microplastics. This represents a significant gap in our knowledge, and one which warrants urgent attention – particularly for key-stone species in the Antarctic marine ecosystems. Microplastics adsorb and concentrate other POPs, acting as a vector for high concentrations of other toxins – toxins which have been detected in the wastewater outlet of some Antarctic stations.

The new data presented in the present study supports other recent findings that microplastic debris does indeed pollute the waters surrounding Antarctica. The CPR does provide a source of information about the abundance and distribution of pelagic microplastics (at 10m depth). The average concentration of microplastics in the Southern Ocean as identified in the SO-CPR is between $1.24x10^{-3}$ and $1.54x10^{-1}$ particles per meter^3. The present findings are of limited value beyond this since the protocols for identifying microplastics by SO-CPR analysts have not yet been well developed.

Nevertheless, the CPR records hold significant potential for ongoing monitoring of pelagic microplastics in the Southern Ocean, and old samples could also be re-analysed to determine historical abundance of microplastics since the time that records commenced. Ideally, a new protocol needs to be developed for all SO-CPR tows that specifically considers the methodology in relation to microplastics as well as for its original purpose of documenting information about plankton. This needs to cover all aspects of sample collection, storage, preparation and identification techniques for optimal results. The most critical aspect of this needs to be the proper training of CPR analysts in the identification of microplastics, ideally from all contributing SO-CPR research teams. Regular standardisation and quality control will also be indispensable to ensure data is comparable. Once effective and standardised protocols have been established, then the CPR should be used for the simultaneous monitoring of microplastics and plankton.

While the CPR will likely prove an invaluable tool for ongoing monitoring of microplastics, there are significant gaps in our current knowledge that need to be addressed with the utmost importance. There is a pressing need for dedicated studies on microplastics in Antarctica. This should include a formal identification of sources through the sampling of wastewater outlets at research stations, field bases, and from all vessels. This will provide the most useful information from which prevention and mitigation strategies could be implemented. The pattern of distribution from point sources must be determined with planned sampling at increasing distances, as well as in the variety of strata or habitats (sediments, surface waters, deep waters, and benthic communities). Finally, the uptake by marine organisms needs to be evaluated, and an attempt to determine the impact on microplastics on key species is essential. At present, there are no known methods for removing microplastics from the environment, thus it is imperative that the utmost care is taken to prevent further pollution, particularly in the unique and fragile Antarctic ecosystems.

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