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Microplastics and other anthropogenic particles in Antarctica: Using penguins as biological samplers

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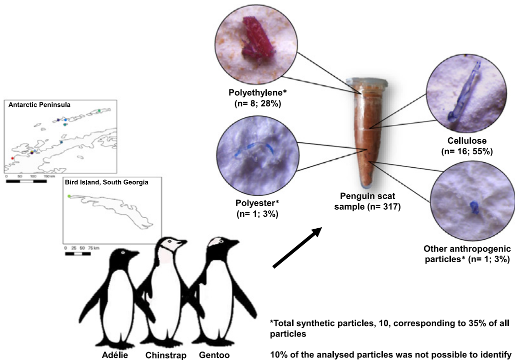
HIGHLIGHTS

- Anthropogenic particles were found in all three pygoscelid penguin species.
- Thirty-five percent were microplastics; these were found in all species.
- Fifty-five percent of the analysed particles were identified as cellulose fibres.
- Polyethylene were the most common synthetic polymer.
- Microplastics were widespread across years and colonies in Antarctic Peninsula.

GRAPHICAL ABSTRACT



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ABSTRACT

Microplastics (< 5 mm in size) are known to be widespread in the marine environment but are still poorly studied in Polar Regions, particularly in the Antarctic. As penguins have a wide distribution around Antarctica, three congeneric species: Adélie (*Pygoscelis adeliae*), chinstrap (*Pygoscelis antarcticus*) and gentoo penguins (*Pygoscelis papua*) were selected to evaluate the occurrence of microplastics across the Antarctic Peninsula and Scotia Sea. Scat samples (used as a proxy of ingestion), were collected from breeding colonies over seven seasons between 2006 and 2016. Antarctic krill (*Euphausia superba*), present in scat samples, contributed 85%, 66% and 54% of the diet in terms of frequency of occurrence to the diet of Adélie, gentoo and chinstrap penguins, respectively. Microplastics were found in 15%, 28% and 29% scats of Adélie, chinstrap and gentoo penguin respectively. A total of 92 particles were extracted from the scats ($n = 317$) and 32% ($n = 29$) were chemically identified via micro-Fourier Transform Infrared Spectroscopy (μ-FTIR). From all the particles extracted, 35% were identified as microplastics, particularly polyethylene (80%) and polyester (10%). It was not possible to ascertain the identification of the remaining 10% of samples. Other anthropogenic particles were identified in 55% of samples, identified as cellulose fibres. The results show a similar frequency of occurrence of particles across all colonies, suggesting there is no particular point source for microplastic pollution in the Scotia Sea. Additionally, no clear temporal variation in the number of microplastics in penguins was observed. Overall, this study reveals the

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presence of microplastics across Antarctica, in three penguin species and offers evidence of other anthropogenic particles in high numbers. Further research is needed to better understand the spatio-temporal dynamics, fate and effect of microplastics on these ecosystems, and improve plastic pollution policies in Antarctica.

1. Introduction

Microplastic pollution, such as films, fragments or fibres less than 5 mm (Arthur and Baker, 2008; Thompson et al., 2004), has become an increasingly hot topic, since they are pervasive and persistent across global ocean ecosystems, from the tropics to the poles, including in the Southern Ocean (Fang et al., 2018; Waller et al., 2017). Most of the microplastics present in aquatic ecosystem come from secondary sources (i.e. plastic litter and debris which breaks down in the ocean) and are expected to continue to fragment until they reach nanometre sizes or mineralize into carbon dioxide and biomass (Dawson et al., 2018). The most abundant microplastic polymers in the marine ecosystem are polyethylene (PE) and polypropylene (PP), polyester (PET), polystyrene (PS), polyvinyl chloride (PVC) and polyamide (PA) (Andrady, 2011; Barboza and Gimenez, 2015). Although microplastics are the most abundant forms of plastic debris, elucidating their biological consequences are challenging and ecosystem-level impacts have not yet been assessed. Some studies already prove that microplastics are present in the waters in certain Antarctic regions, such as South Georgia, the Ross Sea, the Pacific Sector of the Southern Ocean (Lacerda et al., 2019; Reed et al., 2018) and the Weddell Sea (Waller et al., 2017). They have been reported in surface waters (Fang et al., 2018), in sediments (Barnes et al., 2010) and recently in biota, including in gentoo penguins *Pygoscelis papua* (Bessa et al., 2019b), king penguins *Aptenodytes patagonicus* (Le Guen et al., 2020), fur seals *Arctocephalus* spp. (Eriksson and Burton, 2003) and Antarctic toothfish *Dissostichus mawsoni* (Cannon et al., 2016). The presence of microplastics in other biota has not yet been verified in the Southern Ocean region, but microplastics can be easily accessible by a broad range of marine biota due to their small size and widespread occurrence (Fang et al., 2018). A recent laboratory study has proven that Antarctic krill *Euphausia superba* (hereafter krill) is able to ingest microplastic particles when exposed to them, and convert these particles into nanoplastics through digestive fragmentation (Bergami et al., 2020; Dawson et al., 2018).

Antarctica and the Southern Ocean have a relatively low volume of shipping traffic and a very small human presence, which indicates a potentially sparse local source of microplastics (Reed et al., 2018). The highest concentration of microplastic has been found in the Antarctic Peninsula/Scotia Sea region, where many scientific research stations are based, and there is a higher density of maritime traffic (Waller et al., 2017). This indicates that the potential main sources of microplastics are the scientific research stations, fishing vessels, tourist and research vessels. As well as these main sources of microplastics in Antarctica, other potential pathways and long-range sources have been described, such as sea ice melt, the presence and consequent degradation of macroplastics and the action of wind and ocean currents (Rowlands et al., 2020). Pollution by microplastics in the Southern Ocean may be significant on a local scale (Reed et al., 2018), and despite Antarctica being a fairly remote continent, it can be used as a reference for global microplastic pollution assessment and mitigation (Cincinelli et al., 2017).

To evaluate pollution and climate change effects in marine ecosystems, albatrosses (Phillips and Waluda, 2020), seals (Lehnert et al., 2017) and penguins (Bessa et al., 2019b) are commonly used as Antarctic bio-indicators to monitor changes, under the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) monitoring programs (Constable, 2011; Constable et al., 2000). These organisms are top Antarctic predators, and they can record perturbations in Antarctic ecosystems at the upper and lower levels of the food web (Xavier et al., 2016; Xavier and Peck, 2015). Also, top predators, are good for monitoring the health conditions of the marine environment (Clucas et al., 2014; Xavier and Trathan, 2020) through changes in population size, health or breeding success of top predators (Furness and Camphuysen, 1997; Trathan et al., 2015). For example, seabirds, including penguins, have been used to record (macro)plastic pollution for a number of years (Phillips et al., 2010; Phillips and Waluda, 2020).

Penguins from the Antarctic can be regarded as reliable plastic pollution bio-indicators, as they are widely distributed, easy to handle and their ecology (e.g. diet and foraging capacity) and life history are well documented (Furness and Camphuysen, 1997; Trathan et al., 2015). To date, only a small number of research studies have shown that microplastics have entered in the marine food web and are present in penguins. Bessa et al. (2019b) showed that 20% of gentoo penguin scat samples from two islands (South Georgia and Signy Island, Scotia Sea) included microplastics of different types, suggesting potential different contamination sources. Le Guen et al. (2020) also showed the presence of microplastics in king penguin scat samples obtained from Hound Bay, South Georgia. However, those studies did not infer the spatio-temporal dynamics of microplastics in top predators from Antarctica.

Given the important ecological role of Antarctica and the Southern Ocean, and implications for microplastics in ecosystems, this study aims to (1) assess the occurrence of microplastics in Adélie, chinstrap and gentoo penguins, using scat samples (as a proxy of ingestion), and examine the main diet components to assess the likely vectors of microplastics; (2) assess whether the number of microplastics ingested by these three penguin species vary between different colonies, according to their geographical distribution, and over multiple years; and (3) characterize and identify the particles in order to evaluate the potential source of contamination in these environment, contributing to review the present policy measures on plastic pollution under the Antarctic Treaty and to develop and propose potential mitigation measures for the areas where penguins live in the Antarctic Peninsula/Scotia Sea and to other regions.

2. Material and methods

This study took place across the Antarctic Peninsula and Scotia Sea region where Adélie, chinstrap and gentoo penguins breed sympatrically (Fig. 1). Scat (i.e. faecal) samples of penguins were collected from breeding colonies at Paradise Bay A (near Almirante Brown Station) (64°51′S, 62°54′W), Byers Peninsula (62°37′S, 61°04′W), Cierva Cove (64°09′S, 60°57′W), Deception Island (62°58′S, 60°39′W), Hannah Point (62°39′S, 60°36′W), King George Island (62°23′S, 58°27′W), Paradise Bay B (near Gonzalez Videla Station) (64°48′S, 62°51′W), Rongé Island (64°43′S, 62°41′W), Yalour Islands (65°14′S, 64°10′W) and Landing Beach (Bird Island, South Georgia) (54°00′S, 38°05′W) (Fig. 1; Table 1).

Samples of penguin scats were carefully collected by hand from snow or rock immediately after defecation, avoiding collecting substrate remains. Samples were randomly collected across sites, at the colony or between the colony and the sea. Although samples were collected in breeding colonies, it was not possible to confirm the breeding or non-breeding status of birds. All samples were placed into transparent sterile plastic bags, closed tubes or eppendorfs, and frozen at −20 °C prior to further processing in the laboratory.

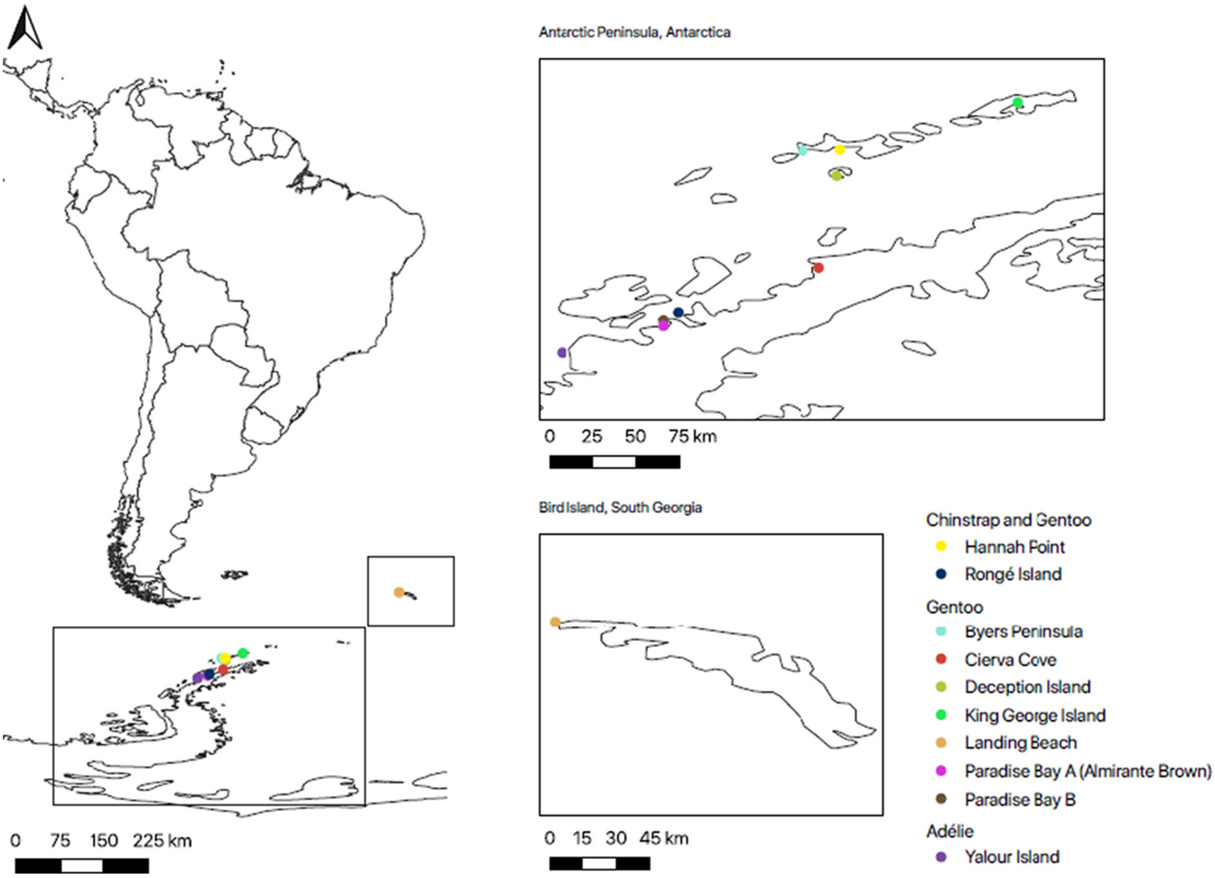

Fig. 1. Antarctic Peninsula and Bird Island (South Georgia) study area, and respective sampling sites.

2.1. Diet analyses

Penguin scat samples were defrosted and analysed at the laboratory (MARE-UC, Portugal). Diet composition of these three penguin species were reconstructed using scat samples collected during December, January and February in various breeding seasons between 2006 and 2016 (Table 1). All scats were examined in order to assess the presence or absence of cephalopod beaks, fish otoliths and krill carapaces. All krill carapaces were measured with a ruler to the nearest mm (scale 20 cm), measured from the tip of the rostrum to the mid-dorsal posterior edge of the carapace (length), following previous studies (Hill, 1990; Mauchline, 1980). These measurements were obtained using a Leica Wild M80 Stereo Microscope (Leica Microsystems GmbH, Wetzlar, Germany), with an IC80 HD camera (software LAS EZ, Wetzlar, Germany) attached to an M80 magnifier used to photograph the carapaces.

Table 1
Penguin scat samples collected (penguin species, location, sampling date, sample size (n)).

Species	Location	Sampling date	Scat samples (n)
Pygoscelis adeliae	Yalour Island	January 2008	20
	Deception Island	January 2006	28
	Hannah Point	January 2008	18
	Rongé Island	January 2008	11
Pygoscelis antarcticus	King George Island	February 2006	19
	Paradise Bay B	February 2006	28
	King George Island	January 2007	18
	Hannah Point	January 2008	18
	Rongé Island	January 2008	20
	Paradise Bay A (Almirante Brown)	February 2008	18
	Cierva Cove	February 2008	13
Pygoscelis papua	King George Island	February 2008	26
	Landing Beach (Bird Island)	January 2012	12
	Landing Beach (Bird Island)	February 2012	11
	Landing Beach (Bird Island)	January 2013	11
	Landing Beach (Bird Island)	February 2013	12
	Landing Beach (Bird Island)	January 2014	10
	Landing Beach (Bird Island)	February 2014	10
	Byers	December 2016	14

Allometric equations were used to determine the total length and body mass based on measured carapace length. To convert removed carapace length (RCL, in mm) of krill to total body length (AT, in mm) the following allometric equation (Hill, 1990) was applied:

$$AT = 11,56 + 2,44 \times RCL$$

Total length measurements were used to extrapolate to the original size of the analysed krill. These data allowed us to assess penguin diet (i.e. whether they feed on juvenile (size classes 20–25, 25–30, 30–35 mm), sub-adult (size classes 35–40, 40–45, 45–50 mm) or adult krill (size classes 50–55, 55–60 mm)) following Siegel and Loeb (1994). In addition, all krill carapaces in each scat sample were counted, in order to analyze the relationship between the number of krill and the number of microplastics, and to ascertain if krill could be a vector of microplastics in the Antarctic marine food chain.

2.2. Microplastics extraction

The extraction of potential microplastics was performed following the procedures described in Bessa et al. (2018) and Bessa et al.

(2019b). Each scat sample was transferred to a clean 0,250 L glass beaker to which a 10% potassium hydroxide (KOH) solution was added. The volume of added solution was 3 times the volume/ ratio, for a total of 0,150 L of biological material. After 72 h of digestion at room temperature, the floating phase was vacuum filtered through a 1.2 μm glass microfibre filter, and the resulting filters were sealed in a Petri dish properly identified and placed to dry in an oven at 50 °C for 24 h. As some filters had a large amount of biological material, hydrogen peroxide was added (H_2O_2, 10%) to the filters to increase the recovery of particles potentially trapped in the residue, after the initial 24 h drying period. The added solution was variable, with a maximum of 0,010 L. For those samples containing large amounts of organic matter, an additional step was added to improve the extraction of microplastics with samples passed through a 63 μm stainless steel sieve. As a result, the lower limit of detection of microplastics was set to 63 μm.

2.3. Observation and identification of microplastics

All extracted particles were analysed using a stereomicroscope LEICA M80 (Leica Microsystems GmbH, Wetzlar, Germany) to identify anthropogenic particles. After examination, all particles suspected to be of anthropogenic origin were kept on filters and photographed, using an image analysis system IC80 HD Camera with Leica Application Suite (LAS) software, and then placed between two microscope slides, until further chemical analysis, in order to determine polymer composition.

All particles were classified and categorized according to their shape into fibres (elongated), fragments (angular and irregular pieces), films (thin and transparent) and by their colour (blue, red, black, green, transparent, and other). In addition, their largest cross-section (size) was measured, using ImageJ software, and sorted according to their size classes (0.63 μm −1 mm, 1–2 mm, 2–3 mm, 3–4 mm and 4–5 mm). Taking into account that there is currently no agreed convention for categorising size classes of microplastics extracted from environmental samples, we follow the classification made in Isobe et al. (2017).

In order to determine the chemical composition of the particles collected ($n = 97$), a sub-sample of 29 particles (32% of the total) randomly selected from among the total penguin scats of the three species from all colonies and years was analysed using micro-Fourier Transform Infrared Spectroscopy (μ-FTIR) in transmittance mode, to confirm their synthetic origin (i.e. microplastics or other) and to determine their chemical composition (i.e. polymer type). Spectra were acquired in a Nicolet® Nexus spectrophotometer coupled to a Continuum microscope (15 x magnification) with a MCT-A detector cooled by liquid nitrogen, as fully described in (Bessa et al., 2018). Spectra were obtained in transmission mode, 4000 and 650 cm^{-1}, with a resolution of 8 cm^{-1} and 128 scans, and are shown as acquired, without corrections or any further manipulations, except for the occasional removal of the CO_2 absorption at ~2300–2400 cm^{-1}. Polymer identification was based on their spectral absorption bands (Cai et al., 2019; Hummel, 2012) and compared with a spectral library database using Thermo Scientific™ OMNIC™ Software.

Due to logistic constraints, it was only possible to analyze this subsample, which is above the minimum required and recommended (10%) for monitoring purposes of microplastics in environmental samples (Hanke et al., 2013).

2.4. Contamination control

In microplastic research it is crucial to reduce and monitor potential airbone cross-contamination. To minimize any contamination of samples, the entire process of extraction, processing and identification of microplastics was performed in a closed laboratory room with restricted access, and nitrile gloves and cotton coats were used during all processing steps. All laboratory materials and equipments used during sample processing were previously decontaminated using distilled water and ethanol. Glass materials were properly decontaminated using a 1% acid nitric bath and cleaned with distilled filtered water. The use of plastic material was avoided whenever possible. In addition, and to avoid potential airbone contamination, all liquids used were previously filtered through a 1.2 μm glass microfibre filter and glass containers rinsed with distilled water before reuse. During the digestion procedure, all glass beakers were properly covered to minimize contamination through airborne particles and open Petri dishes with clean filters were placed on the workbench as contaminant controls. The controls were repeated multiple times, during the extraction and identification stages, and kept close to the working area throughout processing. At the end of the sample analyses, these controls were checked for possible contamination and all fibres found in the samples resembling those found in the controls were discarded. The measures undertaken followed the protocol described in Bessa et al. (2019a).

2.5. Statistical analysis

The length of krill found in penguin scats was classified in 8 size classes at 5 mm increments from 20 to 25 mm to 55 to 60 mm. To determine which length class occurred most frequently in the samples of the three penguin species, frequency of occurrence was used. Data were tested for normality using Kolmogorov-Smirnov and Liliefors tests and tested for homoscedasticity using Levene's test. Analysis of variance (ANOVA) was used to test for significant differences between the lengths of krill ingested by the three penguin species. All statistical analyses were performed using the software STATISTICA 7.

All data describing the number of ingested krill and the number of anthropogenic particles found were tested for normality using Kolmogorov-Smirnov and Liliefors test and tested for homoscedasticity using Levene's test. Statistical analysis was made using $\alpha = 0.05$. For these tests, the software STATISTICA 7 were used. When the data were not normally distributed (Kolmogorov-Smirnov: $p < 0.05$) and not homoscedastic (Levene's test: $p < 0.05$), a permutational multivariate analysis of variance was used (PERMANOVA) (Anderson, 2001).

The number of krill ingested and the number of anthropogenic particles were compared among the factors Species, Locations and Years. Pairwise PERMANOVA between all pairs of groups are provided as post-hoc tests, when the number of unique permutations is small (<100) then one should preferably interpret the Monte-Carlo p value. All statistical analyses were performed using PRIMER v.6 and PERMANOVA+.

Spearman correlation analysis was used to assess possible relationships between the number of microplastics and the number of krill ingested. Statistical tests were considered significant at p-values < 0.05. Data were analysed using the software STATISTICA 7.

3. Results

3.1. Diet of Adélie, chinstrap and gentoo penguins

A total of 317 penguin scat samples, from Adélie, chinstrap and gentoo penguins were collected at ten different breeding sites across the Antarctic Peninsula and Scotia Sea (Fig. 1; Table 1), with all the three penguin species feeding mainly on sub-adult krill (Table 2). Krill was most frequently present in Adélie, followed by gentoo penguin diets, with chinstrap penguin diets having the lowest frequency of occurrence of krill.

In general, significant differences were found in the number of ingested krill for all three penguin species (PERMANOVA: pseudo-F = 5.890 and $p(\text{perm}) = 0.003$). The highest quantities of krill were found in gentoo, average 40.6 ± 6.0 (SD), followed by chinstrap, average 39.3 ± 5.5 (SD) and Adélie, average 35.2 ± 4.9 (SD) (Table 2), where significant differences were found between Adélie and chinstrap penguins (Pair-Wise test: $t = 3.526$; $p(\text{perm}) = 0.001$) and between

Table 2
Antarctic krill *Euphausia superba* and anthropogenic particles found in scats of Adélie (*Pygoscelis adeliae*), chinstrap (*Pygoscelis antarcticus*) and gentoo penguins (*Pygoscelis papua*) (penguin species, scat samples (n), krill (n), krill length (mm), krill F.O.(%), Krill (average ± SD), anthropogenic particles (n), anthropogenic particles F.O. (%), anthropogenic particles (average ± SD) and sample size (n)).

Penguin species	Scat samples (n)	Krill (n)	Krill length, (mm)	Krill F.O. (%)	Krill (average ± SD)	Anthropogenic particles (n)	Anthropogenic particles F.O. (%)	Anthropogenic particles (average ± SD)
Adélie penguins	20	71	35.2 ± 4.9	85	3.55 ± 3.2	3	15	0.15 ± 0.4
Chinstrap penguins	57	62	39.3 ± 5.5	54	1.09 ± 1.6	18	28	0.31 ± 0.5
Gentoo penguins	240	652	40.6 ± 6.0	66	2.72 ± 3.8	71	29	0.29 ± 0.5

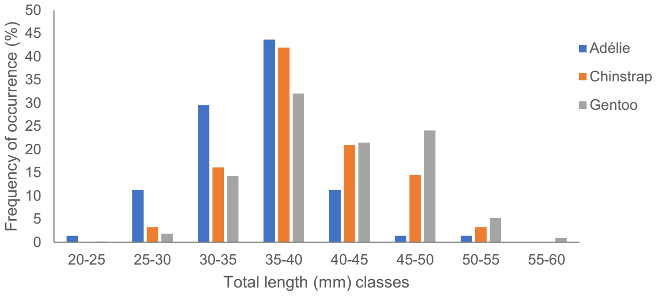

Fig. 2. Frequency of occurrence (%) of krill size classes in the diet of Adélie, chinstrap and gentoo penguins.

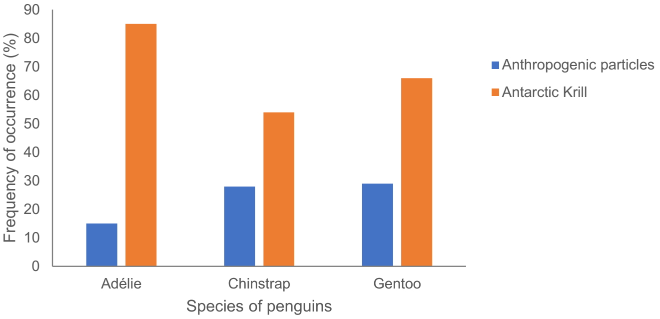

Fig. 3. Frequency of occurrence of anthropogenic particles and Antarctic krill (%) in the scats of Adélie, chinstrap and gentoo penguins.

chinstrap and gentoo penguins (Pair-Wise test: $t = 2.650$; $p(\text{perm}) = 0.009$).

The highest percentage of individuals of krill belonged to the 35-40 mm size class. This was 43.7% for Adélie, 41.9% for chinstrap and 32.1% for gentoo penguins (Fig. 2). Indeed, significant differences were detected between the total length of krill consumed by all penguin species (Kruskal-Wallis: H = 38.075; $p(\text{perm}) < 0.001$). Gentoo penguins ingested larger krill than Adélie penguins (Kruskal-Wallis: H = 38.075; $p(\text{perm}) < 0.001$), while chinstrap penguins ingested the smallest krill of the three penguin species.

3.2. Presence of anthropogenic particles in the scats

All items extracted from samples after digestion were defined as anthropogenic particles (i.e. particles created and/or processed by humans, including synthetic and dyed cellulosic fibres) and were found in all penguin species. These particles were most abundant in chinstrap penguins, with an average of 0.31 ± 0.5 (SD) particles per scat (18 anthropogenic particles in 16 scats of 57 scats analysed) followed by gentoo penguins, with an average of 0.29 ± 0.5 (SD) particles per scat (71 anthropogenic particles in 70 scats of 240 scats analysed). Such particles were less frequent in Adélie penguins, with an average of 0.15 ± 0.4 (SD) particles per scat (3 anthropogenic particles in 3 scats of 20 scats analysed) (Table 2; Fig. 3). In total 92 anthropogenic particles were extracted from the all penguin samples with an average of averaging 0.29 ± 0.5 (SD) particles per scat ($n = 317$).

No significant differences were found regarding the number of particles in the scats from the three penguin species (PERMANOVA test: pseudo-F = 0.922; $p(\text{perm}) = 0.427$). Additionally, there was no significant correlation between the number of particles and the number of krill ingested (Spearman's rank correlation: rho = 0.047; $p > 0.050$).

3.3. Anthropogenic particles in penguins from different colonies

Differences were found in the occurrence of anthropogenic particles in colonies distributed from north to south from South Georgia to the Antarctic Peninsula (Table 3; Fig. 4).

Particles were found in all penguin colonies (Table 3), with the highest number recorded in Rongé Island colony (for gentoo) with an average of 0.55 ± 0.5 (SD) particles per scat (Table 3; Fig. 4). However, there were no significant differences in the number of particles between the colonies (PERMANOVA test: pseudo-F = 1.553; $p(\text{perm}) = 0.175$ and $p(\text{MC}) = 0.175$).

As data from gentoo penguins were available for nine colonies, PERMANOVA tests were performed in order to verify spatial variation

Table 3
Anthropogenic particles found in scats from breeding colonies listed from north to south, of Adélie (*Pygoscelis adeliae*), chinstrap (*Pygoscelis antarcticus*) and gentoo penguins (*Pygoscelis papua*) (colony, penguin species, scat samples (n), anthropogenic particles (n), F.O. (%), average ± SD).

Colony	Penguin specie	Scat sample (n)	Anthropogenic particles (n)	F.O. (%)	Average ± SD
Landing Beach	Gentoo penguins	66	16	24	0.24 ± 0.4
King George Island	Gentoo penguins	63	19	29	0.30 ± 0.5
Hannah Point	Chinstrap penguins	18	4	22	0.22 ± 0.4
Hannah Point	Gentoo penguins	18	2	11	0.11 ± 0.3
Byers Peninsula	Gentoo penguins	14	6	43	0.43 ± 0.5
Deception Island	Chinstrap penguins	28	11	32	0.39 ± 0.6
Cierva Cove	Gentoo penguins	13	3	23	0.23 ± 0.4
Rongé Island	Chinstrap penguins	11	3	27	0.27 ± 0.5
Rongé Island	Gentoo penguins	20	11	55	0.55 ± 0.5
Paradise Bay B	Gentoo penguins	28	10	36	0.36 ± 0.5
Paradise Bay A (Almirante Brown)	Gentoo penguins	18	4	22	0.22 ± 0.4
Yalour Island	Adélie penguins	20	3	15	0.15 ± 0.4

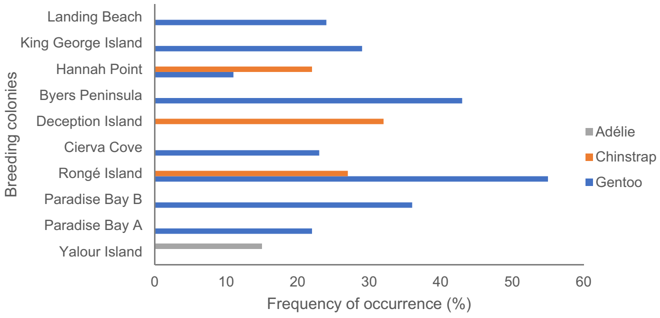

Fig. 4. Frequency of occurrence (%) of anthropogenic particles in breeding colonies of Adélie, chinstrap and gentoo penguins.

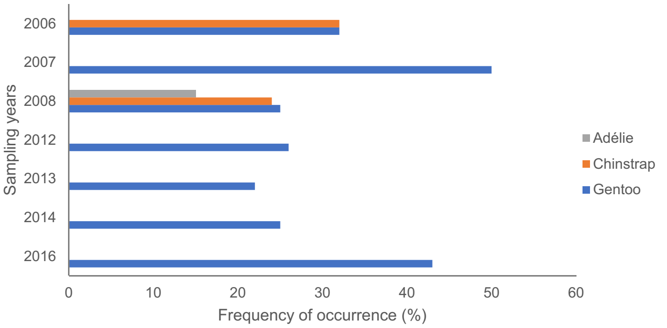

Fig. 5. Frequency of occurrence (%) of anthropogenic particles in Adélie, chinstrap and gentoo penguins, for each sampling year.

of anthropogenic particles for this species among the colonies. Significant differences were detected among these colonies (PERMANOVA test: pseudo-F = 2.471; p(perm) = 0.036), therefore Pair-wise tests were performed for the colonies and differences were found between Rongé Island and Hannah Point (Pair-wise test: $t = 3.125$; p(perm) = 0.006 and p(MC) = 0.004), Rongé Island and King George Island (Pair-wise test: $t = 2.863$; p(perm) = 0.005 and p(MC) = 0.006) and Rongé Island and Paradise Bay A (Almirante Brown) (Pair-wise test: $t = 2.132$; p(perm) = 0.051 and p(MC) = 0.042). The number of anthropogenic particles ingested by penguins was higher in colonies located at south of the geographical range (Rongé Island: average of 0.60 ± 0.5 (SD) particles per scat; Paradise Bay A (Almirante Brown): average of 0.22 ± 0.4 (SD) particles per scat) than in the colonies located at north (Hannah Point: average of 0.11 ± 0.3 (SD) particles per scat; King George Island: 0.30 ± 0.5 SD) particles per scat).

3.4. Temporal variation of anthropogenic particles

As samples from all years were available only for gentoo penguins, temporal comparisons were examined for this species only (Table 4; Fig. 5), with no significant differences among years being found (PERMANOVA test: pseudo-F = 1.844; p(perm) = 0.118).

3.5. Characterization of anthropogenic particles

A total of 92 anthropogenic particles were recovered from the scats of Adélie, chinstrap and gentoo penguins and characterized according to their shape, colour and total length. The particles extracted were categorized as fibres (74%) and fragments (26%). Colour distribution of ingested particles was very similar across all penguin species, where blue particles were the most common (70%), followed by green (10%), and red (9%), while other colours such as brown, transparent, purple and black were rarely found (Fig. 6). The highest percentage of anthropogenic particles found were within the 0.63 μm –1 mm size class (44%) (Fig. 7).

Table 4
Anthropogenic particles found in scats from different years, of Adélie (*Pygoscelis adeliae*), chinstrap (*Pygoscelis antarcticus*) and gentoo penguins (*Pygoscelis papua*) (year, penguin species, scat samples (n), anthropogenic particles (n), F.O. (%), average ± SD).

Year	Penguin specie	Scat sample (n)	Anthropogenic particles		
			n	F.O. (%)	Average ± SD
2006	Chinstrap penguins	28	11	32	0.39 ± 0.6
2006	Gentoo penguins	47	15	32	0.32 ± 0.5
2007	Gentoo penguins	18	10	50	0.56 ± 0.6
2008	Adélie penguins	20	3	15	0.15 ± 0.4
2008	Chinstrap penguins	29	7	24	0.24 ± 0.4
2008	Gentoo penguins	95	24	25	0.25 ± 0.4
2012	Gentoo penguins	23	6	26	0.26 ± 0.5
2013	Gentoo penguins	23	5	22	0.22 ± 0.4
2014	Gentoo penguins	20	5	25	0.25 ± 0.4
2016	Gentoo penguins	14	6	43	0.43 ± 0.5

Particles were identified as microplastics i.e. synthetic ($n = 10$, 35%), cellulose ($n = 16$, 55%) or unidentified (n = 3, 10%). The majority (80%) of synthetic particles were identified as polyethylene, with 10% identified as polyester (Fig. 8) and the remaining 10% confirmed as synthetic though it was not possible to match their polymer identification, and they were classified as "unidentified synthetic particles".

4. Discussion

This study revealed that the main particles found in Adélie, chinstrap and gentoo penguins were microplastics but also other anthropogenic particles were documented. Scat samples were used as a proxy of ingestion, to understand the route of these particles into the Antarctic marine

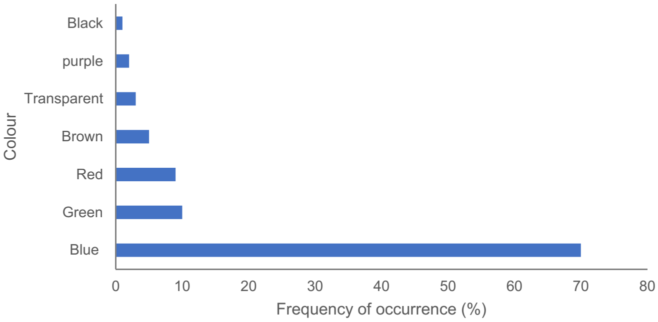

Fig. 6. Frequency of occurrence (%) of colours of anthropogenic particles found in penguins scats.

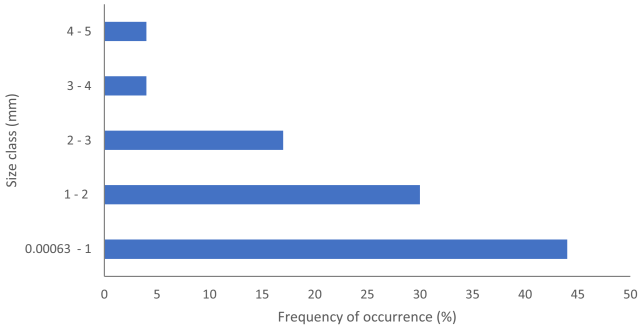

Fig. 7. Frequency of occurrence (%) of size classes of anthropogenic particles found in penguins scats.

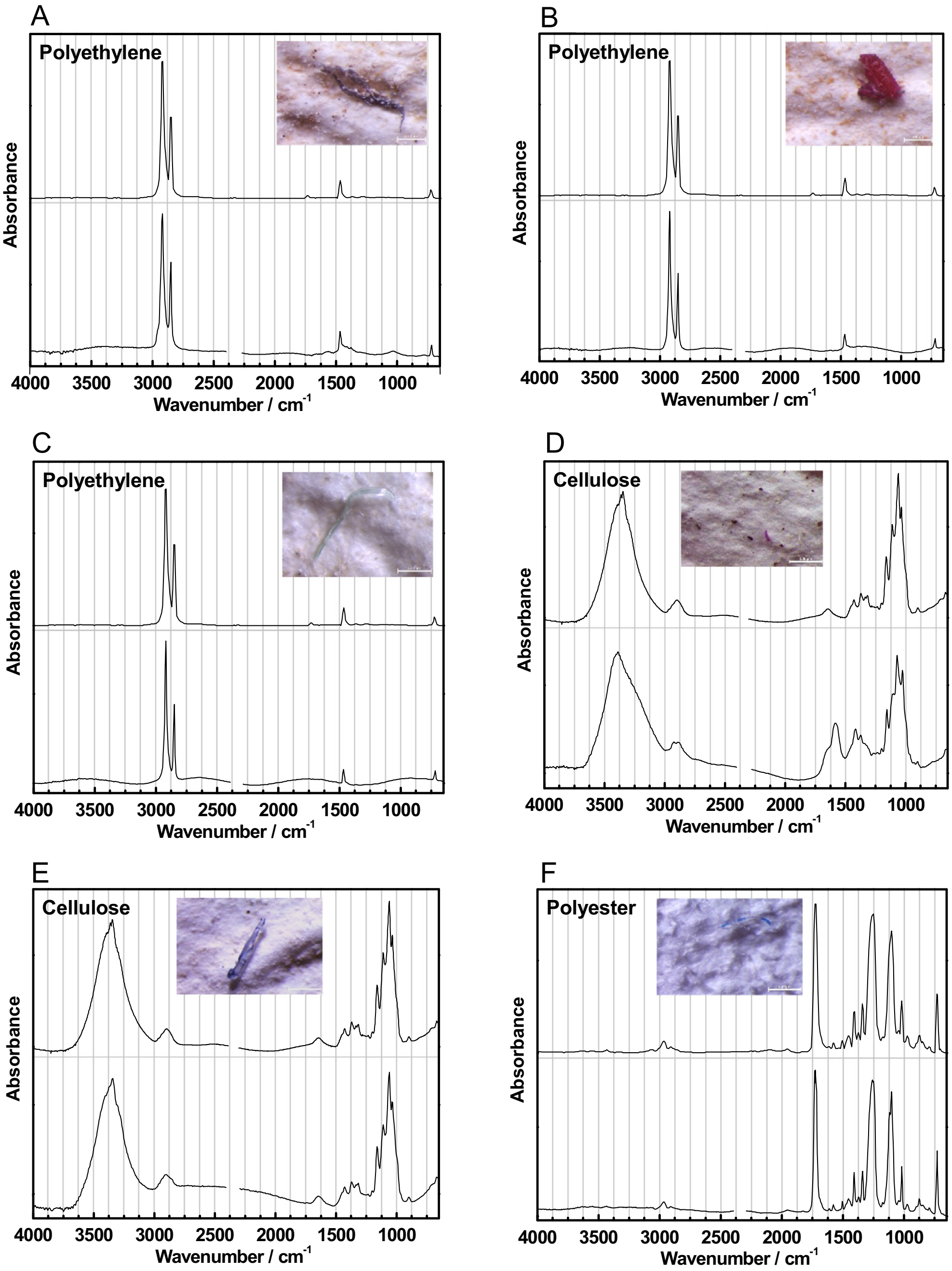

Fig. 8. μ-FTIR spectra of representative anthropogenic particles and fibres found in the scats of Adélie, chinstrap and gentoo penguins: (A) blue polyethylene, (B) red polyethylene, (C) green polyethylene, (D) purple cellulose, (E) blue cellulose, (F) blue polyester (scale bar is 500 μm).

food chain. Our results show the presence of anthropogenic particles in Adélie, chinstrap and gentoo penguins, and that pollution by these particles is widespread throughout our study region. It is unlikely that the particles observed originated from a single source as their frequency of occurrence was found to be similar among the different breeding colonies distributed from north to south. Additionally, our results show that the frequency of occurrence of anthropogenic particles varies over the years (Table 4), with no clear trend in the number of particles over the time series investigated.

4.1. Penguin diet: foraging capacity and microplastics ingestion

Our results are consistent with previous studies examining the presence of microplastics in the Southern Ocean (Suaria et al., 2020; Waller et al., 2017) as well as in gentoo and king penguin scats (Bessa et al., 2019b; Le Guen et al., 2020). Comparing the results obtained in our study with previous studies, the percentage of anthropogenic particles obtained in the present study for gentoo (29% of scat samples with anthropogenic particles; averaging of 0.29 ± 0.5 (SD)) is slightly higher than the percentage obtained by Bessa et al. (2019a, b), who found 20% of scat samples to contain anthropogenic particles. At South Georgia 77% of samples from king penguins were found to contain microplastics, whereas we found only 24% of gentoo penguin samples (collected at Landing beach, Bird Island, South Georgia) to contain anthropogenic particles. These differences may be largely due to the fact that the two penguin species have different diets and foraging ranges, with king penguins largely feeding on mesopelagic fish rather than krill (Le Guen et al., 2020). The temporal and spatial variation of the levels of anthropogenic particles pollution was not assessed in these previous studies.

The diet composition of the three penguin species was mainly krill (Table 2), mostly immature stages (Fig. 2), based on the assessed average range 36–45 mm (Ettershank, 1984).

The frequency of occurrence of krill obtained in this study for Adélie, chinstrap and gentoo penguins is consistent with previous studies which report that krill is the primary component of the diet of these penguins (Alonzo et al., 2003; Croxall, 1987; Trivelpiece et al., 2011). However, there are significant differences in the number of krill among Adélie, chinstrap and gentoo penguins found in this study (Table 2). These differences are probably due to the fact that chinstrap penguins forage more frequently at night when krill is more abundant and Adélie and gentoo penguins during the day (Borboroglu and Boersma, 2015; Croxall et al., 1988). At night, krill tend to be distributed diffusely within 15 to 30 m of the surface as they make vertical migrations into surface waters to find food, which increases their risk of becoming prey for predators like penguins (Knox, 1984; Swadling, 2006). During the day, krill are concentrated below 50 m, thus decreasing their risk of predation by diving seabirds (Swadling, 2006). In addition, gentoos are coastal foragers (Juáres et al., 2016; Xavier et al., 2017), while the other two species make longer foraging trips (Borboroglu and Boersma, 2015; Juáres et al., 2016).

A total of 92 anthropogenic particles, averaging of 0.29 ± 0.5 (SD) particles per scat were extracted from the total scat samples ($N = 317$). Although microplastic pollution is known to be ubiquitous in aquatic habitats, these results show that it occurs much less frequently in Antarctica than in other regions, such as the Arctic, where microplastics have already been found in higher percentages in birds (Bourdages et al., 2020; Hallanger and Gabrielsen, 2018). For example, in the great shearwater (*Ardenna gravis*), 71% of individuals contained at least one plastic piece ($n = 17$) (Provencher et al., 2014), with the white-faced storm petrel (*Pelagodroma marina*), containing volumes of plastic in 84% of the individuals (Furness, 1985).

No significant differences were found in the number of anthropogenic particles between the three studied penguin species, which may indicate that the availability of anthropogenic particles in this region may be relatively homogeneous without any point source of pollution (Suaria et al., 2020). As these three species have a similar diet, this could also explain the similarities observed. Based on these results, anthropogenic particles are likely to have been ingested directly (e.g. accidental consumption of particles through indiscriminate feeding strategies, by mistaking these anthropogenic particles for food) (Sfriso et al., 2020), or indirect via contaminated prey (Bessa et al., 2019b). Krill have been shown to ingest microplastics under laboratory conditions (Dawson et al., 2018), so it is plausible that krill may also ingest them in the wild as is expected for zooplankton (Tirelli et al., 2020), which can also ingest them in the laboratory (Beiras et al., 2018). Although we found that the main component of the diet of the three penguin species was krill and microplastics was found in all species, no significant correlations were found between the number of krill ingested and the number of particles. Thus, it is necessary to investigate whether krill ingest microplastics under natural conditions, in order to test the hypothesis that microplastics are entering the food chain via krill. It is likely that mesopelagic fish, which are the main prey of king penguins could act as a potential source of microplastics (Le Guen et al., 2020), particularly as recent studies have reported the presence of microplastics in mesopelagic fish from other regions including the North Pacific (Davison and Asch, 2011), North Atlantic (Wieczorek et al., 2018) and Indian Oceans (Bernal et al., 2020).

4.2. Spatial variation of anthropogenic particles

While we found a wide variation in anthropogenic particles across all gentoo colonies sampled we found no evidence of a latitudinal pattern in the distribution of microplastics.

Our results suggest that there is a wide distribution of microplastic pollution across the Antarctic Peninsula. The largest source of anthropogenic particles in this region (fishing vessels, research ships and research stations) are located essentially to the north of the Peninsula, however, the existence of the Antarctic Circumpolar Current (ACC), which moves from west to east around Antarctica (Rintoul, 2010), may contribute to a widely dispersed and distribution of microplastic pollution in the Southern Ocean.

The ACC may also retain plastic particles for years, creating a plastic accumulation zone around the continent. That said, a constant source of microplastic pollution is not recognized in the Southern Ocean (Lacerda et al., 2019) and consequently, we might not expect there to be differences between the colonies in the north and south, as indicated in our results. The low numbers of particles and the similar values found among colonies may be due to Antarctica being geographically isolated by this current (Hughes and Ashton, 2017). However, the Antarctic Polar Front (APF) has been noted to be insufficient to safeguard Antarctic waters from plastic pollution (Horton and Barnes, 2020). In fact, the Antarctic region has been hypothesized as a "dead-end" for plastics (Jones-Williams et al., 2020): areas where APF is relatively close to the continent, like the western Antarctic Peninsula, permit a short transfer of anthropogenic particles out to near-shore environments (Waller et al., 2017). This makes possible the transport of surface and suspended microplastics, which are small particles which tend to float, into the Antarctic region.

Due to the limited direct human pressures in the Antarctic and restricted legislation, potential additional sources of anthropogenic particles (Hughes et al., 2018) can be associated with disposal or inadequate management of waste produced by fishing vessels, tourist and research ships and research stations, which are more common in the Antarctic Peninsula region (Lacerda et al., 2019; Waller et al., 2017). Indeed, our study sites are very close to fishing areas, tourism sites and research stations (e.g. at King George Island) (Pertierra et al., 2017; Reid, 2019). An example is the lack of sewage treatment in most research stations of Western Antarctic Peninsula (Hughes, 2004), which may introduce anthropogenic particles via wastewater into the surrounding environment (Reed et al., 2018).

4.3. Temporal variation of anthropogenic particles

This study presents new temporal information on the presence of anthropogenic particles in the Antarctic region, revealing that there is no clear increase in the number of anthropogenic particles over the timespan of our study, as the frequency of occurrence of these particles remains almost constant (Table 4; Fig. 5).

4.4. Type and origin of anthropogenic particles

Although earlier studies have reported the presence of microplastics in gentoo penguin scats (Bessa et al., 2019b) from Bird Island and Signy Island and in king penguin scats (Le Guen et al., 2020) from South Georgia, this is the first study to show that microplastics and other anthropogenic particles are also ingested by Adélie and chinstrap penguins, and in penguin species inhabiting the Antarctic Peninsula.

We found 97 anthropogenic particles in scats of Adélie, chinstrap and gentoo penguins, of which fibres were the main category recorded. These results are consistent with previous findings reported in the Antarctic marine environment, with fibres contributing 77% of the particles found in King penguin scats (Le Guen et al., 2020) and 58% of the particles in gentoo penguin scats (Bessa et al., 2019b).

Fibres are often the main type of microplastic pollution found in aquatic environments, which are likely to derive from textiles and domestic washing machines as well as from the degradation of fishing nets lost in the environment (Cesa et al., 2017). In our study the majority of anthropogenic particles in the samples, are similar to those found in marine species from other habitats, such as fish from the Mondego estuary, Portugal (96%) (Bessa et al., 2018), the North East Atlantic Ocean (54%) (Barboza et al., 2020), Northeast Greenland (88%) (Morgana et al., 2018) and in polar cod from the Arctic Ocean (90.2%) (Kühn et al., 2018).

The colour distribution of detected anthropogenic particles was uniform across all scats analysed, with blue particles most commonly detected (70% frequency of occurrence), followed by green (10%) and red (9%) particles. These results are also in agreement with previous studies, that show that blue microplastics are the most reported and common in the marine environment (de Vries et al., 2020; Jones-Williams et al., 2020). This may be because blue fibres are more easily detected than transparent fibres and may be more likely to be ingested, since they are similar in colour to some plankton organisms (Ory et al., 2017).

Synthetic polymers, such as polyethylene and polyester, are widely used plastics in the production of consumer items (Bellas and Gil, 2020) and are also dispersed throughout the marine ecosystem (Cortes and Otadoy, 2020; Nelms et al., 2019; Neves et al., 2015) and in the Antarctic waters (Jones-Williams et al., 2020; Waller et al., 2017).

All plastics which it was possible to identify were either polyethylene (PE) or polyester (PES). It is important to note that, although the samples were stored in PE transparent plastic bags (during the period of the samples were collected, between 2006 and 2016 was not possible to control the storage of these samples), our results did not find any transparent PE particles similar to those of the plastic bags (i.e. transparent fragments or films of PE). The particles of PE that we found in the scats samples of the three penguin species were blue fibres and a red fragment.

PE is of low density and is likely to float in seawater (Lusher et al., 2017). Possible sources are varied since it is a polymer widely used in many applications such as single-use plastics. One additional source is plastic debris from the fishing industry, such as ropes, since polyolefins are also used the manufacture of fishing gear (Andrady, 2011).

PES is a synthetic polymer that occurs in high densities in aquatic environments (Lusher et al., 2017), and it is likely to be present in the water column. It is commonly associated with the textile industry and in clothing manufacture. PES may enter the marine environment directly from clothing or from wastewater from washing machines, particularly due to the lack of wastewater treatment in some research stations in Antarctic Peninsula (Reed et al., 2018). Also, 15% of the total particles analysed and confirmed as synthetic could not be properly assigned to any polymer, probably due to degradation of the polymer or to the presence of other compounds (Bessa et al., 2019b).

Cellulose-based fibres are made from natural resources such as wood pulp and cotton (Shen et al., 2010) and play an important role in the production of textiles (Frydrych et al., 2002). They are primarily used for high-value applications, account for 6.2% of the global production of fibres (Suaria et al., 2020) and are also used in the production of toilet paper. Despite being of natural origin, they can pose an additional threat to the marine environment since they may have associated contaminants (Graupner et al., 2009; Shen et al., 2010). We found cellulose to be present in 55% of analysed samples, consistent with recent studies which demonstrate that natural cellulose polymers dominate the composition of oceanic fibres in marine environments (Huntington et al., 2020; Suaria et al., 2020). The presence of a high proportion of fibres from natural origin in the Antarctic marine ecosystem might be a consequence of slow degradation rates due to the particular conditions offered by these environments such as low temperatures (Le Guen et al., 2020), however, little is known about the degradation rates of such natural fibres in marine ecosystems (Suaria et al., 2020).

4.5. Relevance to support future decisions in the ecosystem management of the Antarctic

Currently, the level, distribution and environmental impacts of plastic pollution within the Southern Ocean are poorly understood (Rowlands et al., 2020; Waller et al., 2017), and countries are being encouraged to support scientific research efforts on plastics in the Southern Ocean (Resolution 5 (2019) (SAT, 2019)). In response to these gaps in knowledge, the Scientific Committee on Antarctic Research (SCAR) has recently established its cross-disciplinary Action Group 'Plastic in Polar Environments' (Plastics-AG) to examine the presence, origin and biological effects of macro-, micro- and nanoplastics; quantify the scale of the problem; develop standard procedures for plastic sampling and monitoring and propose solutions for minimizing the environmental risk and impacts on Polar ecosystems. Several of the Annexes to the Protocol on Environmental Protection to the Antarctic Treaty (including Annex 1: Environmental Impact Assessment, Annex III: Waste Disposal and Waste Management and Annex IV: Prevention of Marine Pollution) are relevant to plastic pollution within the Antarctic Treaty area (SAT, 2014). Indeed, Annex IV specifically prohibits the disposal into the sea of all plastics, including but not limited to synthetic ropes, synthetic fishing nets and plastic garbage bags. Within this context, it was recommended to the countries of the Antarctic Treaty in 2019 to prohibit the use of personal care products containing microplastic beads within the Treaty area, and to promote the development, use and sharing of methods and technologies to reduce plastic pollution release into the Antarctic environment, including in partnership with CCAMLR marine debris programme as appropriate, and encourage greater monitoring of plastic pollution around Antarctica and in the Southern Ocean (SAT, 2019) (Phillips and Waluda, 2020; Waluda et al., 2020).

Our results show that microplastics, like polyester, polyethylene and also natural cellulose are widespread in all three species of penguins in all study sites from South Georgia to the Antarctic Peninsula, confirming previous studies in penguins but also in other organisms, in oceanic water and sediments from this area (Bessa et al., 2019b; Le Guen et al., 2020; Munari et al., 2017; Sfriso et al., 2020; Waller et al., 2017). To reduce the presence of these particles in the Antarctic environment, mitigation initiatives can be implemented. For example, the inclusion of filters in washing machines at research stations and on ships to reduce the emission of microplastics into the environment. Another measure that could be implemented is to control the use of materials used for fishing gear as they can be discarded and/lost in the environment, and over time can release fibres to the Antarctic marine ecosystem.

This study encourages countries within the Antarctic Treaty System to continue to support research into the potential effects of microplastics on penguins and other Antarctic organisms (i.e. toxic effects, since microplastics are persistent, can adsorb organic contaminants from the water which can accumulate in organisms) and to provide a platform for a detailed assessment of the levels, origins and fate of microplastics within Antarctica in line with Resolution 5(2019) (SAT, 2019). Moreover, quantification of microplastics generated from macroplastic degradation and/or transferred into the Southern Ocean should also be assessed. Further research is also needed to improve our knowledge of the distribution of plastic in the Southern Ocean through temporal analyses and monitoring activities that generate comparable data (i.e., data from other trophic levels, from the environment - water and sediments- to complement data from the biota) and a greater understanding of the impact of plastic upon species across the food chain and in different marine habitats. To show that microplastics are entering in the Antarctic marine food chain, it is important to evaluate and prove that krill, a key specie, ingest microplastics in the wild conditions, and consequently if this species may further convert microplastics into nanoplastics (Dawson et al., 2018), which are becoming a serious global environmental problem (Chang et al., 2020). For that reason, further research is needed to detect, monitor and determine the fate and effects of microplastics as well as nanoparticles in Antarctica.

Ethical aprovement

The sampling methods used were under the recommendations from the Scientific Committee for Antarctic Research (SCAR) and the permission for sampling was issued by the Spanish Polar Committee and the Government of South Georgia and the South Sandwich Islands (GSGSSI).

CRediT authorship contribution statement

Joana Fragão: Investigation, Writing – original draft, Writing – review & editing. **Filipa Bessa:** Supervision, Funding acquisition, Methodology, Writing – review & editing. **Vanessa Otero:** Investigation, Writing – review & editing. **Andrés Barbosa:** Funding acquisition, Writing – review & editing. **Paula Sobral:** Writing – review & editing. **Claire M. Waluda:** Resources, Writing – review & editing. **Hugo R. Guímaro:** Investigation, Writing – review & editing. **José C. Xavier:** Supervision, 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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