Arctic, Antarctic, and Alpine Research
An Interdisciplinary Journal

ISSN: 1523-0430 (Print) 1938-4246 (Online) Journal homepage: www.tandfonline.com/journals/uaar20

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To cite this article: Luis Santillán, Ana D. Forero López, Carolina V. Colombo, Guido N. Rimondino, Fabio E. Malanca & Gabriel E. De-la-Torre (2025) Microplastics in Antarctic penguins and seals in Admiralty Bay, King George Island, Antarctica, Arctic, Antarctic, and Alpine Research, 57:1, 2507463, DOI: 10.1080/15230430.2025.2507463

To link to this article: https://doi.org/10.1080/15230430.2025.2507463

Published online: 09 Jun 2025.

ARCTIC, ANTARCTIC, AND ALPINE RESEARCH
2025, VOL. 57, NO. 1, 2507463
https://doi.org/10.1080/15230430.2025.2507463

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Microplastics in Antarctic penguins and seals in Admiralty Bay, King George Island, Antarctica

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ABSTRACT

The occurrence of microplastics in top predators was evaluated during summer 2020 along Punta Crepín through the analysis of fresh scats of seals and penguins. We collected 37 scats – 23 from penguins, likely *Pygoscelis papua* (the most frequent in the area), and 14 from seals (3 *Mirounga leonina* and 11 likely *Leptonychotes weddellii*, the most frequent). Scats were digested with 20 percent KOH and put at 60°C for 5 days. The solution was vacuum filtered through 4 µm pore size filters. Suspected microplastics were analyzed by micro Fourier Transformed Infrared Spectroscopy (µFTIR). About penguins, 56.5 percent contained suspected particles, mainly fibers (90.5 percent), predominantly blue color (81.0 percent). Among these particles 77.3 percent were cellulose, 9.1% were PET and acrylic resin + kaolin, and 4.5 percent were polyacrylic polymer according the µFTIR analysis. Concerning seals, samples from *Mirounga leonina* did not present MPs while in the scats of likely *L. weddellii*, six had fibers (91.3 percent) of colors blue (56.5 percent) and red (30.4 percent). Overall the µFTIR for suspected particles in seal scats revealed 81.8 percent were cellulose and the rest were PET. Our findings indicated the presence of PET and acrylic resins, as well as cellulose particles, in low incidence, probably derived from the consumption of contaminated prey like Antarctic krill.

ARTICLE HISTORY
Received 7 December 2024
Revised 8 May 2025
Accepted 13 May 2025

KEYWORDS
Pinnipeds; seabirds; *Pygoscelis*; Punta Crepin; McKellar inlet; microplastic; polymer

Introduction

Undoubtedly, plastic in nature has been raised as one of the most concerning environmental issues in recent years. Plastic in the environment is one example of how human beings have the capacity to change the environment on a global scale (Hale et al. 2020). Plastic production worldwide is still increasing; roughly 79 percent has accumulated in the natural environment (Geyer, Jambeck, and Law 2017). Approximately 4.8–12.7 million metric tons of plastic debris has accumulated in the ocean, a figure that is projected to increase 10-fold by 2025 (Van Sebille et al. 2015). Microplastics (MPs; plastic particles in the range of 1 µm to 5 mm in size) (Duis and Coors 2016; Pietrelli, Dodaro, and Pelosi et al. 2024) are primarily considered a transitional stage in the life cycle of plastics, existing between macro debris and nanomaterials. They occur in various forms, such as fibers, spheres, and fragments (Hale et al. 2020). However, MPs are not the only anthropogenic microparticles present in nature. Semisynthetic cellulose (e.g., Cellophane, cellulose acetate, and Rayon) in the form of fibers has also been detected in marine biota (Remy et al. 2015; Aguirre-Sánchez et al. 2024). Semisynthetic cellulosic particles generally have highlighting colors such as blue, red, and green (Remy et al. 2015), although they have been poorly studied in contrast to conventional synthetic polymers.

MPs and semisynthetic cellulose are present in all environmental compartments; however, their ecological impact remains uncertain (De-la-Torre et al. 2023; Walkinshaw et al. 2023). The poles have not been immune to the presence of MPs, despite being remote areas, far from continents and large cities, which would suggest that they are pristine zones (Waller et al. 2017; Peeken et al. 2018; Gross 2022). Plastics are ubiquitous across global ecosystems, including remote regions such as Antarctica (Waller et al. 2017). Marine biota are particularly exposed to these

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pollutants, as documented in various marine environmental matrices, such as seawater (Ruangpanupan et al. 2022), sand (Santillán et al. 2023), and sediments (Okoffo et al. 2024).

Furthermore, MPs and anthropogenic micropollutants have been reported in the components of the marine food web in Antarctica. There are reports in invertebrates like Antarctic Krill *Euphasia superba* (Wilkie Johnston et al. 2023; Zhu et al. 2023a), salps (Wilkie Johnston et al. 2023), sponges (Corti et al. 2023) and benthic organisms (Sfriso et al. 2020). Fishes have also presented MPs pollution (Bottari et al. 2022; Geng, Wang, and Zhu 2023; Zhu et al. 2023b), as well as in penguins (Bessa et al. 2019; Fragão et al. 2021).

Penguins and pinnipeds are common top predators in the Antarctic ecosystem (Blix 2016). Antarctic top predators have been considered as bio-indicators to monitor changes according to the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) (Constable 2011). In addition, top predators are regarded as valuable indicators for monitoring the health of marine ecosystems (Clucas et al. 2014). They also contribute to a variety of ecosystem functions and services, including regulating food webs, cycling nutrients, engineering habitats, transmitting diseases and parasites, mediating ecological invasions, influencing climate, supporting fisheries, and promoting tourism, among others (Atwood and Hammill 2018; Hammerschlag et al. 2019; Fortuna et al. 2024).

Research efforts on MP pollution in Antarctic top predators are limited, with most studies focusing on penguin tissue, such as the gastrointestinal tract, scats, stomach contents, and gizzards, across various species of the genus *Pygoscelis* (*P. papua, P. antacticus, P. adeliae*) and *Aptenodyte forsteri* (Bessa et al. 2019; Panasiuk et al. 2020; Fragão et al. 2021; Leistenschneider et al. 2022; Kim et al. 2023; De-la-Torre et al. 2024a). For pinnipeds, there is only a single study on *Arctocephalus gazella*, which reported no evidence of MPs presence (García-Garín et al, 2020). In the framework of the Peruvian Expeditions to Antarctica XXVII (2019–2020), scats from Antarctic top predators were collected to investigate the presence of MPs. The collection focused on poorly investigated species, such as the Elephant seal and Weddell seal, as well as species that have already been reported in the literature, such as *Pygoscelis* spp.

Methods

Study area

The study was carried out in Almirantazgo bay, specifically along Punta Crepín, on the shore of the McKellar inlet in King George Island in the Antarctic Peninsula (Figure 1). In addition, two areas in the close vicinity were visited once, the east side of Punta Keller, in front of Punta Crepín, and Punta Thomas, where only scats of Elephant seal were obtained. Punta Crepín and Punta Thomas located in the vicinity of the Peruvian base, Machu Picchu, and the Polish station, Henryk Arctowski, respectively.

Sampling

Sampling consisted of an exhaustive search along the beach, looking for fresh feces of penguins and seals. The sampling was performed every two or three days. All the collections were made with bare hands, once a fecal sample was located, the researcher positioned themselves parallel to the sample so as not to disrupt the airflow and potentially influence the deposition of particulate matter on the ground. During the collection, fresh scats were preferred. Each sample was georeferenced, and the material was collected using a metal spatula. The material was stored in glass containers, which were then stored in the laboratory freezer.

Processing of samples

Samples were defrosted at room temperature for 1 day. Then, 12 g were weighed, stored in a clean glass beaker, and covered with aluminum foil. Following the digesting procedures described by Garcia-Garin et al. (2020) and Santillán, Saldaña-Serrano, and De-La-Torre (2020), abpproximately 500 mL of 20 percent KOH was added to the beakers with the samples and manually stirred with a glass rod. Then, the beaker was covered with aluminum foil and heated at 60°C for 5 days. The digestate was then vacuum filtered through 4 µm pore-size cellulose filters (Whatman, GE Healthcare, UK). The filters were stored in glass petri dishes. As quality control measures during the samples processing, the laboratory staff did not use synthetic clothes and a filter was placed in the room close to the processing area to determine cross-contamination with particles in the air.

Optical microscopy

Each filter was scanned using a hund Wetzlar stereomicroscope at ×10 magnification to identify suspected MPs particles. Particles larger than 200 µm were counted due to analytical limitations. Each suspected particle was photographed and classified based on its shape (fragment, fiber, sheet/film, foam, pellet/microbead), and color (De-la-Torre et al. 2024b).

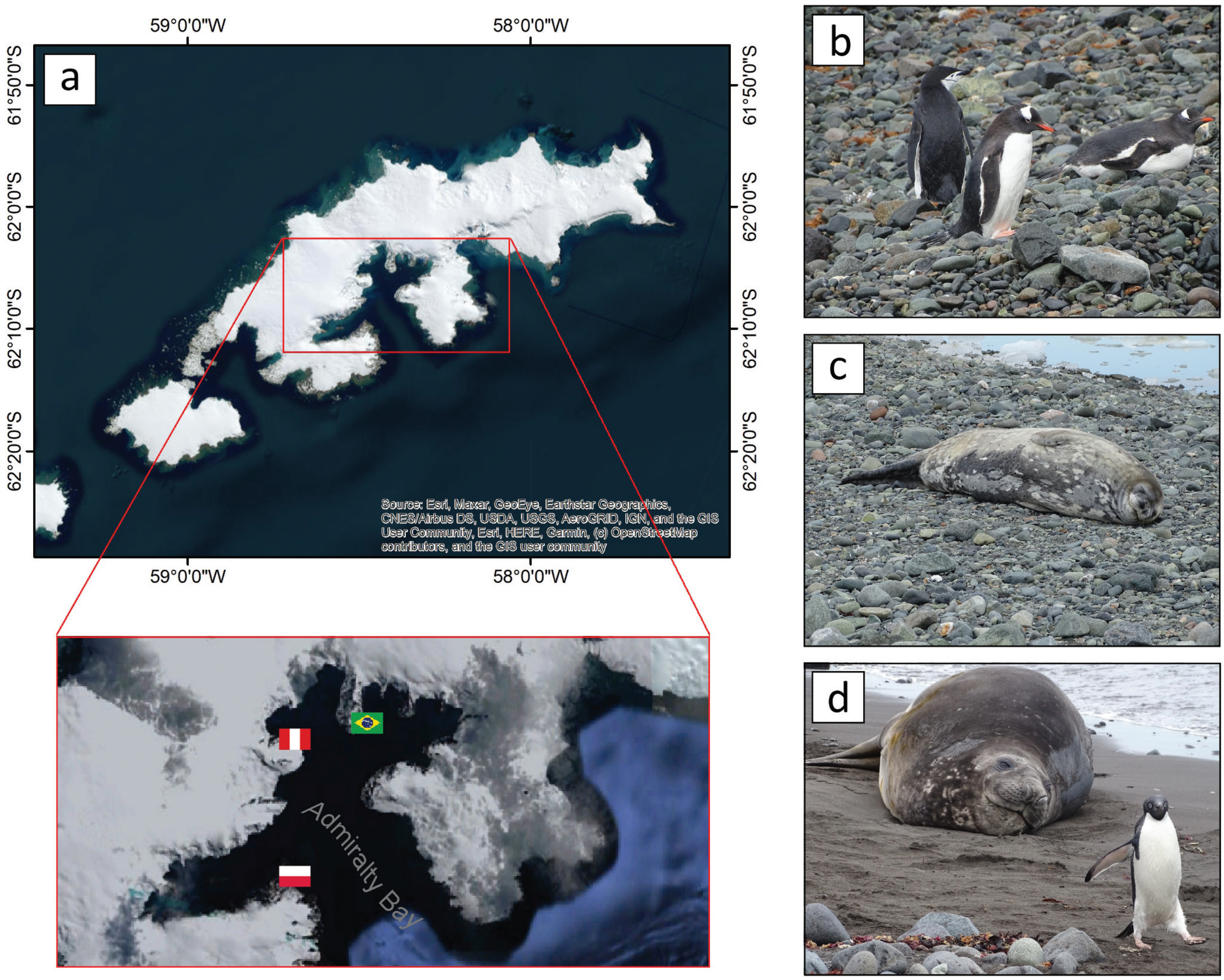

Figure 1. (a) Geographic map of King George Island. Insert shows Admiralty Bay with the flags of Peru, Brazil, and Poland indicating the position of their respective bases. Photographs of (b) Chinstrap and Gentoo penguins in Punta Crepín; (c) a Weddell seal in Punta Crepín; (d) an Elephant seal and Adelie penguin in Punta Thomas. (Photographs taken by Luis Santillán).

Polymer identification

Suspected particles were chemically analyzed in order to confirm their composition. Each filter was carefully rinsed with pre-filtered distilled water into a clean 500 mL beaker to remove any MP particle from cross-contamination. After rinsing the filter was reviewed in a stereoscopy. Then, the water containing the suspected particles was filtered through a 4 µm pore size filter (Whatman, GE Healthcare, UK) and carefully stored in glass petri dishes. The first filtration was to clean the filter and was made with distilled to remove particles that would be present due to cross contamination. The second was to filter the digestion solution. This process was carried out separately for penguin and seal samples. Following Forero López et al. (2021a), Forero López et al. (2021b)), the filters containing the suspected particles were analyzed by micro Fourier Transformed Infrared Spectroscopy (µFTIR). For this purpose, a Nicolet™ iN10 IR Microscope in reflectance mode was used. Spectra were recorded as wavelengths between 4000 and 500 cm^{-1} and 64 scans per reading. The spectra were manually analyzed to determine the most probable polymer identity.

Contamination prevention

Essential contamination prevention measures were taken into account as described by Dehaut, Hermabessiere, and Duflos (2019). In brief, all the liquids and reagents were previously filtered through 4 µm pore size filters. All the surfaces and glassware were rinsed or cleaned at least three times before use and covered with aluminum foil if not in use. The filtration step was carried out under a fume hood to further prevent the sedimentation of airborne particles on the samples. In addition, filters were checked randomly before filtering in order to detect any airborne particle. Procedural controls were carried out for each batch of samples processed. A mean of 0.33 particles/control was found, primarily composed of blue fibers. The data from each batch was normalized by subtracting the same number of particles found in the controls matching the color and shape.

Data analysis

MP concentrations were expressed in terms of MPs per gram of scat (MPs/g) ± *SD*. In order to compare the abundance of MPs in scats of penguins and seals, a Mann–Whitney *U*-test was carried out. Statistical significance was set to 0.05. Graphs and statistical analyses were carried out using GraphPad Prism (v. 8.4.3).

Results

During the 7 days of sampling, 37 scats were collected. Most of the scats were of penguins (n = 23), and the remaining were of seals (n = 14).

The species-specific identification of scats was not possible. However, only penguins of the genus *Pygoscelis* were present in the area. Three species were determined as regular users of the beach, *Pygoscelis antarticus* "Chinstrap penguin," *Pygoscelis adeliae* "Adelie penguin," and *Pygoscelis papua* "Gentoo penguin." The last was the most common, while Adelie penguins were less frequent in our observations. According to this, we cannot assure with confidence the species for each scat, however, there is a high possibility that most of them were dropped by Gentoo penguins. Groups of Gentoo penguins were usually observed close to the areas of scat collection during the whole sampling period.

Suspected 21 MPs were observed in 13 penguin scats, representing 56.5 percent of samples. Most of these showed a fiber shape (90.5 percent), and the rest were fragments (Figure 2a). The majority of these particles were blue in color (81.0 percent), followed by red (9.5 percent), gray, and green (4.8 percent each) (Figure 2c). The concentration of suspected particles ranged between 0.00 and 0.42 particles/g with an average of 0.09 particles/g ± 0.11.

A total of 21 colored particles were observed and analyzed with μFTIR. The majority of the particles were identified as cellulose (77.3 percent), followed by polyethylene terephthalate (PET) and acrylic resin + kaolin (9.1 percent each), and polyacrylic polymer (4.5 percent).

Concerning seals, there was not a species-specific certainty on the origin of the scats. However, three species were recorded in our observations: *Leptonychotes weddellii* "Weddell seal," *Lobodon carcinophagus* "Crabeater seal," and *Mirounga leonina* "Elephant seal." Among them, the Weddell seal was the most frequent on Crepin Point. Three scats from Elephant seals were collected at Thomas Point, while the rest are most likely from Weddell seals, although we cannot be certain.

Among the suspected 23 MPs, the majority were fibers (91.3 percent), followed by fragments (8.7 percent) (Figure 2b). Blue was the most abundant color (56.5 percent), followed by red (30.4 percent), and other colors (13.0 percent) (Figure 2d). The concentration of suspected MPs ranged from 0.00–0.33 particles/g, with a mean of 0.10 ± 0.12 particles/g.

Eleven suspected MPs from six seal's scats (possible Weddell seal's scats) were analyzed by μFTIR. Overall, 81.8 percent of the particles were identified as cellulose, and the rest as PET. None MPs were identified in Elephant seal scats. In contrast, white/transparent particles not originally counted in the MPs pool were randomly analyzed by μFTIR in order to crosscheck the MPs counts from the μFTIR detects. It was revealed that three white MPs particles were identified as PET.

The Mann–Whitney *U*-test revealed no significant difference between the groups ($U = 208.5$, $p = .7995$) (Figure 3).

Discussion

Our results showed that scats of penguins and seals are a useful organic structure for studying the occurrence of anthropogenic pollutants, including MPs. A group of studies about MPs in Antarctic predators have previously used scats, primarily from penguins, as a main element of analysis (Bessa et al. 2019; Fragão et al. 2021). Taurozzi and Scalici (2024) mentioned that the most studied element for MPs research in Antarctic seabirds was pellets and the second category was guano, the matrix that we investigated in penguins.

It is estimated that the majority of our samples were derived from Gentoo penguins, as they were the most frequently observed species. In addition, Chinstrap penguins were also identified, and it is likely that a small portion of the samples can be attributed to this species, species of the genera *Pygoscelis* were among the seabird's species where research has been done in previous studies in MPs (Taurozzi and Scalici 2024). As reported by Bessa et al. (2019) and Fragão et al. (2021), Gentoo and Chinstrap penguins present a significant number of particles in their scats. In accordance with the present and previous studies, it

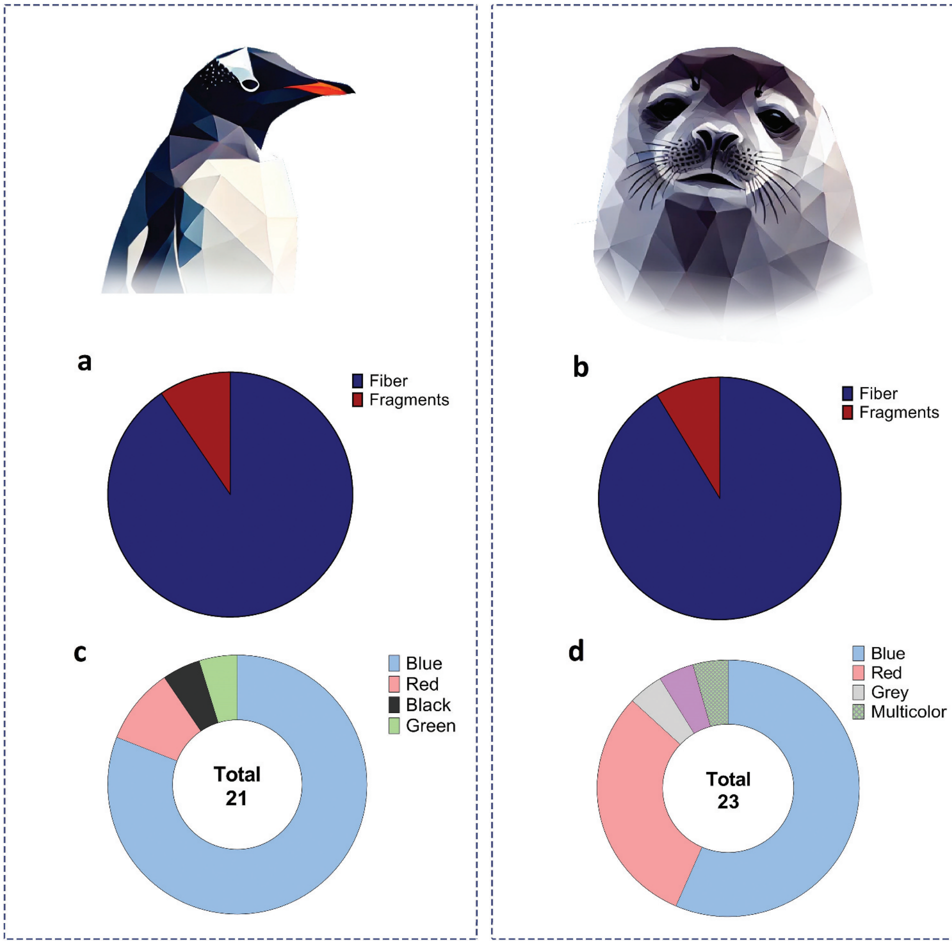

Figure 2. Pie charts showing the portion of suspected microplastic shapes and colors in penguin (a, c) and seal (b, d) samples.

is apparent that blue fibers are among the most common particles found in penguin scats. This is not surprising, however, as these types of particles are some of the most reported in multiple environmental compartments (e.g., Chen et al. 2024; Patidar et al. 2024). Both synthetic and nonsynthetic particles have also been reported in King Penguins from islands in the Southern Ocean (Le Guen et al. 2020). In a general view, studies on microplastics in Antarctic seabirds have revealed a high incidence of particles, Taurozzi and Scalici (2024) found that among the samples of seabirds investigated in several studies in Antarctica, 97 percent of them present MPs. Our results support this comment since in our sample, penguin's scat had more particles than seals

To the best of our knowledge, only one study investigated the presence of MPs in Antarctic mammals, specifically Antarctic fur seals from Deception Island (Garcia-Garin et al. 2020). However, MPs were not detected in their feces. The detection of MPs in the present study may suggest that the contamination of the species that make up the diet of the Elephant seals, Weddell seals, and Crabeater seals differs across geographic locations. In Admiralty Bay, there are three main Antarctic stations: Machu Picchu Base (Peru), Comandante Ferraz Antarctic Station (Brazil), and Henryk Arctowski Station (Poland), which could be important sources of plastic litter and MPs.

MPs have been reported in pinnipeds in other latitudes (Donohue et al. 2019; Nelms et al. 2018; Perez-Venegas et al. 2020; Zantis et al. 2020). The report closest to Antarctica was the early work of Eriksson and Burton (2003), who reported small plastic debris composed of PE in scats of the Antarctic and sub-Antarctic fur seals from Macquarie island.

Although our study did not include an analysis of diet components from fecal samples of either penguins or seals, the vivid red coloration observed in all penguin scat samples could indicate a high concentration of krill. This finding aligns with the dietary habits of penguins from the genus *Pygoscelis*, known to be krill specialist consumers (Pütz et al. 2001; Panasiuk et al. 2020). The occurrence of MPs in the scats can be a consequence of indirect consumption when they are preying on krill

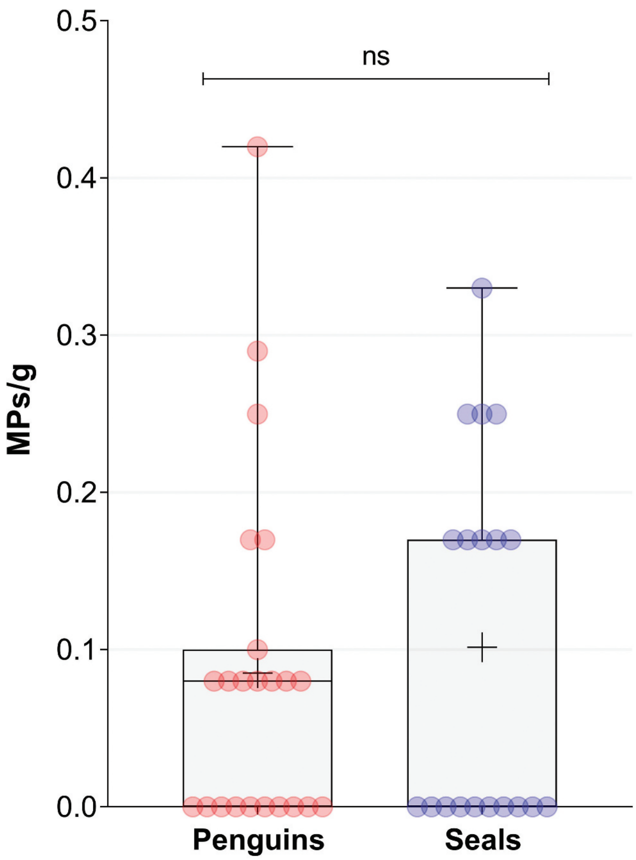

Figure 3. Boxplot with individual values showing the calculated concentration of suspected MPs (MPs/g) in penguins and seals. "+" indicates mean. "ns" indicates not significant (Mann–Whitney *U*-test: $p = .7995$).

(Fragão et al. 2021). MPs contamination has already been reported in krill (Wilkie Johnston et al. 2023; Zhu et al. 2023a). In krill specimens from the South Shetland Islands, MPs primarily consisted of black (32 percent), blue (22 percent), and red (21 percent) fibers composed of PE, PP, or polyester (Zhu et al. 2023a). In our findings, penguin scats presented PET fibers as one of the MPs components, which is concordant with krill's polyester fibers, suggesting that prey is one of the sources of pollution. However, penguins' scats also contained bright blue fragments of acrylic resins in a similar percentage to PET, and also polyacrylic fibers but in a lesser percentage. Acrylic resins probably derived from paint coatings or construction materials, such as sealants and adhesives or coatings. These types of particles are not commonly reported within the MPs pool. However, a previous study in sea surface waters around the Antarctic Peninsula reported a significant presence of paint fragments composed of polyurethane alongside MPs with varying degrees of degradation (Lacerda et al. 2019). While it is not possible to precisely track the source of acrylic resins in the Antarctic environment and, specifically, the penguins' diet, we believe that this type of contaminant may have been released by materials used during the Antarctic expeditions and operation of the Antarctic stations (i.e., Peru, Brazil, and Poland). The continuous observation of large debris and litter (e.g., bottles, painted metal and wooden structures) that may have been accidentally lost during the maintenance or operation of the Antarctic stations supports this idea.

Among the colored particles, these were largely cellulose. Attention to potentially anthropogenic cellulosic particles has increased and is usually reported in studies on MPs (McMullen et al. 2024), even in polar areas (Adams et al. 2021) and Antarctic seabirds (Taurozzi and Scalici 2024). However, there is some debate on whether these particles should be included within the umbrella of "microplastics" (Hartmann et al. 2019). While some studies exclude cellulosic fibers from the MPs counts, others suggest that only cellulosic particles that exhibit a clear color are taken into account (e.g., Aguirre-Sánchez et al. 2024). Regardless, it is very complicated to distinguish naturally occurring cellulose fibers from anthropogenic ones (e.g., Rayon, Cellophane) using, for instance, FTIR spectroscopy, particularly when these are weathered particles (Geminiani, Campione, and Corti et al. 2022). In the present study, only colored cellulosic particles were counted within the reported MPs concentrations, although it is imperative to acknowledge that their anthropogenic origin cannot be confirmed. In this sense, we recommend future studies to report the total number of polymers identified by spectroscopic (or similar) techniques, including those that could be from natural origin, and their color/shape.

Our study focused on two groups of Antarctic predators with different trophic niches. While *Pygoscelis* penguins (among them, Gentoo and Chinstrap as the most common in the study area) are krill predators (Wilkie Johnston et al. 2023; Zhu et al. 2023a), seals are fish, squid or krill predators (Forcada and Staniland 2018; Lowther 2018). Seals' scats derived from Elephant seals (three samples) and probably Weddell seals. In that context, Elephant seal is a cephalopod and fish consumer (Lowther 2018) and even krill by juveniles (Walters et al. 2014), while Weddell seals primarily feed on fish and squid; in addition, small crustaceans (mysids) might be an important diet component (Lake, Burton, and van den Hoff 2003; Lowther 2018). We found differences in the composition of microplastic particles, with PET and colored cellulose being the coincident particles between the two groups. However, it must be highlighted that Elephant seals' scats did not present MPs, and the PET occurrence is exclusive to potential Weddell seals' scats. Particles of paint and coats were only present in penguins. There is not enough evidence to establish that paints and coats and even PET particles might originate from Admiralty Bay or McKellar inlet. However, this is an emerging concern (Ceia and Bessa 2024). The next step in MPs in top predators is to include more information, increase the number of samples, and continue with this effort and correlate it with other environmental matrices in Antarctica.

Limitations

The present study aimed to investigate the presence of MPs in Antarctic top predators, including species that have not been assessed before, such as the Elephant seal. However, several limitations should be acknowledged. First, the choice of sample size and collection sites was limited to the funds and logistics available in the XXVII Peruvian Antarctic Expedition framework. Ideally, a higher number of samples per species should be taken to understand the differences among these species and obtain better representation. This is particularly challenging due to the extreme weather conditions, even during the austral summer. Species-specific identification for each scat was not possible and consequently we could suggest that the samples were from the most common species of penguins (Gentoo) and seals (Weddell) in the area. Conversely, the identification of particles through optical stereomicroscopy is not completely reliable in distinguishing particles that match the color of the filter as it was observed with white/transparent particles identified as PET. In particular, it should be recognized that the normalization of the datasets based on shape and color should be complemented by the chemical analysis of the particles detected in the blanks and determine if, indeed, these are synthetic polymers and whether they are detected in the samples matching shape, color, and polymer composition. However, due to the analytical limitations, only color and shape were taken into account to avoid overestimating the concentration of MPs. These challenges could be overcome by applying imaging techniques, such as a focal plane array.

Conclusions

The presence of MPs in scats from various *Pygoscelis* penguins and seals from Admiralty Bay, Antarctica, was investigated. Our findings indicated the presence of MPs primarily composed of PET and acrylic resins, as well as cellulosic particles that exhibited an intense color. While the concentrations of MPs in scats remain relatively low, it is plausible that contamination may have derived from the consumption of contaminated prey, such as Antarctic krill. Due to the ubiquitous presence and long-range transport of MPs in the environment, it is difficult to estimate the possible sources of contamination. However, fiber-like PET and fragment acrylic resins may likely derive from synthetic textiles and paint coatings, respectively. Future research must focus on tracking down local (Antarctic stations) and long-range (atmospheric transport) sources of MPs in Antarctica, as well as contamination across various trophic webs.

Acknowledgments

This research was done during the XXVII Peruvian Antarctic Expedition organized and financed by the Programa Nacional Antártico del Perú from the Ministerio de Relaciones Exteriores del Perú. This information is part of the Antarctic research program of Universidad San Ignacio de Loyola, which co-financed the research and has the support of Instituto Argentino de Oceanografía (IADO).

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research was co-funded by Ministerio de Relaciones Exteriores and San Ignacio de Loyola University within the framework of the XXVII Peruvian Antarctic Program - Ministry of Foreign Affairs of Peru.

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