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No evidence of microplastic ingestion in emperor penguin chicks (*Aptenodytes forsteri*) from the Atka Bay colony (Dronning Maud Land, Antarctica)

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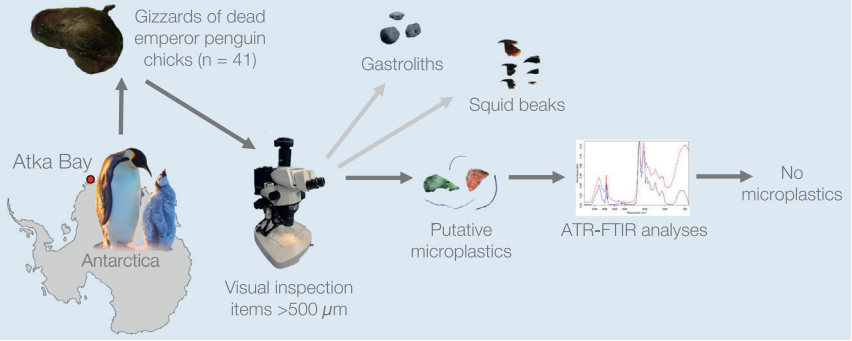
HIGHLIGHTS

- First study on microplastic ingestion in one of the most southerly distributed apex predators.
- Gizzards of dead emperor penguin chicks were screened for microplastics >500 μm.
- No evidence for microplastics applying state-of-the-art analytical methods.
- Microplastic concentrations in the remote study region might still be neglectable.

GRAPHICAL ABSTRACT



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ABSTRACT

Microplastic (<5 mm; MP) pollution has been an emerging threat for marine ecosystems around the globe with increasing evidence that even the world's most remote areas, including Antarctica, are no longer unaffected. Few studies however, have examined MP in Antarctic biota, and especially those from Antarctic regions with low human activity, meaning little is known about the extent to which biota are affected. The aim of this study was to investigate, for the first time, the occurrence of MP in the emperor penguin (*Aptenodytes forsteri*), the only penguin species breeding around Antarctica during the austral winter, and an endemic apex predator in the Southern Ocean. To assess MP ingestion, the gizzards of 41 emperor penguin chicks from Atka Bay colony (Dronning Maud Land, Antarctica), were dissected and analyzed for MP >500 μm using Attenuated Total Reflection Fourier-transform Infrared (ATR-FTIR) spectroscopy. A total of 85 putative particles, mostly in the shape of fibers (65.9 %), were sorted. However, none of the particles were identified as MP applying state-of-the-art methodology. Sorted fibers were further evidenced to originate from contamination during sample processing and analyses. We find that MP concentrations in the local food web of the Weddell Sea and Dronning Maud Land coastal and marginal sea-ice regions; the feeding grounds to chick-rearing emperor penguin adults, are currently at such low levels that no detectable biomagnification is occurring

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via trophic transfer. Being in contrast to MP studies on other Antarctic and sub-Antarctic penguin species, our comparative discussion including these studies, highlights the importance for standardized procedures for sampling, sample processing and analyses to obtain comparable results. We further discuss other stomach contents and their potential role for MP detection, as well as providing a baseline for the long-term monitoring of MP in apex predator species from this region.

1. Introduction

Microplastics (MP; <5 mm; Arthur et al., 2009) were found to be ubiquitous in the marine environment (Bergmann et al., 2017) and pose a potential threat to biota from all levels of the food web (e.g. Besseling et al., 2013; Bobori et al., 2022; Cole et al., 2015; Lusher et al., 2015; Smith and Turner, 2020; Wang et al., 2021). The extensive scientific attention that was raised towards marine MP pollution in the last decades has revealed that not even the remote Arctic Ocean (Bergmann et al., 2022) nor the Southern Ocean surrounding Antarctica, have remained protected from this contaminant. MP contamination was evidenced in Antarctic seawater (Cincinelli et al., 2017; Jones-Williams et al., 2020; Lacerda et al., 2019; Leistenschneider et al., 2021; Suaria et al., 2020), sediments (Cunningham et al., 2020; Munari et al., 2017; Van Cauwenberghe et al., 2013), sea ice cores (Kelly et al., 2020) and glacier surface samples (González-Pleiter et al., 2021). MP pollution in Antarctica has been shown to originate from local human activity (Cincinelli et al., 2017; González-Pleiter et al., 2021; Lacerda et al., 2019; Munari et al., 2017), or might be transported to the Southern Ocean (Waller et al., 2017), by crossing the strong Antarctic Circumpolar Current (ACC) via ocean eddies and storm-forced surface waves (Fraser et al., 2018).

Antarctic biota are considered to be particularly vulnerable to environmental changes and are already threatened by global warming and ocean acidification (Gutt et al., 2021), as well as chemical pollution (Morales et al., 2022; Szumińska et al., 2021) for which MP may also act as an vector (Amelia et al., 2021). The evidence of MP pollution in the Antarctic environment raises the question of the extent to which Antarctic biota are potentially impacted by this pollutant. To date, MP ingestion was reported for benthic biota from the Ross Sea (Sfriso et al., 2020), and in Antarctic apex predators such as Adélie (*Pygoscelis adeliae*), chinstrap (*P. antarctica*), gentoo (*P. papua;* Bessa et al., 2019b; Fragão et al., 2021) and king penguins (*Aptenodytes patagonicus;* Le Guen et al., 2020) from the Antarctic Peninsula and the Scotia Sea region. While plastic fibers and/or fragments have been evidenced in the scat of all penguin species studied so far, Antarctic fur seal (*Arctocephalus gazella*) scat, collected at Deception Island close to the Antarctic Peninsula, were found to be free of MP (Garcia-Garin et al., 2020). In these studies, scat samples collected in the region of the Scotia Sea and the Antarctic Peninsula, were from animals residing in the Antarctic regions with the highest human activity (McCarthy et al., 2022; Waller et al., 2017), thus being possibly more prone to local MP pollution. To better understand the full extent of MP pollution in Antarctica, the degree to which MP has penetrated food webs, in particular for the most remote and southerly-situated regions, must be further investigated.

Seabirds are established bioindicators for pollutants in the marine environment (e.g. Blévin et al., 2013; Burger and Gochfeld, 2004; Carravieri et al., 2020) including for plastic pollution (Amélineau et al., 2016; Orlando-Bonaca et al., 2022; Piatt and Sydeman, 2007; van Franeker et al., 2011). They are proven to ingest, and also retain and accumulate, plastic particles (Carlin et al., 2020; van Franeker and Law, 2015) and to reflect the environmental abundance and distribution of marine plastic litter (Provencher et al., 2017; van Franeker and Law, 2015), making them suitable proxies for the monitoring of marine plastics. In the Southern Ocean, penguins have been used as bioindicators for other environmental pollutants (Calizza et al., 2021; Carravieri et al., 2020, Carravieri et al., 2013; Mwangi et al., 2016), with Fragão et al. (2021) suggesting penguins to be suitable indicators for anthropogenic particles (e.g. MP).

The emperor penguin (*Aptenodytes forsteri*) is, together with the Adélie penguin, the most southerly distributed penguin species and the only one to breed during the harsh Antarctic winter (Prévost, 1961; Stonehouse, 1953), with breeding colonies situated either in coastal areas, or on land-fast ice, between 64°S and 77°S (Fretwell et al., 2012; Fretwell and Trathan, 2021). These pursuit divers mostly target live prey, including fish, squid and crustaceans, which are delivered to their offspring via regurgitation (Cherel and Kooyman, 1998; Kirkwood and Robertson, 1997a; Klages, 1989; Robertson et al., 1994). Being the deepest diver among seabirds, with recorded dive depths of up to 560 m (Wienecke et al., 2007), emperor penguins can forage throughout the whole water column. Their foraging depths have been shown to alternate seasonally, depending on the seasonal prey abundance and distribution (Ancel et al., 1992; Kirkwood and Robertson, 1997b; Zimmer et al., 2008). Other than during the first year after fledging, when juvenile emperor penguins can spend a few weeks north of 60°S (Houstin et al., 2021), emperor penguins spend the remainder of theirs lives in the Southern Ocean. This behaviour is unlike most other penguin species, making the emperor penguin a highly suitable bioindicator for marine pollution on a regional scale (Calizza et al., 2021). For regions with low MP contamination, seabird chicks might be especially suitable indicators, since they are shown to accumulate particularly high amounts of plastic particles in their stomachs (Kühn et al., 2015). This is possibly due to a less developed grinding action in the gizzards, as well as the potential for the transfer of plastic particles accumulated in the proventricular stomach of the parents via regurgitation (Kühn et al., 2015). Moreover, adult emperor penguins have a restricted range for foraging trips during chick rearing, limited to approximately 200 km from the colony. Given this localized food provenance, sampling chicks therefore provides a more thorough indication of MP presence in the regional water masses of the Weddell Sea and Dronning Maud Land region (WS/DML; Houstin, 2020).

In this study, we investigate the ingestion of MP >500 μm in emperor penguins by analyzing 41 gizzards of dead chicks collected at the Atka Bay colony (Dronning Maud Land coast, Antarctica), with the aims of: (1) evaluating the extent to which MP in the remote WS/DML have already penetrated the local food web; (2) assessing and quantifying the hard remains of the prey items which may possibly act as vectors for MP ingestion via trophic transfer; (3) assessing possible MP sources, by means of the particles' characteristics and determination of the polymer composition by applying Attenuated Total Reflection Fourier-transform infrared spectroscopy (ATR-FTIR; Veerasingam et al., 2021).

2. Material and methods

2.1. Study site and sampling procedure

Samples were collected at the Atka Bay emperor penguin colony, near the Ekström Ice Shelf on Dronning Maud Land coast, just east of the eastern boundary of the Weddell Sea (Fig. 1A). The Atka Bay colony (location: 70° 36.664′ S–70° 37.064′ S; 008° 7.709′ W–008°8.769′ W) is among the 10 largest emperor penguin colonies, counting approximately 9600 individuals (Fretwell et al., 2012). The emperor penguins arrive at the colony from April onwards for mating, egg laying and incubating. The chicks hatch in July and August, with fledging occurring in December and January (Prévost, 1961; Stonehouse, 1953; Trathan et al., 2020).

Between 16th of November 2018 and 10th of January 2019, chicks found dead in the colony were collected to estimate mortality during this

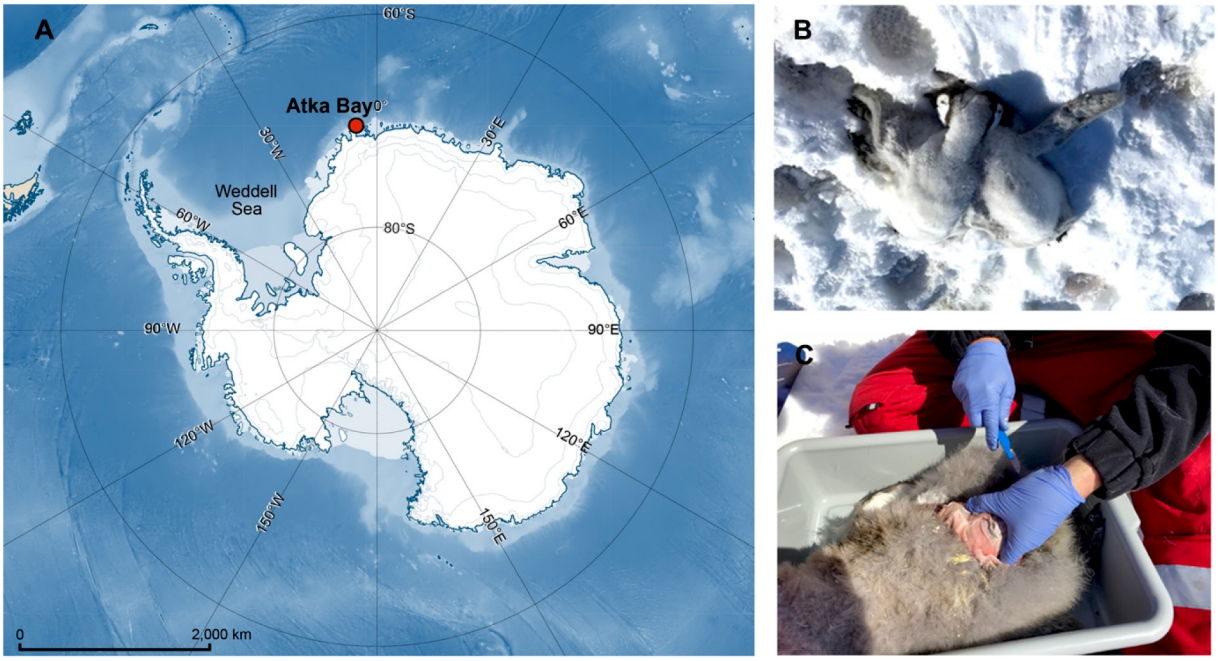

Fig. 1. Sampling location and sampling procedure. (A) Map of Antarctica showing the location of the Atka Bay emperor penguin colony (red dot). The map was created using Quantarctica (v3.1; Matsuoka et al., 2021). (B) Dead emperor penguin chicks collected for sampling the gizzards. (C) Dissection of an emperor penguin chick to isolate the gizzard.

last phase of the breeding season and to perform gizzard sampling. Sampling events were performed shortly after severe storms, increasing the likelihood of finding chicks that lost orientation from their huddle and thus died of exposure on the sea ice. Moreover, collection of dead chicks also took place while performing other observations on the sea ice. At the end of the breeding season, collected carcasses of dead chicks were sorted according to their body size and weight to assign them an approximate age class, as the month in which the chicks died: August (AUG; < 1 month old; $n = 13$), September (SEP; ca. 1 to 2 months old; $n = 3$), October (OCT; ca. 3 months old; $n = 8$), and December (DEC; ca. 4 to 5 months; $n = 17$; Table S1). A dissection was then performed to isolate the gizzards, conducted on-site by trained biologists and medical surgery personnel, avoiding any damage to the stomachs apart from two cuts at the top and bottom (Fig. 1B & C). As the gizzards were initially not taken to study MP ingestion, they were individually packed into PE Ziploc-bags and frozen at −20 °C. Despite the lack of MP contamination prevention measures employed during sampling, the intact stomach wall itself should protect the gizzard contents from external contamination with MP.

2.2. Sample processing

In the laboratory, the frozen gizzards were removed from the zip-lock bags and rinsed with filtered water (Whatman®, Grade GF/C, Sigma-Aldrich, Darmstadt, Germany; pore size: 1.2 μm) to remove potential MP, possibly adhered during sampling and storage. The gizzards were thawed overnight under a fume hood, in glass dishes covered with aluminum foil. The following dissection steps were conducted under a clean bench (ScanLaf Fortuna 1800, Labogene, Lynge, Denmark) to prevent airborne contamination (Wesch et al., 2017). Unless mentioned otherwise, filtered water (1.2 μm, GF/C) and a Polytetrafluorethylene (PTFE) squirt-bottle was used for rinsing of samples and materials used during sample processing and analyses.

Before opening the thawed gizzards, they were, again, rinsed thoroughly from the exterior. They were then opened using stainless-steel dissection scissors and forceps to make a sagittal incision (Fig. S1A). With the aim of targeting 'large' microplastics, >500 μm (Roscher et al., 2021), the gizzard content was transferred to a stainless-steel sieve with a mesh size of 500 μm, using a stainless-steel spoon. The interior surface of the empty gizzard was rinsed to combine any remaining material with the other contents in the sieve. The gizzard tissue was stored in a glass dish covered with aluminum foil for later visual inspection (see Section 2.3.). The gizzard contents were rinsed in the sieve by means of a stainless-steel showerhead, covered with a stainless-steel funnel of the same diameter of the sieve to protect the sample from airborne contamination during rinsing (Fig. S1B). The showerhead was connected to the tap water supply by a silicone hose and the tap water was filtered through a 5 μm mesh stainless-steel cartridge filtration system (Wolftechnik Filtersysteme GmbH & Co. KG, Weil der Stadt, Germany). Using this method, any particles <500 μm were discarded through the sieve, with only particles >500 μm retained for use in this study. The rinsed material was then transferred to clean glass dishes.

Though outside of the scope for this study, filtrate samples containing particles smaller 500 μm in size was stored at −20 °C for three randomly selected gizzards of each age class, respectively (Fig. S1C), for the potential future analyses of "small" MP (<500 μm; Roscher et al., 2021).

2.3. Gizzard content analyses

The weight of the total gizzard content of each sample, to the nearest 0.01 g (Electronic Precision Balance, L610, Sartorius, Göttingen, Germany) was determined using the following equation:

$$W_{gizzard\ content} = W_{gizzard\ full} - W_{gizzard\ empty}$$

Indigestible items and undigested prey remains were sorted and counted during the visual analyses of the samples for MP (see Section 2.4). Items of a biological nature, such as squid beaks, undigested squid parts and fish eyes, were stored in ethanol (96 %, Ph. Eur., extra pure, Carl Roth, Karlsruhe, Germany). The other commonly occurring items found in the gizzards were gastroliths, which were first rinsed with tap water and then with ethanol (≥70 %, denatured, Carl Roth, Karlsruhe, Germany), before being dried and stored in zip-lock bags. These gizzard contents will be subjected to further analyses in future studies, investigating

the geologic provenance of pebbles ingested by the penguins from the seafloor, as well as food analyses.

Making up a substantial portion of the gizzard contents, squid beaks and gastroliths were additionally weighted to the nearest 0.01 g to determine the contribution to the total weight of the gizzard content of each individual. Differences in these contributions between the age classes were compared using a Kruskal-Wallis test with a Dunn's test (Benjamin-Hochberg adjustment) in R (R Core Team, 2021, Vienna, Austria).

2.4. Visual sorting of putative microplastics

For visual inspection, the gizzard content was transferred, in portions, to a Bogorov counting chamber (10.5 × 7.3 cm, Hydro-Bios, Germany; polymethyl methacrylate (PMMA); Löder and Gerdts, 2015) by means of a stainless-steel spoon. Putative MP were identified following criteria defined by Mani and Burkhardt-Holm (2020) and Norén (2007) under a stereomicroscope (SZX 16, Olympus, Hamburg, Germany). In addition to the gizzard content, the gizzard tissue and the rinsing sieves were inspected visually for remaining putative MP particles. Sorted particles were photographed and the longest and smallest dimensions were measured (Simon et al., 2018; Olympus SC50, Tokyo, Japan, CellSens Entry Version 1.17.16030.0).

2.5. Chemical identification of microplastics

Putative MP recovered from the gizzard content were analyzed by means of a Tensor 27 ATR-FTIR coupled to a diamond platinum ATR unit (Bruker Optics GmbH, Ettlingen, Germany). From each particle, three replicate spectra were compiled, with 32 scans in absorbance mode, a resolution of 4 cm^{-1}, a wave number range of 4000–400 cm^{-1}, a Blackman-Harris 3-term apodization, and a zero filling factor of 2. For polymer identification, all resulting spectra were compared to the custom database, BASEMAN for MP analyses (Primpke et al., 2018), using the OPUS 7.5 software (Bruker Optics GmbH, Ettlingen, Germany). Spectra were preprocessed by vector normalization of the first derivative of the data (Primpke et al., 2018) and were considered as successfully assigned to a material with a spectral match >70 % (Thompson et al., 2004). With a match between 60 % and 70 %, the best matches were validated by visual re-evaluation of the spectra (Kroon et al., 2018; Lorenz et al., 2019; Roscher et al., 2021). Particles with spectral matches <60 % were considered as unidentified.

2.6. Quality assurance and quality control (QA/QC)

All steps were conducted within designated MP laboratories equipped with dust filters (DustBox 1000, Möcklinghoff Lufttechnik GmbH, Gelsenkirchen, Germany) and white cotton lab coats were worn at all times. To counteract waterborne contamination, the water used in this study was either filtered through a 5 μm filter (tap water system), a 1.2 μm filter (GFCW) or Milli-Q water was used (Merck Millipore, Milli-Q Biocell 0.22 μm, Darmstadt, Germany). The gizzards were prepared for MP analyses under a flow bench (ScanLaf Fortuna 1800, Labogene, Lynge, Denmark) to prevent airborne contamination (Wesch et al., 2017). Glassware and material made of stainless-steel was used whenever possible and all materials were thoroughly rinsed with Milli-Q before use. The samples were covered with Milli-Q rinsed aluminum foil during thawing or whenever the procedure was paused.

To quantify and account for possible contamination while processing the samples, three procedural blanks were run for the rinsing and transfer process, by using 5 μm-filtered tap water and GF/C-filtered water only. To account for possible airborne contamination during visual analyses of each sample, a glass petri dish filled with Milli-Q water was placed next to the dissection microscope and analyzed for airborne contamination directly after completion of the visual analysis of the sample. For each color of fibers found in the air blanks, as well as in the procedural blanks, three representative fibers were measured using ATR-FTIR, for comparison with fibers found in the gizzards during visual analyses.

Additional to the blank samples, references of plastic materials used during sample storage and processing, were taken and measured by ATR-FTIR. These references included the PE zip-lock bags in which the gizzards were stored, in addition to the silicone hose and a black O-ring from the tap water filtration system. Although 100 % cotton lab coats were worn during the entire analysis to prevent contamination from clothing, fibers from the clothing worn under the lab coats were also taken as references, as well as samples of laboratory cellulose wipes (Kimtech precision wipes, Kimberly-Clark, Kent, UK), used to clean the surfaces. Using these rigorous QA/QC conditions, spectra of all fibers recovered from the gizzard samples were compared to those recovered from the blank samples and reference materials. The spectral matching was performed as described in Section 2.5. Fibers matching with reference or blank sample fibers due to the spectra and the color, were excluded and regarded as contamination deriving from sample processing and analyses.

3. Results

3.1. Putative microplastics

We found 85 putative MP, sorted from 23 out of 41 gizzards, yet none of the putative MP was assigned to a synthetic polymer. Fibers made up 65.9 % of the sorted particles and were found in 15 gizzards, followed by fragments and films with 32.9 %, found in 11 gizzards, and a single spherule (1.2 %). There was, as previously stated, no detection of synthetic polymer particles. A total of 20, out of the 51 measured fibers, were successfully identified as being natural materials with 50 % being natural polyamides (e.g. animal fur; comparative FTIR spectra of a natural and a synthetic polyamide are shown in supplementary Fig. S2 illustrating the differences.); 35 % being cellulose; 10 % natural polysaccharides (plant fibers) and 5 % chitin. For the spectra from the remaining 36 fibers, the best database matches were below the applied threshold of 70 % (or 60 % with visual re-evaluation), and could therefore not be clearly assigned to a material. It should be noted that, though below the threshold value, the first/best matches for these fibers were always for natural materials (33.33 % natural polysaccharides (plant fibers); 33.33 % chitin; 23.33 % cellulose; 10 % natural polyamides (animal fur)). However, as all fibers recovered from the gizzards had similar colors (Fig. 2), and ATR-FTIR spectra (Fig. 3), to fibers found in air and procedural blanks, or to fibers from reference materials, they were considered as contamination from sample processing and analyses steps and therefore excluded (results of the blank samples are described in Section 3.2.).

Fibers were mostly blue (50 %), followed by clear (21 %), black (20 %), red (7 %), and brown (2 %). When comparing the ATR-FTIR spectra of fibers recovered from the samples to fibers from the blank samples, the blue fibers in the gizzard samples closely matched those of blue fibers from clothing worn under the lab coat during the analyses (52 %) or to blue fibers from air and procedural blanks (48 %; Fig. 3). Clear fibers mainly matched with clear fibers from the blank samples (73 %) and the laboratory cellulose wipes (27 %); black fibers matched with black fibers from the blank samples (100 %); and red fibers to fibers from a red item of clothing (100 %; Fig. 3). Only one brown fiber did not match the colors of the fibers from the blank and reference samples, but could be successfully identified as a natural polysaccharide (plant fiber).

The only spherule sorted from the gizzard content as a putative MP was brown in color and assigned to natural polyamides (animal fur), indicating a keratinous material.

The database matching of FTIR-spectra from fragment shaped putative MP safely assigned 13, out of 28 fragments, to a natural material (85 % natural polyamides (animal fur); 15 % chitin; Fig. S3). The best matches for spectra with a match below the threshold were for coal (60 %), natural polyamides (animal fur; 27 %), and chitin (13 %), showing no potential evidence of MP. Data on all putative MP recovered from the gizzards, and blank samples as well as fibers from reference materials are shown in the supplementary material (Table S2 – S6).

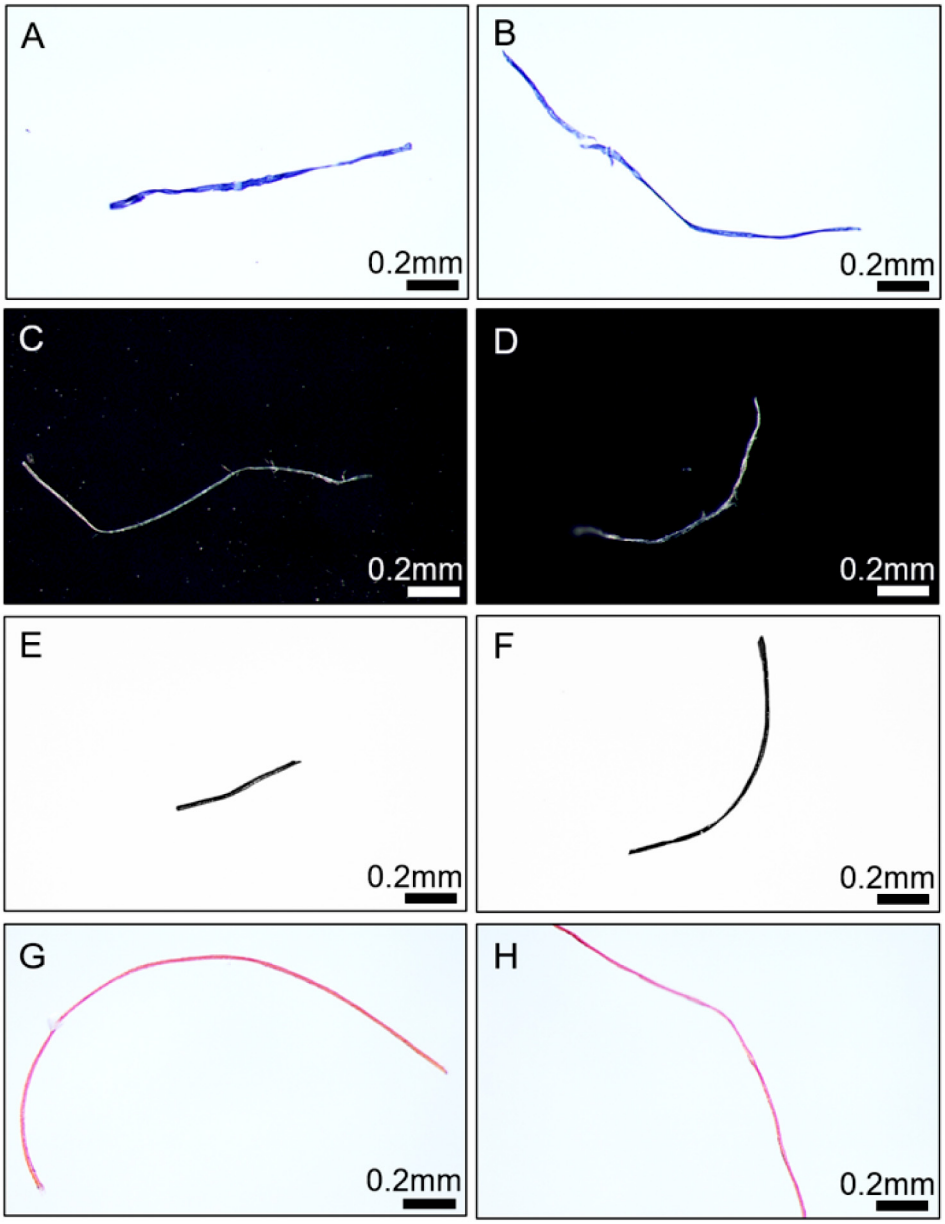

Fig. 2. Examples for fibers recovered from the gizzard-contents and similar fibers from the blank samples and reference material. (A) Blue fiber found in the gizzard-content of emperor penguin #9 and (B) a blue fiber from a cotton-sweatshirt worn during sample processing and analyses. (C) Clear fiber recovered from the gizzard-content of emperor penguin #13 and (D) from a procedural blank. (E) Black fiber recovered from the gizzard-content of emperor penguin #13 and (F) from a procedural blank. (G) Red fiber from the gizzard-content of emperor penguin #61 and (H) a red pullover (made of merino wool and camel hair) worn during sample processing and analyses.

3.2. QA/QC

In total 7 procedural blanks and 41 air blanks were taken during sample processing and visual analysis. From the seven procedural blanks, 51 fibers were recovered, ranging from 1 to 14 fibers per procedural blank with a mean (± SD) of 7.3 (± 5.8) fibers per procedural blank. From the 42 air blanks a total of 132 fibers were recovered, ranging from 0 to 31 fibers per air blank with a mean of 3.2 (±6.1). Fibers in the air blanks were mostly clear (77 %), blue (12 %), black (8 %), and red (3 %). Of the 36 fibers representatively measured from the blank samples (clear, $n = 16$; blue, $n = 10$; black, $n = 6$; red, $n = 4$), 9 could be safely assigned to a material after ATR-FTIR, with two clear fibers identified as being made of synthetic polymers (one polyester and one viscose). From the fibers identified as natural material, three were made of cellulose (clear, $n = 2$; blue, $n = 1$), two of natural polyamides (animal fur; clear and red) and two blue fibers were made of natural polysaccharides (plant fibers). The chemical composition of the other fibers from the blank samples could not be clearly identified, however, all spectra were included, regardless of their match quality, when comparing fibers recovered from the gizzards with those of the blank and reference fibers, and used to exclude potential contamination.

3.3. Other gizzard contents

In all samples, a large portion of the gizzard content was already strongly digested and thus not possible to identify, however, squid beaks were found in all gizzards, while gastroliths were found in all but one gizzard. The mean (± SD) gastrolith count per gizzard was 73 (± 97; range: 0–430), with a mean combined weight of 41.88 g (± 56.01 g; range: 0–238.13 g), accounting for 31.5 % (± 21.1 %; range: 0.9–41.1 %) of the total gizzard content weight. Regarding the occurrence of squid beaks, the mean count per gizzard was 597 (± 616; 6 to 2222), with a mean

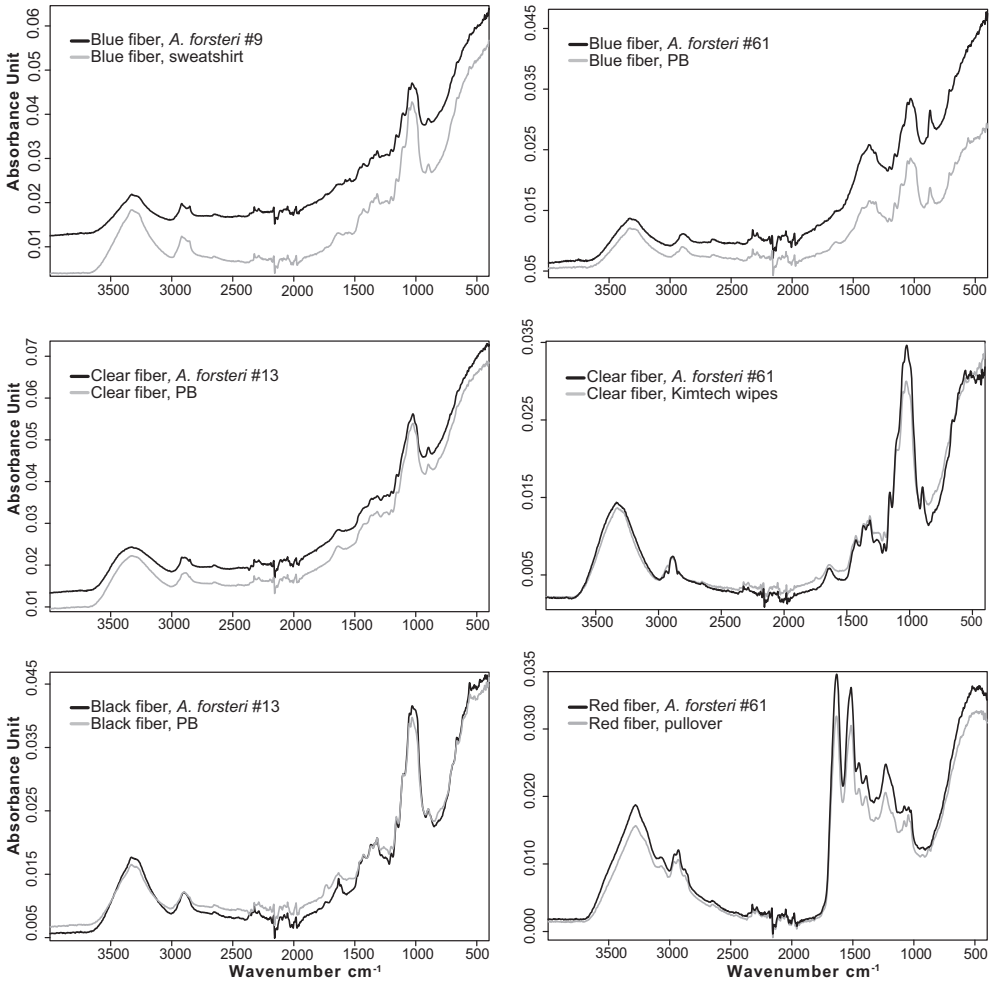

Fig. 3. Examples for spectra of fibers recovered from the gizzard-contents in comparison to spectra of fibers of the same color, respectively, from the blank samples and reference material. Spectra of fibers found in the gizzard contents of the emperor penguins (*A. forsteri*) are displayed as black lines, while spectra of fibers from reference material (cotton sweatshirt and merino wool / camel hair pullover) and procedural banks (PB) are displayed as gray lines.

weight of 12.23 g (± 16.17 g; range: 0.07 g to 68.1 g), accounting for 8.7 % (± 7.3 %; range: 0.9–41.1 %) of the total weight of the gizzard content (Table S7).

The number and combined weight of the gastroliths, as well as squid beaks, increased with age class, with a significant difference between the age class of AUG and SEP, and the age class of DEC ($p < 0.01$). Moreover, a significant increase from AUG to OCT was found for the squid beak number, and from OCT and DEC for the squid beak weight. However, the relative weight of the gastroliths and squid beaks to the weight of the total gizzard content did not reveal any significant differences over the age classes ($p > 0.05$; Fig. S4; Table S8).

Other identifiable items present in the gizzards were fish eyes, found in 34 out of 41 gizzards (mean: 61 (± 63); range: 0–238), undigested squid tentacles and three small entire only slightly digested squid, found in two gizzards of the age class DEC, as well as the exoskeletal remains of a few small crustaceans (e.g. copepods). Photos of the gizzard contents are shown in the supplements (Fig. S5).

4. Discussion

This study is, to our knowledge, the first study investigating MP ingestion in an endemic Antarctic apex predator from a particularly remote region in Antarctica, the WS/DML, while previous studies focused on the Antarctic Peninsula and Scotia Sea region (the region with the highest human activity in Antarctica). The ingestion of macroplastics was, however, evidenced before in an emperor penguin chick from Haswell Island (East Antarctica), where a plastic rope was fed to the chick by an adult emperor penguin (Golubev, 2020).

The analysis of the gizzard contents of emperor penguin chicks should reflect the MP uptake in the adult penguins, as well as indicate localized MP occurrences in the underlying pelagic food web. Considering that the emperor penguin is a pursuit diver that targets live prey, it likely mainly ingests MP indirectly via the consumption of contaminated prey, though other uptake routes could include the incidental uptake via contaminated water or sediments. Although MP has been reported to be ingested by other Antarctic and sub-Antarctic penguin species (Bessa et al., 2019b; Le Guen et al., 2020), we did not find any evidence for ingestion of MP >500 μm in parentally-fed juvenile emperor penguins from the Atka Bay colony. The reasons for this apparent discrepancy may be explained by various geographic, environmental, and methodological factors.

4.1. Emperor penguin diet

The diet of the emperor penguin has been shown to consist of fish (mostly *Pleuragramma antarcticum*), squid (mostly *Psychroteuthis glacialis*) and crustaceans (mostly *Euphausia superba*), with proportions varying strongly depending on location and season (Ratcliffe and Trathan, 2012). For the WS/DML region, squid was dominating the diet (percentage of stomachs: squid 93 %, fish 74 %, krill 67 %, amphipods 55 % and isopods 22 %) in January and February of 1990 and 1992 (Piatkowski and Pütz, 1994). In the diet of emperor penguins from Weddell Sea and the

Weddell–Scotia Sea confluence squid were also dominating with 99 % by mass (Ainleyet al, n.d.).

The predominance of squid we found in the portion of identifiable diet items from the gizzards of emperor penguins from the Atka Bay colony is in accordance with these previous studies. The accumulation of squid beaks in the stomachs could possibly lead to an overestimation of the squid component (Pütz, 1995). Besides squid beaks, we also found fish eyes. Other penguin species have been shown to mainly forage on fish and crustaceans, while squid is of minor importance in the diet of these species (Ratcliffe and Trathan, 2012). Although it has been found that pelagic squid (Gong et al., 2021) and fish (e.g Davison and Asch, 2011; Li et al., 2022; Pereira et al., 2020; Sathish et al., 2020) from other geographical regions are vulnerable to MP ingestion, there are no reports on the in situ ingestion of MP by fish, crustaceans or cephalopods from the region south of the Polar Front (Caruso et al., 2022). It only has been shown, experimentally, that Antarctic krill can ingest and fragment MP into nanoplastics (Dawson et al., 2018). Still, the absence of MP in emperor penguins, in contrast to the other Antarctic/sub-Antarctic penguin species, might be due to dietary differences and the vulnerability of the specific prey organisms to MP ingestion and accumulation.

The absence of MP >500 μm in each of the analyzed gizzards suggests a particularly low occurrence of MP in emperor penguins from the Atka Bay colony, in biota from the underlying pelagic food web, and thus in the WS/DML and the Antarctic coastal current in that region.

4.2. Study region

Located on the northeastern edge of Ekström Ice Shelf in the eastern Weddell Sea, our sampling site in Atka Bay is located in one of the most remote regions of Antarctica. Apart from the fact that the mean MP concentrations are in general significantly lower in marine waters south of the Polar Front (Suaria et al., 2020), the WS/DML further experiences low human activity and has few local MP sources. The region has considerable year-round sea ice coverage in some parts and harsh weather conditions, making the region hard to access. This becomes evident when looking at the numbers of vessels, including fishing, tourism, and research vessels, as well as research stations in the WS/DML, compared to regions, such as the eastern Ross Sea or Antarctic Peninsula, which are particularly affected by human activity (McCarthy et al., 2022; Waller et al., 2017).

The low presence of potential local MP sources might already be a major reason for the absence of MP >500 μm in emperor penguins from the study colony, yet, a recent study reported the presence of MP in surface and subsurface waters within the WS/DML region, with mean concentrations of 0.01 MP m^{-3} and 0.04 MP m^{-3}, respectively (Leistenschneider et al., 2021). These concentrations are not considerably low when compared to other Antarctic regions, being within the range of previously reported values for the Antarctic Peninsula (Lacerda et al., 2019) and Ross Sea (Cincinelli et al., 2017).

To date, MP in the WS/DML have only been reported for surface waters and waters of a depth of approximately 12 m (Leistenschneider et al., 2021), however, MP present within deeper areas of the water column might be more relevant considering that emperor penguin forage within the whole water column: their foraging dives can reach depths of 560 m (Wienecke et al., 2007), but do more commonly occur between the water surface and a depth of 100 m – 150 m (Kirkwood and Robertson, 1997b; Zimmer et al., 2008). By numerically modeling the transport and accumulation of MP in the Southern Ocean, Mountford and Maqueda (2021) demonstrated that the lowest concentration of neutrally buoyant particles within the water column will be found in the WS/DML. The same numerical model, as well as a backtracking dispersal model by Lacerda et al. (2019) showed that MP from other regions may enter the WS/DML due to the cyclonic circulation in the Weddell Sea Gyre and the strong Antarctic coastal current (the counter-current to the Antarctic circumpolar current), representing a major water-mass inflow into the Weddell Sea and the foraging grounds of emperor penguins of the Atka Bay colony. Although the Antarctic coastal current might be a pathway for MP into the WS/DML, the concentration of neutrally buoyant particles in the water column is predicted to be lowest in the coastal region of the WS/DML (Mountford and Maqueda, 2021). This localized distribution pattern could in turn mean that MP concentrations in the foraging region of breeding emperor penguins from Atka Bay are so low that MP did not yet enter the food web at levels relevant to the species.

Moreover, emperor penguin colonies commonly occur near polynyas (areas of open water or persistently loose sea ice) within the fast ice, which provide close access to open water and valuable foraging opportunities during the breeding period (Labrousse et al., 2019). Leistenschneider et al. (2021) reported particularly low MP concentrations in seawater samples taken in coastal polynyas of the WS/DML. This is possibly because MP in polynyas are removed from the sea water by being incorporated into newly forming sea ice, in a process referred to as scavenging (e.g. Alurralde et al., 2022; Obbard et al., 2014; van Sebille et al., 2020).

Though local MP sources are currently only sparsely present within the WS/DML, fishing activities have been increasing in the last decades (CCAMLR, 2021). It can, therefore, be expected that local MP pollution sources, as well as MP input from other regions, will increase in the near future. A long-term monitoring of MP in top-predator species feeding around the Ekström Ice Shelf region might be valuable for observing any potential increase of MP contamination in the WS/DML, a region playing an important ecological role for migrating seabirds and marine mammals, but also for endemic Antarctic species, as the emperor penguin (Fretwell and Trathan, 2009; Teschke et al., 2020; van Franeker, 1996).

4.3. Methodology

Despite the ongoing progress in developing MP research methodologies, studies on the presence of MP in biological samples are based on different sampling procedures, sample processing and detection methods, possibly influencing the results (Bessa et al., 2019a). Additionally, strict QA/QC measures are crucial to provide reliable results and to avoid overestimation of MP contamination (Miller et al., 2021; Song et al., 2021).

4.3.1. QA/QC and putative MP fibers

Although putative MP fibers were recovered from the samples, higher numbers of fibers of the same colors were found in air and procedural blanks, indicating the fibers originated from background contamination during sample processing and analyses. This was confirmed further by comparing the spectra of the fibers found in the gizzard samples to fibers from the blanks and reference samples, resulting in the exclusion of all fibers found in the gizzards as contamination from sample analyses. This result highlights the importance of strict QA/QC measures. Although 100 % cotton lab coats, covering our everyday clothing, were worn at all times in the laboratory, we detected fibers from clothing in the blank samples and gizzard samples, showing the need to expand the QA/QC measures, and to additionally take references of the clothing worn during sample analyses and materials used.

QA/QC measures taken during sampling in the field are just as important as laboratory QA/QC measures (Miller et al., 2021; Scopetani et al., 2020). However, as it is also the case for our study, MP ingestion is often investigated based on opportunistic sampling where samples were collected for other purposes (Provencher et al., 2017), and thus, no QA/QC measures for MP are applied in the field. In the best case, QA/QC in the field should include field blanks, taken, pre-treated and analyzed in the same way as the samples (Miller et al., 2021), as well as collecting references from any possible contamination source (e.g. garments and other plastic materials). Despite these drawbacks, sampling intact gizzards, as was done in our study, has the advantage that the intact stomach wall, protecting the gizzard content sample, mitigates contamination from the field. By thoroughly cleaning the gizzards carefully from the exterior before opening them, any major contamination from sampling, handling and storage should be negated, without interfering with the gizzard content. This is in contrast to other types of samples (e.g. scats or regurgitated material), which might be directly exposed to contamination at the sampling site, during sampling

from the field garment and equipment, and during storage from sampling containers.

In contrast to our study, previous studies using penguin scats as a proxy for MP ingestion, found MP fibers and/or fragments (Bessa et al., 2019a; Fragão et al., 2021; Le Guen et al., 2020). These scat samples might have been directly exposed to background contamination in the field, yet, no field QA/QC measures were reported. The unprotected scat samples were further stored in plastic bags and/or tubes, which could be another overlooked contamination source in these studies, as no reference samples of these materials were taken. For samples collected in less polluted regions, from less affected biota, disregarded background contamination due to inadequate QA/QC measures could lead to a substantial overestimation of MP presence (Song et al., 2021).

4.3.2. Sample type and target MP size

There are further differences that have to be considered, regarding the various sample types, when investigating MP ingestion. Plastics were shown to be retained in the gizzards (as well as the proventriculus), making this part of the gastrointestinal tract (GIT) suitable for plastic accumulation studies (Provencher et al., 2019). In our study, we targeted MP > 500 μm in the gizzards, as smaller particles are likely to pass through the pyloric sphincter (the passage from the stomach to the intestines) and will be excreted (Day et al., 1985; Furness et al., 1984; Provencher et al., 2019). This, in turn, means that scat samples, as analyzed by Bessa et al. (2019a), Fragão et al. (2021) and Le Guen et al. (2020), might be more suitable for the analyses of the excreted MP <500 μm, but will have limitations when it comes to larger ingested plastic items. In accordance with that, these studies used a lower size detection limit of approximately 60 μm. In general, the abundance of environmental MP has been shown to increase with decreasing size (Lorenz et al., 2019; Poulain et al., 2018; Roscher et al., 2021). The different MP target sizes could therefore be another factor influencing our results. Samples of the filtrate from the gizzards, containing particles <500 μm, were stored for potential future analyses and might possibly clarify this discrepancy.

Even when plastic pieces are retained in the gizzards due to their size, they might eventually be ground down by the action of gastroliths until they can pass the pyloric sphincter into the intestines and are excreted (Provencher et al., 2019). We found gastroliths in all but one gizzard. These “stomach stones” are commonly ingested by birds and accumulate in the gizzard to help break down hard food items (Wings, 2007). Due to high proportions of gastroliths we found in the analyzed gizzards, the fragmentation of large plastic particles in the gizzards into small particles, that are not detected with the applied method, might be enhanced further. However, the high number of intact squid beaks found in the gizzards, co-occurring with high numbers of gastroliths, indicates that despite the gastroliths and the grinding action of the gizzards, hard dietary items might be retained in the stomachs for an extended time. Previous studies showed that emperor penguins might retain squid beaks for weeks or even months (Cherel and Kooyman, 1998; Pütz, 1995). Especially in chicks in which the grinding action of the gizzards might be less developed. As shown for gastroliths, emperor penguin chicks may be more vulnerable to the accumulation of hard dietary items, as they may further be less effective in eliminating these by regurgitation or by passing them through the digestive tract (Splettstoesser and Todd, 1999).

Little is known about the retention time of plastics in seabirds and especially in penguins, however, plastics might have similar retention times to other hard dietary items, as shown in studies of albatrosses, where it was shown that they retain squid beaks and plastic items for 50 days and more (Furness et al., 1984; Pettit et al., 1981). For seabirds, such as for albatrosses and petrels, the plastic retention time may vary depending on the size and weathering state of the ingested plastic item, other persistent food items in the stomach, and morphological differences in the gastrointestinal tract relative to the taxon (Day et al., 1985; Ryan, 2015). With regards to juvenile seabirds, Ryan and Jackson (1987) showed that weathered polyethylene pellets collected from beaches that had been fed to fledglings of white-chinned petrels, only lost 1 % of the mass after 12 days, suggesting a retention time of at least one year. Plastic items fed to albatross chicks by their parents were shown to be retained for >31 days, and possibly for even >4 months (Pettit et al., 1981).

Although plastic items could possibly be broken down in the gizzard, with the additional aid by ingested gastroliths, previous studies indicate that plastic particles, large enough to not pass into the intestine, should be detectable in the gizzards of the emperor penguin chicks for an extended period of time. Furthermore, MP of a size <500 μm can possibly still be retained in the stomach by being trapped in gastric folds (Xiong et al., 2018). Future studies should include the analyses of MP larger and smaller than 500 μm, to also detect small MP that were potentially trapped in the gastric folds, were recently ingested, or had been fragmented by grinding. This holistic approach would improve our understanding of the true exposure of seabirds to this pollutant and the dynamics involved. The examination of stomach contents together with scat samples would also be valuable to show patterns of MP accumulation and excretion.

4.3.3. Chemical identification of putative MP

The method for chemical identification applied here is in accordance with a study by Primpke et al. (2017), showing that the applied method will lead to the lowest number of miss-assignments. Applying other pretreatments of the spectral data and routines might influence the results significantly and lead to substantial miss-assignments, including false-positives (Primpke et al., 2017; Renner et al., 2019). Another important factor is the used reference library database, which, even though synthetic polymers are targeted, should include natural materials typically found in the sample matrices (e.g. plant material, animal fur, sand) to reduce the risk of false-positives and misidentification (Primpke et al., 2017; Renner et al., 2019).

A common problem in MP studies, however, is the lack of adequate details regarding the applied methods used for the chemical identification of FTIR-spectra. These missing details make inter-study comparisons difficult, and might be a potential methodological factor affecting the detection or non-detection of MP. For studies concerning MP and Antarctic biota, in which FTIR spectroscopy was applied for polymer identification, three out of five studies did not specify the preprocessing of the spectral data nor the applied threshold (Bessa et al., 2019a; Fragão et al., 2021; Sfriso et al., 2020). The two other studies reported an applied threshold of 60 % and 75–80 %, respectively (Garcia-Garin et al., 2020; Le Guen et al., 2020), but did not provide details on the preprocessing of the data. While Le Guen et al. (2020) used the same reference database library as used in the present study, detailed specifications on the reference library are missing for the other studies (Table S4; (Bessa et al., 2019b; Fragão et al., 2021; Garcia-Garin et al., 2020; Sfriso et al., 2020).

In our study, all particles successfully identified using the spectral database were classed as being made of natural materials; however, the composition of a high number of particles remained unidentified. The best matches for spectra from these particles, although with a match below the threshold, were natural materials, possibly indicating a natural origin of these particles too. The spectral database used in our study, designed particularly for the identification of synthetic polymers in environmental samples (BASEMAN; Primpke et al., 2018), includes 77 entries of natural materials (e.g. different types of plant fibers, algae, animal fur, chitin, and minerals) besides the 248 entries for synthetic polymers. The unsuccessful identification of natural particles could be due to the specific natural materials not being included in the reference database, and/or the spectra of ingested particles might be chemically altered, as they were exposed to the acidic gastric fluid in the stomach. With plastic particles, it has been shown that the treatment with artificial gastric fluids has no pronounced effects on the particle size, shape and surface structure (Stock et al., 2020), however, we did not find any published data on the effects on the spectra of treated MP.

5. Conclusion

Our results reveal the absence of, or a particularly low contamination with, MP >500 μm in the emperor penguin, one of the most southerly

distributed predators of Antarctica. Although no MP >500 μm were found in this study, plastic production, and consequently marine plastic pollution, is projected to further increase in the future (UNEP, 2021). Global warming is already posing a number of other threats to Antarctic ecosystems and biota (Holland et al., 2021; Hughes et al., 2021; Trathan et al., 2015). As sea ice melts, remote regions might become more accessible, potentially leading to higher human activity and more MP sources on a local scale (McCarthy et al., 2022). Long-term monitoring programs using apex predators from the Weddell Sea and other regions of Antarctica, along with environmental samples, should be implemented to track the trends of MP contamination in this unique and sensitive environment. Beyond tracking pollution, such programs will also provide the basis for determining the tipping-point at which exposure levels biota may be affected by environmental MP contamination, and for quantifying the processes and consequences at the individual, species and ecosystem level. Knowledge about MP contamination at lower trophic levels would further be of importance to understand the entry point into the food web. When using apex predators as bioindicators for MP pollution, a more comprehensive insight to better understand MP accumulation and excretion in biota might be obtained by investigating MP in both size fractions (larger and smaller than 500 μm) and in different parts of the gastrointestinal tract together with scat samples. While logistical constraints make long-term sampling in the Antarctic environment particularly challenging, we stress the importance of implementing a more rigorous level of comparability between studies in terms of methodologies, and consider it critical to standardize the sampling, QA/QC, MP extraction and analytical methods for such valuable samples. This standardization will contribute to a more accurate and holistic overview of MP pollution in the Southern Ocean and the unique environments contained within.

CRediT authorship contribution statement

Clara Leistenschneider: Investigation, Methodology, Data curation, Writing - Original Draft, Writing - Review & Editing, Visualization.

Céline Le Bohec: Concept initialization, Sample acquisition, Writing - Review & Editing, MARE PI.

Olaf Eisen: Concept initialization, Sample acquisition & dispatching, Writing - Review & Editing.

Aymeric Houstin: Sample acquisition, Writing - Review & Editing.

Simon Neff: Formal analysis, Investigation, Writing - Review & Editin.

Sebastian Primpke: Formal analysis, Writing - Review & Editing.

Daniel P. Zitterbart: Sample acquisition, Writing - Review & Editing, MARE PI.

Patricia Burkhardt-Holm: Writing - Review & Editing, Supervision.

Gunnar Gerdts: Methodology, Writing - Review & Editing, Supervision.

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Data availability

Data will be made available on request.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.158314.

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