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Do Antarctic bivalves present microdebris? The case of Livingston Island☆

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ABSTRACT

Marine microdebris (MD) seem to be widespread in benthic invertebrates, even in the most remote areas of the planet such as Antarctica, although the information available is still very scarce. Here we provide a detailed quantification and characterization of the MD found on three common bivalve species (*Aequiyoldia eightsii*, *Thracia* cf. *meridionalis*, and *Cyclocardia astartoides)* inhabiting shallow areas in Johnsons' Bay, Livingston Island (South Shetland Islands, Antarctica) as a snapshot of the MD present. On average, these bivalves contained 0.71 ± 0.89 items per individual and 1.49 ± 2.35 items per gram, being comparable to the few previous existing studies in other Antarctic areas. Nearly half of the organisms analysed here (45.6 %), contained at least one item. No significant differences were found in the three bivalve species. As far as we know, this is the first study to analyse and compare MD in three bivalve species in the Antarctic Peninsula. Although our results indicate bivalves are as not as polluted as in other areas of the planet, this is remarkable since this is considered one of the last pristine areas of the world. Our results point to local activities as the main source of MD pollution in Livingston Island, although global pollution cannot be discarded. We believe this research provides a useful baseline for future studies and will contribute to develop policies and strategies to preserve Antarctic marine ecosystems from MD pollution.

1. Introduction

Plastic production increases globally year by year (PlasticsEurope, 2020). In 2019, 368 million tons were produced over the world, and it is estimated that by the year 2039 this number will double (Lebreton and Andrady, 2019; PlasticsEurope, 2020). Plastics are everywhere in the ocean, with total estimates being around 75 to 199 million tons (McGlade et al., 2021). They are categorized by size into macroplastics, mesoplastics, and microplastics (Kershaw et al., 2019; McGlade et al., 2021).

Microplastics (MP) are polymeric particles from 1 μm to 5 mm. Depending on its origin they can be classified as either primary, when the polymer is produced in that size range, or secondary, when a macroplastic deteriorates due to environmental processes into smaller fragments (Frias and Nash, 2019; Kershaw et al., 2019). Microdebris (MD) include synthetic (MP), semi-synthetic, and naturally derived items (Kroon et al., 2018). In the last decade, MD have been found in all parts of the globe including the most pristine and isolated environments (Peng et al., 2018). Antarctica is not an exception despite being a remote continent with limited human impact. In this isolated continent, MD have been found on the snow (Aves et al., 2022), ice (Kelly et al., 2020), freshwater (González-Pleiter et al., 2020), seawater (Cincinelli et al., 2017), marine sediments (Reed et al., 2018; Cunningham et al., 2020; Perfetti-Bolaño et al., 2022) and some biota (Fragão et al., 2021; Zhang, M et al., 2022a). However, there is still very little information on benthic invertebrates from the Southern Ocean.

The interaction between MD and biota poses different ecological and biological risks. MD can be transferred through the trophic chain and bioaccumulate in different organisms, which also play a key role in the transportation of MD on a vertical scale: through the water column to the seafloor and viceversa (Coyle et al., 2020; Miller et al., 2020). The ingestion of MD by marine animals has several health risks such as growth inhibition, infertility and reproduction decrease, blockage of body tracts, an increased vulnerability to illnesses, and a general affectation of the survival rates. (Foley et al., 2018; Gola et al., 2021). In Antarctica, little is known about the effects of MD on marine organisms with just a few studies characterizing their concentration in a few species (Rota et al., 2022). MD have been detected in different marine

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animals in the Southern Ocean, including penguins (Bessa et al., 2019; Le Guen et al., 2020; Fragão et al., 2021), fish (Bottari et al., 2022; Zhang, M. et al., 2022a), krill (Zhu et al., 2023), and a few benthic invertebrates (Sfriso et al., 2020; Bergami et al., 2023; González-Aravena et al., 2024). Local activities such as tourism, fisheries, and research stations alongside plastic production around the globe have been suggested as the main sources of MD contamination in Antarctica (Waller et al., 2017; Bargagli and Rota, 2023).

Everything stated above suggests that there is a significant lack of knowledge regarding MD in benthic invertebrates from the Southern Ocean, with just few studies characterizing MD on different benthic organisms. In the Ross Sea, Sfriso et al. (2020) analysed the MD in one Cnidaria, five Mollusca, two Arthropoda and four Annelida species, whereas Bergami and co-authors (2023) compared the fibres found in a gastropod (*Neobuccinum eatoni* (E. A. Smith, 1875)) with those coming from clothing in the Mario Zucchelli Italian base. Recently, the bivalve, *Laternula elliptica* (P. P. King, 1832) from King George Island has been analysed for MD pollution and suggested to be a potentially useful bioindicator (González-Aravena et al., 2024). Overall, additional research on MD pollution in benthic invertebrates is required to assess the environmental impacts and risks for the marine biota inhabiting these ecosystems.

Bivalves are filter-feeding molluscs, easy to sample in soft-bottom areas, and therefore commonly used as bioindicators for MD pollution around the globe (Ding et al., 2021; Sun et al., 2023). In the Southern Ocean, scientific activity is often limited by harsh environmental conditions, and thus, the collection of these abundant organisms from soft seabed floors, like the bay of this study, together with their feeding biology traits, make them a perfect choice for the study of MD contamination (González-Aravena et al., 2024).

We provide here a detailed quantification and characterization of the MD found in three bivalve species in Johnsons' Bay (Livingston Island, South Shetland Islands) as a snapshot of the MD present. For this purpose, three common bivalve species in the bay were selected: *Aequiyoldia eightsii* (Jay, 1839), *Thracia* cf. *meridionalis* (E. A. Smith, 1885), and *Cyclocardia astartoides,* E. von Martens, 1878. The aims of the research are: (a) to quantify the MD found in these organisms, (b) to characterize the items found in shape, size, colour, and polymer type and, (c) to observe whether there were differences in the MD abundance and characteristics in the three species. As far as we know, this study is the first to characterize and compare the MD in these three bivalve species in the Antarctic Peninsula. Moreover, two species (*Thracia* cf. *meridionalis* and *Cyclocardia astartoides*) are analysed for the first time in Antarctica regarding MD quantification and characterization. Since previous studies on MD pollution have been done only in the Ross Sea and King George Island, this is the first study in Livingston Island, being the southernmost location analysed in the Antarctic Peninsula. Therefore, this work sets a starting point for MD quantification and characterization of benthic species in Livingston Island and contributes to expand knowledge in this topic. This baseline will be crucial to develop management policies to protect Antarctic benthos from MD pollution.

2. Material and methods

2.1. Location and sampling procedure

Bivalves were sampled during the CHALLENGE-1 campaign (January–March 2022) at Johnson's Bay (62°39.618′ S, 060°22.386′ W) in Livingston Island (South Shetland Islands, Antarctica). The study area is situated between two Antarctic stations: the Bulgarian base (St. Kliment Ohridski), located 2 km North from the bay whereas the Spanish Base (JCI) is 1 km South (Fig. 1). Sampling was done in a single day using a Van Veen dredge at 15 m depth, and three bivalve species were targeted as the most abundant in the bay. A total of 69 individuals of the three species were collected.

The species used for this study are, *Aequiyoldia eightsii* (n = 38), *Thracia* cf. *meridionalis* (n = 18), and *Cyclocardia astartoides* (n = 13). The individuals collected were placed in zip bags immediately after

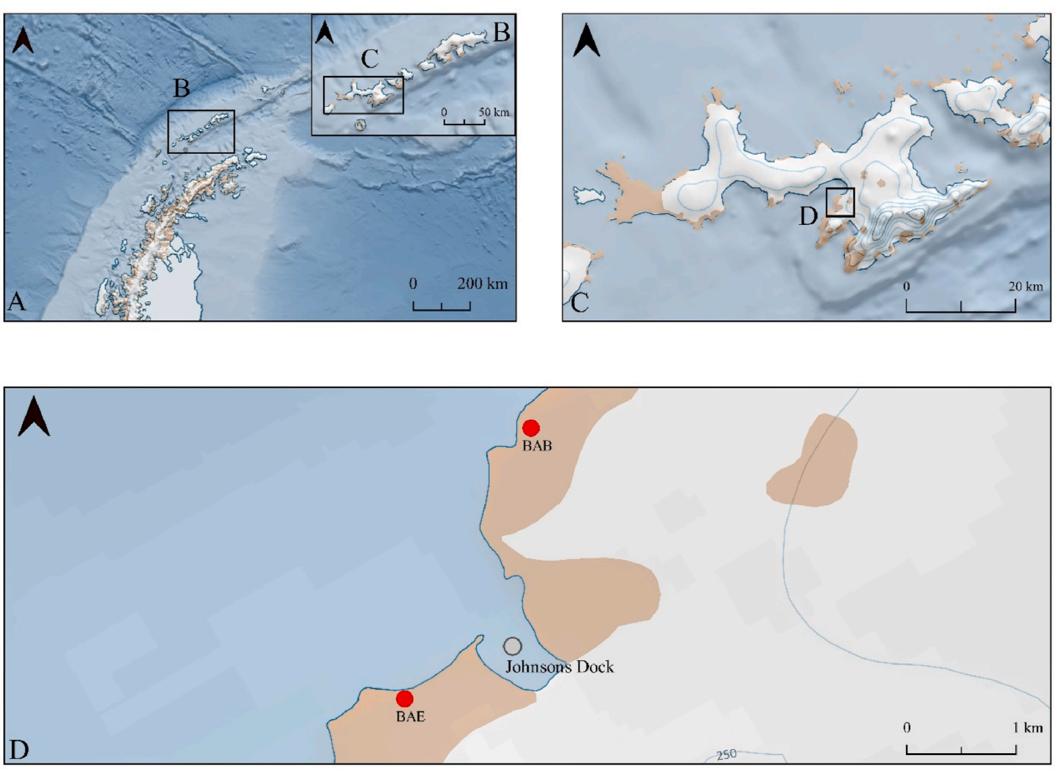

Fig. 1. Study area. A) West Antarctic Peninsula (WAP), B) South Shetland Islands, C) Livingston Island and D) Johnsons dock, between Bulgarian (BAB) and Spanish (BAE) stations.

sampling and preserved at −20 °C for further analysis at the Universitat de Barcelona (Catalonia).

2.2. Digestion procedure and microdebris extraction

Once in the laboratory, samples were kept in the fridge until defrosted. Total weight, soft body weight (wet weight), and shell measures were registered for each specimen. Tissue was extracted from the bivalves with a blade and carefully rinsed with Milli Q water. To digest the animal 3:1 (w/v) KOH 10 % was added to a glass flask in an orbital shaker incubator (Edmund Buehler GmbH Incubator Hood TH 15 with shaker) at 40 °C for 48 h (Dehaut et al., 2016; Karami et al., 2017; Thiele et al., 2019; Bom and Sá, 2021). Then, 10 ml citric acid 1M was poured into the digested solution and immediately filtered on a 1.2 μm pore glass fibre filter (GF/C Whatman 47 mm) with a glassware vacuum filtering system (Glassco®, All-glass filter-holder assembly, ref. 258.284.01) (Thiele et al., 2019). Filters were kept in glass Petri dishes and left to dry at room temperature until analysed.

Filters were examined with a stereomicroscope (Olympus SZX10) connected to a digital camera (Olympus SC30) and MD items were visually identified. Pictures of each MD item were taken and processed through the Olympus CellSens Standard software (RRID:SCR_014551). The shape and the colour of each item was determined through detailed visual identification. For each item, size was measured using the longest axis with the above mentioned software.

2.3. Polymer identification

Identified MD were carefully isolated using stainless steel tweezers and placed between two Calcium Fluoride (CaF_2) slides (CAF2 UV grade 76 × 26 × 1 mm polished window, Crystran®). MD item characterization was carried out at CCIT-UB (Scientific and Technological Centres of the University of Barcelona) services with a μ-FTIR microscope Thermo Nicolet iN10 MX, OMNIC Software. The instrument has an MCT detector, KBr Beamsplitter and a Glowbar source. The spectral range analysed was 4000 to 850 cm-1, 64 accumulations and 4 cm-1 of spectral resolution. Different zoom optics apertures were used to obtain the spectral.

2.4. Quality assurance/quality control (QA/QC)

To prevent airborne contamination, work was done in a laminar flow hood, where all surfaces were cleaned prior to use with ethanol 50 %. Glassware and stainless-steel material were used. The material was further cleaned with HNO_3 1 % and rinsed three times with Milli Q water before usage and covered with aluminium foil. A 100 % cotton lab and nitrile gloves were worn during the processes. All reagents were filtered at 1.2 μm pore glass fibre filter (GF/C Whatman 47 mm) with a vacuum glass filtering system.

Procedural blanks were prepared throughout the dissection, digestion, and filtration processes using filters placed next to the samples on each step of the process to control airborne contamination. Blank procedurals were analysed on shape, colour, size, and polymer type. To discard possible contamination items, blank items were compared to sample ones and normalised if they shared characteristics or visual similarity according to literature protocols (Brander et al., 2020; Crutchett et al., 2020; Bergami et al., 2023).

To evaluate the methodology employed, the digestion efficacy was calculated for all the organisms analysed (n = 69) following Dehaut et al. (2016). Limit of Quantification (LOQ) and limit of detection (LOD) were also calculated based on Bråte et al. (2018) and Brander et al. (2020) (Supplementary material, E.S.1).

2.5. Statistical analysis

Data was analysed through SPSS 27 software for Windows (IBM, New York, United States) and reproduced using GraphPad version 8.0.2 (GraphPad Software, Boston, Massachusetts, USA). MD quantity (items) was analysed per gram and per individual, and their characteristics were evaluated as well (colour, size, polymer, and type). In addition, statistical analysis was used to compare the amount and size of items (dependent variables) per species (factor). Kruskal-Wallis test was performed to test the differences between species as in some data the normal distribution (Shapiro–Wilk, $P > 0.05$) or the variances homogeneity (Levene's test, $P > 0.05$) could not be assumed. A Spearman correlation test was used to detect correlations between the organism's weight, the size of the items found and the items quantity, if any.

3. Results

3.1. QA/QC procedural results

The KOH treatment for organic material removal had an average efficacy of 92.4 ± 5.3 %. *Cyclocardia astartoides* (94.4 ± 2.2 %), which was the best digestion efficiency followed by *Aequyioldia eigthsii* (92.2 ± 5.8 %), and *Thracia* cf. *meridionalis* (91.0 ± 5.5 %).

A total of 10 blanks were conducted for the entire analysis apart from airborne contamination controls. From the 20 items found in the blanks, 17 were excluded in the samples due to matching visual identification or polymer composition. On average, blanks contained between 2 ± 1.05 items per filter and the candidates' size was 610 ± 730 μm. LOD had a 5.2 value and LOQ was 12.5.

3.2. Morphometric data and microdebris abundance

On average, the organisms' soft tissue wet weight was 0.54 ± 0.33 g. For each species, details can be found in Table 1 while the complete morphometric data can be found in the Supplementary Information (T. S.1).

For all the bivalves analysed (n = 69) more than half (54.4 %) had no MD, 25 % contained one item per individual (it/ind), 16.2 % two it/ind, and 4.4 % three it/ind.

For each species studied, the values are similar, in *Aequiyoldia eightsii* items were found in 16 out of 38 individuals (42.11 %), for *Thracia* cf. *meridionalis* 9 out of 18 individuals (50.0 %) while for *Cyclocardia astartoides* 6 out of 13 individuals contained MD (46.15 %). In *Aequiyoldia eightsii* 22 individuals (57.9 %) had no items, eight individuals (21.1 %) contained one item, seven individuals (18.4 %) contained two items and one individual (2.6 %) had three items. Half of the individuals of *Thracia* cf. *meridionalis* were free of MD (n = 9, 50 %), four individuals contained one item (22.2 %), three individuals (16.7 %) contained two items and two individuals (11.1 %) contained three items. For *Cyclocardia astartoides*, more than half of individuals (n = 7, 53.9 %) had no MD, five individuals contained one item (38.4 %) and one individual (7.7 %) had two items.

On average, the organisms had 0.71 ± 0.89 items per individual (it/ind) and the amount of MD per gram of soft tissue wet weight (it/g) was 1.49 ± 2.35 (Table 1, Fig. 2). Kruskal-Wallis test indicated that no significant differences were found between MD quantity and the different species studied.

3.3. Microdebris characteristics

From all the items found through visual sorting (n = 48), 43 were fibres whereas, 5 were fragments. MD found measured between 100 and 2380 μm, average was 745 ± 633 μm (Table 1). Most items ranged between 100 and 500 μm (n = 27, 57.4 %), followed by 1500 and 2000 μm (n = 8, 17 %). Two fractions, 500 to 1000 and 1000–1500 μm had the same proportion (n = 5, 10.6 %) and only 4.3 % (n = 2) of the MD found were larger than 2000 μm. No significant differences were found between species and sizes in the Kruskall Wallis test. Regarding fibres, the proportions are similar, with most of them (n = 23, 54.8 %) measuring up to 500 μm, followed by 1500–2000 μm (n = 8, 19.1 %). Five fibres

Table 1
Wet weight and microdebris abundance and size for each species.

Species	Soft tissue weight (g) $\overline{X} \pm$ s.d.	Minimum weight (g)	Maximum weight (g)	Items per individual $\overline{X} \pm$ s.d.	Items per gram $\overline{X} \pm$ s.d.	Items mean size (μm) $\overline{X} \pm$ s.d.
Aequiyoldia eightsii	0.64 ± 0.40	0.10	1.53	0.66 ± 0.87	1.30 ± 2.27	738 ± 625
Thracia cf. *meridionalis*	0.36 ± 0.13	0.16	0.59	0.94 ± 1.08	2.34 ± 3.13	648 ± 620
Cyclocardia astartoides	0.52 ± 0.18	0.32	0.90	0.54 ± 0.66	1.05 ± 1.21	988 ± 716

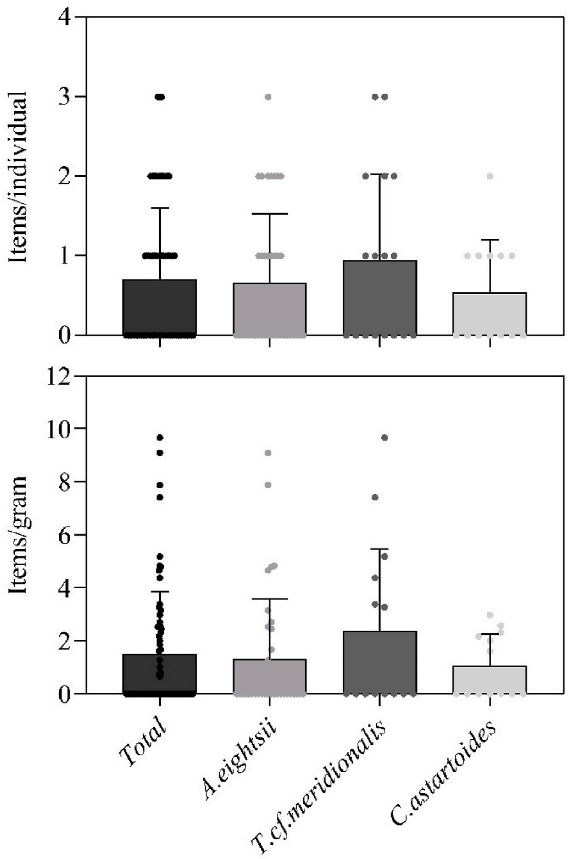

Fig. 2. Items per individual (It/ind) and items per gram (It/g) -wet weight soft tissue-for all three bivalves, *Aequiyoldia eightsii*, *Thracia* cf. *meridionalis* and *Cyclocardia astartoides*. Individual values (dots) are represented, the graph indicates the mean and the line the standard deviation.

(11.9 %) ranged between 500 and 1000 μm, four fibres (9.5 %) between 1000 and 1500 μm, and just two fibres (4.7 %) were larger than 2000 μm. For the five fragments found, four were smaller than 500 μm (80 %) and only one (20 %) was between 1000 and 1500 μm (Fig. 3).

Colour was determined through the stereomicroscope for all the items (n = 48). A total of 19 items were blue (39.6 %), 12 were black (25.0 %), 8 items were transparent (16.6 %), 7 were red (14.6 %), and green and purple were just one item each colour (2.1 %). For fibres, proportions were similar as they represent most of the MD found. Fragments though, present different proportions and only blue (n = 2, 40 %), red (n = 2, 40 %) and green (n = 1, 20 %) items were found (Fig. 3).

Identification of polymers was possible for 58.3 % of the items found (n = 28), as some were lost during the visual identification process and the translocation to the Calcium Fluoride slides. For each species, polymer identification was possible in *Aequiyoldia eightsii* for 14 out of 25 items (56 %), *Thracia* cf. *meridionalis* in 11 out of 16 items (68.75 %), and *Cyclocardia astartoides* in 3 out of 7 items (42.86 %). Cellulose was the most abundant polymer in all cases (n = 16, 57.1 %), followed by Polyethylene terephthalate (PET) (n = 5, 17.9 %), Nylon (n = 4, 14.3 %), Polypropylene (PP) (n = 2, 7.1 %) and Epoxy resin (n = 1, 3.6 %). Most of these proportions account for fibres. Fragments consisted of one PET, one nylon, one epoxy resin and two cellulosic MD (Fig. 3).

No differences were observed between species and MD quantity ($p = 0.602$) and items size found ($p = 0.411$) according to the Kruskal-Wallis test. No correlation was found between organism's weight and items quantity ($p = 0.904$), nor among shell width and items found ($p = 0.712$) when tested with Spearman correlation tests. However, a weak correlation was found between the wet weight of the soft tissue and the size of the found in the organisms ($r = 0.431$ ($p < 0.05$)).

4. Discussion

4.1. MD abundance

Marine pollution in Antarctic benthic invertebrates is a recent area of research with a strong need of new data, with only three studies reported so far (Sfriso et al., 2020; Bergami et al., 2023; González-Aravena et al., 2024). To help filling this knowledge gap, we quantified and characterized MD in three different bivalve species (*Aequiyoldia eightsii*, *Thracia* cf. *meridionalis* and *Cyclocardia astartoides*) in Johnson's Bay, Livingston Island. We report here, for the first time, MD pollution in bivalves in this island, with about half of the analysed individuals containing MD. Furthermore, it is the first study to characterize and compare MD in these three bivalve species. Moreover, it represents the southernmost study of MD in benthic organisms in the Antarctic Peninsula.

From the 69 analysed bivalves, a mean concentration of 0.71 ± 0.89 it/ind and 1.49 ± 2.35 it/g were found. Our results differ from other Antarctic benthic studies, with concentrations about half of those MD reported in the Ross Sea for bivalves (1.9 it/ind) (Sfriso et al., 2020). Conversely, our results show about double concentrations of textile fibres than those reported in a gastropod (0.3 ± 0.53 it/ind) in Terra Nova Bay, Ross Sea (Table 2) (Bergami et al., 2023). In King George Island, the abundance of MD in bivalves was significantly higher in items per individual (42.86 ± 25.36 it/ind), but similar to our results in items per gram of soft tissue (1.82 ± 1.61 it/g) (González-Aravena et al., 2024). *Aequiyoldia eightsii,* the only species that had been previously analysed, presented higher concentrations of MD in the Ross Sea (n = 12, ~2.2 it/ind); Sfriso et al. (2020), compared to our results in Livingston Island (n = 38, 0.66 it/ind). These differences in *A. eightsii* could perhaps be attributed to sample size, as the Ross Sea study indicates that as more individuals were analysed, MD concentration decreased (Sfriso et al., 2020). When comparing our findings to pelagic invertebrates and benthic fish in Antarctica they show higher MD concentrations. In South Georgia, krill (2.13 ± 0.26 MP/ind) and salps (1.38 ± 0.42 MP/ind), tripled and doubled our findings, whereas benthic fish from the South Shetland Islands had five times more MD abundance (3.80 ± 1.95 MP/ind) (Table 2) (Ergas et al., 2023; Johnston et al., 2023). Overall, such differences may be related to a variety of factors such as the diverse locations studied, the sampling depth and the taxa analysed. For example, the gastropod in Terra Nova Bay was sampled at 150 m depth while our samples were collected at 15 m (Bergami et al., 2023). Additionally, our bivalve and this gastropod have different feeding strategies, which may indeed affect MD ingestion (Setälä et al., 2016; Sfriso et al., 2020; Bergami et al., 2023). Differences between items per individual in *Laternula elliptica* and the bivalves studied here may be related to individual's size and soft tissue weight, 66.8 ± 35.51 g versus

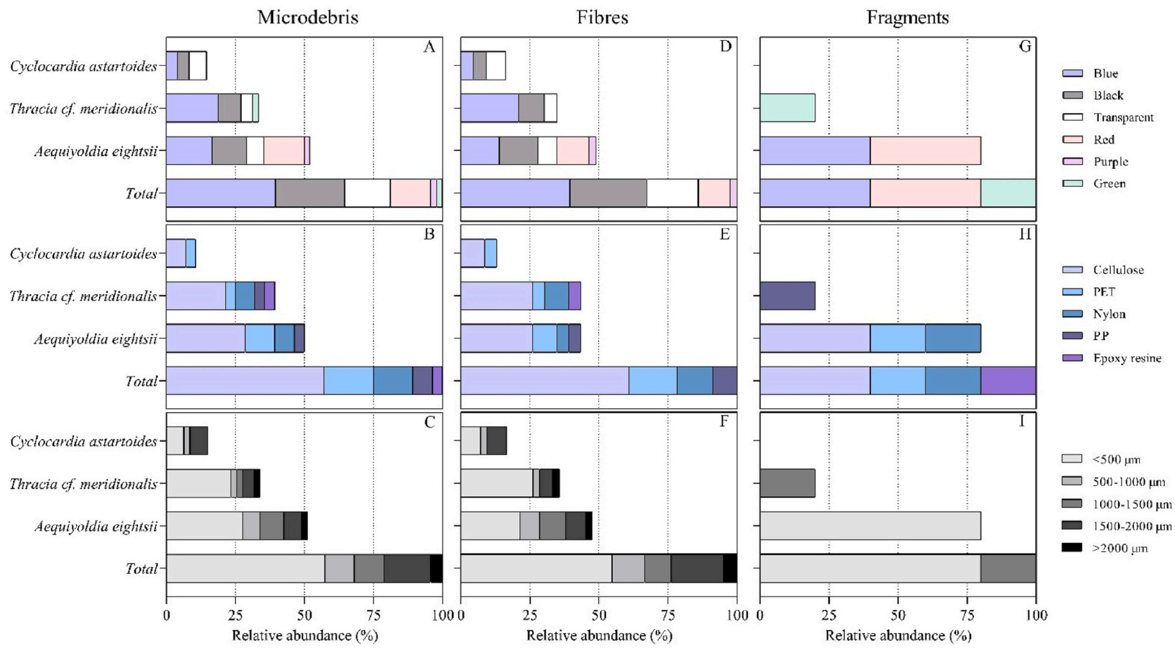

Fig. 3. Relative abundance (%) of the colour (A,D,G), polymer (B,E,H), and size (µm) (C,F,I) of the items (Total-Microdebris, Fibres and Fragments) found in the different species of the study (*Cyclocardia astartoides, Thracia* cf. *meridionalis and Aequiyoldia eigthsii*), and all the bivalves analysed. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Abundances of microdebris in bivalves and other benthic organisms around the world.

Taxons	Species	Location	Abundance (items/ individual)	Reference
Bivalva	*Laternula elliptica*	King George Island, Antarctica	42.86 ± 25.36	González-Aravena et al. (2024)
Bivalva	All Bivalva *Aequiyoldia eightsii*	Ross Sea, Antarctica	1.9 ~2.2	Sfriso et al. (2020)
Gastropoda	*Neobuccinum eatoni*	Ross Sea, Antarctica	0.3 ± 0.53	Bergami et al. (2023)
Several benthic organisms	Several	Arctic and sub-arctic regions	0.17-0.87	Fang et al. (2018)
Bivalva	Several (Digestive system)	Jiazhou Bay, China	0.5-3.3	Ding et al. (2021)
Bivalva	*Mytilus chilensis* Hupé, 1854	Ushuaia Bay, Argentina	8.6 ± 3.53	Pérez et al. (2020)
Fish	*Harpagifer antarcticus* Nybelin, 1947 (Gastrointestinal tract)	King George Island, Antarctica	3.80 ± 1.95	Ergas et al. (2023)
Bivalva	*Aequiyoldia eightsii* *Thracia* cf. *meridionalis* *Cyclocardia astartoides* All Bivalva	Johnsons' Bay, Livingston Island	0.66 ± 0.87 0.94 ± 1.08 0.54 ± 0.66 0.71 ± 0.89	This study

0.54 ± 0.33 g, respectively (González-Aravena et al., 2024). It is important to note as well that in King George Is. bivalves were collected next to the wastewater treatment facility discharge (González-Aravena et al., 2024).

Furthermore, a study in Arctic benthic organisms showed a similar abundance of debris compared to our organisms (0.17–0.87 it/ind) (Fang et al., 2018). Broadly, our results suggest that Johnsons' Bay bivalves present MD, in lower abundances than other Antarctic marine organisms. Also, this work shows that the bivalves here studied are less polluted than those in more populated areas (Pérez et al., 2020; Ding et al., 2021). Contrastingly, a recent study in King George Is., showed equivalent or higher MD abundances, as mentioned above (Table 2) (González-Aravena et al., 2024).

4.2. MD characterization

From the items found here, the main particle size fraction was up to 500 µm, in line with studied bivalves from polar and non-polar regions (Bom and Sá, 2021; González-Aravena et al., 2024). The most common MD found were fibres, in agreement with what is commonly found in the oceans and in marine organisms (Fang et al., 2018; Sfriso et al., 2020; Bom and Sá, 2021; Zhang, S. et al., 2022c). Blue and black fibres were predominant, like also seen in a study on whelks and a bivalve in King George, and within Antarctic and sub-Antarctic surface water (Jones-Williams et al., 2020; Bergami et al., 2023; González-Aravena et al., 2024). Regarding polymers, our items were mainly cellulose and polyethylene terephthalate (PET), representing the 75 % of the fibres. These results are also in agreement with studies in different matrices of the Southern Ocean. In Antarctic biota, the same polymers ratio was found in the gastropod *Neobuccinum eatoni,* the fish *Harpagifer antarcticus,* and the bivalve *Laternula elliptica* (Bergami et al., 2023; Ergas et al., 2023; González-Aravena et al., 2024). In the ocean, cellulosic fibres predominate and are also found at all depths in the Southern Ocean (Suaria et al., 2020; Rowlands et al., 2023). Furthermore, Antarctic surface waters also report PET and cellulose fibres (Jones-Williams et al., 2020; Suaria et al., 2020; Antacli et al., 2024).

In Livingston Island, a mean concentration of 0.26 ± 0.18 items/m^3 in surface waters was previously reported (Monràs-Riera et al., 2023), exhibiting similar characteristics (colour and polymer) to those observed in our bivalves. Black and blue, followed by transparent and red were the predominant colours in line with our data. Nevertheless, the polymeric MD composition, was mostly polyester (62 %), nylon (30 %) and cellulose (27 %) (Monràs-Riera et al., 2023). This different polymer proportions might be due to the material density, as cellulose (1.54–1.63 g/cm^3) is denser than polyester (1.37–1.46 g/cm^3) and nylon (1.14–1.18 g/cm^3) (Suaria et al., 2020). Fragments consisted mostly in polyethylene, nylon, and polypropylene in the surface waters (Monràs-Riera et al., 2023), where in our organisms they were cellulosic, PET, nylon and epoxy resin.

Cellulosic MD may have a natural or a semi-synthetic origin that could not be distinguished in this study due to the inconsistency in the polymer spectra produced by fibre degradation as previously reported (Cai et al., 2019). These polymers can be degraded faster than synthetic fibres, but attention must be given to them since they constitute most of the MD composition in the marine environment and therefore, they are more available for the organisms (Zambrano et al., 2020). Although cellulosic MD may have different origins or sources, these fibres must be considered as MD since they may also have a harmful effect for biota and the environment (Remy et al., 2015).

4.3. Feeding strategies and MD intake

Compared to other previously analysed Antarctic marine biota, MD amounts found here differ. This may be because the species analysed here had not been studied yet in other areas of Antarctica, except for *Aequiyoldia eightsii*. It is also important to note that understanding MD abundance in benthic organisms requires full investigation of their biology and feeding type (Setälä et al., 2016). When comparing different feeding types in the Arctic, concentrations of MD were higher in a starfish (*A. rubens)*, which is a predator, than in bivalves, which are filter-feeders, whereas in Antarctica filter-feeders (bivalves) and grazers (gastropod) had higher abundances of MD than omnivores and predators (Fang et al., 2018; Sfriso et al., 2020). The bivalves in Johnson's Bay have the same feeding type, being filter-feeders, although *Aequiyoldia eigthsii* is both a suspension and a deposit feeder (Davenport, 1988). Nevertheless, the MD results are similar in the three species studied here. Few studies have analysed how feeding type affects MD intake, and almost no information is available for the Southern Ocean (Setälä et al., 2016; D'Avignon et al., 2023).

MD abundance could also be positively correlated with the weight of the organism. However, both in our study and in the King George bivalve, no correlation between shell length or soft tissue weight and items quantity was found (González-Aravena et al., 2024). Contrastingly, previous studies on different bivalves in China have reported a positive correlation between shell length and items per individual, as well as a negative correlation between length and MD per gram (Wu et al., 2022). These discrepancies may be related to the much higher values of MD pollution found in the Chinese study. Our results found a weak positive correlation in organisms' weight and items size. Greater weight is related to a larger filtering surface and size, which may be directly linked to the items' length and the amount of MD that can be captured (Dowarah et al., 2020). Further research should focus on this topic to better understand these relationships in Antarctic organisms.

4.4. Possible MD sources

MD in the Southern Ocean may have local or global origin. It was thought that the Antarctic Circumpolar Current (ACC) was a pollution barrier from the rest of the globe, but recent studies have proved that MD may also have a global connectivity (Cunningham et al., 2022; Lozoya et al., 2022). In our study most items were cellulosic, PET and nylon fibres, which have their origin in the textile industry (Liu et al., 2023). Therefore, in our case, wastewater discharge could be the main local source of MD, as clearly found in the bivalves in King George Island (González-Aravena et al., 2024), and considering that our study area is located between two Antarctic research stations. In this sense, both tourist vessels and research stations may play a crucial role to mitigate MD pollution in the Antarctic waters by improving management of wastewaters (Suaria et al., 2020; Kalnina et al., 2022; Gurumoorthi and Luis, 2023). Fishing activities may also be a relevant local source of PET and nylon fibres (Cunningham et al., 2020; Monràs-Riera et al., 2023). However, a global origin through air transportation and currents cannot be fully discarded as a complementary source of MD in the Southern Ocean (Cunningham et al., 2020; 2022; Aves et al., 2022; Caruso et al., 2022).

4.5. Protocol procedural

Most of the studies carried out so far used KOH digestion treatment for bivalves in MD analysis, as it has been seen as the technique that best preserves polymers quality (Bom and Sá, 2021; Ding et al., 2021; Zhang, T. et al., 2022b; Ribeiro et al., 2023). This methodology worked as well in our study with a mean digestion efficiency value of 92.4 ± 5.3 %. We further recommend, the use of KOH combined with citric acid, as it decreased the filtration time and the filter pore size up to 1.2 μm (Thiele et al., 2019). Although KOH has a bleaching effect in cellulose items (Dawson et al., 2023), no full effect was observed in our fibres. Instead, only some of the fibres bleached, while most items (11 out of 16) maintained their original colour. The use of a stereomicroscope and visual identification for MD detection has some size limitations. In this work, the smallest item found using this technique was 100 μm in length.

The blank correction technique may lead to items underestimation or overestimation and no uniform protocol has been defined yet. In this study, the technique used to discard items was the coincidence on the visual similarity or characteristics for the blank and for the wild samples (Supplementary material, T.S.2). This method removed approximately the 30 % (n = 17) of the original MD quantity found (Brander et al., 2020; Crutchett et al., 2020; Bergami et al., 2023). As we analysed polar specimens, we followed a cautious blank correction to avoid items overestimations following Bergami et al. (2023). Blanks used in all the procedural steps lead to a LOD and LOQ value of 5.2 and 12.5, respectively. These values are higher than the number of MD obtained in the blanks, so the sample procedural methodology is considered well developed (Shruti and Kutralam-Muniasamy, 2023). In addition, LOD and LOQ were similar to other microplastic studies conducted in Antarctica (Bergami et al., 2023).

The use of a laminar flow cabinet and cleaned space plus blank controls has been shown in the literature to be the best option for reducing air bone contamination in environmental samples, and therefore this is the procedure we used. Also, filtering reagents before use, and specifical clothing plus sample cover, are good methods to prevent contamination. Blank correction type and LOD/LOQ values are specifications that all studies should include to evaluate in a more appropriate manner the MD obtained and to standardize QA/QC protocols (Dawson et al., 2023; Munno et al., 2023; Noonan et al., 2023). A potential limitation of the study was the recovery rate, that should also be included in QA/QC protocols to assess the efficiency of MD extraction from the samples. The collection of the organisms analysed here took place in a single sampling day and therefore offers data collected at the same time in the same place for the three species analysed. Therefore, we did not collect samples over time. Overall, this work presents a snapshot of MD characteristics and abundances in the three bivalve species in Johnsons' Bay. The results provide crucial information for the study of MD in Antarctic marine life.

4.6. Future prospects

Around the globe, bivalves have been used as bioindicators of MD pollution due to different reasons, such as their abundances in the natural environment or their easy collection in fisheries. Together with the recent study by González-Aravena et al. (2024), our study sets a baseline for MD study in bivalves in the Antarctic Peninsula, as well as an evaluation of the use of bivalves as bioindicators for MD pollution.

In this study, the bivalves analysed were collected in just one location, Johnsons' Bay in Livingston Island. More sites and more organisms need to be studied to improve MD characterization on benthic organisms and provide a better understanding of the marine system and MD transference. In addition, more information about cellulose polymers should be obtained to assess their possible sources, according to its natural or semi-synthetic origin. Furthermore, the development of techniques to detect particles smaller than those find through visual identification is fundamental to understand MD ingestion by benthic organisms and their possible health risks.

MD studies in the Antarctic benthos are expected to increase in future years. Further studies should focus on the characterization of the MD in different species with diverse feeding types, along with various locations in the Antarctic continent and the peninsula. More information is needed to ascertain all the potential impacts of MD (synthetic, semi-synthetic and natural) in the organism's health and the environment. Standardized protocols and data should be used to allow data comparison and to incorporate data into marine food webs. This would support the implementation of management policies to preserve and mitigate the Antarctic marine ecosystem.

5. Conclusions

This research found that three bivalve species in Johnsons' Bay (Livingston Island, Antarctic Peninsula) contained MD, proving that marine benthic biota are affected by marine MD pollution in the Southern Ocean. No significant MD abundance differences were found between species, being equally polluted. The number of items per individual was lower than in other Antarctic bivalves, probably due to different environmental characteristics, the biology of the studied species, and the sampling area. Local activities may be the main pollution source on the Antarctic marine benthos, but global pollution may also play a minor role. Here we set a baseline for MD studies in bivalves in Antarctica, as well as to evaluate the use of bivalves as bioindicators for MD pollution. We further encourage more studies to have a better picture of MD pollution in the Southern Ocean and its consequences to the marine biota and environment. Finally, standardized protocols and data are key to develop proper management policies for the protection of the Antarctic marine environment.

CRediT authorship contribution statement

Mariona Gonzalez-Pineda: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Humbert Salvadó:** Writing – review & editing, Supervision, Resources, Methodology. **Conxita Avila:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2024.124086.

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