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# Experimental ingestion of microplastics in three common Antarctic benthic species

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#### ABSTRACT

Microplastics (MP) have spread to every corner of the globe, reaching remote areas like Antarctica. Recent studies detected MP in marine environments, including biota. Benthic organisms suffer negative effects upon MP ingestion, leading to impacts on their populations. To address the current knowledge gap on how Antarctic benthic invertebrates interact with MP, we conducted an experiment exposing a bivalve (Aequiyoldia eightsii) and two ascidians (Cnemidocarpa verrucosa and Molgula pedunculata) to polyethylene microbeads (mb). Specimens of each species were exposed for 48 h to two different concentrations of microbeads, a low dose (100 mb/l) and a high dose (1000 mb/l), with the same proportion of four different microbead size fractions (Fine (10-20 μm), Small (45-53 μm), Medium (106-125 μm), and Large (850-1000 μm)). After exposure, all three species had ingested microbeads. Significant differences between doses were observed in A. eightsii and C. verrucosa but not in M. pedunculata. Both ascidians ingested microbeads of all size fractions, whereas the bivalve did not ingest the largest microbeads. No significant differences were found between species in the number nor sizes of microbeads ingested. Minor variations between taxa may be attributed to the specific biology and anatomy of each species. Our study highlights the need for a deeper understanding of Antarctic benthic ecosystems, suggesting that the interaction with MP is species-specific. We believe that this study provides a baseline for assessing MP pollution in Antarctic benthic invertebrates and will help to inform policy-makers in protecting and preserving Antarctic marine ecosystems from MP pollution.

## 1. Introduction

In the context of global change, marine ecosystems face many significant threats, including global warming, acidification, invasive species, and pollutants, among other factors (Gissi et al., 2021; Gutt et al., 2021). The Southern Ocean is particularly sensitive to all these anthropogenic pressures despite Antarctica being considered a continent with minimal human impact (Rogers et al., 2020; Gutt et al., 2021). Both global and local (scientific, military, tourism and fisheries) activities

affect Antarctic marine ecosystems by releasing heavy metals, oils, persistent organic pollutants, and plastics into the environment (Bhardwaj et al., 2018; Bhardwaj and Jindal, 2020; Da Silva et al., 2023). In recent years, microplastics (MP) (<5 mm) have been detected in the Antarctic marine ecosystem, with reports of their presence in water, sediments, and biota (Frias and Nash, 2019; Cunningham et al., 2020; Suaria et al., 2020; Bargagli and Rota, 2022; Perfetti-Bolaño et al., 2022; Bhardwaj, 2023; Monràs-Riera et al., 2023). Recent studies analysed MP content in different benthic Antarctic invertebrates. In

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particular, in the South Shetland Islands, four bivalve species were studied, while in the Ross Sea, a gastropod and some other benthic taxa (five Mollusca, four Annelida, two Arthropoda and, one Cnidaria species) were analysed, with reported abundances ranging from  $0.3 \pm 0.53$  to  $42.86 \pm 25.36$  items per specimen (Sfriso et al., 2020; Bergami et al., 2023; González-Aravena et al., 2024; Gonzalez-Pineda et al., 2024).

Benthic organisms play an important role in provisioning, supporting, and regulating marine ecosystems, and particularly filter-feeders are known to contribute to nutrient cycling and carbon sequestration, productivity, and species diversity in the oceans (Bremner, 2005; Lam-Gordillo et al., 2020). MP, which are widespread in marine environments, reach the sea floor by sinking through the water column or through the faecal pellets of pelagic organisms, where they are ingested by filter-feeding benthic organisms (Bergami et al., 2020; Berlino et al., 2021). MP have detrimental effects on individual organisms, causing alterations in growth and reproduction, that can directly affect population dynamics and, consequently, disrupt the ecological functions and services that these animals provide to marine ecosystems (Berlino et al., 2021; Bargagli and Rota, 2022). In Antarctica, filter-feeders bivalves and ascidians are essential for maintaining ecosystem processes, underscoring the need to assess the impacts of MP on these species, an aspect that has been previously suggested (Rimondino et al., 2015; Servetto

Bivalves (Mollusca) have been widely used to monitor and quantify environmental MP from low latitude areas to the poles (Ding et al., 2021; Teichert et al., 2021; Sun et al., 2023; González-Aravena et al., 2024; Gonzalez-Pineda et al., 2024). It has been documented that MP intake by bivalves leads to alterations in feeding rates, growth, reproduction, and overall physiology of animals (Sussarellu et al., 2016; Zhang et al., 2020; Li et al., 2022). For instance, in the Southern Ocean, a recent study found that exposure to nanoplastics negatively affected the gills microbiome of the bivalve *Laternula elliptica* (King, 1832; Rondon et al., 2024).

Ascidians (Chordata: Tunicata) also play a crucial role in the benthopelagic coupling and in nutrient recycling in the ocean. Their tunic provides a substrate for epibionts, and their branchial structure can serve as a refuge for some amphipods (Lambert, 2005; Dewar-Fowler, 2017). Due to their potent filtering capability, widespread distribution and ecological relevance, some ascidians have been proposed as MP sentinels for environmental management and conservation in the Mediterranean and Brazilian coasts (Vered et al., 2019; Da Silva et al., 2021). Furthermore, in the Mediterranean, several ascidians were found to be polluted with MP, and exposure to these particles has been shown to alter and delay their development (Messinetti et al., 2018; Vered et al., 2019). Additionally, MP have been observed accumulating into the gut cavity and translocating into the hemocoelic cavity, potentially inducing toxicological effects in the organisms (Messinetti et al., 2018, 2019; Vered et al., 2019).

In Antarctica, ecotoxicological research on MP has predominantly focused on nanoplastics and pelagic species with just two studies using benthic invertebrates (Dawson et al., 2018; Bergami et al., 2019, 2020; Da Silva et al., 2023; Rondon et al., 2024). To our knowledge, there are no specifical studies investigating the effects of MP on Antarctic bivalves or ascidians. Moreover, little is known on how MP interact within the Antarctic trophic web with just one study comparing different benthic feeding-types and reporting no bioaccumulation along the food web (Sfriso et al., 2020).

Within this context, it is essential to understand how different benthic taxa and feeding types interact with MP. To address this relevant knowledge gap, we exposed three common Antarctic benthic species: one bivalve (Aequiyoldia eightsii (Jay, 1839)), and two ascidians (Cnemidocarpa verrucosa (Lesson, 1830) and, Molgula pedunculata (Herdman, 1881)) to varying concentrations of a mixture of polyethylene microbeads (mb) of different sizes. We hypothesize that there will be differences in both the abundance and size of microbeads ingested, particularly between the ascidians and the bivalve. Thus, here we aim to:

a) Characterize and quantify the microbeads ingested by each species in the experiment, b) Compare the size and amount of microbeads ingested at different concentrations for each species and, c) Assess whether there are differences between species regarding microbeads ingestion abundance and size.

#### 2. Material and methods

#### 2.1. Experimental design

The experiment was conducted in January 2022 at the Antarctic Spanish Base on Livingston Island. The experimental setup included three tanks, each containing 15 independent glass jars filled with 1.5 l of filtered seawater (Durapore® 0.22  $\mu m$  Capsule Filters). The water in the tank was kept at 1 °C by an external flow-cooling system. Each jar was equipped with its own oxygen and aeration system. Seawater environmental characteristics were checked before introducing the animals, and as pH, oxygen and temperature conditions were similar between jars as well as to the environmental conditions, the experiment was carried out. In each tank, a different species were studied: Cnemidocarpa verrucosa (Ascidiacea, filter feeder), *Molgula pedunculata* (Ascidiacea, filter feeder) and Aequivoldia eightsii (Bivalva, filter and deposit feeder). For each species, five individuals were exposed to a 100 microbeads per liter (mb/l) dose (Low dose-LD), five to a 1000 mb/l dose (High dose-HD) and five were kept as controls (no microbeads added) (Fig. 1). Both doses contained the same proportion (25 %) of polyethylene microbeads (Blue Polyethylene Microspheres 1.00 g/cc, Cospheric ®) from four size fractions: 10–20 μm (Fine), 45–53 μm (Small), 106–125 μm (Medium), and 850-1000  $\mu m$  (Large). Organisms (n = 45) were collected simultaneously at Johnsons' Bay using a Van Veen grab and placed into the jars for acclimation during 72 h. Once the organisms were acclimated to the new environment the microbeads were introduced into the jars for 48 h using a small amount of filtered seawater. After exposure, specimens were wrapped individually in aluminium foil and frozen at  $-20\,^{\circ}$ C.

## 2.2. Digestion procedure and microplastic extraction

Once at the laboratory, the samples were stored in the fridge for 24 h or until defrosted. For Molgula pedunculata and Aequiyoldia eightsii, the total wet soft body tissue was weighed, while for Cnemidocarpa verrucosa, only the digestive tract was weighed due to technical limitations. Afterwards, the organic matter was digested using 3:1 (v/v) solution of 10% KOH at 40 °C for 48 h (Dehaut et al., 2016; Thiele et al., 2019; Vered et al., 2019; Bom and Sá, 2021). The digested solution was transferred into a separatory funnel, 100 ml of a saturated saline solution (NaCl, 1.2 g/cm<sup>3</sup>) was added, and the solution was left overnight (Cutroneo et al., 2021; F.M. Santana et al., 2022; Monteiro & Pinto Da Costa, 2022). Then, 10 ml of citric acid 1M was added to the supernatant and filtered through a 0.8 µm gold-coated polycarbonate filter (i3 Membrane GmbH, Germany) using a glass vacuum filtering system (Thiele et al., 2019). The filters were placed in glass Petri dishes and allowed to dry at room temperature until analysis. All procedures were carried out under a laminar flow hood, glassware and stainless steel material were used, and the researcher used a cotton lab coat and nitrile gloves during the sample manipulation.

## 2.3. Microplastic quantification

The filters were visually examined using a stereomicroscope (Nikon SMZ-745) and a microscope (Nikon Eclipse 50i) to detect most of the microbeads size fractions (Small, Medium, and Large). For quantifying the fine size fraction (10–20  $\mu m$ ), Raman spectroscopy was used. Confocal Raman microscopy measurements were performed on a WITec Alpha 300-RA Raman confocal imaging system. Raman spectra were collected using an average of 100 measurements with an integration time of 0.5 s for each spectrum. The excitation laser wavelength was

Fig. 1. A) Experimental set-up with the different treatments: 1) Control 2) Low dose treatment (100 mb/l) 3) High dose treatment (1000 mb/l) 4) Air pump and cooling system. B) Aequiyoldia eightsii (Jay, 1839), C) Cnemidocarpa verrucosa (Lesson, 1830) and D) Molgula pedunculata (Herdman, 1881) in the glassware treatment jars.

532 nm, and the laser power was set to 45 mW.

After examining the filters, the amount of particles in each specimen was represented in items per wet gram of the analysed tissue, representing the microbead load in the organism/tissue after 48 h of exposure.

#### 2.4. Statistical analysis

Data were analysed using the statistical software R Studio and graphics were created with GraphPad version 8.0.2 (GraphPad Software, Boston, Massachusetts, USA). Normality and homogeneity of variances were assessed using Shapiro-Wilk and Levene's test, respectively. Comparisons between treatments (factor), low dose and high dose, and between the quantity or sizes of microbeads (dependent variables) were conducted using either a t-student (t) or a Wilcoxon test (W), depending on whether normality assumptions were met. In the case of non-normality, a Kruskal-Wallis (H) test was used to compare the microbeads load and size between species. Also, a Spearman correlation test was applied to evaluate the relationship between the number of microbeads and the organism's/tissue wet weight.

## 3. Results

For each species, the wet weights of the specimens were similar across treatments, with no significant differences found (t-student or Wilcoxon test, p > 0.05). The bivalve had an average soft body wet weight of 1.81  $\pm$  0.60 g, the ascidians without the tunic of 15.21  $\pm$  13.11 g for M. pedunculata and 29.81  $\pm$  23.52 g for C. verrucosa. For the latter species, the digestive tract weighted on average 5.83  $\pm$  3.20 g.

After the experiment, specimens from all three species had ingested microbeads. On average, A. eightsii had 5.6  $\pm$  4.65 microbeads per individual (mb/ind) and 3.91  $\pm$  3.90 microbeads per gram of wet weight (mb/g), C. verrucosa had 24.30  $\pm$  53.55 mb/ind and 6.36  $\pm$  17.21 mb/g and, M. pedunculata 49.20  $\pm$  128.33 mb/ind and 1.81  $\pm$  3.31 mb/g. From the total microbeads found, A. eightsii primarily accumulated the small size fraction (45–53  $\mu m$ , 75%) with no large items observed (Table 1). In the bivalve, a positive correlation was observed between

Table 1 Total microbeads load (items n) and total microbeads per size fraction (% items and items n) found in each species for both doses together.

Species	Total load (items)	Fine 10–20 μm	Small 45–53 μm	Medium 106–125 μm	Large 850–1000 μm
Aequiyoldia eightsii (Jay, 1839)	n = 56	7.14 %, (n = 4)	75 %, (n = 42)	17.86 %, (n = 10)	0 %, (n = 0)
Cnemidocarpa verrucosa (Lesson, 1830)	n = 243	1.65 %, (n = 4)	8.64 %, (n = 21)	31.28 %, (n = 76)	58.43 %, (n = 142)
Molgula pedunculata (Herdman, 1881)	n = 492	0.81%, (n = 4)	16.06 %, (n = 79)	9.96 %, (n = 49)	73.17 %, (n = 360)

the amount of microbeads and weight ((r = 0.447, p < 0.05), which was confirmed for both the small (r = 0.54, p < 0.05) and fine (r = 0.51, p < 0.05) size categories. For the ascidians, one individual of each species ingested large quantities of the large size fraction microbeads, increasing the proportion of large particles found (Table 1). In both ascidians, no significant correlation was found between microbeads quantity and weight once these extreme values were removed. In the control treatment (0 mb/l), no microbeads were observed in any of the organisms used in the experiment.

Regarding treatments, higher doses were associated with higher MP load in the organisms in all three species (Fig. 2). Significant differences were found in microbeads amounts between the high and low doses in both A. eightsii (t (8) = 4, p = 0.0039) and C. verrucosa (W = 22.5, p = 0.025) but not in M. pedunculata (W = 15.5, p = 0.75). When analysing the microbeads amounts of the different size fractions within the two treatments, it was noted that in A. eightsii the finest size fraction was ingested only in the highest dose (HD). In fact, in the bivalve, no large particles were ingested in any of the treatments, and for the small and medium particles, a higher dose meant a larger load of microbeads. In the case of C. verrucosa, no microbeads of any size fraction was observed to accumulate in the low dose (LD) treatment, while in the high dose, all sizes were ingested. In the other ascidan, M. pedunculata, the fine, the small and the medium size fractions were found in higher quantities in the low dose treatment. For the largest particles, the highest doses were equivalent to more microbeads ingested (Fig. 3). There were no significant differences between size fractions and the applied doses except for the small fraction in A. eightsii (H (2) = 1.90, p = 0.0058) (Fig. 3). Between species, no significant differences were found regarding the microbeads quantity found (p = 0. 38), nor the size fractions (Fine, (H (2) = 0, p = 1); Small, (H(2) = 3.83, p = 0.16); Medium, (H(2) = 0.56, p = 0.16);

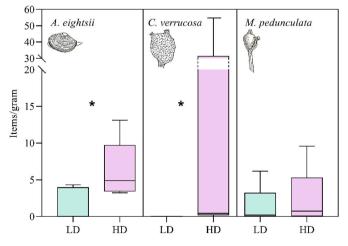


Fig. 2. Boxplot of the microbeads load per wet weight found in the low dose (LD) and the high dose (HD) for all the size fracions together and for each species: Aequiyoldia eightsii, Cnemidocarpa verrucosa and, Molgula pedunculata (Line represents the median, whiskers represent minimum and maximum values, and the asterisk represents statistical differences between treatments).

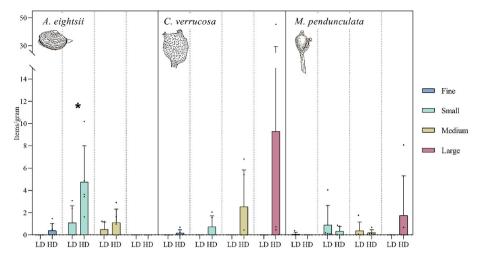


Fig. 3. Barplot indicating the items per gram for the different particle size fractions (fine: 10–20 μm, small: 45–53 μm, medium: 106–125 μm, and large: 850–1000 μm) in the two treatments (LD: low dose, HD: high dose) and the three species: *Aequiyoldia eightsii, Cnemidocarpa verrucosa*, and *Molgula pedunculata*. (Bars represent the mean, whiskers the standard deviation, dots represent the value for each replicate, and the asterisk represents statistical differences between treatments in a size fraction).

p=0.36); Large, (H (2)  $=3.56,\ p=0.55$ )) encountered using the Kruskal-Wallis test.

## 4. Discussion

After exposing specimens of three benthic species to microbeads in our experiment, all three species had incorporated microbeads, with some quantitative and size differences among them. As far as we know, our study is the first to expose Antarctic benthic invertebrates, specifically a bivalve and two ascidians, to MP. In the current global change context, our results contribute to indicate that plastic pollution poses a significant threat to Antarctic benthic ecosystems in agreement with recent studies conducted in the Southern Ocean (Sfriso et al., 2020; Bergami et al., 2023; González-Aravena et al., 2024; Gonzalez-Pineda et al., 2024). Therefore, there is a pressing need to better understand how benthic organisms interact with MP in these fragile ecosystems.

The bivalve, A. eightsii, ingested microbeads of different size categories (Fine, Small, and Medium particles (10–125 µm)) except the large one (>850  $\mu$ m), with the small size (45–53  $\mu$ m) being the most abundant. As protobranchs, these organisms can select the grain size they process, which may explain the diverse size fractions encountered (Zardus, 2002). This bivalve is both a deposit and a suspension feeder, ingesting sediment particles and sorting out the inorganic fraction, which is expelled through the inhalant siphon every 6-35 min (Davenport and Fogg, 1997). Smaller and lighter particles are expelled through the exhalant siphon every 12–15 s (Davenport and Fogg, 1997). Additionally, the anatomy of the siphons plays an important role, particularly for the largest particle size ingested, as the inhalant siphon has a maximum diameter of 1 mm, while the exhalant siphon is approximately four times smaller (Davenport and Fogg, 1997; Batistão et al., 2023). Our results also agree with a previous study of our group on A. eightsii, where just fragments smaller than 500 µm were found in specimens collected from the sea bottom (Gonzalez-Pineda et al., 2024). Hence, a combination of grain size selection and the subsequent expulsion of most of the inorganic matter could explain the abundances and sizes of microbeads found in A. eightsii in our experiment.

Regarding the ascidians and differing from the bivalve, all the size fractions of microbeads were ingested during the experiment. Previous studies observed that *C. verrucosa* may ingest food particles ranging from 1.3 µm to several millimetres in size, while *M. pedunculata* has been reported to ingest particles larger than 1.2 µm (Kowalke, 1999; Tatián et al., 2002, 2004, 2008). In these ascidians, the inhalant siphon is several millimetres wide, allowing even the largest microbeads to enter

the organisms without any anatomical size limitation (Tatian et al., 1998). In our experiment, in both species, only one individual among the five replicates ingested hundreds of 1 mm microbeads (n = 133 for C. verrucosa, and n = 348 for M. pedunculata), increasing the average load of MP observed. In comparison, more microbeads were ingested by specimens of M. pedunculata than for C. verrucosa, although the mean density per organism was higher in the latter. When exposed to sediments, C. verrucosa squirts, ejecting the inorganic material whereas, M. pedunculata is unable to squirt and cannot distinguish between organic and inorganic material (Torre et al., 2014). Furthermore, when the concentration of suspended inorganic matter in the environment increases, C. verrucosa does not increase ingestion rates whereas M. pedunculata due to its inability to squirt, ingests more inorganic matter, and increases its filtration and respiration rates (Tatián et al., 2002; Torre et al., 2014). Moreover, it has been reported that Antarctic ascidians produce more mucus in response to an increase in sediment particulate matter (Kowalke, 1999; Torre et al., 2012, 2014). Usually, mucus helps the organisms retain the food particles and process them, before being expelled through the atrial siphon (Da Silva et al., 2021). Since M. pedunculata cannot squirt, it could be possible that more organisms of this species ingested MP through mucus and filtering activity compared to *C. verrucosa*, which likely increased its squirting frequency. Therefore, the number of individuals with a high microbeads load was higher in M. pedunculata than in C. verrucosa in our experiment. Also, the squirting ability of C. verrucosa may explain why no microbeads were found in the low dose treatment. In particular, the increase in ingestion and filtration rates when exposed to inorganic particles may account for the lack of significant differences between doses in M. pedunculata (Torre et al., 2014). Another relevant aspect to consider is that the analysed tissues differed between species, since in C. verrucosa only the digestive system was dissected, while in M. pedunculata the entire animal was used due to the difficulty of separating each organ.

Our results demonstrate that these three common Antarctic benthic species ingested microbeads after a two-day exposure. In the South Shetland Islands, specifically in our study area on Livingston Island, superficial waters contain MP in an average of  $0.264 \pm 0.185$  items/m³ (Monràs-Riera et al., 2023). As mentioned above, Antarctic wild benthic invertebrates have already shown evidence of MP pollution. The bivalve used in our study was previously studied in the Ross Sea and on Livingston Island (South Shetland Islands) where the environmental mean density of MP was ~2.2 items/ind and 0.66 items/ind, respectively (Sfriso et al., 2020; Gonzalez-Pineda et al., 2024). In the Ross Sea, Sfriso et al. (2020) analysed several different benthic species and found a mean

MP content of 0.7 items/mg (Sfriso et al., 2020). Microplastic pollution in ascidians has been poorly studied globally, with no previous studies regarding Antarctic species (Dewar-Fowler, 2017; Messinetti et al., 2019; Vered et al., 2019). MP ingestion has been shown to negatively impact marine benthic organisms in many biological processes. For instance, a false sense of feeding satisfaction can reduce nutritional intake causing problems in growth and development, reproduction, and survival rates in some benthic organisms (Messinetti et al., 2018; Mason et al., 2022; Harmon et al., 2024). In addition to physical effects, plastic additives, along with the release of toxic monomers and the accumulation of persistent organic pollutants, may disrupt the endocrine system, cause reproductive and development abnormalities, and may be carcinogenic in ascidians and other marine biota (Dewar-Fowler, 2017; Messinetti et al., 2018; Vered et al., 2019; Bhardwaj, 2023). Furthermore, the surface of MP serves as a niche for microorganisms (the "plastisphere") that can alter the microbiome of the benthic organisms and cause new diseases (Bargagli and Rota, 2022; Ballesté et al., 2024; Harmon et al., 2024). Thus, MP pollution induces harmful effects on organisms and their responses appear to be species-specific, making it fundamental to know how a species interacts with MP before assuming it to be a potential bioindicator (Berlino et al., 2021). In this study, our results agree with the species specificity of the microbeads load and

Microplastic accumulation in benthic organisms is influenced by many factors beyond just the amount of MP in the environment. Factors such as the organisms' feeding type, food availability, particle and size selection, anatomy of the filtering systems, and the intrinsic MP characteristics, among others, play a crucial role in determining the MP organisms load (Bour et al., 2018; Porter et al., 2023; Sfriso et al., 2024). Contrary to what could be expected, bioaccumulation of MP does not seem to occur along the benthic food web, although more studies across diverse ecosystems are still needed to confirm that (Setälä et al., 2016; Bour et al., 2018; Sfriso et al., 2020, 2024). In Antarctic benthic organisms, no bioaccumulation of MP was observed in the trophic chain either, as filter-feeders had higher abundances of MP than predators (Sfriso et al., 2020; Bargagli and Rota, 2022). In Antarctica, however, the ecological impact of MP is still not well understood. One study found that sea ice acts as a reservoir for MP, which could be an important source of exposure for marine species, especially krill (Kelly et al., 2020). Krill may break MP into nanoplastics and their egestion has been linked to a decrease in the sinking rate of faecal pellets (Dawson et al., 2018; Bergami et al., 2020). This alteration could potentially modify the biogeochemical cycles of the Southern Ocean, affecting the carbon input and sequestration in the deep-sea sediments (Bergami et al., 2020). Both ascidians and bivalves contribute to this bentho-pelagic coupling and could play an important role in the transfer of these MP into the sea floor (Dewar-Fowler, 2017; Vaughn and Hoellein, 2018; Filipa Mesquita et al., 2024).

In our study, three common Antarctic benthic species had different MP burden after 48 h according to their biological traits. While this research sets a baseline for understanding Antarctic bivalves and ascidians interactions with MP, our experiment did not allow to determine whether the organisms can expel the ingested MP, offering instead a snapshot of the organisms' microbeads load after 48h. Moreover, although variations in the quantity and size fractions of microplastics were observed among species, no significant differences were detected, probably due to the variance among individuals within each species and the limited number of replicates. We specifically used polyethylene microbeads in this study, as it is one of the most common plastic polymers found in the studied area (Suaria et al., 2020; Ergas et al., 2023; Monràs-Riera et al., 2023; Gonzalez-Pineda et al., 2024). Future experiments should consider using other materials, such as cellulose fibres, to better understand their impact on marine biota, as they are prevalent globally, as well as in the Southern Ocean, and their effects remain still unclear (Suaria et al., 2020; Gonzalez-Pineda et al., 2024). Additionally, further studies are needed to assess the interactions, with MP across

different feeding types and trophic levels, particularly in Antarctic benthic marine ecosystems. This information is key for developing policies and management strategies to protect marine life from microplastic pollution.

#### 5. Conclusions

To contribute to the knowledge of microplastics in this remote Antarctic area and its interaction with benthic life, we conducted an experiment with marine invertebrates by using microbeads. This study shows that three common Antarctic benthic invertebrates ingested polyethylene microbeads after being exposed during 48 h. When increasing the concentration of MP in the water, more particles were found in the organisms although there were no significant differences between species and items quantity, nor between the size fractions ingested. Between taxa, there were differences in the size fractions ingested probably due to biological and anatomical reasons. Each species had different behaviour probably due to their different feeding-type, their specific biology, and their ability to process the inorganic material filtered. Thus, the studied organisms' interaction with MP seems to be species-specific, depending on the feeding-type, the trophic level, the MP characteristics, and the MP amount in the environment, among others. We believe that this study sets a baseline for MP experimental research in Antarctic benthic invertebrates. Further studies should analyse MP variation and effects according to the above-mentioned variables in Antarctic marine biota.

#### CRediT authorship contribution statement

Mariona Gonzalez-Pineda: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Conxita Avila: Writing - review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. Gissell Lacerot: Writing - review & editing, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation. Juan Pablo Lozoya: Writing - review & editing, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation. Franco Teixeira de Mello: Writing – review & editing, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation. Ricardo Faccio: Methodology, Investigation, Data curation. Fernando Pignanelli: Methodology, Investigation, Data curation. Humbert Salvadó: Writing - review & editing, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis, 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.

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## Data availability

Data will be made available on request.

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