



Textile microfibers in wild Antarctic whelk *Neobuccinum eatoni* (Smith, 1875) from Terra Nova Bay (Ross Sea, Antarctica)



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ABSTRACT

Antarctica has been affected directly and indirectly by human pressure for more than two centuries and recently plastic pollution has been recognized as a further potential threat for its unique biodiversity. Global long-range transport as well as local input from anthropogenic activities are potential sources of plastic pollution in both terrestrial and marine Antarctic territories. The present study evaluated the presence of microplastics in specimens of the Antarctic whelk *Neobuccinum eatoni*, a key species in benthic communities of the Ross Sea, one of the largest marine protected areas worldwide. To this aim, a thermo-oxidative extraction method was applied for microplastic isolation and quantification, and polymer identification was performed by manual μ -FTIR spectroscopy. Textile (semi-)synthetic or composite microfibers (length range: 0.8–5.7 mm) were found in 27.3% of whelk specimens, suggesting a low risk of bioaccumulation along Antarctic benthic food webs in the Ross Sea. Their polymer composition (of polyethylene terephthalate and cellulose-polyamide composites) matched those of outdoor technical clothing in use by the personnel of the Italian “Mario Zucchelli” station near Terra Nova Bay in the Ross Sea. Such findings indicate that sewage from base stations may act as potential local sources of textile microplastic fibers in this remote environment. More in-depth monitoring studies aiming at defining the extent of microplastic contamination related to such sources in Antarctica are encouraged.

1. Introduction

Although Antarctica is perceived as a symbol of the last great wilderness, anthropogenic pressure in the Southern Ocean began in the 1790s with fur seal hunting and whaling activities (CCAMLR, 2019). In the last 50 years, increasing tourism (up to 74,401 visitors in 2019–2020 season) (IAATO, 2019) as well as investments in logistics and scientific research facilities (76 research stations are currently operative below 60°S) have caused an increase in local inputs of chemicals (Bargagli, 2008; Wild et al., 2015) and wastes in Antarctica (Aronson et al., 2011), including the most recently documented plastic pollution (Waller et al., 2017; Corsi et al., 2021a). First records of plastic in Antarctica date back to the 1980s (reviewed in Ivar do Sul et al., 2011), with observations of plastic debris floating in surface waters or stranded along the shores of Sub-Antarctic and Antarctic Islands (Barnes et al., 2010; Convey et al., 2002). More recently, the occurrence of meso- (1–10 mm) and

microplastics (<1 mm, Hartmann et al., 2019) has further been reported in Antarctic environments (Corsi et al., 2021a; Caruso et al., 2022), including remote areas such as East Antarctica (Kelly et al., 2020) and the Ross Sea. Recent estimates report an average of 1794 plastic debris/km² in the surface waters of the Antarctic Peninsula (Lacerda et al., 2019), raising concerns on their potential impact on Southern Ocean biodiversity, already coping with other sources of anthropogenic disturbances such as climate change (Stark et al., 2019). Microplastics may have diversified ecological impacts in the polar regions compared to lower latitudes due to the peculiar climatic and bio-geographical characteristics of polar ecosystems, which can affect their fate and distribution giving rise to unknown impacts.

The unique harsh Antarctic climate (e.g., sea surface T varying approx. from -1.8 to +4 °C, UV radiation, ice formation) could hamper the fragmentation of plastic debris, thus exposing local communities to the smallest plastic fraction, the nanoplastics, known to exert a higher

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toxicity to biota (Dawson et al., 2018a; Waller et al., 2017; Bergami et al., 2020a; Corsi et al., 2021a,b). Microplastics have been found in Southern Ocean surface waters North and South of the Polar front both in the most anthropized and pristine territories (e.g., Antarctic Peninsula and Ross Sea, respectively); therefore their routes of entrance either through surface and sub-surface ocean currents or atmospheric inputs via wind transport by crossing the Antarctic Circumpolar Current are still uncertain (Cózar et al., 2017; Isobe et al., 2017; Waller et al., 2017; Fraser et al., 2018; Bergmann et al., 2019; Lacerda et al., 2019; Marsh and van Sebille, 2021). Point sources of plastic pollution have been recognized in terrestrial and marine environments adjacent to scientific base stations with the highest occurrence in the Western Peninsula but also in the Ross Sea (Waller et al., 2017; Reed et al., 2018; Bergami et al., 2020b).

Since 2017, the Ross Sea hosts one of the largest marine protected areas in the world (CCAMLR, 2016), being characterized by high biodiversity and ecological value but extremely vulnerable to climate change and other stressors, including increasing local anthropogenic impact (e.g., construction of new base stations and the new airfield at Boulder Clay (Final CEE, 2017)). Therefore, long-term protection actions and monitoring measures to conserve biodiversity and protect such habitats are indeed required to prevent any detrimental consequences on local marine living resources (Smith et al., 2007).

Microplastics in the Ross Sea have been documented in seawater (Cincinelli et al., 2017; Zhang et al., 2022), sediments (Munari et al., 2017) and biota (Sfriso et al., 2020) and very recently in snow (Aves et al., 2022) and in sea ice as nanoplastics <1 µm (Materić et al., 2022). Global long-range transport by air as well as local anthropogenic inputs, for instance the outfall of wastewater treatment plants (WWTPs) of scientific base stations into Terra Nova Bay have been recognized as potential sources of contamination in the region (Cincinelli et al., 2017; Munari et al., 2017; Sfriso et al., 2020; Zhang et al., 2022). The wide range of microplastic concentration (0.002–0.1 microfibers/m³), shape (fibers vs beads or films) and polymer composition (e.g., cellulosic such as cotton vs synthetic polymers) found in seawater and sediments in the proximity of scientific base stations across Antarctica clearly addressed a point source coming from logistics, field-based activities as well as treated or untreated sewage (Reed et al., 2018; Suaria et al., 2020; Waluda et al., 2020). Sfriso and co-authors (2020) first documented the presence of microplastics in Ross Sea macrobenthos representative of 12 different species (Crustacea, Cnidaria, Mollusca and Annelida) belonging to different trophic levels and feeding strategies. With an average microplastic content of 0.01–3.29 items/mg dry weight (DW) and approximately 1 item/individual, the highest amount of microplastics was found in specimens collected near the WWTP outfall of the Italian “Mario Zucchelli” station (Mzs) in Terra Nova Bay, followed by those from Camp Icarus and the lowest in Adelie Cove.

Benthic food webs in the Ross Sea are characterized by complex structures, depending on resources availability, local environmental conditions and disturbances, such as sea ice cover (Rossi et al., 2019). Such biodiverse benthic communities are often dominated by gastropod mollusks, as the Antarctic whelk *Neobuccinum eatoni* (E. A. Smith, 1875, belonging to the Order Neogastropoda, Family Buccinidae), found on both hard and soft substrates from 4 to 2350 m depth (Norkko et al., 2007). This species is endemic to the Southern Ocean and has a Circum-Antarctic distribution, characterizing both Antarctic benthic communities below 60° S, such as in the Ross Sea, and Sub-Antarctic ones, up to the South Sandwich Islands in the Scotia Sea, South Atlantic Ocean sector as well as Kerguelen and Heard Islands in the Indian Ocean sector (Schiaparelli et al., 2006; Aldea and Troncoso, 2008; Fraussen, 2020).

The Antarctic whelk acts both as a scavenger, feeding on carcasses on the seabed, and as an active predator, mainly preying on filter-feeding bivalves such as *Adamussium colbecki*, *Laternula elliptica* and *Limatula Hodgsoni* (Norkko et al., 2007). In turn, *N. eatoni* is predated by starfish, brittle stars, isopods and demersal fish (Marina et al., 2018). Given its

role in Antarctic benthic food webs, as recently addressed in Terra Nova Bay, Ross Sea, by Rossi et al. (2019), *N. eatoni* is a suitable candidate to monitor contaminants within Antarctic benthic communities, as done in the past for trace metals, such as mercury and cadmium (Bargagli et al., 1996, 1998).

Based on recent evidence of microplastic occurrence in marine waters, sediments and biota from the Ross Sea and the need to protect the biodiversity of the marine protected area from anthropogenic disturbances, the aim of this study was to analyze the microplastic content in specimens of the Antarctic whelk *N. eatoni* from Terra Nova Bay, giving its key ecological role in Antarctic benthic communities of the Ross Sea marine protected area. The types and polymeric composition of clothing and equipment in use at the nearby Italian Mzs was also analyzed to unravel potential local sources of microplastic released from wastewater outfall reaching Terra Nova Bay.

2. Materials and methods

2.1. Field sampling

Specimens of the Antarctic whelk *N. eatoni* ($n = 50$) were collected using a nylon-based bottom trawl at 150 m depth in the Gerlache Inlet at Terra Nova Bay (74°38.485' S, 164°03.726' E), near Mzs (Fig. 1). Sampling was conducted during the XXXI Italian Antarctic expedition in the 2015–2016 austral summer. Following sorting and taxonomic identification, specimens with intact shell and operculum were placed in sealed clean plastic bags and stored at –80 °C until analysis, conducted at the laboratory of ecotoxicology of emergent contaminants and nanomaterials of the University of Siena (Italy).

2.2. Morphometric measures of *N. eatoni* specimens

For this study, only well-preserved specimens of *N. eatoni* were considered (i.e., intact shell, presence of operculum, no sign of deterioration in the soft body). Prior to the analysis, *N. eatoni* specimens were defrosted and wet weight (WW) and morphometric parameters were recorded according to Malvé et al. (2018) as reported in the Supplementary material 1 and in Fig. S1.

2.3. Microplastic extraction

To determine the microplastic content in the Antarctic whelk *N. eatoni* ($n = 36$), individual specimens underwent a thermo-oxidative treatment, based on recently published methodologies for bivalve mollusks (Li et al., 2015, 2016, 2018; Akoueson et al., 2020; Sui et al., 2020). Quality assurance/quality control (QA/QC) procedures adopted at each step of the study are described in paragraph 2.6, protocols used to test the efficiency of microplastic extraction are detailed in the Supplementary material 1.

Once removed from the shell (Fig. S1), the soft body of each specimen was first washed with ultrapure Milli-Q water, cut in half and transferred to a 500 mL glass flask to which 200 mL of 30% hydrogen peroxide (H₂O₂) was added. Flasks, covered with a glass stopper were then incubated in a thermostatic water bath (KW W.82/O) at 60 °C for 24 h with gentle shaking (30 rpm), and left for 24 h at room temperature, in the dark under a chemical fume hood to allow organic matter to dissolve. Some samples, still showing some residues of organic matter in suspension, underwent a further treatment with 1:10 of (100%): sample (v/v), based on Dawson and collaborators (2020). Following treatment, 250 mL of a saturated sodium chloride solution (NaCl, density of 1.2 g/cm³) was added to each flask, which was shaken vigorously by hand and left under the fume hood overnight, to allow flotation of any microplastics present in suspension.

The samples were then filtered on Whatman quantitative filter papers (Grade 41, pore size of 20 µm, diameter of 47 mm) using a vacuum glass filtration apparatus kit (Supelco® 58061), rinsed extensively with

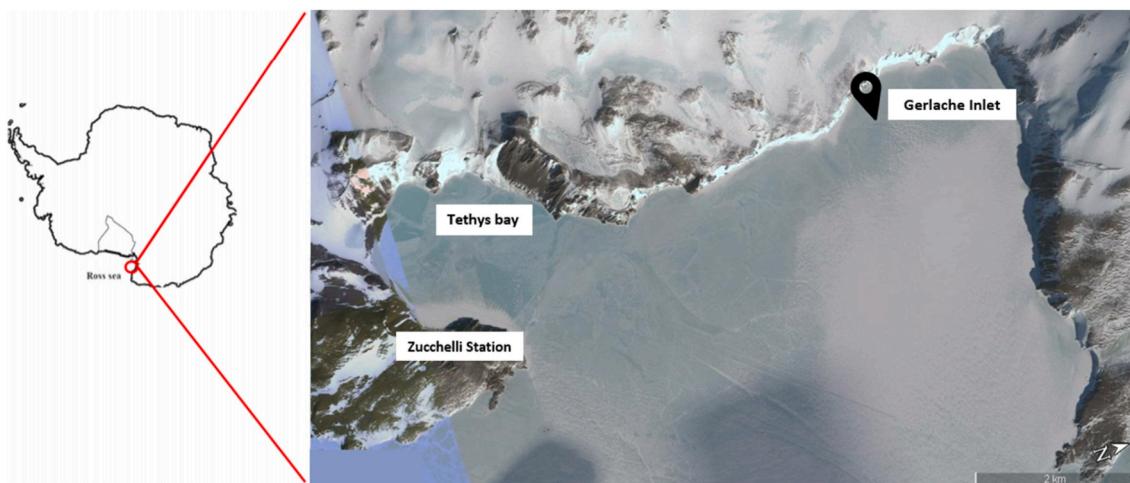


Fig. 1. Map showing the study area in the Ross Sea region (Antarctica) with position of the sampling site near the Gerlache Inlet and the Italian MZS in front of Tethys Bay in Terra Nova Bay.

Milli-Q water and stored in covered and labelled glass petri dishes. The filters were first observed under stereomicroscope and then under optical microscope (Olympus BX51, coupled with a DP50 camera) to assess the presence of microplastic candidates, which were imaged and classified according to their shapes and colors (Fig. 2). Microplastic sizes were also measured from images using ImageJ software (<https://imagej.nih.gov/ij/>).

2.4. Polymer characterization by FTIR spectroscopic analysis

Microplastic candidates found in blanks and *N. eatoni* specimens were isolated in a small glass Petri dish under a stereomicroscope, rinsed with filtered Milli-Q water using a glass Pasteur pipette and analyzed individually by microscope-coupled Fourier Transformed Infrared spectroscopy (manual μ -FTIR) at the facilities of SISSI-Bio beamline, ELETTRA Synchrotron Trieste (Birarda et al., 2022). For the measurements, single microplastic candidate was carefully transferred to a diamond cell (Diamond EXPress Compression Cell, ST-Japan) using the tip of a wet wooden toothpick, compressed and examined under the

Hyperion 3000 microscope, connected to a Vertex 70v interferometer (Bruker Optics) and to a Mercury–Cadmium–Tellurium detector. To determine sample homogeneity, measurements were taken at different points of the microplastic, with each measurement corresponding to 256 scans at a frequency of 40 KHz. Prior to each measurement, a reference spectrum was acquired in a region of the diamond cell without the sample. Furthermore, the polymeric composition of the outdoor technical clothing in use by researchers and logistic personnel at MZS was characterized by Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy and considered as a reference for possible local source of textile microfibers and fragments (e.g., rubbers) in the wastewaters and surrounding waters in the sampling region (see Supplementary material 1).

Spectra acquired through μ -FTIR and ATR-FTIR were analyzed using Wiley KnowItAll Software & Spectral Libraries (trial version) to determine the polymeric composition of the microplastic candidates. Match rates $>70\%$ were considered acceptable based on Kroon et al. (2018). Cellulosic-based materials were further assigned to natural or semi-synthetic cellulose (Rayon) by spectral analysis considering the guidelines set in Cai et al. (2019), primarily based on the sharpness of the C–O asymmetric in plane stretching band centered at about 1105 cm^{-1} .

2.5. QA/QC procedures

Airborne contamination during sampling and transport was limited as intact organisms were immediately transferred at $-80\text{ }^{\circ}\text{C}$ in new transparent PE bags soon after sorting. As researchers were wearing outdoor technical clothes during sampling operations, we processed only specimens displaying intact shells and operculum. Once defrosted, *N. eatoni* soft bodies extracted from shells (Fig. S1) were washed in 0.2 μm filtered Milli-Q water to avoid any potential sample contamination. In order to prevent contamination of samples during analysis and overestimation of microplastics content, QA/QC procedures were adopted following guidelines from previous studies (Li et al., 2015; Bråte et al., 2018; von Friesen et al., 2019; Dawson et al., 2020; Cho et al., 2021), as detailed below. Microplastic extraction was carried out under a chemical fume hood, in a closed and clean environment, with limited access to few operators only wearing a white lab coat (100% cotton) and latex gloves. Before and after analysis, all work surfaces were cleaned with ethanol and paper. All the solutions (Milli-Q water, H_2O_2 , NaCl) were filtered at 0.2 μm (Whatman cellulose nitrate membrane filters, diameter of 47 mm) using a vacuum glass filtration apparatus kit prior to use, while 100% ethanol was pre-filtered at 100 μm . For the analysis, only glassware or metal containers (i.e., flasks, beakers, petri dishes,

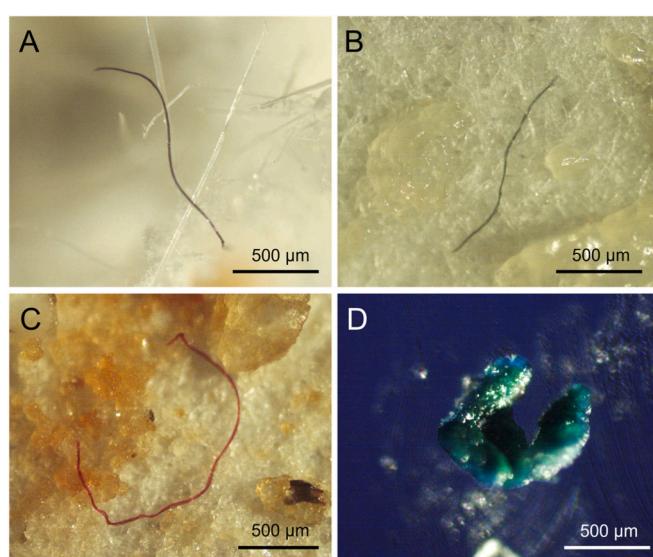


Fig. 2. Examples of microplastic candidates extracted from the Antarctic whelk *N. eatoni* from Terra Nova Bay, Ross Sea. Black (A), blue (B) and red (C) fibers and a green-blue fragment (D). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

filtration apparatus) were used and washed in filtered Milli-Q water before use. During the analysis, the samples were kept as much as possible under a chemical fume hood, in flasks covered with glass stoppers.

To establish any procedural contamination, at each step, an analytical laboratory blank was also added (with 1 blank each 5 samples on average), containing all the reagents used for the extraction except the biological sample. The use of laboratory blanks was essential to normalize the amount of microplastics extracted from the gastropods. Normalization was carried out by subtracting from the total of microplastic candidates found in each sample all those found in procedural blanks sharing shape (e.g., microfibers), color and polymer (see [Supplementary material 2](#)), to allow a highly conservative estimate of the microplastic content in the Antarctic gastropods.

The extraction method was validated assessing the digestion efficiency (%), the level of coverage of the filters and the recovery rate (%) of red polyethylene terephthalate (PET) microfibers and weathered polyethylene (PE) fragments, see the [Supplementary material 1](#) for further details ([von Friesen et al., 2019](#); [Dawson et al., 2020](#)). The buoyancy of 7 reference plastic polymers (PET, Low/High density PE, Polypropylene (PP), polystyrene (PS), Polyamides (PA-6, PA-6.6)) in NaCl (1.2 g/cm³) was also evaluated as a reference for the floatation step. Limit of detection (LOD) and limit of quantification (LOQ) were calculated based on [Bråte et al. \(2018\)](#) using 8 laboratory blanks. These QA/QC procedures are further described in the [Supplementary material 1](#).

3. Results

3.1. Morphometric data

For the present study, 36 specimens of *N. eatoni* were processed using the thermo-oxidative treatment; 3 of these were used to calculate the digestion efficiency and 3 for the recovery rate. Values (mean \pm Standard Deviation (SD), min, max and coefficient of variation) of the morphometric parameters measured in the Antarctic whelk are reported in [Table S1](#). Morphometric data show little variation among the individuals collected, with average WW of 12 ± 2 g (min – max, 8.4–15 g) and total shell length of 54 ± 3 mm (min – max, 49–61 mm).

3.2. Evaluation of the extraction method

The results of QA/QC procedures are reported in [Table S2](#). Loss of organic matter following thermo-oxidative treatment (i.e., digestion efficiency) referred to 3 whole specimens of *N. eatoni* was equal to $93.7 \pm 2.1\%$ based on DW and $98.3 \pm 0.5\%$ when calculated based on the initial WW (see [Supplementary material 1](#)).

The average level of coverage of the filters, as a qualitative assessment of the extraction process, was equal to 2.4 ± 1 . This indicates that, although the digestion efficiency exceeded 90%, some organic residues (mostly red and orange in color) were still found on filters after treatment, attributed to the variable content of natural organic material present in the digestive gland of the wild specimens. The average recovery rate of PET microfibers ([Fig. S2 A](#)) was $94.4 \pm 1.8\%$, whereas the recovery calculated using environmentally relevant PE micro-sized fragments ([Fig. S2 B](#)) was equal to $74 \pm 3.1\%$.

In the analytical blanks, an average of 1 ± 1.6 microplastic candidates was found (8 in total), with 5 out of 8 blanks showing no contamination. LOD and LOQ ([Table S3](#)) were equal to 5.8 and 17, respectively. The only type of microplastic candidates present in blanks was textile microfibers of dark colors (black, blue) with mean length of 2118 ± 1886 μm (median of 1345 μm , range 556 – 6285 μm) and average diameter of 12 ± 3 μm . Polymer identification initially showed a prevalence of rayon, with the exception of one PET, based on the match rates of the Wiley IR spectral library. However, further spectral analysis of cellulosic microfibers ([Cai et al., 2019](#)) allowed to distinguish

between natural cellulose ($n = 4$) and semi-synthetic rayon ($n = 3$) in the blanks.

3.3. Microplastic candidates in the specimens and polymeric characterization

A total of 19 microplastic candidates was found in 33 specimens of *N. eatoni* ([Fig. 2](#)), classified as fibers ($n = 17$), with blue/black (71%) or red (29%) color ([Fig. 3 A](#)), and fragments ($n = 2$) of green-blue and purple color. No other shapes, such as films or spheres, were identified. As fragments were lost during polymer characterization, they were not taken into account in the microplastic count.

Following blank normalization ([Supplementary material 2](#)), textile microfibers extracted from *N. eatoni* specimens were characterized by an average length of 1899 ± 1753 μm (median of 1111 μm , range 253 – 5768 μm) and an average diameter of 13.5 ± 6 μm , with 12 fibers less than 1500 μm in length (70.6%), and 3 fibers with length higher than 4000 μm (17.6%) ([Fig. 3 B](#)). As far as their composition, polymeric identification of the microfibers extracted from *N. eatoni* specimens ($n = 14$ analyzed by μ -FTIR) showed at first the prevalence of synthetic and semi-synthetic (rayon) microplastic fibers ($n = 10$) followed by natural cellulose ($n = 4$), based on the scores of the Wiley IR spectral library. Further in-depth spectral analysis led to a different attribution, with most of the fibers being of natural cellulose and cellulose-PA composite ($n = 8$, 47.1%), followed by polyester (PET, $n = 5$, 29.4%) and rayon ($n = 1$, 5.9%) ([Figs. 3 C](#), [Fig. 4](#)). Three microfibers were referred to as of unknown composition, as they were lost during sample loading onto μ -FTIR diamond cell and could not be analyzed. Overall, considering the additional spectral analysis of the celluloses (semi-)synthetic microplastics (i.e., PET and rayon), accounted for 35.3% of the total textile microfibers extracted from the Antarctic whelks.

Following blank correction, 27.3% of the whelks contained textile microfibers of PET, rayon and cellulose-PA composition (average of 0.3 ± 0.53 microfibers per individual and 0.03 ± 0.04 microfibers per g WW of the specimens) (Semi-)synthetic microplastics (i.e., PET and rayon). were found in 18.2% of the specimens, with an average of 0.18 ± 0.39

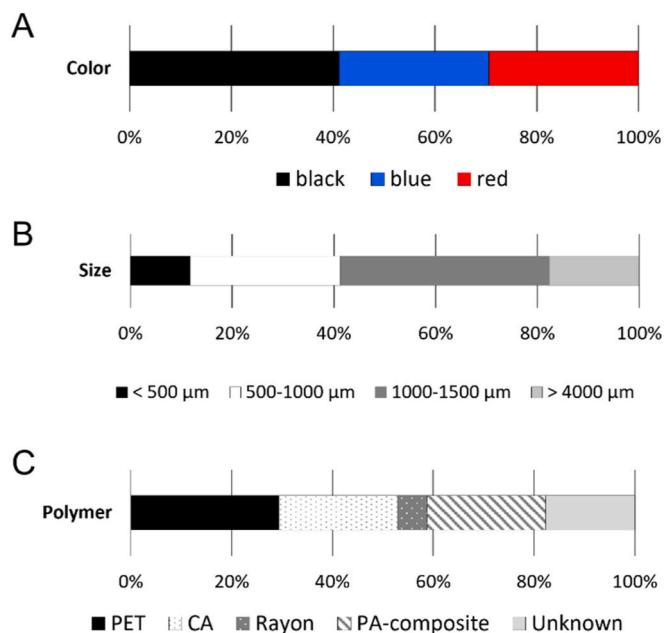


Fig. 3. Features of the textile microfibers retrieved from the Antarctic whelks: color (A), length (B) and polymer composition (C): polyethylene terephthalate (PET), natural cellulose (CA), rayon, polyamide (PA)-composite, unknown. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

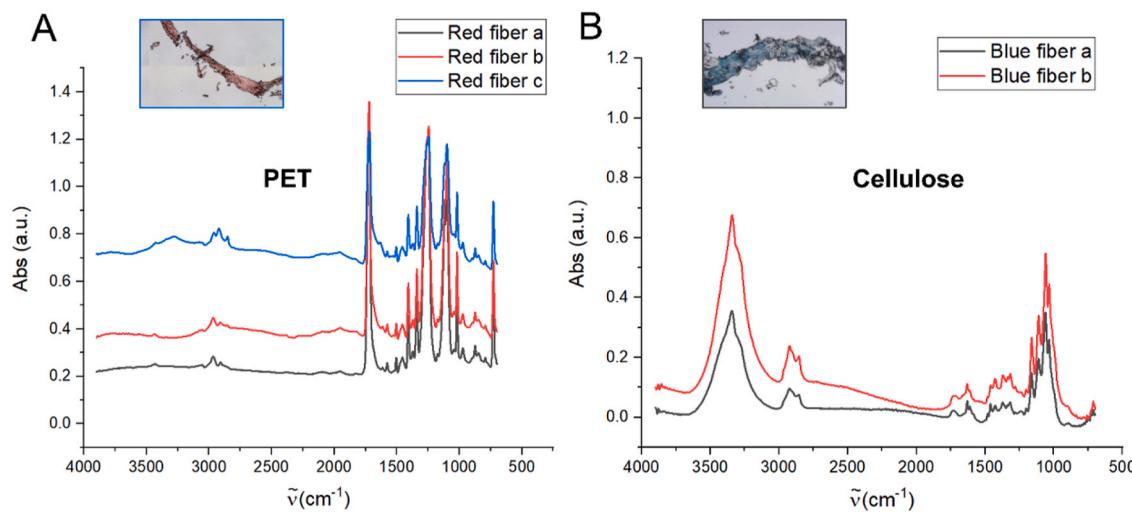


Fig. 4. Characterization of microfibers retrieved from Antarctic whelks by μ -FTIR. Absorption spectra as absorbance units (Abs, a.u., Y-axis) as a function of wavenumbers (v , cm^{-1} , X-axis) of red polyester (PET) fibers (A) and blue cellulose-based fibers (B), displayed in the images as compressed in the diamond cell. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

microplastics per individual and 0.02 ± 0.03 microplastics per g WW of the specimens.

ATR-FTIR measurements showed that the outdoor technical clothing and equipment ($n = 10$) used by Antarctic personnel operating at MZS contained a wide range of polymers, including PA, PET, cellulose and composites (Table S4). For example, the blue overalls were made of nylon (PA), the red full winter suit was characterized by an outer layer of natural cellulose-PA composite (reported in the label as 70% cotton and 30% PA) and a lining of PET, while the sole of Antarctic winter boots contained a poly(styrene-co-butadiene-co-isoprene) rubber.

Comparison of the absorption spectra of the microfibers from the Antarctic whelk and those from the technical clothing and equipment used by the Antarctic personnel operating at MZS (Table S4) disclosed a similar polymeric composition, as shown in Fig. 5. Red PET fibers extracted from the Antarctic whelk shared spectral features with two red garments (i.e., the winter jacket and the Dermizax® boiler suit), while blue PET fibers were comparable with the inner lining (i.e., blue pile fabric) of the full winter suit (Fig. 5A and B), but not with another blue pile jacket (listed in Table S4). In the case of the red cellulose-PA composite fiber, its absorption spectra matched with the one of the red outer fabric of the full winter suit (Fig. 5 C).

4. Discussion

4.1. Microplastic extraction from the Antarctic whelks

The extraction method was chosen based on protocols largely adopted in microplastic biomonitoring studies using mollusks, such as mussels (Li et al., 2015, 2016, 2018) and pectinids (Akoueson et al., 2020; Thiele et al., 2019; Sui et al., 2020). The thermo-oxidative treatment showed high efficiency in the removal of the organic matter (digestion efficiency >90% for DW, digestion efficiency >95% for WW) in the soft body of the gastropods, in agreement with other studies on Arctic bivalves (von Friesen et al., 2019, enzymatic treatment) and fish tissues (Dawson et al., 2020, KOH-ethanol treatment). In some of the digested samples, few organic residues were still found, attributed to the variable content of the digestive gland and/or gonads of the wild specimens. This is indicated by average level of coverage values (2.43 ± 1 , scale 1–4), which was considered acceptable, as in line with the ones reported by von Friesen et al. (2019): i.e., level of coverage from 2.5 ± 0.7 to 3 ± 0 following enzymatic treatment and filtration at $20 \mu\text{m}$.

Strictly related to the level of coverage, the recovery rate ($74 \pm 3\%$) of PE microplastics was slightly below optimal threshold, usually set as > 75% (Cho et al., 2021) or $\geq 80\%$ (Brander et al., 2020), whereas the recovery of PET microfibers was >94%. However, it should be ascribed

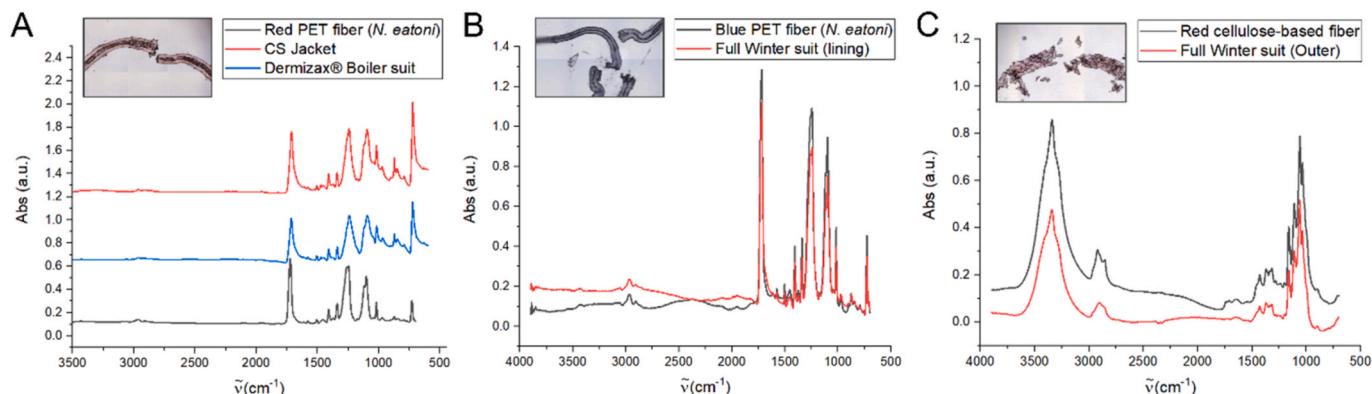


Fig. 5. Comparison of the absorption spectra of red (A) and blue (B) polyester (PET) microfibers and a red cellulose-polyamide composite (C) found in the Antarctic whelk *N. eatoni* (black lines) and those of the textiles from technical clothing (red and blue lines) used by Antarctic personnel in the study area. Absorption spectra are shown as absorbance units (Abs, a.u., Y-axis) as a function of wavenumbers (v , cm^{-1} , X-axis). Fibers are displayed in the images as compressed in the diamond cell. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

that here the recovery test was conducted as a blind trial using weathered micro-sized fragments of various colors and sizes to ensure a more truthful and environmentally relevant outcome. Recovery tests are usually conducted using virgin uniform plastic particles/fragments, with lack of details of the characteristics of the spiked microplastics (shape, color, size) and either absence or poor description of the protocol used, which does not allow a comparison among different studies.

As proposed by Brander et al. (2020), the use of standard reference materials to conduct recovery tests would ensure a proper evaluation of individual methods used in different laboratories, allow a better comparison among studies and solve the dispute about suitable extraction protocols (e.g., enzymatic, oxidative and alkaline treatments). However, such reference materials should also represent the diversity of microplastics found in environmental and biological matrices, in terms of shape, color, composition and ageing.

To evaluate microplastic extraction methods in the Greenland Smoothcockle (*Serripes groenlandicus*), von Friesen et al. (2019) performed recovery tests using more realistic pre-weathered PE microplastics derived from environmental samples and of various sizes. Likewise, the authors found lower recovery compared to the literature, with values of $75 \pm 11\%$ and $87 \pm 6\%$ following KOH and enzymatic treatment, respectively.

The QA/QC procedures here adopted during sample processing including laboratory blanks confirmed the limited airborne contamination from the laboratory, with the only microplastic candidates found in the procedural blanks corresponding to textile microfibers of dark color (black, blue). In microplastic research, there is still no consensus upon standardized methodology for blank correction (Brander et al., 2020), as this varies greatly among studies: some authors do not provide details (e.g., Cossi et al., 2021), some define an acceptable threshold of microplastics in the blanks (e.g., Sfriso et al., 2020), while others perform blank correction based on visual similarity with the samples (e.g., Crutchett et al., 2020) or they subtract the daily mean value of the blanks for each plastic type (e.g., Bråte et al., 2018). In this study, we adopted a conservative method to avoid overestimates of microplastic candidates or even contamination of microplastic-free samples in line with Tirelli et al. (2020), considering that the organisms were collected in a remote polar region. Notwithstanding, since the method used for blank normalization can affect data analysis, we cannot also exclude a possible underestimate of the microplastic content in the Antarctic whelk, for example related to PET fragments, which did not pass the floatation test in NaCl solution (see *Supplementary material 1*).

LOD and LOQ values obtained for microfibers are in line with data from Bråte et al. (2018, see *Supplementary material 1* for further details), stressing the need for a new and shared definition of LOD/LOQ estimates in microplastic analysis.

4.2. Abundance and features of textile microfibers

To date, limited studies have investigated the presence of microplastics in Antarctic marine biota and even less in the benthic species inhabiting marine coastal areas of the Ross Sea marine protected area. In this study, we investigated the abundance and features of textile microfibers in one of the key species of Ross Sea benthic communities, the Antarctic whelk *N. eatoni*, collected from an area close to MZS, in Terra Nova Bay.

The low number of microfibers and more specifically of synthetic and semi-synthetic fibers (0.18 ± 0.39 n/ind) found in *N. eatoni* individuals is in line with a previous report on marine whelks from the Arctic, including a species of Buccinidae (*Latisiphon hypolipis*: ~ 0.2 n/ind) of similar size and weight (Fang et al., 2018). reported a higher microplastic content in 5 mollusks from the same study region, including a gastropod species, the Antarctic sea snail *Eatoniella* sp. (average content of 1.2 n/ind). Such difference could arise from the following different aspects: (I) the extraction method used by Sfriso et al. (2020) by homogenizing the samples in an alkaline solution with pestle and mortar,

may have potentially cause plastic fragmentation and thus overestimating the number of extracted microplastics; (II) the different method used for blank correction could have led to changes in the estimates of microplastic content; (III) the different numerosity of samples processed ($n = 36$ *N. eatoni* in this study, $n = 14$ *Eatoniella* sp.) as the authors showed that n/ind of microplastics decreased at increasing numbers of individuals considered; (IV) the variability related to the sampling site in terms of exposure to various contamination sources and the biological/ecological characteristics of the species itself (e.g., size, life stage, feeding strategies).

Age of the individuals could affect microplastic accumulation as a function of time of exposure during life period. Morphometric measurements of the *N. eatoni* specimens here analyzed (in terms of WW, shell length and width), showed a little variation among individuals, classified as sub-adults (Fraussen, 2020), indicating that they could have been exposed to microplastics for a relatively short time. Although the life cycle of *N. eatoni* has not been fully described, detailed information on life stage, age and feeding behavior should be included in future studies for a better comparison of microplastic content among species including other mollusks from the Ross Sea. Trophic level and feeding strategy have been considered as key factors affecting microplastic content in macroinvertebrates representative of polar benthic communities. Fang et al. (2018) reported relatively high amounts of microplastics in carnivorous or omnivorous predators, such as the starfish *Asterias rubens* and the crab *Chionoecetes opilio*, compared to other Arctic and Sub-Arctic benthic species ranging from echinoderms to mollusks and crustaceans. The authors suggest that microplastics are likely to be transferred along Arctic benthic food webs. Sfriso et al. (2020) reached a different conclusion as Antarctic filter-feeders and grazer/detritivore mollusks were the most contaminated compared to omnivores and predator species, indicating no bioaccumulation trend within Ross Sea benthic communities.

Being both scavenger and active predator (Norkko et al., 2007), the Antarctic whelk represents a key intermediate link within the benthic food web structure of the Ross Sea region (Rossi et al., 2019). The contamination from microfibers found in this species suggests exposure to both natural and man-made textiles in their natural environments, but overall a low risk of accumulation and trophic transfer to higher predators, including echinoderms and fish. Nevertheless, the paucity of data on the effects of textile microfibers and associated contaminants on Antarctic benthos limits the assessment of their ecological impact on Antarctic benthic marine biodiversity of the Ross Sea.

Microfibers as prevalent microplastic found in biological samples has been highlighted in several reports on marine mollusks (mostly bivalves) both from markets and coastal monitoring worldwide (reviewed in Cho et al., 2019), including Arctic wild specimens (Fang et al., 2018). Textile fibers were also the most abundant microplastic type reported in surface waters and sediments of Terra Nova Bay (Cincinelli et al., 2017; Munari et al., 2017). However, our findings are in contrast with Sfriso et al. (2020), who reported a majority of small plastic fragments (size <300 μm) in benthic organisms from the same study area. Compared to the size of the microplastics from this latter study, the length of the microfibers extracted from *N. eatoni* was higher but in line to data available on other whelk species (i.e., *R. daphnelloides* and *Euspira nana*, but lower than *L. hypolipis*) (Fang et al., 2018).

Regarding their polymeric composition, only PET or cellulosic fibers were found in *N. eatoni* specimens, in agreement with data on microfiber contamination recently obtained through a global scale survey, including the Southern Ocean (Suaria et al., 2020). The authors revealed that most of the microfibers ($\sim 80\%$) were of natural cellulosic composition and among the fraction of synthetic fibers, PET was the prevalent polymer.

Here, natural and semi-synthetic celluloses were further distinguished by in-depth spectral analysis (Cai et al., 2019) preventing the overestimation of rayon, which was the main outcome of the IR polymer library for cellulosic fibers. Man-made celluloses such as rayon, viscose

and cellophane are often included in microplastic monitoring studies, although they can be easily mismatched with natural cellulose by commercial IR libraries. Therefore, a careful examination of the cellulosic fibers (or a sub-sample, with large numbers of microfibers found) is necessary to avoid discrepancy in microplastic counts (Cai et al., 2019; Suaria et al., 2020).

Considering the importance to protect Ross Sea marine protected area and its unique biodiversity, further monitoring studies should be performed to assess the impact posed by textile microfibers on species belonging to different trophic levels and having an important ecological role in benthic communities.

4.3. Antarctic facilities as a local source of microplastic contamination

The high similarity between the spectral signatures of the textile fibers extracted from *N. eatoni* and those of technical clothing used by Antarctic personnel operating at MZS allowed us to hypothesize that the wastewater outfalls of the MZS act as potential source of fiber contamination in the study region, the Ross Sea.

As a further confirmation of our hypothesis, the polymeric characterization performed by ATR-FTIR of technical clothing and equipment in use at MZS, corresponds to the main polymers identified in previous studies in the Ross Sea. Cincinelli et al. (2017) reported microplastics made of PE and PP (both 57.1%), followed by PET (28.6%), polytetrafluoroethylene (5.7%), polymethyl methacrylate (5.7%) and PA (2.9%) in historic samples of surface waters from the Ross Sea (see Table S4 for comparison). In a more recent survey, Zhang et al. (2022) showed that microplastic contamination in surface and sub-surface waters of this region was mainly due to lines/microfibers of PET, having lower abundance at increasing distance from the coast. Similarly, Munari et al. (2017) documented the presence of fibers, films and fragments made of PA-6.6, polystyrene-butadiene-styrene and ethylene-PP rubber in sediments of Terra Nova Bay, with concentrations increasing approaching MZS (see Table S4 for comparison).

Recent findings on nanoplastics (<1 µm) in landfast sea ice cores from the Ross Sea region further confirmed the presence of PE (50% by mass), followed by PP and PET, in the samples. Sea currents were indicated as the main drivers for nanoplastic pollution, although the authors did not exclude local anthropogenic contamination especially in the top layer of the sea ice cores probably influenced from surface waters (Materić et al., 2022). Furthermore, a recent study on Antarctic snow samples collected near McMurdo scientific station in the Ross Sea, revealed the prevalence of microfibers, mostly composed of PET (41%) and other polymers matching those from textile garments and equipment in use at the station (e.g., flags used for routes signaling) (Aves et al., 2022).

Sewage carrying wastewaters containing fibers from washing clothes has been widely recognized as an important source of contamination in the receiving marine coastal waters and seafood (Browne et al., 2011; Rochman et al., 2015; Cesa et al., 2017; De Falco et al., 2018; 2019). Mechanical, thermal and chemical stresses are the main responsible for the detachment of fibers from fabrics during laundry in a washing machine. Resulting textile fibers released during wash are barely retained in WWTPs whatever present, thus ending up into coastal waters (Napper and Thompson, 2016; Hernandez et al., 2017; Gaylarde et al., 2021). Very little information is available on the status and presence of WWTPs in Antarctic research stations, but more important no information on their ability to retain textile fibers or in general microplastics from wastewaters has been provided until now (Gröndahl et al., 2009; Stark et al., 2016).

Although natural fibers released from textiles as cotton or wool are considered biodegradable, little is known about their behavior, fate and more important degradation into the environment and seawater (Arshad et al., 2014; Henry et al., 2019). Rayon and cotton are often processed, dyed and coated with chemicals such as resins, softeners and flame retardants, which can significantly slow their remineralization and

increase their residence time in the marine environment (Li et al., 2010; Suaria et al., 2020). Ingestion of textile microfibers by marine organisms and consequent biological impacts depend on several factors, including the intrinsic features of the microfiber themselves, such as size, density, color and above all adsorption of harmful substances. Likewise, physiology, feeding strategies and position in the trophic web of the exposed species could affect microplastic accumulation, translocation and/or excretion as well as ecotoxicological effects. In terms of toxicity, the size of microfibers represents a critical factor influencing not only absorption but also biodistribution in the exposed organisms (Phuong et al., 2018; Ziajahromi et al., 2018). In this study, the sizes of the microfibers fall in the range of 0.5–6 mm, with most <1.5 mm ($n = 12$), consistent with previous studies (Lusher et al., 2017; Bellas et al., 2016; Naji et al., 2018; Phuong et al., 2018) and suggesting that fibers with smaller sizes, likely resulting from the fragmentation of larger textile fibers, are more readily available for benthic organisms. Recent studies reported negative toxic effects due to the ingestion of synthetic and natural fibers in aquatic and terrestrial species. Song et al. (2019) showed that PET microfibers can be ingested by land snails (*Achatina fulica*) and altered through passage in its digestive system. The authors also reported significant damages in the gastrointestinal villi, a reduction in the activity of antioxidant enzymes and lower food intake over prolonged exposure to microfibers. Remy and collaborators (2015) investigated the ingestion and effects of synthetic cellulose-based microfibers (such as viscose) coated with carcinogenic dyes in marine detritivorous invertebrates populating Neptune grass (*Posidonia oceanica*) meadows in Mediterranean coastal areas, indicating potential risk for the macrobenthic community.

Concerning Antarctic species, previous lab-bench studies on Antarctic krill (*Euphausia superba*) showed ingestion and excretion of PE microspheres, although with no direct effect on the exposed organisms (Dawson et al., 2018a,b). The authors also demonstrated that internalization of the PE microplastics in the digestive tract of Antarctic krill was able to fragment the microplastics down to nano-sized debris (Dawson et al., 2018a). This process could progressively facilitate the release of organic and inorganic plastic additives which could represent an ecotoxicological risk for the species itself but also for the receiving ecosystems through the release of smaller plastic debris in the fecal pellets (Hermabessiere et al., 2017). Our previous findings on Antarctic krill *E. superba* juveniles experimentally exposed to nanoplastics revealed a significant impact on krill molting and sinking velocities of fecal pellets containing nanoplastic agglomerates (Bergami et al., 2020a).

Recommendations by the Antarctic Treaty Consultative Meeting to limit the use of personal care products containing microplastic beads inside the Antarctic base stations have arisen only recently, in 2019, while no discussion is available on WWTPs as a significant source of textile microfibers in Antarctic marine coastal waters.

Following the first observations of marine debris in Antarctica, in 1989 the Convention on the Conservation of Antarctic Marine Living Resources established an international monitoring program to report stranded plastic debris and fishing gears along Antarctic shores recognizing how routine monitoring is essential to identify threats, future trends and impacts on Antarctic marine biota (CCAMLR Antarctic Treaty Secretariat, 2019; Waluda et al., 2020). Our study indicates that technical clothing and equipment used in Antarctica and washed at base stations is a potential local source of synthetic and natural microfibers into marine coastal areas of the Ross Sea region. Such phenomenon needs to be investigated more in depth with focused monitoring and mitigation strategies to limit or prevent any detrimental impact on Antarctic marine biodiversity.

5. Conclusions

The occurrence of microplastics in specimens of the Antarctic whelk *N. eatoni* from Terra Nova Bay, in the Ross Sea, was assessed here for the first time. As far as the extraction method used, it showed an efficient removal of the organic material and QA/QC protocols led to a minimal

laboratory airborne contamination. Apart from two fragments of unknown composition, all the microplastic candidates extracted from *N. eatoni* were textile fibers of black, blue or red color. The number of textile fibers found was in line with previous reports on Arctic whelks, including other *Buccinidae*, but low compared to data available on Antarctic mollusks (including the gastropod *Eatoniella* sp.) from the Ross Sea. The low content of microplastics in *N. eatoni* specimens suggest a low risk of bioaccumulation along Antarctic benthic food webs in the Ross Sea. The in-depth polymeric characterization through μ -FTIR showed that the fibers found in *N. eatoni* were of synthetic (PET) or cellulosic nature (natural cellulose, rayon and PA composite), sharing main spectral features with some of the textile fibers of clothing commonly used by the personnel of a scientific research station nearby. Our findings suggest that base stations could represent important local input sources of textile microfibers in the marine coastal waters of the Ross Sea. Future studies should assess the amount of microplastic released from WWTPs of Antarctic facilities to determine to what extent they contribute to plastic pollution in Antarctica and affect marine biodiversity.

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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.envres.2022.114487>.

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