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Monitoring of anthropogenic microplastic pollution in antarctic fish (emerald rockcod) from the Terranova Bay after a quarter of century

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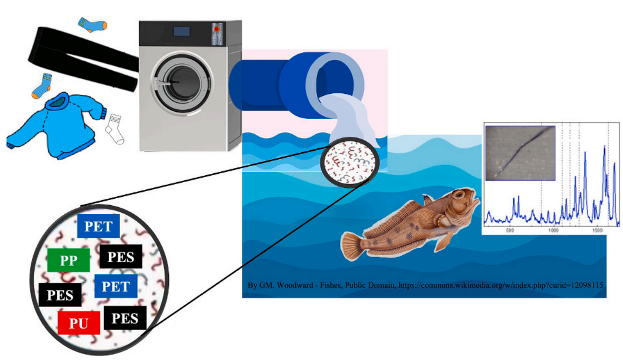
HIGHLIGHTS

- Anthropogenic microparticles were found in *Trematomus bernacchii* specimens from Antarctica.
- Polyester (PES) was the main polymer found (52 %).
- Indigo was the main industrial dye found (56 %).
- The polymers found are compatible with most of the clothing used by research personnel in MZS.

GRAPHICAL ABSTRACT



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ABSTRACT

Monitoring the occurrence of microplastic contamination in the Antarctic area is the key to implement policy measures for waste regulations in the research stations. Antarctic fish *Trematomus bernacchii* is a suitable species for establishing microplastic contamination and for investigating changes over time in the concentration and type of microplastics in the Antarctic region. In this paper a total of 78 fish, caught during the 37th Italian Antarctic expedition (2021–2022) in the Ross Sea (Antarctica) were analysed. Different microfibers and dyes were identified by Raman spectroscopy and the results were compared with those obtained for fish sampled in 1998. Differences in polymer type emerged with a predominance of synthetic fibers with respect to natural ones. These changes appear to be related to the increased human activities in the Antarctica over the last twenty years

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and highlights the need to improve the environmental sustainability of the numerous research stations operating throughout that area.

1. Introduction

In last years, there was an increase in the presence of anthropogenic microparticles (AMs), such as microplastics (MPs, items <5 mm), in marine environments worldwide (Cózar et al., 2014; Ng and Obbard, 2006; Van Cauwenberghe et al., 2013; Yuan et al., 2023; Hansani et al., 2023). The presence of these micro-particles has also been reported in Antarctica, in the marine biota, seawater, and sediments (Anfuso et al., 2020; Bottari et al., 2022a; Kelly et al., 2020; Munari et al., 2017; Reed et al., 2018).

Antarctica protection is regulated by the strict guidelines of the Antarctic (Antarctic Treaty Secretariat 2014). In addition, in 2018 microplastic pollution was recognized by the Scientific Committee on Antarctic Research (SCAR) as a "serious and emerging threat" in the Southern Ocean, (Alurralde et al., 2022); therefore, the Antarctic Treaty System (ATS) began to increase its attention on the actions to prevent this issue (Zhang et al., 2020).

The most important sources of MPs pollution come from activities related to the research stations (logistics, field activities, etc.), and above all from wastewater systems (Bottari et al., 2022a; Reed et al., 2018; Waller et al., 2017; Waluda et al., 2020).

The Italian Base "Mario Zucchelli Station" (MZS) is close to the Antarctic Specially Protected Area (ASPA) n. 161 and overlooks the Ross Sea, where one of the largest marine protected areas worldwide is located. MZS is open from mid-October to mid-February, corresponding to the Antarctic summer. The base can accommodate up to 100 people and is equipped with a 30-year-old wastewater treatment plant for domestic waters. This means that it is unable to retain AMs such as textile fibers and microplastics. In fact, Sfriso et al. (2020) noted the presence of microplastics in macrobenthic species next to MZS, and Bottari et al. (2022a) reported the presence of AMs coming from the MZS washing machines, into the gastrointestinal tract of the emerald rock cod *Trematomus bernacchii* (Boulenger, 1902) caught in 1998.

Several studies have reported the presence of microplastics in various key components of the ecosystem, including mollusks, fish, and penguins (Bessa et al., 2019; Bottari et al., 2022a; Dawson et al., 2020; Fragão et al., 2021; Le Guen et al., 2020; Sfriso et al., 2020; Zhang et al., 2022).

Gurumoorthi and Luis (2023) carried out an ecological risk assessment of MPs in the water, sediments, and marine biota around Antarctica by the use of some pollution indexes (Pollution Load Index, Polymer Hazard Index, and Potential Ecological Risk Index) finding that the Ross Sea and Antarctic Peninsula have high levels of plastic microfibers. At the moment, the adverse effects of microparticles ingestion have not been yet clarified and quantified on wild organisms (Porcino et al., 2023).

MPs can also enter Antarctic waters coming from sub-Antarctic waters. In fact, although in the past it was believed that the Antarctic Polar Front (APF) constituted an efficient circumpolar barrier preventing the southward transport of microplastics, recent studies highlighted that the APF is not continuous and can carry plastic items (Bargagli and Rota, 2022).

Trematomus bernacchii, commonly called emerald rock cod, is an extreme stenotherm fish that lives in the cold, thermally stable waters of coastal Antarctica, where temperatures range from −1.86 °C to +0.3 °C (Eastman, 1993). *T. bernacchii* lives in shallow waters down to 700 m depth. This is an opportunist feeder with a wide niche breadth composed almost exclusively of benthic organisms and small fish (La Mesa et al., 2004a). The species is very common in the shallow waters of the High-Antarctic Zone (Froese and Pauly, 2023), and it is commercially and ecologically important (La Mesa et al., 2004b).

The aims of this study were: i) to detect and characterize anthropogenic microparticles in the emerald rock cod caught near the wastewater treatment plant (WWTP) of MZS and in a control site, located further from the base, and ii) to evaluate the differences in polymer composition among fish caught on 1998 (Bottari et al., 2022a) and those caught in during the 37th Italian Antarctic expedition (2021–2022). From this study, the authors expect to detect a different impact of AMs on emerald rock cods sampled in the two different sites and, more interestingly, a higher occurrence of AMs compared to the samples caught in 1998. This is quite reasonable since, in the last 25 years, despite an even increasing use of polyester - based technical clothes, WWTP has neither been replaced nor implemented thus significantly limiting the efficiency of WWTP for microplastics removal.

2. Materials and methods

2.1. Study area and samplings

Mario Zucchelli Station is located at Terra Nova Bay (Ross Sea, Antarctica) along the coast of the Northern Foothills to the north-east of Gerlache Inlet (74° 41′ S; 164° 6′ E) (Fig. 1). This site is an important littoral area for long-term scientific investigations, and in 2003 Italy proposed it as an Antarctic Specially Protected Area (ASPA n. 161). During the austral summer, up to a maximum of 100 people (both scientists and technicians) are present at the MZS.

Samplings were carried out during the XXXVII Italian Antarctic expedition in the 2021–2022 austral summer. Two sampling sites were chosen, site A, namely Road Bay (Lat. 74°41′45.9″ S, Long. 164°07′37.5″ E), close to the base, heavily impacted because located next to the wastewater treatment plant (WWTP), and site B, namely Tethys Bay (Lat. 74°42.05.5″ S, Long. 164°02′28.6″ E), furthest from the base, about 2.5 km, which will be considered as control. Were carried out 5 samplings for each site, from 3rd November and 13th December 2021. A total of 8 fish were caught during each sampling. Furthermore, the main chemical-physical parameters were measured using the multiparametric probe, i.e. oxygen, temperature, salinity, pH, and depth (Table 1S).

T. bernacchii were caught using fishing rods and baits. Following taxonomic identification, specimens were stored at −20 °C until analysis, that were carried out at the laboratory of plastics and microplastics of the Institute for Biological Resources and Marine Biotechnology (IRBIM) – National Research Council (CNR) of Messina.

2.2. AMs isolation

Prior to the analysis, samples were slowly thawed at 4 °C overnight, subsequently, each sampled specimen was measured (TL: total length, mm), weighed (TW: total weight, g), and sexed.

The gastrointestinal tract (GIT), from the oesophagus to the end of the intestine, was removed, weighed, placed in a conical glass flask, and treated with 10 % KOH solution, in a ratio of 1:5 (*w/v*). Each flask was stirred at 50 °C for 48 h, after this period each sample was placed in a glass cylinder, and a hypersaline 15 % NaCl solution was added in order to obtain density separation of the two phases and to collect the floating microparticles. In some cases, GITs were particularly rich in lipid content, probably due to the fish diet, so following the Dawson et al. (2020) protocol, ethanol (EtOH), in a ratio of 1:4, was added to the solution KOH/NaCl, to avoid the saponification process. Then, the supernatant was collected in a glass beaker and filtered through fiberglass filters (1.6 μm Whatman GF/F, UK) using a vacuum system (Millipore). After that, the filters were placed in sterile Petri glass dishes and observed under a stereomicroscope (Leica M205C). All isolated microparticles were counted, measured, and photographed. The found items were classified based on their size (small-microparticle: 0.1–1 mm; large-microparticle: 1–5 mm; meso-particles: 5–25 mm), shape (pellets, fibers, foams, fragments, sheets, and spheres), and colour according to the protocol of the Marine Strategy Framework Directive (Galgani et al., 2013).

2.3. Preventing contamination

To avoid contamination during laboratory analysis, rigorous precautions were carried out according to Bottari et al. (2022b). The samples were processed in a room with restricted access, to prevent any accidental external contamination. All workspaces and tools were cleaned with ethanol and filtered deionized water from any particle contamination. Only glassware was used. The operators wore cotton coats (100 % cotton) and latex gloves. All the solutions (Milli-Q water, KOH, NaCl) were pre-filtered at 0.2 μm (Whatman cellulose nitrate membrane filters, diameter of 47 mm) using a vacuum glass filtration apparatus kit prior to use. The procedures were executed under a laminar hood. As a safeguard against airborne contamination, procedural blanks were incorporated using a 10 % KOH solution. To ensure accuracy and prevent an overestimation of fibrous content, filters dampened with moisture were placed in Petri dishes and positioned under the fume hood and in close proximity to the stereomicroscope. If any indications of airborne contamination emerged, the associated sample was discarded.

2.4. Polymer characterization by Raman spectroscopic analysis

The identification assays were carried out at the Laboratory of Optical Spectroscopy of the Department of Physics -University of Messina. Raman spectra were measured with an HR Evolution micro confocal Raman system (Horiba Scientific) using a 532 nm laser diode, 20× or 50× objective lens, and a grating with 600 grooves/mm. A CCD detector cooled to 77 K was used to collect Raman signals over the range of 200–4000 cm^{-1}, with an integration time varying in the range of 5–20 s and a number of accumulations changing between 2 and 100, depending on the Raman activity of the sample measured and the fluorescence generated by it. The laser power was maintained below 5 mW to avoid sample photodegradation. Several measurements were taken for each sample to establish the reproducibility of spectra. Furthermore, different points of the same MP were tested to eliminate the influence of local impurities on the MPs Raman spectra. Spectral data were first processed using Labspec 6 software to remove the fluorescence background and perform baseline subtraction and noise filtration. Then they were analysed using the Bio-Rad KnowItAll Spectral Library and the Spectral Library of Plastic Particles (SLoPP and SLoPP-E) to determine the polymeric composition of investigated samples. The Hit Quality Index (HQI) was used to describe the correlation between the spectrum of the unknown samples and spectra of known compounds in reference databases. Spectral matches with an HQI value of 80 % or greater have been considered.

2.5. Data analysis

The comparison between sexes and sampling sites was performed considering data on the frequency of occurrence (FO). Chi-squared test ($\chi 2$) was performed in order to check potential significant differences between the occurrence of AMs in males and females and in sites A and B.

Spearman correlation has been performed to assess the correlation between fish body size and fish weight with respect to AMs abundance, as well as between fish body size and fish weight with respect to the number of items/g GIT (Pastorino et al., 2023).

Abundance of data, expressed as the number of items/specimens, were tested for homoscedasticity and normality by the Levene and Shapiro–Wilk tests. Because the data did not satisfy the assumption required to perform a parametric analysis of variance (ANOVA), even after log transformation, the Kruskal–Wallis non-parametric test was used to test whether there were any significant differences in the number of items/specimens among sexes and sampling sites.

The number of items/g of GIT was also estimated for different sexes and sampling sites.

The relative condition factor (Kn) was chosen as a general indicator of fish health, according to Sbrana et al. (2020) and Bottari et al. (2022b). Kn was calculated by comparing the observed weight of the fish (TW) to an expected weight based on the fish's observed lengths (TL; Bottari et al., 2014). Length and weight data were log-transformed and the linearized relationships were fitted by least squares regression to estimate “a” (intercept) and “b” (allometry) coefficients. The isometric condition (H0: b = 3) was tested by Student's *t*-test. The relative condition factor (Kn) was calculated, for positive and negative specimens to

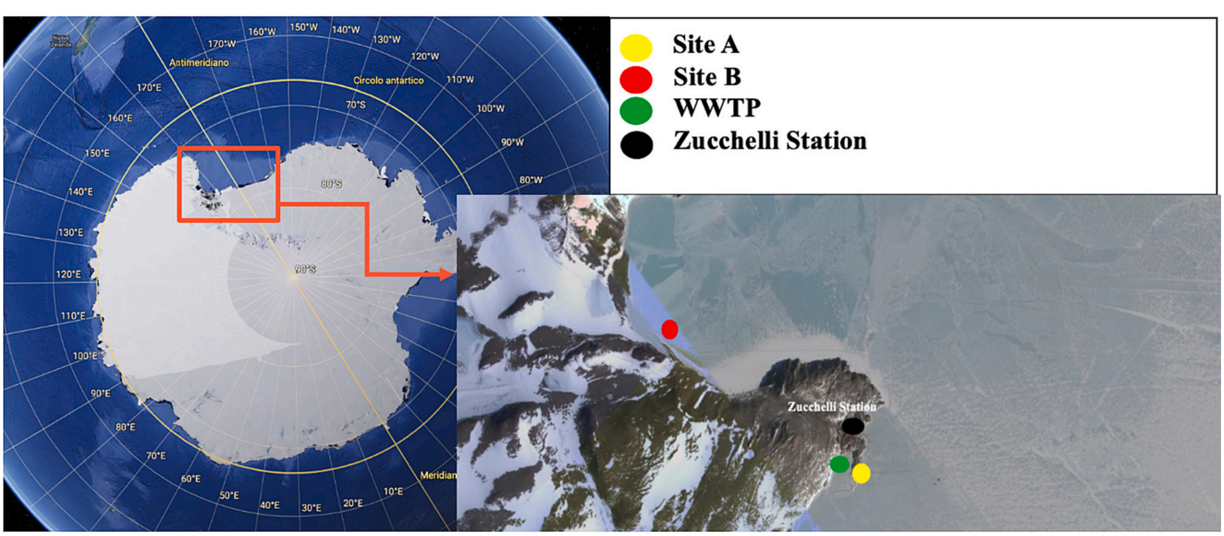

Fig. 1. Study area (Terranova Bay, Ross Sea). Map was created using Google Earth (https://earth.google.com/web/search/74°42%27+05%2e5%27%27+S+164°02%2728%2e6%27%27+E/@74.70162494,164.0414348,1.90516998a,251.18435904d,35y,50.91498231h,44.99998024t,0.00000005r/data=CmUaOxI1GQeP29TlrFLAIaAh0iVSgWRAKiE3NMKwNDInIDA1LjUnJyBTIDE2NMKwMDInMjguNicnIEUYAiABIiYKJAmadV7pwKxSwBFzfWQ0-6xSwBlhwIAKm4FkQCEWqozFLoFkQA) on which sampling sites and wastewater treatment plant were indicated.

AMs occurrence, according to the expression:

$$K_n = \frac{TW}{a' \bullet TL^b}$$

where a′ (antilog of a) and b are the power length-weight relationship parameters (Le Cren, 1951).

Spearman correlation was used to assess the correlation between MPs abundance vs. fish body size and fish weight. PAST software was used for all statistical analysis (Hammer et al., 2001). Differences were considered significant at $p < 0.05$.

2.6. Risk assessment of MPs: induced risk index and pollution load index

There is no standard method to assess the MPs risk, but several authors (Chen et al., 2021; Gurumoorthi and Luis, 2023; Pan et al., 2021; Xu et al., 2018) consider the concentration and the chemical composition of MPs. Particularly, the MPs-pollution load index (PLI) and the MPs-polymer hazard index (PHI) were used to assess MPs risk. The first indicator was calculated according to Xu et al. (2018) as follows:

$$PLI = \sqrt{CF_i} = \sqrt{\frac{C_i}{C_{0i}}}$$

The PLI was based on the contamination factor (CF_i) which is the ratio of MPs concentration in biota (C_i) to the background value (C_{0i}) at each sampling location, where the background value is determined by the lowest concentration of MPs value detected in biota samples.

The PHI was calculated according to the following formula:

$$PHI = \sum S_n{}^{*}P_n$$

where S_n is the hazard score of polymers present in our samples derived from the published tables (Chen et al., 2021; Gurumoorthi and Luis, 2023; Lithner et al., 2011; Pan et al., 2021; Xu et al., 2018), P_n is the percent of MPs polymer types collected in each sample. The criteria for the risk level of MPs pollution are reported in Table 4 provides the degree of MPs risk and could support for the risk management of MPs pollution. Briefly, Level I (0–1) very little harm to humans; Level II (1–10) may cause respiratory irritation, skin irritation, serious eye irritation, severe skin burns, or eye damage; Level III (10–100) may be toxic if inhaled, swallowed, or in contact with skin; Level IV (100–1000) may cause an allergic skin reaction; Level V (1000–10,000) that is the most hazardous may cause cancer.

3. Results

Two samples were excluded from the analysis due to airborne contamination of the corresponding environmental filter.

3.1. AMs abundance

A total of 78 specimens of *T. bernacchii* (total length: from 150 to 335 cm; total weight: from 39 to 557 g) were analysed. In Table 1 data for each sex are reported in greater detail. Thirty-nine individuals (FO: 43.2 %) had ingested AMs. A total of 73 items (0.9 items/specimen), mainly belonging to small AMs (53.8 %) and large AMs (44.9 %) were found (Fig. 2a). A single meso- particle was also isolated (1.3 %). The length range of AMs varied from 0.1 to 7 mm. Fibers (92.3 %) resulted the predominant shape category, followed by fragments (7.7 % - Fig. 2b). No pellets, foams, or spheres were observed. Black particles were the most abundant (42 %), followed by blue (28 %), red (9 %), transparent (7 %), light blue (6 %), yellow (4 %), and white (4 %; Fig. 2c).

A total of 53 females and 23 males were examined for the occurrence of AMs (Table 1). AMs were found in both sexes. The FO was higher in females (49.1 %) than in males (30.4 %) with no significant differences ($\chi2$: 2.3; $p > 0.05$). Similarly, the number of items was higher in females (1.0 items/specimen) than in males (0.9 items/specimen), but the Kruskal–Wallis test showed no statistically significant differences (H: 0.6, p > 0.05). The number of items/g of GIT was 0.05 and 0.08 for females and males, respectively. No correlation has been found between fish size and weight versus number of items/g of GIT (r_s = 0.04 and 0.07, p > 0.05) (Table 1).

Two sites were studied for AMs occurrence (site A: Road Bay and site B: Thetys Bay). AMs were found in both sites. The FO was higher in site B (44.7 %) than in site A (40 %) without significant differences ($\chi2$: 0.18, p > 0.05). The items/specimens ranged from 0.9 in site B to 1.0 in site A. No significant differences were observed between sampling sites (H:

Table 1
Environmental parameters of the two sites (Table 1S).

Site	Date	Depth/ m	pH	Oxygen/ mg/l	Salinity/ psu	Temperature/ °C
A1	03/11/ 2021	0	7.72	8.41	33.79	−1.98
A1	03/11/ 2021	10	7.82	9.44	33.78	−1.99
A1	03/11/ 2021	16	7.83	8.70	33.76	−1.95
A2	13/11/ 2021	0	7.94	8.58	33.94	−1.72
A2	13/11/ 2021	10	7.95	9.42	33.84	−1.95
A2	13/11/ 2021	16	7.89	9.2	33.78	−1.95
A3	23/11/ 2021	0	8.09	10.47	33.92	−1.81
A3	23/11/ 2021	10	8.03	9.22	33.81	−1.93
A3	23/11/ 2021	16	8	10.72	33.75	−1.94
A4	03/12/ 2021	0	8.14	10.1	32.74	−1.62
A4	03/12/ 2021	10	8.1	8.64	33.74	−1.89
A4	03/12/ 2021	16	8.1	9.52	33.7	−1.81
A5	13/12/ 2021	0	8.33	11.38	33.62	−1.34
A5	13/12/ 2021	10	8.31	11.66	33.65	−1.33
A5	13/12/ 2021	16	8.28	10.04	33.51	−1.19
B1	01/11/ 2021	0	7.68	9.23	25.86	−1.99
B1	01/11/ 2021	10	7.73	10.6	33.74	−2
B1	01/11/ 2021	16	7.74	8.4	33.71	−2.02
B2	11/11/ 2021	0	7.88	9.98	32.77	−1.98
B2	11/11/ 2021	10	7.9	9.48	33.78	−2
B2	11/11/ 2021	16	7.88	9.6	33.81	−1.97
B3	21/11/ 2021	0	7.99	8.58	33.94	−1.72
B3	21/11/ 2021	10	8	9.42	33.84	−1.95
B3	21/11/ 2021	16	7.98	9.2	33.78	−2
B4	30/11/ 2021	0	8.17	10.66	33.57	−1.54
B4	30/11/ 2021	10	8.1	11.76	33.75	−1.81
B4	30/11/ 2021	16	8.09	8.79	33.7	−1.82
B5	11/12/ 2021	0	8.8	12.45	29.93	−1.28
B5	11/12/ 2021	10	8.2	9.03	33.7	−1.31
B5	11/12/ 2021	16	8.28	7.96	33.64	−1.28

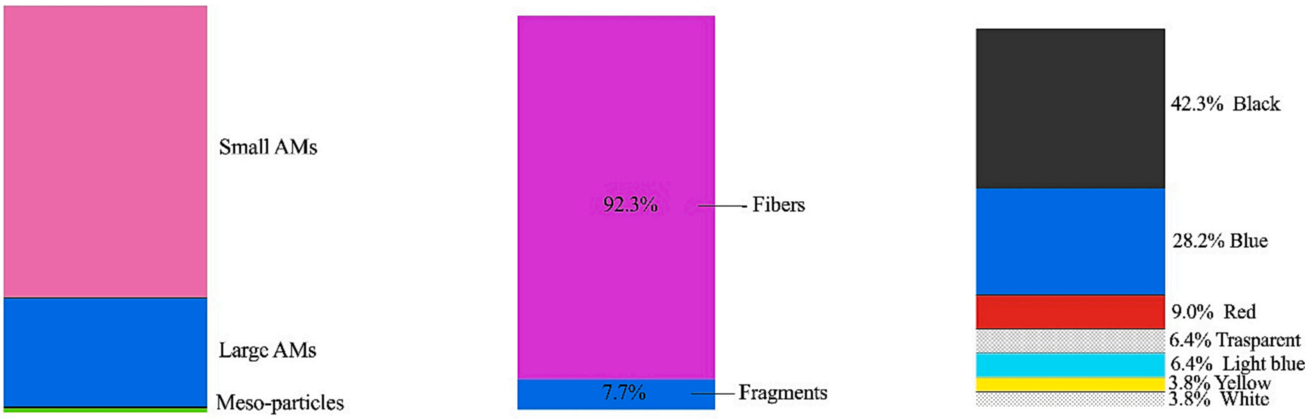

Fig. 2. Categorization (%) by size (a), shape (b) and colour (c) of anthropogenic particles isolated from *Trematomus bernacchii.*

0.002, $p > 0.05$). No correlation has been found between the AMs abundance and the fish body size ($r_s = 0.15$; $p > 0.05$) and between the AMs abundance and the fish body weight ($r_s = 0.12$; $p > 0.05$).

Table 2 provides the Kn data for AMs positive and negative samples; Kn was very similar in the groups considered with small fluctuations between 1.0 in the positive samples and 1.04 in the negative samples and an average value equal to 1.01 (F: 0.06, $p > 0.05$).

3.2. Polymer characterization

A total of 31 AMs were analysed using micro-Raman spectroscopy. Of them, ten showed spectra completely dominated by fluorescence or of poor quality with substantial peak overlap and peaks missing due to enormous line broadening, which made impossible their identification. The remained 21 AMs (29 %) were processed for the identification of their components by comparison to spectral libraries. Polyester (PES), Polyethylene terephthalate (PET), Polypropylene (PP), Polyurethane (PU), PES + cotton, and cellulose microfibers were the polymers identified.

In almost all cases, the Raman spectra showed the contribution of dyes or pigments used as additives for staining plastics and textile fibers. In some samples, Raman signals of colorant prevailed over the contribution of the polymeric matrix. In such cases, the identification of their polymeric components was based on a thorough analysis of specific vibrational bands weakly emerging from the Raman pigment spectrum. Then by using the KnowItAll software both the pigments and the polymeric component were identified. For example, we identified a blue microfiber as a mixture of polypropylene (PP) and the dye Hostopen violet, as its Raman spectrum showed the main bands of PP (397 cm^{-1}, arising from the wagging of CH_2 moieties, and 1455 cm^{-1}, arising to the bending of CH_3 and CH_2 moieties) and the band of violet pigment (1193 cm − 1, 1335 cm − 1, and 1381 cm − 1) (Andreassen, 1999; Scherrer et al., 2009). Similarly, many blue fibers, of which an example is shown in Fig. 3., were identified as a blend of polystyrene (Stuart, 1996, bands at: 859 cm^{-1} attributed to C—C and COC bending, 1096 cm^{-1} attributed to C—C stretch, 1182 cm^{-1} attributed to C—C ring stretch, 1287 cm^{-1} attributed to CO-O stretch, 1626 cm^{-1} assigned to CH_2 bending) and indigo dye (Baran et al., 2010; Stuart, 1996). The Raman spectra of selected identified items are shown in Fig. 3.

The main type of polymer found was PES (52 %), followed by PES + cotton and cellulose (14 % both), PP, and PU (10 % both). Moreover, in the examined AMs, 4 industrial dyes were identified Indigo and Hostopen violet in blue fibers, Vine black in black fibers, and Basic red 46 in red fibers, respectively). Indigo occurred in most of them (56 %). Notably, similar combinations of polymer and colour are present in the technical equipment of personnel working in Antarctica (see Table 3).

Concerning the two sampling sites, in site A PES (89 %), PP (11 %), Indigo (78 %), Vine black, and Hostopen violet dyes (11 % both) were found. In site B PET (43 %), PES (29 %), PU and PP (14 % both), Basic red 46 (60 %), and Vine black dyes (40 %) were detected.

Table 2
Number (n) of *Trematomus bernacchii* specimens, mean, standard deviation (SD) and ranges of total length (TL, cm) and total weight (TW, g), and relative condition factor (Kn).

Trematomus bernacchii	Sample size	Total length (mm)		Total weight (g)		Kn
	N	Range	mean ± SD	Range	mean ± SD	
Positive	33	150–335	234.4 ± 43.8	29–557	204.7 ± 120.1	1.0
Negative	45	150–290	216.1 ± 38.4	39–413	152.7 ± 86.8	1.04

3.3. Risk assessment: pollution load index and polymer hazard index

PLI found in *T. bernacchii* had low pollution load (Level $I < 10$), in fact, PLI in our samples ranged from 1.0 to 3.8 in site A, and from 1.0 to 5.8 in site B, with an average of 2.1 and 2.2 respectively.

The PHI was 4.8 and 1480.2, respectively, for site A and site B. According to the criteria for the risk level of microplastic pollution, site A is classified as Level II, while site B is classified as Level V."

4. Discussion

In the present study, anthropogenic microparticles ingestion by 78 *T. bernacchii* inhabiting marine coastal areas of Terra Nova Bay (Ross Sea, Antarctica) was evaluated. To date, only two studies have investigated the presence of AMs in Antarctic fish species, but a very limited number of specimens was examined (Bottari et al., 2022a; Zhang et al., 2022).

4.1. AMs abundance

The frequency of occurrence (43 %) and abundance value (0.9 items/specimen) reported in this study are slightly lower than those reported for Perciformes of the Ross Sea by Zhang et al. (2022) FO = 50 %, 1.3 items/specimen). In *Trematomus scotti*, a frequency of occurrence of 33.3 % with an average abundance of 0.83 items/individual was reported (Zhang et al., 2022), very close to our results. As concern the shape, microfibers were the most frequent microparticles in *T. bernacchii* (92.3 %), as well as reported for the sediments close to the MZS (Munari et al., 2017) and the waters close to the WWTP of MZS (Cincinelli et al., 2017). In this study, the predominant colour was black (42 %), in

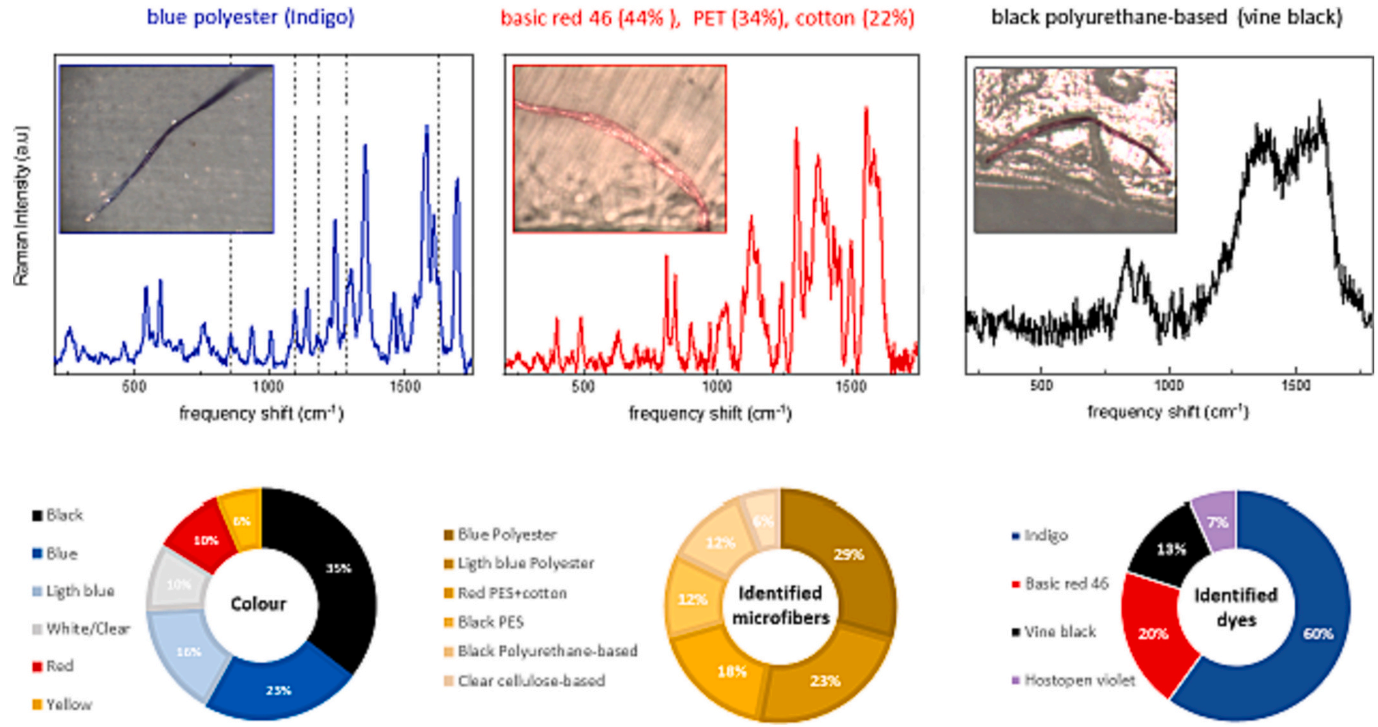

Fig. 3. Selected set of anthropogenic microparticles (AMs) isolated from the gastrointestinal tract (GIT) of *Trematomus bernacchii* samples caught in 2022 in the Antarctica (Terra Nova Bay, Ross Sea). AMs have been identified analysing the spectra measured by means of micro-Raman spectroscopy (top panels), after image acquisition with a Raman confocal microscopy (insets of top panels). Distribution percentage of colour, identified microfibers and identified are reported in the bottom panel.

accordance with Zhang et al. (2022; 39%).

In this study, no significant correlation emerged between the size/ weight of the samples and the number of items/g in the GIT. However, Pastorino et al. (2023) reported a noteworthy negative correlation in *Salvelinus frontalis*, indicating that both weight and fish size were inversely related to the number of items/g in the GIT.

4.2. Synthetic microparticles

The synthetic polymers identified in this study were PES, PU, and PP. PP was also found in the sediments of the Ross Sea, as well as in the seawater, sediment, and sea ice in other Antarctic areas (Munari et al., 2017). In accordance with our data, the most common polymer in Perciformes of the Ross Sea was PES (30 %; Zhang et al., 2022). Finally, PU, one of the constituents of both gloves (second coating) and shoes (midsole) used by personnel in Antarctica, was found also. Polyurethane is a very versatile material and due to the great elasticity of its fibers, is widely used.

Munari et al. (2017) found in sediments next to the MZS (Ross Sea) fibers of thermoplastic polyurethane (TPU). Moreover, PU microfibers were found in zooplankton (Absher et al., 2019; Jones-Williams et al., 2020) and PU fragments were found in oceanic surface waters and deep-sea sediments (Cunningham et al., 2020; Lacerda et al., 2019).

The polymers isolated from the GITs of *T. bernacchii*, PES, PU, and PP are present in the composition of technical clothing and equipment used by personnel operating at MZS in Antarctica (Table 3). The industrial dyes identified in this study (Indigo, Hostopen violet, Vine black, and Basic red 46) are compatible with the technical equipment (see Table 3).

The PLI level found in *T. bernacchii* (average 2.1) was lower than that reported by Sfriso et al. (2020) in the macro-benthic species (8.4) in Sea Ross.

4.3. Sites comparison

Statistical analysis reported no differences between the two sites although we expected not to find AMs in the control site. The presence of AMs in the control site can be linked to the currents that are able to transport the microplastic items and textiles fibers even in places far from their release (Petersen and Hubbart, 2021).

4.4. Comparison with Trematomus bernacchii caught in 1998

Comparing these results with those of 1998 (Bottari et al., 2022a), we found a higher occurrence in the first study (75 %) than in this one (41 %). The abundance value was equal to 3.3 items/specimen in 1998, while in this study were 0.93 items/individual. For what concerns the size range in 1998 large AMs, (size range 1.1–4.2 mm) were the most abundant 63 %, while in this study small MPs (size range 0.1–0.9 mm) were the majority 72 %. For both studies, the highest percentage of microparticles found were fibers 95 % and 92 % respectively for Bottari et al. (2022a) and the current study.

Bottari et al. (2022a), showed that natural fibers were the most abundant items found, in particular, cotton (45 %) and cellulose (18 %); some synthetic polymers were also found as polyester, polypropylene, and cellulose acetate. Further, industrial dyes were found such as Indigo, Cromophtal Violet B, Drimaren Navy Bue R-2RL, Vine black dye, and Sirius light green. In Table 5 are shown the chemical differences of the anthropogenic microparticles from *Trematomus bernacchii* caught in the Ross Sea (Antarctica) specimens of the current study and that of 1998 Bottari et al. (2022a).

The presence of dyed fibers in *T. bernacchii* caught in 1998 indicated that the textiles pollution originating from the base was present already 25 years ago when the wastewater treatment plant (WWP) was new. The results found in this work are different from those found in 1998, where a high percentage of natural fibers was found. On the contrary, the obtained results highlighted that most of the fibers are made up of

Table 3
Polymeric composition of technical clothing in use by personnel at Mario Zucchelli Italian Station and polymeric identification.

Antarctic equipment	Colour	Manifacturer	Polymers according to manufacturer	Polymer identification	Dye
Outer jacket	Black	Polartec	100 % PES	–	–
Long trousers	Red/grey or black	Thermolite	100 % PES	–	–
Shoes	Black	Pedula	Lining: GORE-TEX	–	–
			Mid sole: PU	PU	Vine black
			Sole: Vibram Rubber	–	–
Boiler suit	Red	Dermizax®	Outer: Nylon, PU, Kevlar	–	–
	Black		Lining: PES + Kevlar	PES	
	Black		Padding: 100 % PES	PES	
	Blue	Thermore®	Overalls: 100 % PA	–	–
Anorak	Red	–	Outer: 70 % PET - 30 % Cotton	PET, Cotton	Basic red 46
		–	Lining: 100 % Cotton	–	–
Pile jacket	Blue	Tecnopile®	100 % PET	–	–
Down Jacket	Red	Ligron®	Outer: 65 % PES, 35 % cotton	–	–
			Inside: 55 % cotton, 45 % PA	–	–
Gloves	black	Primaltoft®	First coating: 86 % nylon, 14 % spandex	–	–
			Second coating: 75 % PES, 25 % PU	PES, PU	
			Lining: 100 % PES	PES	Vine black
			Insulation: 100 % aerogel	–	–
Sweater	black	Mico®	60 % PES, 40 % PP	PP	–
Socks	yellow, orange, grey, light grey, black	Micotex®	Antistress cuff: lycra®	–	–
			Inside: micotex sponge	–	–
			Outer: wool	–	–
Zip up sweater	Blue	–	100 % PA		
Zip up sweater	Blue	–	100 % PES	PES	Indigo
Turtleneck sweater	Blue	–	100 % PES	PES	Indigo
Padded pants	Red/Black	FWD	94 % PES, 6 % Spandex	–	–
Padded jacket	Red/Black	Dermizax®	Outer: 100 % Nylon	–	–
			Lining: 100 % PES	–	–
			Inside 100 % PES	–	–
Light boiler suit	Red	Dermizax®	Outer:100 % Nylon	–	–
			Inside 100 % PES	–	–
Suit pants	Blue	–	60 % PES, 40 % Spandex	PES	Indigo, Hostopen violet, Vine black
Suit pants	Blue	–	90 % PES, 10 % Spandex	PES	Indigo
Suit pants	Blue	–	94 % PES, 6 % Spandex	PES	Indigo
Suit pants	Blue	FWD	95 % PES, 5 % Spandex	PES	Indigo

Table 4
Risk level category for microplastic pollution. Pollution Load Index: PLI; Polymer Hazard Index: PHI.

	I	II	III	IV	V
PLI	<10	10–20	20–30	>30	
PHI	0–1	1–10	10–100	100–1000	1000–10,000

Table 5
Chemical composition of anthropogenic microparticles isolated from the gastrointestinal tract of *Trematomus bernacchii* caught in the Ross Sea (Antarctica). N: Number of anthropogenic microparticles.

Year	N	Composition	Dye	Reference
1998	20	cellulose, cotton, polyester, polypropylene, polypropylene + polyester, cellulose acetate.	Indigo, Cromophtal Violet B, Drimaren Navy Bue R-2RL, Vine black, Sirius light green	Bottari et al., 2022a
2021	21	polyester, polyethylene terephthalate, polypropylene, polyurethane, polyester + cotton, cellulose)	Indigo, Hostopen violet, Vine black, Basic red 46	Present study

polyester, a constituent of many garments used by the personnel working at the Italian base in Antarctica.

4.5. Origin of the microfiber contamination

The wastewater treatment plant present at the MZS base is old, and obviously, its retention efficiency is greatly decreased over time; therefore, it releases huge quantities of microfibers deriving from washing suits, equipment, and clothing into the Ross Sea. The latter are continuously used by the staff throughout the Antarctic summer season.

This study further stresses the importance of anthropogenic microparticles and textile dyes monitoring in Antarctica and underlines the urgency of actions to mitigate this phenomenon. The problem needs to be tackled from several points of view first of all it emerges the need for a new wastewater treatment plant equipped with specific filters to prevent microplastics from reaching the sea. Not less important is the design of new and sustainable clothing for personnel working in Antarctica that are technically feasible and ecologically more fair.

4.6. Risk assessment: pollution load index and polymer hazard index

The low PLI indicates that Antarctica was generally less polluted with MPs, in accordance with data of Gurumoorthi and Luis (2023) that obtained level of PLI in *Pygoscelis papua* ranged from 1.0 to 8.4 with an average value of 2.7. Moreover, Gurumoorthi and Luis (2023) deduced the PLI from several studies concerning Antarctic benthic macroinvertebrates (Sfriso et al., 2020) and penguin species (Bessa et al., 2019; Le Guen et al., 2020; Fragão et al., 2021). In all cases, they obtained low PLI belonging to Category I. Overall, the low PLI indicates that in Antarctica there is a low pollution load due to the remoteness, less population, and less industrialized. In specific Antarctic geographical areas (Dronning Maud Land and South Shetland Islands), the examination of excrement from predators like the Antarctic fur seal (*Arctocephalus gazella*) and the gizzard of the emperor penguin (*Aptenodytes forsteri*) has indicated the lack (or extremely low levels) of microplastics (Garcia-Garin et al., 2020; Leistenschneider et al., 2022). The high PHI reported in the present study was related to the presence of PU, which has a high hazard score. The opposite results of PLI and PHI, shown in this study, let us state that the risk of MP pollution is not only due to the accumulation of MPs, but especially it is due to the polymer types. The suggestion to improve our planet is to reduce the use of chemical and polymers with high hazard score values.

5. Conclusions

This paper reports on the presence of textile microparticles in the gastrointestinal tract of a key fish species of the Ross Sea, *T. bernacchii*. The data here reported highlights how important is to monitor sensitive species. It could be interesting to create a database of anthropogenic microparticles ingested by Antarctic organisms in order to have a precise idea of AMs pollution in this delicate marine ecosystem.

Our study highlights how important is: 1) to monitor the wastewater treatment plant of the Italian research station and 2) to strengthen, in terms of efficiency, the wastewater plants of the Italian research station. Moreover, the use of commercial microfiber filters, inserted before the water discharged from the washing machines could partially reduce the entry of these particles into the sea. The use of commercial filters could reduce the emission of microfibers for a period of time necessary for the adaptation of the treatment plant.

CRediT authorship contribution statement

M.M. Conceptualization, funding acquisition, Methodology, Investigation, Data curation, Formal analysis, Writing – original draft, Writing, and reviewing. V.C.N. formal analysis, curating data, Writing, and reviewing. C.B. and N.P. Investigation, data curation, formal analysis, writing, reviewing, and editing; S.N. data curation, formal analysis; F.S. and M.A. sampling, reviewing, and editing. G.D. formal analysis, data, and writing curation, supervision. T.B. Conceptualization, formal analysis, Writing – original draft, Writing – review, editing, Funding acquisition, Project administration, and supervision. All authors provided approval for publication of the content and contributed to drafting the work and critically revisiting the manuscript.

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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

No data was used for the research described in the article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.167244.

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