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Cellulosic and microplastic fibers in the Antarctic fish *Harpagifer antarcticus* and Sub-Antarctic *Harpagifer bispinis*

Mauricio Ergas [a], Daniela Figueroa [a], Kurt Paschke [b,c,d], Mauricio A. Urbina [e,f], Jorge M. Navarro [a,b], Luis Vargas-Chacoff [a,b,d,g,*]

[a] *Instituto de Ciencias Marinas y Limnológicas, Universidad Austral de Chile, Valdivia, Chile*
[b] *Centro Fondap de Investigación de Altas Latitudes (IDEAL), Universidad Austral de Chile, Valdivia, Chile*
[c] *Instituto de Acuicultura, Universidad Austral de Chile, Puerto Montt, Chile*
[d] *Millennium Institute Biodiversity of Antarctic and Subantarctic Ecosystems, BASE, University Austral of Chile, Valdivia, Chile*
[e] *Departamento de Zoología, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Concepción, Chile*
[f] *Instituto Milenio de Oceanografía (IMO), Universidad de Concepción, Concepción, Chile*
[g] *Integrative Biology Group, Universidad Austral de Chile, Valdivia, Chile*

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ABSTRACT

Human settlements within the Antarctic continent have caused significant coastal pollution by littering plastic. The present study assessed the potential presence of microplastics in the gastrointestinal tract of the Antarctic fish *Harpagifer antarcticus*, endemic to the polar region, and in the sub-Antarctic fish *Harpagifer bispinis*. *H. antarcticus*. A total of 358 microfibers of multiple colors were found in 89 % of *H. antarcticus* and 73 % of *H. bispinis* gastrointestinal track. A Micro-FTIR analysis characterized a sub-group ($n = 42$) of microfibers. It revealed that most of the fibers were cellulose (69 %). Manmade fibers such as microplastics polyethylene terephtalate, acrylics, and semisynthetic/natural cellulosic fibers were present in the fish samples. All the microfibers extracted were textile fibers of blue, black, red, green, and violet color. Our results suggest that laundry greywater discharges of human settlements near coastal waters in Antarctica are a major source of these pollutants in the Antarctic fish.

1. Introduction

The production of synthetic polymers has grown exponentially since the mid-20th century, reaching 390 annual million tons in 2021 (*PlasticsEurope*, 2022). It has become a major pollution problem in aquatic ecosystems worldwide (Espinosa et al., 2016). Countless pieces and fibers of plastic accumulates in the marine environment worldwide and may persist there for centuries (Chen and Jakes, 2001; Courtene-Jones et al., 2017; da Costa and Duarte, 2020). These plastics are degraded to microscopic pieces by the combination of mechanical and photochemical action in the marine ambient (Espinosa et al., 2016). However, small fragments and mainly microfibers (MFs) are also directly incorporated into the environment mainly by atmospheric deposition and wastewater discharge in all kind of urban, rural and remote areas around the world (Mazurais et al., 2015; De Falco et al., 2020; Napper et al., 2023). The calculations suggest that primary microplastics could be the source of half of the total plastic waste accumulating in the oceans (Boucher and Friot, 2017).

Microplastics (MPs) are plastic fibers, pellets, and fragments ranging from 100 μm to 5 mm in size (Arthur et al., 2009; Bergmann et al., 2015). MPs are harmful to aquatic organisms (Thompson et al., 2004; Lönnstedt and Eklöv, 2016; Wang et al., 2019; Tang et al., 2021). Their accumulation and persistence in animals at all trophic levels have been studied. It affects the survival, reproduction, and growth of invertebrates and fish (Watts et al., 2015; Hermabessiere et al., 2017; Urbina et al., 2023). Microfibers (MFs) are a major component of MPs (Woodall et al., 2014), they can be classified as natural, such as cotton, wool, silk, and manmade, where we found synthetic fibers (nylon, polyester, acrylic) and semisynthetic, regenerated cellulose fibers (viscose/rayon) (Liu et al., 2019). MFs are an emerging pollutant that comprise an enormous fraction of MP debris found in shorelines worldwide (Henry et al., 2019; Suaria et al., 2020). They have been found multiple times as the most common form of MP in different environmental compartments, even in the Arctic (Athey and Erdle, 2022). Both natural and manmade fibers are

* Corresponding author at: Instituto de Ciencias Marinas y Limnológicas, Universidad Austral de Chile, Valdivia, Chile.
E-mail address: luis.vargas@uach.cl (L. Vargas-Chacoff).

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vastly used in clothing. Even though natural and regenerated cellulose fibers are biodegradable, it can take up to four years to fully biodegrade (Le et al., 2022). Natural fibers are not considered plastics. Nevertheless, they represent a large volume of textile pollution, and cellulose is believed to be the most abundant non-plastic microfiber in water samples (Suaria et al., 2020; Mateos-Cárdenas et al., 2021). Textile fibers may contain numerous industrial, both residual and additive, compounds that potentially harm aquatic organisms (Almroth et al., 2021).

Nothothenioid fishes of the genus *Harpagifer* inhabit shallow coastal waters, underneath stones and crevices (Hureau, 1990). *H. antarcticus* inhabits only the Antarctic continent and islands. It eats almost exclusively gammarid amphipods (Casaux, 1998), whereas *H. bispinis* lives in the Patagonian fjords, and its diet is more generalist, including isopods and polychaetes (Hüne and Vega, 2016). The life cycle of *H. antarcticus* is characterized by a long-lived larvae and very restricted movement in adult individuals (White and Burren, 1992). Both species are genetically and ecologically related (Schwob et al., 2023), with their divergence estimated to have occurred more recently than anticipated from other Antarctic species, coinciding with the Last Glacial Maximum during the Quaternary era (Hüne et al., 2015). The presence of microfibers in the prey of both species has been reported (Sfriso et al., 2020; Jones-Williams et al., 2020).

Plastic litter drifts and accumulates due to oceanographic and atmospheric processes (Jorquera et al., 2022; Prata et al., 2022; Napper et al., 2023). Studies by Waller et al. (2017) confirm the presence of macro and microplastics on the coast of the Antarctic continent, particularly the Antarctic Peninsula. The source of these plastics is still a debate, but it could be coming from local population, land-based sources or sea-based sources, for instance MPs derived from enormous fishing net contamination in the Southern Ocean (Tirelli et al., 2022). Nevertheless, enormous gaps of information about microplastic contamination exist in the Southern Ocean (Castillo et al., 2020), especially in the rocky intertidal zones (Ehlers et al., 2022; Klein et al., 2022) where *Harpagifer* spp. lives.

The Antarctic environment is changing fast, and organisms are exposed to multiple stressors. MFs can affect the energy balance (feeding, immune system, basal metabolism) of organisms (Urbina et al., 2023), thus decreasing their resilience to other environmental changes they face, such as increasing temperatures and decreasing salinity (Vargas-Chacoff et al., 2021a, 2021b; Navarro et al., 2019; Courtene-Jones et al., 2017).

A high share of the species diversity and endemic biomass composition of Antarctic fishes are mostly benthic ichthyofauna, especially from the suborder Notothenioidei (Barrera-Oro, 2002). This comparative study between the Antarctic endemic species and its Patagonian relative provides new insight into the actual microfiber contamination occurring on King George Island and highlights the need for new strategies for minimizing human impact on the island. This study aims to quantify the potential presence of MFs in the gastrointestinal tract of both species. Results are discussed and compared among species, habitat, gut portion (stomach and intestine), and microfiber characterization.

2. Materials and methods

2.1. Ethics statement

Sampling and experimental procedures complied with ethical guidelines regulating the use of laboratory animals established by the *Comisión Nacional de Investigación Científica y Tecnológica, Agencia Nacional de Investigación y Desarrollo* (CONICYT, now ANID) and Universidad Austral de Chile. Fish were anesthetized with a lethal dose of 2-phenoxyethanol (1 mL L^{-1}), followed by spinal cord sectioning after they were captured (Vargas-Chacoff et al., 2021a, 2021b).

2.2. Fish and sampling

The fish (*H. antarcticus*, $n = 55$ and *H. bispinis* $n = 57$) were caught by turning over rocks from the lower intertidal zone at the lowest tide. *H. antarcticus* were collected at the South Shetland Islands, King George Island, Antarctic (62° 11′ S, 58° 59′ W) (Fig. 1.A) and *H. bispinis* from Bulnes fort (53°37′ S, 70° 55′60″ W), a natural area 50 km away from Punta Arenas, Chile (Fig. 1.B). They were kept in 7 % formaldehyde for further lab analysis (da Costa et al., 2022).

2.3. QA/QC

Fish arrived at "*Laboratorio Fisiología de Peces*" in Universidad Austral de Chile, Valdivia. QA/QC protocols were performed to avoid air contamination, following the approach of different authors by the time fibers were counted in 2018 (Alomar and Deudero, 2017; Lusher et al., 2014; Nadal et al., 2016; Peters et al., 2017; Woodall et al., 2015). The whole process was done by one experimenter, on a working table that was used exclusively for the counting process and was thoroughly cleaned several times, with EtOH70%/Milli-Q water and white paper towels until there was no sign of particles on the paper surface. All the materials and the stereoscope were cleaned and disinfected with the same procedure as the working space. All instruments were specially cleaned and examined for contamination under the stereoscope (Lusher et al., 2014). Latex gloves, face masks, and a single 100 % cotton lab coat were used at all times when performing the dissection (Lusher et al., 2013; Neves et al., 2015). The lab coat did not leave the processing laboratory (Woodall et al., 2015). To minimize the risk of contamination, the stomach, and the intestine were transferred directly from the sample fish into a clean glass petri dish for visualization (Peters et al., 2017). The dissection was made in a metal tray, with no plastic instruments. During analysis, samples were covered at all times except during analysis under the stereoscope, at which point samples were exposed to the air (Lusher et al., 2014). As a control, we left an open Petri dish with Milli-Q water next to the working tray and the stereoscope (Bellas et al., 2016). After the stomach and intestine plates were sorted, samples were observed, as recommended by Bellas et al. (2016). They were revised under the stereoscope, MFs were counted, and water was changed after each dissection, so each sample had its own control blank. Minimum air contamination was found in our sample blanks, indicating a largely clean environment. Nevertheless, since each sample was associated with its own blank, we corrected for this by reducing the number of MFs corresponding to each sample (McIlwraith et al., 2021). Detailed data for each sample can be found in Supplementary Table 3.

2.4. Dissection protocol

First, fish were measured (*H. antarcticus*: mean = 8.98 cm; se = 1.22 cm – *H. bispinis*: mean = 6.67 cm; se = 0.82 cm). Then the stomach and intestine were removed, avoiding damaging the organs and trying not to lose any of its content. Stomach contents were obtained by performing a V-shaped cut and scraping the inside with a scalpel. The intestine was cut longitudinally and opened, then held with tweezers and scraped with a dull scalpel to get everything from the inside (McGoran et al., 2017). The Petri dish was then analyzed under stereomicroscope Olympus ZJ61 (0.67× – 4,5×) with 10× eyepiece (6.7× – 45×) and the MFs were counted (Supplementary Fig. 5) and isolated into glass tubes with Milli-Q water and stored for microfiber characterization analysis.

2.5. Measure of microfibers

To measure the size of MFs, they were imaged with a digital camera attached to the stereomicroscope Olympus ZJ61 (0.67× – 4,5×) with 10× eyepiece (6.7× – 45×). The length of each fiber was measured with the ImageJ 1.53 software (Cossi et al., 2021) using graph paper as a reference.

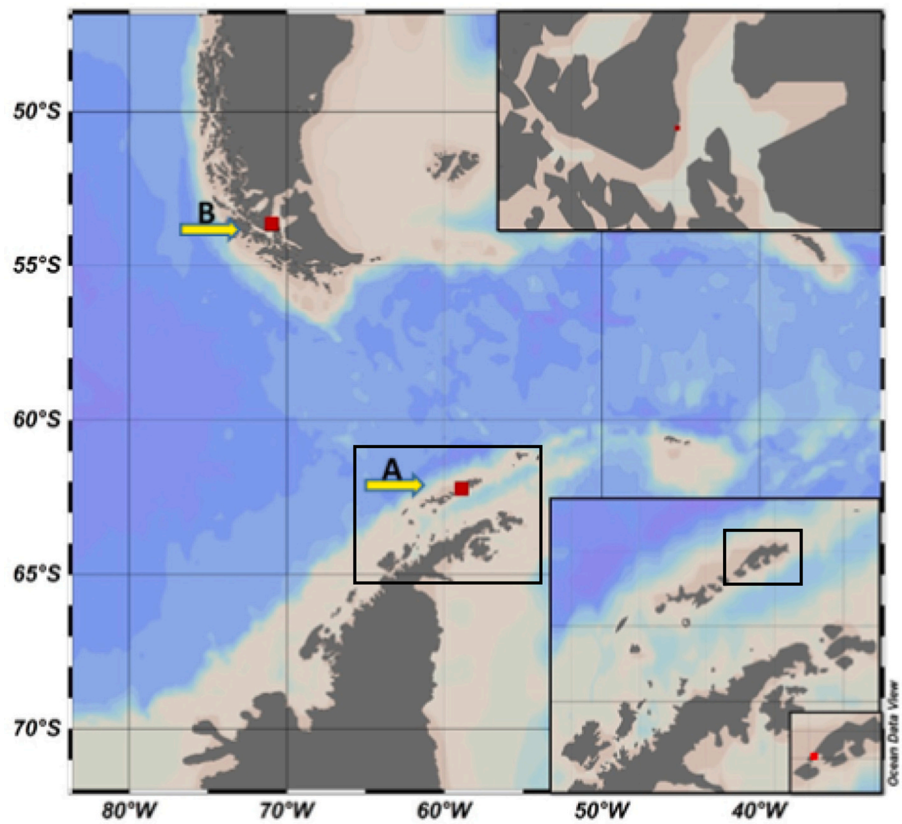


Fig. 1. A) King George Island where *H. antarcticus* were collected in the Fildes Bay, B) The Bulnes Fort where *H. bispinis* were collected, 50 km away from the regional capital Punta Arenas. Red dots represent the sampling site. Maps were made in Ocean Data View 4. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.6. Spectral analysis (Micro-FTIR)

Potential MPs, carefully isolated from *H. bispinis* and *H. antarcticus* samples were sent to the "*Laboratorio de Fisiología Animal Comparada*" at Universidad de Concepción for Micro-FTIR analysis ($n = 42$ MFs). Fiber's spectra (Fig. 2) were obtained by diffuse reflectance, and attenuated total reflectance for fragments (Jorquera et al., 2022; Correa-Araneda et al., 2022), on a Spotlight 400 FT-IR Imaging System PerkinElmer. Microplastics were extracted and placed on a Potassium bromide disc under a stereomicroscope (Nikon SMZ18). Each isolated polymer spectra between 4000 cm^{-1} and 750 cm^{-1} (4 cm^{-1} resolution) were obtained and compared against polymer libraries (FDM ATR Polymers Germanium, Paints, Polymers, Biological Samples Sigma among others) to identify whether they were polymers and their type (only when the match was above 70 %). A baseline correction, subtracting CO_2 and humidity (H_2O) signals, was performed automatically on the equipment.

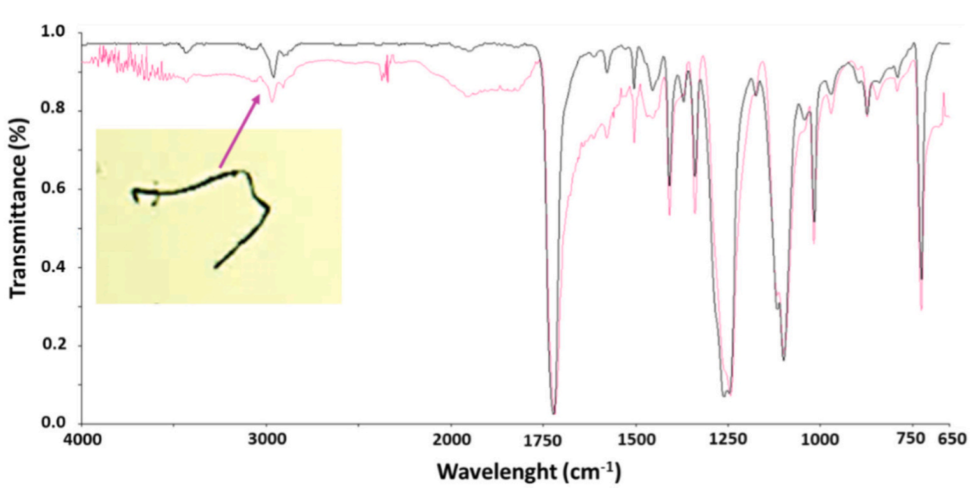


Fig. 2. Spectrum (Pink) of a blue fiber found in *H. bispinis* (sample HUB 66), compared with a polyethylene terephthalate (PET) reference spectrum (Black). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.7. Statistical analyses

The abundance of MFs in fish was expressed as the average ± standard error (SE). Assumptions of normality and homogeneity were tested for the detected variances with Shapiro and Levene's test correspondingly. The data did not follow a normal distribution. Consequently, we employed the *Kruskal-Wallis Test* to identify any statistical differences among species and between organs using R 4.2.2 and RStudio. Following this, we conducted a *Post-hoc Dunn Test* to pinpoint which groups were statistically different. All the tests were executed using confidence intervals of 99.5 % and a *p-value* of 0.005 or less. Spearman correlation was used Number of fibers vs length of fish. Spearman correlation was used Number of fibers vs length of fish.

3. Results

3.1. Contamination measured on control plates

The control plates showed insignificant signs of atmospheric contamination during the dissection. It should not have affected microfiber count, since no fibers were found in 76 % of the control plate, 20 % of the plates reported only one blue fiber, and the rest 4 % had two blue fibers. In total 29 blue fibers were recovered from the control plates ($n = 112$) (Supplementary Table 3). Given that each sample has its own corresponding blank, the fiber count identified in the blanks was directly subtracted from the total plastic count in our presented results.

3.2. Microfiber counting

Overall, 112 fish (*H. antarcticus*, $n = 55$ and *H. bispinis*, $n = 57$) were analyzed in this study (Table 1). Fibers of multiple colors (Fig. 2) were found in 89 % of *H. antarcticus* samples and 73 % of *H. bispinis* samples. A significant correlation between size of *H. antarcticus* and MFs ind^{-1} was observed ($R^2 = 0.13$) (Fig. 3). 358 MFs and 1 irregular shape MP were found, all the fibers extracted from the fish were mostly blue and black, with some occurrences of red, green, and violet. Color frequency was not assessed. Results show that *H. antarcticus* (3.80 ± 1.95 MFs ind^{-1}) had significantly more fibers than *H. bispinis* (1.26 ± 1.12 MFs ind^{-1}) ($p < 0.005$) (Fig. 4.a). When comparing fibers between stomach (*H. antarcticus*: 2.89 ± 1.72 MFs ind^{-1}; *H. bispinis*: 0.78 ± 0.91 MFs ind^{-1}) and intestine (*H. antarcticus*: 2.07 ± 1.27 MFs ind^{-1}; *H. bispinis*: 0.68 ± 0.88 MFs ind^{-1}) (Fig. 4.b), no significant difference was observed between organs in both the species ($p < 0.005$).

3.3. Microfiber characterization

$n = 42$ MFs were carefully isolated and identified through the Micro-FTIR assay from $n = 4$ *H. antarcticus* and $n = 4$ *H. bispinis*, individuals with the most MFs found. Cellulose was the predominant type of material, making 67.7 % and 72.7 % of the total particles isolated in *H. antarcticus* and *H. bispinis* respectively (Table 2). In contrast, the presence of polymers was significantly less, with only three found in *H. antarcticus* and two found in *H. bispinis*. Among the polymers, polyethylene terephthalate (PET) was the most common polymer with three MFs, trailed by acrylic, represented by two MFs.

A total of 24 MFs were measured in length, ranging between 0.32 and 2.2 mm, and averaging 0.94 ± 0.49 mm (Fig. 5). Most abundant MFs ranged between 0.5 and 1 mm.

Table 1
Summary of sampling data and biological aspects. SE = standard error.

Species	Sampling	Biometry		Manmade microfiber occurrence		
	n	Length (cm) range	Length (cm) average	MF mean ± SE	Min	Max
Harpagifer antarcticus	55	6.1–12	8.98	3.80 ± 1.95	0	18
Harpagifer bispinis	57	5.9–8.9	6.76	1.26 ± 1.12	0	5

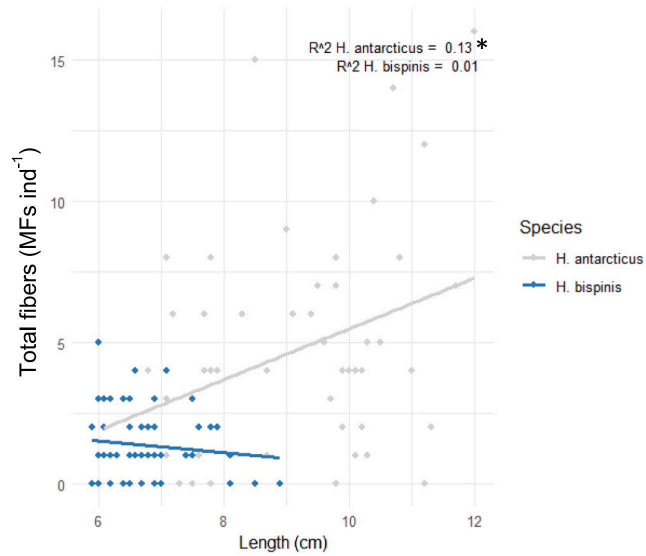
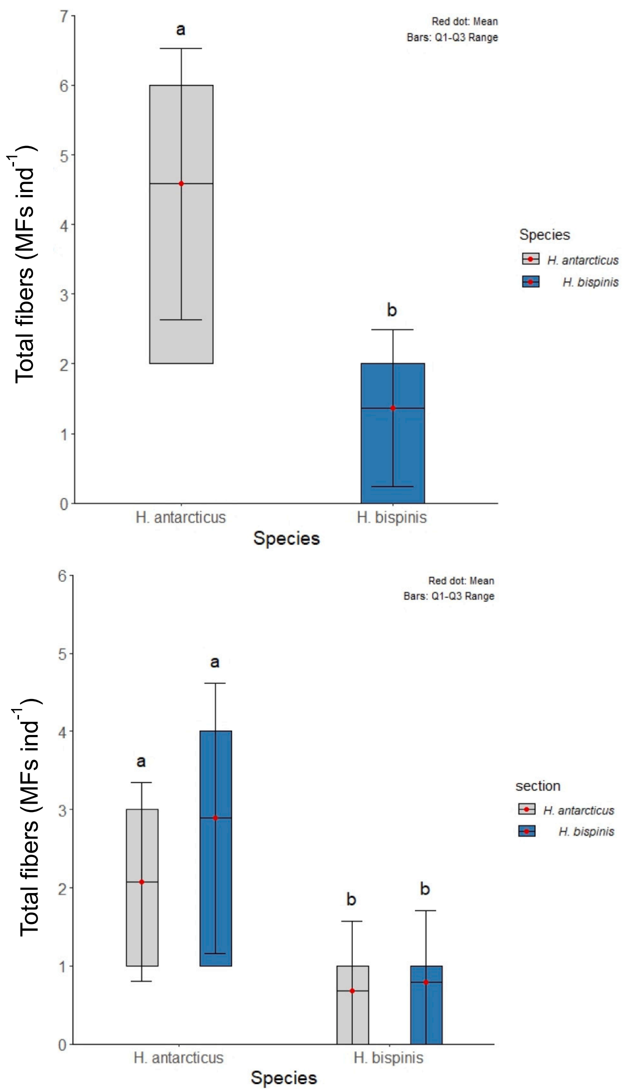


Fig. 3. Correlation analysis using Spearman Method showed a tendency of more MFs in bigger individuals in *H. antarcticus* ($p = 0.02$).

4. Discussion

The presence of plastics has been detected in seawater, freshwater, glaciers, pelagic and benthonic organisms in Antarctic waters (González-Pleiter et al., 2021). *H. antarcticus* and *H. bispinis* eat mostly blue and black fibers. This is consistent with other studies assessing MP count in fish gastrointestinal tract, as it has been observed that fibers are the main component of ingested MPs (Bellas et al., 2016; Halstead et al., 2018; Thiele et al., 2021), and blue/black as the prevalent colors (Peters et al., 2017; Compa et al., 2018). In Zhang et al. (2022), the gastrointestinal tract of 5 families of Antarctic *Perciformes* were analyzed. Results showed that in the Amundsen Sea, MF size was similar to that observed in this study (0.5–1 mm), but in the Ross Sea, the finest fibers (<0.2 mm) were dominant. Also, near several research stations in the Ross Sea, the rayon fibers were the most abundant, with up to 30 % of the MPs detected. They were also the second most abundant polymer in the suborder *Nototheniidae* (that averaged 0.83 items individual^{-1}). However, the notothenіids fish in this study have no coastal-benthic behavior, and they are less exposed to MFs than *H. antarcticus* in Fildes Bay, as it has been noted that intertidal species are especially susceptible to MP and MF pollution (Ehlers et al., 2022; Klein et al., 2022). This vulnerability arises from the considerable mixing of waters, which can intensify the resuspension of particles that have accumulated in sediments (Wright et al., 2013; Mizraji et al., 2017). Pinho et al. (2022) studied the MP uptake of 142 individuals of *Urobatis halleri* in the Gulf of California. They found an average of 2.72 individual^{-1} items with a total of 386 MPs, of which 381 were fibers, and 61 % were blue. Even though they are completely different fish species, our results have a similar tendency, where benthic fish consume massive amounts of fibers instead of other kinds of MPs (Halstead et al., 2018; Neves et al., 2015). This explains the similarity in MFs of *H. antarcticus* with this species, compared with the other members of the *Nototheniidae* family in Antarctic waters (Zhang et al., 2022). All benthic fish found in an estuarine river in Goa, India had MPs (1.4 ± 0.3–7.8 ± 4.4 items individual^{-1}). The water column samples had 48 ± 19.4 MPs L^{-1}, while the sediment samples had 3950 ± 930 MPs kg^{-1} (Saha et al., 2021). Fibers are also the



a.

b.

Fig. 4. a. Microfiber ingestion of the two species sampled (*H. antarcticus/ H. bispinis*). The average of total microfibers counted in the gastrointestinal tract of fishes with corrections made with control plates analysis is shown as the mean ± SE (n = 112). Different letters show statistical differences between species (Kruskal-Wallis Test, $p < 0.005$); b. Average microfibers counted in the stomach and intestine in the two species with corrections made after the micro-FTIR analysis. Different letters indicated statistical differences between tissues and species (Kruskal-Wallis Test; Dunn Test, $p < 0.005$).

Table 2
Summary of particle types following micro-FTIR analysis.

	H. antarcticus	*H. bispinis*
Cellulose	21	8
PET	2	1
Acrylic	1	1
Hair like/wool	1	1
Undetermined	6	0
Total	31	11

dominant shape of all plastic samples in this study. With a similar QA/ QC method, Nadal et al. (2016) reported ingestion in 68 % of *Boops boops* sampled around the Balearic Islands (Mediterranean Sea), with 3.75 ± 0.25 MFs ind.$^{-1}$, which is very close to our results on *H. antarcticus*. The studies mentioned above derive from densely human-populated zones, and major fiber contamination comes from city dust,

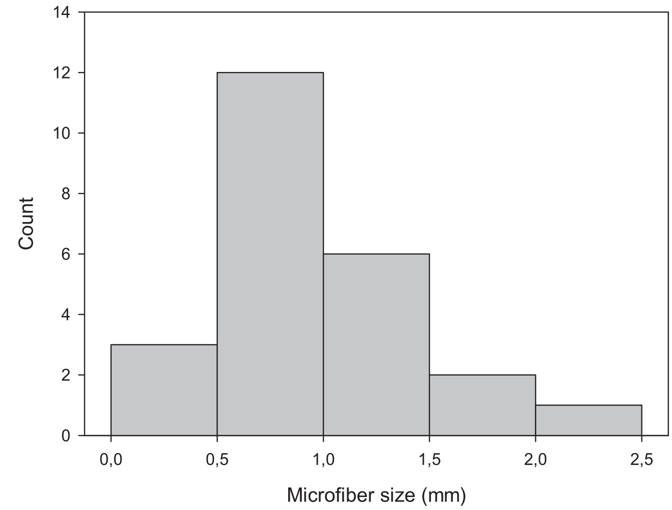


Fig. 5. Histogram of 24 microfibers measured by length using the ImageJ software. To measure the size of MFs, a sub-sample of 24 MFs from $n = 12$ *H. antarcticus* and n = 4 *H. bispinis* were imaged with a digital camera attached to the stereomicroscope.

airborne deposition and discharged wastewater (De Falco et al., 2020). *H. antarcticus* in Fildes Bay had over 4 times more fibers than *H. bispinis* in the Strait of Magellan, near the city of Punta Arenas. Signs of major contamination were also observed in other studies in Fildes Bay (Perfetti-Bolaño et al., 2022; Tapia et al., 2021). In Cossi et al. (2021), in two species of sea stars near Falkland Islands (*Henricia obesa* and *Odontaster penicillatus*), fibers were predominantly blue, with a mean length of 555.18 ± 354.81 μm ($n = 23$), and all of them were semisynthetic cellulosic fibers, that were associated with clothing textiles through sewage discharges (Cossi et al., 2021).

Most fibers floating in the world's oceans are not plastic but are dyed cellulose (Suaria et al., 2020). Cellulose is considered biodegradable, but little is known about their degradation rate in water and inside aquatic organisms (Henry et al., 2019; Zambrano et al., 2020). Zhao et al. (2016) proposed that a possible path inside the organism is to be digested, since fewer natural and semisynthetic fibers were found in the intestine than in the stomach of terrestrial birds. This pattern was also observed in our work, as more MFs were found on average in the stomach than in the intestine of *H. antarcticus*. Natural and manmade semisynthetic MFs are processed and dyed with chemicals, and they can still adsorb and be vectors of additives and bacterial films in organisms (Zambrano et al., 2020; Athey and Erdle, 2022; Bergami et al., 2023). Faster degradation also increases the bioavailability risk of these pollutants in their bodies (Zhao et al., 2016). Nevertheless, few studies have been made about the toxicological effects of natural and regenerated cellulose fibers (Mateos-Cárdenas et al., 2021). The level of attention given to these MFs is lower than their plastic counterparts because they are "biodegradable" as they have been found to endure in marine ecosystems for extended periods, potentially spanning several decades. (Athey and Erdle, 2022). Studies that count natural and semisynthetic fibers in animal gastrointestinal tract have a lot of variation, and a tendency is not clear yet, as standardized methods are still being developed for recovery rates of synthetic and non-synthetic materials (Athey and Erdle, 2022). Overall, half of the studies considering natural and semisynthetic MFs reported more of these MFs than synthetic ones in samples (Athey and Erdle, 2022). In the sub-sample sent to the FTIR, the number of cellulose fibers in this study was 69 % (sub-sample of $n = 42$ fibers of 8 fishes). Natural and semisynthetic forms of cellulose are hard to distinguish in Micro-FTIR assays (Comnea-Stancu et al., 2017; Suaria et al., 2020); therefore, they were counted as a single "cellulose" group for comparative purposes. Bergami et al. (2023) assessed up to 47 % of their fibers as cellulose in the Antarctic sea snail *Neobuccinum eatoni*. In the feces of King

Penguins, on South Georgia island, natural fibers comprised up to 88 % of MFs (Le Guen et al., 2020). In Compa et al. (2018), *Sardina pichauris* mostly ingested MPs in blue microfibers, but, *Engraulis encrasicolus* primarily ingested natural blue MFs (mostly cotton). The authors suggest that this difference may be due to different foraging strategies. Woodall et al. (2014) stablished that half of the plastic in deep marine sediments were semisynthetic rayon fibers in their samples. In Song et al. (2015), up to 85 % of fibers initially counted as MPs in water samples, were cotton and cellulosic fibers. They suggest that natural, and non-synthetic manmade fibers are a major marine anthropogenic debris, and as suggested in further studies (See Athey and Erdle, 2022; Belzagui et al., 2021; Bergami et al., 2023; Henry et al., 2019) they should also be considered as part of the MP problem (Song et al., 2015).

QA/QC protocols were made to assure no airborne contamination. No white fibers that could have come from cotton lab coats were found in samples or control plates. Transparent fibers were not counted. Since there was no digestion method, it wasn't possible to assure that the transparent fibers observed were not organic debris, so they weren't counted at all. Textile cellulose fibers are a major component of MFs in indoor environments such as offices and laboratories (Dris et al., 2017). Since only 29 blue fibers were recovered from 112 control plates, we suggest that airborne contamination was minimum, but each fiber was discounted from its correspondent sample. We encourage future studies to use strict QA/QC protocols, as the lack of standardization of the protocols for MP extraction is the main issue for comparing studies on plastic contamination (da Costa et al., 2022; Justino et al., 2022). The methods to enumerate and characterize MFs in the environment were originally designed to recover synthetic materials (Athey and Erdle, 2022). Compa et al. (2018) suggest that the method of chemical digestion to find microplastics in the gastrointestinal tract of fishes does not consider natural and cellulosic fibers, that can be destroyed in this process (Compa et al., 2018). Thus, recent studies targeting polymers generate a false absence of natural and semisynthetic fibers (Henry et al., 2019). Studies show that H_2O_2 at even 15 % *v*/v caused degradation of rayon and natural MFs (Prata et al., 2020), and 10 % KOH (*v/w*) 60 °C resulted in a major degradation of PET MFs (Treilles et al., 2020). Thiele et al. (2021) argued that the KOH method could not recover small MPs in fishmeal for aquaculture. With their proposed method of NaCl density separation, 62.5 % of fibers recovered were rayon. Jones-Williams et al. (2020) searched for MPs in Antarctic and Sub-Antarctic amphipods. With an excellent QA/QC protocol, they discarded up 31 % of fibers inside pelagic amphipods in Antarctic waters, as they were cellulosic. They highlighted that it was a necessary step to not take in account natural fibers in the search of MPs (Jones-Williams et al., 2020). Since MFs are a new trending pollutant, two divided paths of investigation are developing. The determination of these pathways hinges on the quantification of both semi-synthetic and natural microfibers, which makes comparisons of such discoveries challenging (Comnea-Stancu et al., 2017). The results might vary if alternate methods such as KOH, or H_2O_2 digestion were employed, specifically in the search for MPs. This study tried to consider natural and semisynthetic fibers, discovered and characterized inside Antarctic fish, by referring to MFs (including cellulosic ones) as the major pollutant. More comparative studies between fiber types, and the ecotoxicological implications cellulose could have, are necessary to know if they are harmless to organisms. As the composition of only 42 out of 358 fibers has been confirmed by Micro-FTIR, results and interpretations must be taken with care. Our study results emphasize the need for chemical analysis to explore and confirm fiber composition. The proportions of the composition of these fibers inside the fish are closely related to the high number of cellulosic fibers found in water samples all over the world, including the Southern Ocean (Suaria et al., 2020). Since cellulose is denser than water (Zhu et al., 2019), it is expected to sink close to the source, which makes it available for benthic feeders such as *Harpagifer antarcticus* for consumption. Fish inhabiting between 230 and 600 m deep on the Brazilian coast contain higher MP amounts than shallower dwellers, likely due to water/MP densities (Justino et al., 2022). We suggest that the results in this study are a sign of major pollution on the sampling site, Fildes Bay. Major human settlements and activities, such as tourism and scientific bases, are being developed in the Fildes Peninsula that could generate plastic debris (González-Pleiter et al., 2021). In Tapia et al. (2021), the highest concentrations of heavy metal pollutants in Fildes Bay were at the sites near human settlements.

Textile fibers, one of the most abundant MFs, are being reported near several Antarctic research stations (Bergami et al., 2023; Le Guen et al., 2020; Reed et al., 2018; Zhang et al., 2022; Tirelli et al., 2022). The prevalence of multi-colored fibers, along with a majority of cellulose fibers and other microplastics frequently employed in textile production, such as PET and acrylic, discovered with Antarctic organisms (Wilkie Johnston et al., 2023), implies a substantial influx of MFs into the shallow coastal waters surrounding King George island, most likely originating from sewage discharge and atmospheric deposition occurring from human activities on the island (Browne et al., 2011; Comnea-Stancu et al., 2017; Gao et al., 2019; De Falco et al., 2020; Prata et al., 2022; Napper et al., 2023). that both could possibly be occurring from scientific bases and tourism ships, as other studies are reporting major contamination in remote islands (Cossi et al., 2021; Bergami et al., 2023). MFs will persist in shallow waters and are vastly eaten by coastal benthic organisms (Halstead et al., 2018; Neves et al., 2015). However, there is not much information about wastewater treatment in research Antarctic stations, or their ability to retain textile fibers or MP from wastewaters (Bergami et al., 2023). There are also few studies of atmospheric deposition and wind-derived microfibers in the Antarctic continent, but it has been widely seen as a primary vector for MFs transport (Alurralde et al., 2022), and Allen et al. (2019) confirmed that MFs can travel up to 100 km reaching non-populated environments. More information is needed to properly stablish a source of microfibers vastly reported in King George Island ecosystems (González-Pleiter et al., 2021; Perfetti-Bolaño et al., 2022). In the study from Perfetti-Bolaño et al. (2022) they searched for microplastics in Antarctic soils and intertidal sediments near several Antarctic scientific stations, in Fildes Bay. In the two intertidal sampling points, near the station effluent and locally close from our collected *H. bispinis*, major fiber contamination was seen, where 50 %–67 % of them were identified as cellulose, and the remaining fibers were identified as PET (Perfetti-Bolaño et al., 2022). In Alurralde et al. (2022), MFs in sediment traps were detected in King George Island, also 83 % of MFs characterized were semisynthetic cellulose. These two studies on the island, that have found cellulose, acrylics and PET in the same proportion as we did, is a very good hint that our results in *H. antarcticus* are valid, calling for attention on microfiber contamination sourced from scientific stations surrounding Fildes Bay and tourism ships that visits the island every summer.

Attempts to establish Antarctic Specially Protected Areas (ASPAs) in the island have been made, but with little international support (Padeiro et al., 2016). Dialogue between bases and tourism companies must start in order to reduce the impacts on the local environment and resident species and communities that inhabit King George Island. (Padeiro et al., 2016). The scientific effort demonstrated the exponentiality of the plastic problem in Antarctic waters, a pollutant that is already summing on other anthropogenic disturbances affecting the biota in the southernmost continent (Caruso et al., 2022). This study's results urge larger efforts to prevent and repair human-derived contamination on King George Island. They also present the importance of evaluating the natural and semisynthetic MF pollution problem, their degradation rate in nature and animals, the pollutants they could adsorb, and the potential toxicity they could represent to Antarctic organisms.

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CRediT authorship contribution statement

M.E., D.F., K. P., J. M. N., and L.V.-CH. Conceptualization of this study

M.E., D.F., K. P., M. A. U., and L.V.-CH.: Assays using different methodology

M.E., D.F., K. P., M. A. U., and L.V.-CH.: Analysis of data

M.E., D.F., K. P., M. A. U., J. M. N., and L.V.-CH.: To write the draft

M.E., D.F., K. P., M. A. U., J. M. N., and L.V.-CH.: To check the final version

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2023.115380.

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