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Micropollutants in the deep sea: influence of feeding mode on microplastic intake by benthic organisms and detection of persistent organic pollutants in biological and sediment samples

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Corrected Version

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To all the little critters down in the deep dark sea.

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RESUMO

STEFANELLI SILVA, Gabriel. **Micropoluentes no mar profundo**: influência do modo alimentar na ingestão de microplásticos em organismos bentônicos e detecção de poluentes orgânicos persistentes em amostras biológicas e de sedimento. 2024. Tese (Doutorado) – Instituto Oceanográfico, Universidade de São Paulo, São Paulo, 2024.

O mar profundo já foi considerado a última fronteira para a chegada dos impactos antrópicos no oceano. Estudos recentes, realizados sobretudo no hemisfério norte, já constatam lixo marinho e poluentes químicos em organismos, no sedimento e na coluna d'água desse que é o maior ecossistema do planeta. Considerando os esforços recentes para documentar a poluição oceânica de profundidade no hemisfério sul, o primeiro capítulo deste trabalho mostra que plásticos já estavam presentes na comunidade bentônica do continente Antártico ainda na década de 1980, sendo apresentado aqui o registro mais antigo de microplásticos no continente, proveniente de invertebrados depositados em coleções biológicas. Os níveis de contaminação são similares aos obtidos em estudos que analisaram amostras mais recentes e em profundidades mais rasas, possivelmente indicando uma entrada consistente de microplásticos há pelo menos quatro décadas na região. Já no segundo capítulo, este trabalho relata o primeiro registro de microplásticos e poluentes orgânicos persistentes em uma comunidade bentônica de invertebrados de profundidade e no sedimento associado no Atlântico Sudoeste, especificamente na Bacia de Santos. Em ambos os capítulos, organismos que se alimentam da matéria orgânica depositada sobre o sedimento foram os que mais ingeriram microplásticos tanto em quantidade de partículas quanto em termos de frequência de ingestão, indicando o papel dessa guilda como concentradora de poluentes ambientais.

Palavras-chave: Polímeros sintéticos. POPs. Detritivoria. Oceano Austral. Oceano Atlântico Sudoeste.

ABSTRACT

STEFANELLI SILVA, Gabriel. **Micropollutants in the deep sea:** influence of feeding mode on microplastic intake by benthic organisms and detection of persistent organic pollutants in biological and environmental samples. 2024. Tese (Doutorado) – Instituto Oceanográfico, Universidade de São Paulo, São Paulo, 2024.

The deep sea has been known as the last marine environment to face the impacts of human action. Recent studies, carried out predominantly in the northern hemisphere, have found marine debris and chemical pollutants in the fauna, sediments and water column of this ecosystem which is the largest on Earth. Considering ongoing efforts to document deep-sea pollution in the southern hemisphere, the first chapter in this thesis shows that microplastics have been present in the benthic community of Antarctica since the 1980s, presenting here the earliest record of microplastics in the continent, originating from invertebrates deposited in biological collections. Levels of contamination are similar in specimens which were recently sampled or were originally caught at shallower depths, which could indicate a consistent entry of microplastics in Antarctica for at least the past four decades. In the second chapter, this work presents the first record of microplastics and persistent organic pollutants in a deep-sea benthic community and in the surrounding sediment in the Southwestern Atlantic, within the Santos Basin. In both chapters, organisms feeding off organic matter deposited in surface sediments had the highest ingestion values, both regarding number of particles and ingestion frequency, indicating the role of this feeding mode in the concentration of environmental pollutants.

Keywords: Synthetic polymers. POPs. Deposit-feeding. Southern Ocean. Southwest Atlantic Ocean.

LIST OF ABBREVIATIONS AND ACRONYMS

ColBIO	Biological Collection “Prof. Edmundo F. Nonato”
DFP	Dampened filter paper
μFTIR	Micro Fourier-transformed infrared spectroscopy
MP	Microplastics
PA	Polyamide
PAEK	Polyaryletherketone
PAN	Polyacrylonitrile
PBDE	Polybrominated diphenyl ether
PC	Polycarbonate
PCB	Polychlorinated biphenyl
PE	Polyethylene
PET	Polyethylene terephthalate
POP	Persistent organic pollutant
PP	Polypropylene
PS	Polystyrene
PSU	Polysulfone
PVC	Polyvinyl chloride
RV	Research Vessel
SR	Synthetic rubber
TLS	Tape lift screening

CONTENTS

1 GENERAL INTRODUCTION

1.1 Theoretical background

The issue of marine pollution is not exclusive to shallow waters. Our deep sea – the entire tridimensional region of the ocean at depths greater than 200 m (Thistle, 2003) – is one of the great silent recipients of human waste (see Woodall et al., 2014). Between the dumping of burnt coal from steamships at the height of the Industrial Revolution and the worldwide ban on overboard littering in the 1970s, the "out of sight, out of mind" approach to littering the ocean has introduced antibiotics and even radioactive material to the deep seafloor (Ramirez-Llodra et al., 2011). But far from only receiving exotic waste and following industrial developments in the 20th century, plastics are, today, one of the most ubiquitous types of refuse to reach the deep ocean (Chiba et al., 2018).

Plastics are cheap, light, durable polymers being produced at a rate of almost 400 million tons a year (Plastics Europe, 2023). Plastic particles smaller than 5 mm are known as microplastics (MPs) (GESAMP, 2019). These can be categorized as primary, i.e. purposefully manufactured to carry out a function, or secondary, i.e. resulting from the wear or fragmentation of larger objects. Upon entering the ocean, MPs may be subjected to biofouling, turbidity currents and storms (Kooi et al., 2017; Kane et al., 2020), thus becoming negatively buoyant. And once they reach the seafloor, due to their minute size, MPs may be ingested by benthic organisms.

Several studies have shown consumption of MPs by coastal benthic invertebrates including bivalves (Santana et al. 2016), crabs (Watts et al., 2015; Brennecke et al., 2015), shrimp (Devriese et al., 2015), lobsters (Murray & Cowie, 2011) and polychaetes (Setälä et al, 2016; Jang et al., 2018). Detrimental effects of MP ingestion include starvation (reviewed in Secretariat of the Convention on Biological Diversity and the Scientific and Technical Advisory Panel GEF, 2012), increased immune response (von Moos et al., 2012), lower reproductive potential (Sussarellu et al., 2016), and decreased growth rate (Huerta-Lwanga et al., 2016). However, only a few studies (Taylor et al., 2016; Courtene-Jones et al., 2017; La Beur et al., 2019) have examined MP ingestion in deep-sea benthic communities encompassing more than a single phylum and more than one feeding mode.

The physical and chemical properties of MPs also facilitate the sorption of organic contaminants, from the moment these particles are produced to well after their release into the environment (Woodwell et al., 1971; Mato et al., 2001; Hartmann et al., 2017). One such class of toxins are persistent organic pollutants (POPs): compounds invulnerable to natural degradation and highly hazardous due to their disruptive effects to hormonal balance (Teuten et al., 2009). In addition to their association with plastics, POPs are found in other matrices across all oceanic environments, contaminating biota, sediments, and water (Allchin et al., 1999).

Polychlorinated biphenyls (PCBs), used as dielectric fluids (Schulz et al., 1989), and polybrominated diphenyl ethers (PBDEs), used as flame retardants (De Wit et al., 2002), are two classes of POPs commonly found in deep-sea environments. Recent studies have shown contamination in sharks (Nakajima et al., 2022), echinoderms (Lawson et al., 2021), and sediment (Zhang et al., 2020). Given the potential for bioaccumulation of these contaminants in shallow water marine food webs (Haukås et al., 2007; Sobek et al., 2010), this trend could eventually result in human consumption of contaminated specimens caught in deep waters.

So considering how human actions have impacted regions of the ocean which are well beyond our coastal environments, the assessment of micropollutant levels in the deep sea is of the utmost importance. Thus, the aim of the collective efforts which are part of this thesis was to characterize the occurrence and concentration of two classes of such pollutants, namely MPs and POPs, in remote and undocumented environments along the deep seafloor. In order to accomplish this, we analyzed both biotic and environmental samples. Samples were obtained through the endeavors of many researchers and crew members and were subjected to thorough quality assurance/quality control protocols to minimize contamination.

1.2 Thesis description

This thesis is the product of my work at the Instituto Oceanográfico da Universidade de São Paulo, with financial support from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (grant 88882.330940/2019-01), and the Fundação de Amparo à Pesquisa do Estado de São Paulo (grant 2018/19278-2). For the past five years, I sought to determine the occurrence of microplastics and characterize their polymeric composition in samples obtained from the deep waters of the Southwest Atlantic Ocean and along the shelf off the Antarctic

Peninsula. Following the successful undertaking of my research, I have (i) studied the occurrence of MPs and POPs in the marine environment, an issue which has interested me since my early years as an undergraduate, and (ii) carried out oceanographic research centered on the deep sea, a topic which has fascinated me from a very young age. With these results, I hope to inform future research and the general public on the prevalence of micropollutants even in remote environments of the ocean. The two chapters which make up this thesis (#2 and #3) are presented in manuscript form.

Chapter two presents the earliest documented record of MPs in Antarctica, isolated from invertebrates caught in 1986 and which are deposited in the Biological Collection "Prof. Edmundo F. Nonato" (ColBIO) at the Instituto Oceanográfico. Additionally, we investigated samples belonging to the University of Hawai'i at Mānoa, which, along with ColBIO samples, result in 30 years of Antarctic research efforts. Organisms including holothurians, ophiuroids, decapods, mysids, amphipods, polychaetes and gastropods were classified according to their predominant feeding mode. Our results indicate higher quantities of ingested MPs in deposit-feeding echinoderms, as opposed to predacious and scavenging organisms. The possible implications are a lack of trophic transfer of MPs in the Antarctic deep shelf, but further studies are necessary in this environment.

Chapter three showcases the first record of MPs and POPs in biological and sediment samples from the deep waters of the Southwest Atlantic Ocean, specifically along the Brazilian continental slope. These results were made possible following two 2019 cruises to the inner portion of the Santos Basin, aboard the RV Alpha Crucis, 140 km away from the nearest coast. Regarding the sampled invertebrates, deposit-feeding echinoderms were the taxon which attained the highest level of MP ingestion. Nine of the ten sediment samples were contaminated with MPs. Polyaryletherketone (PAEK) and synthetic rubber fibers were found in both sediment and biological samples. Finally, levels of polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) were assessed in fish and sediment samples and compared with contamination reports from other deep-sea locations.

2 EARLIEST HISTORICAL RECORD OF MICROPLASTICS IN ANTARCTICA AND INFLUENCE OF FEEDING MODE ON TEXTILE FIBER INTAKE BY DEEP-SEA BENTHIC INVERTEBRATES

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Abstract

Inefficient recycling and waste management policies have yielded similar concentrations of microplastics (<5 mm) in shallow-water and deep-sea sediments, with microplastic fibers being found in all studied oceanic environments. Here, we report microplastic ingestion in benthic organisms obtained along the Antarctic Peninsula's shelf between 212 and 805 m depth, over the period of 1986 to 2016. A total of 146 fibers were found following analysis of 169 organisms. We provide the earliest documented record of microplastics in Antarctica, ingested by *Boreomysis* sp. mysids caught in 1986. Ingested polymers include polyamide, polybutylene terephthalate, polycarbonate, polyester, polyethylene terephthalate, polysulfone, polyvinyl chloride and synthetic rubber. Out of the 15 species studied, 14 ingested fibers, in 43.2% of individuals. The highest ingestion percentages were recorded in the sea cucumbers *Molpadia violacea* (n=6) (100%), *Heterocucumis steineni* (n=5) (100%), *Scotoplanes globosa* (n=4)

(75%), *Echinopsolus koehleri* (n=6) (66%) and *Protelpidia murrayi* (n=3) (66%), and in the brittle star *Amphiura joubini* (n=17) (70%). The number of ingested fibers had a tendency to be higher in deposit feeders compared to predators, indicating a possible lack of trophic transfer of microplastics and a historical occurrence of microplastics in the sediment of the world's most remote continental margin.

Keywords: synthetic polymers, marine debris, megabenthos, feeding guild, Southern Ocean.

Synopsis: Microplastic fibers were ingested by deep-sea megabenthos from different trophic levels and sampled since 1986 in the Antarctic shelf. These results renew concerns regarding human impact on this supposedly pristine environment.

2.1 Introduction

Worldwide plastic production has substantially increased from 0.5 million tons in the 1950s to close to 390 million tons in 2021 (Plastics Europe, 2023). Plastics are inexpensive, durable and versatile materials, yet over 30% of them are ultimately destined for disposal, mainly as packaging (Andrady, 2003). Microplastics (MPs), in turn, are small particles (<5 mm) which can be categorized as primary, purposefully manufactured to carry out a function, or secondary, resulting from the wear or fragmentation of larger objects (GESAMP, 2019). Because of indiscriminate waste disposal, MPs eventually reach the ocean as fibers, fragments and pellets and may be transported great distances (Barnes et al., 2009), eventually entering marine food webs.

Marine species of different sizes and niches, from salps to whales, ingest MPs (Moore et al. 2001; Besseling et al. 2015; Romeo et al., 2015), which may then cause blockage of the digestive tract, injuries to stomach lining and reduced feeding, leading to starvation (reviewed in Secretariat of the Convention on Biological Diversity and the Scientific and Technical Advisory Panel GEF, 2012). Several studies have observed consumption of these particles by coastal benthic invertebrates including bivalves (Santana et al. 2016), crabs (Watts et al., 2015; Brennecke et al., 2015), shrimp (Devriese et al., 2015), lobsters (Murray & Cowie, 2011) and polychaetes (Setälä et al, 2016; Jang et al., 2018). MPs are widely distributed in the oceans, including polar deep-sea regions in part due to their minute size (Bergmann et al., 2017; Kanhai et al., 2019; Cunningham et al., 2020).

The downward transport of MPs through the water column in marine snow, via convection and saline subduction, or even due to storm-induced mixing (Woodall et al., 2014; Kane et al., 2020) conveys MPs to the world's deepest environments as a likely final sink (Woodall et al., 2014; Lobelle et al., 2021). However, only a few studies (Taylor et al., 2016; Courtene-Jones et al., 2017; La Beur et al., 2019) have examined MP ingestion in deep-sea benthic communities encompassing more than a single phylum, and more than one feeding mode, mainly because of financial and logistic constraints. Given that detritus-based deep-sea benthic communities are dependent on sinking or advected material from the surface ocean, the issue of plastic ingestion by benthic organisms is extremely relevant, even in seemingly pristine environments such as Antarctica. Research explicitly dedicated to Antarctic MPs has only been conducted in the last five years (reviewed in Tirelli et al., 2022), despite growing interest in the study of plastic distribution and sources in this frozen continent. A recent study by Sfriso et al. (2020) is the first to assess MP ingestion in shallow-water benthic invertebrates in Antarctica.

Samples from the late 1950s constituted the first record of MP ingestion in Antarctic biota (Harper & Fowler, 1987), but true MP origin could not be determined due to the migratory nature of the model organisms in that study (*Pachyptila* spp. prions). Given the possibility of past contamination during handling, one must be careful when considering working with archived specimens. With some caveats, examination of particles obtained from inside the gastrointestinal tracts of intact specimens is a possible way to remedy methodological limitations of decades-old specimens, as effectively done by Courtene-Jones et al. (2019). Thus, our aims were (i) to detect MPs in deep-sea sediment-dwelling invertebrates from Antarctica, especially whether specimens preserved in biological collections could provide the oldest record of MPs in that marine polar region, and (ii) to verify whether the number of ingested MPs was influenced by feeding mode and body mass of the studied organisms.

2.2 Materials and methods

2.2.1 Study area and field work

Organisms were obtained during eight expeditions carried out in the austral summers of 1986, 1987, 2000, 2009, 2012, 2014, 2015 and 2016 along the Bransfield Strait and in the vicinity of Elephant and King George islands, west and southwest of Anvers Island and in Andvord Bay, and in the former Larsen A Ice Shelf (Figure 2.1). The Bransfield Strait comprises a body of water extending between the Antarctic Peninsula and the South Shetland Islands, and runs in a northeast-southwest direction. Elephant and King George are two of the South Shetland Islands, located north of the Antarctic Peninsula. Sampling near King George Island also took place within Admiralty Bay, south-southeast of the island. Anvers Island sits on the western shore of the Antarctic Peninsula, being the largest island in the Palmer Archipelago. Andvord Bay is situated east of Anvers Island, also in the West Antarctic Peninsula. Lastly, area A of the former Larsen Ice Shelf, eastern Antarctic Peninsula, is a region which has been undergoing major ice shelf break-ups since the mid-1800s.

In the 1980s, beam- (1.55 x 0.5 m, 5 mm mesh) and otter-trawls (14 m wide, 45 mm mesh) were used for benthic sampling. During cruises from 2000 and onwards, either a smaller 0.6 x 0.4 m, 20 mm mesh beam trawl, or a 0.6 x 0.4 m, 20 mm mesh Agassiz trawl were used. All collected organisms were initially stored intact in 4% or 10% formalin solution, and then in 70% alcohol in glass jars once on land. Organisms were subsequently identified by specialists. In this work, a subset of megafauna (>10 mm total length/diameter) samples obtained below 200 m were dissected and underwent analysis. For more information, see Table 2.1.

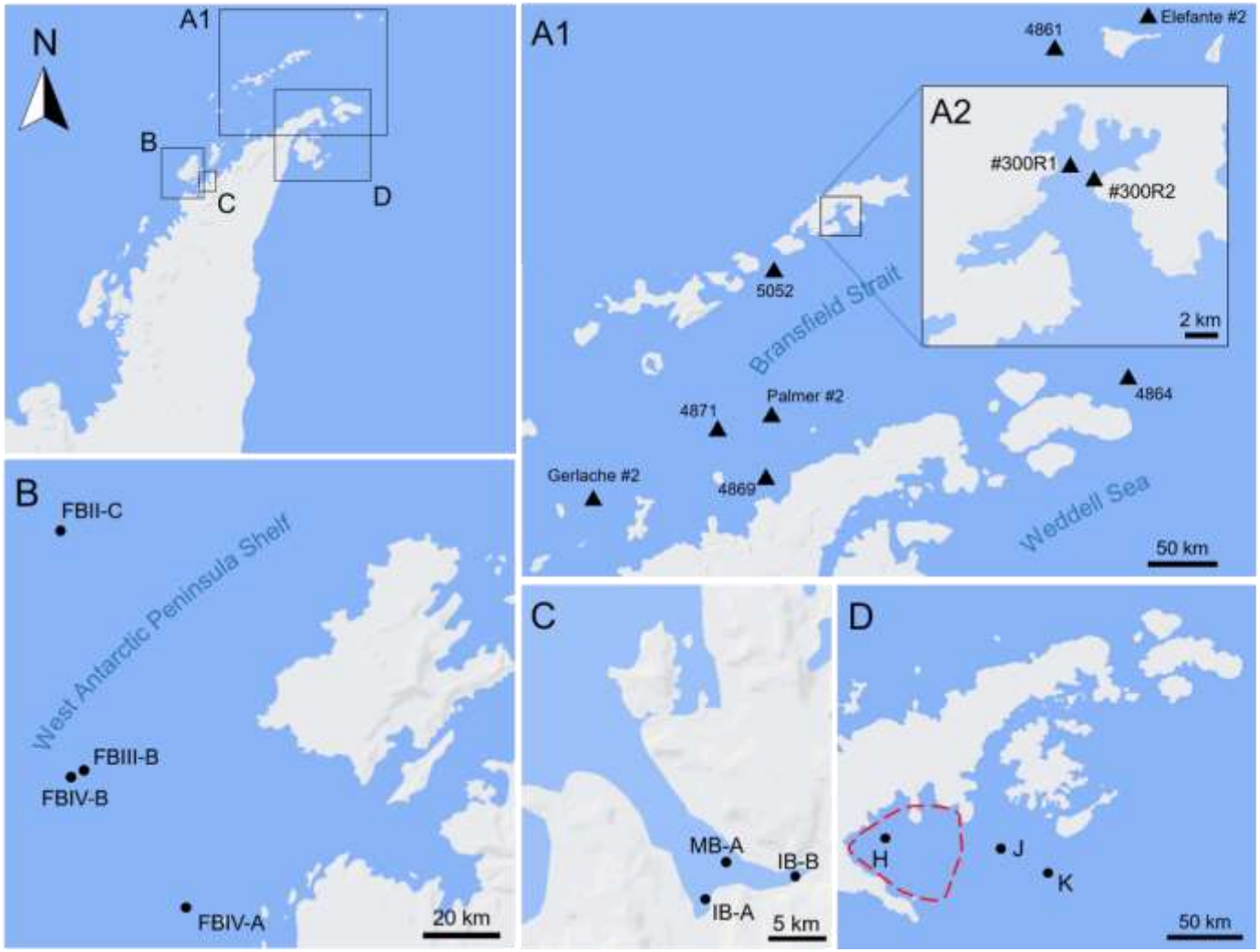

Figure 2.1. Study area depicting three decades of Antarctic research. A1. South Shetland Islands and northern Antarctic Peninsula. A2. Admiralty Bay, King George Island. B. West of Anvers Island, western Antarctic Peninsula. C. Andvord Bay, western Antarctic Peninsula. D. Tip of the Antarctic Peninsula and area of the former Larsen A Ice Shelf (dashed red polygon). Triangles indicate ColBIO (Universidade de São Paulo) sampling stations, circles indicate University of Hawai'i at Mānoa stations. More details regarding sampling stations can be found in Table 2.1.

Table 2.1. Station identifiers, coordinates, number of dissected organisms in a given station, date of collection, and trawling depth for sampled megafauna. FOODBANCS – Food for Benthos on the Antarctic Continental Shelf, MABIREH – Antarctic Marine Life: Biodiversity in Relation to Environmental Heterogeneity, LARISSA – Larsen Ice Shelf System, Antarctica, SOBE – Sistemas de Observação Bentônicos no Oceano Austral, FjordEco – Fjord Ecosystem Structure and Function on the West Antarctic Peninsula.

Station	Coordinates	Organisms (n)	Date (dd/mm/yy)	Depth (m)	Project	Vessel
4861	61°08’ S; 55°52’ W	*Echinopsolus koehleri* (Vaney, 1914), Holothuroidea (6)	01/02/86	362	Bionomy of Antarctic Benthic Fauna	RV Prof. W. Besnard
4864	63°01’ S; 54°49’ W	*Boreomysis* sp. G.O. Sars, 1869, Mysida (10)	02/02/86	275	Bionomy of Antarctic Benthic Fauna	RV Prof. W. Besnard
4869	63°33’ S; 59°15’ W	*Heterocucumis steineni* (Ludwig, 1898), Holothuroidea (5)	08/02/86	240	Bionomy of Antarctic Benthic Fauna	RV Prof. W. Besnard
4871	63°16’ S; 59°55’ W	*Chorismus antarcticus* (Pfeffer, 1887), Decapoda (6)	08/02/86	264	Bionomy of Antarctic Benthic Fauna	RV Prof. W. Besnard
5052	62°26’ S; 59°16’ W	*Notocrangon antarcticus* (Pfeffer, 1887), Decapoda (13)	24/02/87	212	Bionomy of Antarctic Benthic Fauna	RV Prof. W. Besnard
5052	62°26’ S; 59°16’ W	*Chorismus antarcticus* (Pfeffer, 1887), Decapoda (2)	24/02/87	212	Bionomy of Antarctic Benthic Fauna	RV Prof. W. Besnard
5052	62°26’ S; 59°16’ W	*Eusirus perdentatus* Chevreux, 1912, Amphipoda (3)	24/02/87	212	Bionomy of Antarctic Benthic Fauna	RV Prof. W. Besnard
5052	62°26’ S; 59°16’ W	*Laetmonice producta* Grube, 1877, Polychaeta (4)	24/02/87	212	Bionomy of Antarctic Benthic Fauna	RV Prof. W. Besnard
5052	62°26’ S; 59°16’ W	*Harpovoluta charcoti* (E. Lamy, 1910), Gastropoda (10)	24/02/87	212	Bionomy of Antarctic Benthic Fauna	RV Prof. W. Besnard
FBII-C	64°13’ S; 65°25’ W	*Protelpidia murrayi* (Théel, 1879), Holothuroidea (3)	10/03/00	550-569	FOODBANCS	RV Laurence M. Gould
FBIII-B	64°48’ S; 65°17’ W	*Scotoplanes globosa* (Théel, 1879), Holothuroidea (2)	16/06/00	609-628	FOODBANCS	RV Nathaniel B. Palmer
FBIV-B	64°49’ S; 65°21’ W	*Scotoplanes globosa* (Théel, 1879), Holothuroidea (2)	??/10/00	602-700	FOODBANCS	RV Laurence M. Gould
FBIV-A	65°07’ S; 64°42’ W	*Molpadia violacea* Studer, 1876, Holothuroidea (3)	29/10/00	444-658	FOODBANCS	RV Laurence M. Gould
#300R1	62°06’ S; 58°26’ W	*Ophionotus victoriae* Bell, 1902, Ophiuroidea (10)	11/12/09	338	MABIREH	RV Ary Rongel
#300R2	61°08’ S; 55°52’ W	*Laetmonice producta* Grube, 1877, Polychaeta (6)	11/12/09	316	MABIREH	RV Ary Rongel
K	64°58’ S; 57°46’ W	*Ophiosparte gigas* Koehler, 1922, Ophiuroidea (5)	22/03/12	422-445	LARISSA	RV Nathaniel B. Palmer
H	64°48’ S; 60°12’ W	*Ophionotus victoriae* Bell, 1902, Ophiuroidea (5)	31/03/12	510-592	LARISSA	RV Nathaniel B. Palmer
J	64°39’ S; 58°20’ W	*Ophiosparte gigas* Koehler, 1922, Ophiuroidea (5)	07/04/12	542-543	LARISSA	RV Nathaniel B. Palmer
Palmer #2	63°12’ S; 59°17’ W	*Molpadia violacea* Studer, 1876, Holothuroidea (3)	14/01/14	805	SOBE	RV Almirante Maximiano
Elefante #2	60°55’ S; 54°52’ W	*Amphiura joubini* Koehler, 1912, Ophiuroidea (7)	17/01/14	734	SOBE	RV Almirante Maximiano
Elefante #2	60°55’ S; 54°52’ W	*Nematocarcinus lanceopes* Spence Bate, 1888, Decapoda (4)	17/01/14	734	SOBE	RV Almirante Maximiano
Gerlache #2	63°41’ S; 61°22’ W	*Amphiura joubini* Koehler, 1912, Ophiuroidea (10)	20/01/14	749	SOBE	RV Almirante Maximiano

IB-B	64°52’ S; 62°26’ W	*Ophionotus victoriae* Bell, 1902, Ophiuroidea (10)	06/12/15	557	FjordEco	RV Laurence M. Gould
IB-A	64°53’ S; 62°34’ W	*Ophionotus victoriae* Bell, 1902, Ophiuroidea (10)	16/12/15	534	FjordEco	RV Laurence M. Gould
MB-A	64°51’ S; 62°34’ W	*Notocrangon antarcticus* (Pfeffer, 1887), Decapoda (10)	07/04/16	530	FjordEco	RV Nathaniel B. Palmer
MB-A	64°51’ S; 62°34’ W	*Chorismus antarcticus* (Pfeffer, 1887), Decapoda (5)	07/04/16	530	FjordEco	RV Nathaniel B. Palmer
MB-A	64°50’ S; 62°35’ W	*Notocrangon antarcticus* (Pfeffer, 1887), Decapoda (10)	24/04/16	433-450	FjordEco	RV Nathaniel B. Palmer

2.2.2. Biological collections

Our research takes advantage of samples obtained as early as 1986 and catalogued in two biological collections: the Biological Collection “Prof. Edmundo F. Nonato” (ColBIO) at the Instituto Oceanográfico of the Universidade de São Paulo, obtained via the Brazilian Antarctic Expeditions, as well as samples belonging to the University of Hawai’i at Mānoa and collected by Prof. Craig Smith. Fifteen species were analyzed in our work. These were selected according to availability of multiple organisms per station and the largest possible variation in sampling locations and years. Feeding guilds were determined according to the dominant feeding mode following the literature for these species. When records were unavailable, feeding strategy was inferred based on literature records from either the same species found elsewhere or evidence from congeneric species.

2.2.3. Laboratory work

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

To minimize contamination, samples were handled in an isolated laboratory. During analysis, the air conditioner was turned off, and doors and windows remained shut. All surfaces on the worktable were cleaned with 70% alcohol on non-shredding paper three times prior to analysis. Natural fiber clothes were worn under a clean cotton laboratory coat which never left the laboratory. Primarily metal and glass instruments were used, with as little plastic apparatus as possible. All instruments were thoroughly rinsed with ultrapure water (resistivity of 18.2 MΩ·cm, Thermo Scientific Barnstead Easypure II) prior to being used. Tape lift screenings (TLS) were employed to check for background fibers on the table's surface during each work session: following alcohol cleanup, three 5 x 4 cm (60 cm2 total surface) pieces of clean adhesive tape were randomly placed face down on the table and any particles found were kept for further analysis (see next section). Additionally, a single fiberglass filter (Whatman, 47 mm in diameter, 0.7 μm pore size) dampened with ultrapure water (dampened filter paper, DFP) was placed in a Petri dish during each work session to check for airborne contamination, and any particles found were kept for further analysis. Both protocols were based on Woodall et al. (2015).

2.2.3.2 Microplastic isolation

Invertebrates were first rinsed with ultrapure water and placed on a clean Petri dish. They were then precautiously weighed while still dampened (see Supplementary material) using a precision scale (Shimadzu UX620H), as measured mass of preserved specimens may be highly variable due to ethanol evaporation (minimized here by room temperature being kept constant), and then photographed. Photographs were later used for measurements of total length (most organisms) or central disk length (ophiuroids) using ImageJ 1.53n (National Institutes of Health). Whole gastrointestinal tracts – or central disk content in the case of ophiuroids – were carefully removed with small incisions so not to cause excessive damage to the preserved specimens, and then analyzed under a stereomicroscope (Nikon SMZ-1B).

All particles found within the organic content as well as with TLS and DFP protocols were photographed and then measured using ImageJ. The identification key presented in Lusher et al. (2020) was followed to aid in the classification of particles as possibly anthropogenic, and all particles were classified by shape, size, color and elasticity. Following characterization, particles were wrapped in fiberglass filters held shut using a metal paperclip and immersed in 15% hydrogen peroxide (H_2O_2) (Êxodo) for eight days to remove excess

organic matter (Zhao et al, 2017), before drying off in an oven (60 °C, Nova Ética 400 ND). Finally, particles were transferred to an Anodisc filter membrane (Whatman, 25 mm, 0.02 µm) and individualized in a Petri dish.

2.2.3.3 Microplastic identification

Raman spectra were obtained using either an alpha300 R (WITec) microscope or an inVia (Renishaw) microscope with varying laser wavelengths and objective lenses (633 nm/50 x; 785 nm/100 x, respectively). µFTIR spectra were obtained using a HYPERION (Bruker Optics) microscope in the 1250-4000 cm^{-1} range, coupled with a 400-4000 cm^{-1} KBr beamsplitter and a DLaTGS high sensitivity detector. Spectra were registered with either 128 or 256 accumulations and a resolution of 4 cm^{-1}. Finally, µFTIR spectra were also obtained via ATR mode with germanium as the reflection element, in the 700-4000 cm^{-1} range, with 64 accumulations.

2.2.4 Statistical analysis

We tested whether specimen size and feeding mode could predict the number of ingested particles. We had the option to choose mass or length data to describe size, but we decided to only include length in the analysis given the two variables were correlated, and to avoid problems related to possible variations in the weight of preserved specimens. We tested for normality using the Shapiro-Wilk test and used a log transformation to decrease bias as length was not normally distributed. Generalized Linear Models (GLMs) were conducted to test the response variable (number of ingested particles) as a function of feeding mode and length, using the Poisson distribution. Single- and multi-predictor models were used by: 1. taking into account only length or feeding mode as the predictor variable, and 2. taking into account length plus feeding mode as predictor variables, respectively. We determined outliers in the number of particles by using Grubbs' test, and models were conducted with and without the outliers. We detected one outlier in the number of particles (G=6.395, U=0.750, P=6.191e-10), from one of the largest specimens. Pair-wise differences regarding feeding modes were

then tested using a post-hoc Tukey's test. All statistics were carried out in R 4.1.1 (R Core Team, 2022).

2.3 Results

2.3.1 QA/QC

The contamination controls were 100% composed of fibers. Number of fibers found using both DFP and TLS protocols was 1.04±0.17 (mean ± S.E.) per day. Following immersion in H_2O_2, control fibers were totally degraded or became susceptible to crushing under pressure. Thus, these were considered organic, with the exception of ten fibers, which underwent polymeric determination. These were classified as polyacrylonitrile (PAN), polyamide (PA) and polyester blend, polyamide and polypropylene (PP) blend, polycarbonate (PC), polyester, polyethylene (PE) (n=2 fibers), polysulfone (PSU), polypropylene, and synthetic rubber (SR). Contamination was accounted for by subtracting MP candidates that shared similar characteristics from each daily batch.

2.3.2 Microplastics in deep-sea invertebrates

A total of 148 particles were recovered from 74 of the 169 (43.79%) sampled organisms. From these particles, 146 were classified as fibers, corresponding to 98.6% (n=100) of particle shapes (Figure 2.2), and the other two (1.4%) being fragments. Fiber morphology and color are shown in Table S2.1 in accordance with the visual identification key. Fibers in megafauna were mostly blue (53.4%, n=78), followed by black (19.9%, n=29), green (10.3%, n=15), red (8.2%, n=12), white (3.4%, n=5), light blue (2%, n=3), blue/white (2.1%, n=3) and orange (0.7%, n=1). Fragments were yellowish. Following H_2O_2 immersion, while fibers displayed malleable and elastic behavior, fragments showed a propensity to crushing under pressure and were not considered possible plastics.

Fibers ranged in size from 0.17 to 5.91 mm, and most fibers were placed within the MP range, i.e. smaller than 5 mm (99.87%, n=144) (Figure 2.3). Length was used as the standard measurement for most fibers. Only two fibers exceeded the 5 mm threshold, but these were also grouped as possible MP candidates by using diameter measurements instead. Even though diameter is not usually considered the most appropriate measurement for the fibrous shape – due to a fiber's elongated nature and small diameter not accurately representing its true size -, we chose this approach for the sake of simplicity. Therefore, taking into account their maximum Feret's diameter, i.e. length of the shortest line joining two points of an object, these two above-mentioned fibers larger than 5 mm were considered MPs. Finally, smaller fibers were more abundant and >90% of fibers were smaller than 3.5 mm in length. Overall mean fiber length was 1.36±0.09 mm across all species.

Fifty-seven fibers ingested by invertebrates were subjected to FTIR/Raman spectroscopy. Nine polymers were identified across samples, and all of them classified as plastics (n=14). The remaining fibers were either unidentified, identified as natural or yielded dye spectra. Polymers were identified as PA (n=3), PA and polyester blend, PAN, PC, PC and polyethylene terephthalate (PET) blend (n=3), PET, PET and polybutylene terephthalate (PBT) blend, PSU, polyvinyl chloride (PVC), and SR. MPs found in the control treatments did not match confirmed MP occurrences in the examined specimens within each sampling day, with the exception of a PAN fiber found in *Amphiura joubini*. Thus, this single occurrence was not considered in our work. Polyester (in this generic form, further identified as PET, or even as a PET blend) was the most common polymer in our samples, present in five fibers, followed by PA, which occurred in four fibers.

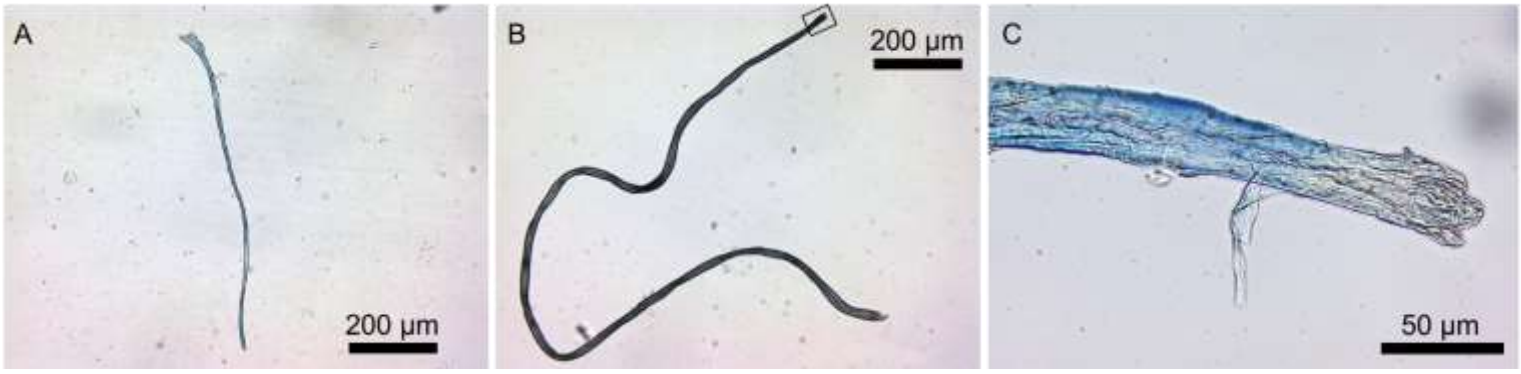

Figure 2.2. Microplastics ingested by deep-sea Antarctic invertebrates. A. Aromatic polyamide and polyester blend fiber found in one individual of the sea cucumber *Molpadia violacea* (Echinodermata: Holothuroidea) sampled south of Anvers Island, western Antarctic Peninsula, in October 2000. B. Polycarbonate fiber found in one individual of the brittle star *Ophiosparte gigas* (Echinodermata: Ophiuroidea) sampled in the area of the former Larsen A shelf, eastern Antarctic Peninsula, in April 2012. C. Close-up of image 2B showing details of the fiber's tip.

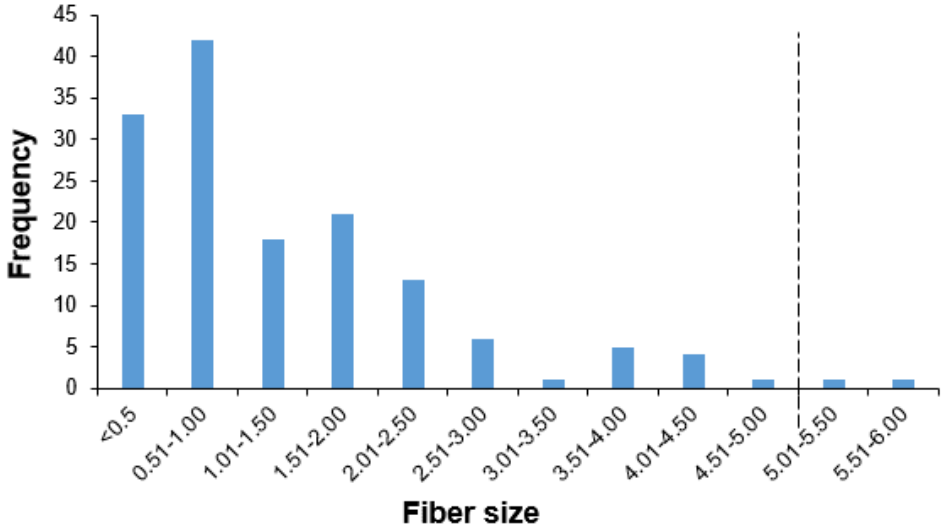

Figure 2.3. Size distribution of fibers (length, in mm) isolated from Antarctic invertebrates. The dotted line delimitates the upper limit for classifying fibers as putative microplastics (<5 mm).

The organisms studied here are generally considered predators (39%), deposit feeders (38.5%), suspension feeders (12.4%) and scavengers (10%). Sea cucumbers and brittle stars concentrated 78% of fibers (n=114), 54 and 60 fibers respectively. All *Heterocucumis steineni* and *Molpadia violacea* sea cucumber individuals had at least one fiber within their gastrointestinal tracts. Only one individual was found to have ingested fibers in *Chorismus antarcticus* (n=13) shrimps, *Harpovoluta charcoti* (n=10) gastropods and *Laetmonice producta* (n=10) polychaetes. Conversely, *Eusirus perdentatus* (n=3) amphipods had no fibers within their digestive tracts.

Invertebrate mass (0.26-58.22 g) and size ranges (10.79-229.73 mm), feeding modes, number of fibers per individual and polymer type are shown in Table 2.2. The oldest confirmed MP records were obtained from *Boreomysis* sp. mysids sampled on February 2nd, 1986, which ingested PET, PSU and PSU fibers. Out of the four feeding modes analyzed, deposit/suspension-feeding yielded the highest values for number of ingested fibers (in descending order) in *M. violacea* (4.17±1.4 fibers/ind), *Protelpidia murrayi* (2±1.15 fibers/ind), *Scotoplanes globosa* (1.75±0.75 fiber/ind), *Echinopsolus koehleri* (1.5±0.67 fiber/ind), *H. steineni* (1.4±0.24 fiber/ind) and *A. joubini* (1.29±0.33 fiber/ind). The lowest values were associated (in ascending order) with the predacious shrimp *C. antarcticus* (0.08±0.08 fiber/ind), the predacious polychaete *L. producta* (0.17±0.1 fiber/ind), the scavenger gastropod *H. charcoti* (0.4±0.4 fiber/ind), and the predacious shrimp *Notocrangon*

antarcticus (0.42±0.18 fiber/ind). Average fiber ingestion for all invertebrate species was 0.86±0.11 fiber/ind.

The single predictor models with and without the outlier were similar (Table 2.3). The model considering only length showed no association with the number of ingested fibers. The single-predictor model with "feeding mode" showed that feeding strategy was associated with the number of ingested fibers. In the models including the outlier, the only significant difference found occurred between deposit-feeding and predation (Tukey's post hoc test: $p<0.001$), the former having the highest number of ingested fibers. Meanwhile, in the model without the outlier, predation was significantly different from both deposit and suspension-feeding (Tukey's post hoc test: $p<0.001$). The multi-predictor models followed a similar pattern, in which the number of ingested fibers was associated with feeding mode and not with invertebrate size. Comparable results were found for the multi-predictor model when we excluded the specimen with the highest number of ingested fibers, a *M. violacea* individual which ingested 10 fibers. Figure 2.4 depicts the variation in the number of ingested fibers across all feeding modes surveyed in our study, with each species represented by a unique color. Deposit feeders ingested 63.7% of MPs, and displayed the highest variation in MP ingestion.

Table 2.2. Antarctic deep-sea specimens and their ingested fibers. Fibers are shown in relation to corresponding invertebrate species, year of sampling, species mass and length ranges, feeding strategy, number of fibers found per species, percentage of individuals which ingested at least one fiber, number of fibers ingested per individual, and plastic polymer type, when identified. PET = polyethylene terephthalate, PVC = polyvinyl chloride, PSU = polysulfone, PC = polycarbonate, PA = polyamide, SR = synthetic rubber, PAN = polyacrylonitrile, PBT = polybutylene terephthalate. + indicates polymer blend, * indicates occurrence not considered in our work.

Species (n)	Year	Mass (g)	Length (mm)	Feeding mode	Fibers (n)	Fiber(s)/ind (mean ± S.E.)	Ingestion (%)	Polymer
Echinopsolus koehleri (6)	1986	1.12-3.08	23.18-31.26	Suspension feeder (Massin, 1982; Moura, 2009)	9	1.5±0.67	66	-
Boreomysis sp. (10)	1986	0.65-1.00	52.37-66.92	Filter feeder, occasional predator (Fanelli et al., 2009)	7	0.7±0.34	40	PET, PSU, PVC
Heterocucumis steineni (5)	1986	2.65-9.14	30.78-59.40	Suspension feeder (Fraser et al., 2004)	7	1.4±0.24	100	PET+PC
Notocrangon antarcticus (33)	1987, 2016	2.3-11.9	69.18-134.23	Predator (Clarke, 1979)	14	0.42±0.18	33	-
Chorismus antarcticus (13)	1987, 2016	2-7.65	84.18-101.30	Predator (Gorny, 1992)	1	0.08±0.08	7.7	-
Harpovoluta charcoti (10)	1987	3.94-12.55	32.46-50.59	Scavenger (Siciński et al., 2011)	4	0.4±0.4	10	PA
Protelpidia murrayi (3)	2000	14-19.4	49.11-73.71	Deposit feeder (Mincks et al., 2008)	6	2±1.15	66	-
Scotoplanes globosa (4)	2000	22.5-50.7	56.26-91.26	Deposit feeder (Millet et al., 2000)	7	1.75±0.75	75	PA, SR
Molpadia violacea (6)	2000, 2014	4.7-58.22	67.23-229.73	Deposit feeder (Féral & Magniez, 1985; Hudson et al., 2004)	25	4.17±1.4	100	PA+polyester
Ophionotus victoriae (35)	2009, 2012, 2015	0.66-9.4	13.81-35.47	Deposit feeder, occasional predator (Dearborn, 1977; Kellogg et al., 1982; Fratt & Dearborn, 1984)	33	0.94±0.22	48	PET+PC
Laetmonice producta (6)	2009	0.26-37.24	20.54-173.12	Predator, occasional scavenger/deposit feeder (Day, 1967; Hutchings, 2000; Parapar et al., 2013)	1	0.17±0.1	16	-
Amphiura joubini (17)	2014	0.81-2.22	10.79-18.45	Deposit feeder (Buchanan et al., 1964; Mansour et al., 2005)	22	1.29±0.33	70	PAN*, PET+PBT
Ophiosparte gigas (10)	2012	6.7-19.4	31.46-42.22	Predator, occasional scavenger (Arnaud, 1970; Dearborn et al., 1996)	5	0.5±0.17	50	PA, PC
Nematocarcinus lanceopes (4)	2014	3.05-4.63	111.18-119.73	Scavenger, occasional predator (Allen et al., 2000; Bluhm et al., 2002)	5	1.25±0.95	50	-

Table 2.3. GLMs conducted in our study to test for the influence of animal size and feeding mode on the number of fibers ingested by deep-sea Antarctic invertebrates. We include the single and multi-predictor models. We also show testes with and without the outlier. Details indicate excluded outliers and asterisks denote statistical significance.

Model	Parameters	df	*F* test	*p*	Individuals included details
1. Fibers ~ log(length)	log(length)	1	0.001	0.973	All individuals included
	Residuals	163	-		
2. Fibers ~ log(length) + feeding	log(length)	1	0.001	0.97	All individuals included
	Feeding mode	4	11.301	<0.001*	
	Residuals	159	-	-	
3. Fibers ~ feeding	Feeding mode	3	8.14	<0.001*	All individuals included
	Residuals	165	-	-	
4. Fibers ~ log(length)	log(length)	1	1.344	0.248	Without the outlier
	Residuals	162	-	-	
5. Fibers ~ log(length) + feeding	log(length)	1	1.57	0.212	Without the outlier
	feeding	3	10.11	<0.001*	
	Residuals	159	-	-	
6. Fibers ~ feeding	Feeding mode	3	8.505	<0.001*	Without the outlier
	Residuals	164	-	-	

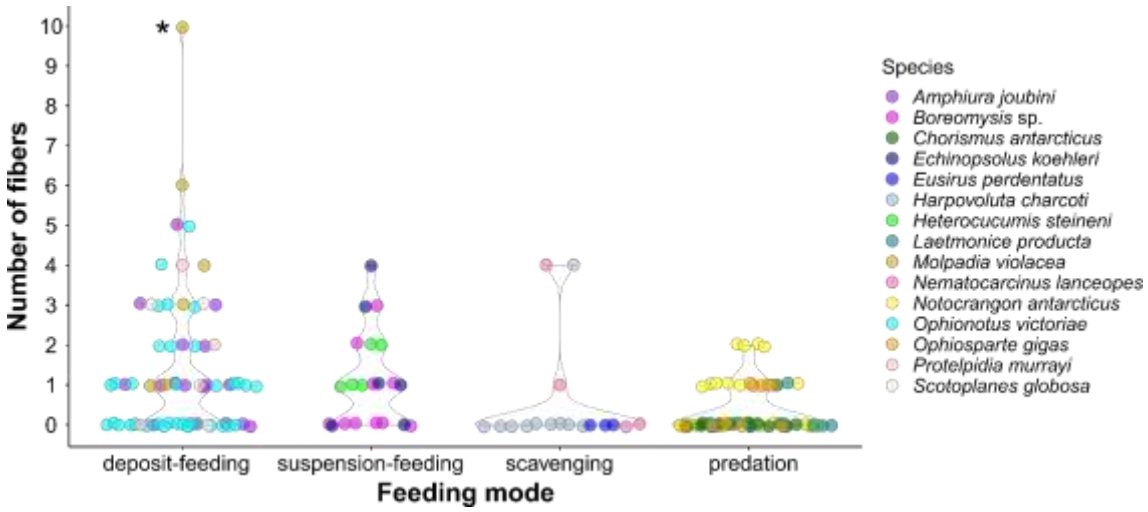

Figure 2.4. Scatterplot of the number of fibers found across the four feeding modes commonly associated with sampled benthic invertebrates from the Antarctic shelf. Each circle represents one individual and the number of fibers found in their stomach. The color of the circles indicates the species. The asterisk indicates the outlier.

2.4 Discussion

We confirm the presence of MPs in deep-sea Antarctic benthic invertebrates originally collected between 1986 and 2016 and available in the biological collections at

the Universidade de São Paulo and the University of Hawai'i at Mānoa. The work of curators and researchers in providing precious environmental insights even decades after sampling and the importance of biological collections (discussed in Borges et al., 2022) is evidenced by our results, which show the oldest record of MPs found in deep-sea benthic organisms from Antarctica. Our results also indicate a prevalence of fibers in this marine environment and offers more data on the widespread impact of human activities even in seemingly pristine regions. This study thus: (i) provides data on the occurrence of MPs in the deep-sea invertebrate megafauna of the Southern Ocean, and (ii) highlights deposit feeders as the group of benthic organisms ingesting the largest quantity of MPs dispersed in the sediment, giving further evidence on the lack of trophic transfer of MPs, given low ingestion rates in predators and scavengers.

Information on the feeding ecology of invertebrate species is critical to marine pollution studies, being more common for commercial species and fish (e.g. Lusher et al., 2013; Van Cauwenberghe & Janssen, 2014; Devriese et al., 2015). Deposit-feeding was the feeding mode which concentrated the highest portion of MPs (63.7%), a similar result found by Bour et al. (2018) in a Norwegian fjord and Rios-Fuster et al. (2022) in the western Mediterranean Sea. Contrary to Bour et al. (2018), however, we did not observe a comparably high percentage of MP ingestion in (epi) benthic predators, which could be related to differences in food type and abundance, and availability of MPs between that region and our study area. Echinoderms, represented by the sea cucumbers *M. violacea*, *P. murrayi*, *S. globosa*, *H. steineni* and *E. koehleri*, and the brittle stars *O. victoriae*, *A. joubini* and *O. gigas*, contained 78% of all fibers found in our study, further indicating the role of this phylum as a reservoir for historical anthropogenic impacts, as seen in Courtene-Jones et al. (2019).

Molpadia violacea was the most contaminated species in our study, both in terms of number of ingested fibers (4.17±1.4 fibers/ind) and percentage of affected individuals, with a 100% ingestion rate. These results come as no surprise given how holothurians are capable of concentrating MPs available in the sediment (Mohsen et al., 2019; Plee & Pomory, 2020) and may even selectively ingest MPs (Graham & Thompson, 2009; Renzi et al., 2018). While studies on the ingestion of MPs by deep-water benthic organisms in Antarctica are scarce, Sfriso et al. (2020) reported a maximum value of 1.9 MP items/ind for the suspension-feeding bivalve *Thyasira debilis*, and an overall average ingestion of 1.0 MP/ind across bivalves, gastropods, amphipods, polychaetes and actinarians. Similarly, Bergami et al. (2023) reported 0.3±0.53 textile fiber/ind in the Antarctic whelk

Neobuccinum eatoni. Such studies did not evaluate ingestion of MPs by holothurians, but values can still be compared, with *M. violacea* ingesting over twice the maximum amount in the most contaminated species in these earlier studies.

Conversely, predacious species consistently showed the lowest values of MPs per individual, despite being as numerous as the deposit feeders in our samples. The highest ingestion percentage in this guild was observed in *O. gigas*, which is the only opportunistic predacious echinoderm in our study. A likely reason could be its ventral-facing mouth in intimate association with the seafloor, whereby small amounts of contaminated sediment could be ingested while this brittle star forages on other benthic organisms. We found no apparent transfer of MPs between trophic levels in the benthic megafauna from the deep waters surrounding the Antarctic shelf, same as reported for other benthic marine organisms in high latitudes (Bour et al., 2018; Sfriso et al., 2020; Bergami et al., 2023) and those from a well-known deep-sea food web (Hamilton et al., 2021). These field observations offer some interesting insights, especially when compared to laboratorial settings, where predacious crustaceans have been shown to accumulate MP particles ingested by their prey (Farrell & Nelson, 2013; Setälä et al., 2014; reviewed in Avio et al., 2017). Regarding the lower levels of MP in scavengers and suspension feeders, our results could be biased by the low number of analyzed specimens for these particular guilds.

The dominance of fibers in our MP samples is no surprise given the previously documented environmental prevalence of such items. Woodall et al. (2014) showed that fibers were up to four orders of magnitude more abundant in deep-sea sediments than in contaminated sea surface waters, and fibers are indeed generated by many aspects of everyday life (Owen et al., 1992; Grieve & Biermann, 1997; Palmer & Burch, 2009). Given how studies in Antarctica usually focus on samples collected in proximity to scientific stations (reviewed in Rota et al., 2022), MP fibers are likely originating from laundry-released wastewater. Textile fibers have also been found in sediments (Munari et al., 2017; Waller et al., 2017; Reed et al., 2018; Cunningham et al., 2020) and water samples (Absher et al., 2019; Suaria et al., 2020). Regarding MPs in Antarctic marine organisms, fibers were the most common MP shape found by Bergami et al. (2023), Johnston et al. (2023) and Zhu et al. (2023), while Sfriso et al. (2020) reported ingestion of fragments.

Particle shape and size are also relevant when considering organism morphology. The largest fibers were found in sea cucumbers and brittle stars (≤5.91 mm), while smaller

ones were retained in the crustaceans (≤2.6 mm). Gut morphology in crustaceans, the presence of a gastric mill, and a complex filtering system of setae could be an impediment for the passage of larger fibers from the stomach to the intestine, as found by Welden & Cowie (2016) and Dawson et al. (2018) in laboratory studies, and Cau et al. (2020) in the field. This is possibly why larger fibers could not be found in crustaceans analyzed in our study. Conversely, echinoderms possess very distinct functional morphologies. In case of the sea cucumbers studied here, *M. violacea* grab sediment particles with their digitated tentacles, while *E. koehleri* use their dendritic tentacles to capture suspended particles. Regarding the studied brittle stars, their features favor either deposit feeding, scavenging, or both. Therefore, the morphological characteristics of the echinoderms included here, and their corresponding feeding mechanisms could explain the presence of the largest MP fibers found in their guts.

Polyester was the most prevalent material found in our samples. PET, a thermoplastic in the polyester family, is the most common constituent of MP fibers according to a global survey on surface waters (Suaria et al., 2020), and was likewise the principal material found ingested by gastropods in Terra Nova Bay (Bergami et al., 2023). Our second most common polymer, polyamide, was also the most abundant material found in zooplanktonic Antarctic krill and salps sampled around South Georgia (Johnston et al., 2023) and in the macrobenthic invertebrate community of Terra Nova Bay (Sfriso et al., 2020). Effluents from research stations and ships appear to be the main source of MPs in Antarctic environmental samples (Gröndahl et al., 2009; Jones-Williams et al., 2020; Suaria et al., 2020), but ocean and atmospheric circulation also play a role in the transport of MP fibers to the Southern Ocean (Waller et al., 2017; Aves et al., 2022; Cunningham et al., 2022).

This study shows, to the best of our knowledge, both the first and oldest occurrence of MPs in deep-water biological samples in maritime Antarctica. Our findings indicate that microplastics may have reached waters – and sediments – from multiple locations in the Southern Ocean even before the 1980s. Future work should continue to assess MP ingestion in organisms from distinct feeding modes and trophic levels, such as predators, scavengers and suspension feeders. This would provide a fuller view on how feeding strategies contribute to plastic intake, and which organisms could be more affected by MP pollution in deeper waters in Antarctica. MPs are also capable of adsorbing persistent organic pollutants and carrying plasticizers whose effects on the endocrine system have been known for decades (Haukås et al., 2007; Teuten et al., 2009;

Sobek et al., 2010; Wang et al., 2023). Finally, this dataset is a testimony to the broad scale of anthropogenic impacts on remote ecosystems and demonstrates the value of archiving and documenting biological samples for posterity.

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3 CONTAMINATION OF DEEP-SEA BENTHIC FAUNA AND SEDIMENTS BY MICROPLASTICS AND PERSISTENT ORGANIC POLLUTANTS IN THE SOUTHWEST ATLANTIC OCEAN

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Abstract

Micropollutants are a pervasive threat to our ocean. Here, we present the first report of microplastics (MPs) and persistent organic pollutants (POPs) in the deep waters of the Southwest Atlantic Ocean. Ingestion of MPs by benthic invertebrates and contamination by POPs in deep-sea fish and sediment samples were recorded along the southwestern Brazilian continental margin, within the Santos Basin, between 400 and 1503 m depth. A total of 64 MPs, all fibers, were extracted from nine invertebrate species and ten sediment samples. Eighteen individuals ingested at least one fiber, resulting in a 24.65% ingestion rate. Fibers were found in nine of the ten sediment samples. Detected polymers include polyamide (PA), polyacrylonitrile (PAN), polyaryletherketone (PAEK), polyester, polyimide (PI), polypropylene (PP), polystyrene (PS) and synthetic rubber. The holothurian *Deima validum validum* was the most contaminated species in our study, with 2.36±0.75 fibers/individual, and an ingestion rate of 63.63%. Ingestion of fibers, especially by sediment dwellers, is a natural consequence of the ubiquitous prevalence of MPs along the seafloor, even in deep waters. In fish muscle samples, total polychlorinated biphenyls (PCBs) ranged from 519.29 to 7636.36 ng/g lw; tetra- and penta-PCBs were the main contributors to contamination. Regarding sediment samples, total PCBs ranged

from 1.28 to 3.96 ng/g dw; tri- and tetra-PCBs were the main contributors to contamination. Prevalence of lower-chlorinated PCBs in fauna and sediment samples points to an off-site source of contamination. Finally, among the seven polybrominated diphenyl (PBDE) congeners investigated, only BDE-47 and BDE-99 were found in fish samples. PBDE concentration was below the quantitation limit across all sediment samples. PBDE congeners found in our study are the main components of the pentabromodiphenyl ether commercial mixture, with both congeners displaying a greater bioavailability in relation to other PBDE components.

Keywords: synthetic polymers; toxins; marine litter; deposit feeding; Santos Basin.

3.1 Introduction

Despite being a source of myths and mysteries for as long as our species has existed, the deep sea – the entire tridimensional region of the ocean at depths greater than 200 m (Thistle, 2003) – is no strange to human waste (see Woodall et al., 2014; Chiba et al., 2018). Between the dumping of burnt coal from steamships at the height of the Industrial Revolution and the worldwide ban on overboard littering in the 1970s (London Convention), the "out of sight, out of mind" approach to littering the ocean has introduced domestic waste, antibiotics, and even radioactive material to the deep seafloor (Ramirez-Llodra et al., 2011).

Plastics, for instance, are one of the ubiquitously notorious types of refuse continually reaching the ocean through urban runoff (Frias et al., 2014) and illegal dumping (Law et al., 2020). These are cheap, light, durable polymers with seemingly endless potential produced at a rate of almost 400 million tons a year (Plastics Europe, 2023). Deleterious effects of plastics such as entanglement and smothering (see Kühn et al., 2015) are obvious when considering larger, more conspicuous organisms; the interaction between smaller critters and even smaller plastics, however, is especially concerning and not as commonly addressed (Wright et al., 2013a).

Upon reaching the ocean's surface, microplastics (MPs) – particles smaller than 5 mm in their longest dimension (GESAMP, 2019) – may be incorporated into marine snow and subjected to biofouling, turbidity currents and storms (Kooi et al., 2017; Kane et al., 2020), thus becoming negatively buoyant. Once MPs eventually reach the ocean's

deepest environments (Van Cauwenberghe et al., 2013), their ingestion by bottom-dwelling organisms becomes a possible pathway. Such a phenomenon was first observed recently (Taylor et al., 2016), but has been ongoing for several decades (Courtene-Jones et al., 2019) and quite likely since the global dissemination of plastics in the mid-twentieth century. The detrimental effects of ingested MPs include increased immune response (von Moos et al., 2012), lower reproductive potential (Sussarellu et al., 2016), and decreased growth rate (Huerta-Lwanga et al., 2016), albeit in laboratory conditions.

The high area-volume ratio and hydrophobic nature of MPs also facilitate the sorption of contaminants from the moment these particles are produced to well after their release into the environment (Woodwell et al., 1971; Mato et al., 2001; Hartmann et al., 2017). One such class of toxins are persistent organic pollutants (POPs): compounds invulnerable to natural degradation and highly hazardous due to their disruptive effects in hormonal balance (Teuten et al., 2009). But far from being exclusively found associated with plastics, POPs are found in all oceanic environments, contaminating biota, sediments, and the water, having entered the environment via industrial discharge, landfill leakage, or waste incineration (Allchin et al., 1999).

Polychlorinated biphenyls (PCBs), used as dielectric fluids (Schulz et al., 1989), and polybrominated diphenyl ethers (PBDEs), used as flame retardants (De Wit et al., 2002), are two classes of POPs commonly found in deep-sea environments. Recent studies have shown contamination in sharks (Nakajima et al., 2022), echinoderms (Lawson et al., 2021), and sediment (Zhang et al., 2020). Given the potential for bioaccumulation of these contaminants in shallow water marine food webs (Haukås et al., 2007; Sobek et al., 2010), this trend could eventually result in human consumption of contaminated specimens caught in deep waters.

Considering the importance of determining levels of contamination in deep-sea benthic organisms and sediments obtained from the southwestern Atlantic continental slope, our objectives were (i) to determine the occurrence and characterize the composition of microplastics in the benthic megafauna and soft sediments in the deep waters of the Santos Basin, the largest offshore sedimentary basin in Brazil, and (ii) to determine the types and concentration of PCBs and PBDEs found in both fauna and sediment samples.

3.2 Materials and methods

3.2.1 Study area and sampling procedure

The study area encompasses two subareas in the south-southeastern Brazilian continental slope, off the coasts of São Paulo and Santa Catarina (Figure 3.1, Table S3.1), within the Santos Basin, southwestern Atlantic. Sampling depth varied between 400 and 1503 m. Sediment collection (top 10 cm layer) was carried out via box corer (OSIL, area of 0.25 m^2/Ocean Instruments, area of 0.25 m^2) and megafauna collection was done via bottom trawling. Once on board, all samples were secured within an aluminum bucket and then wrapped in pre-muffled aluminum containers/foil (550 °C, 5 hours) before being frozen at -20 °C. All collection efforts took place on board the RV Alpha Crucis as part of the BIOIL (Biology and Geochemistry of Oil and Gas Seepages, SW Atlantic, Shell Petróleo Brasil LTDA and Agência Nacional do Petróleo), and DEEP-OCEAN (Diversidade E Evolução de Peixes de Oceano Profundo, FAPESP 2017/12909-4) projects. Megafaunal feeding guild was determined according to the dominant feeding mode following the literature for this region. For the remaining species, the diet was inferred based on literature records from either the same species found elsewhere or evidence from congeneric species. Collection permits were issued by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBIO; #28054) and the Secretaria da Comissão Interministerial para Recursos do Mar da Marinha do Brasil (SECIRM; Portaria #223) to MRSM. Handling of megafauna was approved by the Instituto Oceanográfico's Comitê de Ética em Uso de Animais (CEUA-IOUSP) – authorization #008-Pesq. to GSS.

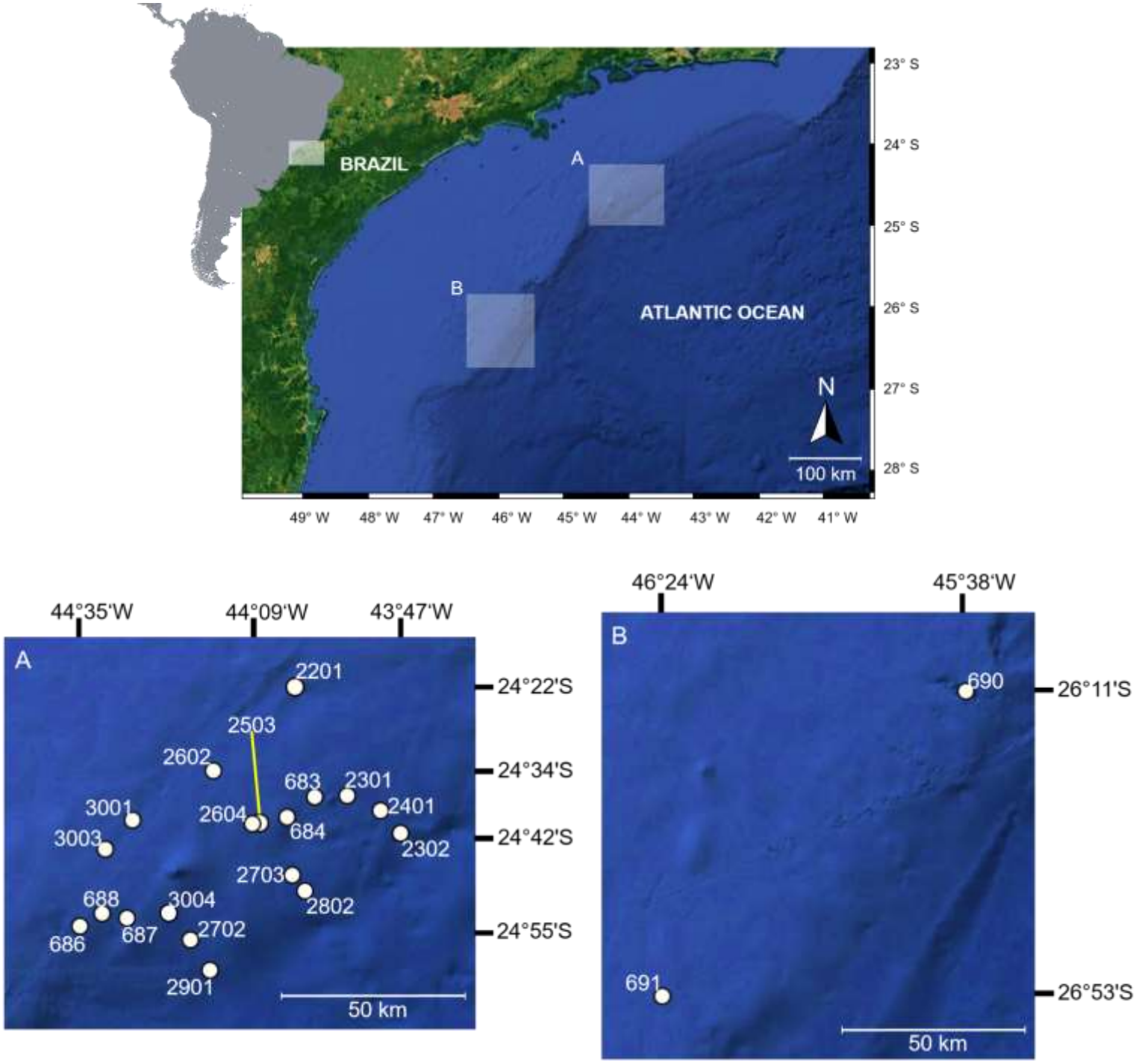

Figure 3.1. Map depicting the two subareas surveyed (A and B) in central Santos Basin, along the southwestern Brazilian continental margin, investigated in September and November 2019 aboard the RV Alpha Crucis. White circles indicate sampling sites, white numbers are site identifiers.

3.2.2 Microplastic isolation

Back on land, laboratory procedures were carried out after fauna samples thawed out. Only invertebrates were considered for this analysis. The whole gastrointestinal tract

– or central disk content in the case of asteroids – was carefully removed from each specimen and analyzed under a stereomicroscope (Nikon SMZ-1B). All particles found within the organic content were placed in Anodisc (Whatman, 25 mm in diameter, pore size 0.02 μm) membranes individualized in Petri dishes.

Sediment samples were also freeze-dried and homogenized with a mortar and pestle (Fries et al., 2013). Then, a subsample (up to 175 g per sample) was introduced to a 2000 ml separatory funnel filled with a saturated solution of NaCl (food grade, 1.2 g/cm^3) prepared with ultrapure water (resistivity of 18.2 MΩ cm, Thermo Scientific Barnstead Easypure II). The funnel was vigorously agitated to ensure homogenization, and left to settle for one hour. The deposited sediment in the lower half of the solution was drained off, and the entire process was repeated twice more. Lastly, the funnel was rinsed with 1000 ml of ultrapure water, and the content of the funnel was vacuum-filtered through a Whatman® fiberglass filter (47 mm in diameter, 0.7 μm pore size). Filters were dried at room temperature and, if present, their resulting particles were transferred to a Whatman® Anodisc filter membrane (25 mm, 0.02 μm) and individualized in a Petri dish.

3.2.3 Quality assurance/quality control (QA/QC)

To minimize contamination, samples were handled in an isolated laboratory, according to the protocol suggested by Woodall et al. (2015), as follows. During analysis, the air conditioner was turned off, and all doors and windows remained shut. All surfaces on the worktable were cleaned with 70% ethanol on non-shredding paper three times prior to analysis. Natural fiber clothes were worn under a clean cotton laboratory coat which never left the laboratory. Whenever possible, metal and glass instruments were used, with minimum use of plastic utensils. All instruments were thoroughly rinsed with ultrapure water prior to being used.

Tape lift screenings (TLS) were employed to check for background particles on the table's surface during each work session: following ethanol cleanup, three 5 x 4 cm (60 cm^2 total surface) pieces of clean adhesive tape were randomly placed face down on the table and any particles found were kept for further analysis. Additionally, a single fiberglass filter (Whatman, 47 mm in diameter, 0.7 μm pore size) dampened with ultrapure water (dampened filter paper, DFP) was placed in a Petri dish during each work

session to check for airborne contamination, and any particles found were also kept for further analysis.

3.2.4 Microplastic identification

Raman spectra were obtained via a LabRAM HR Evolution HORIBA microscope with varying laser wavelengths (473 nm, 532 nm, 633 nm, and 785 nm) and a 50x long-range objective lens (NA=0.55). Resistance of particles to each laser type was tested so as not to damage samples, and laser wavelength and power – as well as integration time, number of accumulations, slit diameter and detector sensitivity – were optimized accordingly. Following optimization, the detector was checked for saturation over the entire spectral collection width, and signals were obtained within 1-3 min. A filter was employed to identify and automatically remove eventual spikes. An additional filter and a baseline correction algorithm were applied via LabSpec 6 and MATLAB 9.13.0 (The MathWorks Inc, 2022). Spectra were identified using the KnowItAll® database with a minimum similarity index of 60%, excluding artifact regions.

3.2.5 POP extraction and analysis

The following protocol was adapted from MacLeod et al. (1986). After dissection procedures, a subsample of muscle (approximately 10 g per individual) was removed from ten fish specimens and freeze-dried. Approximately 0.25 g of each freeze-dried muscle sample was further desiccated with sodium sulphate, and then homogenized and ground with mortar and pestle. Samples were then extracted in a Sohxlet apparatus with a 50% (v/v) hexane-dichloromethane mixture for eight hours. Solvent purity followed the requirement for “organic residue analysis” by Panreac Química S.L.U. For sediment samples, PCBs and PBDEs were extracted for eight hours with a 50% (v/v) hexane-dichloromethane mixture following UNEP (1992) and Combi et al. (2013). Before the extraction, a 100 µl mixture containing PCB-103 and 198 surrogate standards (1 ng/µl) were added to the blanks, samples and reference material for both tissue and sediment samples (organics in whale blubber NIST® SRM® 1945 and organics in marine sediments NIST® SRM® 1941b, respectively).

For tissue samples, the evaporated extract was analyzed using a chromatography column containing 8 g of silica gel over 16 g of alumina (both Merck), 5% deactivated with pre-extracted water (five times) eluted with n-hexane, and 1 g of sodium sulphate (J. T. Baker) on top. Elution was achieved with an 80 ml mixture of n-hexane and dichloromethane (50%). For further purification, the eluate was concentrated to 0.9 ml and injected in an Agilent Technologies HPLC equipped with two exclusion columns (gel permeation). Dichloromethane was used as the mobile phase. The eluate was further concentrated, and the tetrachloro-m-xylene standard was added. Final volume was 1 ml. Similarly, for sediment samples, the extract was purified in a glass chromatography column containing 3.2 g of deactivated alumina (5%) where PCBs and PBDEs were eluted with a 20 ml mixture of dichloromethane in n-hexane (30%, v/v).

For both sample classes, an aliquot of the final extract was injected onto the gas chromatograph equipped with a triple quadrupole mass spectrometer (GC/MS/MS) (7890/7010B, Agilent Technologies). GC/MS/MS temperatures in the injector, interface and ion source were maintained at 300 °C. A 30 m x 0.25 mm, 0.25 μm film thickness HP-5MS ultra inert (5%-Phenyl)-methylpolysiloxane column (Agilent J&W) was used. Multiple reaction monitoring (MRM) was used as the mode of acquisition. For tissue samples, oven temperature started at 50 °C for three minutes increasing at a rate of 15 °C up to 150 °C, a rate of 2 °C up to 260 °C, and a rate of 20 °C up to 300 °C, remaining constant for one minute. Likewise, for sediment samples, temperature started at 50 °C for one minute increasing at a rate of 20 °C up to 200 °C, and a rate of 10 °C up to 300 °C, remaining constant for five minutes.

Quantification was performed via the ratio between surrogates and compounds of interest, based on the analytical curves following Accustandard references, with at least five different concentrations for each group of compounds for tissue sample and at least nine different concentrations for sediment samples. The following compounds were investigated: CBs- (IUPAC No.) 8, 18, 28, 31, 33, 44, 49, 52, 56/60, 66, 70, 74, 77, 81, 87, 95, 97, 99, 101, 105, 110, 114, 118, 123, 126, 128/167, 132, 138, 141, 149, 151, 153, 156, 157, 158, 169, 170, 174, 177, 180, 183, 187, 189, 194, 195, 199, 203, 206 and 209, including the ICES (International Council for Exploration of the Sea) 7 PCB congeners, and BDEs- (IUPAC No.) 28, 47, 99, 100, 153, 154 and 183.

3.3 Results

In total, 64 particles were extracted from the nine invertebrate species surveyed here (Table 3.1) as well as the sediment samples. All particles were classified as fibers. Fiber abundance in invertebrate megafauna (n=61 individuals, n=39 fibers) varied between 0-26 ingested fibers (mean ± standard error 0.07±0.02 fiber/g total wet weight for all species). Twenty-five fibers were found in the sediment samples. Fiber abundance in the sediment varied between 0-0.11 fiber/g dry sediment (0.03±0.01 fiber). Eighteen individuals ingested at least one fiber (24.65%). Fibers were found in nine of the ten sediment samples, except for site 691. An additional 20 fibers were captured using DFP (n=15) and TLS (n=4) during screenings. Quantity of fibers found in the contamination controls varied, with three fibers being the maximum amount found for DFP (0.66±0.18 fiber), and only one for TLS (0.19±0.08 fiber). Fibers found in fauna and sediment samples were blue (n=36, 56% of total), black (n=16, 25%), white (n=5, 8%), red (n=4, 6%), green (n=2, 3%) and blue/white (n=1, 2%). QA/QC fibers were blue (n=11, 55%), black (n=4, 20%), green (n=3, 15%), blue/white (n=1, 5%) and red/black (n=1, 5%). Out of the total 84 particles, 50 were subjected to Raman spectroscopy: 18 fibers were characterized as synthetic, nine were identified as natural and the remaining ones did not produce usable polymeric data and instead yielded mostly dye spectra. All contamination control fibers that underwent analysis were classified as natural.

Table 3.1. Santos Basin megafauna, sampled along the southwestern Brazilian continental margin, (n) in relation to sampling site and trawling depth (m).

Species	Site	Depth
Acanthocarpus alexandri Stimpson, 1871 (1) (Decapoda), *Astropecten* sp. Gray, 1840 (1) (Asteroidea), *Rochinia* sp. A. Milne-Edwards, 1875 (1) (Decapoda)	2201	400
Nymphaster arenatus (Perrier, 1881) (5) (Asteroidea)	2301	1000
Deima validum validum Théel, 1879 (1) (Holothuroidea), *Nymphaster arenatus* (2) (Asteroidea)	2302	1408
Allocyttus verrucosus (Gilchrist, 1906) (1) (Zeiformes), *Deima validum validum* (3) (Holothuroidea), *Nymphaster arenatus* (4) (Asteroidea)	2401	1142
Aristaeopsis edwardsiana (Johnson, 1868) (1) (Decapoda), *Benthodesmus tenuis* (Günther, 1877) (2) (Scombriformes), *Chaceon ramosae* Manning, Tavares & Albuquerque, 1989 (1) (Decapoda)	2503	655

Chaceon ramosae (1) (Decapoda), *Coelorinchus marinii* Hubbs, 1934 (2) (Gadiformes), *Hoplostethus occidentalis* Woods, 1973 (1) (Trachichthyiformes), *Polymixia* sp. Lowe, 1836 (1) (Polymixiiformes)	2602	500
Benthodesmus tenuis (1) (Scombriformes), *Neoscopelus macrolepidotus* Johnson, 1863 (1) (Myctophiformes)	2604	737
Ceramaster sp. Verrill, 1899 (1) (Asteroidea), *Deima validum validum* (1) (Holothuroidea), *Nymphaster arenatus* (13) (Asteroidea)	2702	1190
Astropecten sp. (1) (Asteroidea), *Deima validum validum* (2) (Holothuroidea), *Nymphaster arenatus* (6) (Asteroidea), *Pentacheles validus* A. Milne-Edwards, 1880 (1) (Decapoda)	2703	1220
Deima validum validum (2) (Holothuroidea), *Nymphaster arenatus* (2) (Asteroidea)	2802	1342
Deima validum validum (2) (Holothuroidea), *Nymphaster arenatus* (2) (Asteroidea), *Pentacheles validus* (1) (Decapoda)	2901	1503
Acanthocarpus alexandri (1) (Decapoda), *Parasudis truculenta* (Goode & Bean, 1896) (1) (Aulopiformes)	3001	400
Astropecten sp. (1) (Asteroidea), *Aristaeopsis edwardsiana* (1) (Decapoda), *Monomitopus americanus* (Nielsen, 1971) (2) (Ophidiiformes), *Neoscopelus macrolepidotus* (2) (Myctophiformes), *Nymphaster arenatus* (2) (Asteroidea), *Pentacheles validus* (1) (Decapoda)	3004	794

Twelve polymers were identified across all samples, with eight characterized as microplastics (n=13 fibers, Figure 3.2). Fauna samples yielded six distinct plastic spectra: polyamide (PA), polyaryletherketone (PAEK), polyacrylonitrile (PAN), polyester, polystyrene (PS) and synthetic rubber (Table 3.2), while sediment samples yielded five plastic spectra: PA, PAEK, polyimide (PI) (n=2 fibers), polypropylene (PP) (n=2) and synthetic rubber (Table S3.2). A summary of findings regarding microplastics in deep-sea sediments from around the globe can be found in Table S3.3. Non-plastic synthetic polymers found in fauna samples were identified as dimethicone, polyacrylic acid (PAA) and poly(sodium 4-styrenesulfonate) (PSS), while PAA and polyvinylpyridine were found in the sediment samples.

Microplastics were found in seventeen individuals, in seven *Deima validum validum* sea cucumbers, eight *Nymphaster arenatus* sea stars, one *Astropecten* sp. sea star, and one *Pentacheles validum* blind lobster. Polymers in *D. validum validum* were

identified as PAN, polyester, PS and synthetic rubber. PA and PAEK were found in *N. arenatus*, but given that PA is also a component of the trawling net, this single occurrence was not considered. Considering the two more commonly sampled species, 63.63% of *D. validum validum* individuals ingested at least one fiber, followed by 22.22% of ingestion in *N. arenatus.*

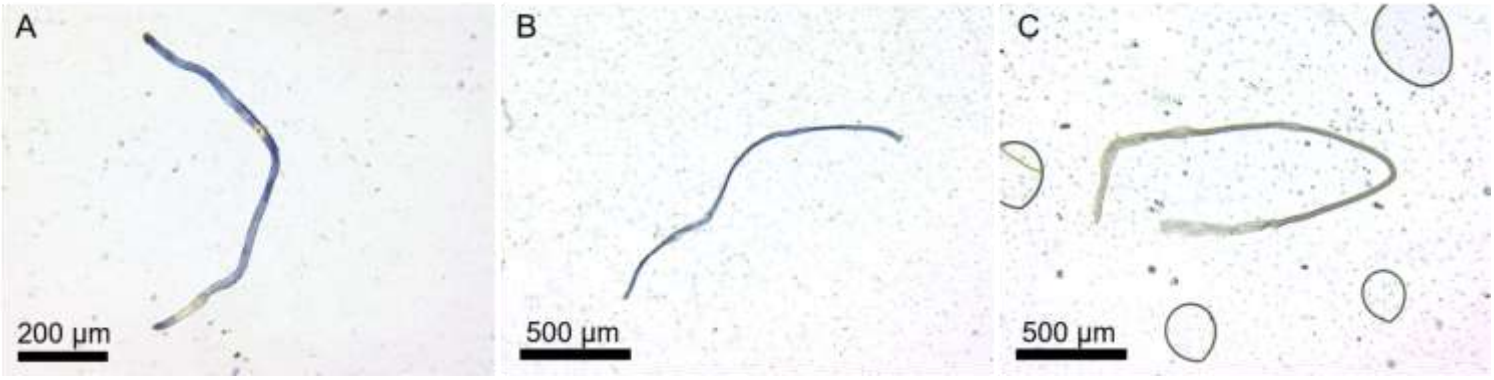

Figure 3.2. Microplastics found in deep-sea samples from the Santos Basin, southwestern Brazilian continental margin. A. Polyaryletherketone (PAEK) fiber ingested by *Nymphaster arenatus* sea star. B Polyacrylonitrile (PAN) fiber ingested by *Deima validum validum* sea cucumber. C. Polypropylene (PP) fiber found in a sediment sample.

Considering the plastic polymers identified across both fauna and sediment samples, PA, PAEK, PI, PP and SR accounts for 15.4% of samples each, while PAN, polyester and PS represent 7.7% each. Additionally, Raman spectroscopy identified the plasticizer bis(2-ethylhexyl) adipate (DEHA) in a PAEK fiber ingested by *N. arenatus*, the aromatic hydrocarbon perylene in an unidentified fiber ingested by *D. validum validum*, and the varnish component β-carboxyethyl acrylate (β-CEA) in an unidentified sediment fiber.

Table 3.2. Fibers ingested by Santos Basin megafauna, sampled along the southwestern Brazilian continental margin. Fibers are presented in relation to corresponding species, prevalent feeding mode, percentage of individuals which ingested at least one fiber, number of fibers ingested, fiber color (n), polymer (if applicable), number of ingested fibers per total weight for the species, number of ingested fibers per individual, and site of sampling. PA = polyamide; PAA = polyacrylic acid; PP = polypropylene; PAN = polyacrylonitrile; PS = polystyrene; Poly(sodium 4-styrenesulfonate) = PSS; SR = synthetic rubber; PAEK = polyaryletherketone; PET = polyethylene terephthalate. *PA fiber excluded due to also being trawling net material.

Species (n)	Feeding mode	Ingestion	Fibers (n)	Color (n)	Polymer	Fibers/g	Fibers/individual (mean ± S.E.)	Site
Astropecten sp. (3)	Predator (Beddingfield & McClintock, 1993; Guilherme & Rosa, 2014)	33.33%	4	Blue (1), black (2), white (1)	dimethicone	0.04	1.33±0.88	2201, 2703
Deima validum validum (11)	Deposit feeder (Alberic & Khripounoff, 1984)	63.63%	26	Blue (12), black (11), green (2), red	PAA, PAN, polyester, PS, PSS SR	0.1	2.36±0.75	2302, 2401, 2702, 2703, 2802, 2901
Nymphaster arenatus (36)	Predator (Pequegnat, 1983; Wagstaff et al., 2014), scavenger (da Costa et al., 2015)	22.22%	8	Blue (6), red, blue/white	PA*, PAEK	0.03	0.22±0.07	2301, 2702, 2703, 2901
Pentacheles validus (3)	Predator (Cruz et al., 2021)	33.33%	1	Blue	-	0.03	0.33±0.33	2703

In fish muscle samples, total PCBs ranged from 519.29 to 7636.36 ng/g (lipid weight), and ICES 7 PCBs ranged from 173.57 to 2779.55 ng/g lw (Table S3.4). Tetra-PCBs (44, 49, 52, 56, 60, 66, 70, 74, 77, 81) and penta-PCBs (87, 95, 97, 99, 101, 105, 110, 114, 118, 123, 126) were the main contributors to contamination across most species, except for *H. occidentalis* where the concentration of tri- and tetra- chlorine substituents was higher (Figure 3.3A). When expressing results as lipid weight, both *N. macrolepidotus* individuals had the largest variation in contamination values, with simultaneously the highest and lowest concentration of total PCBs for all fishes. When expressing results as dry weight, however, both *B. tenuis* individuals were the most contaminated specimens in our study. Regarding sediment samples, total PCBs ranged from 1.28 to 3.96 ng/g (dry weight) (Table S3.5). Tri-PCBs (18, 28, 31, 33) and tetra-PCBs were the main contributors to contamination (Figure 3.3B). CBs-18, 44, 49, 52, 66, 95 and 97 were found in all fish samples.

Among the seven PBDE congeners investigated, only BDE-47 (2,2'4,4'-tetrabromodiphenyl ether) and BDE-99 (2,2',4,4',5-pentabromodiphenyl ether) were found in fish samples (Table S3.6). BDE-47 was found in *Polymixia* sp. and *B. tenuis* at a concentration of 22.38 and 11.19 ng/g lw, respectively, and BDE-99 was present in *H. occidentalis*, *B. tenuis* and *M. americanus* samples at a concentration of 20.71, 1.27 and 5.69 ng/g lw, respectively. The same *B. tenuis* individual was contaminated with both PBDE congeners. Total PBDEs thus ranged from 5.69 to 22.38 ng/g lw in *M. americanus* and *Polymixia* sp., and 0.29 to 3.34 ng/g dw in *H. occidentalis* and *B. tenuis*, respectively. PBDE concentration was below the quantitation limit across all sediment samples.

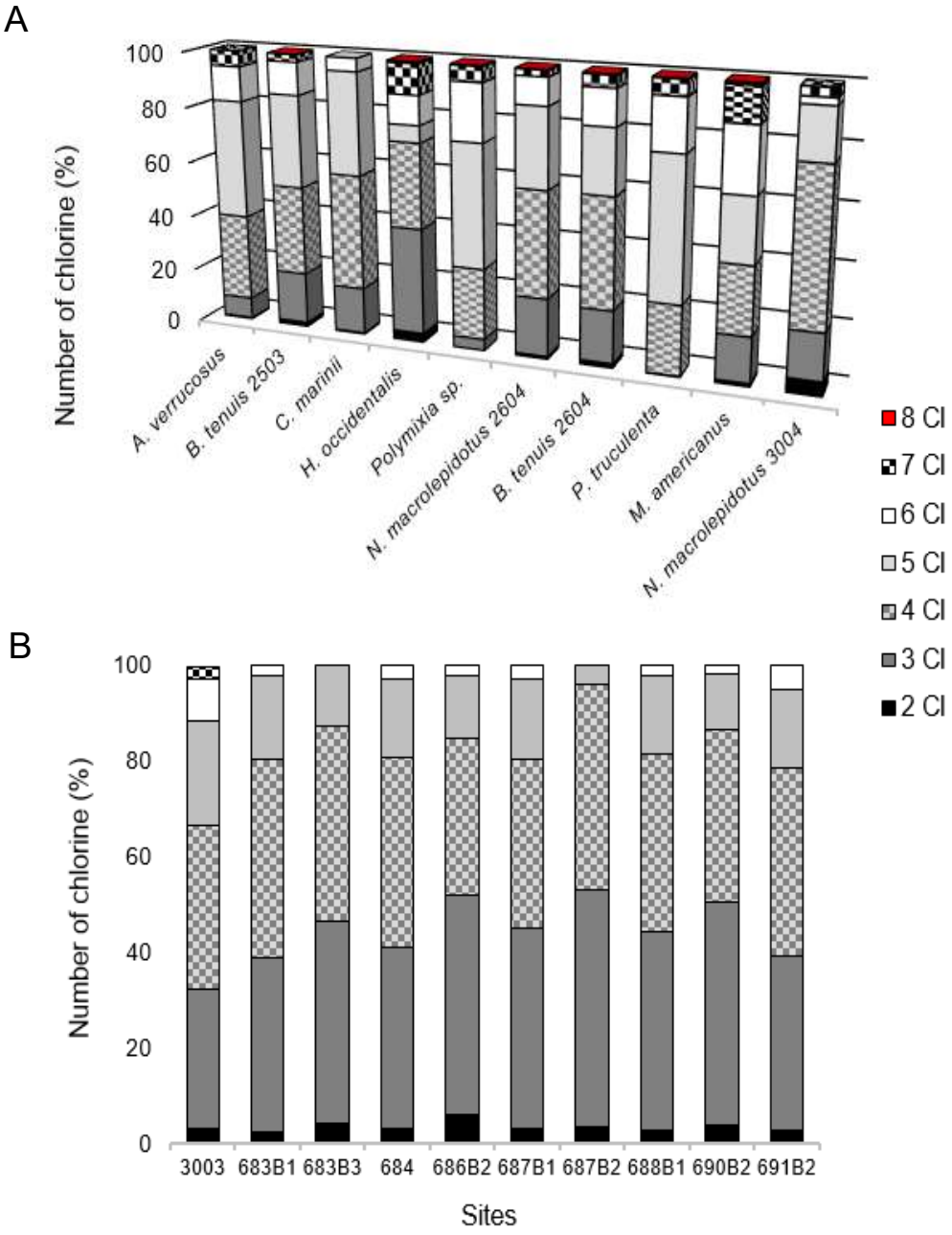

Figure 3.3. Concentration of PCBs arranged by number of chlorine (%) in deep-sea samples originating from the Santos Basin, southwestern Brazilian continental margin. A. fish muscle samples and B. sediment samples. B# = box number.

3.4 Discussion

This is, to the best of our knowledge, the first study in the southwest Atlantic to document contamination by MPs and POPs in deep-sea benthic megafauna, as well as in

the upper sediment layer of the continental slope off Brazil. The presence of these contaminants in an area over 140 km away from the nearest coast is an indicative of the potential for transport of microplastics via oceanic circulation (Kane et al., 2020), through the bottom layer of the Brazil Current (Stramma et al., 1989) and via North Atlantic Deep Water (Zangenberg & Siedle, 1998). Likewise, PCBs and PBDEs originating from coastal industrial activity and dredged up sediment in the Santos Bay (Buruaem et al., 2013) also reach the ocean basin via sewage outfall (Abessa et al., 2005), eventually being incorporated into the food web. Meanwhile, ingestion of fibers, especially by sediment dwellers, is a natural consequence of the ubiquitous prevalence of MPs along the seafloor, even in deep waters (Van Cauwenberghe et al., 2013).

The holothurian *Deima validum validum* (Deimatidae) was the most contaminated species in our study, in terms of total number of fibers ingested (27 fibers, 2.36±0.75 fibers/individual) and percentage of multiple affected organisms (63.63%). Such results are likely related to its deposit-feeding habit, as holothurians are known reservoirs of MPs found in the sediment (Mohsen et al., 2019; Plee & Pomory, 2020) and may even selectively ingest MPs (Graham & Thompson, 2009; Renzi et al., 2018). Similarly, the bottom-dwelling sea star *Nymphaster arenatus* (Goniasteridae) was the second most affected invertebrate species regarding fiber ingestion (22.22%, 0.22±0.07 fiber/individual). The sea star's opportunistic predatory feeding mode and its ability to actively search for and consume prey (Mah, 2020), while still ingesting small amounts of contaminated sediment, probably facilitates MP ingestion. The association between bottom-feeding and MP intake is well documented in marine organisms (Thompson et al., 2004; Wright et al., 2013b; Zhang et al., 2020) and further illustrated here given that PAEK and rubber fibers were found in both these species and in the sediment.

Records of microplastic ingestion by deep-sea fauna in the west Atlantic are currently restricted to the northeast portion of Brazil, in the mesopelagic cephalopods *Vampyroteuthis infernalis* (9.58±8.25 MPs/individual) and *Abralia veranyi* (2.37±2.13 MPs/individual; Ferreira et al., 2022), in lanternfishes (1.27 MP/individual; Ferreira et al., 2023), as well as in a combination of lanternfishes and hatchetfishes (1.25 MP/individual; Justino et al., 2022). The highest value reported here, obtained in *D. validum validum*, is twice as high as the maximum average MP count in these fishes. Given how mesopelagic fish actively prey on larvae and small invertebrates, an opportunistic predator habit could explain such lower values, thus indicating a lack of

trophic transfer of MPs (Hamilton et al., 2021). The maximum ingestion record in our study is four times lower than in *V. infernalis*, possibly due to this cephalopod's main ecological trait of actively selecting and feeding on sinking aggregates of marine snow and fecal pellets (Hoving & Robison, 2012; Golikov et al., 2019; Ferreira et al., 2022), which could in turn be contaminated with MPs.

While there are no other studies documenting MPs in sediments in the southwest Atlantic, we can still draw a comparison with data from coastal environments in southeast Brazil by adjusting our results. Considering an average marine sediment density of 1.7 g/cm^3 (Tenzer & Gladkikh, 2014), our mean (± S.E) and maximum values of 0.03±0.01 MP/g and 0.11 MP/g can be converted to 0.05±0.02 MP/cm^3 and 0.18 MP/cm^3, respectively. For instance, Turra et al. (2014) found an average of 5385 MPs/m^3 (0.005 pellet/cm^3) in the top sediment layer of Santos Beach, 10 times lower than our values. That study, however, focused on pellets, so MP abundance for all plastic shapes would be much higher. Likewise, De Carvalho & Baptista Neto (2016) found a maximum of 2468 MP particles/m^2 (0.12 MP/cm^3) at Galeão Beach, 1.5 times less than the maximum amount found in this study. Finally, in the Vitória Bay estuary, Baptista Neto et al. (2019) and Zamprogno et al. (2021) respectively found a maximum of 38 particles per sample (0.12 MP/g), similar to maximum values found here, and 2175 MPs across all samples (0.68 MP/g), over six times higher than our reported values.

Regarding MP contamination in deep-sea sediments, maximum values reported here are similar to those found in the Arctic Sea (Kanhai et al, 2019), Black Sea (Cincinelli et al., 2021) and South China Sea (Ding et al., 2022), and in the eastern Indian Ocean (Cordova & Wahyudi, 2016) and west/northwest Pacific Ocean (Peng et al., 2020; Zhang et al., 2020) (Table S3.3). Maximum values observed elsewhere were especially high in the Great Australian Bight (Barrett et al., 2020), in the Arctic Ocean (Bergmann et al., 2017; Tekman et al., 2020), in the eastern Indian Ocean (Qi et al., 2022), in the northwest Pacific Ocean (Peng et al., 2018; Tsuchiya et al., 2023), and in the Mediterranean Sea (Kane et al., 2020), where particle abundance was up to 123 times higher than our findings. Abundance of MPs found here is higher than observed amounts in the north Atlantic Ocean (Jones et al., 2022; Nash et al., 2023), in the northwest Pacific Ocean (Fischer et al., 2015) and in the multi-location precursor studies by Van Cauwenberghe et al. (2013) and Woodall et al. (2014). Such a broad spectrum of different abundance records even within the same general location is to be expected, given differences in exact

sampling coordinates, depth, year and technology, and presence of contamination procedures or polymer identification analysis in some of these studies.

Polyamide, PAN, PET, polyester, PS and PP are all used in textile items and/or packaging containers. Although it is impossible to determine the original purpose and source of the materials these particles came from, it is not too far-fetched to assume a domestic origin for most of these, as synthetic fibers are typically released from textile washing cycles (reviewed by Cesa et al., 2017) and can even undergo atmospheric transportation (De Falco et al., 2020). While a domestic origin is also possible for PAEK, PI and synthetic rubber fibers, these materials are mostly used in the oil drilling, electronics and automotive industries, and can thus reach the deep seafloor either through wastewater effluents or directly from oil platforms (Osten et al., 2023). Regarding non plastic polymers, the perylene fiber ingested by *D. validum validum* is a possibly carcinogenic polycyclic aromatic hydrocarbon (PAH) with the potential to bioaccumulate (Zhang et al., 2015).

While production, commercialization and use of PCBs in Brazil were banned in 1981, use of dielectric fluids containing these components is permitted in older appliances. Additionally, their high environmental persistence means they can still be detected at high concentrations regardless of location. By also considering values in the literature expressed as lipid weight, our maximum value of 2779.55 ng/g lw for ICES 7 indicator PCBs in fish species was up to 26% higher than reports from deep water fishes of the Mediterranean Sea (145.2-2125 ng/g lw – Koenig et al., 2013), the south (265-440 ng/g lw – Froescheis et al., 2000) and northeast Atlantic (188-792 ng/g lw – Webster et al., 2009; 1.5-961 ng/g lw – Romero-Romero et al., 2017) and northwestern Pacific Ocean (19-110 ng/g lw – Ramu et al., 2006; n.d.-2200 ng/g lw – de Brito et al., 2002; 34-390 ng/g lw – Takahashi et al., 2010) including dry weight Σ_{10}PCB values observed by Ohkouchi et al. (2016) off-Tohoku (0.12-51 ng/g dw). Meanwhile, the sediment contamination range of 1.28-3.96 ng/g dw in our study is roughly 2.5 times lower than contamination records in superficial sediments in the Santos-São Vicente Estuarine System (0.36-9.8 ng/g dw – de Souza et al., 2018), the closest available evidence in the literature. As for other deep-sea sediment studies, most efforts have concentrated on the western Pacific: our values are over five times lower than density-adjusted (Tenzer & Gladkikh, 2014) records found by Zhang et al. (2020) (0.02-20.61 ng/g dw) and similar to those found by Dasgupta et al. (2018) (0.93-4.19 ng/g dw). Contamination records for

that region, however, have proven to be highly heterogeneous, considering the lack of PCB congeners reported by Cui et al. (2020) and Ge et al. (2021) in Pacific hadal trenches.

Expressing results as dry or lipid weight can influence the reporting of contamination levels in toxicological studies, but one must be careful as the relationship between lipid percentage and hydrophobic contaminants is not always isometric (Hebert & Keenleyside, 1995). In our samples, for instance, *B. tenuis* had the highest level of muscle lipid tissue and the highest contamination values when considering PCBs as dry weight. In contrast, *N. macrolepidotus* had a much lower percentage of muscle fat and the highest PCB concentration when considering values normalized to lipid weight. Following Annasawmy et al. (2022) and Zhang et al. (2022), the higher nitrogen stable isotope ratio of *N. macrolepidotus* and thus higher trophic level could explain the lipid-normalized contaminant levels in this species. So, while the normalization of results may result in a different contamination profile for these species, additional data may help elucidate actual trends, especially in organisms which have not yet been studied regarding organic contaminants, such as the ones in our study.

While similar to PCBs in both structure and properties (de Boer & Cofino, 2002), no legislation concerning PBDEs exists currently in Brazil. Regarding global deep-sea records, our maximum PBDE record of 22.38 ng/g lw in fish samples was up to 10 times higher than maximum values observed in the northwest Pacific (0.85-2.1 ng/g lw – Ramu et al., 2006; 1.3-8.5 ng/g lw – Takahashi et al., 2010) and northeast Atlantic within the Bay of Biscay (0.074-15 ng/g lw – Romero-Romero et al., 2017). In contrast, Mediterranean Sea (11.8-27.3 ng/g lw – Covaci et al., 2008; 14.5-501 ng/g lw – Koenig et al., 2013) and Scottish northeast Atlantic (11.7-50.5 ng/g lw – Webster et al., 2009) maximum records were up to 22 times higher than our results. As for observations in the south Atlantic, values were 9.4 times higher in lipid-adjusted fish muscle samples from northeastern Rio de Janeiro (120-210 ng/g lw – Quinete et al., 2011), and below the detection limits for the same species in the southern portion of that state (Levandier et al., 2013). No records of PBDEs were obtained from our sediment samples, which contrasts with values (albeit low) found in deep-sea environments elsewhere, e.g. in the Indian Ocean, on the outer edge of the Bay of Bengal (49-152 pg/g – Cheng et al., 2015) and in the Mariana Trench (~136 pg/g dw – Dasgupta et al., 2018).

As expected, the prevalence of lower-chlorinated (tri-, tetra-) PCBs in fauna and sediment samples is indicative of an off-site source of contamination, as higher-

chlorinated congeners are typically deposited close to the source (de Souza et al., 2018). A major point of origin for pollutants in the basin lies on the southeastern coast of Brazil, specifically in the Baixada Santista metropolitan area. The Santos-São Vicente Estuarine Complex houses the largest city in the region and the largest port in Latin America, and the city of Cubatão was, up until the 1990s, considered the most polluted place on Earth, owing to its large industrial complex (CETESB, 1989). Additionally, higher vapor pressure values in lower-chlorinated congeners also indicate a possible atmospheric transportation for these compounds (Harvey & Steinhau, 1974). Presence of PBDE congeners in fauna samples alone is a testament to the bioaccumulative potential of these substances. Similarly, BDE-47 and BDE-99, the only congeners found in this study, have also been the only PBDEs found in shallow-water fish in the Baixada area (Magalhães et al., 2017). These are the main components of the pentabromodiphenyl ether commercial mixture, and both congeners display a greater bioavailability in relation to other PBDE components (De Wit, 2002).

3.5 Conclusions

This study is the first to assess pollution by MPs and POPs in a benthic deep-sea environment within the southwest Atlantic Ocean. Invertebrates in our study are either opportunistic predators and scavengers or deposit feeders, and are thus recipients of micropollutants via direct uptake or bioaccumulation. While not targeted by commercial fishing in Brazilian waters, except for the royal crab *C. ramosae* and the scarlet shrimp *A. edwardsiana* (Pezzuto et al., 2006; Dallagnolo et al., 2009), all these species are impacted by manmade waste and can provide invaluable information regarding the health of our deep-sea. Sediment-dwelling echinoderms like *D. validum validum* and *N. arenatus* are especially relevant indicators of sediment pollution, as they appear to concentrate MP particles due to their feeding ecology. Frequency of MP ingestion and organic contaminant values reported here, while highly variable, are several times higher than in other studies from around the world, including shallow-water coastal environments. While macroplastic debris has been recently reported in the Brazilian continental slope (Masumoto et al., 2023), more work is necessary to offer a fuller picture of contamination levels in the offshore Santos Basin.

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4. CLOSING REMARKS

The findings presented here offer a glimpse into how micropollution has reached two seemingly pristine marine environments: the continental shelf surrounding the tip of the Antarctic Peninsula and the slope off the southeastern coast of Brazil. Following our results, MPs pollution in Antarctica dates back to at least the mid-1980s, with MP fibers being ingested in larger quantities by deposit-feeding echinoderms. Similarly, holothurians in the Santos Basin seem to be acting as MP reservoirs. Finally, while PCB levels were up to 26% higher than in deep-sea fishes from other parts of the world, levels of both PCBs and PBDEs were highly variable when compared to fish and sediment samples in other studies.

As with most of the deep seafloor, researching these regions is both onerous and financially demanding. So while the studies enclosed in this thesis paint a not entirely optimistic picture of human impact in remote environments, the difficulty in accessing these regions still raises many questions. For instance, are deposit feeders being affected the most by ingesting MPs? What are the main origin points for MP fibers reaching the Southern Ocean as well as the Southwest Atlantic? And what about the concentration of MPs in the deep-sea pelagic communities across both locations? What are the population-level impacts of PCBs and PBDEs on commercial and non-commercial deep-sea fishes? Filling these gaps is paramount for the conservation of our deep-sea biodiversity.

The exploitation of the ocean leads to impacts that go well beyond the local sphere. Our results demonstrate how far human waste can go, in both temporal and spatial scales. Research efforts focusing on the deep sea cannot keep up with anthropogenic pollution, and at this rate these environments will continue to undergo degradation. Benefits that could arise from ecosystem services will, likewise, be diminished or completely lost. And as entire environments are compromised before being truly understood, no longer being able to feel that wondrous sense of fascination for the large and unknown deep sea is something that I will truly miss.

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APPENDIX A – Supplementary material for chapter 2

Table S2.1. Fibers ingested by deep-sea Antarctic specimens in our study. Fibers are presented in relation to corresponding invertebrate species, particle size range, color (n) and sampling site origin.

Species	Fiber length (mm)	Color	Site
Echinopsolus koehleri	0.35-5.91	Blue (4), black (2), white (2), blue/white	4861
Boreomysis sp.	0.58-2.33	Blue (6), green	4864
Heterocucumis steineni	0.56-3.79	Blue (4), black, blue/white, orange	4869
Notocrangon antarcticus	0.24-2.60	Blue (7), black (3), green (3), red	5052, MB-A
Chorismus antarcticus	2.32	Blue	5052, MB-A
Harpovoluta charcoti	0.66-4.30	Blue (2), light blue, black	5052
Protelpidia murrayi	0.182-1.33	Blue (2), green (2), blue/white, white	FBII-C
Scotoplanes globosa	0.31-1.64	Green (3), red (4)	FBIII-B, FBIV-B
Molpadia violacea	0.25-4.70	Blue (17), black (4), green (2), red, white	FBIV-A, Palmer #2
Ophionotus victoriae	0.14-4.03	Blue (16), black (8), red (4), green (3), light blue, white	#300R1, H, IB-A
Laetmonice producta	2.65	Blue	#300R2
Amphiura joubini	0. 71-5.05	Blue (13), black (7), light blue, red	Elefante #2
Ophiosparte gigas	0.74-3.97	Blue (3), black, red	K, J
Nematocarcinus lanceopes	0.46-1.80	Blue (2), black (2), green	Elefante #2

APPENDIX B – Supplementary material for chapter 3

Table S3.1. Sampling sites, method of sample collection, sampling coordinates, depth, sediment type and dates for megafauna sampled along the southwestern Brazilian continental margin. MCS = muddy carbonate sand.

Site	Sampling method	Coordinates	Depth (m)	Sediment	Date (dd/mm/yy)
2201	Trawling	24°22’ S; 44°04’ W	400	MCS	22/09/19
2301	Trawling	24°37’ S; 43°56’ W	1000	Mud	23/09/19
2302	Trawling	24°42’ S; 43°48’ W	1408	Mud	23/09/19
2401	Trawling	24°39’ S; 43°51’ W	1142	Mud	24/09/19
2503	Trawling	24°41’ S; 44°09’ W	655	Mud	25/09/19
2602	Trawling	24°34’ S; 44°16’ W	500	Mud	26/09/19
2604	Trawling	24°41’ S; 44°10’ W	737	Mud	26/09/19
2702	Trawling	24°57’ S; 44°19’ W	1190	Mud	27/09/19
2703	Trawling	24°48’ S; 44°04’ W	1220	Mud	27/09/19
2802	Trawling	24°50’ S; 44°02’ W	1342	Mud	28/09/19
2901	Trawling	25°01’ S; 44°16’ W	1503	Mud	29/09/19
3001	Trawling	24°41’ S; 44°28’ W	400	Mud	30/09/19
3003	Box-corer	24°45’ S; 44°32’ W	351	Mud	30/09/19
3004	Trawling	24°53’ S; 44°24’ W	794	Mud	30/09/19
683	Box-corer	24°37’ S; 44°00’ W	850	MCS	18/11/19
684	Box-corer	24°40’ S; 44°04’ W	829	MCS	18/11/19
686	Box-corer	24°55’ S; 44°35’ W	581	Sandy mud	19/11/19
687	Box-corer	24°54’ S; 44°28’ W	675	Mud	20/11/19
688	Box-corer	24°53’ S; 44°32’ W	564	Mud	20/11/19
690	Box-corer	26°11’ S; 45°38’ W	739	Mud	21/11/19
691	Box-corer	26°53’ S; 46°24’ W	519	Mud	21/11/19

Table S3.2. Fibers in sediments sampled along the southwestern Brazilian continental margin. Particles are presented in relation to sampling site, quantity, color, mass of sediment for each site and abundance of particles per sediment mass.

Station	MP quantity	MP color	Sediment mass	MP/g
683 B1	1	Blue	101.734 g	0.0098296
683 B3	7	Blue	64.555 g	0.1084347
684 BQ	3	Black, white, blue	92.358 g	0.0324823
686 B2	6	Blue (5), red (2)	117.571 g	0.051033
687	1	Blue	72.703 g	0.0137546
687 B2	2	Blue	104.006 g	0.0192297
688 B1	2	Blue, red	81.375 g	0.0245776
690 B2	2	Black, white	114.99 g	0.0173928
691 B2		-	67.015 g	0
3002	1	Black	175.013 g	0.0057139

Table S3.3. Studies reporting abundance of microplastics (MP) in deep-sea marine sediments around the world, and MP characteristics. MP abundance is expressed as original values of range/average number of particles per mass of dry sediment/sediment volume, and then adjusted to MP abundance per mass (g) of dry sediment, if necessary, following Tenzer & Gladkikh's (2014) average marine sediment density of 1.7 g/cm^3. ABS = acrylonitrile butadiene styrene, PA = polyamide, PAN = polyacrylonitrile, PAEK = polyaryletherketone, PE = polyethylene, PET = polyethylene terephthalate, PI = polyimide, PP = polypropylene, PS = polystyrene, PU = polyurethane, PVC = polyvinyl chloride, SR = synthetic rubber. *includes values for depths <200 m.

Reference	Location	Depth range	MP abundance	MP/g	MP shape	Main materials
This study	Southwest Atlantic Ocean	400-1503 m	0-0.11 MP/g	0-0.11 MP/g	Fibers (100%)	PI, PP, PA, PAEK
Abel et al., 2021	Northwest Pacific Ocean	5142-8250 m	14-209 MPs/kg	0.01-0.2 MP/g	-	PP, acrylic, PU
Abel et al., 2022	Northwest Pacific Ocean	5740-9750 m	215-1596 MPs/kg	0.22-1.6 MP/g	Fragments (71-97%), fibers (3-29%),	Acrylic, PU, PP, PE, PA
Angiolillo et al., 2021	Mediterranean Sea	350-2200 m	0.12-1.04 MP/g	0.12-1.04 MP/g	Fibers (80%), fragments (17%), pellets (1.2%), foam (0.6%), film (0.4%)	-
Barrett et al., 2020	Great Australian Bight	1670-3060 m	0-13.6 MPs/g	0-13.6 MPs/g	Fragments (fibers excluded)	SR, PU, polyester, PP
Bergmann et al., 2017	Arctic Ocean	2340-5570 m	42-6595 MPs/kg	0.04-6.6 MPs/g	- (fibers excluded)	PE, PA, PP
Cincinelli et al., 2021	Black Sea	22-62 m, 1165-2131 m	106.7 MPs/kg*	0.11 MP/g*	Fibers	PE, PP, acrylic*
Cordova & Wahyudi, 2016	Eastern Indian Ocean	69-2182 m	0-6 MPs/100 g	0-0.06 MP/g	Granule (85%), fiber (15%)*	-
Courtene-Jones et al., 2020	North Atlantic Ocean	2200 m	0-0.2 MP/g	0-0.2 MP/g	Fibers (89%), fragments (10%), film (1%)	Polyester, PP

Cunningham et al., 2020	Southern Ocean	201-3342 m	1.04-1.30 MP/g	1.04-1.30 MP/g	Fragments (56%), fibers (39%), film (5%)	Polyester, PP, PS, PU, PVC, SR, acrylic
Cutroneo et al., 2022	Mediterranean Sea	2443 m	80 MPs/l	0.24 MP/g	Fragments (67%), fibers (13%), granules (11%), beads (4%)	PU, PVC
Dhineka et al., 2022	Bay of Bengal	225-1070 m	2-12 MPs/50 g	0.04-0.24 MP/g	Fibers (67%), fragments (25%), film (8%)	PP, PE, PS
Ding et al., 2022	South China Sea	3-3980 m	128.64 MPs/kg, 100.42 MPs/kg	0.13 MP/g, 0.1 MP/g	Fragments (37%), fibers (32%), granules (25%), film (4%), foam (2%)*	PE, PP, rayon, PS
Feng et al., 2022	South China Sea	~1300 m	1412.25 MPs/kg	1.41 MP/g	Lines, granules, fibers, film	PU, PA, polyacetal, PET
Fischer et al., 2015	Northwest Pacific Ocean	4870-5770 m	60-2020 MPs/m^2	0-0.01 MP/g	Fibers (75%), fragments (25%)	-
Jones et al., 2022	North Atlantic Ocean	196-1135 m	0-2.95 MPs/50 cm^3	0-0.03 MP/g	Fragments, fibers	-
Kane et al., 2020	Mediterranean Sea	~150-1400 m	0-182 MPs/50 g*	0-3.64 MPs/g*	Fibers (70-100%), fragments (0-30%)*	PA, PE, PET*
Kanhai et al., 2019	Arctic Ocean	855-4353 m	0-9 MPs/100 g	0-0.09 MP/g	Fibers (55%), fragments (45%)	Polyester, PS, PAN, PP, PVC, PA
Lechthaler et al., 2020	Northeast Atlantic Ocean	72-625 m	0-0.29 MP/g*	0-0.29 MP/g*	Fibers (100%)	-
Loughlin et al., 2021	Northwest Atlantic Ocean	241-1628 m	0.12-0.77 MP/g	0.12-0.77 MP/g	Fibers (100%)	ABS, PVC, PE
Nash et al., 2023	Northeast Atlantic Ocean	147-2998 m	0-33 MPs/kg	0-0.03 MP/g	Fibers (90%), fragments (10%)*	PA, PU*

Peng et al., 2018	Northwest Pacific Ocean	5108-10908 m	0.27-6.2 MPs/g	0.27-6.2 MPs/g	Fibers, fragments	Polyester, PP, PU, PA, PVC, rayon, PE
Peng et al., 2020	Northwest Pacific Ocean	4800-10890 m	71.1 MPs/kg	0.07 MP/g	Fibers (57%), fragments (43%)	Polyester, PA, PP, PET
Qi et al., 2022	Eastern Indian Ocean	2161-4666 m	30.30-701.7 MPs/kg	0.03-7.02 MPs/g	Fragments (47.5%), fibers (45.6%), film (4.4%), beads (2.5%)	Rayon, polyester, PE, PS, PP
Sanchez-Vidal et al., 2018	Southern European seas	42-3500 m	10-70 MPs/50 g*	0.2-1.4 MP/g*	Fibers (100%)	PET, acrylic, PA, PE, PP*
Tekman et al., 2020	Arctic Ocean	272-5569 m	239-13331 MPs/kg	0.2-13.33 MPs/g	- (fibers excluded)	PE, SR, PP
Tsuchiya et al., 2023	Northwest Pacific Ocean	855-9232 m	5.1-2080.9 MPs/g	5.1-2080.9 MPs/g	Fragments (fibers excluded)	PE, PP, PET, vinyl, acrylic, PS, PA
Van Cauwenberghe et al., 2013	Gulf of Guinea	4785 m	0 MP/25 cm^3	-	-	-
	Mediterranean Sea	1176 m	0.5 MP/25 cm^3	0.01 MP/g	Fragment (100%)	-
	North Atlantic Ocean	4842-4844 m	1 MP/25 cm^3	0.02 MP/g	Fragments (100%)	-
	Southern Ocean	2749-4881 m	0.3 MP/25 cm^3	<0.01 MP/g	Fragment (100%)	-
Woodall et al., 2014	Mediterranean Sea	300-3500 m	70 MPs/200 cm^3	0.2 MP/g	Fibers (100%)	Rayon, polyester, PA, acetate, acrylic
	North Atlantic Ocean	1400-2200 m	81 MPs/250 cm^3	0.19 MP/g	Fibers (100%)	

	Southwest Indian Ocean	900-1000 m	3.4 MPs/100 cm^3	0.02 MP/g	Fibers (100%)	
Zhang et al., 2020	West Pacific Ocean	4601-5732 m	0-1042 MPs/kg	0-0.1 MP/g	Fibers (52.5%), film (30%), fragments (17.5%)	PP, PE, PET
Zhang et al., 2022	South China Sea	457-2727 m	19-347 MPs/kg	0.02-0.35 MP/g	Fragments (72%), fibers (22%), beads (6%)	PC, PE, polyester, PVC, PP

Table S3.4. PCB concentrations, sum of PCBs (ICES7 and total, ng/g dry weight) and lipid content in muscle samples from fish caught along the southwestern Brazilian continental margin. MQL = method quantitation limit (0.1 ng/g). Sum of congeners expressed as ng/g lipid weight are highlighted in bold.

	Allocyttus verrucosus	*Benthodesmus tenuis*	*Coelorinchus marinii*	*Hoplostethus occidentalis*	*Polymixia* sp.	*Neoscopelus macrolepidotus*	*Benthodesmus tenuis*	*Parasudis truculenta*	*Monomitopus* americanus	*Neoscopelus macrolepidotus*
Site	2401	2503	2602	2602	2602	2604	2604	3001	3004	3004
PCB 8	0.10	4.20	0.16	0.56	<MQL	1.43	4.48	<MQL	0.44	0.36
PCB 18	0.29	5.99	0.79	1.16	0.32	3.78	4.96	0.15	0.99	0.40
PCBs 28 and 31	1.49	31.01	3.82	3.46	2.51	18.95	33.15	<MQL	4.03	0.78
PCB 33	0.32	18.05	2.51	2.14	1.57	12.03	19.39	<MQL	1.60	<MQL
PCB 44	1.60	13.55	1.71	1.31	2.68	11.20	11.72	1.93	2.09	0.16
PCB 49	0.83	6.73	2.08	1.27	2.84	8.41	10.09	1.89	1.67	1.14
PCB 52	1.57	15.12	3.15	1.85	4.68	15.58	15.52	2.61	2.62	1.43
PCBs 56 and 60	0.45	10.93	1.34	0.11	3.30	6.41	16.11	<MQL	<MQL	<MQL
PCB 66	2.89	18.85	4.95	0.74	3.31	8.13	24.89	3.43	2.79	1.21
PCB 70	1.17	21.33	2.83	<MQL	8.60	9.27	26.51	4.93	0.59	<MQL
PCB 74	0.18	7.97	1.63	0.13	2.42	4.60	13.21	1.93	0.22	<MQL
PCB 77	<MQL	0.39	<MQL	<MQL	<MQL	0.25	2.83	0.21	<MQL	<MQL

PCB 81	<MQL	0.39	<MQL	<MQL	0.16	0.14	0.45	0.27	<MQL	<MQL
PCB 87	2.01	8.60	1.60	<MQL	5.25	4.94	7.36	3.75	1.24	<MQL
PCB 95	2.03	18.54	2.24	0.32	6.61	10.96	9.37	8.15	1.34	0.41
PCB 97	0.77	7.30	1.90	0.76	3.89	2.77	5.32	2.97	1.87	0.88
PCB 99	0.30	9.65	1.05	<MQL	3.70	4.04	7.85	2.66	0.68	<MQL
PCB 101	2.00	21.58	2.76	<MQL	8.83	11.16	16.10	7.14	1.50	<MQL
PCB 105	0.24	4.13	0.40	<MQL	2.63	1.45	2.23	1.51	0.34	<MQL
PCB 110	2.31	15.42	3.01	<MQL	8.92	6.96	11.24	6.16	1.58	<MQL
PCB 114	<MQL	0.24	<MQL	<MQL	0.13	0.11	0.26	<MQL	<MQL	<MQL
PCB 118	2.01	13.52	2.83	<MQL	8.98	5.50	11.37	2.84	0.91	<MQL
PCB 123	0.18	0.38	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
PCB 126	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
PCBs 128 and 167	0.30	1.32	<MQL	<MQL	1.18	0.74	2.01	0.63	0.77	<MQL
PCB 132	<MQL	4.82	0.31	<MQL	2.60	1.92	2.25	0.14	<MQL	<MQL
PCB 138	0.29	8.63	0.36	0.77	6.78	4.12	12.27	3.67	2.83	<MQL
PCB 141	0.11	1.61	<MQL	<MQL	0.98	0.66	1.59	0.62	0.40	<MQL

PCB 149	1.09	7.52	0.96	<MQL	3.61	3.11	6.90	3.48	0.99	0.18
PCB 151	0.12	1.59	<MQL	<MQL	0.75	0.63	2.28	0.46	0.21	<MQL
PCB 153	1.42	7.94	0.40	0.95	5.44	4.28	10.24	3.16	3.55	<MQL
PCB 156	0.18	0.66	<MQL	0.13	0.61	0.39	0.81	0.42	0.39	<MQL
PCB 157	<MQL	0.19	<MQL	<MQL	0.10	0.11	0.27	<MQL	0.20	<MQL
PCB 158	<MQL	0.64	<MQL	<MQL	0.62	0.33	0.71	0.22	<MQL	<MQL
PCB 169	<MQL	<MQL	<MQL	<MQL	<MQL	0.11	0.14	<MQL	0.15	<MQL
PCB 170	0.39	1.18	<MQL	0.48	1.30	0.66	2.22	0.84	0.90	<MQL
PCB 174	0.13	0.64	<MQL	<MQL	0.45	0.29	0.96	0.30	0.36	<MQL
PCB 177	0.11	0.49	<MQL	<MQL	0.38	0.24	1.08	0.24	0.31	<MQL
PCB 180	0.68	3.10	<MQL	1.09	2.20	1.56	4.51	1.58	2.15	0.22
PCB 183	<MQL	0.72	<MQL	<MQL	0.48	0.34	1.03	0.34	0.25	<MQL
PCB 187	0.27	1.85	<MQL	0.34	1.19	0.63	3.04	0.55	0.99	<MQL
PCB 189	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	0.13	<MQL	0.15	<MQL
PCB 194	<MQL	0.15	<MQL	0.12	0.23	0.19	0.40	0.20	0.23	<MQL
PCB 195	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	0.11	<MQL

PCB 199	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	0.47	<MQL	<MQL	<MQL
PCB 203	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	0.17	<MQL	<MQL	<MQL
PCB 206	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	0.12	<MQL	<MQL	<MQL
PCB 209	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
Σ_7PCBs	9.46	100.9	13.32	8.12	39.42	61.15	103.16	21	17.59	2.43
	450.48	**720.71**	**1210.91**	**580**	**1877.14**	**2779.55**	**384.93**	**1615.38**	**303.28**	**173.57**
Σ_{TOTAL}PCBs	27.9	297	42.8	17.7	110	168	308	69.4	41.4	7.2
	1328.57	**2121.43**	**3890.91**	**1264.29**	**5238.1**	**7636.36**	**1149.25**	**5338.46**	**713.79**	**514.29**
Lipids (%)	2.1	14	1.1	1.4	2.1	2.2	26.8	1.3	5.8	1.4

Table S3.5. PCB concentrations and sum of PCBs (ICES7 and total ng/g dry weight) in sediments sampled along the southwestern Brazilian continental margin. B# = box number; MQL = method quantitation limit (0.01 ng/g).

Site	3003	683B1	683B3	684BQ	686B2	687B1	687B2	688B1	690B2	691B2
PCB 8	0.107	0.069	0.084	0.065	0.109	0.124	0.046	0.113	0.130	0.084
PCB 18	0.126	0.067	0.083	0.068	0.096	0.153	0.042	0.111	0.189	0.084
PCB 28	0.353	0.421	0.319	0.289	0.298	0.594	0.225	0.606	0.582	0.387
PCB 31	0.293	0.349	0.265	0.240	0.248	0.493	0.187	0.503	0.483	0.321
PCB 33	0.207	0.324	0.200	0.218	0.214	0.397	0.180	0.415	0.421	0.322
PCB 44	0.167	0.207	0.100	0.099	0.074	0.207	0.095	0.212	0.167	0.164
PCB 49	0.113	0.129	0.069	0.070	0.057	0.171	0.052	0.184	0.145	0.112
PCB 52	0.197	0.184	0.087	0.111	0.100	0.280	0.089	0.303	0.242	0.172
PCBs 56 and 60	0.094	0.117	0.089	0.078	0.064	0.094	0.052	0.103	0.096	0.093
PCB 66	0.208	0.268	0.179	0.188	0.132	0.240	0.099	0.248	0.237	0.260
PCB 70	0.172	0.244	0.143	0.148	0.070	0.220	0.098	0.274	0.253	0.218
PCB 74	0.113	0.108	0.101	0.096	0.067	0.128	0.042	0.118	0.110	0.120
PCB 77	0.013	0.017	0.018	0.013	0.013	0.011	<MQL	0.010	0.018	0.019

PCB 81	0.065	0.049	0.049	0.045	0.038	0.035	0.025	0.031	0.024	0.044
PCB 87	0.069	0.055	0.026	0.042	0.025	0.060	<MQL	0.066	0.040	0.036
PCB 95	0.110	0.094	0.031	0.056	0.037	0.141	0.022	0.114	0.069	0.094
PCB 97	0.040	0.041	0.011	0.024	0.014	0.040	<MQL	0.044	0.031	0.020
PCB 99	0.070	0.055	0.030	0.035	0.026	0.064	0.010	0.067	0.042	0.054
PCB 101	0.190	0.129	0.053	0.075	0.059	0.166	0.020	0.166	0.091	0.111
PCB 105	0.049	0.033	0.021	0.022	0.017	0.031	<MQL	0.030	0.024	0.037
PCB 110	0.109	0.086	0.042	0.052	0.051	0.101	<MQL	0.092	0.058	0.093
PCB 114	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
PCB 118	0.094	0.065	0.046	0.039	0.015	0.059	<MQL	0.061	0.068	0.055
PCB 123	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
PCB 126	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
PCBs 128 and 167	0.015	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
PCB 132	0.028	0.010	<MQL	<MQL	<MQL	0.016	<MQL	0.013	<MQL	0.023
PCB 138	0.082	0.019	<MQL	0.026	0.011	0.039	<MQL	0.025	0.028	0.038
PCB 141	0.019	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL

PCB 149	0.062	0.022	<MQL	0.024	0.014	0.032	<MQL	0.030	0.019	0.047
PCB 151	0.017	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	0.011
PCB 153	0.073	0.015	<MQL	0.015	0.014	0.029	<MQL	0.021	0.013	0.037
PCB 156	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
PCB 157	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
PCB 158	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
PCB 169	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
PCB 170	0.020	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
PCB 174	0.012	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
PCB 177	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
PCB 180	0.030	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
PCB 183	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
PCB 187	0.024	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
PCB 189	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
PCB 194	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
PCB 195	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL

PCB 199	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
PCB 203	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
PCB 206	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
PCB 209	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL
Σ_7PCBs	1.019	0.833	0.505	0.555	.497	1.167	0.334	1.182	1.024	0.8
ΣPCBs	3.34	3.18	2.04	2.14	1.86	3.93	1.28	3.96	3.58	3.06

Table S3.6. PBDE concentrations (ng/g dry weight) in muscle samples from fish caught along the southwestern Brazilian continental margin. MQL = method quantitation limit (0.2 ng/g). Sum of congeners expressed as ng/g lipid weight are highlighted in bold.

	Hoplostethus occidentalis	*Polymixia* sp.	*Benthodesmus tenuis*	*Monomitopus* americanus
Site	2602	2602	2604	3004
PBDE 28	<MQL	<MQL	<MQL	<MQL
PBDE 47	<MQL	0.47	3.00	<MQL
PBDE 99	0.29	<MQL	0.34	0.33
PBDE 100	<MQL	<MQL	<MQL	<MQL
PBDE 153	<MQL	<MQL	<MQL	<MQL
PBDE 154	<MQL	<MQL	<MQL	<MQL
PBDE 183	<MQL	<MQL	<MQL	<MQL
ΣPBDEs	0.29	0.47	3.34	0.33
	20.71	**22.38**	**12.46**	**5.69**