Natural substrates and amphipods epibiontes contaminated by microplastic

Substratos naturais e anfípodes epibiontes contaminados por microplástico

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ABSTRACT

Amphipods inhabit microhabitats that protect them against predation, provide nutrition and an area for reproduction and development. However, the components of the ecosystem are impacted by the presence of microplastics (MPs), which are a threat to marine communities. Since the organisms interact/ingest/filter the different quantities of MPs, and making them available to the associated epibionts, three natural substrates (algae, sponges, and ascidians) were sampled to compare the number of MPs in them and in their associated amphipods. This study was conducted on rocky shores from Rio de Janeiro (Brazil), differently from other studies that have been focused on experimental tests. The samples were submitted to density separation to extract the microplastics and acid degradation to access MP in amphipods. Algae retained a greater concentration of MPs as a sink of particles, as well as its associated amphipods. Sponges and ascidians have different mechanisms to filter water, which influences the MPs accumulation in amphipods. Those associated with sponges and ascidians had fewer microplastic particles. This is the first study comparing the retention of MP in different natural substrates and their amphipod epibionts, which gives information about microplastic contamination in these microhabitats and their associated organisms.

Keywords: microplastic contamination, natural substrates, Brazil

RESUMO

Os anfípodes habitam microhabitats que os protegem contra a predação, fornecem nutrição e uma área para reprodução e desenvolvimento. No entanto, os componentes do ecossistema são impactados pela presença de microplásticos (MPs), que são uma ameaça para as comunidades marinhas. Uma vez que os organismos interagem/ingerem/filtram as diferentes quantidades de MPs, os disponibilizando aos epibiontes associados, três substratos naturais (algas, esponjas e ascídias) foram amostrados para comparar o número de MPs neles e em seus associados anfípodes. Este estudo foi realizado em costões rochosos do Rio de Janeiro (Brasil), diferentemente de outros estudos que foram focados em testes experimentais. As amostras foram submetidas à separação de densidade para extração dos microplásticos e degradação ácida para acessar MP em anfípodes. As algas retiveram uma maior concentração de MPs como sumidouro de partículas, assim como seus anfípodes associados. Esponjas e ascídias possuem diferentes mecanismos para filtrar a água, o que influencia o acúmulo de MPs em anfípodes. Aqueles associados a esponjas e ascídias tinham menos partículas microplásticas. Este é o primeiro estudo comparando a retenção de MP em diferentes substratos naturais e seus epibiontes de anfípodes, o que fornece informações sobre a contaminação microplástica nesses microhabitats e seus organismos associados.

Palavras-chave: contaminação microplástica, substratos naturais, Brasil.

INTRODUCTION

Microplastics (< 5 mm), small particles that originate from beads and fragmentation of macroplastics, are accumulating in natural environment and being ingested, inhaled, and filtrated by different organisms (Silva et al., 2021). After contamination, their persistence in the inside the animal body brings many threats to physiology, including oxidative stress, inflammation, reduced body growth, bioaccumulation, and magnification of contaminants through food chain (Alimba & Faggio, 2019). There is a great number of papers focusing on MP contamination in particular species (fish, mussel, crabs) and few studies approach it in communities (Browne et al., 2015), especially in phytoplankton, macroalgae and sessile organisms (Li et al., 2020). Many benthic organisms are microhabitats (substrates) to polychaetas, mollusks, crustaceans, and are colonized by diatomaceous, algae, and bryozoans (Jones et al., 2020). The transfer of MPs and other contaminants of primary producers to invertebrates may occur as these microhabitats are deposit to MP (Goss et al., 2018). Filter organisms, such as ascidians and sponges, absorb many particles that are translocated to tissues (Girard et al., 2020), and these materials may reach their epibionts, although, this transference is yet not well explored.

Among the associated organisms of algae, ascidians and sponges, the amphipods are important components of benthic communities. They are diverse, abundant, have different feeding habits, and are essential primary and secondary consumers in the trophic chain. It is well demonstrated that amphipods ingest microplastic in nature and in lab experiments, which decreases the nutritional rate (Carrasco et al., 2019). However, only Jones et al. (2020) related microplastics in epibionts amphipods and the seagrass. Therefore, the aim of this study was to analyze the presence of MPs in three different natural substrates (algae, sponge, and ascidian) and their associated amphipods

MATERIALS AND METHODS

The survey was performed in five (5) coastal sites (Figure 1) in the State of Rio de Janeiro (Brazil). The state lies in the southeast of Brazil and its littoral has a 633 km extension from Barra de Itabapoana to Trindade (south). According to the coast orientation, this region is separated by Cabo Frio city into two macro-regions, north and south. The northern region has a delta shape and configures the regions of the Itabapoana River, Paraíba do Sul, Macaé to the cape Búzios-Cabo Frio embayment. The southern is defined by the Região dos Lagos, Guanabara Bay, Jacarepaguá, Sepetiba Bay to Ilha Grande and Paraty Bay (Muehe and Lins- de-Barros, 2016; Muehe and Valentini, 1998). The regions have rivers, lakes, sandy beaches, dunes, bays, and rocky shores (Dias and Kjerfve, 2009). Samples were performed in João Fernandinho Beach (Armação de Búzios), Urca and Boa Viagem Beach (Baía de Guanabara), and Ilhas Maricás, which are described below.

João Fernandinho (JF) is one of the 23 beaches in Armação de Búzios (22°44'20.0"S 41°52'26.5"W), which receives the Brazilian Current, from the South-Equatorial Current, and the Malvinas Current, originating from the Upwelling effect. The deep waters bring and mix the nutrients to the surface that contribute to the phytoplankton blooms, and consequently plankton, and several invertebrates and vertebrates (Carborel 1998). In 2009 a Protected Area of Armação de Búzios (MPAAB) was established, although there are only few studies regarding its marine diversity (Pedrini et al. 2016; Silva et al. 2019). According to Oigman-Pszczol and Creed (2007), the density of litter on the beaches of Búzios is significantly lower compared to other regions of the world. However, this scenario may have changed, as there is a gap in data on contamination and pollution in the region, and tourism and population density have been growing considerably (IBGE).

Two other sampled beaches were Urca (UR) (22°56'51.8"S 43°09'47.2"W) and Boa Viagem (BV) (22°54'31.0"S 43°07'48.3"W) sited in Guanabara Bay. This is the second biggest bay in Brazil, located in one of the most populated regions of the world (Fistarol et al., 2015). The BG is considered one of the most important geographical regions in the state for having an oil refinery (REDUC), two commercial ports (Niterói and Rio de Janeiro), two naval bases and a Brazilian Navy shipyard, marinas and two large airports (Santos Dumont Airport and Tom Jobim International Airport). Due to intense maritime and tourist activity, it is considered one of the most degraded marine and estuarine systems in the country (Soares-Gomes et al. 2016). The bay receives drainage discharges from more than 11 million inhabitants, where organic and metallic contamination has been evident in recent years (Kjerfve et al. 1997; Carreira et al. 2002; Machado et al. 2008).

In the oceanic part, east of Guanabara Bay in the city of Niterói, is the embayment of Itaipú (22°58'09.2"S 43°02'47.9"W), which has four beaches, Piratininga, Sossego, Camboinhas and Itaipú (ITA). The region has less hydrodynamics due to the presence of Morro das Andorinhas and the Ilhas Menina, Mãe and Pai. The oceanic region does not have a high population density when compared to Guanabara Bay, but the occupation process since the late 1970s and the construction of buildings on the shores of the lagoon and beaches have been causing destruction of sandbank vegetation and dunes. As a result, the increase in pollution from garbage and sewage discharges has intensified over time (Eccard et al. 2017).

The last sampled site is Maricás Islands (MA) (23°00'50.4"S 42°55'09.1"W), which is composed of 3 islands under the domain of the Brazilian Navy and uninhabited. They are 28 km from the coast, sheltered and consequently preserved. It is still an unexplored area by science and monitoring programs. However, these programs become necessary given the construction of effluent outfalls from the petrochemical complex in the region (Moraes et al. 2013). Because they are exposed to different levels of contamination, Búzios (Lagos Region), Baía de Guanabara and Ilhas Maricás (Maricá) were defined as collection locations, where the regions were treated as areas of medium, high, and low contamination levels, respectively.

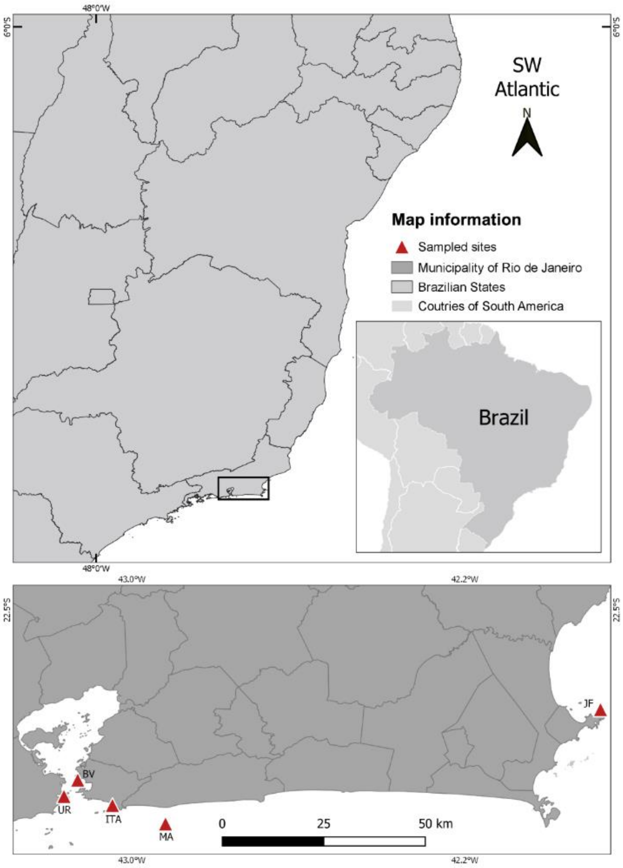

Figure 1: Samples sites in Rio de Janeiro (Brazil). JF: João Fernandinho Beach, MA: Maricás Island, ITA: Itaipu Beach, BV: Boa Viagem Beach, UR: Urca Beach. Software Qgis 3.4. Coordinates SIRGAS 2000. Author: Rayane Sorrentino.

The survey was performed in September, October, December 2019, and January 2020 on the 5 beaches (more samples would be performed in 2020 and 2021, but it was impossible due to COVID-19 pandemic). Triplicates of each substrate, algae, sponge, ascidians were sampled manually by scuba and free diving. During low tide, organisms were collected in rocky shores, 1 – 6 meters deep, following an arbitrary method. As the distribution of organisms is scattered, especially of ascidians, transects or quadracts were not utilized. The organisms were carefully covered by pre-cleaned pots and collected from the rock shores, which was conducted fast to avoid amphipods escaping and exclusion of MPs from the sample. After sampling the material was fixed and preserved with 96% ethanol (Setälä et al., 2016) and taken to Carcinology Lab at Universidade do Estado do Rio de Janeiro/FFP to further analysis.

Under a stereomicroscope, when possible, the organisms were identified (tuffs of algae including different species were labelled as algae), and sponges and ascidians were shattered to obtain the amphipods. To extract the microplastics, a hypersaline solution with a density of 1.2 g cm-3 (358.9g of NaCl in 1L of demineralized water) and each substrate was added, mixed, and held for 24 hours in a Becker. This is a cheap and eco-friendly method, although it only separates polymers up to 1.2 g cm-3 (PP, PU, PE, PA, and PS), so denser polymers were not counted (Montagner et al., 2021). The amphipods were removed from samples with tweezers, and the material was filtered with cellulose filters (0,45µm Whatman AE98) using a vacuum bomb Prismatec 121. The amphipods from each sample were screened, identified, separated. To observe a possible difference of ingestion between feeding habits, the amphipods were pooled by family. Amphipods caprellids were excluded from counting due to the morphological difference. Their abdomens are reduced and consequently, only rounded and curved body shapes were selected for the analyses. Additionally, only entire, adults or those bigger than 5 mm (< 10 mm) were analysed. For MP detection, the animals were rinsed with distillate water to avoid external plastics and degraded using nitric acid (HNO3). In a Becker, 0.5 mL of acid was added with the amphipods for 30 min, then 50 mL of distillate was added and filtered. The filters of the substrates and amphipods were analysed using stereoscopic microscopy with a camera (Bel Photonics, STEREO-ZOOM SÉRIE SZ/SZT). MPs were quantified and classified by the shape of fiber and fragment. Due to methodological limitations, FT-IR was not available for polymer identification. As amphipods were pooled by family, results of plastic were MP divided by number of individuals.

To avoid external sources of contamination, there was a preference for use of metallic and glass materials. For sampling we have used plastic pots, glass pots were unavailable, and their use would be dangerous to dive. However, the pots were rinsed and cleaned with filtered distillate water, as well as other materials. Besides, the staff and students wore cotton coats, samples were covered with lid or aluminium foil. Solutions, as saline water, were pre-filtered before use. A petri dish with filtered distillate water, free of contamination was exposed during the processing of samples to avoid airborne contamination. This contamination was quantified, and the same type of material found in samples was removed to avoid the overestimation of data (Bogdanowicz et al., 2021).

Correlation was computed to examine the relationship between the density of MP in substrates and amphipods (MP/individual) using R Studio Software (Version 1.3.959). Normality test was examined with Shapiro-Wilk ($p > 0.05$). Since the data was not normally distributed and had a low number of n, Kendall`s correlation was calculated. P-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Microplastics in sites from Rio de Janeiro coast

This study focused on the presence of MP contamination in rocky shore benthic organisms and correlating its presence in natural substrates and their associated amphipods. Regarding the concentration of MP around sites in Rio de Janeiro, fibers were more common than fragments, which is align with other studies (Goss et al. 2018; Jones et al., 2020; Li et al., 2020; Seng et al., 2020). Comparing the sites, João Fernandinho beach (JF) presented a greater quantity of plastics. João Fernandinho beach had higher abundance of MPs in algae (51%) followed by ITA (18%), MA (16%), BV (8%) and UR (7%) (Figure 2). Length of fibers ranged from 0.09 to 4.9 and fragments from 0.04 to 3.0 mm, both with an average of 1.1 mm. In respect to the colours of MPs, there was a predominance of blue microplastics (67.7%), followed by black (10%), pink (5.7%) and purple (4.8%). Blue particles, generally are related to fishery gear, which is widely used by locals, and other colours originated mainly from macroplastics fragmented and colourful clothes (Cole et al. 2018) (Table 1 Supplementary Material).

The number of MP in João Fernandinho was unexpected because more polluted and densely populated areas are related to a high concentration of MP (Browne et al., 2015), as Guanabara Bay. Intense fishing, recreational and the predatory tourism activities (Oigman-Pszczol and Creed, 2007) could contribute to this elevated number of MP, although more samples are needed to confirm the spatial distribution of MPs in these areas. Maricás, Boa Viagem and Urca also presented unexpected densities of MP. MA is a well-preserved area and with a low human activity which we believed having fewer particles of plastic. But limitation and absence of studies in the area make it difficult to compare and discuss. On the other hand, BV and UR lie inside Guanabara Bay, an area surrounded by a big metropolitan area, with severe environmental disturbance (presence of organic pollutants, heavy metals, and litter) (Carvalho and Baptista Neto, 2016). Due to this characteristic, it was expected that both sites would contribute more to the microplastic density. The absence of sponges and ascidians in Boa Viagem beach contributed to the lowest number of MP. Also, there are not studies about MP in organisms from rocky shore on these two beaches, which make impossible to compare results. Some studies about MP in beaches close to UR presented a lower concentration of MP than inside the bay (Carvalho and Baptista Neto, 2016; Olivatto et al., 2019). Urca lies at the entrance of the bay, far from rives and with more wave movement. These factors could contribute to the lower concentration of MP reported in the work. In addition, the lack of sponge and ascidians samples from BV also contributed to the lower concentration of MPs.

Microplastics in benthic organisms

The comparison and analyses of retention of MP in benthic fauna are important to understand the pathway of this contamination and the animals that are susceptible to ingest more plastics. Firstly, regarding the identified species, *Sargassum* sp. was the most common algae, also including *Jania* sp. and tuffs of unidentified algae. The collected sponges were Aplysina sp., Chalinidae, and an unidentified species, and ascidians identified as *Phallusia nigra, Clavelina oblonga*, and *Styela plicata*. A total of 3,063 amphipods was analysed and identified in eleven families. The highest number of individuals and diversity of families was reported in Maricás (8 families registered).

Microplastics were registered in all substrates from all studied sites (232 items), with a predominance of fibers, 146 items. For all observations, algae retained more MPs than other substrates, 56% of the total items, followed by ascidians (27%) and sponges (17%). Comparing the abundance of MP retained in ascidians species, *P. nigra* contained more particles (40 items) than *C. oblonga* (19) and *S. plicata* (4). Since these substrates are inhabited by a diversity of animals, microplastics are directly transferred through trophic chain. It was reported 20 fragments and 17 fibers in all amphipods, and a mean of 0.01 per individual. Maeridae and Synopiidae (0.2 MP/ind.) presented more microplastics ingested per individual, followed by Ischyroceridae (0.18 MP/ind.) (Figure 3). Regarding characteristics of MPs, it was observed an average of 0.8 mm length and there was also a representativity of blue microplastics (32%), and purple, pink, black representing 16%, followed by green and colourful (8%) (Table 2 Supplementary Material).

Site	**Substrate**	**MP substrate**		**MP total**
		Frag	**Fiber**	
JF	*Sargassum* sp.	15	52	67
	Aplysina sp.	10	1	11

	Phallusia nigra	13	27	40
UR	Algae	9	3	12
	Porifera	3	1	4
BV	Algae	7	12	19
ITA	Algae	3	14	17
	Porifera	10	2	12
	Clavelina oblonga	3	6	9
	Styela plicata	3	1	4
MA	*Sargassum sp.*	1	9	10
	Jania sp.	3	1	4
	Chalinidae	2	11	13
	Clavelina oblonga	4	6	10
	TOTAL	86	146	232

Table 1: Substrates collected and the total of microplastics (fragment and fiber) retained (JF: João Fernandinho; UR: Urca; BV: Boa Viagem; ITA: Itaipú; MA: Maricás Islands).

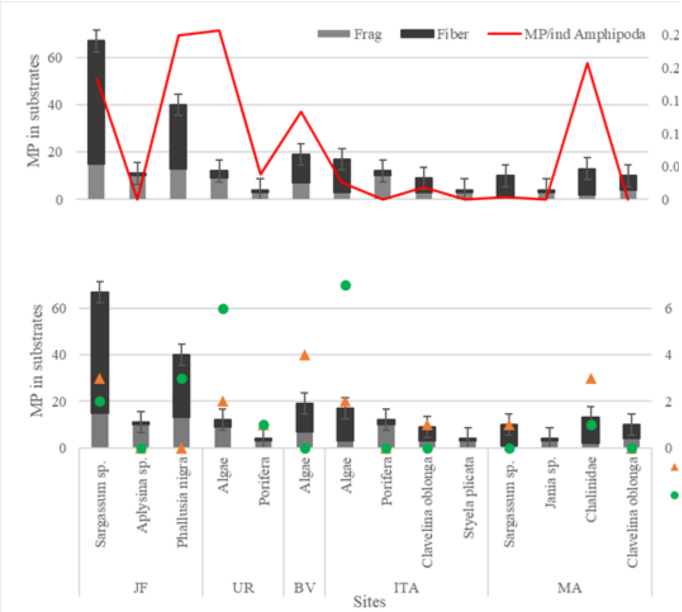

Figure 2: Presence of MPs in the substrates and associated Amphipoda (MP per individual and types of MP in amphipods) from sampled sites. Gray column represents fragments and black column represents fiber. Red line shows number of MP per individual of amphipods. Green circle represents fiber in amphipods and orange triangle fragments. (*MP in amphipods). JF: João Fernandinho Beach, MA: Maricás Island, ITA: Itaipu Beach, BV: Boa Viagem Beach, UR: Urca Beach.

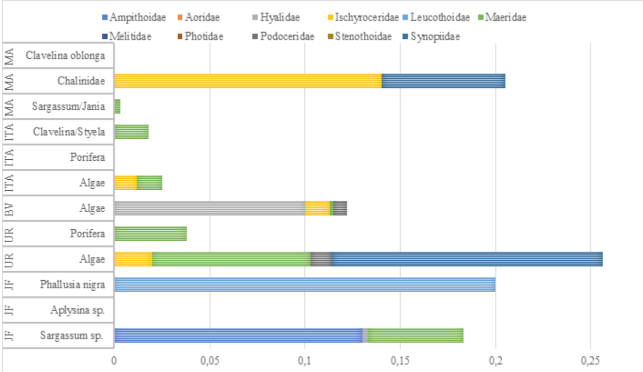

Figure 3: Quantity of microplastic per individual of amphipod among families from sampled sites. No MP in amphipods of ascidian *Clavelina oblonga* (MA), porifera (ITA) and *Aplysina* sp. and in Aoridae, Melitidae, Photidae and Stenothoidae families (these families are in the graphic because they were analysed through degradation method). JF: João Fernandinho Beach, MA: Maricás Islands, ITA: Itaipu Beach, BV: Boa Viagem Beach, UR: Urca Beach.

Correlation between MP density in algae (p = 0.07), sponges, and ascidians (p = 0.09) and MP in amphipods was not observed (Table 3 Supplementary Material). Although, epibionts from JF had more MPs per individual (39%), supporting the previous result (Figure 2). As opposed to the abundance of MPs in substrates from Urca, amphipods from this site represented the second-highest MP quantity (26% of total MP/individual). It was observed that those epibionts associated with algae retained more MP/individual (54%) (ascidians 24% and sponges 22%). Amphipods associated with sponges from JF and ITA and associated with Clavelina oblonga (Ascidiacea) from MA had no ingested plastics.

There was no correlation between MPs found in substrates and amphipods. The ingestion of MPs by freshwater, marine intertidal, pelagic, and benthic amphipods have been commonly reported (Bruck and Ford, 2018; Weber et al., 2018; Yardy and Callaghan, 2020; Mateos-Cárdenas et al., 2021). Few studies investigate ingestion of MP in natural contexts (Jamieson et al., 2019; Jones-Williams et al., 2019). No effects on survival, food consumption, body development, and metabolism were reported (Buck and Ford, 2018; Weber et al., 2018), and amphipods consume more food when the concentration of MP is zero (Carrasco et al., 2019). The mechanisms that amphipods have selecting food in a presence of microplastics needs further investigation. But amphipods seem to prefer food with high nutritional quality (Carrasco et al., 2019). In this study, it may explain a possible preference for real food and consequently low consumption of MP by amphipods. In addition, there was no difference in particles ingestion among families. Many factors may influence MP ingestion, such as the size, shape of plastics and animal`s mouthparts (Wu et al., 2021). These amphipod families are detritivorous, herbivorous, and carnivorous, and further analysis are necessary to understand whether feeding habits affect microplastic ingestion.

More than 50% of the MPs were retained by algae and its associated amphipods. These findings affirm that structures of algae such as branches, stipes, and thallus, trap plastic that become available to associated organisms. The adherence in macroalgae is the most common interaction with MPs, followed by entanglement and wrapping (Li et al., 2022). It has also been reported in seaweed Fucus vesiculosus through laboratory experiments (Gutow et al., 2016), and testing extrapolated concentration of polystyrene particles (Sundaek et al., 2018), in fresh nori Pyropia sp. (Li et al., 2020), as well as in blades of macroalgae and seagrass from natural environment (Goss et al., 2018; Feng et al., 2020; Seng et al., 2020). Jones et al. (2020) also analysed MP in seagrass and associated biota, however, MPs ingested by amphipods were less frequent than here, even quantifying more animals. Nevertheless, these results show that primary producers are potential sink and carriers MPs to its associated organisms.

The retention of MPs in marine sponges and ascidians are still less common. Laboratorial experiments demonstrated uptake of plastic beads in sponges (Turon et al., 1997; Leys and Eerkes-Medrano, 2006), and Modica et al. (2020) and Girard et al. (2021) reported incorporated MPs of sponges from natural contexts (Fallow and Freeman 2021). As these studies measured the MP concentration in MP g-1 of dry tissue, our results are not comparable. However, the length of MPs aligns with ours results (minimum length 0.1 mm (Modica et al., 2020), and especially regards the few large fibers (3 – 5 mm) retained in sponges (Fallon and Freeman, 2021). It is still difficult to confirm how sponges interact with microplastics, actively or passively, due to the absence of laboratory experiments (Saliu et al. 2022). However, following what is suggested, MPs enter the sponge tissue by active filtration, and the organism may select the size, preferring small particles (0.01 – 0.02 mm). For food fragments, porocytes, choanocytes and vacuoles internalize and retain the particles for posterior digestion. It could explain the retention of MPs in sponges, without the digestion process (Saliu et al., 2022). Larger fibers would incorporate the sponge body by endocytose process, but experiments inducing MP uptake by exopinacocytes are necessary.

Experimental studies analysing the filtration of MPs by sponges would confirm a possibility of particles selection, whether the water flow would transport all the particles, or how the fiber would get embedded and incorporated in tissues as showed in sponges from museum collections (Fallon and Freeman, 2021; Modica et al., 2021). In fact, sponges may gather fibers through shallow to deep water, as fibers are distributed and accumulated in shallow rocky shores and deep sediments. In addition, associated amphipods of sponges inhabit the spongocoel where the water is already filtered by the host (Thiel 1999). Experiments are necessary, but perhaps this may justify the lower concentration of ingested MP by these associated organisms.

At the present, only four studies reported MPs in ascidians (Messinetti et al., 2018; 2019; Vered et al., 2019; Silva et al., 2021), two of those reporting juvenile growth effects through laboratory experiments. It is not clear whether the ascidians select the particles and distinguish the real food from plastic. As showed by Messinetti et al. (2018), some specimens may sense the microbead and rejected it from oral siphon with body contractions. Although the MPs were accumulated, translocated to the gut circulatory system, and caused slow development of juveniles, the process of uptake and translocation through tissues and organs are still insufficiently investigated. Regarding the analysis of ascidians from natural environment, *Herdmania momus* and *Microcosmus exasperatus* (Vered et al., 2019) and Phallusia nigra (Silva et al., 2021) were reported ingesting MP. Here, *P. nigra* retained two-thirds of the MP concentration in ascidians (40), followed by colonial ascidian *Clavelina oblonga* (19) and *Styela plicata* (4). As the process of uptake of MP by ascidians is still unclear, it is difficult to suggest factors that influence this ingestion. Nevertheless, in solitary and colonial species, the water flow level, tentacles, structures in oral siphon and branchial baskets may influence the uptake and retention of MP in the ascidian’s bodies. Species of amphipods dwell in the ascidian body, generally the branchial baskets, where the incoming water is not yet filtered (Thiel, 1999). In this way, MP accumulated or translocated to branchial basket become available to associated amphipods.

These findings provide important information on the interaction of benthic organisms to MP contamination, especially to sites in Rio de Janeiro previously not reported. Natural substrates in rocky shores are excellent microhabitats to many organisms. They provide to epibionts a protected place to develop and recruit and food resources. The presence of MP in these substrates contribute to the passage of this contamination through the trophic chain. Although amphipods have not presented an intense abundance of MP, other organisms may be vulnerable to these particles, such as crabs and molluscs. Future research is necessary to clarify the mechanisms that influence the MP uptake by sponges, ascidians, and amphipods. More attention should be applied to understand the process of water filtering of these filter-feeding animals and the accumulation of particles in the tissue and the bioavailability of MP from these organisms to the associated amphipod.

The current scenario of ocean plastic contamination should concern the population and politicians. However, only scientists and a few environmental organizations show these matters and contributions to reducing this pollution. It is necessary to link these social, political, and scientific areas to collaborate in monitoring studies of plastic in the ocean and to create environmental planning and solid residue management to preserve marine coasts.

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