Scientia Marina 89(1)
March 2025, e094
ISSN-L: 0214-8358, eISSN: 1886-8134
https://doi.org/10.3989/scimar.05569.094

Assessment of macro-, meso- and microplastics in wild and cultivated *Perna perna* mussels (Mollusca: Bivalvia)

Lucas Aguiar Leal Rocha[1], Helena Antunes Portela[2],
Mônica Regina da Costa Marques Calderari[3], Fábio Vieira de Araújo[1,4]

[1]Universidade Federal Fluminense, Programa de Pós Graduação em Biologia Marinha e Ambientes Costeiros,
Instituto de Biologia, Bloco M, Rua Prof. Marcos Waldemar de Freitas Reis - São Domingos, Niterói - RJ, Brazil, 24210-201.
(LALR) E-mail: lucas.lealrocha@gmail.com. ORCID: https://orcid.org/0009-0007-5104-4619
(FVA) (Corresponding author) E-mail: araujofv.bio@gmail.com. ORCID: https://orcid.org/0000-0002-1931-7302
[2]Universidade do Estado do Rio de Janeiro, Faculdade de Geologia,
Rua São Francisco Xavier 524, Maracanã, Rio de Janeiro, RJ, Brazil, 20550-900.
(HAP) E-mail: helenaportela@gmail.com. ORCID: https://orcid.org/0009-0007-7789-8460
[3]Universidade do Estado do Rio de Janeiro, Instituto de Química,
Rua São Francisco Xavier 524, Maracanã, Rio de Janeiro, RJ, Brazil, 20550-900.
(MRCMC) E-mail: monicamarquesuerj@gmail.com. ORCID: https://orcid.org/0000-0001-6906-8327
[4]Universidade do Estado do Rio de Janeiro, Faculdade de Formação de Professores,
Rua Francisco Portela 1470, Patronato, São Gonçalo, RJ, Brazil, 24435-005.

Summary: Microplastics have drawn significant interest within the scientific community due to their environmental impact. In marine ecosystems, these particles readily absorb toxic substances and, because of their microscopic size, are consumed by various organisms that are eventually incorporated into the human food chain. Several studies emphasize this unintentional transfer to human foods. To investigate microplastic contamination in *Perna perna* mussels sold commercially, wild (harvested from the coastal environment) and farm-raised (cultivated) samples were purchased from local markets in Niterói, RJ, Brazil. One kilogram of each type was processed through digestion in an NaOH 5% solution and then analysed to assess the quantity and types of microplastics present. Both wild and cultivated mussels contained microplastic film and fibres, with 145 items (0.145 items g^{-1}) identified in wild specimens [67% nylon (polyamide) and 33% polyethylene] and 120 items (0.120 items g^{-1}) in cultivated mussels (100% polyamide). The microplastics varied in size, ranging from 0.45 µm to 28 mm, with the most common colours being black, blue and transparent. These findings highlight the potential risks of consuming these organisms regardless of their source and the need for further investigation into the effects of these pollutants on human health.

Keywords: mussel culture, Itaipu Beach, Cedro Beach, marine litter, sodium hydroxide, coastal environments, seafood contamination, human health.

Evaluación de macro, meso y microplásticos en mejillones *Perna perna* silvestres y cultivados (Mollusca: Bivalvia)

Resumen: Los microplásticos han despertado un gran interés dentro de la comunidad científica debido a su impacto medioambiental. En los ecosistemas marinos, estas partículas absorben fácilmente sustancias tóxicas y, debido a su tamaño microscópico, son consumidas por diversos organismos que eventualmente se incorporan a la cadena alimentaria humana. Varios estudios enfatizan esta transferencia involuntaria a los alimentos humanos. Para investigar la contaminación por microplásticos en los mejillones *Perna perna* vendidos comercialmente, se compraron muestras silvestres (cosechadas del ambiente costero) y criadas en granjas (cultivadas) en los mercados locales de Niterói, RJ, Brasil. Se procesó un kilogramo de cada tipo mediante digestión en una solución de NaOH al 5% y luego se analizó para evaluar la cantidad y los tipos de microplásticos presentes. Tanto los mejillones silvestres como los cultivados contenían películas y fibras microplásticas, con 145 artículos (0,145 artículos g^{-1}) identificados en especímenes silvestres (67% nailon (PA) y 33% polietileno (PE)) y 120 artículos (0,120 artículos g^{-1}) en mejillones de cultivo (100% PA). Los microplásticos variaban en tamaño, desde 0,45 µm hasta 28 mm, siendo los colores más comunes el negro, el azul y el transparente. Estos hallazgos resaltan los riesgos potenciales de consumir estos organismos independientemente de su fuente y la necesidad de realizar más investigaciones sobre los efectos de estos contaminantes en la salud humana.

Palabras clave: mitilicultura, playa de Itaipú, playa del Cedro, basura marina, hidróxido de sodio, ambientes costeros, contaminación de mariscos, salud humana.

Citation/Como citar este artículo: Aguiar Leal Rocha L., Antunes Portela H., da Costa Marques Calderari M.R., Vieira de Araújo F. 2025. Assessment of macro-, meso- and microplastics in wild and cultivated *Perna perna* mussels (Mollusca: Bivalvia). Sci. Mar. 89(1): e094. https://doi.org/10.3989/scimar.05569.094

Editor: A. Sanchez Vidal.

Received: August 02, 2024. **Accepted:** December 09, 2024. **Published:** March 28, 2025.

INTRODUCTION

When improperly discarded or released into the environment, solid waste can travel great distances, contaminating various ecosystems, spreading invasive species, creating socioeconomic challenges, harming human health and negatively affecting tourism (Hirata et al. 2017, Olivatto et al. 2018, Silva et al. 2018, Machado et al. 2021). Research has consistently shown that plastic is the most prevalent type of waste on beaches and coastal areas (Silva et al. 2018, Olivatto et al. 2019, da Silva et al. 2022). This is largely due to plastic's durability, resistance and low cost, which have made it integral across numerous sectors such as single-use products, food packaging, cosmetics (e.g. scrubs and abrasives), medical equipment, construction materials and textiles, effectively replacing traditional materials in these industries (Dehaut et al. 2018, Olivatto et al. 2018, Machado et al. 2021).

According to Plastics Europe (2023) estimates, global plastic production reached 400 million t in 2022, including thermoplastics, highlighting its widespread use. Given its high production and disposal rates, plastic is considered a highly contaminating product and a global threat to environmental issues, food safety and human health (Sharma et al. 2017, Bessa et al. 2018, Peixoto et al. 2019, da Silva et al. 2022).

Once in the environment, plastics undergo weathering processes such as photodegradation, chemical breakdown and mechanical wear, leading to fragmentation and changes in properties such as colour. When these fragments measure between 5 mm and 0.1 µm, they are classified as microplastics, either secondary (from the breakdown of larger plastics) or primary (originally manufactured at this size, such as pellets and microspheres). Particles smaller than 0.1 µm are categorized as nanoplastics (Castro et al. 2016, Olivatto et al. 2018, Mercogliano et al. 2020). Studies have widely documented microplastics and nanoplastics across aquatic, terrestrial and aerial environments, highlighting their extensive dispersion and ingestion across trophic levels, from plankton to humans (Sobral et al. 2011, Hirata et al. 2017, Peixoto et al. 2019, Zurier et al. 2020).

Microplastics are known to carry pathogens and absorb various pollutants present in the marine environment, including persistent organochlorine pesticides, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, pharmaceuticals and polybrominated diphenyl ethers. When these pollutants detach from ingested microplastics, they can be absorbed by marine organisms, potentially compromising their health and survival. Furthermore, the risk of biomagnification poses a hazard to top predators in the food chain, including humans, as prolonged exposure to these contaminants accumulates over time through the food web (Araújo et al. 2021).

When microplastics come into contact with skin or are inhaled or ingested, they can trigger oxidative stress and inflammatory lesions, affecting various human bodily systems, including cardiovascular, renal, respiratory, reproductive, gastrointestinal and neurological systems. This exposure can lead to serious health issues such as cancer and diabetes (Prata et al. 2020, UNEP, 2021). As a result, the inadvertent consumption of microplastics by a range of marine organisms poses a potential health risk to humans, even if the viscera are removed before consumption (Olivatto et al. 2018, De-la-Torre, 2020, Kutralam-Muniasamy et al. 2020, Zurier et al. 2020).

Brazil's average seafood consumption is around 5-10 kg per capita yearly (FAO, 2020). While mussels constitute a relatively small fraction of this consumption, their sessile and filter-feeding nature allows them to accumulate pollutants in the water, potentially amplifying health risks when ingested. These characteristics enable mussels to reveal environmental impacts that may otherwise go unnoticed (Resgalla et al. 2008, Pierri et al. 2016, Cho et al. 2018, Mercogliano et al. 2020, Machado et al. 2021). Li et al. (2019) note that numerous researchers have suggested using mussels as indicators for microplastics in coastal areas.

Given their prevalence along the Brazilian coast and their popularity as a food source, especially in the southern and southeastern regions, the *Perna perna* mussel was chosen for this study. These mussels are harvested from natural beds and cultivated through aquaculture. According to IBGE (2023), Brazil's total mussel production reached 15700 t, with the southeastern region alone contributing 82896 kg of oysters, mussels and scallops. This highlights the significance of mussel farming in the region's seafood industry.

Mussel farming in Brazil utilizes longline systems, which involve nylon cables ranging from 50 to 100 m anchored to floats, typically large plastic barrels, to support cultivation nets. This method can support approximately 4 t of mussels (Valenti et al. 2021). Once harvested, farmed mussels typically undergo a depuration process before being packaged for sale, ensuring they are clean and safe for consumption.

Given the prevalence of plastic waste in the environment and the plastic materials used in mussel farming, there is a critical need for research to assess the potential presence of microplastics in mussels available to consumers. We hypothesize that farmed mussels, which undergo a depuration process before sale, are less likely to be contaminated with microplastics than those harvested directly from natural sources.

Additionally, the findings from this study could inform future research aimed at establishing maximum permissible concentrations of these pollutants in water and mussels, ultimately reducing the risks associated with consuming these organisms.

MATERIALS AND METHODS

Sample processing

Mussels of the *Perna perna* species were purchased from two locations in Niterói, RJ: Mercado São Pedro, a seafood market, and Mercado Dom Atacadista, a retail supermarket chain. At Mercado São Pedro, the mussels were sold in 1 kg bags, cooled and without shells. According to the sellers, these mussels were collected artisanally from the rocky shores of Itaipu Beach, Niterói, RJ. In contrast, those purchased at Mercado Dom Atacadista were in 1 kg commercial packaging, without shells, frozen and sourced from a cultivation farm at Cedro Beach, Palhoça, SC, as indicated on the packaging.

The samples were kept frozen at -20°C until the moment of analysis. A total of 180 individuals of the species were analysed, 90 from artisanal extraction and 90 from cultivation farms. The average size and weight of mussels from both sources were approximately 5 cm and 11 g, respectively.

Sources of Mussels

Itaipu Beach (22°58'15"S, 43°2'47"W), located at the eastern end of Itaipu Cove in Niterói, is just 800 m long (Fig. 1A) (da Silva et al. 2021). It is bounded by the Itaipu Channel to the north and Andorinhas Hill to the south, formed by Precambrian gneiss. Due to its inland location, Itaipu Beach is the least susceptible to wave dynamics (Eccard et al. 2017). The beach and its surroundings experience high levels of real estate speculation and intense economic activity, including restaurants, bars and street vendors. This activity intensifies during the high season with the influx of tourists, serving nearby neighbourhoods that are home to families from various economic backgrounds (Timbó et al. 2019, da Silva et al. 2021).

The mussel cultivation area is located at Cedro Beach, Palhoça, SC (27°44'93"S and 48°36'54"W), in a region between 6 to 8 m deep (Fig. 1B). Palhoça is situated in the Cubatão do Sul River Basin, which covers approximately 1451 km², consists of 51 micro-basins and is delimited by lands drained by the Cubatão do Sul River and its tributaries, such as the Vargem do Braço, Salto, Bugres, Cedro, Caldas do Norte (or das Forquilhas) and Matias rivers. The municipality's coastal margin is also influenced by the Rio da Madre Basin covering an area of approximately 545 km² and consisting of 17 micro-basins (Novaes et al. 2010).

Sample analysis

The mussels were not analysed individually but in a single pooled sample per location, one from a natural bank (wild mussels) and one from a cultivation area (cultivated mussels).

To facilitate the dissolution of the samples by increasing the surface area of the mussels in contact with the reagent, each sample was divided into portions of approximately 25g and gently crushed by hand before being placed in beakers with 100 mL of a 5% solution of sodium hydroxide (NaOH). To avoid contamination, these beakers were covered with aluminium foil and incubated for 24 h in a water bath at 60°C to degrade the organic matter (mussel body) (adapted from Catarino et al. 2017). After 24 h, the solution was diluted with distilled water and vacuum-filtered through 8 μm cellulose acetate filters. The filters were examined under a stereomicroscope to quantify and characterize the microplastics by shape, size and colour, based on the methodology adapted from Castro et al. (2020).

The NaOH solution and distilled water were previously filtered to ensure the accuracy of the results. Contamination-free Petri dishes filled with previously filtered distilled water were exposed during the sample processing to monitor for laboratory contamination. Contamination in the control samples was analysed and quantified, and any microplastic observed was identified and excluded from the data for each sampling site.

The chemical composition of the microplastics was analysed using a Fourier transform infrared spectrometer with an attenuated total reflection diamond crystal (FTIR-ATR), specifically the Perkin

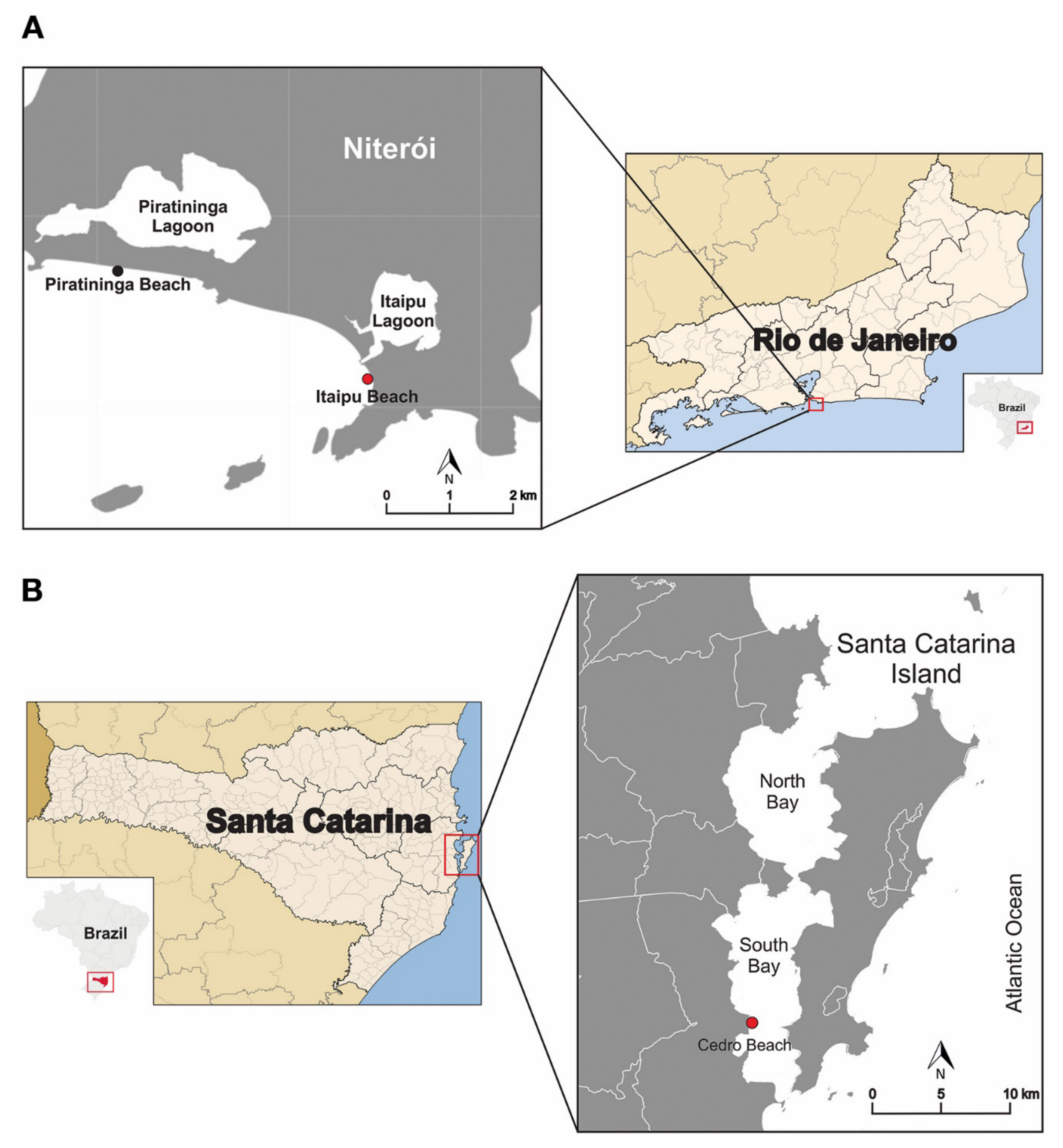

Fig. 1. - Map showing the mussels' collection sites (red dot) analysed in this study.
A - Itaipu Beach in Niterói, RJ, Brazil, from where the wild mussels were collected, and
B, Cedro Beach, Santa Catarina, SC, Brazil, where the farmed mussels were collected.

Elmer Frontier FTIR model. Two plastic control samples of polyethylene and polypropylene were used to check the spectrum-matching capability of the equipment and software. All spectra were recorded in the wavelength range from 500 to 4000 cm^{-1} at a 4 cm^{-1} resolution. The particles were selected respecting the limit of 500 µm because the analysis of FTIR-ATR needs to put the samples in contact with the ATR crystal (Castro et al. 2020).

RESULTS

Quantification and characterization of microplastics

In total, 34 microplastics (all white fibres) were observed in the control samples (20 during the processing of the wild samples and 14 during the processing of the cultivated samples). These values were subtracted from the total value found in the respective samples.

The result (after deducting the values of the control experiments) showed fibre and film microplastic found in all analysed samples (Fig. 2). In the 1 kg portion of mussels purchased at Mercado São Pedro, sourced from Itaipu Beach, 145 microplastic particles were detected (0.145 particles g^{-1}), comprising 123 fibres (0.123 fibers g^{-1}) and 22 films (0.022 films g^{-1}). Most fibres were black (90%), followed by blue (4%), brown (2%) and red (0.8%). The films were mostly transparent (45.5%), with others being pink (27.3%) and green (13.6%) (Fig. 3).

A total of 120 microplastic particles (0.12 particles g^{-1}) were found in the cultivated mussels purchased from supermarkets, consisting of 86 fibres (0.086 fibers g^{-1}) and 34 films (0.034 films g^{-1}). Among fibres, blue colour was prevalent, accounting for 44%. In the film particles, transparent microplastics predominated, making up 76.5% (Fig. 3).

The sizes of the recovered plastic particles were classified according to the standards set by UNEP (2021) and EFSA (2016) (Table 1).

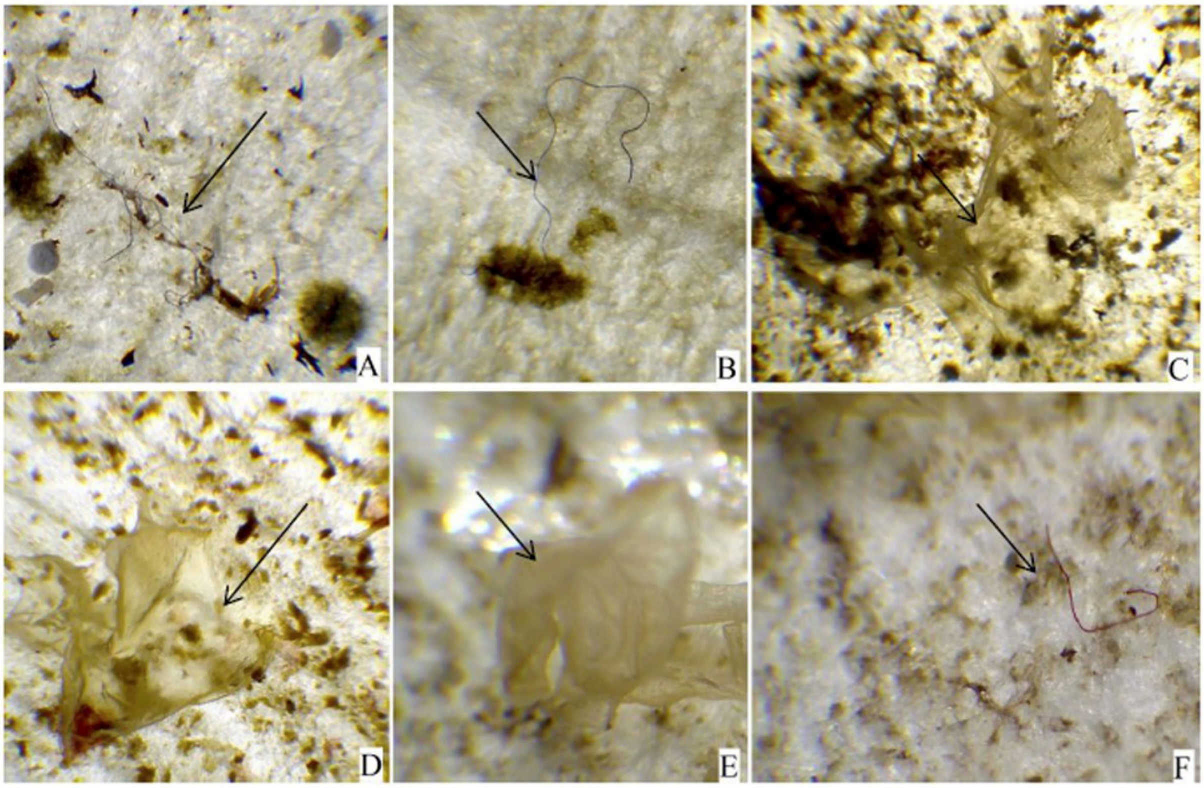

Fig. 2. - Microplastic particles recovered from wild and cultivated mussels. A, B and F, fibres; C, D and E, films.

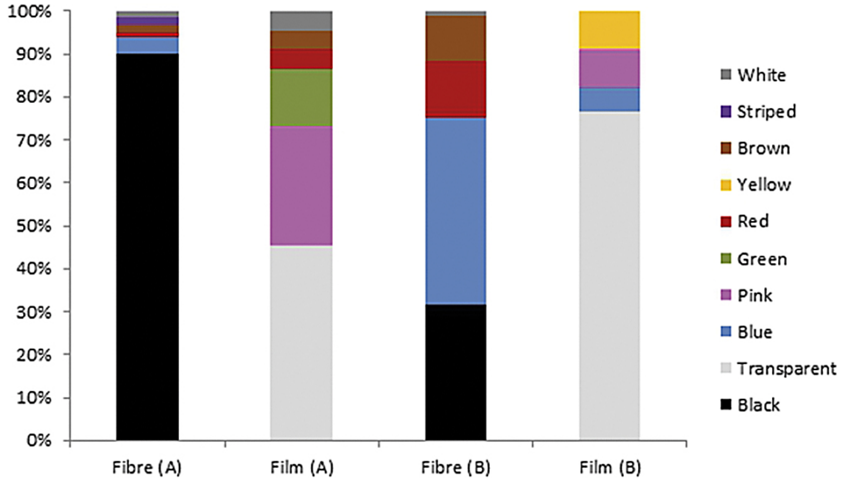

Fig. 3. - Percentage of colours found in microplastic fibres and films found in wild (A) and cultivated (B) mussels.

Chemical composition of microplastics

Due to the small size, difficulty of removal from the filter and similarity between the particles, only 23 plastic particles were submitted to FTIR (8.67% of the total). Nine were fibres from the cultivated sample and 14 (6 fibres and 8 films) were from the wild sample. Examination of particles collected from cultivated mussels revealed that all analysed particles were chemically similar to nylon (polyamide). In contrast, among the wild mussels, 42% of the particles were chemically like nylon (polyamide), while the remaining 58% were identified as chlorinated polyethylene and chlorosulphonated polyethylene.

DISCUSSION

The presence of microplastics in both wild and farmed mussels is widely documented in the literature (Li et al. 2015, Li et al. 2018, Barboza et al. 2018, Cho et al. 2018, Mercogliano et al. 2020), aligning with our findings. This occurrence can be attributed to the behaviour of mussels, which can filter up to 2 L of surrounding water per hour, as well as to the increasing prevalence of these pollutants in coastal waters, where natural beds and aquaculture farms are located (Van Cauwenberghe et al. 2015, Cho et al. 2018, Mayoma et al. 2020, Machado et al. 2021). Mussels feed on what is carried by currents, including plankton and pollutants such as microplastics.

As in our results, the literature generally reports higher amounts of microplastics in wild mussels than in cultivated ones. Some authors (Van Cauwenbergh et al. 2015, Catarino et al. 2018, Cho et al. 2018)

Table 1. - Number of plastic particles recovered per kilogram found in wild and cultivated mussels

Classification	Wild		Cultivated	
	Fibre	Film	Fibre	Film
Microplastic (0.001-<5 mm)	116	18	80	20
Mesoplastic (5-10 mm)	07	03	06	12
Macroplastic (>20 mm)	-	01	-	02
TOTAL	123	22	86	34
	145		120	

suggest that this difference may be due to pollution in the surrounding waters and intertidal zones, where bivalves commonly settle, exposing them to suspended microplastic particles. In contrast, cultivated mussels are typically located at depths greater than one metre and remain submerged for extended periods (or constantly) as a result of aquaculture setups.

The slight difference in microplastic values between wild (0.145 particles g^{-1}) and cultivated mussels (0.120 particles g^{-1}) in our study may be primarily due to the low levels found in wild mussels compared with other regions. For instance, Li et al. (2018) reported concentrations of 0.7 to 2.9 particles g^{-1} in mussels from United Kingdom coastal areas. Renzi (2018) also found higher levels in four Italian locations, ranging from 3.0 to 9.2 particles g^{-1}. Similarly, Li et al (2015) reported concentrations ranging from 2.1 to 10.5 particles g^{-1} in nine Chinese commercial bivalve species, while another study reported 1.52 to 5.36 particles g^{-1} (Qu et al. 2018). All these authors commonly suggest that the main source of microplastics is the contamination of nearby areas, which is directly related to human activities.

In Niterói, Birnstiel et al. (2019) recorded 6.67 particles g^{-1} in *Perna perna* mussels collected from Eva Beach and 4.12 particles g^{-1} from Jurujuba Beach. Although these beaches are close to Itaipu Beach, they are in sheltered coves within Guanabara Bay, a known polluted area with high microplastic loads (Castro et al. 2016, Birnstiel et al. 2019, Castro et al. 2020). According to Castro et al. (2020), the waters of Itaipu, where wild mussels were collected, exhibited relatively low microplastic concentrations, ranging from 1.2 particles m^{-3} in winter to 2.86 particles m^{-3} in summer. The Itaipu Beach area is significantly influenced by ocean waters, which promote the easy dispersal of microplastic particles due to local hydrodynamics, potentially explaining the lower concentration of mussels from this area.

Another possible explanation for the lower difference in values between wild and cultivated mussels in this study could be the depuration process, which the latter undergo after harvesting and before packaging. Birnstiel et al. (2019) demonstrated that microplastic concentrations in cultivated mussels in Niterói decreased by 28.95% after 93 hours of depuration. This process can reduce larger particle concentrations (Van Cauwenberghe and Janssen, 2014). These authors found that depuration with HNO_3 lowered the microplastic concentration by around 30% after three days, although smaller particles persisted, suggesting potential translocation through the intestinal wall into tissues and the circulatory system. This is corroborated by the continued presence of microplastics in organisms even post-depuration, but differs from our results, as we found a greater quantity of larger particles in cultivated mussels.

The values obtained in this study for cultivated mussels were below those reported by Van Cauwenberghe and Janssen (2014), who found microplastic concentrations in *Mytilus edulis* of 0.36±0.07 particles g^{-1} before depuration and 0.24±0.07 particles g^{-1} afterward, and in *Crassostrea gigas* (0.47±0.16 particles g^{-1} before depuration and 0.35±0.05 particles g^{-1} afterward). Likewise, Li et al. (2018) observed concentrations ranging from 0.9 to 1.4 particles g^{-1} in mussels purchased from supermarkets and Cho et al. (2018) reported values up to 1.08 particles g^{-1} in mussels from a fish market in southern Korea.

The lower value found in this study (0.12 items g^{-1} in cultivated mussels) corresponds to approximately 2% of the average daily microplastic intake per person (100 to 140 particles/day according to Cox et al. 2019), assuming a daily seafood intake of 20 g in Brazil (FAO, 2020). These values are only estimates, as mussel consumption in Brazil constitutes only a portion of total seafood consumption, but in the south and southeast regions where this consumption is higher, this daily intake may represent a higher percentage, increasing the risk of consuming these molluscs.

It is important to note that the varying concentrations of microplastics reported in various studies may also be influenced by the methodologies used to dissolve organisms. There is no standardized protocol for recovering microplastics in seafood (EFSA, 2016, Catarino et al. 2018, Cho et al. 2018, Digka et al. 2018, Li et al. 2018). The choice of chemical agents and their concentrations can partially or completely damage microplastics, affecting their size and colour.

Our study used 5% NaOH, a concentration that falls outside the range specified by Lusher et al. (2017). This concentration is unlikely to damage the main types of microplastic particles frequently reported in the literature, including polypropylene, polyethylene, polyvinyl chloride and polyethylene terephthalate.

The microplastic particles shown in this study displayed chemical similarities to nylon (polyamide), chlorinated polyethylene and chlorosulphonated polyethylene, with fibres and films identified as the main morphological types. Plastic fibres and films are commonly found in marine environments and were predominantly described in Guanabara Bay, a region close to Itaipu, by Baptista Neto et al. (2019). Similarly, Brocardo (2022) reported that fibres (75%) and fragments (25%) were the predominant microplastic forms in mussels from farming sites on Santa Catarina Island, close to the area of the current study. Fibres found in our study were all characterized as polyamide and were the total samples from farmed mussels. Birnstiel et al. (2019), studying microplastics in a farming area in Jurujuba, Niterói, RJ, also reported a high prevalence of polyamide in fibre form. These authors proposed that these detected fibres may have secondary sources, potentially originating from synthetic fabrics or maritime ropes, given the higher density of polyamide relative to polyethylene, the fact that mussels are farmed at depths greater than one metre and remain submerged for long periods, and the use of nylon ropes in these aquaculture structures.

In the Itaipu area, where wild mussels were collected, there is a fishing community, and the presence of polyamide fibres in the mussels from this area can be explained by fibre contamination from fishing gear used by these fishermen. The films analysed in mussels from Itaipu were characterized as chlorinated polyethylene and chlorosulphonated polyethylene. These compounds were also described by Castro et al. (2020) when they analysed the waters of this location. These compounds are used in various products and provide these products with resistance and durability against environmental stressors such as UV degradation. These characteristics may explain their availability in Itaipu's waters, influenced by Guanabara Bay, a region heavily contaminated with microplastics, and their subsequent ingestion by mussels from natural beds.

These results support the hypothesis that improper waste disposal and fishing materials are sources of plastic particle contamination in the marine environment.

In this study, the most prevalent colours of microplastic particles were black and transparent, with blue also being common in mussels from cultivated sources. The prevalence of blue particles aligns with findings from Digka et al. (2018) and Brocardo (2022), which indicate that blue is characteristic of plastics used in longline structures such as ropes and suspension buoys. This blue coloration was consistently observed in the studies by Birnstiel et al. (2019) and Castro et al. (2020) carried out in the same areas as the present study.

Regardless of their origin, our results show that mussels accumulate microplastics and most likely reflect the presence of these pollutants in their environment, highlighting their suitability for monitoring pollution levels. This reinforces the importance of using mussels as indicators of plastic contamination in marine ecosystems, providing valuable data that can inform both environmental management practices and public health concerns.

CONCLUSION

This study aimed to quantify and characterize microplastic particles and their chemical composition in *Perna perna* mussels sold in Niterói and sourced from aquaculture farms and natural habitats. According to previous studies conducted in the regions studied, the quantity, colour and chemical composition of the microplastics found in wild and cultivated mussels suggest a strong influence from the surrounding environment.

Although the values found in wild and cultivated mussels were lower than those reported in other studies, it is evident that the population consumes contaminated mussels, regardless of whether they are wild or cultivated, putting their health at risk. Management measures, especially in areas designated for mussel cultivation, must be implemented to prevent the presence of plastic waste in these environments. Additionally, given the use of plastic materials in cultivation structures, mandatory depuration of these mussels before commercialization should be enforced.

ACKNOWLEDGEMENTS

Thanks to Dr Gisela Mandali de Figueiredo and Dr Rebeca de Oliveira Castro for the revisions made to the original draft and to the reviewers of the first version of the article for their suggestions and comments.

DECLARATION OF COMPETING INTERESTS

The authors of this article declare that they have no financial, professional or personal conflicts of interest that could have inappropriately influenced this work.

FUNDING SOURCES

Thanks to FAPERJ (Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro), CAPES (Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for the grants given to the authors.

AUTHORSHIP CONTRIBUTION STATEMENT

Lucas A. L. Rocha: Formal analysis, Investigation, Methodology, Project administration, Writing - original draft. **Helena A. Portela:** Project administration, Writing - review & editing. **Mônica R. C. M. Calderari:** Methodology, Writing - review & editing. **Fábio V. Araújo:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Writing - review & editing.

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