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Experimental assessment of microplastic selectivity in the brown mussel *Perna perna* (Linnaeus, 1758)

Eshelley T. Pires[1], Beatriz Louveira[1], Maria Eduarda S. Manso[1], Isabela Maria B. F. Mendes[1], Cibele X. Cenciani[1] and Raquel A. F. Neves[1,2*]

[1] Departamento de Ecologia e Recursos Marinhos – Instituto de Biociências (IBIO) - Universidade Federal do Estado do Rio de Janeiro (UNIRIO) (Rio de Janeiro - Avenida Pasteur 458, Botafogo - CEP: 22.290-240 - RJ – Brazil).

[2] Departamento de Ecologia e Recursos Marinhos – Instituto de Biociências (IBIO) - Grupo de Pesquisa em Ecologia Aquática Experimental e Aplicada - Universidade Federal do Estado do Rio de Janeiro (UNIRIO) (Rio de Janeiro - Avenida Pasteur 458 Lab 410, Botafogo - CEP: 22.290-240 -RJ, , Brazil).

* Corresponding author: raquel.neves@unirio.br

ABSTRACT

Microplastics are ubiquitous in aquatic ecosystems and raise concerns due to their persistence and potential toxicity. Given their known impacts on marine species and the use of bivalves as sentinel species for contamination monitoring, this study aimed to experimentally investigate the selectivity of the brown mussel *Perna perna* (Linnaeus, 1758) when exposed to the chlorophyte microalga *Tetraselmis* sp. F. Stein, 1878 and plastic microbeads of similar morphology and size. The study hypothesis is that when microplastics resemble natural food in shape and size, *P. perna* can discriminate between particles, preferentially ingesting microalgae and rejecting microplastics. Experiments included three treatments in triplicate: microalgae only (MA), polystyrene microbeads only (MB), and a 1:1 mixture of microalgae and polystyrene microbeads (MA+MB). Aliquots were collected at the beginning and end of the incubation period and quantified using an optical microscope. Clearance and ingestion rates were calculated for each replicate, and mean values were determined. Although ingestion rates did not differ significantly among treatments, the clearance rate was significantly higher in the MB treatment (627.07 mL g dw^{-1} h^{-1}) than in MA (270.59 mL g dw^{-1} h^{-1}) and MA+MB (518.39 mL g dw^{-1} h^{-1}), suggesting increased filtering activity. Ingestion rates ranged from 6.80 x 10^4 to 1.22 x 10^5 particles g dw^{-1} h^{-1} across treatments. Mussels ingested microbeads regardless of microalgal availability, indicating no particle selectivity. Hence, the study hypothesis was not supported. Ecologically, this inability to discriminate among particles may have detrimental consequences for mussels. A similar pattern may occur in natural environments, where microplastics are present in the water column of coastal areas and interact with plankton and suspended organic particles, facilitating ingestion and subsequent contamination.

Keywords: Clearance rate, Chlorophyte, Ingestion rate, Microbeads, Polystyrene

Plastics have become indispensable in contemporary society due to their versatility, durability, and wide range of applications (Andrady and Neal, 2009; Khoshoue et al., 2026). Although first synthesized in the early 20th century, their large-scale production and use intensified significantly after World War II. Today, it is challenging to envision modern life without plastics—synthetic organic polymers whose mass production has led to a dramatic increase in waste generation (Geyer et al., 2017; Yang et al., 2021). Their ability to be molded under heat and pressure has enabled the replacement of numerous natural

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resources and the development of products for diverse sectors, including medicine, textiles, agriculture, technology, packaging, construction, and transportation (Khoshoue et al., 2026). Common applications range from disposable food and beverage containers to medical devices, toys, insulation materials, and household goods (Hale et al., 2020). However, because most plastics are designed for single use (e.g., plastic bottles and packaging), they account for an estimated 61–87% of marine litter (Bellou et al., 2021; Neves et al., 2022, 2024). As plastics are synthetic and derived from fossil hydrocarbons, they are not fully biodegradable; instead, macro- and mesoplastics fragment into smaller particles that persist in ecosystems (Geyer et al., 2017; Ghobish et al., 2025). The United Nations estimates that marine environments worldwide contain around 75–199 million tons of plastic debris (Ghobish et al., 2025).

Microplastics (MPs), defined as plastic particles ranging from 5 mm to 1 µm in size, originate either as primary particles, manufactured for specific applications, or as secondary fragments derived from the breakdown of larger plastic items (Geyer et al., 2017; Yang et al., 2021). MPs are of increasing concern due to their persistence, resistance to degradation, and potential toxic effects (Eerkes-Medrano et al., 2015; Ghobish et al., 2025; Law and Thompson, 2014). MPs can affect a wide range of marine organisms, from zooplankton and meiofauna to invertebrates and mammals, via trophic transfer within food webs (Botterell et al., 2019; Gusmão et al., 2016; Santana et al., 2016; Tansel 2026; Zantis et al., 2021). For example, MP exposure has been shown to impair the embryonic development of mollusks, with larval stages exhibiting high mortality rates (Gandara e Silva et al., 2016).

Bivalves—benthic, mostly sessile, filter-feeding organisms—are particularly vulnerable to MP exposure due to their feeding strategy (Lima et al., 2022). By filtering large volumes of water, they indiscriminately accumulate particles and substances regardless of nutritional value, making them effective bioindicators of contaminants (e.g., metals, hydrocarbons, and emerging contaminants) and environmental stressors (Azizi et al., 2018; Morley, 2010). Consequently, bivalves are frequently employed in ecotoxicological research, including studies on MP bioaccumulation (reviewed in Silva dos Santos et al., 2022a). Mussels have been proposed as sentinel species for MP contamination in marine ecosystems (Beyer et al., 2017; Bråte et al., 2018), including the brown mussel *Perna perna* (Linnaeus, 1758) (Staichak et al., 2021).

Perna perna is abundant along the Brazilian coast, especially between the states of Rio de Janeiro and Santa Catarina (Fernandes et al., 2008; Klappenbach, 1965). This species exhibits characteristics that make it suitable for biomonitoring, including tolerance to environmental variability and the capacity to bioaccumulate numerous pollutants (Birnstiel et al., 2019; Cortez et al., 2019; Dailianis, 2011; Oliveira et al., 2016; Silva dos Santos et al., 2018). As filter feeders, *P. perna* may experience adverse physiological effects when exposed to environmental stressors. For instance, exposure to phycotoxins produced during harmful algal blooms can cause histopathological damage in mussels and pose health risks to human consumers (Neves et al., 2021). Fecal contamination has also been reported as a threat to mussel farming in coastal areas with limited water circulation, where elevated coliform levels have been detected in both aquaculture systems and mussel hemolymph (Silva dos Santos et al., 2022b). Regarding MPs, mussel embryos were shown to be sensitive to leachates from both virgin and beached pellets (Gandara e Silva et al., 2016). However, 48 h exposure to additive-free polyethylene (PE), polystyrene (PS), polypropylene (PP), and PP with additives (PPa) MP particles did not significantly affect larval development of *P. perna* (Palanch et al., 2026). Additionally, no significant physiological effects were observed in adult *P. perna* following long-term exposure to polyvinyl chloride (PVC) nano- and microparticles (Santana et al., 2018). Despite declines in wild populations in areas such as Santos Bay (Henriques et al., 2004) and along the Santa Catarina coast (Suplicy, 2018) due to overexploitation, this species holds substantial social and economic value for both aquaculture and artisanal harvesting (Lage & Jablonski, 2008; Valenti et al., 2021).

MP contamination has been reported in wild *P. perna* from the states of Santa Catarina (microfibers only) (Gusmão et al., 2016), Paraná (Machado et al., 2021), São Paulo (Santana et al., 2016; Ribeiro et al., 2023), Rio de Janeiro (Birnstiel et al., 2019; Carvalho et al., 2024; Rocha et al., 2025), and Espírito Santo (Bom et al., 2022; Costa et al., 2023) coasts, as well as in farmed mussels from Guarapari, Espírito Santo State (Bom and Sá, 2022) and at the Island Bay, Santa Catarina State (Brocardo et al., 2025). Although the ecological impacts of MPs in marine systems have become a critical global issue, research in Brazil remains limited given the country's extensive coastline. Studies have focused primarily on the occurrence, distribution, and chemical characterization of MPs and plasticizers (i.e., additives incorporated into plastic polymers to confer specific properties) in sediments and coastal waters along the northeastern and southeastern coasts (e.g., Araújo et al., 2018; Carvalho and Baptista Neto, 2016; Castro et al., 2020; Olivatto et al., 2019; Neves et al., 2023a, 2024), as well as in invertebrates (e.g., Neves et al., 2023b; Pantoja et al., 2024; Ribeiro et al., 2024) and vertebrates (e.g., Justino et al., 2021; Nunes et al., 2021).

Considering the global concern over MP contamination and the suitability of bivalves as sentinel species, this study aimed to experimentally investigate the particle selectivity ability of *P. perna* when exposed to a chlorophyte microalga and plastic microbeads of similar morphology and size. To this end, clearance and feeding rates were determined for mussels incubated under three treatments: microalgae only (MA), microbeads only (MB), and a 1:1 mixture of microalgae and microbeads (MA+MB). The study hypothesis is that when MPs resemble natural food in shape and size, *P. perna* can discriminate between particles, preferentially ingesting microalgal cells while rejecting MPs.

Adult specimens of *P. perna* were manually collected using stainless steel spatulas from the rocky shore of Vermelha Beach, located in the Urca neighborhood at the city of Rio de Janeiro, Brazil (Figure 1). Sampling was conducted on May 22, 2023, during low tide, when part of the rocky shore was exposed. A total of 80 individuals were collected and placed in a 20 L container filled with seawater from Vermelha Beach to keep the organisms fully submerged. Simultaneously, approximately 40 L of seawater was collected using buckets and stored in a thermal container. Both the specimens and seawater were transported to the laboratory. Scientific research and collecting permits authorizing field studies were obtained from the Chico Mendes Institute for Biodiversity Conservation (ICMBio) (permit numbers: 35192-3 and 56897-1).

Mussel shells were cleaned using tweezers and soft biodegradable coconut-fiber sponges to remove surface biofilm, algae, barnacles attached to the valves, and excess byssus threads. For acclimation, 20 individuals measuring 53.01–75.72 mm in total length (mean ± standard deviation = 65.31 ± 4.93 mm) were placed in a previously decontaminated glass aquarium with constant aeration (290 L h^{-1}) and a biological filtration system (Boyu ZJ-401). Glass decontamination for MP analysis followed a standardized protocol involving thorough rinsing with filtered ultrapure water and filtered denatured alcohol (Frias et al., 2018). The aquarium was filled with 40 L of pre-filtered seawater using a stainless-steel mesh (100 μm). Mussels were acclimated to the experimental conditions at 20 ºC for 72 h prior to the trials and were fed the chlorophyte *Tetraselmis* sp. F. Stein, 1878 ad libitum until 24 h before the assays.

The chlorophyte *Tetraselmis* sp. (10–20 μm) used for mussel acclimation and assays was isolated using the single-cell method from a water sample collected in Guanabara Bay (22°46′05.73′ S, 43°10′04.31′ W), Rio de Janeiro State, Brazil. This microalga is commonly used for feeding invertebrates and as a control in assays with filter-feeding mollusks (Neves et al., 2021). Cultures were maintained in filtered seawater (FSW; 0.7 μm, AP-40 glass-fiber filter, Millipore, Brazil) at salinity 34 and enriched with L2 medium (Guillard and Morton, 1995), modified by omitting silicate, nickel, vanadium, and chromium. Stock culture were kept in a temperature-controlled cabinet at 24 ± 2 ºC under a 12:12 h light–dark cycle, with a photon flux density of 60 μmol $m^{-2} s^{-1}$ provided by cool-white fluorescent tubes. Photosynthetically active radiation was measured with a QSL-100 quantum sensor (Biospherical Instruments, San Diego, CA, USA). Microalgal cells were harvested during the exponential growth phase to ensure optimal nutritional quality. Before the assay, an aliquot of the culture was preserved in buffered Lugol's solution for cell counting and to estimate the volume required to achieve the target experimental concentration. The sample was quantified using a Neubauer chamber under an optical microscope, yielding an estimated concentration of 2.15×10^6 cells mL^{-1}.

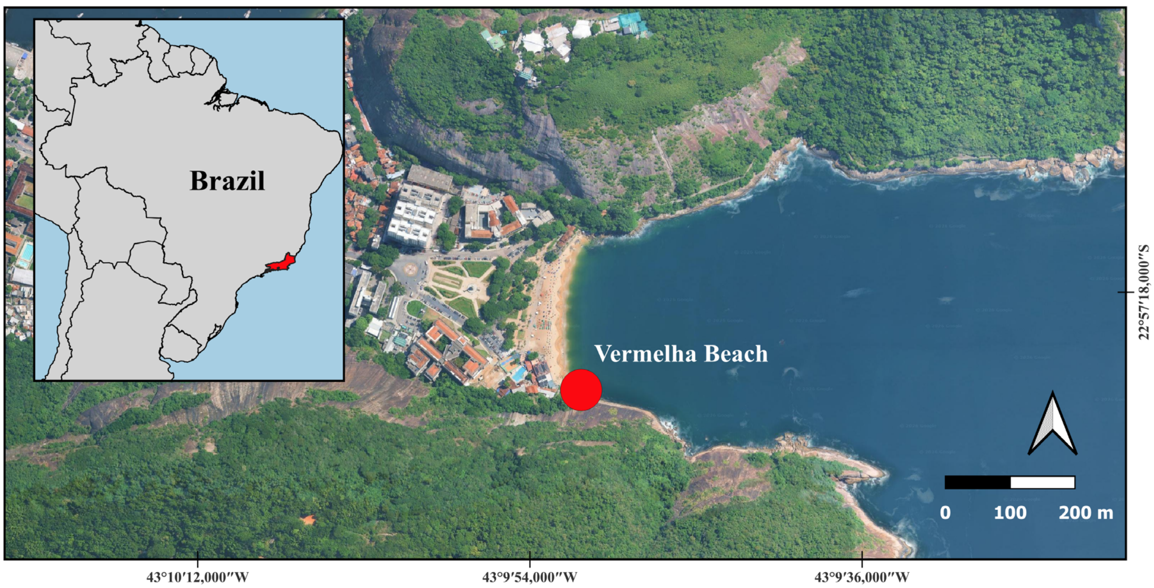

Figure 1. Geographical location of Vermelha Beach, Rio de Janeiro City, southeastern Brazil. The sampling site is indicated by a red circle. Google satellite image.

For the MP solution, additive- and pigment-free polystyrene microbeads (15 ± 0.2 μm), similar in shape and size to the microalga, were purchased from Sigma (74964). A stock solution using the particles in aqueous suspension (10% solids) was prepared in filtered seawater (FSW) to obtain a final concentration of 1.15×10^6 particles mL^{-1}. Polystyrene MPs have previously been reported in coastal systems in Rio de Janeiro, including the Cagarras Islands (Neves et al., 2024) and beaches in Guanabara Bay (Alonso, 2014; Pegado et al., 2024), as well as Vermelha Beach, where mussels were collected for the present assays.

Experimental trials comprised three treatments. Working solutions (2.8 L each) were prepared in 6 L glass aquariums as follows:

MA = FSW + *Tetraselmis* sp. (1×10^3 cells mL^{-1});

MB = FSW + polystyrene microbeads (1×10^3 particles mL^{-1});

MA + MB = FSW + *Tetraselmis* sp. (5×10^2 cells mL^{-1}) + polystyrene microbeads (5×10^2 particles mL^{-1}).

Solutions were homogenized and transferred to clean 1 L glass aquariums, with three replicates per treatment, each receiving 500 mL of working solution. Two previously acclimated *P. perna* individuals were added to each replicate. Additionally, two negative controls per treatment (working solution without bivalves) were included to detect changes in microalgal and/or microbead concentrations unrelated to mussel activity. During the 60 min of incubation, all 15 aquariums (experimental replicates and negative controls) were maintained at 20 ºC under constant illumination (light photoperiod). Based on a pilot assay, the incubation time was selected to prevent fecal production, which could affect food selectivity. Aliquots (10 mL) were collected at the beginning and end of each incubation using an automatic pipette and preserved in buffered Lugol's solution. After incubation, mussels were removed, placed in labeled zip-lock bags, and frozen for further analysis.

Aliquots (1 mL) collected at the beginning and end of the incubation period from each aquarium (experimental replicates and negative controls) were quantified using a Sedgewick–Rafter chamber under an optical microscope. Mussel shells were opened, soft tissues were removed, and wet tissues were weighed using a semi-analytical balance (0.0001 g). Tissues were then oven-dried at 80 °C for 48 h to determine dry weight, also measured using a semi-analytical balance. The combined dry weight (dw) of individuals from each replicate was used to calculate feeding rates.

Feeding rates were estimated based on changes in particle concentrations between the beginning and end of the incubation period. Clearance (mL $g_{dw}{}^{-1}h^{-1}$) and ingestion rates (particles $g_{dw}{}^{-1}$ h^{-1}) were calculated independently for each replicate and corrected using mean values of the corresponding negative controls (Coughlan, 1969), according to the following equations:

Clearance rate (mL $g_{dw}{}^{-1}$ h^{-1}) = $\frac{K\,x\,V}{w\,x\,t}$

Ingestion rate (particles $g_{dw}{}^{-1}$ h^{-1}) = $clearance\ rate\ x\ [initial]$

in which, K = $[Log\left(\frac{Final\ concentration\ of\ replicate}{Initial\ concentration\ of\ replicate}\right) - Log\left(\frac{Final\ concentration\ of\ negative\ control}{Initial\ concentration\ of\ negative\ control}\right)]$, V = suspension volume (mL), w = total dry weight of individuals in each aquarium (g), t = incubation time (h), [initial] = geometric mean of the initial and final particle concentrations in the replicate.

Mean values and standard deviations (SD) were calculated for each treatment. Data normality and homogeneity of variances were assessed using the Shapiro–Wilk and Levene tests, respectively. Differences in clearance and ingestion rates among treatments were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test, as the assumptions of parametric testing were met (Shapiro–Wilk test: $p = 0.049$ and $p = 0.037$ for clearance and ingestion rates, respectively; Levene's test: $p = 0.093$ and $p = 0.245$, respectively). Differences in the average proportion of microbead and microalgal particles in the mixture treatment (MA + MB) were analyzed using a paired t-test comparing samples collected at the beginning and end of the incubation period, as well as the corresponding negative controls. Statistical analyses were conducted using Statistica 10.0 (StatSoft). The map was produced using QGIS 3.34.8.

Feeding rates of *P. perna* were successfully estimated for all experimental replicates (n = 3 per treatment). Data on feeding rates are shown in Table 1. Clearance rate was significantly affected by treatments (one-way ANOVA, $F_{2,6} = 9.27$, $p = 0.0146$; Figure 2). Specifically, clearance was significantly higher in MB than in MA (Tukey's test, $p = 0.0135$). In MA + MB, clearance rates did not differ significantly from those observed in either MA or MB (Tukey's test, $p > 0.060$). The higher clearance rate observed in the MB treatment may suggest that, in the absence of organic particles, mussels increased their filtering activity in response to limited food availability.

Table 1. Clearance (mL g dw^{-1} h^{-1}) and ingestion (particles g dw^{-1} h^{-1}) rates of the brown mussel *Perna perna* under the following treatments: chlorophyte microalga *Tetraselmis* sp. (MA), a 1:1 mixture of microalga and polystyrene microbeads (MA + MB), and polystyrene microbeads only (MB). Values are presented as mean ± standard deviation (SD) of replicates.

	Clearance rate		Ingestion rate	
Treatment	**Mean**	**SD**	**Mean**	**SD**
MA	270.59	72.61	6.80×10^4	1.49×10^4
MA + MB	518.39	254.05	8.10×10^4	2.08×10^4
MB	627.07	169.62	1.22×10^5	3.40×10^4

Despite the significant treatment effect on clearance rate and a slight increasing trend in feeding activity under the MB treatment, no significant effect of treatment was observed on ingestion rates of *P. perna* (one-way ANOVA, $F_{2,6} = 3.88$, $p = 0.0831$; Figure 2). This result indicates that, although mussels increased their particle clearance in the MB treatment, ingestion rates of MPs were not significantly higher than in the other treatments. This behavior diverges from the expected pattern, as filter-feeding organisms typically show parallel trends in clearance and ingestion rates, with an initial increase followed by stabilization (Resgalla Jr. and Piovezan, 2009; Rodrigues et al., 2023a,2023b). These findings are consistent with a previous experimental study on *Mytilus galloprovincialis* Lamarck, 1819, which reported clearance rates of high-density polyethylene MPs comparable to those observed for similarly sized microalgae (Fernández and Albentosa, 2019).

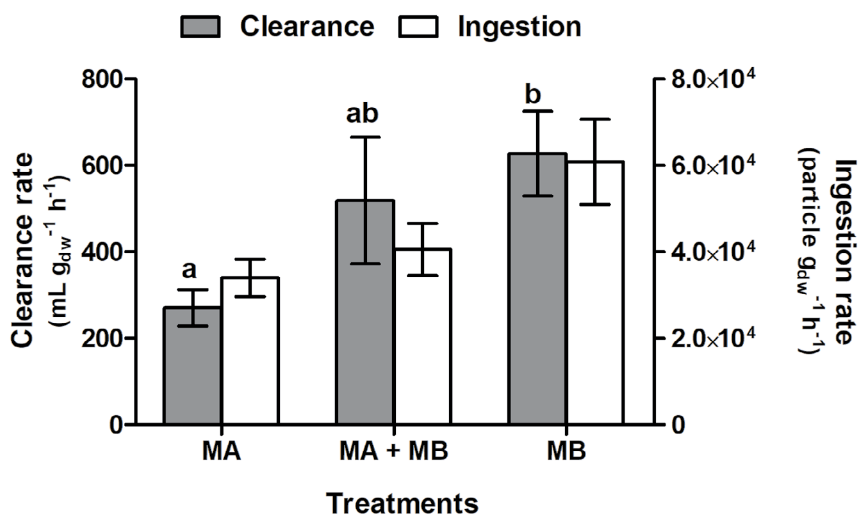

Figure 2. Clearance (mL g dw^{-1} h^{-1}) and ingestion (particle g dw^{-1} h^{-1}) rates of the brown mussel *Perna perna* under experimental treatments with the chlorophyte microalga *Tetraselmis* sp. (MA), a mixture of the microalga and polystyrene microbeads (MA + MB), and polystyrene microbeads only (MB). Data are presented as mean ± standard deviation. Different letters denote significant statistical differences (Tukey's test, $p = 0.0135$).

Bivalves may avoid ingesting previously cleared particles by means of pre-ingestive mechanisms such as the production of pseudo-feces (reviewed in Neves et al., 2021). However, in this study, mussels did not produce feces or pseudo-feces during incubations, indicating the absence of a pre-ingestive mechanism during short-term experimental conditions. It is well established that most MPs cleared by bivalves are later eliminated in biodeposits (feces and pseudo-feces) (e.g., Birnstiel et al., 2019; Fernández and Albentosa, 2019). In *Mytilus galloprovincialis* exposed to polyethylene MPs and microalgae, pseudo-feces production during the first four hours eliminated MPs larger than 10 µm; after six days of depuration, approximately 85% of the cleared MPs were eliminated (Fernández and Albentosa, 2019).

The relative abundances of microalgae and microbeads were assessed to test for selective ingestion in the MA + MB treatment. After incubation, 47.99% of the residual particles were microalgae and 52% were microbeads, with no significant difference between them (t-test, $p = 1.00$). Moreover, the final relative abundance of particles in the MA + MB treatment was very similar to that of the negative control and in the initial working solution (Figure 3). These results suggest that mussels ingested microbeads regardless of organic particle availability, showing no particle selection. Hence, the study hypothesis was fully refuted. Although bivalves may reduce clearance and ingestion rates as an active avoidance response via particle recognition—such as in the presence of harmful algal cells (reviewed in Neves et al., 2021)—under the present experimental conditions *P. perna* was unable to distinguish polystyrene microbeads from chlorophyte microalga of similar shape and size, whether offered in mixture or alone. Ecologically, this inability to sort particles has harmful implications. In natural environments, *P. perna* also appears unable to discriminate between MPs and organic matter, which is consistent with reports of MP contamination in the species (e.g., Carvalho et al., 2024; Costa et al., 2023; Machado et al., 2021; Ribeiro et al., 2023; Rocha et al., 2025). This limitation may be associated with the relatively recent emergence of plastic pollution in marine environments, for which no evolutionary adaptations for detection and avoidance are known. Consequently, increasing evidence documents MP contamination in mussels, including *P. perna*. Although most cleared MPs may be eliminated, a fraction of microparticles may remain in their tissues, especially smaller particles (< 6 µm) (Fernández and Albentosa, 2019). Accumulated MPs may induce physiological responses such as oxidative stress, histological inflammation, metabolic alterations, and reduce fitness (Afeniforo et al., 2026; Wei et al., 2021). Therefore, this study raises concerns about contamination dynamics in shellfish destined for human consumption, both in natural environments and aquaculture systems where microalgae, suspended organic particles, and MPs coexist. Further research is needed to clarify uptake and depuration dynamics across different polymer types, shapes, and particle sizes commonly found in coastal systems. The findings align with Sustainable Development Goal 14 (Life Below Water), reinforcing the need to advance research efforts to improve ocean health and environmental and human safety.

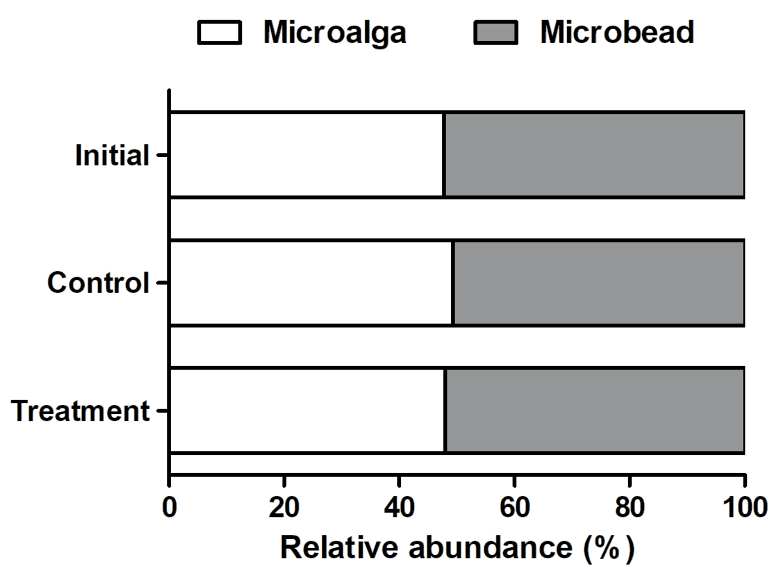

Figure 3. Relative abundance (%) of particles in the mixture treatment containing the chlorophyte microalga *Tetraselmis* sp. and polystyrene microbead (MA + MB) at the beginning of incubation (initial aliquot), in the negative control (without mussels), and after one hour of incubation.

AI Use statement

The authors declare that no generative artificial intelligence (AI) tools were used in the preparation, writing, or editing of this manuscript.

Data availability statement

All data are available from the corresponding author upon reasonable request.

Supplementary material

This article does not include any supplementary materials.

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Author contributions

E.T.P.: Conceptualization; Investigation; Writing – original draft; Writing – review & editing.

B.L., M.E.S.M., I.M.B.F.M.: Conceptualization; Investigation; Writing – original draft.

C.X.C.: Investigation; Writing – original draft.

R.A.F.N.: Conceptualization; Methodology; Formal Analysis; Investigation; Supervision; Project Administration; Funding Acquisition; Writing – review & editing.

Conflicts of interest

The authors declare no conflicts of interest.

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