Marine Pollution Bulletin 188 (2023) 114613

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Particle uptake by filter-feeding macrofoulers from the Mar Grande of Taranto (Mediterranean Sea, Italy): potential as microplastic pollution bioremediators

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ARTICLE INFO

Keywords:
Bioremediation
Filter-feeders
Microplastics
Digestion method
Clearance rate
Particle retention

ABSTRACT

Microplastics (MPs) are a serious threat to the marine environment affecting ecosystem functioning and biodiversity. There is a vast literature about the uptake of MPs at different trophic levels, mainly focused on ecotoxicological effects in commercially relevant species. Little is still known about possible strategies to face MP pollution. Bioremediation is recently gaining attention in this framework. The clearance rate and particle retention of *Sabella spallanzanii*, *Mytilus galloprovincialis*, *Phallusia mammillata*, *Paraleucilla magna* at three MP concentrations (C1: $1.4 \cdot 10^1$ p/L; C2: $1.4 \cdot 10^2$ p/L; C3: $1.4 \cdot 10^3$ p/L) were investigated to test their potential as MP remover. Digestion protocol removed 98 % of tissues simplifying the MP quantification. *P. magna* clearance rate decreased with increasing concentration while *P. mammillata* showed no significant variations. *S. spallanzanii* and *M. galloprovincialis* instead exhibited the highest values of clearance rate. Yet, unlike mussels, *S. spallanzanii* can inhibit particle return to the surrounding water storing them in the tube, resulting to be the best candidate for bioremediation purposes.

1. Introduction

Plastic is an everyday material comprised of various polymers, shapes and sizes to meet human needs. It is also a major anthropogenic impact threatening terrestrial, freshwater and marine ecosystems (Thushari and Senevirathna, 2020). Plastic debris eventually reach the marine environment as primary and/or secondary MPs, tiny items within a size range from 1 μm to 5 mm (Frias and Nash, 2019). MPs interact with marine organisms affecting trophic webs (Andrady, 2011; da Costa et al., 2017; De Sá et al., 2018; Franzellitti et al., 2019; Hidalgo-Ruz et al., 2012; De Oliveira Soares et al., 2020; Troost et al., 2018) and potentially threatening human health by eating and concentrating polluted sea products (Barboza et al., 2018; Dehaut et al., 2016; Wu et al., 2020). In 2004, Thompson and coworkers performed the first laboratory experiments assessing MP ingestion by marine animals with different feeding strategies. Thereafter, an increasing interest of the scientific community in this topic has resulted in a wide knowledge on the uptake of MPs in several marine animals at different trophic levels (see Prokić et al., 2021 for a review). However, most of the studies were focused on ecotoxicological effects in commercially relevant species (De Sá et al., 2018) and the MP concentrations used to perform feeding laboratory-controlled experiments were often much higher than real environmental conditions (Phuong et al., 2016; Lenz et al., 2016; Pinheiro et al., 2020).

Generally, MPs may be identified as food and ingested by marine organisms entering marine food webs. The combination of several factors such as MP size, shape, density and, the presence of biofilm on their surface, results in misidentification of MPs as food (Franzellitti et al., 2019; Thompson et al., 2004). The degree of microplastic ingestion in marine environment depends both on MP characteristics, and on feeding strategy of marine organisms (Setälä et al., 2016; Wesch et al., 2016). Indeed, density, size, and chemical composition of MPs influence the sinking or floating of these micropollutants (Arienzo et al., 2021) and, in turn, their concentration in the different marine compartments. For

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https://doi.org/10.1016/j.marpolbul.2023.114613
Received 16 August 2022; Received in revised form 5 January 2023; Accepted 9 January 2023
Available online 20 January 2023

Table 1
Literature clearance rate values and preferred range of particle size of the target species (see Rossi et al., 2017 for a comparative drawing).

Species	Clearance rate (L h^{-1} DWg^{-1})	Particle size range (μm)	Reference
Sabella spallanzanii	5.50 ± 1.60	1–1000	Licciano et al., 2005
Mytilus galloprovincialis	3.95 ± 2.17	1–100	Gardner, 2002
Paraleucilla magna	Not available	0.1–100	Rossi et al., 2017
Phallusia mammillata	4.78 ± 0.17	1–500	Fiala-Médioni, 1978

example, filter feeders are more likely to uptake low-density MPs floating in the water column, while deposit feeders tend to collect high-density MPs that sink and accumulate in the sediments (Wright et al., 2013). Benthic filter-feeding invertebrates consume suspended particles (Gili and Coma, 1998) being thus passively exposed to MP contamination (Arienzo et al., 2021; Van Cauwenberghe et al., 2015; Wesch et al., 2016).

The most abundant polymers in the marine environment are polyethylene (PE), polypropylene (PP), polystyrene (PS), nylon (PA), polyethylene terephthalate (PET), polyvinyl chloride (PVC), and cellulose acetate (CA) (Andrady, 2011). Most of those polymers are used in manufacturing of several plastic materials widely used in marine aquaculture sector (e.g., boats, fish cages, buoys, nets, ropes etc.) and considered as sources of MPs (FAO, 2017). Moreover, farming sites are commonly located in sheltered areas, with typically low water exchange, where MPs tend to accumulate both in the water column and in the sediments, exposing commercial species to a high risk of ingestion (Chen et al., 2021). Similarly, mariculture environment is characterized by high organic and nutrients loads, derived from the fish wastes (e.g. uneaten feed, feces, excretions; Black, 2001; Wu, 1995), whose long-term accumulation affects both the water column and the benthic functioning (Dalsgaard and Krause-Jensen, 2006; Karakassis et al., 2000). Such eutrophic conditions along with the availability of several submerged infrastructures, suitable to be colonized, allow biofouling to proliferate (Arduini et al., 2022; Cook et al., 2006; Fernandez-Gonzalez and Sanchez-Jerez, 2017).

Marine biofouling refers to any association of marine organisms that accumulate on artificial or natural hard submerged substratum. According to the size of the organisms, biofouling is classified into two distinct forms, “microfouling” (biofilm) and “macrofouling” (hereafter the term biofouling will refer to macrofouling component). Biofouling assemblages vary spatially and temporally (Relini, 1977). Variations of biofouling biomass and composition are driven by the interaction of several abiotic and biotic factors as seasonality, seston concentration and quality, depth, water flow, elapsed time of substratum immersion and larval pool availability (Cook et al., 2006; Cowie, 2010; Cronin et al., 1999; Greene and Grizzle, 2007; Lezzi et al., 2018). Yet, biofouling assemblages developing in mariculture farms are generally dominated by sessile filter-feeding organisms (Fitridge et al., 2012). Even though the growth of biofouling on these structures represents an expensive problem to be eliminated for the mariculture industry (De Nys and Guenther, 2009; Dürr and Watson, 2010; Fitridge et al., 2012), several authors demonstrated the ability of such assemblages to act as “biofilters” by assimilating the surplus of exogenous matter (i.e., organic and pollutants) deriving from fish cages and thereby reducing its negative impact on the surrounding environment (Angel and Spanier, 2002; Gonzalez-Silvera et al., 2015; Hughes et al., 2005; Sarà et al., 2007).

An innovative IMTA (Integrated Multi-Trophic Aquaculture) system was developed in the Mar Grande of Taranto, where the local biofouling assemblage, with a dominance of few target species, is cultured by “natural” recruitment on eco-friendly collectors placed around fish cages (Giangrande et al., 2020a). The novel species employed as bioremediators were chosen after years of research on their life cycle and development in the area (Lezzi et al., 2018; Lezzi and Giangrande, 2018), as well as on their filtering efficiency and ability to remove organic and microbial waste from the water column (Licciano et al., 2005; Longo et al., 2016; Stabili et al., 2006, 2010).

Table 2
MP concentrations (C1, C2, C3) used in the experiments given as number of particles per 1.5 liter (p/1.5 L), number of particles per liter (p/L) and number of particles per cube meter (p/m^3).

	MP concentration		
	p/1.5 L	p/L	p/m^3
C1	$2.10 \cdot 10^1$	$1.4 \cdot 10^1$	$1.4 \cdot 10^4$
C2	$2.10 \cdot 10^2$	$1.4 \cdot 10^2$	$1.4 \cdot 10^5$
C3	$2.10 \cdot 10^3$	$1.4 \cdot 10^3$	$1.4 \cdot 10^6$

We took advantage of this IMTA system selecting four dominant filter-feeding species composing the biofouling assemblage developed on bioremediating collectors: *Sabella spallanzanii* (Gmelin, 1791); *Mytilus galloprovincialis* Lamarck, 1819; *Phallusia mammillata* (Cuvier, 1815); and *Paraleucilla magna* Klautau et al., 2004, characterized by different filtration mechanisms and capacities. In the present paper, we report the results of particle retention and clearance rate of these four species on plastic microparticles in laboratory-controlled feeding experiments in order to compare their performances in the uptake of MPs and to explore their potential role in removing also MPs from seawater, with the aim of providing a starting point for a new perspective on bioremediation of MP pollution in aquaculture environment.

2. Materials and methods

2.1. Target species selection

The four target species account collectively for more than the 80 % of the biofouling biomass developed on the collectors of the IMTA system in the Mar Grande of Taranto (Arduini et al., 2022; Giangrande et al., 2020a). Their ability to tolerate eutrophic conditions, allows for easy access to numerous specimens attached on different submerged substrates in harbors (e.g., quays, ropes, boat hulls etc.). All of them are common components of Mediterranean biofouling communities in enclosed areas, as occurs in the Mar Grande and Mar Piccolo of Taranto (Arduini et al., 2022; Longo et al., 2007; Pierri et al., 2010).

S. spallanzanii is a gregarious tube-dwelling polychaete widely distributed along the Italian coasts, both in the open sea from 1 to 30 m depth and in shallow confined areas, where it can reach high densities (Giangrande et al., 2000). *M. galloprovincialis* is a mollusk bivalve native of the Mediterranean and North-east Atlantic areas, commonly cultured for human consumption (Wonham, 2004). *P. mammillata* is a large (up to 20 cm in height) solitary ascidian, commonly known as white sea-squirt, living on rocky substrates in the northeastern Atlantic Ocean, the North Sea and Mediterranean Sea. *P. magna* is a calcareous sponge native of the Atlantic coasts of Brazil (Klautau et al., 2004) and recently introduced also in Italian waters (Longo et al., 2007), showing different morphologies from tubular to irregular massive shapes.

With the exception of the sponge, *P. magna*, for which literature information is lacking, all the other species are characterized by high filtration rates (Table 1; Gardner, 2002; Fiala-Médioni, 1978; Licciano et al., 2005). Yet, these four organisms belong to different phyla and, in order to perform the same function, have evolved different anatomical structures, adapted to different diets and energetic needs, allowing to capture particles with different mechanisms: *M. galloprovincialis*, after valve-opening actively pumps water, by beating numerous cilia along the gills, towards the latero-frontal cirri involved in nutrient uptake (Riisgård et al., 1996); the sea squirt *P. mammillata* pumps water through the inhalant siphon into the pharyngeal basket, where particles remain entrapped on a mucus net (Holley, 1986); *P. magna* is a calcareous

Table 3
Experimental design.

Sabella spallanzanii				*Mytilus galloprovincialis*				*Paraleucilla magna*				*Phallusia mammillata*			
Ctrl	C1	C2	C3	Ctrl	C1	C2	C3	Ctrl	C1	C2	C3	Ctrl	C1	C2	C3
S1	S1	S1	S1	M1	M1	M1	M1	P1	P1	P1	P1	Ph1	Ph1	Ph1	Ph1
S2	S2	S2	S2	M2	M2	M2	M2	P2	P2	P2	P2	Ph2	Ph2	Ph2	Ph2
S3	S3	S3	S3	M3	M3	M3	M3	P3	P3	P3	P3	Ph3	Ph3	Ph3	Ph3

sponge with a leuconoid aquiferous system in which water enters through numerous small openings (ostia) into a system of inhalant canals leading to several spherical or subspherical "chambers" (average diameter 87 μm), where specialized cells named choanocytes retain particles (Longo et al., 2007); the Mediterranean fan worm *S. spallanzanii* holds a structure extruding out of the tube, called branchial crown, that is constituted by several "tentacles", named radioles, bearing in turn numerous microfilaments (pinnules) which direct suspended particles along grooved radioles to the mouth (Clapin, 1996). Having specific structures and mechanisms to filter enables these species to feed within a different preferred range of particle size (Table 1).

2.2. Sampling

All specimens were collected at the Mariculture Mar Grande in the Mar Grande of Taranto. The sampling activity was carried out between February and May 2021 every time an experiment was planned to avoid keeping animals in the aquarium more than the necessary time. All the animals were collected from the bioremediating ropes: *S. spallanzanii* and *M. galloprovincialis* were collected directly from the boat while *P. magna* and *P. mammillata* by scuba diving operators. The animals were measured in length and selected according to their typical length size in the sampling site. Then, the animals were placed in tanks already filled with prefiltered natural seawater. Then they were transported to the laboratory and acclimatized in a 30 L aquarium at 20 °C for 48 h before the experiment. The transport took less than an hour and animals were kept at temperature ranged between 15 and 18 °C in a cooler with natural seawater. Pre-filtered (pore size 250 μm) natural seawater from the sampling site was used at all stages of the experiment.

2.3. MP concentration

Red-dyed polystyrene (PS) microbeads (Polyscience Inc.) were used to assess the particle retention and the clearance rate of the animals. The size of the microspheres (nominal mean diameter of 6 μm) was chosen to be captured by all the four species according to their preferred range of particle size (Table 1). PS microspheres were provided as water suspension to be easily dispersed in the beaker without aggregating. The suspension with a concentration of $2.10 \cdot 10^8$ particles/mL (provided by the dealer) was diluted allowing to work with three different concentrations of microparticles (Table 2). MP concentration in the marine environment shows a great variability among different areas due also to different sampling methodology (Cincinelli et al., 2019). In the Atlantic Ocean values ranging from 13 to 501 items/m^3 were reported (Enders et al., 2015), while along the Swedish coast values vary between 7000 and 10,000 plastic items/m^3 (Lönnstedt and Eklöv, 2016). In the Mediterranean Sea, the average concentration of microplastics in the water column is 0.0058 items/m^3 (Sbrana et al., 2021) (values converted using the relationship proposed by Lusher, 2015), while in the Taranto Gulf this concentration is two orders of magnitude higher, with 0.62 items/m^3 and no differences between inshore and offshore because of an anticyclonic gyre in the open sea (Sbrana et al., 2021). The following MP concentrations were calculated to be a tradeoff between as realistic as possible representation of pollution conditions in the Taranto Gulf water column and the instrument limitation to detect and count the particles. All the suspensions were freshly prepared each time and vortexed for 30 s before using to ensure a homogenous distribution of the beads in the medium.

2.4. Feeding experiment

Twelve specimens of similar length size for each species were selected to perform the feeding experiments: *S. spallanzanii* average length (tube) 20.5 ± 0.5 cm; *M. galloprovincialis* average length (shell) 5.7 ± 0.2 cm; *P. magna* average length (longest axis of tubular shape individuals) 7.0 ± 0.2 cm; *P. mammillata* (tunic) 8.9 ± 0.3 cm. The experiments were carried out in three replicates separately for each species and for each MP concentration, for a total of 16 batches. Three individuals of each species were used for each MP concentration and for the control experiment (without MPs), for a total of 48 animals (Table 3).

2 L beakers were filled up to a volume of 1,5 L with the natural seawater (20 °C) pre-filtered at 250 μm used to acclimatize the organisms. To ensure uniform distribution of MPs and avoid their sinking, a magnetic stirrer was used to maintain a slow and steady water movement. The animals were gently placed in the beakers paying attention to kept them fully submersed and preventing any disturbance between the magnetic bar and the animal itself. To do so for each animal a small support system was developed to locate it in the beaker in a functional way: mussels were fixed in a falcon tube with flat bottom filled with a small rock to keep it fixed to the bottom; the polychaetes tube was gently folded following the circular beaker shape; ascidians and sponges were initially simply placed on the beaker bottom but during the experiment, the water flux was too strong and they were violently moved with the water. Therefore, these animals were fixed with a colourless polyvinyl chloride (PVC) custom-made holder adapting to the different animal's shapes (Fig. S1 a, b, c, d). After placing the organisms in the beaker, the proper MP concentration and phytoplankton were added.

The experiment started as the animals begun to filter; for example, once mussels have opened the valves or polychaetes have extruded the crown from the tube or ascidians have opened the inhalant siphon. Since that moment the experiment lasted 1 h of filtering activity, which means that every time the animals ceased to filter (e.g., some noise causing the closure of the *P. mammillata* inhalant siphon or inducing *S. spallanzanii* to go back in the tube), the timer was stopped until they started again to filter (open the valves, get off the tube, open the siphon).

At the end of the experiment each beaker was moved from the stirrer to the table to let the water stop shaking. The animals were gently taken from the water and thoroughly washed with prefiltered (0.1 μm) Millipore water on their own beakers to remove any particles stuck on their surface, as to recover them in the water and not in the animals. Then, they were placed on an aluminum foil and left in the oven to dry for 48 h at 50°. Before being dried the animals were deprived of all the parts which could not be digested with the alkaline solution:

- mussels were removed from the shells paying attention to save the inner water;
- polychaetes were gently removed from the tubes;
- ascidians were deprived of the tunics saving the inner water;
- sponges were simply placed on the aluminum foil.

Then 10 mL of H_2O_2 were added to the water to remove the organic matter and to make faster the subsequent treatment. The beakers were covered with an aluminum foil and left in the dark for 24 h. The inner

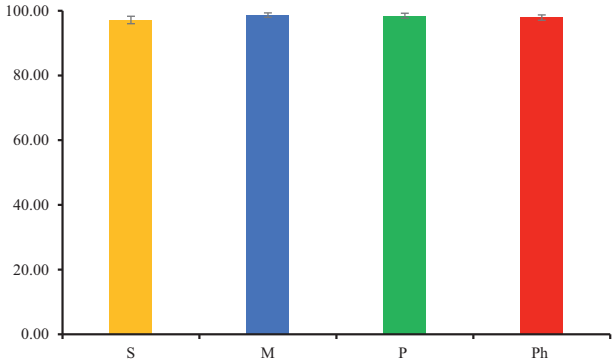

Fig. 1. Percentage of digestion efficiency achieved for each species (S = *S. spallanzanii*, yellow; M = *M. galloprovincialis*, blue; P = *P. magna*, green; Ph = *P. mammillata*, red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

water of mussels and ascidians was treated with 2 mL of H_2O_2 and left in the fridge for 24 h.

2.5. Sample preparation

2.5.1. Animal tissues

Once constant weight is achieved the dried tissues were removed from the aluminum foil, shredded to powders, and then transferred into glass bottles. The alkaline digestion solution was prepared with KOH (2.5 %) + H_2O_2 (5 %) and added to the powdered tissues of all the animals except for *P. mammillata* which required KOH (5 %) + H_2O_2 (10 %) to be digested. The bottles were then placed at 75° for 3 h. After 3 h the bottles were left cooling and the solution was transferred in 50 mL falcon and centrifugated for 5 min at 3000 rpm. The supernatant was removed and neutralized with some drops of citric acid while the precipitate was treated for 30 min with 5 mL of formic acid (25 %) and sodium citrate (10 %) solution (Vered et al., 2019) to dissolve calcium carbonate spicules very abundant in *P. magna* but also present in the other animals. After 30 mins the acid solution was neutralized with a few drops of ammonia solution (25 %). The neutralized solution was filtered on 47 mm glass fibers filters (Whatman pore size 0.7 μm) previously weighted using a vacuum pump apparatus. Each bottle was then rinsed with Millipore water three times to recover all the red plastics microspheres possibly stuck on the glass surfaces and the rinsing water was filtered. Each filter was left in a drier overnight. All the filters were weighted before and after filtration to quantify the digestion efficiency (DE%) using the following formula (Karami et al., 2017)

$$\%DE = \left[1 - \frac{(\mathrm{Wfa} - \mathrm{Wfb})}{\mathrm{Wm}}\right] \cdot 100 \quad (1)$$

where: Wm corresponds to the tissues dry weight, Wfa and Wfb are the filter dry weights after and before filtration of the digested tissue, respectively.

2.5.2. Water treatment

After 24 h the water of the beakers was filtered on 25 mm diameter glass fibers filters (Whatman pore size 0.7 μm), using a 60 mL syringe set up on the filtration apparatus connected to the vacuum pump. The water was directly spilled in the syringe and every 250 mL of seawater filtered the syringe was filled with Millipore water to clean the filter from salts. Everything that came in contact with the plastic particles during the experiments (beaker, syringe, magnetic stirrer) was cleaned three times with Millipore water to recover any microspheres adhering to the surfaces. Then the rinsing water was filtered and the filters were left in the drier overnight. The inner water of mussels and ascidians was treated in the same way.

Fig. 2. Red microparticles on filter surface after animal digestion highlighted by rings (C3 = 1.4 particles/mL). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.6. Count of MPs

Each filter (Whatman pore size 0.7 μm) was analysed using Nikon Eclipse 80i microscope equipped with Nikon camera. Particles were counted at 20× magnification and relevant photos were collected using ACT-2 U acquisition software. For water samples (WS) the filters (25 mm diameter) were ideally divided into two halves, and one was randomly selected to count MPs. For animal samples (AS) MPs were counted on a quarter of filters (47 mm diameter). All the filters were analysed for a total of 48 filters for *S. spallanzanii* and *P. magna* (12 WS filters and 12 AS filters for each of them) and 72 filters for *M. galloprovincialis* and *P. mammillata* (12 WS filters and 24 AS filters for each of them), due to the presence of the inner water.

2.7. Particle retention and clearance rate calculation

The number of particles present in the water after 1 h of filtering activity allowed to calculate the percentage of particle retention (PR%):

$$\%PR = \left(\frac{C0 - Ct}{C0}\right) \cdot 100 \quad (2)$$

where: C_0 = particle concentration at time 0 and C_t corresponds to the particle concentration after 1 h of filtering activity.

The clearance rate was calculated following the formula (Coughlan, 1969)

$$CR = \left(\frac{V}{DW \times t}\right) \cdot ln\left(\frac{C0}{Ct}\right) \quad (3)$$

where V = volume of water in the beaker; DW = individual dry weight; t = time of filtering activity; C_0 = particle concentration at time 0; C_t = particle concentration after 1 h of filtering activity.

2.8. Data analysis

Two-way analysis of variance (ANOVA) was applied to test for differences in particle retention and clearance rate of the animals, using species (S, M, P, Ph; 4 levels) and concentration (C1, C2, C3; 3 levels) as factors. Significance was set at critical level of 95 % (p-value < 0.05). When significance p-values were found, Tukey's Honestly Significant Difference (HSD) post hoc test was utilized to evaluate differences across factors' levels. Prior, Shapiro-Wilk test was performed to verify normal distribution of the residuals of particle retention (SW-W = 0.95; p-value = 0.08) and clearance rate (SW-W = 0.98; p-value = 0.70). Statistical analyses were performed using STATISTICA 10.0 software package.

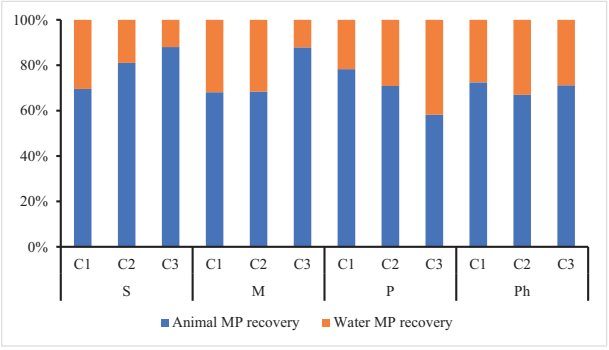

Fig. 3. Percentage of MPs recovered in the animals and in the water for each species (S = *S. spallanzanii*; M = *M. galloprovincialis*; P = *P. magna*; Ph = *P. mammillata*) at the three different MP concentrations (C1; C2; C3).

3. Results

3.1. Digestion efficiency

The applied digestion method allowed reaching very high %DE, making red PS particles easily to be counted. The average digestion efficiency was reported in Fig. 1. All the %DE values were above 95 %, but the highest %DE values were registered for mussels and sponges (above 97 %).

All the filters were analysed and red microparticles were counted, for a total of 48 WS filters and 72 AS filters. Particles were found in each animal but control experiments. The digestion protocol was able to remove almost all the organic matter from the biota samples without damaging plastic microparticles. All the microspheres were well preserved in shape and color with no damaging signs after the basic protocol and the acidic step, making possible and easy their quantification by using a microscope (Fig. 2). Quantifying the microspheres both in the animals and in the water enabled to estimate more precisely the particle's recovery, resulting also as a double-check for the quality of digestion. All the red MPs injected in the beakers were recovered, partly in the animals and partly in the water (Fig. 3), although the majority was detected in animal tissues.

3.2. Particle retention and clearance rate

All four filter-feeding species were able to retain 6 μm MPs put in the beakers, regardless of their concentration. However, they showed different retention trends in relation to particle concentration (Fig. 4A). For example, *P. magna* and *P. mammillata* had a negative and quite constant trend, respectively, as a response to higher concentrations, while particle retention of *S. spallanzanii* and *M. galloprovincialis* increased with increasing concentration. Notably, at C3 level *S. spallanzanii* and *M. galloprovincialis* showed the highest percentage of retained particles (88.01 % and 87.88 %, respectively), resulting statistically separated from *P. magna* (58.16 %) (Table 4).

Table 4
Results from two-way ANOVA test on particle retention of filter-feeders (S = *S. spallanzanii*; M = *M. galloprovincialis*; P = *P. magna*; Ph = *P. mammillata*) using species (Sp) and concentration (Conc) as factors.

Source	df	SS	MS	F-ratio	p-value
Sp	3	0.06	0.02	2.15	0.12
Conc	2	0.02	0.01	0.79	0.46
Sp * Conc	6	0.18	0.03	3.15	**0.02**
Total	24	0.23	0.01		
Pairwise comparisons (Tukey HSD test; Homogenous groups)					
Within level C1 of "Conc"		Within level C2 of "Conc"		Within level C3 of "Conc"	
S^{ab} - P^{ab} - Ph^{ab} - M^{ab}		S^{ab} - P^{ab} - Ph^{ab} - M^{ab}		S^{a} - P^{b} - Ph^{ab} - M^{a}	

Homogenous groups are given as superscript letters. Significant p-values (<0.05) are given in bold.

The clearance rate in *M. galloprovincialis* showed on average the higher values with a maximum at C3 (6.95 $Lh^{-1}gDW^{-1}$) while in *P. magna* showed the lower values with a minimum at C3 (0.95 $Lh^{-1}gDW^{-1}$) (Fig. 5A). Unlike particle retention, the clearance rate was significantly affected by species and concentration and their interaction (Table 5). Tukey post hoc test revealed that the levels *M. galloprovincialis* and C3 were the responsible for these differences (Table 5).

4. Discussion

Thanks to the efficient protocol after digestion the filters were clear and the particles could be easily counted. The mean values of the clearance rate calculated on red polystyrene (PS) MPs resulted to be lower for *S. spallanzanii* and *P. mammillata*, whereas higher for *M. galloprovincialis* and *P. magna*, when compared to the reference values (Table 6). *P. mammillata* showed no differences among the three concentrations underlining its high efficiency as an active suspension feeder. *S. spallanzanii* showed highest clearance rate mean value at C3 and no differences between C1 and C2. *M. galloprovincialis* clearance rate increased with increasing concentration. In fact, as expected, at higher concentration more particles were retained (Valsesia et al., 2021), except for *P. magna*, which showed the lowest values of clearance at higher concentrations (Table 6). With the exception of *M. galloprovincialis*, there are no lab-controlled experiments in the literature feeding our target species with MPs (Table 7).

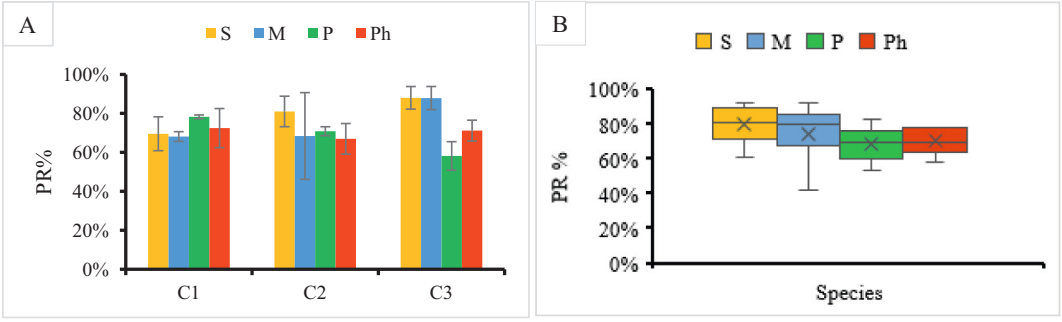

Fig. 4. Mean percentage ± SD (A) and boxplot (B) of particle retention of each species after 1 h of filtering activity (C1, C2, C3 = MP concentrations; S = *S. spallanzanii*, yellow; M = *M. galloprovincialis*, blue; P = *P. magna*, green; Ph = *P. mammillata*, red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

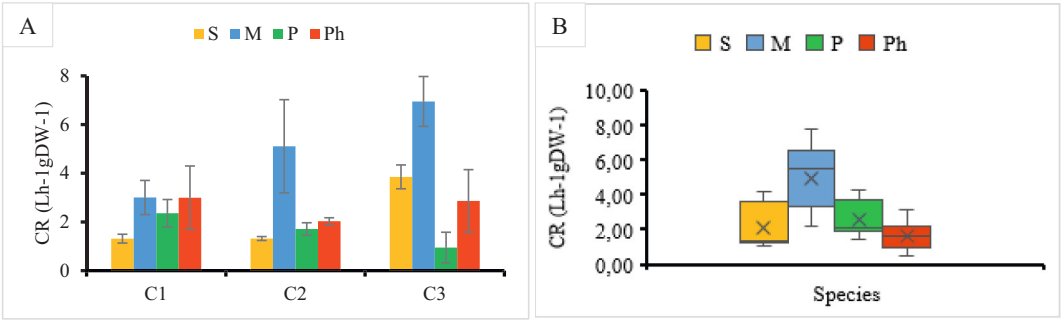

Fig. 5. Mean percentage ± SD (A) and boxplot (B) of clearance rate of each species after 1 h of filtering activity (C1, C2, C3 = MP concentrations; S = *S. spallanzanii*, yellow; M = *M. galloprovincialis*, blue; P = *P. magna*, green; Ph = *P. mammillata*, red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4.1. *Mytilus galloprovincialis*

M. galloprovincialis is one of the first animals used to assess the environmental quality of seawater thanks to its characteristics such as: widespread distribution, sedentary lifestyle, accessibility and high tolerance to pollutants (Goldberg, 1986), making it an ideal biological indicator in the monitoring of anthropogenic pollution trends in coastal waters (Beyer et al., 2017). Mussels have been consumed by people all over the world for thousands of years (Beyer et al., 2017), so it is really concerning their interaction with micropollutants. It is not surprising, though, that this is the most studied animal in MP pollution framework (Table 7). Numerous experiments were performed exposing *M. galloprovincialis* to MPs (Cappello et al., 2021; Paul-Pont et al., 2016; Gonçalves et al., 2019; Trestrail et al., 2021) and various work focused on the presence of these pollutants in mussel tissues from the field (Fraissinet et al., 2021; Qu et al., 2018; Masiá et al., 2022). It is then well known that mussels filtrate and retain MPs. From our experiment, *M. galloprovincialis* showed the best and alarming response to MP exposure, with the higher values of clearance rate at all the concentrations and with the second highest mean value of particle retention, behind only *S. spallanzanii* (Figs. 4, 5). Due to its filtering activity, *Mytilus* is able to capture MPs from the seawater entering the gills and then they are assimilated or transported to the digestive system (Von Moos et al., 2012; Beyer et al., 2017; Li et al., 2019). Not all the particles which stick to the gills are ingested (Santana et al., 2018) since mussels are able to separate and reject unwanted particles as pseudofeces (Li et al., 2019). Mussels shows size-selection in particle uptake and egestion (Li et al., 2019). After three days of gut clearance, just bigger particles (>20 μm) were completely egested while the smaller ones (5–20 μm) can be detected still in the animal (Van Cauwenberghe and Janssen, 2014). Knowing that the particle size used in the experiments can be retained by mussels for days, the analysis of pseudofeces was not performed. There are many proofs of the capability of mussels to catch and retain MPs, as well as of their toxic effects (Franzellitti et al., 2019), and moreover, mussels are one of the most common seafood products for human consumption. For these reasons, despite the high efficiency in clearing water by retaining a large part of microparticles at each concentration, mussels cannot be proposed as bioremediators and it is, indeed, preferable culture these organisms in cleaner water. Their performance in the uptake MPs was still evaluated to be used as benchmark for the other tested species and as a confirmation of previous studies.

4.2. *Sabella spallanzanii*

The filtering capacity *S. spallanzanii* has been well studied (Clapin, 1996; Licciano et al., 2005), and laboratory experiments report very high capability in removing particles from the water (Giangrande et al., 2005; Stabili et al., 2006). Such capability combined with a great tolerance to polluted environment and with its food plasticity makes this organism a great candidate for bioremediation purposes (Giangrande et al., 2005). The filtering activity of this worm is quite high and constant at a particle concentration of ca. 10^3 p/mL and then declines at ca. 10^4 p/mL (Clapin, 1996), probably because this amount represents its gut saturation point (Riisgård and Ivarsson, 1990). Even though our concentrations are far lower than this point (Table 2), the measured values of *S. spallanzanii*'s clearance rate in the present experiment were lower than the reported ones in literature (Table 6). This may be due to the lower temperature (20 °C vs 22 °C) and/or the different particle size (6 μm vs 2–3 μm) utilized in our experiment. The response to MP exposure is however positive, showing an increasing trend both for CR and PR% with the increase of MP concentration. Mayer and coworkers in 1994 found that Sabellid polychaetes are able to retain 100 % of 3–8 μm particles and that the retention capability of these fan worms depends on particle size. The branchial crown can capture and allocate suspended material of diverse sizes: small particles (3–8 μm) go to the mouth;

Table 5
Results from two-way ANOVA test on clearance rate of filter-feeders (S = *Sabella spallanzanii*; M = *Mytilus galloprovincialis*; P = *Paraleucilla magna*; Ph = *Phallusia mammillata*) using species (Sp) and concentration (Conc) as factors.

Source	df	SS	MS	F-ratio	p-value
Sp	3	59.5684	19.8561	24.7390	**0.0001**
Conc	2	11.1165	5.5583	6.9251	**0.0004**
Sp * Conc	6	29.7544	4.9591	6.1786	**0.0001**
Total	24	19.2630	0.8026		
Pairwise comparisons (Tukey HSD test; Homogenous groups)					
Within "Species"	Within "Concentration"				
S^a - P^a - Ph^a - M^b	$C1^a$ - $C2^a$ - $C3^b$				
Within level C1 of "Conc"		Within level C2 of "Conc"		Within level C3 of "Conc"	
S^{ab} - P^{ab} - Ph^{abc} - M^{abc}		S^{ab} - P^{ab} - Ph^{ab} - M^{cd}		S^{bc} - P^a - Ph^{abc} - M^d	

Homogenous groups are given as superscript letters. Significant p-values (<0.05) are given in bold.

Table 6
Comparison among literature data and measured averaged experimental values.

Species	Mean clearance rate (L h^{-1} DWg^{-1})			
	Literature	C1	C2	C3
Sabella spallanzanii	5.50 ± 1.60	1.31 ± 0.18	1.32 ± 0.08	3.48 ± 0.49
Mytilus galloprovincialis	3.95 ± 2.17	3.03 ± 0.70	5.11 ± 1.91	7.11 ± 1.02
Paraleucilla magna	0.60 ± 0.70[a]	2.36 ± 0.57	1.71 ± 0.26	0.95 ± 0.63
Phallusia mammillata	4.78 ± 0.17	3.00 ± 1.30	2.10 ± 0.14	2.95 ± 1.28

[a] Giangrande et al., 2020b.

medium-size particles are kept in the ventral part and then used for tube building mixing them with mucus; and big particles are pushed away into the water (Piazzolla et al., 2020). Further, even a large part of feces and pseudofeces is compacted with the mucus to build the tube, resulting removed from the system (Giangrande et al., 2005). Recently, MPs were found in *S. spallanzanii* tissues from the field (Vecchi et al., 2021). The authors found fragments and film of different colors, most of them bigger than 330 μm, with a mean of 0.1 ± 0.2 particles/g of tissue. It is interesting to note that some authors found numerous MPs of different shape and chemical composition also in the tube of *S. spallanzanii,* from the Mediterranean Sea (Piazzolla et al., 2020), and of *S. pavonina,* collected in the North and Barents Seas (Knutsen et al., 2020). Piazzolla et al. (2020) classified the found MPs by shape and size and the higher percentage of items were filaments of 100–500 μm in length. This evidence and our experiment data support the theory that this animal tolerates MPs, already acting as bioremediator, and its capabilities could be more exploited in this framework.

4.3. Paraleucilla magna

P. magna is abundant in eutrophic environments as in transparent waters and, prefers semi-enclosed basins probably due to its fragile structure (Longo et al., 2007). In Rio de Janeiro it is the most abundant calcareous sponge and is considered resistant to pollution (Klautau et al., 2004). There is very little information about MP laboratory-controlled feeding experiments performed with calcareous sponges since most of the studies deal with demosponges (Baird, 2016; De Marchi et al., 2022). However, it is reported that marine sponges contain MPs (Girard et al., 2021; Fallon and Freeman, 2021) and since the studied species have a leuconoid body organization as *P. magna* (Leys and Eerkes-Medrano, 2006), we used them as a comparison. The clearance rate, as well as particle retention, of *P. magna* decreased with increasing MP concentrations (Fig. 4). De Marchi and coworkers found that the filtration rate of *P. ficiformis* (even if with a different method from this work) is not affected by MP presence after 24 h, but it starts to decrease after 72 h. They also underlined that egestion does not occur in the short-term, but the result might be a consequence of the reduction of sponge pumping activity due to plastic particles presence. Due to the spiculate body organization of *P. magna* this sponge likely accumulates MPs <50 μm in its ectosome (Girard et al., 2021). Because these studies can confirm the capability of sponges to retain particles, our data could be explained with the high concentration used. Higher concentrations of particles may lead to clogging of the aquiferous system and choanocyte chambers causing the reduction of sponge pumping activity as previously observed by Baird (2016). The more concentrated solution we tested (C3) corresponds to ca. 10^6 p/m^3 higher than the real marine environment contamination. By contrast, the lower concentration (C1, ca. 10^4 p/m^3), even if it is still higher than the values reported for the Mediterranean Sea (Sbrana et al., 2021), is closer to a realistic scenario (Lönnstedt and Eklöv, 2016). It is challenging to use realistic particle concentration to perform manipulative experiments because MP concentration in marine environment is quite low. Comparing the *P. magna* behaviour in these two different conditions (C1 and C3), data showed that *P. magna* has higher clearance rate calculated on MPs with respect the reported value (Table 6). This confirms the sponge's ability to remove plastic particles from the water column until saturation. This is evident also from the particle recovery data (Fig. 3) in which at C3 we found the highest number of particles in water (over 40 %), underlining how in these saturated conditions *P. magna* reduced its pumping activity leaving the particles in excess in the water or egesting them. Basing on its ecological features, and unpublished data, we can assume that this species can handle micropollutants present in the water column consenting us to suggest its possible use for bioremediation purposes.

4.4. Phallusia mammillata

Solitary ascidians like *P. mammillata* are widespread organisms that can form tridimensional structures (Rossi, 2013). *P. mammillata* is used to live in eutrophic coastal habitat in dense populations (Zega et al., 2009). The mucus filter produced by endostyle plays an important role in the particle capture activity in ascidians (Randløv and Riisgård, 1979). From our experiment, this organism showed no significant differences in CR and PR% among the three tested concentration confirming as already reported by Jacobi et al. (2018) for various ascidians: their capture efficiency seems to be concentration-independent. The clearance rate calculated on PS microsphere in this study and the literature value are instead quite comparable (Table 6). Only in the experiment performed with the intermediate concentration (C2) the CR slightly decreased, but this may be related to a spontaneous closure of the siphons reducing the filtering activity or more probably it was just natural variability because *P. mammillata* is not so sensitive to external stressors (Fiala-Médioni, 1978). Some studies evaluated the effect of polystyrene MPs on different ascidian species at various life stages. Messinetti et al. (2018) reported that *Ciona robusta* larvae seem to show an adaptive behaviour to high concentration of MPs and that juveniles were not affected during development by 10 μm PS spheres. Vered et al. (2019) reported the occurrence of MPs in *M. exasperates* along Israelian coastline with no differences in the measured MP quantities among sites, underlining the important role of solitary ascidians as potential biological indicators for these pollutants in marine environment. From recent investigation it has been proved that ascidians are able to filter and capture MPs from water column with higher retention from 10 μm up to the submicron range (Jacobi et al., 2018; Anderson and Shenkar, 2021). It's not completely clear how long MPs stays in the organisms but some authors suggested that probably ascidians can accumulate particles for more than a week and that these particles may be translocated to the gut cavity or in the gonads (Messinetti et al., 2018; Anderson and Shenkar, 2021; Valsesia et al., 2021). The low ecotoxicological consequences reported for ascidians after MP ingestion, their plasticity to environmental eutrophic conditions and their abundance in MP contaminated environment, combined with their capability to capture and retain microparticles regardless of concentration - as confirmed by our study - make *P. mammillata* a great option for MP bioremediation. Our results are quite promising since our lower concentration is higher

Table 7
Literature MP laboratory-controlled feeding experiments involving our target and/or taxonomically related species and relative tested MP concentrations.

Target species	MP laboratory-controlled feeding experiment				
	Reference	MP concentration (p/L)	Related species	Reference	MP concentration (p/L)
S. spallanzanii	N. A.	–	N. A	N. A.	–
M. galloprovincialis	Paul-Pont et al., 2016	$1.8 \cdot 10^6$	*M. edulis*	Browne et al., 2008,	$1.0 \cdot 10^{12}$
	Gonçalves et al., 2019	$1.0 \cdot 10^6$	*M. edulis*	Van Cauwenberghe et al., 2015	$1.1 \cdot 10^5$
	Cappello et al., 2021	$5.0 \cdot 10^4$	*M. edulis*	Green et al., 2019	$1.3 \cdot 10^3$
	Trestrail et al., 2021	$5.0 \cdot 10^4$	*M. edulis*	Li et al., 2020	$1.2 \cdot 10^8$
P. magna	N.A.	–	*Crella incrustans*, *Tethya bergquistae*	Baird, 2016	$4.0 \cdot 10^8$
			Petrosia ficiformis	De Marchi et al., 2022	$2.6 \cdot 10$
P. mammillata	N.A.	–	*Ciona robusta*	Messinetti et al., 2018	$4.6 \cdot 10^7$
			Ciona intestinalis	Messinetti et al., 2019	$4.6 \cdot 10^8$

with respect environmental conditions further underlining the *P. mammillata* potential in efficiently removing particles from the water column in highly stressful conditions.

5. Conclusions

Following the purpose of this work the bioremediation potential referred to MPs of each filter feeders was evaluated. Bioremediation in marine systems is a sustainable tool to be applied in waters subjected to pollutant inputs. Recently, it is gaining attention also in MP pollution framework and principally bacteria are proposed to remove by degradation these pollutants from the environment (Kučić Grgić et al., 2021; Kumar et al., 2020), while the use of higher eucaryotes as MP bioremediators is an alternative still underrated. Indeed, according to Masiá et al. (2020), it is urgent the efficiency evaluation of potential candidate species that should possess the following features: 1) respect the animal welfare legislation (Directive 2010/63/UE, http://data.europa.eu/eli/dir/2010/63/oj); 2) high capture, retention and filtration/ingestion rates of MPs and, they should not be returned to the environment; 3) species should be selected and used only within their native range; 4) preferably species with widespread distribution and easy to manage. Filter feeders seems to be the best candidates (Masiá et al., 2020), especially *Mytilus* spp., as this work has proved, that fulfill almost all the requirement but due to the proven toxicity effect and especially to its direct consumption by humans cannot be a candidate for this purpose. *P. magna* is not the best option because: 1) is not efficient at removing particles; 2) it is a calcareous sponge with a fragile structure; 3) is an invasive species in Mediterranean Sea; these features make it not easy to set up in a specific place for bioremediate with effective results. Nevertheless, other sponge species could be further tested in this context to evaluate their potential role as bioremediators in aquaculture farms. Sponges are already farmed for several purposes (like extraction of useful metabolites) and within this cultivation they can be raised to be used as biofilter in polluted sites (Milanese et al., 2003).

Otherwise, *P. mammillata* fulfills all the requirement to be a good remediators of MP contamination, with high clearance rate and high pollutant tolerance, but there is still a gap of knowledge about its real interaction with MPs in the environment and further investigations, about its ability to retain particles and how long these particles remain inside the body, are needed. Lastly, *S. spallanzanii* is a widespread species, living in polluted sites, and thanks to its high filtration rate it was already tested and used as bioremediator of organic enrichment in aquaculture implants with successful results (Giangrande et al., 2005, 2020a). Since there is evidence of its capability to sequester MPs from sea water and to use a large amount of feces and pseudofeces to build its tube (Giangrande et al., 2005), it can be assumed that even "egested" MPs can be considered removed from the water system. *S. spallanzanii,* by our point of view, represents the best candidate for this bioremediation role. However, it is interesting to note that all the species resulted to be equally efficient in removing MPs at C1, the most environmentally relevant concentration. Long-term experiments both at field and laboratory level to improve knowledge about the interaction of MPs with less studied benthic species as ascidians and sponges are needed. Studies focused on what happens seasonally in the IMTA facilities, or what could be the result of combining different species even at different density, will open new cues to understand whether these organisms may be used as bioremediators in MP polluted areas. The IMTA concept may be improved by exploiting different species as bioremediators to improve the overall bioremediating performance removing even MPs from the water system.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2023.114613.

CRediT authorship contribution statement

Silvia Fraissinet: Conceptualization, Methodology, Investigation, Data curation, Visualization, Writing – original draft. **Daniele Arduini:** Methodology, Investigation, Data curation, Visualization, Writing – original draft, Formal analysis. **Olaya Vidal:** Investigation, Data curation. **Antonio Pennetta:** Resources, Data curation. **Giuseppe Egidio De Benedetto:** Validation, Writing – review & editing, Supervision. **Cosimino Malitesta:** Validation, Writing – review & editing, Supervision. **Adriana Giangrande:** Writing – review & editing, Supervision, Funding acquisition. **Sergio Rossi:** Conceptualization, Formal analysis, Validation, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

This study is part of the project "i-Plastics: Dispersion and impacts of micro- and nano-plastics in the tropical and temperate oceans: from regional land-ocean interface to open ocean". The authors would like to acknowledge the funding from the JPI Oceans International Consortium. The authors would like to acknowledge also "REMEDIALife: REmediation of Marine Environment and Development of Innovative Aquaculture" project.

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