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Microplastics Uptake by Four Filter Feeders

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Abstract: Microplastics (MPs) are insidious plastic particles with sizes ranging from 1 to 5000 μm. Their presence has been reported all over the world. Recently, bioremediation to remove MPs from water columns using filter feeders as biofilters has been proposed. In a previous lab experiment, the MP bioremediation potential of four fouling organisms from a mariculture facility (*Mytilus galloprovincialis*, *Sabella spallanzanii*, *Phallusia mammillata*, *Paraleucilla magna*) was separately assessed in single-species experiment. Herein, a follow-up of the work is presented using a multi-species approach. The four organisms were placed together in the same 5 L beaker and fed with a concentration of 250 p/L 6 μm red polystyrene discernible particles. After digesting the organisms and counting the MPs in both the water and the organisms, the results of the two experiments were compared. In the previous experiment, *S. spallanzanii* had the highest particle retention (PR) value (PR = 88.01%), while in this experiment, *P. mammillata* has the lowest PR value (PR = 31%). The multi-species approach resulted in a higher number of plastics being removed from the water (88%) compared to the single-species experiments. These fouling organisms naturally exist as a community, acting as an efficient filter with complex morphologies and hydrodynamic features. Here, this simple marine animal forest is re-evaluated by exploiting the ecosystem services provided by these organisms as a solution to MP pollution problem in a mariculture environment.

Keywords: microplastics; bioremediation; IMTA; filter feeders

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1. Introduction

Microplastics (MPs) are tiny pieces of plastic, with sizes ranging between 1 and 5000 μm [1]. These micropollutants are ubiquitous in the world's oceans and seas, appearing from the offshore to the shoreline, the surface microlayer to the deep sea, and even in the deepest places in the ocean [2,3], showing the enormous impact of humans on the planet. Activities like intensive fishery and aquaculture are considered possible sources of microplastics in the marine environment [4]. In the aquaculture sector, floatable structures, cages, boat varnishes, garments, and tools are made of plastic [4]. The flexibility, weight, durability, and low price of this material make it perfect for these activities. Unfortunately, prolonged exposure to the chemical and physical features of the marine environment, such as irradiation, temperature, biofilm formation, and mechanical abrasion, can induce a weathering process which leads to macroplastics' fragmentation into microplastics [5,6]. In Europe, various important directives have been developed regarding plastic waste since the Marine Strategy Framework Directive (MSFD) [7] introduced the problem, with Descriptor 10 focused on marine litter and specifically plastic litter. Among them, there is the Waste Framework Directive [8], the EU Strategy on Plastics [9], and the Single-Use Plastics Directive [10]. In September and October 2023, the EU deliberated on restricting the intentional addition of MPs to products (cosmetics, detergents, glitter, fertilizers, and medicines, to name a few), and proposed a measure to prevent pellet losses [11]. However, there are no guidelines for managing and removing MPs already in the marine ecosystem.

In the sea, MPs of different sizes and forms interact with marine fauna with dangerous consequences that have been widely reported [12–15], including the potential risk of these pollutants to humans through eating seafood [16–18]. Filter feeders are widely studied in the MP pollution framework. These animals contribute to ecosystem function, regulating primary production by controlling nutrient cycling and phyto- and zooplankton population structures, cleaning the water column, increasing habitat heterogeneity, regulating the seston composition and concentration, and playing a key role in the benthic–pelagic coupling process [19–23]. These characteristic features and their trophic strategy make filter feeders among the most affected organisms by the MP pollution [24]. The more complex the ecosystem–engineering species combination, the more turbulent the patterns and potential particle retention [25,26].

Finding reliable and sustainable solutions to counteract the microplastic spread is an urgent priority for decision-makers and policymakers. The scientific community is making efforts to provide solutions. Recently, a new sustainable option has been suggested to address the plastic presence in marine environments using macrobenthic organisms as natural biofilters [27–29]. This perspective is already being applied to the aquaculture sector, where a promising strategy is represented by the Integrated Multi Trophic Aquaculture (IMTA) approach [30]. In this system, multiple species are combined with farmed animals to reduce algal blooms or surplus organic waste (Borghese et al., submitted). The organisms selected, indeed, can use these resources to improve their growth and thus their biomass, giving the IMTA system a double-positive effect by producing exploitable biomass and providing bio-mitigative services [30–33].

In the Mar Grande of Taranto (Ionian Sea), at the Mariculture facility “Mar Grande”, an Integrated Multitrophic Aquaculture (IMTA) system has been developed within the framework of RemediaLife Project [34]. Placing natural rope collectors near the fish cages allowed for the development of an abundant and healthy fouling community principally composed of filter feeders such as mussels, polychaetas, ascidians, and sponges [35,36]. This bioremediation concept was applied to the MP pollution framework in a previous experiment, where the MP removal potential of four filter feeders macrofoulers from the fouling community associated with IMTA (*Mytilus galloprovincialis*, *Sabella spallanzanii*, *Phallusia mammillata*, *Paraleucilla magna*) was assessed [28]. This previous laboratory experiment was performed using a single-species approach (evaluating the particle removal efficiency one species at the time), as also recently conducted by other authors [27–29].

However, there is an important aspect to consider: the animals naturally co-occur in the environment. After exploring the MP bioremediation potential of these organisms in the initial single-species experiment, here, “community” bioremediation potential was estimated: the four filter feeders were exposed to the microplastic concentration at the same time, following a multispecies approach. The experimental setup for the selected species was kept the same as in the previous study in order to compare the data. The aim of this study, which can be considered a follow-up of the previous study, is to compare the uptake of particles by the “community” (EXP2) and individual species (EXP1). This, providing further data to improve our knowledge regarding the ecosystem services provided by marine animal forests such as fouling communities, especially in IMTA systems where they can act as bioremediators.

2. Materials and Methods

The methodological section will be shortly explained, and a more detailed explanation of the whole theoretical and practical approach to the topic can be found in Fraissinet et al., 2023 [28].

2.1. Sampling

All specimens were collected from the natural ropes used as fouling collectors in the IMTA system hosted at the 'Maricoltura Mar Grande' in the Mar Grande of Taranto (40°25′56″ N;17°14′19″ E, Ionian Sea). The sampling activity was carried out between May and June 2022. Animal selection was based on physiological features and density on the rope collectors. *Sabella spallanzanii* is a tube-dwelling polychaete widely distributed throughout the open seas. The animal can live at depths between 1 and 30 m in both open and confined areas, reaching high densities [37]. *M. galloprovincialis* is a bivalve mollusk with a high distribution in the Mediterranean Sea and northeastern areas of the Atlantic Ocean. It is widely farmed and is one of the most commonly consumed seafood in Europe [38,39]. *Phallusia mammillata* is a solitary ascidian, commonly called White Seaquirt. This can grow up to 20 cm in height. It is a benthic organism that lives on hard substrates, typically in the North Sea, the Mediterranean Sea, and the northeastern part of the Atlantic Ocean. *P. magna* is a calcareous sponge native to the Brazilian coast. It has recently been introduced into the Mediterranean Sea, where it exhibits different morphologies (tubular, massive, or irregular shapes) [40,41]. These organisms are part of the fouling community, living in enclosed areas such as the Mar Grande and Mar Piccolo in Taranto, and are able to tolerate eutrophic and polluted conditions [41–43]. The four selected species collectively represent more than 80% of the fouling biomass established on the rope collectors near the IMTA cages [36,42].

2.2. MP Concentration

Red-dyed polystyrene (PS) microbeads (Polyscience Inc; Hirschberg an der Bergstrasse, Germany) were used to assess particle uptake of the animals. Microsphere size (nominal mean diameter 6 µm) was selected based on the animal's prey size range that is desired to be captured for all four species. PS microspheres were purchased as water suspensions to ensure easy dispersal in the beaker, reducing the risk of aggregation. The suspension with a concentration of 2.10×10^8 particles/mL (provided by the dealer) was diluted to obtain a specific concentration of microparticles corresponding to ca. 250 p/L in order to perform the feeding experiment.

2.3. Feeding Experiment

Six specimens of similar size were selected for each species (six groups of four animals) in order to perform the experiment. *Sabella spallanzanii* average length (tube) 21.3 ± 0.8 cm; *Mytilus galloprovincialis* average length (shell) 6.01 ± 0.7 cm; *Paraleucilla magna* average length (longest axis of tubular shape individuals) 4.5 ± 1.1 cm; *Phallusia mammillata* (tunic) 8.1 ± 1.06 cm. The experiment was divided into five feeding treatments plus one control. A different individual was selected for each trial, including the control. In the control experiment, the animals were placed in the beaker, but no particles were added to the water to prove the absence of red beads in animal tissues before the experiment.

Each 5 L beaker was filled with 250 µm pre-filtered natural seawater at 20 °C to acclimatize the organisms. A magnetic stirrer was used to avoid MPs sinking and ensure their uniform distribution, keeping the water in a gentle, steady motion. Four animals were gently placed together in the same beaker on customized supports, paying attention to prevent any disturbance due to the magnetic bar and keeping them fully submersed. Plastics were added to the water in the proper concentration, and the experiment lasted 1 h (Supplementary Materials Figure S1).

2.4. Sample Preparation

Mussels, sponges, and polychaete tissues were digested using KOH (2.5%) + H_2O_2 (5%), while reagent concentrations were doubled to digest ascidian tissues. The digestion was performed at 75 °C for 3 h. After centrifugation, the precipitate was treated at room temperature for max. 30 min, using a solution of formic acid (25%) and sodium citrate (10%) to dissolve carbonate parts. Then, the digestate and acid solution were filtered using

47 mm glass fiber filters (Whatman pore size 0.7 µm). More details about the digestion procedure can be found in [28,44]. To reduce the presence of organic matter, H_2O_2 was added to water to achieve a final concentration of 5%. After 24 h, the water was filtered using 47 mm glass fiber filters (Whatman pore size 0.7 µm). Thus, MPs were counted at 20× magnification using a Nikon Eclipse 80i microscope (NIKON Europe Badhoevedorp, Neatherlands). Photos were captured through the microscope using an integrated Nikon camera with ACT-2 U acquisition software v. 1.6. Each filter was divided into four parts, with one quarter randomly selected for counting the red particles. The total number of filters used was 24 for the animal tissues (5 treatments + 1 control per species), and 6 for the water (1 per beaker).

2.5. Particle Retention Rate Calculation

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

$$\%\mathrm{PR} = \left(\frac{C_0 - C_t}{C_0}\right)\cdot 100, \quad (1)$$

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

2.6. QC/QA

During both field sampling and sample preparation in the laboratory, precautions were taken to reduce contamination. During field activities, divers did not wear gloves during animal collection, and immediately after sampling the animals were stored in aluminum foil directly on the boat. In the lab, each animal was rinsed multiple times with ultra-pure, pre-filtered (0.1 µm) MilliQ water to remove any possible debris or particles from their surface. All operators wore cotton gowns and/or cotton clothing, and nitrile gloves were used for all laboratory activities. All operations were performed in glass containers using metal tools and tweezers. The filtration step, which represents the riskiest stage, was executed inside a fume hood while covering the top of the filtration apparatus with aluminum foil to minimize airborne contamination.

2.7. Data Analysis

One-way ANOVA was performed to test for differences between species (*M. galloprovincialis*, M; *P. magna*, P; *P. mammillata*, Ph; *S. spallanzanii*, S) in the percentage of particle retention (PR%). Previously, Shapiro–Wilk and Levene's tests were performed to verify the ANOVA assumptions of normal distributions of data ($W = 0.93$; $p = 0.13$) and homogeneity of variance ($p = 0.85$) respectively. Significance was set at a critical level of 99% ($p < 0.01$). Tukey's HSD (honestly significant difference) post hoc test was used to determine the group(s) that differed significantly from each other.

3. Results

3.1. Recovery of Particles

All the particles added to the beakers were recovered in both water and the animal tissues, and no particles were found in the control experiment after 1 h. The organisms filtered higher amounts in all replicas (Figure 1). The best-performing organism was *P. mammillata*, followed by *M. galloprovincialis* and *S. spallanzanii*. The ANOVA test showed significant differences in PR% between the four species ($F_{3,16} = 8.89$; $p = 0.001$). The post hoc comparison Tukey's HSD test revealed that *P. magna* had a significantly lower PR than *M. galloprovincialis* and *P. mammillata*, while *S. spallanzanii* showed no significant differences.

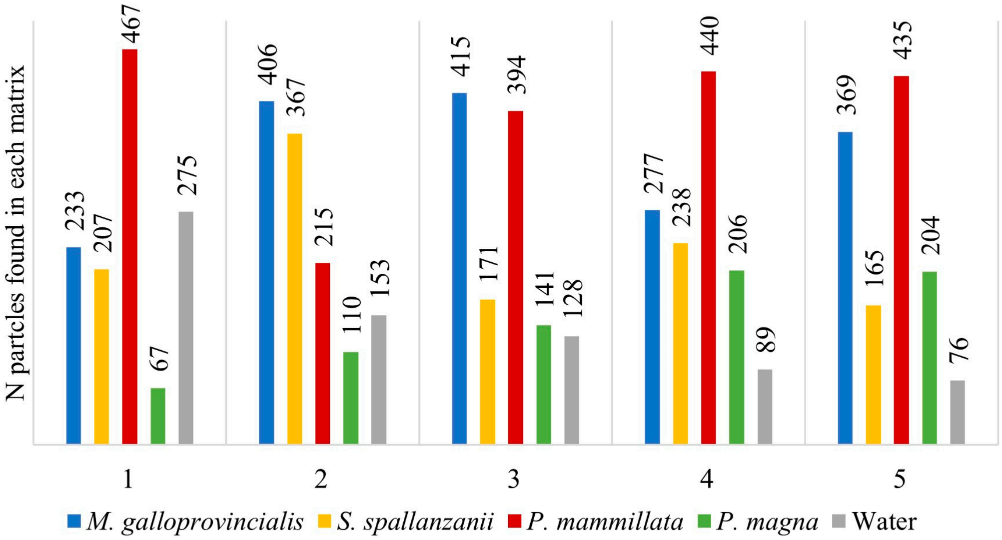

Figure 1. Number of particles found in animal tissues and water after each experiment. The different colors refer to the different matrices. Numbers 1 to 5 identify each treatment.

3.2. Single-Species Approach vs. Multi-Species Approach

Comparing data from the two experiments, the particle retention per species in the EXP1 is higher than that registered in the animals tested in this work, as expected. The average particle retention per animal in the single-species approach was 68 ± 27 (*M. galloprovincialis*), 81 ± 18 (*S. spallanzanii*), 67 ± 31 (*P. mammillata*), and 71 ± 12 (*P. magna*). In the multi-species approach, average PR% was 27 ± 6% (*M. galloprovincialis*), 18 ± 7% (*S. spallanzanii*), 31 ± 8% (*P. mammillata*), and 12 ± 5% (*P. magna*) (Figure 2).

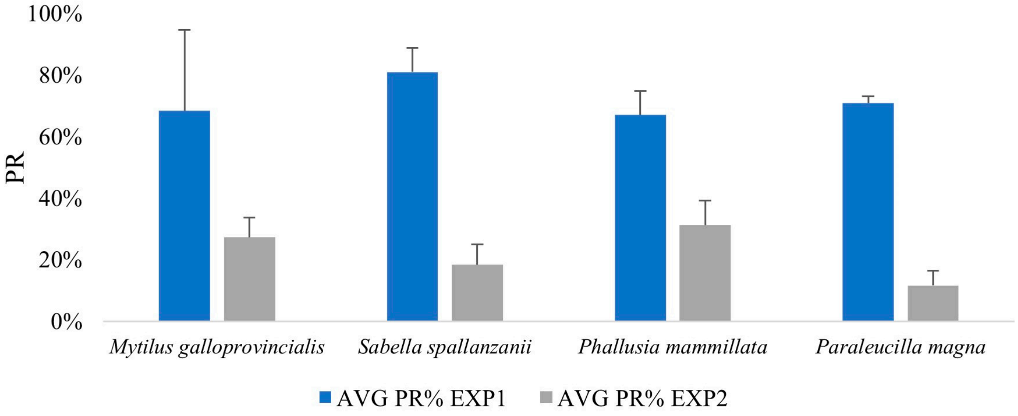

Figure 2. Average particle retention in the single-species experiment (EXP1, blue) compared to average particle retention in the multi-species experiment (EXP2, grey).

However, the picture changes slightly when comparing the average PR% of the individually tested species (EXP1) with the PR% of the “community” (88%) in the present experiment (EXP2), where the PR% of the individual species are taken together, showing that the “community” is more efficient at removing particles than individual species (Figure 3).

The mean PR% for the community was 88%, while for *S. spallanzanii* in EXP1 the mean PR% was 81% in similar conditions. In the previous experiment, it was concluded that *Sabella spallanzanii* has the potential to be the best candidate for MP bioremediation compared to the other three filter feeders tested. These data support the proposal of the EXP1, confirming *S. spallanzanii* as a suitable bioremediator for MPs in the marine environment, while also highlighting the PR% positive increment induced in the multi-species approach.

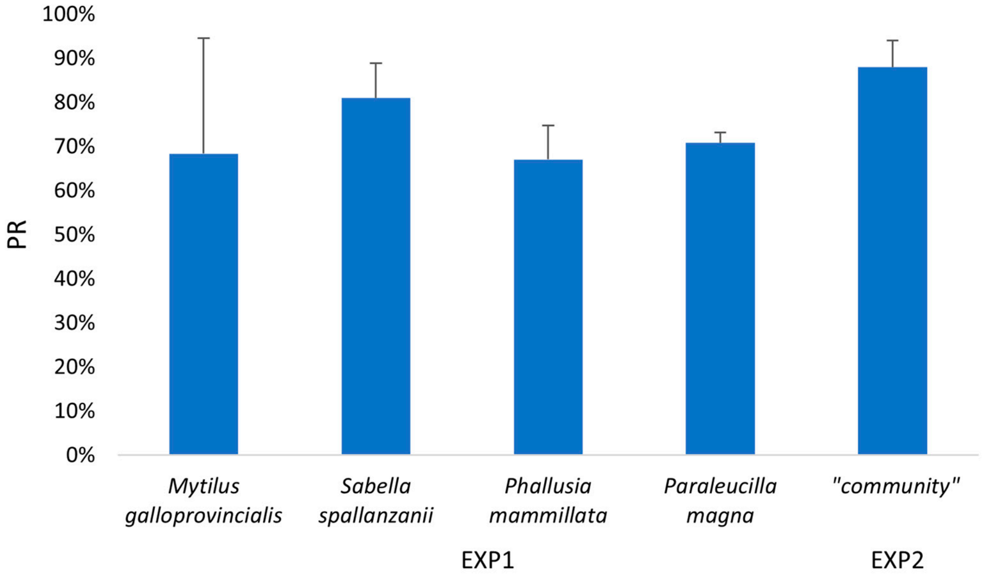

Figure 3. Comparison among the PR% of each species in the EXP1 (single-species approach) and the average PR% of the "community" in EXP2 (multi-species approach).

4. Discussion

MPs are widespread in marine environments, and their concentration is highly variable among different areas due to different environmental conditions and sampling methodologies [45]. The Mediterranean Sea is a hot spot for plastic pollution, with an average concentration of 0.0058 items/m^3 microplastics in the water column [46–48], as per the relationship suggested by Lusher (2015) [49]. In the IMTA area of the Taranto Gulf, where the species were collected, this concentration is two orders of magnitude higher, at 0.62 items/m^3 [46].

The concentration of MPs chosen for this work struck a compromise between realistic and operative conditions. The selected species observed living on the natural rope collectors of the IMTA system collectively correspond to almost the entire biofouling biomass in the Mar Grande of Taranto [36,42], and the same organisms form parts of biofouling communities in confined areas all over the Mediterranean Sea [41–43].

In the EXP2 (multispecies approach), *P. mammillata* showed the best performance. Ascidians are powerful filter feeders [50], and the associated concentration of MP found in the literature contamination values is in a similar range as other wild marine organisms [51]. A recent study proposed ascidians as MP bioremediators [29], reporting that a 1 m^3 specimen cage can remove hundreds of grams of administered microplastics in one day [29]. However, to the best of our knowledge, studies about ascidian adult individuals and their interactions with MP are still scarce, making it difficult to make comparisons and evaluations.

Mytilus galloprovincialis registered a high PR% in the EXP1 and the EXP2. The presence of mussels can improve the water quality by acting as biofilters [52,53], and recently, these organisms have been proposed as natural-based solutions for remove plastic particles from the marine ecosystem [27]. Also, it has to be considered that the size of the MPs used in the experiment is in the same range as the particles retained by active suspension feeders like mussels [54,55]. MPs can be compacted into mussel pseudofaeces and mechanically removed [27], or in the case of the IMTA, they can be intercepted by placing *S. spallanzanii* below *M. galloprovincialis* on the collectors and stored in the tubes [28,56,57].

Sabella spallanzanii achieved a lower PR% in this experiment with respect to previous one but comparing the PR% of the "community" (88%) with the PR% achieved by *S. spallanzanii* in the EXP1 (81%), the particle retention rate of this tubeworm is slightly lower than that of the community itself. For this reason, and due to the capability to store particles (including plastics) removed from water in their tube, these polychaetes are still proposed as the best candidates for bioremediation. *Sabella spallanzanii* and *P. magna* from the same study area were recently analyzed to assess plastic contamination in their tissues in field conditions [44], resulting in higher contamination levels than generally reported for the polychaete [24,56,58,59]. regarding the sponge, data were consistent with [60,61], but also different from those in other studies [62,63]. However, the lack of a method of standardization for biota MP analysis in field and lab conditions makes it difficult to compare data among studies and define threshold values for the amount of microlitter ingested by animals, despite the MSFD Descriptor 10 criteria 3 and 4 (D10C3 and D10C4). This requires the establishment of threshold values and the assessment of consequent adverse effects, respectively [64].

Sponges are excellent filter feeders and are quite affected by MP pollution, with pieces of evidence found worldwide also in historical samples, highlighting high exposure of these animals to water column particle pollution in spatial and temporal terms over the past decade [60–62,65]. The concentration of the particles within their tissues is related to their environmental concentration. Generally, in the animals, we found lower quantities of MP than in the surrounding waters, this was probably because their tissues can be saturated by the presence of plastic particles in high quantities, or because they developed some sort of resistance [28,61,62,66,67], making them an interesting product, especially for remediation purposes [66]. This resistance could be ascribed to the holobiont nature of sponges: the associated symbiont microorganisms can accumulate and resist heavy metals, or, considering sponges an IMTA by-product, this microbiota can be used to produce biosurfactants that are able to degrade heavy metals and MPs [66,68–70]. However, *P. magna* appeared to be the least efficient particle remover among these tested species, registering the lowest PR%. Comparing the EXP1 with the EXP2 in the same conditions (C2 in the EXP1), *P. magna* showed an intense decrease in PR% value. This could be ascribed to the ability of sponges to excrete non-edible microparticles in a time frame that is consistent with our experimental time (1 h). For example, it was recently observed that sponges can filter and subsequently expel MPs of 2 and 10 µm in a time frame of 58 ± 34 min and 95 ± 36 min, respectively [71]. It is possible that *P. magna* expelled some of the filtered MPs during the experiment that became available in the beaker water to the other filter feeders, so that the number of MPs retained in the sponges was lower than at the end of the experiment. This could not happen in the EXP1, as the individual sponge could at most refilter the same microparticles that were expelled by itself, resulting in higher retention levels. Placing the animals near (or close) to the pollution input could improve the amount of plastic removed [27]. However, prior to setting up and placing a high number of organisms in a selected site, it is mandatory to

1. check the environmental conditions related to the possible biosecurity risks posed by placing the species in that specific site and [27];
2. set the protocol for recovering microplastics after removal [27,72].

These issues could be reduced by exploiting the ecosystem services already provided by one of the simplest examples of marine animal forests: fouling assemblages [73]. The fouling community, when the colonizable substrate is not limiting, can increase in density and abundance with the increase in food input, and on the other hand, the species richness is less prone to change [74]. These features make these species coexist in a stable and complex structure. In real conditions, the presence of the rope collectors creates new natural substrata for the recruitment of species, allowing the fouling community in the IMTA facility in the Mar Grande of Taranto to proliferate and making it suitable for bioremediation purposes [36]. An effective polyculture needs to be designed in a way that considers and promotes the complementarity of the selected species in a biodiversity-centered framework [75]. In this case, placing the animals close to fish cages improved the clearance of the water column from both organic matter and MPs, allowing the quality of fish and water to increase [32,76]. A fundamental requirement for bioremediation is blocking the removed particles from returning to the environment [77]. The whole fouling biomass should be managed to gain economic profit and ecological benefits. Some options have been proposed and include providing ornamental animals for aquaria [78], or the production of fish food using *Sabella* [79].

The benthic fouling animal forest established in the IMTA system is anything but monospecific and can tolerate eutrophication and pollution while providing numerous ecosystem services such as cleaning from the water column the metabolic waste of farmed fish [36,80]. It has already been demonstrated that the presence of this fouling community as part of the IMTA system of Taranto provided significant benefits and led to the overall amelioration of the environmental conditions, as both the soft- and hard-bottom macrozoobenthic assemblages, the microbiological standards, and the local biodiversity showed evidence of recovery [32,42,76]. In this positive scenario, another ecosystem service can be achieved: the removal of microplastics.

5. Conclusions

To the best of our knowledge, this is one of the first attempts at assessing the MP bioremediation potential of filter feeders using a multi-species approach. In our opinion, this is the best way to deal with this problem while following the bioremediation requirements [67]. Furthermore, with this approach, animals such as *M. galloprovicialis* and *P. magna*, previously considered unsuitable on their own, can instead contribute as part of the community to capture and/or concentrate MPs for the other species (e.g., the pseudofaeces of mussels containing undigested MPs may become available for *P. mammillata* and *S. spallanzanii*). The selected species showed a high capability to clean water from plastic particles, and *P. mammillata* achieved higher performance in this experiment. Comparing data from EXP1 to EXP2, the community is more efficient than single specimens, even if there are not many differences with *S. spallanzanii* alone (which is, in this case, also the most abundant biomass in the IMTA facilities of Taranto) [35]. This tube worm is still proposed as the best candidate for the bioremediation of MP. However, the fouling community is naturally present in polluted sites and naturally tolerant to stressful conditions. This auto-sufficient community can generate big biomasses, which can achieve economic profits, closing the cycle of an eco-friendly cleaning water biofilter pump. This work can be considered a starting point, while further experiments will be needed to advance the knowledge in this field and to evaluate other possible species suitable for this purpose.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jmse12061000/s1, Figure S1: Animals positioned in the 5 L beaker were gently placed on the bottom to be fully submerged. In the center, a magnetic stirrer kept the plastic particles in motion during the experiment.

Author Contributions: Conceptualization, S.F. and D.A.; Methodology, S.F., D.A. and A.M.; Formal analysis, D.A.; Investigation, A.M., S.F. and D.A.; Resources, S.R., A.G., G.E.D.B. and C.M. Writing—original draft, S.F.; Writing—review and editing, S.R., A.G., S.F. and D.A.; Supervision, S.R., A.G., G.E.D.B. and C.M.; Project administration, S.R.; Funding acquisition, S.R. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding authors.

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