Polyethylene microplastics reduce filtration and respiration rates in the Mediterranean sponge *Petrosia ficiformis*

Lucia De Marchi [a,*], Monia Renzi [b], Serena Anselmi [c], Carlo Pretti [d,e], Elisa Guazzelli [f], Elisa Martinelli [f], Alessia Cuccaro [a], Matteo Oliva [e], Michele Magri [g], Fabio Bulleri [h]

[a] *Departamento de Biologia, Universidade de Aveiro, Aveiro, Portugal*
[b] *Dipartimento di Scienze della Vita, Università di Trieste, Trieste, Italy*
[c] *Bioscience Research Center, Orbetello, Italy*
[d] *Dipartimento di Scienze Veterinarie, Università di Pisa, Pisa, Italy*
[e] *Consorzio per il Centro Interuniversitario di Biologia Marina ed Ecologia Applicata "G. Bacci" (CIBM), Livorno, Italy*
[f] *Dipartimento di Chimica e Chimica Industriale, Università di Pisa, Pisa, Italy*
[g] *Ambienti Magri, Livorno, Italy*
[h] *Dipartimento di Biologia – Unità di Ecologia e Biologia Marina, Università di Pisa, Pisa, Italy*

ARTICLE INFO

Keywords:
Microplastics
Porifera
Particle characterization
Potential microplastic concentrations
Filtration rate
Respiration rate

ABSTRACT

Microplastic (MP) pollution represents a distinctive mark of the Anthropocene. Despite the increasing efforts to determine the ecological impacts of MP on marine biodiversity, our understanding of their toxicological effects on invertebrate species is still limited. Despite their key functional roles, sponges (Phylum Porifera) are particularly understudied in MP research. These filter-feeders extract and retain particles from the water column, across a broad size range. In this study, we carried out a laboratory experiment to assess the uptake of MPs (polyethylene, PE) by the Mediterranean sponge *Petrosia ficiformis*, how MPs influence key biological process after different times of exposure (24h and 72h) and whether they can be subsequently eliminated. MP uptake increased with time of exposure, with 30.6% of the inoculated MP particles found in sponge samples after 72h. MPs impaired filtration and respiration rates and these effects were still evident 72h after sponges had been transferred in uncontaminated water. Our study shows that time of exposure represents a key factor in determining MP toxicity in sponges. In addition, our results suggest that sponges are able to incorporate foreign particles and may thus be a potential bioindicator for MP pollutants.

1. Introduction

Floating plastic debris are currently one of the most abundant items of marine litter, making up the 80–90% of all litter found in the aquatic environment (Borrelle et al., 2020). Their release has increased substantially over the last 60 years, from 0.5 million tonnes (MT) in 1960 to 311 MT in 2014 and it is projected to reach around 1800 MT in 2050 (UNEP, 2016). Beaches, rivers, wastewater discharges and transport of land litter by wind are the most frequent sources of plastic debris to the ocean (Guen et al., 2021). Despite the fact that microplastics (MPs) definition has been introduced since 2004, recently, they were described as any synthetic, solid particle with a size range from 1 μm to 5 mm of either primary or secondary origin (Bessa et al., 2019). Primary MPs, derived from synthetic textiles, cosmetics, industrial and medical applications, are introduced directly into the ocean (Bessa et al., 2019; Le Guen et al., 2020; Bajt, 2021); secondary MPs are generated from larger items through natural weathering processes (Bessa et al., 2019; Bajt, 2021). Both types of MPs have been found to accumulate and persist in natural aquatic ecosystems due to their resistance to degradation processes (Besseling et al., 2019). A recent meta-analysis identified polyethylene (PE), a common commodity plastic, mainly used for packaging (Pabortsava and Lampitt, 2020), as one of the most abundant MP in surface waters (Erni-Cassola et al., 2019), contributing significantly to the content of the global plastic waste in the oceans (~60%) (Geyer et al., 2017; Pabortsava and Lampitt, 2020).

It is now widely recognized that the potential biological implications of MPs are related to their small size, making them easily ingested by invertebrates and fish (Ding et al., 2018) and to the fact that they can act

* Corresponding author. Department of Biology & CESAM, University of Aveiro, 3810-193, Aveiro, Portugal.
E-mail address: lucia.marchi@ua.pt (L. De Marchi).

Accepted 5 March 2022

as vectors for chemical contaminants (Zantis et al., 2020) and pathogen transport, in particular marine bacteria such as vibrios and antimicrobial-resistant bacteria (Bowley et al., 2021). Moreover, selective binding between secretory molecules (e.g., *infochemicals* produced by fouling communities) and MPs may also increase the frequency of plastic ingestion by invertebrates (Procter et al., 2019) and vertebrates (Savoca et al., 2016). A variety of toxicological effects of MP ingestion by invertebrate species have been documented, including: tissue inflammation, neurotoxicity, energy depletion, reduced survival, growth, reproduction and immune function (Besseling et al., 2013; Avio et al., 2015; Cole et al., 2015; Sussarellu et al., 2015; Chapron et al., 2018; Hankins et al., 2018; Foley et al., 2018; Reichert et al., 2018; Tang et al., 2018; Ziajahromi et al., 2018; Rotjan et al., 2019; Piccardo et al., 2020; Cole et al., 2020; Xu et al., 2020).

The effects of MPs can, however, vary across species, depending on their size, feeding mode and anatomies of their mouth and digestive tracts (Fallon and Freeman, 2021). Among filter-feeders, marine sponges (Phylum Porifera) are particularly understudied, despite their key functional role in ecosystem functioning (Bell, 2008) and the fact that they are considered as good sentinel species due to their ability to uptake and accumulate organic and inorganic compounds (Efremova et al., 2002; Perez et al., 2002, 2003; Rao et al., 2006; Cebrian et al., 2007; Marques et al., 2007; Batista et al., 2013; Gentric et al., 2016). Their capability of absorbing up to ~ 80% of suspended particles is directly related to their high pumping rates (0.005–0.6 L of seawater s^{-1} L^{-1} of sponge tissue) (Fallon and Freeman, 2021). Although few studies have investigated MP ingestion rates by sponges, there is increasing evidence that they can uptake MPs (6 ± 4 and 169 ± 71 MP g^{-1} of dry tissue) (Fallon and Freeman, 2021) and incorporate them into their skeletons and other internal tissues (Girard et al., 2021).

We carried out a laboratory experiment to assess uptake of pristine PE-MPs (polyethylene microplastics) and their biological effects on the Mediterranean sponge, *Petrosia ficiformis* (Poiret, 1789). In particular, we evaluate if: I) the aggregation state of PE-MPs may alter the uptake by sponges; II) pristine PE-MPs, not associated with contaminants or colonized by a biofilm, affect the physiology of the sponge; III) the sponge responses are dependent upon the time of exposure to PE-MPs and IV) physiological functions recover with time after a period of exposure to PE-MPs.

2. Materials & methods

2.1. Sponge collection and processing

Petrosia ficiformis specimens were collected at the end of April 2021 by SCUBA diving at 10–12.5 m depth, along the north-east coast of Elba Island (Nisportino, 42°50.160′N; 10°23.152′E, Italy; salinity 40, temperature 14 °C, pH 8.10, oxygen saturation >90%). Twenty-five fragments (~15 × 10 cm^2; ~30–50 g wet mass each) were removed using a steel blade, inserted into a sterile plastic bag filled in with seawater and immediately transported to the laboratory. Before the start of the experiment, sponges were acclimated in 0.45 μm filtered natural seawater (NSW) (3L each fragment) for 7 days at temperature 14 ± 1 °C, oxygen saturation >90%, salinity 40, pH 8.1 ± 0.1 and photoperiod – 13 h light: 11 h darkness. Organisms were fed with *Isochrysis galbana* algal suspension (1 × 10^4 cells mL^{-1}) just once during this period. All water quality parameters were determined by multi parameter sensor (CTD: Conductivity-Temperature-Depth-SeaBird SBE911plus). The seawater in tanks was completely changed at the end of the acclimation period.

2.2. Microplastic preparation

Pristine polyethylene (PE) MPs (ø 40–48 μm) were purchased by Sigma Aldrich (9002-88-4, C_2H_4, white powder, ultra-high molecular weight). PE particles were suspended in ultrapure water and kept in the dark to prevent microbial growth. PE-MP stocks were then vortexed for 10 s to resuspend particles prior to use. Test MPs were selected for their bioavailability to filter-feeder organisms and their density (d = 0.910–0.941 g cm^{-3}). A concentration of 500 ng mL^{-1}, equivalents to ~26.1 PE-MPs L^{-1}, was chosen based on Cole et al. (2020).

2.3. Experimental set-up

The study included three complimentary experimental set-ups: *Exposure 1*: sponge fragments (with one osculum *per* fragment) (N = 4) were individually exposed for 24h to PE-MPs in 3L glass containers (26 x 17.5 × 15 cm) filled with NSW. Negative controls (fragments not exposed to MPs) (N = 4) and positive controls (N = 3) (3L glass water containers (26 x 17.5 × 15 cm) contaminated with MPs and without organisms) were used to verify the correct functioning of the test. *Exposure 2*: sponge fragments (N = 4) were individually exposed for 72h to PE-MPs in 3L glass containers (26 x 17.5 × 15 cm) filled with NSW. As for *Exposure 1* test, four negative and three positive controls were simultaneously used. *Exposure 3*: after 72h of exposure to PE-MPs, sponge fragments (N = 4) were allowed to depurate keeping them separately in 3L non-contaminated NSW for another 72h (recovery time); no positive controls were used at that time. For all treatments, air was pumped in *via* plastic pipette equipped with a porous stone, to enhance aeration and to maintain MPs dispersed through the water column, guaranteeing their re-circulation during entire feeding experiments. Abiotic factors were maintained as for the acclimation period and daily checked. During the test, no additional food was provided to sponges.

2.4. Chemical analyses

2.4.1. Characterization of PE microparticles suspension

To verify if the aggregation state of PE-MPs may alter their uptake by sponges, dynamic light scattering (DLS) measurements on particles suspension in 0.45 μm filtered NSW (26 μg mL^{-1}) were taken with a Beckman Coulter Delsa Nano C particle analyzer (detection angle = 166.22°). Intensity, volume and number particle distributions were obtained from the signal autocorrelation function through CONTIN analysis in the instrument software. Average hydrodynamic diameter (nm) and polydispersity index (PDI) were evaluated from cumulants analysis of the autocorrelation function and averaged over at least 10 measurements.

2.4.2. Potential microplastic (PMP) concentrations

PMP concentrations were analysed in both water column and sponge tissues. For each *Exposure* test, 3L of water were filtered by vacuum on 0.45 μm pore paper disk filters (Whatman®, Sigma-Aldrich, St. Louis, MO, USA) and stored in plastic petri dishes at room temperature. To ensure the recovery of PE-MPs, accurate rinsing of all aquarium was performed with NSW. All filters were dried and sorted by stereomicroscopy (P-DSL32, 10–80X Nikon, NS-Elements v. D.4.60) under air-controlled conditions (HEPA double-filtered purified air by fume hood); counted white items ranging within the target dimension (<63 μm) that were collected on filters were verified by chemical analysis using μFT-IR (iN10 Nicolet, Thermo, N_2 cooled reflection). PMP calculation was determined using Eq. (1):

$$PMP = p_w / pT$$

where p_w is the MP particle numbers detected in 3 L of filtered water samples and pT is the total MP particle numbers in 500 ng mL^{-1} (equivalent to 78.4 ± 2.07 PE-MPs 3 L^{-1}). Data were expressed as percentage and number of PE particles *per* L.

One sponge fragment *per Exposure* test was used to quantify PMP concentrations in their tissues; external surfaces of sponge tissues were rinsed before the extraction to ensure that collected data were referred

only to ingested MPs. Pre-filtered (0.45 μm) distilled water was used for rinsing to avoid sample contamination by external source. Subsequently, tissues were digested according to literature (Enders et al., 2020), first by KOH + NaClO sonicated for 30 min at 30 °C (40 KHz, ArgoLab, mod. AU-32), after this phase samples were added by H_2O_2 30% and were sonicated again under the same conditions. Digested samples were filtered on 0.45 μm pore paper disk filters (Whatman®, Sigma-Aldrich, St. Louis, MO, USA), stored in glass Petri dishes and dried under room temperature till complete exsiccation. Even in this case, filters were checked to perform counts of items and collected particles were verified by μFT-IR as reported for water samples. PMP calculation was determined using Eq. (2):

$$PMP = p_k / pT$$

where p_k is the MP particle numbers detected in sponge tissues and pT is the total MP particle numbers in 500 ng mL^{-1} (equivalent to 78.4 ± 2.07 PE-MPs 3 L^{-1}). Results were expressed as percentage and number of PE particles *per* kg of dry tissue (DW).

2.5. Biological analyses

2.5.1. Filtration rate (FR)

The pumping activity of sponge fragments ($N = 3$ *per* treatment *per* each *Exposure* test) was assessed as the filtration rate (FR). *Dunaliella tertiolecta* algal cells were added to each of the experimental chamber (300 mL NSW) containing a single fragment. The subsequent decrease of *D. tertiolecta* cell concentration was measured during the next 30–90 min by taking water samples (1 mL) at fixed time intervals (30 min) and measuring algal concentration spectrophotometrically at 670 nm (Abs670). Algal concentration (Cells mL^{-1}) was calculated from absorbance using the following Eq. (3):

$$\text{Cells } mL^{-1} = \text{Abs } 670 / 3 \times 10^{-7}$$

This kind of assay is under certification control (ISO 17025), assuring the quality of reported data and the scientific rationale. The FR (mL min^{-1}) was determined from the exponential decrease in algal concentration as a function of time using Eq. (4) (Thomassen and Riisgård, 1995):

$$FR = V / t \times \ln(C0 / Ct) = V \times b$$

where V is the volume of water in the experimental chamber in mL, and b is the slope of the regression line in a semi-ln plot for the reduction in algal concentration (C, cells mL^{-1}) from time 0 (C0) to time t (Ct) in min.

2.5.2. Respiration rate (RR)

Respiration rate (RR) analysis was based on the McGrath et al. (2017) method, with some modifications. Each individual sponge fragment (N = 3 *per* treatment *per* each *Exposure* test) was placed in 1 L watertight chamber. To minimize the oxygen consumption due to photosynthesis by symbionts, the chamber was blackened by aluminium foil. At the beginning and at the end of the experiment (120 min), water samples were collected with a 35 mL syringe through a fitted rubber stopper to prevent oxygen exchange, measuring sponge oxygen consumption. The measure was performed using The Oxytherm + R system (Hansatech Instruments Ltd) equipped by S1 Clark-type polarographic oxygen electrode. The oxygen electrode was calibrated prior to each oxygen measurement to 0% oxygen saturation (water containing 1 g of sodium dithionite [$Na_2S_2O_4$]) and 100% oxygen saturation (water bubbled for 10 min) in 100 mL of seawater. The RR (nM min^{-1} g DW^{-1}) was calculated based on the oxygen consumption (nM min^{-1}) at the end of the analysis and the DW (g) of sponge tissue.

2.6. Statistical analysis

Data of filtration (FR) and respiration (RR) rates were checked for normal distributions (Kolmogorov-Smirnov tests) passing normality test ($\alpha = 0.05$). Thus, effects of PE-MPs on both FR and RR were tested separately at different time of exposure tests for each time of detection by means of *t*-test (GraphPad Prism version 6.00 for Windows, GraphPad Software, La Jolla California USA, http://www.graphpad.com).

3. Results

3.1. Chemical analyses

3.1.1. Characterization of PE microparticles suspension

As a consequence of the low MP concentration (500 ng mL^{-1}) used for *Exposure 1* and *2* tests, the size distribution profile of particle suspension in seawater media was detected by DLS of more concentrated suspensions (26 μg mL^{-1}), by determining the mean hydrodynamic diameter (nm) and polydispersity index (PDI) of PE particles (Table 1).

3.1.2. Potential microplastic (PMP) concentrations

After 24h, similar numbers of PE-MPs in water samples were found between positive control (B 24h) and exposure condition (PE 24h) (Table 2). By contrast, in the *Exposure 2* test, a higher number of MPs was counted in the PE treatment (PE 72h) compared with the respective positive control (B 72h). PE-MPs were also found in sponge tissues at the end of the 72h-long period of depuration (*Exposure 3* test) (R 72h). No MPs were detected in all negative controls, indicating no contamination of samples.

3.2. Biological analyses

3.2.1. Filtration rate (FR)

The filtration rate (FR) of sponge fragments was not affected by PE-MPs after 24h (Fig. 1A). By contrast, after 72h exposure, sponges exposed to MPs had a lower FR (Fig. 1B). Likewise, in the 72h-long recovery test, fragments exposed to MPs and then allowed to depurate exhibited lower FR than controls (Fig. 1C) (Table 3). In both tests after 72h, FR in control fragments tended to decrease through time, likely as a result of the progressive depletion of algal cells in the medium. By contrast, FR in fragments exposed to MPs were lower throughout the duration of the test.

3.2.2. Respiration rate (RR)

There was a significant decrease of respiration rate (RR) in fragments exposed to PE-MPs after 72h, while there were no differences between treatments after 24h (Fig. 2). After 72h, a reduction of RR was also evident in depurated fragments in comparison to controls (Fig. 2) (Table 3).

4. Discussion

This study provides evidence of PE-MP uptake by sponges, as well as of the elicitation of negative biological responses. The uptake of MPs by organisms may depend on particles stability in water media, since it determines the aggregation behaviour of particles (Singh et al., 2019; Issac and Kandasubramanian, 2021). Polyethylene and polypropylene,

Table 1
Intensity size distribution, average hydrodynamic diameter (Mean D) and polydispersity index (PDI) of PE-MP suspension at 26 μg mL^{-1} by DLS. **D n%**: Diameter (nm) at 10%, 50% and 90% of the intensity size distribution from CONTIN analysis; **Mean D:** Mean diameter (from cumulants analysis) **PDI**: polydispersity index (from cumulants analysis). Values are expressed as mean ± standard deviation (SD).

Sample	D 10%	D 50%	D 90%	Mean D	PDI
PE	134.13 ± 59.77	3733.97 ± 8499.23	4925.72 ± 1683.36	2513.96 ± 527.88	0.91 ± 0.16

Table 2
Number (#) and percentage (%) of recovered MPs with respect to the initial exposure concentration (500 ng mL^{-1} corresponding to 26.1 particles L^{-1}) identified *per* L of water as well as *per* kg of individual dry sponge' tissue and respective positive (B) and negative (CTRL) controls following 24h (B 24h; CTRL 24h and PE 24h), 72h (B 72h; CTRL 72h and PE 72h) exposures and 72h of depuration (CTRL 72 hR and R72h). **CTRL:** negative control; **PE:** sponge fragments exposed to PE-MPs; **R:** sponge fragments depurated after PE-MP exposure (recovery time).

Sample	#PE L^{-1}	%	#PE kg^{-1}	%
CTRL 24h	0	0	0	0
B 24h	22.0	84.2	–	–
PE 24h	23.0	88.0	2.7	10.2
CTRL 72h	0	0	0	0
B 72h	54.3	100	–	–
PE 72h	29.7	100	8.0	30.6
CTRL 72 hR	0	0	0	0
R 72h	1.7	6.4	4	15.3

due to their low density, are more stable in water columns in comparison to higher density MPs, which have the tendency to sink (Issac and Kandasubramanian, 2021). Accordingly, a mean of 2500 nm PE aggregates was detected in water columns through DLS analysis, confirming their aggregation kinetics and stability. These characteristics may have fostered PE-MP bioavailability for *P. ficiformis* specimens, explaining the high number of particles counted in the tissues of exposed sponge.

Some studies have documented that MP uptake in sponge may depend on their selectivity in particles ingestion (Fallon and Freeman, 2021; Girard et al., 2021). These authors suggested that non-spiculate sponges tend to incorporate larger (>50 μm) particles into their skeletons, whereas spiculate sponges generally incorporate smaller (<50 μm) particles into their ectosome (Girard et al., 2021). Although we did not perform histological analyses, preventing us to determine the location of PE-MPs within sponge tissues, it is possible that *P. ficiformis* species may incorporate them into their ectosome as suggested by Girard et al. (2021). Indeed, phagocytosis of larger particles (>50 μm) by pseudopodia has been shown in many demosponges (Leys and Eerkes-Medrano, 2006). This hypothesis may justify not only the 30.6% of MPs in sponge tissues after a 72h exposure, but also the 15.3% still present after the 72h of depuration, supporting the idea that sponges have some capacity to retain MPs. In addition, the accumulation of MPs increased with time of exposure, with a percentage of PE particles 3 times higher in tissue after 72h exposure, compared to the 24h exposure. Similarly, Cole et al. (2020) found that MP abundance in mussels' tissue were dependent by the exposure time, with 5.3 ± 2.4 PS-MPs mg^{-1} (polystyrene-MPs) counted after 24h and 34.0 ± 15. mg^{-1} after 7d.

Upon ingestion, MPs are either retained in the organism, accumulated and translocated into the tissue or egested (Prinz and Korez, 2020). The potential for sponges to egest MPs has been also documented. The calcareous sponge *Sycon coactum* egested microbeads (up to 1.0 mm) by choanocytes action, engulfing and carrying them into excurrent chambers (Leys and Eerkes-Medrano, 2006). *P. ficiformis* was able to expel some of the experimental PE-MPs, as demonstrated by their presence in the water from containers where sponges were left depurating for 72h. Assessing what is the ability of MP egestion over longer periods of time will be crucial for a more comprehensive understanding of the effects of PE-MPs on this sponge. Nonetheless, our study brings compelling evidence that PE-MPs egestion does not occur over a short period of time, likely also as a consequence of particles reducing sponge pumping activity.

Particle size, shape, concentration, composition, weathering and erosional status as well as exposure and retention times, are assumed to influence the impact of MPs on marine organisms (Cole et al., 2011), but the role of most of these aspects in generating harmful effects remains to be investigated. Although pristine MPs are not chemically harmful to aquatic organisms, their ingestion can cause mechanical damage to filter

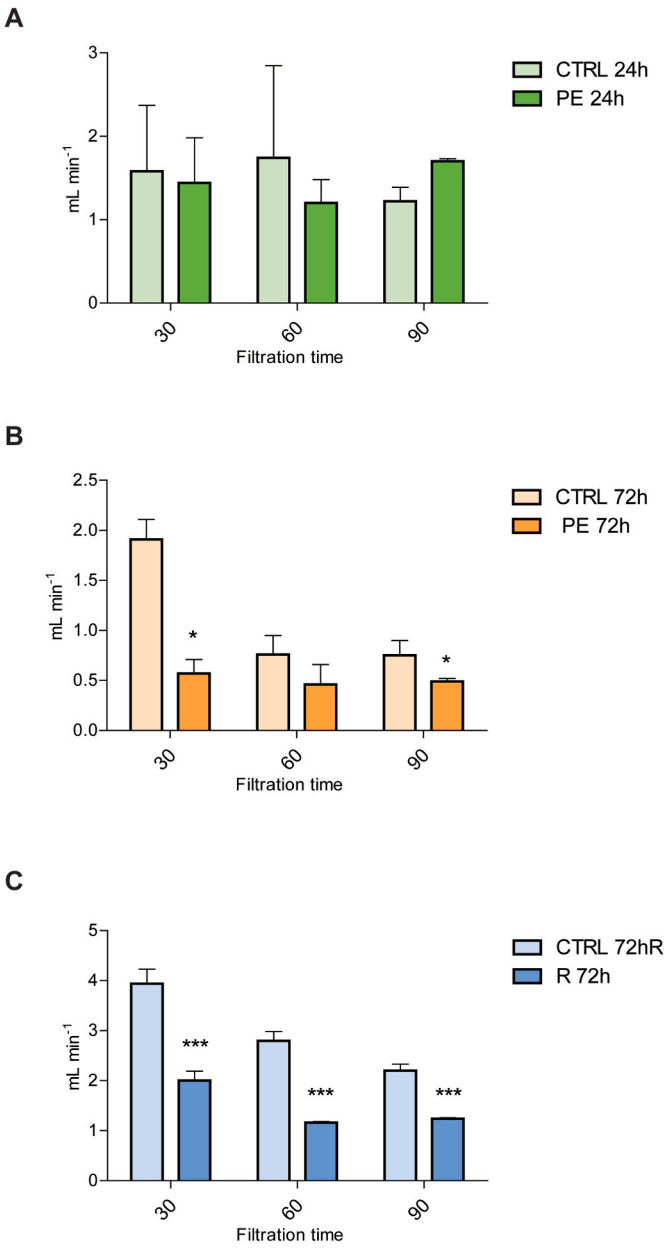

Fig. 1. A. Results (mean ± standard deviation (SD)) of filtration rate (FR) performed at different time of detection (30, 60 and 90 min) for: **A.** *Exposure 1 test* (negative control (CTRL 24h), PE-MP exposure (PE 24h)); **B**: *Exposure 2 test* (negative control (CTRL 72h), PE-MP exposure (PE 72h)); **C.** *Exposure 3 test* (negative control (CTRL 72 hR), recovery time after 72h PE-MP exposure (R 72h). $p < 0.05$ (*); $p < 0.01$ (**); $p < 0.001$ (***).

feeders (Prinz and Korez, 2020). MPs can cause obstruction of feeding organs (Cole et al., 2013), reduction of feeding capacity (Xu et al., 2017), reduction in growth rate and reproductive ability (Sussarellu et al., 2015; Barboza et al., 2018). By exposing sponges to pristine PE-MPs, produced with defined properties for laboratory applications, our results suggest a mechanical alteration of the functionality of tissues. For example, O'Donovan et al. (2018) found that PE-MP ingestion could alter biochemical responses (oxidative stress related biomarkers) in the clam *Scrobicularia plana* through the mechanical damage of gills. Similar mechanisms may explain PE-MP impairment of sponge physiology.

Large MP sizes have been proposed to cause abrasion to sponge assemblages, while small particles (<50 μm) are more likely to clog filtration systems of these organisms (Baird, 2016). Our results showed a time-dependent decrease of filtration rate (FR) in exposed sponges,

Table 3
t-test output for filtration (FR) and respiration (RR) rates performed for all treatments, separately for each time of exposure time and time of detection. Source of variations; df; t; *p* value. **CTRL**: negative control; **PE**: sponge fragments exposed to PE-MPs; **R**: sponge fragments depurated after PE-MP exposure (recovery time). **24h-72h**: time of exposure tests; **72 hR**: time of recovery test.

Variable	Time exposure	Factor	Detection time	df	t	*p* value
FR	24h	CTRL *vs* PE	30 min	2	0.26	0.81
			60 min	2	0.82	0.46
			90 min	2	1.69	0.31
	72h	CTRL *vs* PE	30 min	2	3.71	0.02
			60 min	2	1.83	0.14
			90 min	2	2.97	0.04
	72 hR	CTRL *vs* R	30 min	2	9.64	0.0006
			60 min	2	15.76	<0.0001
			90 min	2	12.79	0.0002
RR	24h	CTRL *vs* PE	120 min	2	0.18	0.86
	72h	CTRL *vs* PE	120 min	2	84.00	<0.0001
	72 hR	CTRL *vs* R	120 min	2	189.00	<0.0001

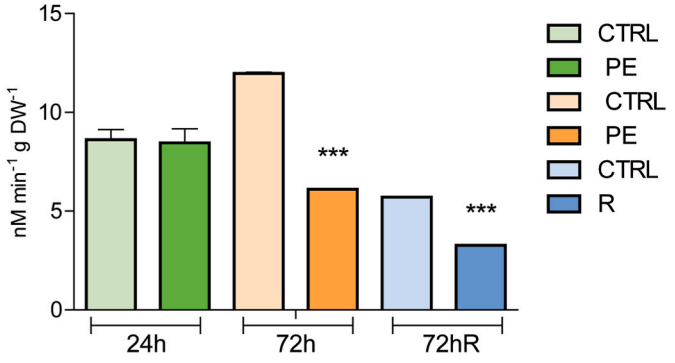

Fig. 2. Results (mean ± standard deviation (SD)) of respiration rate (RR) performed for all treatments at different time of exposure tests. $p < 0.05$ (*); $p < 0.01$ (**); $p < 0.001$ (***). **CTRL**: negative control; **PE**: sponge' fragments exposed to PE-MP; **R**: sponge fragments depurated after PE-MP exposure (recovery time). **24h-72h**: time of exposure tests; **72 hR**: time of recovery test.

demonstrating that MPs may impact the pumping capacity on filter-feeders. Like us, Girard et al. (2021) also documented a decrease in pumping activity of different sponge species following the exposure to MPs. In our study, FR remained lower in depurated *P. ficiformis* fragments, suggesting that prolonged residence time of MPs may lead to more severe effects on organisms (Wright et al., 2013). Active mechanisms of sponges, including expulsion of particles from the aquiferous system and pumping cessation, involve additional energy expenditure and may not be sustainable in the long term (Strehlow et al., 2017). At high contaminant levels, all available energy and metabolic capacity is devoted to sustain the survival of individuals (Sokolova et al., 2012). Respiration responds directly to metabolic needs and the oxygen consumption is one of the main proxies for determining changes in metabolic rates. Most toxicants studied have been found to reduce the metabolic rate and, thus, the respiration of many organisms (Weis, 2014). Accordingly, our results showed that negative effects of MPs on respiration responses (RR) were time-dependent. A reduction in the RR was also detected in depurated sponges, suggesting that decreased food uptake could have impaired sponge metabolic capacity. Indeed, reduced food uptake was also discussed as a possible cause of metabolic impairment by Rist et al. (2016). These authors observed that mussel clearance and respiration rates decreased after 40–44 days of exposure to different suspended polyvinylchloride (PVC) MPs (1–50 μm), supporting the hypothesis that reduced feeding rates, due to a MP-induced decrease in FR, could slow down metabolism.

Our results show that sponges are able to incorporate foreign particles and thus may be a potential bioindicator for MP pollutants. In addition, sponges play key functional roles in both temperate and tropical environments, including primary production, nitrification processes, carbonate cycling, extraction of particles from the water column and antifouling through production of secondary metabolites (Diaz and Rützler, 2001; Bell, 2008; De Marchi et al., 2021). Under these circumstances, reduced sponge diversity and abundance, or functionality, are likely to influence the functioning of entire marine communities (Powell et al., 2010).

5. Conclusions

This study demonstrates that the uptake of PE-MPs from the water column alters filtration and respiration rates of *P. ficiformis*. Ability to expel some of the ingested MPs suggests that this sponge may be somewhat resistant to PE-MP pollution. Nonetheless, 72h of depuration were not sufficient for the sponge to recover. Sponges play key function roles in temperate and tropical environments and assessing their response to MP pollution appears paramount for sustaining marine biodiversity. In particular, further work is necessary to assess MP content in sponge populations under natural settings and whether MPs affect sponges directly, through physical effects (e.g., clogging, use of resources for the isolations of particles) or, indirectly, by favouring the uptake of contaminants.

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.

Acknowledgment

Lucia De Marchi and Fabio Bulleri benefited from a grant from the European Funds for Regional Development through a convention with Comune of Portoferraio within the framework of the Interreg Marittimo-IT FR-Maritime 2014/2020 Project "QUALIPORTI" (F99C18000050006). While writing the manuscript Lucia De Marchi (CDL-CTTRI-17-ARH/2021) benefited from an assistant researchers' contract in the scope of BISPECIAl project (POCI-01-0145-FEDER-028425). We acknowledge also the financial support to CESAM by FCT/MCTES (UIDP/50017/2020+UIDB/50017/2020+ LA/P/0094/2020), through national funds. Alessia Cuccaro benefited from a PhD grant (PD/BD/150609/2020) given by the National Funds through the Portuguese Science Foundation (Fundação para a Ciência e a Tecnologia, FCT).

Referneces

Avio, C.G., Gorbi, S., Milan, M., Benedetti, M., Fattorini, D., d'Errico, G., Pauletto, M., Bargelloni, L., Regoli, F., 2015. Pollutants bioavailability and toxicological risk from microplastics to marine mussels. Environ. Pollut. 198 (12), 211–222. https://doi.org/10.1016/j.envpol.2014.12.021.

Baird, C.A., 2016. Measuring the Effects of Microplastics on Sponges.

Bajt, O., 2021. From plastics to microplastics and organisms. FEBS Open Bio. 11 (4), 954–966. https://doi.org/10.1002/2211-5463.13120.

Barboza, L.G.A., Vieira, L.R., Branco, V., Figueiredo, N., Carvalho, F., Carvalho, C., Guilhermino, L., 2018. Microplastics cause neurotoxicity, oxidative damage and energy-related changes and interact with the bioaccumulation of mercury in the European seabass, *Dicentrarchus labrax* (Linnaeus, 1758). Aquat. Toxicol. 195, 49–57. https://doi.org/10.1016/j.aquatox.2017.12.008.

Batista, D., Tellini, K., Nudi, A.H., Massone, T.P., Scofield, A.D.L., de Lr Wagener, A., 2013. Marine sponges as bioindicators of oil and combustion derived PAH in coastal waters. Mar. Environ. Res. 92, 234–243. https://doi.org/10.1016/j.marenvres.2013.09.022.

Bell, J.J., 2008. The functional roles of marine sponges. Estuar. Coast Shelf Sci. 79 (3), 341–353. https://doi.org/10.1016/j.ecss.2008.05.002.

Bessa, F., Frias, J., Kögel, T., Lusher, A., Andrade, J.M., Antunes, J., Gerdts, G., 2019. Harmonized protocol for monitoring microplastics in biota. Deliverable 4, 3.
Besseling, B., Wegner, A., Foekema, E.M., Heuvel-Greve, M.J., Koelmans, A.A., 2013. Effects of microplastic on fitness and PCB bioaccumulation by the lugworm *Arenicola marina* (L.). Environ. Sci. Technol. 47 (1), 593–600. https://doi.org/10.1021/es302763x.
Besseling, E., Redondo-Hasselerharm, P., Foekema, E.M., Koelmans, A.A., 2019. Quantifying ecological risks of aquatic micro-and nanoplastic. Crit. Rev. Environ. Sci. Technol. 49 (1), 32–80. https://doi.org/10.1080/10643389.2018.1531688.
Borrelle, S.B., Ringma, J., Law, K.L., Monnahan, C.C., Lebreton, L., McGivern, A., Murphy, E., Jambeck, J., Leonard, G.H., Hilleary, M.A., Eriksen, M., Possingham, H. P., De Frond, H., Gerber, L.R., Polidoro, B., Tahir, A., Bernard, M., Mallos, N., Barnes, M., Rochman, C.M., 2020. Predicted growth in plastic waste exceeds efforts to mitigate plastic pollution. Science 369 (6510), 1515–1518. https://doi.org/10.1126/science.aba3656.
Bowley, J., Baker-Austin, C., Porter, A., Hartnell, R., Lewis, C., 2021. Oceanic hitchhikers–assessing pathogen risks from marine microplastic. Trends Microbiol. 29 (2), 107–116. https://doi.org/10.1016/j.tim.2020.06.011.
Cebrian, E., Uriz, M.J., Turon, X., 2007. Sponges as biomonitors of heavy metals in spatial and temporal surveys in northwestern mediterranean: multispecies comparison. Environ. Toxicol. Chem. 26 (11), 2430–2439. https://doi.org/10.1897/07-292.1.
Chapron, L., Peru, E., Engler, A., Ghiglione, J.F., Meistertzheim, A.L., Pruski, A.M., Purser, A., Vétion, G., Galand, P.E., Lartaud, F., 2018. Macro- and microplastics affect cold-water corals growth, feeding and behaviour. Sci. Rep. 8 (1), 1–8. https://doi.org/10.1038/s41598-018-33683-6.
Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2015. The impact of polystyrene microplastics on feeding, function and fecundity in the marine copepod *Calanus helgolandicus*. Environ. Sci. Technol. 49 (2), 1130–1137. https://doi.org/10.1021/es504525u.
Cole, M., Lindeque, P., Fileman, E., Halsband, C., Goodhead, R., Moger, J., Galloway, T. S., 2013. Microplastic ingestion by zooplankton. Environ. Sci. Technol. 47, 6646–6655. https://doi.org/10.1021/es400663f.
Cole, M., Liddle, C., Consolandi, G., Drago, C., Hird, C., Lindeque, P.K., Galloway, T.S., 2020. Microplastics, microfibres and nanoplastics cause variable sub-lethal responses in mussels (*Mytilus spp.*). Mar. Pollut. Bull. 160, 111552. https://doi.org/10.1016/j.envpol.2020.115851.
Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: a review. Mar. Pollut. Bull. 62 (12), 2588–2597. https://doi.org/10.1016/j.marpolbul.2011.09.025.
De Marchi, L., Pretti, C., Cuccaro, A., Oliva, M., Tardelli, F., Monni, G., Magri, M., Bulleri, F., 2021. A multi-bioassay integrated approach to assess antifouling potential of extracts from the Mediterranean sponge *Ircinia oros*. Environ. Sci. Pollut. Control Ser. 1–11. https://doi.org/10.1007/s11356-021-15683-8.
Diaz, M.C., Rützler, K., 2001. Sponges: an essential component of Caribbean coral reefs. Bull. Mar. Sci. 69 (2), 535–546.
Efremova, S.M., Margulis, B.A., Guzhova, I.V., Itskovich, V.B., Lauenroth, S., Müller, W. E., Schröder, H.C., 2002. Heat shock protein Hsp70 expression and DNA damage in Baikalian sponges exposed to model pollutants and wastewater from Baikalsk Pulp and Paper Plant. Aquat. Toxicol. 57, 267–280. https://doi.org/10.1016/s0166-445x(01)00209-0.
Enders, K., Lenz, R., Javar do Sul, J.A., Tagg, A.S., Labrenz, M., 2020. When every particle matter: a QuEChERS approach to extract microplastics from environmental samples. MethodsX 7, 100784. https://doi.org/10.1016/j.mex.2020.100784.
Erni-Cassola, G., Zadjelovic, V., Gibson, M.I., Christie-Oleza, J.A., 2019. Distribution of plastic polymer types in the marine environment; a meta-analysis. J. Hazard Mater. 369, 691–698. https://doi.org/10.1016/j.jhazmat.2019.02.067.
Fallon, B.R., Freeman, C.J., 2021. Plastics in Porifera: the occurrence of potential microplastics in marine sponges and seawater from Bocas del Toro, Panamá. PeerJ 9, e11638. https://doi.org/10.7717/peerj.11638.
Foley, C.J., Feiner, Z.S., Malinich, T.D., Höök, T.O., 2018. A meta-analysis of the effects of exposure to microplastics on fish and aquatic invertebrates. Sci. Total Environ. 631, 550–559. https://doi.org/10.1016/j.scitotenv.2018.03.046.
Gentric, C., Rehel, K., Dufour, A., Sauleau, P., 2016. Bioaccumulation of metallic trace elements and organic pollutants in marine sponges from the South Brittany Coast, France. J. Environ. Sci. Health 51 (3), 213–219. https://doi.org/10.1080/10934529.2015.1094327.
Geyer, R., Jambeck, J.R., Law, K.L., 2017. Production, use, and fate of all plastics ever made. Sci. Adv. 3 (7), e1700782 https://doi.org/10.1126/sciadv.1700782.
Girard, E.B., Fuchs, A., Kaliwoda, M., Lasut, M., Ploetz, E., Schmahl, W.W., Wörheide, G., 2021. Sponges as bioindicators for microparticulate pollutants? Environ. Pollut. 268, 115851. https://doi.org/10.1016/j.envpol.2020.115851.
Hankins, C., Duffy, A., Drisco, K., 2018. Scleractinian coral microplastic ingestion: potential calcification effects, size limits, and retention. Mar. Pollut. Bull. 135 (3), 587–593. https://doi.org/10.1016/j.marpolbul.2018.07.067.
Issac, M.N., Kandasubramanian, B., 2021. Effect of microplastics in water and aquatic systems. Environ. Sci. Pollut. Control Ser. 1–19. https://doi.org/10.1007/s11356-021-13184-2.
Le Guen, C., Suaria, G., Sherley, R.B., Ryan, P.G., Aliani, S., Boehme, L., Brierley, A.S., 2020. Microplastic study reveals the presence of natural and synthetic fibres in the diet of King Penguins (*Aptenodytes patagonicus*) foraging from South Georgia. Environ. Int. 134, 105303. https://doi.org/10.1016/j.envint.2019.105303.
Leys, S.P., Eerkes-Medrano, D.I., 2006. Feeding in a calcareous sponge: particle uptake by pseudopodia. Biol. Bull. 211 (2), 157–171. https://doi.org/10.2307/4134590.
Marques, D., Almeida, M., Xavier, J., Humanes, M., 2007. Biomarkers in Marine Sponges: Acetylcholinesterase in the Sponge *Cliona Celata*. Porifera Research: Biodiversity, Innovation and Sustainability. Museu Nacional Rio de Janeiro, Rio de Janeiro, pp. 427–432.
McGrath, E.C., Smith, D.J., Jompa, J., Bell, J.J., 2017. Adaptive mechanisms and physiological effects of suspended and settled sediment on barrel sponges. J. Exp. Mar. Biol. Ecol. 496, 74–83. https://doi.org/10.1016/j.jembe.2017.07.013.
O'Donovan, S., Mestre, N.C., Abel, S., Fonseca, T.G., Carteny, C.C., Cormier, B., Keiter, S. H., Bebianno, M.J., 2018. Ecotoxicological effects of chemical contaminants adsorbed to microplastics in the clam *Scrobicularia plana*. Front. Mar. Sci. 5, 143. https://doi.org/10.3389/fmars.2018.00143.
Pabortsava, K., Lampitt, R.S., 2020. High concentrations of plastic hidden beneath the surface of the Atlantic Ocean. Nat. Commun. 11 (1), 1–11. https://doi.org/10.1038/s41467-020-17932-9.
Perez, T., Sarrazin, L., Rebouillon, P., Vacelet, J., 2002. First evidences of surfactant biodegradation by marine sponges (Porifera): an experimental study with a linear alkylbenzenesulfonate. Hydrobiologia 489, 225–233. https://doi.org/10.1023/A:1023217218585.
Perez, T., Wafo, E., Fourt, M., Vacelet, J., 2003. Marine sponges as biomonitor of polychlorobiphenyl contamination: concentration and fate of 24 congeners. Environ. Sci. Technol. 37, 2152–2158. https://doi.org/10.1021/es026234v.
Piccardo, M., Provenza, F., Grazioli, E., Cavallo, A., Terlizzi, A., Renzi, M., 2020. PET microplastics toxicity on marine key species is influenced by pH, particle size and food variations. Sci. Total Environ. 715, 136947. https://doi.org/10.1016/j.scitotenv.2020.136947.
Powell, A.L., Hepburn, L.J., Smith, D.J., Bell, J.J., 2010. Patterns of sponge abundance across a gradient of habitat quality in the Wakatobi Marine National Park, Indonesia. Open Mar. Biol. J. 4 (1), 31–38. https://doi.org/10.2174/1874450801004010031.
Prinz, N., Korez, Š., 2020. Understanding How Microplastics Affect Marine Biota on the Cellular Level Is Important for Assessing Ecosystem Function: a Review, pp. 101–120. https://doi.org/10.1007/978-3-030-20389-4. YOUMARES 9-The Oceans: Our Research, Our Future.
Procter, J., Hopkins, F.E., Fileman, E.S., Lindeque, P.K., 2019. Smells good enough to eat: dimethyl sulfide (DMS) enhances copepod ingestion of microplastics. Mar. Pollut. Bull. 138, 1–6. https://doi.org/10.1016/j.marpolbul.2018.11.014.
Rao, J.V., Kavitha, P., Reddy, N.C., Rao, T.G., 2006. *Petrosia testudinaria* as a biomarker for metal contamination at Gulf of Mannar, southeast coast of India. Chemosphere 65 (4), 634–638. https://doi.org/10.1016/j.chemosphere.2006.01.072.
Reichert, J., Schellenberg, J., Schubert, P., Wilke, T., 2018. Responses of reef building corals to microplastic exposure. Environ. Pollut. 237, 955–960. https://doi.org/10.1016/j.envpol.2017.11.006.
Rist, S.E., Assidqi, K., Zamani, N.P., Appel, D., Perschke, M., Huhn, M., Lenz, M., 2016. Suspended micro-sized PVC particles impair the performance and decrease survival in the Asian green mussel *Perna viridis*. Mar. Pollut. Bull. 111 (1–2), 213–220. https://doi.org/10.1016/j.marpolbul.2016.07.006.
Rotjan, R.D., Sharp, K.H., Gauthier, A.E., Yelton, R., Lopez, E.M.B., Carilli, J., Kagan, J. C., Urban-Rich, J., 2019. Patterns, dynamics and consequences of microplastic ingestion by the temperate coral, *Astrangia poculata*. Proc. Royal Soc. 286 (1905), 20190726. https://doi.org/10.1098/rspb.2019.0726.
Savoca, M.S., Wohlfeil, M.E., Ebeler, S.E., Nevitt, G.A., 2016. Marine plastic debris emits a keystone infochemical for olfactory foraging seabirds. Sci. Adv. 2 (11), e1600395 https://doi.org/10.1126/sciadv.1600395.
Singh, N., Tiwari, E., Khandelwal, N., Darbha, G.K., 2019. Understanding the stability of nanoplastics in aqueous environments: effect of ionic strength, temperature, dissolved organic matter, clay, and heavy metals. Environ. Sci.: Nano 6 (10), 2968–2976. https://doi.org/10.1039/c9en00557a.
Strehlow, B.W., Pineda, M.C., Duckworth, A., Kendrick, G.A., Renton, M., Wahab, M.A. A., Clode, P.L., 2017. Sediment tolerance mechanisms identified in sponges using advanced imaging techniques. PeerJ 5, e3904. https://doi.org/10.7717/peerj.3904.
Sussarellu, R., Suquet, M., Thomas, Y., Lambert, C., Fabioux, C., Pernet, M.E.J., Goïc, N. L., Quillien, V., Mingant, C., Epelboin, Y., Corporeau, C., Guyomarch, J., Robbens, J., Paul-Pont, I., Soudant, P., Huvet, A., 2015. Oyster reproduction is affected by exposure to polystyrene microplastics. Proc. Natl. Acad. Sci. U. S. A. 113 (9), 2430–2435. https://doi.org/10.1073/pnas.1519019113.
Tang, J., Ni, X., Zhou, Z., Wang, L., Lin, S., 2018. Acute microplastic exposure raises stress response and suppresses detoxification and immune capacities in the scleractinian coral *Pocillopora damicornis*. Environ. Pollut. 243, 66–74. https://doi.org/10.1016/j.envpol.2018.08.045.
Thomassen, S., Riisgård, H.U., 1995. Growth and energetics of the sponge *Halichondria panicea*. Mar. Ecol. Prog. Ser. 128, 239–246. https://doi.org/10.3354/meps128239.
UNEP, 2016. Marine Plastic Debris and Microplastics. Global Lessons and Research to Inspire Action and Guide Policy Change. United Nations Environment Programme, Nairobi.
Weis, J.S., 2014. Physiological, Developmental and Behavioral Effects of Marine Pollution, ISBN 978-94-007-6948-9.
Wright, L., Rowe, D., Thompson, R.C., Galloway, T.S., 2013. Microplastic ingestion decreases energy reserves in marine worms. Curr. Biol. 23 (23), R1031–R1033. https://doi.org/10.1016/j.cub.2013.10.068.
Xu, X.Y., Lee, W.T., Chan, A.K.Y., Lo, H.S., Shin, P.K.S., Cheung, S.G., 2017. Microplastic ingestion reduces energy intake in the clam *Atactodea striata*. Mar. Pollut. Bull. 124 (2), 798–802. https://doi.org/10.1016/j.marpolbul.2016.12.027.
Xu, X., Wong, C.Y., Tam, N.F., Lo, H.S., Cheung, S.G., 2020. Microplastics in invertebrates on soft shores in Hong Kong: influence of habitat, taxa and feeding

mode. Sci. Total Environ. 715, 136999. https://doi.org/10.1016/j.scitotenv.2020.136999.
Zantis, L., Carroll, E.L., Nelms, S.E., Bosker, T., 2020. Marine mammals and microplastics: a systematic review and call for standardisation. Environ. Pollut. 116142. https://doi.org/10.1016/j.envpol.2020.116142.
Ziajahromi, S., Kumar, A., Neale, P.A., Leusch, F.D., 2018. Environmentally relevant concentrations of polyethylene microplastics negatively impact the survival, growth and emergence of sediment-dwelling invertebrates. Environ. Pollut. 236, 425–431. https://doi.org/10.1016/j.envpol.2018.01.094.