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RESEARCH ARTICLE

Effects of environmentally relevant levels of polyethylene microplastic on *Mytilus galloprovincialis* (Mollusca: Bivalvia): filtration rate and oxidative stress

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Abstract

The objective of the present study was to evaluate the potential toxic effects of polyethylene microplastics (PE-MPs) (40–48 μm) on the Mediterranean mussel *Mytilus galloprovincialis* in controlled laboratory conditions. The exposure was carried out for 14 days with three environmentally relevant PE-MPs concentrations of 1, 10, and 100 and a high concentration of 1000 μg/L. Effects of PE-MPs were assessed by evaluating the filtration rate (FR) after 7 and 14 days of exposure and by analyzing biochemical biomarkers of oxidative stress (catalase - CAT, glutathione S-transferase - GST, and the levels of lipid peroxidation - LPO) in the *M. galloprovincialis* digestive gland after 14 days of exposure. Results showed that *M. galloprovincialis* does not accumulate PE-MPs of 40–48 μm size in its whole tissues. The filtration rate was significantly reduced with the increase of PE-MPs concentrations. The biochemical biomarkers indicated that PE-MPs induced oxidative damage (LPO) at low concentrations (1 and 10 μg/L) with a significant reduction in females of 1000 μg/L treated group and inactivate antioxidative system (CAT and GST) in the digestive gland of both sexes at high concentrations (100 and 1000 μg/L). This study demonstrates that PE-MPs have biological effects on *M. galloprovincialis* at environmentally relevant concentrations thus brings new insights on the potential impacts of PE-MPs in marine bivalves.

Keywords Microplastics · Polyethylene · *Mytilus galloprovincialis* · Filtration rate · Biomarkers

Introduction

The global production of plastics has increased exponentially, reaching almost 359 million tons in 2018 (PlasticsEurope 2019). It has been estimated that by 2050, demand for plastic will exceed three times the current levels (WEF 2016). Recently, plastic waste has become a global challenge because it is uploaded in the aquatic environments (Cole et al. 2011; Wright et al. 2013; Abidli et al. 2017, 2018, 2019; Allen et al. 2019; Toumi et al. 2019) through many pathways such as wastewater treatment plants and atmospheric transport. Plastic materials enter the environment directly as primary (manufactured) materials (medical applications, electronics, coatings, adhesives), or as secondary materials following the degradation of larger plastic debris (Duis and Coors 2016).

The effects of this pollution on ecosystems and biota are becoming a growing concern, especially in the aquatic ecosystems (Ivar do Sul and Costa 2014). In fact, most plastic waste is not absolutely biodegradable and sometimes takes hundreds and even thousands of years to degrade (Barnes et al. 2009; Eriksen et al. 2013). Based on size, small plastics are divided into microplastics (MPs) (100 nm to 5 mm) and nanoplastics (<100 nm) (Ng et al. 2018). MPs have received significant attention as an emerging contaminant of concern in the world (Eriksen et al. 2013).

The ecological impact of plastics can be physical (entanglement and injuries of animals (Allsopp et al. 2006; Cole et al. 2011)), chemical (MPs act as reservoirs of toxic

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chemicals in the environment (Cole et al. 2011)), and biological (colonization of the MPs surface by microorganisms and geographic transfer (Oberbeckmann et al. 2015)).

The ingestion of MPs by marine organisms via various pathways such as filtration, trophic transfer, and confusion with prey (Güven et al. 2017; Nelms et al. 2019; Rist et al. 2019) represents a real danger that demands greater understanding. Wright et al. (2013) showed that MPs are widely available to the entire marine food web because they are similar in size to many organisms in the benthos and plankton communities.

There are different types of plastic polymers and the most commons are polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinylchloride (PVC), polyamide (PA), polyethylene terephthalate (PET), and polyvinyl alcohol (PVA) (Avio et al. 2017). PE and PP are more widely produced and discarded (Andrady 2011, 2015) which led to their high concentrations in marine environments (Abidli et al. 2018, 2019; James et al. 2020). Erni-Cassola et al. (2019) reported that these two polymer types dominate sea surface samples (42% PE and 25% PP) because of their lower density and their abundance decrease with depth (2% and 3% in the deep sea, respectively).

Current studies show that MPs can cause adverse health effects, including weight loss, growth rate, and food consumption decreases, immune response increase (Lusher et al. 2017; Rochman et al. 2015), and respiration and reproduction impacts (Avio et al. 2015a, 2015b; Cole et al. 2015).

Mussels are one of the most suitable indicator organisms, which are widely used in ecotoxicological studies (Castro et al. 2004; Orescanin et al. 2006; Li et al. 2019). They are sedentary filter–feeding organisms that have a large capacity to accumulate pollutants in their tissues, providing a strong and specific response to pollutants (Livingstone et al. 2000; Catsiki and Florou 2006). They have ecological importance (a key player in the food web) and socio-economic value (cultivated and consumed as seafood worldwide) (Beyer et al. 2017). Moreover, mussels have been suggested as a bioindictor species for MP pollution monitoring in the marine environment (OSPAR 2015). *Mytilus galloprovincialis* is native to the Mediterranean coast and the Black and Adriatic Seas but has extended its range in many parts of the world (Branch and Steffani 2004). It is largely consumed by the local populations and considered an important marine organism due to its nutritional relevance. It has a high adaptation capacity to the large fluctuations of environmental conditions and can filter huge volume of seawater. It has been used as sentinel species for monitoring marine environment and identified as a species susceptible to MP uptake (Digka et al. 2018; Renzi et al. 2018; Abidli et al. 2019). Therefore, *M. galloprovincialis* was chosen as the model organism in this study.

Most toxicity studies with MPs, conducted on bivalves, used exposure concentrations often very high and not representative of MP concentrations in coastal waters (Revel et al. 2020). Laboratory exposures conducted at environmentally realistic MP concentrations are scarce on bivalves (Revel et al. 2020; Tlili et al. 2020). In fact, Ivar do Sul et al. (2014) showed that MP concentrations below 23 μg/L can be considered representative of areas on the coast and Goldstein et al. (2012) showed that concentrations between 10 and 100 μg/L can be considered representative of polluted sites located off the coast.

The ecotoxicological consequences of pollutants are generally based on the analysis of changes in various physiological and biochemical parameters in aquatic biota (Palanikumar et al. 2012), in addition to ecological endpoints. Among these parameters, the filtration rate was considered an important parameter because it indicates the ability of the bivalves to intake food from the water column, a crucial function for individual fitness (Oliveira et al. 2018; Pinheiro et al. 2019).

In addition, measuring the levels of oxidative stress biomarkers is an informative approach to evaluate general toxicity (Regoli and Giuliani 2014; Faggio et al. 2016). Studies dealing with the induction of oxidative stress by MPs have in most cases included two components: antioxidative defenses (as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione S-transferase (GST), glutathione reductase (GR) enzymes), and oxidative damage (as lipid peroxidation (LPO)) (Prokić et al. 2018).

Thus, to evaluate the effects of MPs on mussels, a multimarker approach was implemented. The filtration rate and biochemical biomarkers of oxidative stress (GST, CAT activities, and the levels of LPO) were measured in the digestive gland tissues of both sexes of the mussel *M. galloprovincialis* after 2 weeks of exposure to Polyethylene MPs (PE-MPs) at environmentally concentrations of 1 μg/L, 10 μg/L, and 100 μg/L and a high concentration of 1000 μg/L.

Materials and methods

Animal sampling and experimental design

M. galloprovincialis adults were collected from the open Sea of Bizerte (Northern Tunisia) at the beginning of November 2019 at a depth of 3–4 m and immediately brought to the laboratory inside an ice chest container. At the laboratory, the shells were cleaned by gently scraping to remove epibionts and biometric measurements were determined. Then, animals were randomly distributed in three glass aquaria filled with filtered seawater (Whatman® GF/C filters ~10 μm pore size) taken from the sampling site and provided with continuous aeration to acclimate for 2 weeks prior to the onset of the experiment. The use of filtered seawater is based on a

preliminary characterization of the MPs in the seawater of the sampling station which showed a value of 5 items/L and sizes varying from 0.5 to 2 mm in length (Abidli, unpublished data). For this reason, the seawater used during the acclimatization period was filtered with 10 μm filters to eliminate MPs from the seawater and allow the passage of micro-algae such as *Phaeodactylum tricornutum*, *Isochrysis galbana*, and *Tetraselmis* sp. which were consumed by mussels. There was no mortality during the acclimation period.

The average size and weight of mussels were 65.36 ± 6.16 mm and 19.62 ± 5.14 g, respectively. On November 20th, following acclimation, a total of 60 adult animals per treatment (20 individuals per replicate) of both sexes were assigned to 15 L glass aquaria filled with 10 L of filtered seawater and provided with continuous aeration through air diffusers, located in the middle of the aquarium. The temperature was maintained at 20 °C in an acclimatized room under natural photoperiod. The seawater in the aquaria was renewed and contaminated with PE-MPs three times a week. The exposure period lasted for 14 days.

In this study, mussels were exposed to ultra-high molecular weight PE-MPs (40–48 μm), the most produced plastic polymer (PlasticsEurope 2019), commercially purchased from Sigma Aldrich, USA. A stock solution of MPs (1 g/L) was prepared by adding 100 mg PE-MP to 100 mL of filtered seawater and mixing for 30 min. The following experimental treatments were established: 1 μg/L, 10 μg/L, 100 μg/L, and 1000 μg/L. Additionally, a control group was run in parallel with no PE-MPs (filtered seawater only). No mortality of mussels was observed throughout the PE-MPs exposures for any of the treatments.

Each of the five treatments was performed in triplicate. To minimize the possible cross-contamination, especially from airborne contaminants such as fibers, all aquaria were covered with glass lids during the experiment.

Microplastic analyses in mussel's tissues

To detect PE-MPs ingestion in mussels following exposure at day 14, the soft tissues of 9 mussels per treatment (3 individuals per replicate) were weighted (wet weight) after removal of the shell and byssus threads. The extraction method and analysis of MPs from mussels were carried out based on the earlier protocol (Li et al. 2018; Abidli et al. 2019). Briefly, the soft tissues were placed into a 1-L bottle. Approximately 100 ml of 10% KOH was added in the bottle to digest the tissues, covered (with tin foil), and incubated at 60 °C in an oven for 24 h. Then, depending on the digestion effect on the tissue, the bottle was left at room temperature for 24–48 h. After complete digestion, 400 ml of filtered solution of NaCl (140 g L^{-1}) (Galgani et al. 2013) was added to each bottle to float the MPs. The overlying water was then filtered, after 24 h of floatation at room temperature, with a Millipore vacuum pump, onto Whatman® GF/C filters (~1 μm pore size) and dried overnight at room temperature in covered glass Petri dishes. The filters were analyzed under a stereomicroscope (ZEISS KL 1500 LCD) with caution.

In order to avoid external contamination, especially airborne contaminants, the equipment used for the preparation and extraction of MPs were rinsed with bidistilled water prior to use and all solutions were filtered. In addition, procedural blanks were performed with the digestion solutions to evaluate the airborne contamination and filters were analyzed under a stereomicroscope. Any type of airborne contamination, in these blanks, was detected.

Filtration rate

The FR rate was performed according to Coughlan (1969) based on the loss of neutral red dye particles from the water column. It was determined in each mussel individually immediately after 7 and 14 days of exposure. Briefly, 9 mussels from each treatment (3 animals (confused sex) per replicate) were sampled from aquaria on days 7 and 14 carefully by cutting the byssus threads and placed in 200-mL beakers (1 mussel per beaker) containing 100 mL of neutral red solution (1 g/L) and left protected from light for 2 h. Just prior to placing the mussels in the solution, an aliquot of water was removed from each beaker to determine the initial concentration, C0 by reading the optical density (OD) at 550 nm. After 2 h, mussels were removed from beakers and the remaining solutions (Ct), along with the initial aliquot (C0), were acidified to pH 5 with HCl. Neutral red concentrations were determined by measuring the absorbance in triplicate at 550 nm using a Thermo Scientific™ Multiskan™ FC Microplate spectrophotometer. Standards of neutral red were measured along with the samples and used to establish a standard curve, from which dye concentrations could be extrapolated.

The FR (mL/h/individual) was calculated as FR = [*M*/*nt*] log (C0/Ct)

where *M* is the volume of the test solution (mL), *n* is the number of mussels used, *t* is the time (hours), and C0 and Ct are the concentrations of the dye at the beginning and after 2 h, respectively.

Oxidative stress biomarkers

Mussels used to study oxidative stress biomarkers were only sampled at the end of the exposure study (day 14). At day 14, 30 mussels per treatment (10 per replicate) were carefully removed from aquaria by cutting the byssus threads. Then, the digestive gland tissues were dissected on ice, weighted, frozen in liquid nitrogen, and subsequently stored at − 80 °C. At the same time, the gonad of each individual was examined under microscope to determine the sex of the mussels. For biochemical analyses, the digestive gland tissues were

homogenized in k-phosphate buffer (100 mM; pH 7.4). Protein concentration in the supernatant of tissue homogenate was determined by the Lowry method (Lowry et al. 1951) and normalized to 1 mg/L. The samples were divided in aliquots for further biochemical studies according to Capela et al. (2016).

CAT activity was determined by measuring the consumption of H_2O_2 at 240 nm during 2 min at 15-s intervals. It was expressed in μmol/min/mg protein and was measured in 96-well UV microplates (Thermo Fisher Scientific) adapted from Aebi (1974) and Ferreira et al. (2007).

GST activity was assessed at 340 nm for 5 min in 20-s intervals according to Habig et al. (1974). It was measured using a reaction mixture of glutathione (GSH) 10 mM in HB (0.1 M, pH 6.5) and 1-chloro-2,4-dinitrobenzene (CDNB) 60 mM in ethanol and was expressed in nmol/min/mg protein.

LPO levels were determined through the quantification of malondialdehyde (MDA), the result of lipid peroxidation. MDA level was measured based on the thiobarbituric acid (TBA) method described by Ferreira et al. (2008), was measured at 530 nm and expressed in nmol MDA/mg protein. Biochemical analyses were performed in triplicate in a spectrophotometer (Synergy HT, Biotek, USA) at 25 °C.

Statistical analysis

All statistical analyses were performed by using the software Statistica 8.0. All data were tested for homogeneity and normality using Levene's and Kolmogorov-Smirnov tests. The differences in filtration rate and oxidative stress biomarkers among treatments and between males and females were analyzed by one-way ANOVA followed by a post hoc HSD Tukey test (THSD). The statistical significance was set at $p < 0.05$ and all results are given as mean ± S.D.

Results

Analysis of MPs in *M. galloprovincialis* tissues

Results did not show PE-MPs in whole tissues of *M. galloprovincialis* exposed for 14 days in all MP treatments which means that no accumulation of MPs in soft tissues occurred.

Filtration rate

No mortality was observed throughout the exposures for any of the treatments.

After 7 and 14 days of exposure to PE-MPs, the FR of *M. galloprovincialis* at the five different treatments is shown in Fig. 1. PE-MPs have reduced significantly the FR of mussels at the three concentrations 10 μg/L ($F = 0.42$, p = 0.006), 100 μg/L ($F = 3.04$, $p = 0.0004$), and 1000 μg/L ($F = 0.35$, $p = 0.0008$) after 7 days of exposure and only at 100 μg/L ($F = 9.93$, $p = 0.01$) and 1000 μg/L ($F = 7.94$, $p = 0.003$) after 14 days exposure compared to control groups.

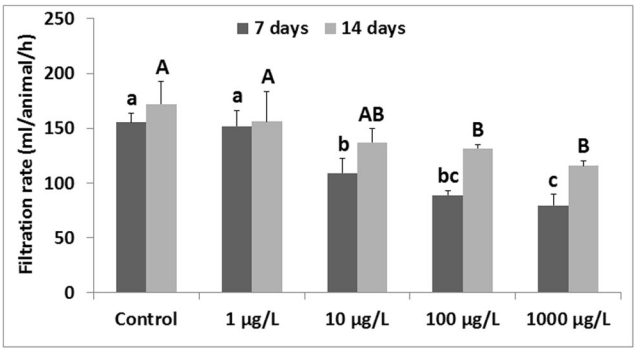

Fig. 1 Filtration rate in *M. galloprovincialis* exposed for 7 and 14 days to PE-MPS. Letters indicate significant differences between different treatments (a, b, c for 7 days and A, B, C for 14 days) ($p < 0.05$: significant difference (ANOVA, Tukey's HSD)), $n = 9$

Oxidative stress biomarkers

CAT and GST activity and LPO levels were evaluated in the digestive gland of mussels. In females, CAT activity (Fig. 2) decreased significantly in the highest PE-MPs concentrations (100 and 1000 μg/L) when compared to control groups ($F = 2.61$, $p = 0.003$ and $F = 0.74$, $p = 0.005$, respectively). In males, despite the gradual decrease in CAT activity, no significant differences were recorded between the different treatments. No differences ($p > 0.05$) were observed between sexes at any to the treatment groups.

After 14 days, a significant effect of PE-MPs exposure was registered on GST activity with a reduced activity in mussels exposed to the highest concentrations (100 and 1000 μg/L) (16.19 ± 2.07 nmol/min/mg protein in females of 100 μg/L

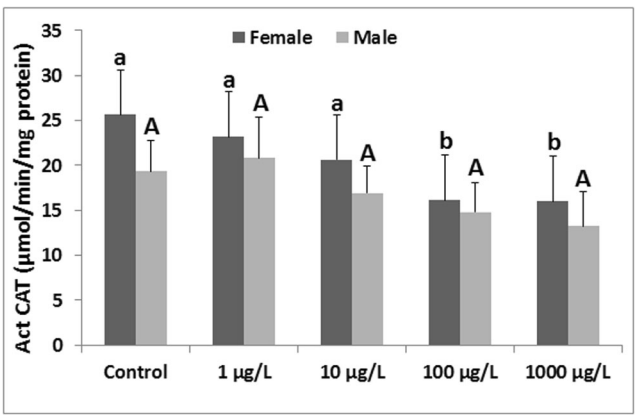

Fig. 2 CAT activity *in M. galloprovincialis* exposed for 14 days to PE-MPs concentrations. Letters (a, b) indicate significant differences between females at different treatments. Letter (A) was used for males. $p < 0.05$: significant difference (ANOVA, Tukey's HSD), $n = 30$ (females/males) per treatments (control (18/12), 1 μg/L (17/13), 10 μg/L (21/9), 100 μg/L (17/13), and 1000 μg/L (20/10))

treatment and 14.02 ± 5.19 and 11.49 ± 2.44 nmol/min/mg protein respectively in females and males of 1000 μg/L treatment) in comparison with controls (24.14 ± 3.52 in females and 21.80 ± 5.21 nmol/min/mg protein in males) (Fig. 3). No differences ($p > 0.05$) were observed between sexes at any to the treatment groups.

Lipid peroxidation (LPO) in the digestive gland tissues of mussels treated with PE-MPs showed a significant reduction between control and 1000 μg/L treated group (1.52 ± 0.36 nmol MDA/mg protein) ($F = 0.016$, $p = 0.0008$) in females (Fig. 4). In males, despite a decrease in LPO levels from control to the highest treated groups, no differences were observed among treatments.

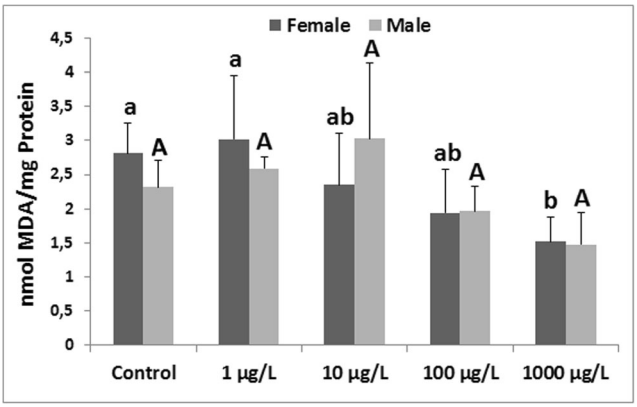

Fig. 4 LPO in *M. galloprovincialis* exposed for 14 days to PE-MPs concentrations. Letters (a, b) indicate significant differences between females at different treatments. Letter (A) was used for males. $p < 0.05$: significant difference (ANOVA, Tukey's HSD), $n = 30$ (females/males) per treatments (control (18/12), 1 μg/L (17/13), 10 μg/L (21/9), 100 μg/L (17/13), and 1000 μg/L (20/10))

Discussion

The study of interactions between MPs and living organisms is of great importance to improve the understanding of the toxicity of this group of pollutant. In most of the ecotoxicological studies conducted on bivalves, the selected concentrations of MPs used for the contamination of organisms are often very high (frequently several orders of magnitude above environmental relevance) and therefore are not representative of the expected MPs concentrations in coastal waters (Lenz et al. 2016; Revel et al. 2020). For this reason, in the present study, we selected three environmentally relevant MP concentrations (1 μg/L, 10 μg/L, and 100 μg/L) (Ivar do Sul et al. 2014; Goldstein et al. 2012; Revel et al. 2019, 2020) and one high concentration (1000 μg/L). In addition, in accordance with previous results in the three bivalves species *M. galloprovincialis*, *Ruditapes decussatus*, and *Crassostrea gigas* from the lagoon of Bizerte (Northern Tunisia), the majority of detected MPs in their tissues were made of PE

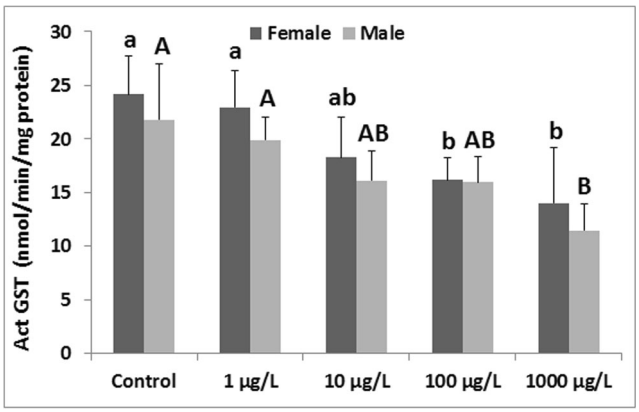

Fig. 3 GST activity *in M. galloprovincialis* exposed for 14 days to PE-MPs concentrations. Letters (a, b) indicate significant differences between females at different treatments. Letters (A, B) were used for males. $p < 0.05$: significant difference (ANOVA, Tukey's HSD), $n = 30$ (females/males) per treatments (control (18/12), 1 μg/L (17/13), 10 μg/L (21/9), 100 μg/L (17/13), and 1000 μg/L (20/10))

polymer (Abidli et al. 2019). Therefore, PE polymer was selected for this study.

After 14 days of exposure, no PE-MPs were observed in the soft tissue of mussels. This result suggests that *M. galloprovincialis* does not accumulate 40–48 μm MPs in tissues as they potentially eliminate them with feces. In the oyster *C. gigas*, Revel et al. (2020) showed that after PE-PP-MPs exposure (0.008, 10, 100 μg/L) during 10 days, no particles of MPs (< 400 μm) were found in the digestive gland, gills, and other tissues of this bivalve. However, in biodeposits, the same authors identified MPs. Similar result was reported in the same species (*C. gigas*) exposed to PS-MPs (2–6 μm, 23 μg/L) (Sussarellu et al. 2016). In contrast, in *Mytilus* spp. tissues, particles of PE and PP (< 400 μm) were only observed in the digestive gland of organisms exposed to 100 μg/L with a very low number of particles (Revel et al. 2019). The same authors revealed that whatever the exposure concentrations of PE and PP, particles between 50 and 200 μm were filtered and excreted by mussels, thus supporting the findings of the present study. In biodeposits, PE-PP-MPs were observed in mussels exposed to all concentrations of MPs (Revel et al. 2019). Ward and Shumway (2004) and Rosa et al. (2018) showed that although the exact mechanisms of selection are still being resolved, it is known that particle size, type, and surface properties (both specific and non-specific) affect preferential rejection and ingestion of material as MPs. In the same context, Moreschi et al. (2020) showed that, under environmental concentrations, accumulation and assimilation of MPs in bivalve tissues might be much slower than at the high experimental concentrations, resulting in insignificant contamination levels during their life span.

The evaluation of PE-MPs effects in *M. galloprovincialis* was carried out using a multi-marker approach. The filtration rate and biochemical biomarkers of oxidative stress such as CAT, GST activities, and the levels of LPO (measured in the

digestive gland tissues of both sexes) were determined. The selection of the digestive gland to analyze biomarkers is based in the fact that this organ is the main organ for xenobiotic biotransformation (Livingstone et al. 1992) and it has therefore been extensively used for toxicity assessment (Bråte et al. 2018). In the present study, PE-MPs decreased significantly the FR of *M. galloprovincialis* at 10 μg/L, 100 μg/L, and 1000 μg/L after 7 days of exposure and only at 100 μg/L and 1000 μg/L after 14 days of exposure. From these results, it seems that *M. galloprovincialis* is highly sensitive to PE-MPs treatments. Due to this loss of function, the capability of bivalves of getting food items from the water column through filtration decreased (Oliveira et al. 2018), which could lead to potential starvation and a decrease of the condition factor in a long-term exposure (Yin et al. 2018). Penning et al. (2013) also showed that the FR was a very important parameter in filter-feeding molluscs and the decrease in this parameter is associated with growth impairment and reduced survival. In addition, changes in FR between the first and the second weeks seem to be depending on the exposure time, which could be related with mussel's adaptation, especially for the two lowest treatment groups. The reduction of FR in *M. galloprovincialis* after MPs exposure is in agreement with previous findings in bivalves *Corbicula fluminea* (Oliveira et al. 2018), *Atactodea striata* (Xu et al. 2017), and *Ruditapes philippinarum* (Sıkdokur et al. 2020). This reduction of the FR by bivalves exposed to MPs compared to the control groups can be related to the tendency of these animals to close their valves to avoid them from exposure and represents a potential adaptive strategy that *M. galloprovincialis* used to reduce ingestion of MPs as reduction in FR effectively reduced the number of particles to be processed. Reduction in filtering activity following MPs exposure was also found in several previous studies after exposure to different stressors as sediments, pressure, Antracene, Hg, and plastics (Pinheiro et al. 2019; Sellami et al. 2015; Tran et al. 2007; Wegner et al. 2012). In contrast, in other previous studies, Revel et al. (2019, 2020) found that MPs contamination (mixture of PE and PP) had no significant impact on the FR of the blue mussel *Mytilus* spp. and on the Pacific oyster *C. gigas* compared to control groups. The authors suggested that the two tested bivalve species do not close their valves to avoid contamination by MPs but they purify this contaminant by egestion in biodeposits. In addition, the difference in response can be also related to the size and the concentration of used MPs.

The analysis of biomarkers of oxidative stress is a very informative tool for studying the effects of contaminants in marine organisms (Capela et al. 2016). In fact, the oxidative stress occurs due to an imbalance between the production of damaging reactive oxygen species (ROS) and an organism's capacity to deal with them. This imbalance can impact an organism's development, reproduction, and life span and that is why the oxidative stress investigation has become increasingly popular from different applied aspects including medicine, sport science, toxicology, and environmental science (Lushchak 2011). To protect against ROS attack, organisms have developed a biochemical antioxidant system (AOS) that includes a number of enzymatic and non-enzymatic components, as well as pathways designed to prevent cellular damage (Halliwell and Gutteridge 2015). In this study, the evaluation of changes in activity of antioxidant enzymes like CAT, GST, and LPO levels, in digestive glands, was a good approach to address the physiological disturbance associated with PE-MPs contamination.

CAT is considered an enzyme of the first line of defense that directly eliminate ROS (Prokić et al. 2018). Its activity was evaluated in the digestive gland tissues of both sexes of *M. galloprovincialis* and showed a significant decrease only in females at 100 and 1000 μg/L compared to control groups. The same result was found in the digestive gland of *R. philippinarum* exposed to 25 μg/L of PE-MPs beads (10–45 μm) for 1 week (Sıkdokur et al. 2020). However, in *Mytilus* spp., a significant increase of CAT activity was measured in the digestive gland of this blue mussel exposed to PE-PP MPs (< 400 μm) at 0.008 μg/L and 10 μg/L for 10 days (Revel et al. 2019). In addition, in the bivalve *Donax trunculus* exposed to PE-PP-MPs (100–400 μm) for 15 days, CAT activity was induced significantly during all exposure period in the flesh. Otherwise, induction of this enzyme was observed from the 3rd and 7th days in gills and the digestive gland, respectively (Tlili et al. 2020). The difference in response of animals to MPs contamination can be associated to the size of the particles, polymer type, MPs concentration, exposure duration, and species-specific differences. In fact, Jeong et al. (2016, 2017) examined the effects of acute exposure to PS microbeads on the AOS of the marine copepod *Paracyclopina nana* and the rotifer *Brachionus koreanus* and reported size-dependent effects. Moreover, it has been shown that a stressful condition can either induce or inhibit the AOS, depending on the time of exposure and intensity of the stressor (Lushchak 2011). Pinheiro et al. (2019) showed that if an organism is under oxidative stress, CAT can become overwhelmed. In the same context, Winston and Di Giulio (1991) showed that ROS are extremely potent oxidants capable of reacting with critical cellular macromolecules, possibly leading to enzyme inactivation.

GST allows the conjugation of the reduced form of glutathione (GSH) to pollutant compounds. It is associated with phase II biotransformation process. In the present study, GST activity in the mussel *M. galloprovincialis* showed practically the same pattern as CAT activity. It decreased according to the increase of PE-MPs concentration in both sexes. The inactivation of GST in the high concentrations of PE-MPs can be related to ROS that react with this enzyme or it is possible that the activation of the GST enzyme could be occurring in a tissue other than the digestive gland. In previous

studies, Revel et al. (2019) reported a decrease in GST activity in gills and the digestive gland of the mussel *Mytilus* spp. exposed to PE-PP-MPs for 10 days. The same result was registered by Paul-Pont et al. (2016) in gills and the digestive gland of the mussel *M. edulis* exposed for 7 days to PS at 32 μg/L. Barata et al. (2005) reported the decrease in GST activity to the inactivation of this enzyme, especially when an important ROS production takes place and the system becomes overwhelmed. In contrast to the results of the present study, Tlili et al. (2020) showed that the exposure to PE-PP-MPs (100–400 μm) for 15 days induced important and rapid GST enzymatic activities in gills and digestive gland of the bivalve *D. trunculus*. As suggested for CAT, it seems that several factors can influence the variability of biomarker responses of animals exposed to MPs.

LPO is a chain reaction of molecular events in ROS that result in the oxidative deterioration of lipids. This may significantly affect cell membrane structure, lipoproteins, and other lipid-containing structures (Nam 2011). The oxidative damage to cell membranes changes membrane permeability and promotes penetration of cells by toxic agents (Ayala et al. 2014). Therefore, measurement of malondialdehyde as the product of LPO is a widely used biomarker of exposure to environmental contaminants. In the present study, LPO in the digestive gland tissues of *M. galloprovincialis* was reduced significantly in females at the highest treatment group of PE-MPs (1000 μg/L). LPO was also significantly reduced in the mussel *Mytilus* spp. exposed for 7 days to PS-MPs (mix of 2 and 6 mm) at 32 μg/L compared to controls (Paul-Pont et al. 2016). In the same context, Pinheiro et al. (2019) noted that if an organism is under oxidative stress, CAT can become overwhelmed and trigger LPO which could explain the observed trend of an initial LPO increase in the 1 and 10 μg/L exposure groups.

Conclusion

In the present study, *M. galloprovincialis* exposed to PE-MPs (40–48 μm) for 14 days showed no accumulation in soft tissues despite a reduction of filtration rate in all treatments. Moreover, PE-MPs are able to induce after 14 days of exposure biochemical stress in mussels expressed by an oxidative damage (LPO) at low concentrations (1 and 10 μg/L) and the alteration in the activity of antioxidant enzymes CAT and GST, which is a reason for concern as effects were observed at environmental and high relevant levels of PE-MPs. More studies on the uptake and depuration of different types MPs by a range species of bivalves, longer-term exposure, characterization of MPs in biodeposits, and analysis of biochemical biomarkers of oxidative stress and molecular endpoints in other tissues, as gills, should be conducted to investigate potential chronic effects of MPs on mussels.

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Authors' contributions All authors contributed to the study conception and design.

SA: sampling of bivalves, analysis of microplastics in samples, contamination of mussels in laboratory conditions, dissection of organs, filtration rate and biomarkers analyses, and writing the first draft of the manuscript.

MP: dissection of organs, biomarkers analysis, and correction of the first draft of the manuscript.

YL: sampling of bivalves and statistic analysis.

TN: correction of the first draft of the manuscript and English revision.

MMS: correction of the first draft of the manuscript and English revision.

NTM: correction of the first draft of the manuscript.

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Data availability All data analyzed during this study are included in the published article.

Declarations

Ethical approval and consent to participate No approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with an unregulated invertebrate species.

Consent to publish Not applicable.

Competing interests The authors declare that they have no competing interests

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