Do polyethylene terephthalate microparticles (PET-µPs) affect the oxidative status of the clam *Ruditapes Philippinarum*?

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Abstract
Microplastics represent a major concern in the marine ecosystems because of their widespread distribution and potential hazard towards organisms. In the present study we investigated the effects induced by two concentrations of micronized polyethylene terephthalate microparticles (PET-µPs) on the oxidative status of the clam *Ruditapes philippinarum*. Although PET-µPs were ingested and egested by organisms, they did not imbalance the oxidative status and did not cause oxidative damage on clam digestive gland. Our results suggest a low risk related to PET-µPs towards clams, at least at the tested concentrations and for short-term exposure periods.
Keywords: clam; microplastics; polyethylene terephthalate; oxidative stress

1. Introduction

Polyethylene terephthalate (PET) is the most favorable polymer used as packaging material (e.g. drinking water bottle). PET items continue to be marketed for four decades but their incorrect disposal represents an underestimated environmental problem. Moreover, in environment PET can be degraded by diverse environmental factors, resulting in the formation of PET microplastics (PET-µPs). Microplastics are plastic particles smaller than 5 mm that are considered as emerging contaminants of aquatic ecosystems (Avio et al., 2017). Several studies have demonstrated the width of microplastic contamination in marine environments, investigating the presence of different plastic polymers in both abiotic (water and sediments) and biotic (zooplankton, mussels and fish) matrices (Cole et al., 2011). Moreover, a growing number of studies has investigated the potential effects induced by microplastic made by different polymers, mainly polyethylene and polystyrene on diverse marine species (Cole et al., 2013). However, no study has been focused on the toxicity of PET-µPs towards any marine species. Thus, the present study aimed at exploring the ingestion and potential effects on oxidative status caused by a 7-days exposure to PET-µPs in the clam *Ruditapes philippinarum*.

2. Materials and Methods

Clams were exposed to two concentrations (0.125 and 12.5 µg/mL) of micronized PET-µPs having irregular shape and a size ranging between 20 and 300 µm in length. Fifteen clams (about 4 cm in length) were seeded in 5 L beakers filled with artificial saltwater. Three independent experimental replicates per treatment were planned. Exposures were performed under semi-static, controlled laboratory conditions (temperature = 14 °C; photoperiod 16 hrs light: 8 hrs dark; oxygen at saturation). Considering the high density of PET, an aerator was placed at the bottom of the exposure tank in order to guarantee the resuspension of microparticles. Saltwater was renewed every single day and the selected amount of PET-µPs was added. Before the renewal of exposure conditions, clams were fed for 1 h by Algamac2000®. At the end of the exposure, eight clams per treatment, including controls, were dissected, the digestive gland isolated and quickly frozen in liquid nitrogen. Samples were maintained at – 80 °C until the execution of biochemical analyses. The digestive gland of three clams were pooled and homogenized. Three pools per treatment were analyzed. Raw or centrifuged homogenate was used to biochemical to perform a suite of six different biomarkers of oxidative stress, depending of specific protocol. The amount of reactive oxygen species was measured according to a fluorimetric method relying on the change in fluorescence of dichlorofluorescein-diacetate (Deng et al., 2009). The activity of antioxidant enzymes, namely superoxide dismutase – SOD, catalase – CAT and glutathione peroxidase – GPx, as well as of the phase II detoxifying enzyme glutathione S-transferase – GST – was assessed according to the spectrophotometric methods, while lipid peroxidation was assessed by the thiobarbituric acid reactive substances (TBARS)

method (Parolini et al., 2010). The effect of PET-µPs on biochemical endpoints was assessed by linear mixed models (LMMs), including the treatment as a fixed affect factor and the tank of exposure as a random factor.

3. Results

Our results showed that clams were able to ingest and to egest PET-µPs at all the tested concentrations, as demonstrated by the presence of such microparticles wrapped within the faeces. However, the analysis of the suite of oxidative stress biomarkers did not show significant effects in clam digestive gland (Figure 1). In detail, the exposure to PET-µPs did not induce a significant overproduction of reactive oxygen species (ROS; F = 1.555; p = 0.316), neither at low, environmentally-similar concentration nor at high, unrealistic one (Figure 1a). Accordingly, the activity of antioxidant enzymes, was not modulated by the exposure to the tested PET-µPs concentrations. Except a significant effect found on GPx (Figure 1d; F = 39.086; p = 0.002) showing a significant activity inhibition at the end of the exposure to the highest tested concentration (p = 0.003), the activity of SOD (Figure 1a; F = 0.156; p = 0.859), CAT (Figure 1b; F = 0.160; p = 0.587) and GST (Figure 1e; F = 4.071; p = 0.109) were not affected by PET-µPs exposure and did not differed in comparison with controls. These data suggest that a 7-days exposure to two different concentrations of PET-µPs was not able to imbalance the equilibrium between pro-oxidant and antioxidants in the digestive gland. Consequently, clams did not suffer and oxidative stress situation, as revealed by the lack of significant alterations of lipid peroxidation levels occurring between treated and control specimens (Figure 1f; F = 1.345; p = 0.347). The results of our short-term exposure suggest that micronized PET-µPs should not represent a threat for the health status of clams. However, further studies assessing the potential effects of PET-µPs on other organs and/or after long-term exposure should be necessary to confirm the limited hazard of these microparticles on filter-feeders marine organisms.

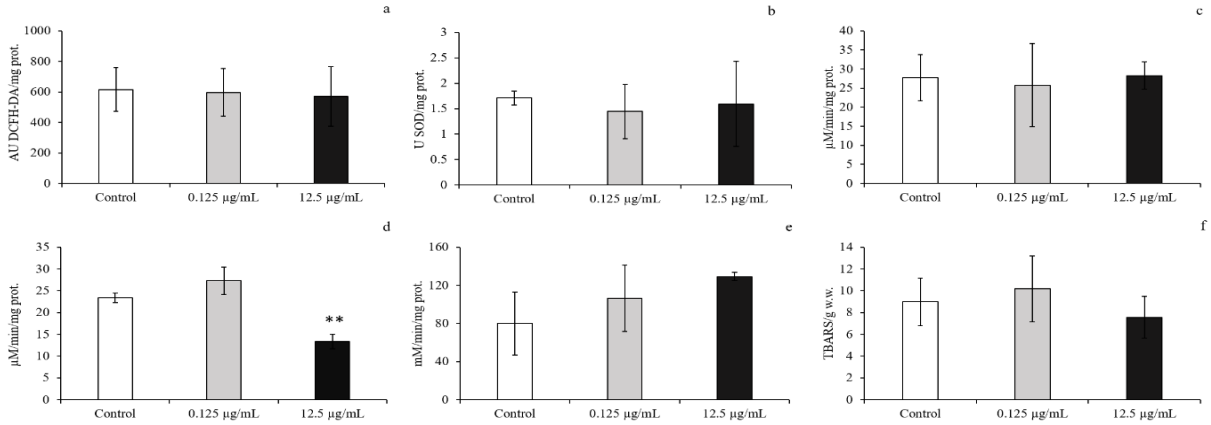

Figure 1. Mean (± standard deviation) of the total amount of reactive oxygen species (a), superoxide dismutase (b), catalase (c), glutathione peroxidase (d), glutathione S-transferase (e) and lipid peroxidation (f) measured in homogenates of clam digestive gland exposed to two concentrations of micronized PET-µPs. Asterisks above the histograms show significant differences in the biomarker response between treated and control group (** $p < 0.01$).

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