Continuum of size from microplastics to nanoplastics: effects on the estuarine bivalve Scrobicularia plana at different levels of biological organization.

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Abstract

Plastic has been largely detected in estuarine environments and represent major concern towards aquatic living organisms. Whereas the majority of previous studies analyze the effects of standard particles, mainly polystyrene (PS), not representative to what is found in natural environments, the present study evaluates the impact of microplastics (MPs) and nanoplastics (NPs) under realistic exposure conditions. *Scrobicularia plana* individuals were exposed to low concentrations (0.008, 10 and 100 µg L^{-1}) of environmental MPs and NPs as well as to standard PS NPs, as a comparison condition. The aim of this study was to understand the ecotoxicological effects of environmental plastic particles on *S. plana* gills and digestive glands but also to compare the effects of plastic polymers size in order to highlight if the size could induce different toxicity profiles within this model organism, at different levels of biological organization. Results showed a differential induction of detoxification enzymes (CAT, GST), immunity (AcP), DNA damage processes as well as differential effect on behavior and condition index of animals depending upon the type of plastic, the size, the concentration tested and the type of organ. This study underlines the necessity of testing *i)* plastics collected from the environment as compared to standard ones and *ii)* the effect of size using plastics coming from the same batch of macrosized plastics. This study concludes on the future need directions that plastic-based studies must take in order to be able to generate large quantity of relevant data that could be used for future regulatory needs on the use of plastic.

Introduction

Plastics, considered as a wide range of synthetic or semi-synthetic organic materials, became essential to our society due, among other things, to their lightweight, strength, durability, corrosion-resistant properties and low cost (Thompson et al. 2009). Different chemicals, named ‘additives’ are added to plastic polymers to improve their properties and extend their lifespan (Hahladakis et al. 2018). Plastics are thus increasingly being used worldwide for countless applications (Cole et al. 2011; Andrady 2011). The annual production of resin and fibers rose from 2 million tons in 1950 to 380 million tons in 2015 (Geyer et al. 2017). Massive production of plastics highly resistant to degradation combined with poor mechanisms for recycling (Sardon and Dove 2018) led to significant accumulation and dispersion of plastic waste in terrestrial, freshwater and marine environments (de Souza Machado et al. 2018; Horton et al. 2017; Li et al. 2016).

Plastics are a major threat in marine and coastal environments (Li et al. 2016; Moore 2008) because of their persistence and their negative impacts on marine life (Andrady 2015; Worm et al. 2017; Derraik 2002). Plastic debris in marine environment originate from both land- (80%) and marine-based sources (20%) (Derraik 2002; Li et al. 2016). Representing 60–80% of marine debris (Derraik 2002), they are mostly composed of polyethylene (PE), polypropylene (PP), polystyrene (PS) and also of polyvinyl chloride (PVC), polyurethane (PU), polyethylene terephthalate (PET) and polycarbonate (PC) (Worm et al. 2017). These plastic debris are widespread and have been found in coastal waters (Doyle et al. 2011), shorelines (Browne et al. 2011), estuaries (Vermeiren et al. 2016; Wright et al. 2013a; Sadri and

Thompson 2014) and oceans (Law et al. 2010; Andrady 2015). These past decades, plastic debris in the marine environment has received increasing research interest.

Plastic particles whose size is less than 5 mm have been commonly described as microplastics (MPs) (Fendall and Sewell 2009; Browne et al. 2007; Arthur et al. 2008). These MPs are classified as primary or secondary ones, based on their sources. Most of the MPs detected in the marine environment are secondary MPs which derived from the fragmentation of larger plastics. Indeed, it has been shown that plastic debris fragment due to photodegradation, the effect of wave, wind and microorganisms action as well as the abrasion from sediment particles (Andrady 2011, 2015; Kale et al. 2015). Primary MPs come from direct sources such as cosmetics, hand and facial cleaners, or production waste from plastic manufacturing (Wang et al. 2016; Gregory 1996). Recently, studies have focused on an even smaller range of plastics, named nanoplastics (NPs) (Gigault et al. 2016, 2018; Lambert and Wagner 2016). These NPs can be defined as particles having colloidal properties in aqueous systems and whose size varies from 1 nm to 1000 nm (Gigault et al. 2018). Although nanoparticles are difficult to evidence in the marine environment because of methodological challenges; studies have already demonstrated their occurrence from microplastics degradation under laboratory environmentally representative conditions (Gigault et al. 2016; Ter Halle et al. 2017; Lambert and Wagner 2016).

A large research effort has been conducted to characterize the capture and ingestion of MPs by numerous marine organisms from small invertebrates to large vertebrates (Galloway et al. 2017). MPs ingestion concerns all modes of nutrition for invertebrates including detritivores, deposit feeders, planktivores, filter feeders and suspension-feeders (Wright et al. 2013a). This ingestion causes harmful effects such as injury (Gall and Thompson 2015), inflammation and intestinal damage (Jin et al. 2018; Lei et al. 2018), reduced food consumption (Watts et al. 2015; Cole et al. 2015), decreased energy reserves (Gardon et al. 2018; Bour et al. 2018; Wright et al. 2013b), reduced growth (Besselin et al. 2014) and decreased reproductive performance (Sussarellu et al. 2016). Moreover, ingested MPs have been evidenced to translocate into the circulatory system of the mussel *Mytilus edulis* (Browne et al. 2008). Recent studies have demonstrated the impacts and the bioaccumulation of NPs in marine organisms (Arini et al. 2022; Lebordais et al. 2021; Baudrimont et al. 2019; Chae and An 2017; Bergami et al. 2017). NPs could easily permeate lipid membranes, which may induce cell damages (Rossi and Monticelli 2014). In addition, a particular concern relates to the chemical effects of NPs. Having a high surface area, NPs may cause stronger sorption affinities for chemicals than MPs (Koelmans et al. 2015; da Costa et al. 2016; Velzeboer et al. 2014), representing a significant risk for marine life. Moreover, the additives incorporated in the plastics may also induce deleterious effects, however, most of the studies do not or little characterize these additives, inducing potential bias in the interpretation of the observed responses (Ateia et al. 2020).

Given the ubiquitous nature and small sizes of MPs and NPs, their ingestion by filter feeders and its adverse effects is of increasing concern. Indeed, these species may be highly impacted by MPs and NPs since they filter large amounts of water. Several studies have highlighted MPs and NPs ingestion by bivalves under environmental conditions (Ward et al. 2019). The species *Scrobicularia plana* is an

endobenthic bivalve that play a key role in the structure and functioning of estuarine and coastal ecosystems (Mouneyrac et al. 2008). *S. plana* is an ecologically relevant species for ecotoxicology studies and has been used as a bio-indicator for assessing the health status of ecosystems (Mouneyrac et al. 2008, 2014; Buffet et al. 2014; Châtel et al 2017). Indeed, this species is widely distributed, has a sedentary lifestyle, is sensitive to pollutants, and is robust under controlled conditions. In addition, this species is relevant for the use of a number of biochemical or behavioral biomarkers (Solé et al. 2009). *S.plana* may be exposed to plastic particles in the water as well as in the sediment. At low tide, this species ingests sediment particles holding its inhalant siphon above the mud surface whereas at high tide it becomes a suspension filter feeder. Surprisingly, there are only very few studies on the effect of plastics on *S. plana*. A study showed that polystyrene MPs (20µm; 1 mg L^{-1}) induce effects on *S. plana*, after 14 days of exposure followed by 7 days of depuration reducing antioxidant capacity, and increasing neurotoxicity, DNA and oxidative damages (Ribeiro et al. 2017). Nevertheless, the concentration of MPs tested was higher than the concentrations measured in seawater in highly contaminated areas (Ribeiro et al. 2017). Another study demonstrated a negative influence of low-density polyethylene MPs (11–13µm; 1 mg L^{-1}) with adsorbed contaminants over the assessed biomarkers in the digestive gland of *S. plana* after 14 days of exposure (O'Donovan et al. 2018). To our knowledge, there is no study, to date, on the effects of NPs on this species.

Most studies on the effects of plastic particles concern nanoplastics or microplastics, but few studies relate to the size continuum from NPs to MPs. Likewise, many studies have focused on the effects of MPs on bivalves, while few studies have investigated the effects of NPs (Wegner et al. 2012; Chae and An 2017; Baudrimont et al. 2019; Lebordais et al. 2021; Arini et al. 2022). One of the findings that can be highlighted from studies on NPs is that the shape, size and stability of NPs are the main factors affecting their availability. In most NPs studies, standard polystyrene beads nanoparticles have a regular size, a homogenous surface and are spherical. As the shape of NPs may induce toxic impacts, standard ones lack environmental representativeness (Phuong et al. 2016). Indeed, they do not show any similarity with the MPs/NPs from plastic waste degradation whose sizes, shapes and compositions are heterogeneous (Gigault et al. 2016, 2018). Moreover, the exposure concentrations in the various studies (in the order of 1 to a few hundred mg L^{-1}) largely exceed MPs concentrations encountered in the environment: 0.4–34 ng L^{-1} in freshwaters in the USA and Europe and 0.51 mg L^{-1} in marine waters (Koelmans et al. 2015). Finally, when using MPs and NPs obtained from mechanically degraded environmental samples, little is known about the characterization of additives and organic compounds contained in plastics. In this light, further studies are needed to characterize MPs and NPs extracted from environmental samples and to investigate their eco-toxicological effects on bivalves.

The objective of this study was to test the effects of environmental MPs and NPs as well as standard PS NPs on different tissues (gills, digestive glands) and cells of *S.* plana at three different concentrations similar to those estimated in the environment. Environmental MPs and NPs size and associated metals were characterized. Biological responses of organisms were assessed from the sub-individual (biochemical activities) to the individual level (burrowing behavior, condition index).

Material And Methods

Collection, preparation and characterization of environmental derived MPs and NPs

Plastic wastes were collected by hand with pliers on the right bank of the Garonne River at low tide, near the Langoiran bridge (44°42'14.56"N, 0°24'3.91"W). The most oxidized plastic debris were sampled, rinsed in the Lab with ultra-pure water, dried at 45°C during 48h before preparation for micro and nanoplastic solutions.

Environmental micro and nanoplastics production

Environmental microplastics (ENV MPs) and nanoplastics (ENV NPs) were generated from macro-sized plastic debris according to the protocol described by Blancho et al. (2021). Briefly, NPs and MPs were produced through a process coupling agitation and sonification in aquatic media. Size range was between 1 and 1200 nm for ENV NPs and between 1.2 and 300 µm for ENV MPs.

ENV NPs and ENV MPs were characterized in terms of composition, size, shape and surface properties by Pyrolysis (Pyrolyzer PY-3030 Frontier Lab) coupled to gas chromatography-mass spectrometry (Py-GC-MS) (5977B, Agilent Technologies). Plastic analysis showed that ENV NPs and ENV MPs were mainly composed of polyethylene (PE) (95%), that they were anisotropic, polydisperse in term of size and possessed high levels of carboxylic groups onto their surface.

Carboxylated polystyrene nanobeads (200 nm) were used as reference material.

Acidic digestion and ICP-MS measurements

In order to optimize the total digestion, 100 mg of microplastics and nanoplastics powder were acid-digested (12 N HNO_3 sub grade) using a multi-step procedure with a microwave oven (MW7000 system from Anton-Paar; increasing ramp of temperature of 6.6°C per minute until reaching 250°C, then 25 min at 250°C under 140 bar of pressure). The solution of three tubes were mixed, evaporated at 90°C and solubilized in 0.37 N HNO_3 prior to ICP-MS measurements. Metal concentrations were measured by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) from Agilent Technologies (7700x Model, Agilent) (Supplementary Information Table A). The digestion and analyses process were validated using reference materials (ERM-EC 680 and ERM-EC 681) from the Joint Research Centre of the European Commission (JRC, Ispra, Italy).

Scrobicularia plana collection and laboratory exposure assay

Individuals of *Scrobicularia plana* were hand-collected in the intertidal mudflat located on the French Atlantic coast (47°01’48”N; 1°59’02.8”W; bay of Bourgneuf). Clams were transported to the laboratory in boxes with sediment from the collection site. Bivalves were immediately transferred into aquaria (30L)

containing 27L of aerated artificial seawater (Tropic Marine) at 25 psu in a temperature-controlled room at 15°C for an acclimatization period of 7 days. Photoperiod was maintained at 12:12. A total of 600 clams were divided among ten glass aquaria. The sea-water was completely renewed every three days before adding the MPs and NPs solutions (see section 2.1.2). Clams were fed once a week with the marine micro-alga *Tetraselmis suesica* (Teramer). At the end of the acclimatization period, 12 individuals were sampled to provide the physiological status of the clams at T0.

Clams were then exposed for 21 days to ten conditions (one condition/aquarium): three plastics conditions: field derived microplastics (ENV MPs, 1-300 µm), field derived nanoplastics (ENV NPs, 235 ± 70 nm) and standard polystyrene nanoplastics (PS NPs, 200 nm) at three different concentrations: 0.008 µg L^{-1}, 10 µg L^{-1} and 100 µg L^{-1} and a negative control without any added plastic. The three tested concentrations were chosen according to Revel et al. (2020) in order to expose clams to environmental relevant concentrations of NPs and MPs which are representative of coastal regions and gyres (Goldstein et al. 2013). To prevent any plastic contamination, the use of plastic material was avoided during all the experimental exposure.

Suspensions of microplastics

For each type of plastic particles (ENV MPs, ENV NPs and PS NPs), stock suspensions at concentrations of 1 and 0.1 g L^{-1} were prepared in ultra-pure (milliQ) water at pH 7. A working solution at 0.1 mg L^{-1} was obtained for each type of plastic particles by three serial dilutions of the stock suspension at 0.1 g L^{-1} in ultra-pure water (milliQ) at pH 7 as performed in Revel et al. (2019). A specific volume of the stock suspension (1 g L^{-1} or 0.1 g L^{-1}) or the working solution (0.1 mg L^{-1}) was distributed in the aquaria to obtain the final concentrations of 0.008, 10 and 100 µg L^{-1}. No surfactant was used during MPs and NPs preparation to prevent any additional effect. Each solution was well mixed before adding it to the aquaria. All MPs and NPs solutions were prepared and spilled in the aquaria every 3 days.

Clam sampling for analysis

After 7 days (T7) of exposure to the ten conditions, 8 individuals per condition were sampled: 5 for biomarker analysis and 3 for genotoxicity. At the end of the experiment at 21 days (T21), 18 individuals per condition were sampled: 5 for biomarker analysis, 3 for genotoxicity and 10 used to determine burial rate and condition index. Gills and digestive glands were dissected and immediately frozen in liquid nitrogen for enzymatic activity analysis.

Condition index

At the end of the exposure, 10 clams per condition were weighted and the soft tissues were individually weighted. Tissues and shells were oven-dried at 70°C for 48h. After 48h dried tissue and shell samples were weighted to determine the CI. The condition index was calculated according to the following formula (Walne 1976).

$$\text{Condition Index (CI)} = \frac{Driedtissuesweight(g)}{Driedshellweight(g)} \times 100$$

Burrowing test

Burrowing tests were performed as described in Bonnard et al. (2009) at T21. The burrowing experiments were conducted in glass crystallizers containing 5 cm of artificial sediments (90% sand, 9% kaolinite, 1% calcium carbonate supplemented with salt water (2 cm above the sediment). The sediment was hand prepared and placed in the crystallizers 5 days before the experimentation. The burial behavior was evaluated by placing 10 individuals per condition at the surface of the sediment in the dark and observing the number of them that had burrowed at frequent intervals. The interval was 5 min within the first hour, every 10 min in the second hour and then every 20 min from the third to the sixth hour. Percentages of unburrowed individuals over time were determined for each condition.

Biomarkers

Enzyme activities (CAT, GST, and phosphatase acid) were quantified in gills and digestive gland tissues. First, the tissues were thawed, weighed and homogenized, on ice, in Tris buffer at pH 7.2 (Tris 0.8M (Trizma-base Sigma® T6791); NaCl 2M (Sigma® S9888); DTT 100mM (Sigma® 8349-12-3); CIP (Protease Inhibitor Cocktail, Sigma® P8340)). The homogenates were then centrifuged at 9000 g at 4°C for 30 min. The amount of total protein was quantified using the Bradford method (Bradford 1976). CAT activity was measured spectrophotometrically at 240 nm (ε = 0.04 $mM^{-1}.cm^{-1}$; UviLine® 9600), following the dismutation of hydrogen peroxide (H_2O_2) (Claiborne et al. 1985). Specific activity was expressed in $nmoles.min^{-1}.mg^{-1}$ of protein. GST activity was measured spectrophotometrically at 340 nm (ε = 9.6 $mM^{-1}.cm^{-1}$ TECAN® Sunrise) by observing the conjugation of the 1-chloro-2,4-dinitrobenzene (CDNB) and the L-glutathione reduced (GSH) (Habig et al. 1974). Specific activity was expressed in $\mu moles.min^{-1}.mg^{-1}$ of protein. Phosphatase acid (AcP) activity was determined spectrophotometrically at 340 nm (ε = 18.3 $mM^{-1}.cm^{-1}$; TECAN® Sunrise; Acid phosphatase Assay kit Sigma® CS0740), measuring the appearance of p-nitrophenol (pNP) by the hydrolysis of p-nitrophenylphosphate (4-NPP) and expressed in $nmoles.min^{-1}.mg^{-1}$ of protein.

Genotoxicity (comet assay)

The comet assay is a method for measuring the amount of DNA damage present in cells. This test was carried out as previously described (Barranger et al. 2014; Akcha et al. 2000). After exposure to the 10 conditions, gills and digestive glands were dissected and then immersed in a solution of dispase II ($2U.mL^{-1}$ – 30 min – 37°C). Cell viability was checked using the Trypan Blue test. Cells were resuspended into low melting point agarose 0.5% and the mixture was placed on microscope slides, previously covered with agarose 0.8%. After gel solidification, slides were submerged in a lysis buffer (NaCl 2.5M (*Sigma® S9888)*, EDTA 1M (*Sigma® E5134)*, Tris 0.001M *(Trizma-base Sigma® T6791)*, Triton (*Sigma® TritonX* 100)) overnight at 4°C. Following lysis, slides were washed 3 times 5 minutes with PBS 1X and the DNA denaturation was carried out (15 min) following by the electrophoresis (10 min – 300 mA). At the end of the electrophoresis, slides were rinsed 3 x 5 min in a neutralization buffer (Tris-base 0.4 M *(Trizma-base Sigma® T6791)* pH 7.5) and then dehydrated 10 min with 70% ethanol. DNA is revealed by adding 30 µL

of ethidium bromide and observed using an optical fluorescence microscope (Olympus BX60, x 40) equipped with a CDD camera (Lucas-S, Andor Technology) and a Komet 6 image analysis system (Kinetic Imaging Ltd). 150 nuclei were analyzed per slide and DNA damage were expressed in percentage of the DNA present in the Comet tail (% Tail DNA) for each nucleus.

Statistical analysis

Normality of data distribution and homogeneity of variance were tested using the Shapiro-Wilk test and Bartlett test, respectively. Data analysis was performed at 5% alpha level using XLSTAT 2019 (version 21.4.63762). If the data did not meet the conditions for parametric tests, Kruskal-Wallis tests were therefore used to test for differences between the treatments. If the overall test was significant, a Dunn procedure was performed to determine which means were significantly different. If the data followed the conditions for application of parametric tests, an ANOVA was used to test for differences between the treatments. If the overall test was significant, a Tukey post hoc test was performed to determine which means were significantly different. For the burrowing test, percentages of unburrowed individuals were first ln-transformed for linearization, then the regression coefficients of the least-square regression lines were compared using analysis of covariance (ANCOVA). In all cases, p values ≤ 0.05 were considered statistically significant.

Results

Condition index (CI)

Mean CI of the organisms were comprised between 12.56 and 16.16 (Table 1) and were not significantly different between the ten conditions.

Table 1
Condition Index (mean and standard deviation)

	Concentrations (µg L^{-1})	CI mean	CI SD
Control	-	12.56	3.89
PS NPs	0.008	14.04	3.46
	10	13.72	3.46
	100	14.20	4.16
ENV NPs	0.008	15.18	5.38
	10	16.16	8.52
	100	15.65	6.25
ENV MPs	0.008	14.15	3.82
	10	13.83	2.96
	100	14.60	5.09

Biomarkers

Oxidative stress and detoxication

After 7 days of exposure, no significant difference was observed for catalase (CAT) activity in gills of *S.plana* exposed to plastic compared to the non-exposed animals (control) (Fig. 1). The CAT specific activity (SA) decreased significantly in gills after 7 days of exposure to 100 µg L^{-1} of PS NPs compared to the two lowest PS NPs concentrations (0.008 and 10 µg L^{-1}). The CAT SA was significantly lower in gills of clams exposed to 10 µg L^{-1} of ENV MPs compared to those exposed to 0.008 µg L^{-1} ENV MPs. The CAT SA decreased significantly after exposure to the highest concentration of ENV NPs (100 µg L^{-1}) compared to both lowest concentrations of PS NPs (0.008 and 10 µg L^{-1}). Comparison of CAT SA between animals exposed 7 days to environmental micro and nanoplastics showed a significant higher level after exposure to 0.008 µg L^{-1} of ENV MPs compared 100 µg L^{-1} of ENV NPs.

After 21 days of exposure, significant higher levels of CAT activity were observed in the gills of *S. plana* exposed to 0.008 and 10 µg L^{-1} of ENV NPs and to 100 µg L^{-1} of ENV MPs compared to the control ones (Fig. 1). A significant inhibition of CAT activity was depicted in gills after clam exposure to 100 µg L^{-1} of PS NPs compared to the two lowest PS NPs concentrations (0.008 and 10 µg L^{-1}). The exposure of clams to 100 µg L^{-1} ENV MPs increased significantly CAT SA in gills compared to 0.008 µg L^{-1} ENV MPs. The lowest concentrations of ENV NPs (0.008 and 10 µg L^{-1}) induced a significant increase of the CAT SA compared to the both 10 and 100 µg L^{-1} PS NPs concentrations. and the CAT activity was significantly increased after 21 days exposure to 100 µg L^{-1} of ENV NPs compared to the same

concentration of PS NPs. Comparing CAT activity in gills of clams exposed to ENV NPs and MPs, only the lowest concentration of ENV MPs (0.008 µg L^{-1}) induced a significant decrease of the enzyme activity compared to the lowest ones of ENV NPs.

In the digestive glands of *S. plana* (Fig. 1), after 7 days, exposure to 10 and 100 µg L^{-1} of ENV NPs induced a significant increase of the CAT activity compared to the control. No significant difference in CAT activity was depicted between the three studied concentrations of each condition (PS NPs; ENV NPs; ENV MPs). The exposure to the three concentrations of ENV NPs induced a significant increase in comparison to the exposure to 100 µg L^{-1} of PS NPs. The two highest concentrations of ENV NPs (10 and 100 µg L^{-1}) caused also a significant increase of the CAT SA in comparison to both 0.008 and 100 µg L^{-1} of PS NPs and ENV MPs. After 21 days, CAT activity decreased significantly in digestive glands of clams exposed to 10 and 100 µg L^{-1} of both PS NPs and ENV NPs compared to control (Fig. 1). No significant difference in CAT SA was depicted between the three concentrations of each exposure condition.

The Glutathione-S-transferase (GST) activity decreased significantly in the clam gills after 7 days of exposure to 0.008 and 10 µg L^{-1} of ENV MPs compared to the control (Fig. 2). No significant difference in GST SA was depicted between the three concentrations of PS NPs and ENV NPs. Comparing exposure to the three concentrations of ENV MPs, GST activity is significantly inhibited in gills of clams exposed to 0.008 and 10 µg L^{-1}. The exposure to 100 µg L^{-1} of ENV NPs decreased significantly the GST activity compared to exposure at the same concentration of PS NPs. The comparison of GST SA in gills of clams exposed to ENV NPs and ENV MPs showed a significant inhibition after exposure to 0.008 µg L^{-1} ENV NPs, 100 µg L^{-1} ENV NPs, 0.008 µg L^{-1} and 10 µg L^{-1} ENV MPs compared to exposure to 100 µg L^{-1} of ENV MPs. After 21 days, a significant decrease of GST activity was depicted in gills of clams exposed to the lowest concentration (0.008 µg L^{-1}) of ENV MPs compared to the control (Fig. 2). Comparing the three concentrations of exposure for each condition, a significant inhibition of GST SA was depicted in gills for the two highest concentrations (10 and 100 µg L^{-1}) of PS NPs; for 0.008 µg L^{-1} ENV NPs compared to 10 µg L^{-1} ENV NPs and for 0.008 µg L^{-1} ENV MPs compared to 100 µg L^{-1} ENV MPs. Comparing exposure to PS NPs and ENV NPs, a significant inhibition was observed after exposure to 10 and 100 µg L^{-1} of PS NPs. The exposure of clams to ENV NPs and ENV MPs led to an inhibition of the GST SA in gills after exposure to 0.008 µg L^{-1} of ENV NPs and 0.008 and 10 µg L^{-1} of ENV MPs.

Regarding GST SA in digestive glands after 7 days of exposure, a significant inhibition was shown only after exposure to 0.008 µg L^{-1} of ENV MPs compared to the control (Fig. 2). Some effects related to the concentrations in digestive glands were observed with a significant decrease of GST SA after exposure to 10 and 100 µg L^{-1} of ENV NPs compared to 0.008 µg L^{-1} and after exposure to 0.008 µg L^{-1} ENV MPs compared to 10µg L^{-1}. Comparing exposure of clams to PS NPs and ENV NPs, the lowest concentrations (0.008 and 10 µg L^{-1}) of ENV NPs induced a significant higher level of GST SA compared to the three concentrations (0.008, 10 and 100 µg L^{-1}) of PS NPs. A significant higher level of GST SA was observed

in digestive glands after exposure to the three ENV NPs concentrations and to ENV MPs 10 µg L^{-1} in comparison to 0.008 µg L^{-1} ENV MPs. After 21 days, a significant decrease of GST SA was shown in digestive glands after clam exposure to 10 and 100 µg L^{-1} PS NPs, 10 µg L^{-1} ENV NPs and 0.008 µg L^{-1} ENV MPs compared to the control (Fig. 2). Exposure of clams to 10 µg L^{-1} ENV NPs and 0.008 µg L^{-1} ENV MPs induced in digestive glands an inhibition of the GST SA compared to 0.008 µg L^{-1} ENV NPs and 10 and 100 µg L^{-1} ENV MPs respectively. Comparing exposure to ENV NPs and ENV MPs, significant lower levels of GST activity were depicted for both 10 µg L^{-1} ENV NPs and 0.008 µg L^{-1} ENV MPs compared to the other studied concentrations.

Immunity

After 7 days, a significant increase of acid phosphatase (AcP) activity was observed in gills of *S. plana* exposed to 0.008 µg L^{-1} ENV NPs compared to the control (Fig. 3). This later concentration induced a significant higher level of AcP SA compared to both other studied ENV NPs concentrations. Concerning ENV MPs, a significant higher level of AcP SA was depicted after exposure to the lowest concentration (0.008 µg L^{-1}) compared to 10 µg L^{-1}. Comparing PS NPs and ENV NPs, exposure to 100µg.L^{-1} ENV NPs led to a significant lower level of AcP SA compared to 10 and 100 µg L^{-1} PS NPs. Concerning exposure to ENV NPs and MPs, the higher level of AcP activity was depicted for 0.008 µg L^{-1} of ENV NPs significantly different from all other studied concentration except 0.008 µg L^{-} ENV MPs. After 21 days, AcP SA increased significantly in gills of clams exposed to 0.008 µg L^{-1} ENV NPs and 10 µg L^{-1} ENV MPs compared to the control (Fig. 3). No significant difference in AcP SA was observed in clam gills exposed to the different concentration of PS NPs or ENV NPs whereas a significant lower AcP activity was depicted after exposure to 0.008 µg L^{-1} of ENV MPs compared to both higher concentrations. Comparing exposures to PS NPs and ENV NPs, the clams exposed to 0.008 µg L^{-1} ENV NPs showed a significant increase in AcP SA compared to the ones exposed to 10 and 100 µg L^{-1} PS NPs. Comparing ENV MPs and NPs, a significant lower level of AcP activity was observed in gills of bivalves exposed to 0.008 µg L^{-1} of ENV MPs compared to all other conditions.

In digestive glands, after 7 days of exposure, results showed a significant increase in AcP SA for all the conditions involving environmental plastics except 0.008 µg L^{-1} ENV MPs when compared to control. No concentration-dependent differences were observed for each condition. Exposure to 10 and 100 µg L^{-1} of ENV NPs induced a significant increase of the AcP activity compared to all the concentrations of PS NPs. Concerning the comparison between ENV NPs and MPs, the clam exposure to 0.008 µg L^{-1} ENV MPs induced a significant decrease of AcP SA compared to 10 µg L^{-1} ENV NPs.

After 21 days, AcP SA decreased significantly in digestive glands of clams exposed to 0.008 µg L^{-1} PS NPs and also to all the concentrations of environmental plastics compared to the control (Fig. 3). Concerning ENV NPs, a significant decrease was shown after exposure of bivalves to 100 µg L^{-1} ENV NPs compared to 0.008 µg L^{-1} ENV NPs. Comparison between the effects of concentrations of ENV MPs

showed a significant increase after exposure to 100 µg L^{-1} ENV MPs compared to 0.008 µg L^{-1} ENV MPs. Concerning the comparison between PS NPs and ENV NPs, a significant decrease of AcP SA was depicted for all concentrations of ENV NPs versus all concentrations of PS NPs. Comparing ENV NPs and MPs, the decrease of AcP activity was higher after exposure to 100 µg L^{-1} ENV NPs and 0.008 µg L^{-1} ENV MPs vs respectively 0.008 µg L^{-1} ENV NPs and 100 µg L^{-1} ENV MPs.

Genotoxicity

After 7 days of exposure of *S. plana*, DNA damages were significantly induced in gills for all conditions in comparison to the control (Fig. 4). Exposure to 0.008 µg L^{-1} PS NPs induced a significant increase of DNA damages compared to 100 µg L^{-1} PS NPs whereas exposure to 0.008 µg L^{-1} ENV NPs induced a significant decrease of DNA damages compared to 10 and 100 µg L^{-1} ENV NPs. Concerning ENV MPs, a higher significant level of DNA damages was depicted after exposure to 100 µg L^{-1} ENV MPs. Comparing the effect of PS NPs and ENV NPs, at the concentration of 0.008 µg L^{-1}, the higher level of DNA damages was provoked by exposure to PS NPs and the lower level by exposure to ENV NPs. Concerning ENV NPs and MPs, DNA damages were significantly higher after exposure to 100 µg L^{-1} ENV MPs and lower after exposure to 0.008 µg L^{-1} ENV NPs compared to the 4 other tested concentrations. After 21 days of exposure (Fig. 4), the three concentrations of environmental NPs and MPs as well as the concentration of 100 µg L^{-1} PS NPs caused in gills a significant decrease of DNA damages compared to the control. Significative differences were shown as a decrease between 0.008 µg L^{-1} and 100 µg L^{-1} PS NPs and an increase of DNA damages between 0.008 µg L^{-1} and 100 µg L^{-1} ENV NPs. Comparing ENV NPs and MPs, all the DNA damages levels were similar except a significant higher value after exposure to 100 µg L^{-1} ENV NPs.

In the digestive glands of *S. plana*, after 7 days of exposure, when compared to control DNA damages were similar for all the conditions except after exposure to 10 µg L^{-1} PS NPs which revealed a significant lower level. After 21 days, the exposure of clams to 0.008 and 10 µg L^{-1} ENV MPs caused significant lower DNA damages compared to the control (Fig. 4). The exposure of bivalves to 10 and 100 µg L^{-1} PS NPs induced a significant decrease of the DNA damages compared to those exposed to 0.008 µg L^{-1} PS NPs. Comparing PS NPs and ENV NPs, higher significant values of DNA damages were depicted after exposure to 0.008 µg L^{-1} PS NPs and to 10 and 100 µg L^{-1} ENV NPs. Concerning ENV NPs and MPs, significant higher levels of DNA damages were observed after exposure to 10 and 100 µg L^{-1} ENV NPs as well as 100 µg L^{-1} ENV MPs compared to the other concentrations.

Burrowing test

Results of burrowing test for *Scrobicularia plana* after 21 days of exposure to the different type of plastics (PS NPs, ENV NPs and ENV MPs) at different concentrations (0.008, 10 and 100 µg L^{-1}) are reported in Table 2. Burrowing kinetic was significantly impaired in clams exposed to 10 and 100 µg L^{-1} PS NPs and to 0.008 µg L^{-1} ENV MPs. Regarding concentration effect, there was no significant

difference in burrowing behavior when concentration increased for PS NPs. The burrowing kinetic was more impaired when *S. plana* was exposed to the higher concentrations of ENV NPs (10 and 100 µg L^{-1}) and to the lower concentration of ENV MPs (0.008 µg L^{-1}). Comparison between exposure to PS NPs and ENV NPs at the same concentrations showed that PS NPs impacted more significantly the burrowing behavior of clams at 0.008 and 100 µg L^{-1}. Concerning ENV NPs and MPs, the burrowing kinetic of clams exposed to 0.008 µg L^{-1} ENV MPs was the most impacted whereas behavior was less impacted after exposure to 0.008 µg L^{-1} ENV NPs.

Table 2
Slopes of burrowing tests for *S. plana* in control condition and after exposure to standard polystyrene nanoplastics (PS NPs) and environmental micro- (ENV MPs) and nanoplastics (ENV NPs) at 0.008, 10 and 100 µg L^{-1}, (N = 10). *[Superscripts letters a, b, c, d for significant differences between conditions and in bold for significant differences between control and conditions p < 0.005).*

	Concentrations (µg L^{-1})	Slopes
Control	-	-0.0021 $^{a, b}$
PS NPs	**0.008**	-0.0006 $^{b, c}$
	10	**-0.0008** $^{c, d}$
	100	**-0.0006** $^{c, d}$
ENV NPs	**0.008**	-0.0035 ^{a}
	10	-0.0018 $^{b, c}$
	100	-0.0025 ^{b}
ENV MPs	**0.008**	**-0.0002** ^{d}
	10	-0.0019 $^{b, c}$
	100	-0.0024 $^{a, b}$

Discussion

The objectives of this study were to evaluate the toxicity of environmental MPs and NPs towards the estuarine bivalve *S. plana*, in comparison to standard PS NPs. The relevance of this study was i) the use of realistic environmental conditions of exposure such as the concentration and the field-derived MPs and NPs, long time exposure and ii) the assessment of the effects on the bivalve at different levels of biological organisation, from molecular to individual levels on both relevant tissues (gills and digestive

glands). Results obtained in this study allowed to highlight some differences in toxicity profiles of bivalves when they were exposed to i) NPs as compared to MPs, both generated from environmental macroplastics, indicating the relevance of assessing a size continuum in ecotoxicological plastic studies and ii) environmental NPs compared to standard NPs, demonstrating the importance of investigating the impacts of plastic with environmental relevance in form and composition.

Detoxification processes

In this study, the oxidative status of *S. plana* after 7 and 21 days of exposure to environmental NPs and MPs was investigated through the enzymatic activity of catalase (CAT) and glutathione-S-transferase (GST). CAT activity was only enhanced in gills after 21 days of exposure to 0.008 and 10 µg L^{-1} ENV NPs and 100 µg L^{-1} ENV MPs. The response profile of the CAT activity in gills is in accordance with the study of Ribeiro et al. (2017) showing a CAT activity increase in gills of *S.plana* after 21 days (14 days of exposure and 7 days of depuration) to PS MPs (20 µm, 1 mg L^{-1}). In our study, an opposite result was observed in digestive glands in which CAT activity was enhanced after 7 days then inhibited after 21 days of exposure by both ENV NPs 10 and 100 µg L^{-1} conditions. A similar increase of CAT activity in digestive glands has also been demonstrated in the mussel *Mytilus spp* after 10 days of exposure to 0.008 and 10 µg L of PE and PP (400µm) (Revel et al. 2019) but not in the oyster *C.gigas* after exposure to the same conditions (Revel et al. 2020). Catalase appears to contribute to a differential tissue response to counteract the oxidative stress. Catalase is an enzyme involved in the cellular first line of defence against oxidative damage catalysing the conversion of H_2O_2 - the main precursor of hydroxyl radicals in aquatic organisms (Regoli and Giuliani 2014) - into H_2O and O_2. As regards to GST activity, it was significantly reduced in gills after 7 days of clam exposure to 0.008 and 10 µg L^{-1} ENV MPs, whereas in digestive glands, GST activity was inhibited for both duration (7 and 21 days) after exposure to 0.008 µg L^{-1} ENV MPs and only at 21 days for 10 µg L^{-1} ENV NPs. In both organs, gills and digestive glands and for both duration exposure (7 and 21 days) GST is significantly inhibited after exposure to 0.008 µg L^{-1} ENV MPs but also at 21 days in digestive glands after exposure to 10 µg L^{-1} ENV NPs. GST is the main phase II enzyme involved in the oxidative stress response which catalyses the conjugation of xenobiotics and endogenous compounds to the GSH (reduced glutathione), allowing the cellular detoxification of their more soluble form and limiting their oxidative impact on cellular molecules and structures. Results suggest a stronger response of the antioxidant system to both ENV NPs and MPs in the digestive gland than in the gills. This is in accordance with the literature on the accumulation of MPs and NPs in bivalves showing that the target organ of these particles is the digestive gland so called a sentinel tissue (Sendra et al. 2021). MPs ingested and accumulated in gills, through microvilli and endocytosis may then be transferred to others organs *via* the haemolymph (Ribeiro et al. 2017). MPs are also ingested through the inhalant siphon and then transported through the digestive gland where the injury could be size dependant (Islam et al. 2021; Ribeiro et al. 2017). Ribeiro et al. (2017) noted the presence of PS MPs essentially in the digestive gland which could explain the higher detoxification activity observed in digestive glands in our study. Li et al. (2021a) used fluorescent tracing to demonstrate that NPs mainly

accumulate in gills, intestine and stomach of *Corbicula fluminea* with a stronger response to oxidative stress expressed in the visceral mass. A meta-analysis conducted by Li et al. (2021b) specifically on bivalves and their response to oxidative stress induced by MPs showed the strong response of the anti-oxidant system during MPs short-term exposure to counteract the imbalance of ROS production. Our study is in accordance with this report for both ENV NPs and ENV MPs and showed that a longer exposure to plastic particles whatever their size – nano or micro - as it could be found in environmental ecosystems (chronical exposure) led to an oxidative imbalance that the antioxidant system can no longer counteract resulting in weaker CAT and GST activities in the exposed organisms than in the control organisms after 21 days. Furthermore, based on their review, Li et al. (2021b) suggested to favour catalase activity rather than GST one as a reliable biomarker for long-term studies of exposure to MPs. Concerning *S. plana* PS NPs exposure, it is important to highlight in our study that only the longer-term exposure (21 days) caused a response: a weaker activity of the antioxidant system in the digestive glands. These results show the importance of studying the effects of environmental plastic particles since the responses of organisms differ from standard NPs used in numerous studies.

Immune response

Baroja et al. (2021) reported that the most frequently investigated responses of bivalves to MPs exposure concerned the immunotoxicity. Numerous studies have demonstrated that MPs and NPs hampered immune responses in exposed bivalves as the mussel *Mytilus galloprovincialis* (Avio et al. 2015; Cole et al. 2020) or the blood clam *Tegillarca granosa* (Tang et al. 2020; Zhou et al. 2021). In our study, the immune response follows the same pattern as the antioxidant response. The acid phosphatase activity in *S. plana* was less impacted in gills than in digestive glands. In this later organ, the AcP activity is enhanced after 7 days of exposure to both ENV NPs and ENV MPs and inhibited after 21 days when compared to the activity in non-exposed organisms. No difference of response was evidenced in relation to size whereas in the literature MPs appear to be more immunotoxic to the bivalves than NPs (Sendra et al. 2020).

Genotoxicity

DNA damages can be produced by the accumulation of MPs and NPs in the organs of bivalves leading to alterations at the cellular level linked to physical interactions between cellular structures including nucleus and NPs. However, these interactions may also be caused by the increased intracellular ROS concentration in relation with the size or the genotoxic substances as metals present within or absorb on plastic particles. In our study, after 7 days of *S. plana* exposure, all the organisms exposed to all tested MPs and NPs presented significant higher DNA damages in gills compared to the control group and between plastics type at 0.008 and 100µg.L^{-1}. Damages are similarly to the ones induced by the exposure for 7 days to 1 mg L^{-1} of PS MPs (Ribeiro et al. 2017) and also after 7 days exposure to 1 mg L^{-1} of LDPE MPs (low-density polyethylene microplastics) oxybenzone-associated (O'Donovan et al. 2020). Genotoxicity could be related to an oxidative stress non supported by the antioxidant system, which would be consistent with the lack of GST activity in gills after 7 days exposure.

Individual markers

Behavior and condition index

Among the most frequently affected biochemical endpoints, regardless of the exposure period or contaminant (MPs, NPs, pharmaceutical drugs) are oxidative stress related endpoints and behavior (Silva et al. 2020b; Li et al. 2021). Thus, besides biochemical response category of biomarkers, behavioral biomarkers are sensitive tools in assessing the impact of the contaminants at concentrations far below the lethal effect (Amiard-Triquet 2009; Bonnard et al. 2009)

This study shows that after 21 days of exposure to plastic particles, standard PS NPs exhibit more alterations in burrowing activity compared to environmental NPs and MPs. Our findings highlighted also that for *S. plana* PS NPs exposure, only the longer-term exposure (21 days) caused a response: a weaker activity of the antioxidant system in the digestive glands. Uptake of nano-PS beads (~ 30 nm) was found to alter the behavior of the blue mussel, reflected by a significant reduction in the filtering activity, valve opening, production of pseudofaeces (Wegner et al. 2012) and clearance rate (Van Cauwenberghe et al. 2013). Exposed *H. diversicolor* to PS NPs concentrations from 0.005 to 0.5 mg L^{-1} demonstrated an increase in burrowing time associated with an overall decrease in acetylcholinesterase (AChE) activity (Silva et al. 2020a). As reported by Oliveira et al. (2013), MPs were able to significantly inhibit AChE (by an average of 22%), an inhibition rate that has been considered high enough to induce adverse effects in neurofunction (Ludke et al. 1975) and thus on burrowing and feeding activity as demonstrated by the ingestion of sediments containing 7.4% polystyrene MPs by the lugworm *Arenicola marina* (Besseling et al. 2013).

Difference between groups with a significant effect of PS NPs compared to environmental NPs and MPs could be explained by clams adjusting their ingestion rate by an active recognition of MPs as for the mussel *Mytilus galloprovincialis* (Masia et al. 2021). The absence of effect on burrowing behavior of two sediment-dwelling bivalve species *Ennucula tenuis* and *Abra nitida* exposed to polyethylene MPs (1; 10 and 25 mg kg^{-1} of sediment) for four weeks suggests that the observed decrease in energy was not sufficient to impact burrowing (Bour et al. 2018) in accordance with other studies showing that condition indices were not altered in bivalves exposed to microplastic, despite toxic effects observed at the cellular level (Ribeiro et al. 2017; von Moos et al. 2012).

Importance of testing environmental plastics versus standard ones

The originality of this study was to test MPs and NPs generated from macro sized plastic collected from the environment. In this sense, the diversity of type of plastic, shape, composition allow to evaluate the complexity of these contaminants in the context of risk assessment. This study underlined differences in *S. plana* exposed to environmental NPs as compared to PS NPs both at sub-individual and individual levels. In particular, results showed no effects on detoxification and immune response of PS NPs in clams

exposed for 7 days as compared to organisms exposed to ENV NPs. After 21 days of exposure, while in digestive glands similar profiles were observed after exposures to PS NPs and ENV NPs, in gills only ENV NPs induced toxicity through induction of detoxification, immunity and DNA damage processes. These results are in accordance with previous studies lead on oysters (Arini et al. 2022). This shows the necessity of investigating the impact of realistic plastic polymers (in terms of composition, size and form), found in natural environments to characterize their toxicity towards aquatic organisms (Piccardo et al. 2020).

The concentration of additives in plastic is considerably greater (orders of magnitude) than that of anthropogenic chemicals that are 'sorbed' to plastics in the environment (Hermabessiere et al. 2017; Hahladakis et al. 2018). Therefore, the chemical additives in plastic could present a considerably higher potential risk to organisms exposed to them in the environment (Murphy 2017). To our knowledge, studies that fully characterize plastics collected in natural environments before exposing organisms to related microplastics are scarce. In the present study, a particular attention was made on the evaluation of chemical contents on the environmental MPs and NPs tested. Among the chemicals present in the MPs and NPs, metals have been shown to be highly abundant. Peculiar attention must also be accorded to the standard ones which can contain potentially toxic stabilizing additives.

Metals are present in MPs and NPs as additives incorporated during their production or after being adsorbed on the surface of plastic particles (Godoy et al. 2019; Liu et al. 2021; Huang et al. 2021). These metals might be transferred from the MPs and NPs to the aquatic organisms. Some studies have shown that plastic particles associated with metals are more toxic than virgin plastic particles (Baudrimont et al. 2019). For instance, the co-exposure of MPs and Hg reduced levels of several biological parameters (filtration rate, cholinesterase enzymes, S-transferases, and the levels of lipid peroxidation) in *Corbicula fluminea* (Fernández et al. 2020; Oliveira et al. 2018). In addition, Zhang et al. (2020) and Cheng et al. (2021) showed antagonistic or synergistic toxicity when zebrafish embryos were exposed both to MPs and Cadmium, depending upon the concentration and the form of tested MPs.

Importance of investigating NPs – MPs continuum in plastic risk assessment

Another aspect to take into consideration is the continuum in size of plastics (Latchere et al. 2020). Indeed, testing the effects of NPs and MPs coming from a common set of macro sized plastics towards organisms will allow to understand the only influence of size on their toxicity mechanisms, with the same initial characteristics (composition, adsorbed contaminants). In the present study, while quite similar effects were observed at 7 days of exposure on DNA damage and immunity for gills and digestive glands, induction of detoxification processes were different between ENV NPs and ENV MPs for both organs. On the opposite, after 21 days of exposure, differences between environmental NPs and MPs were only observed for CAT activity and DNA damage measurements in digestive glands and for GST activity in gills. This suggests a more marked effect of size on toxicity mechanisms at 7 days as compared to 21 days. These findings are in accordance with the study reported by Islam et al. (2021) revealing that *S.*

plana exposed to two sizes (4–6 µm and 20–25 µm) of virgin and PFOS-adsorbed MPs 1 mg L^{-1} increase their ingestion with time (7 and 14 days) in whole soft tissues. As shown in Arini et al. (2022), this study underlined the importance of testing NPs from environmental sources because their aggregation state could modulate their toxicity. In this sense, Baudrimont et al. (2019) showed on bivalve and microalgae that the aggregation state of PE NPs reduced their bioavailability and their toxicity when the concentration of exposure was increasing.

Conclusion

This study showed the importance of taking the plastic pollution as a whole for a better evaluation of risk assessment. In this context, assessing the continuum of size, from MPs to NPs coming from the same batch of macro-sized plastics collected from the environment allow to efficiently compare the effect of size on the estuarine bivalve *S. plana*. Considering different tissues (gills and digestive glands) and biological levels of responses (from individual to sub-individual levels), under realistic concentrations of exposure, it was possible to define difference in toxicity profiles depending on the size of plastics. Moreover, standard PS NP microbeads did not present any similar toxicity pattern as compared to ENV NPs indicating that studying the effects of this kind of plastic will not allow to fully understand the impact of realistic plastic polymers on biota.

This study also allows to understand the effects of MPs and NPs on *S. plana* that is a key species for the functioning of estuarine ecosystem. Whereas many studies investigate the effects of MPs and NPs in marine and freshwater environments, it is also important to consider effects on estuarine medium that present a mixture of freshwater and marine water and particular sediment characteristics (Latchere et al. 2021).

Finally, the plastic pollution represents a global pollution affecting different ecosystems (soil, water, air) and it appears necessary to investigate the impact of MPs and NPs at a larger scale, such as through a trophic chain, through a continuum from the soil to the water in order to understand their effect under more realistic conditions.

Declarations

Compliance with Ethical Standards

Ethical Approval: Not applicable. This article does not contain any studies with human participants. The animal used in this article is an aquatic invertebrate.

Consent to Participate: All authors agreed with the content of this article.

Consent to Publish: All authors gave explicit consent to submit this article.

Authors Contributions: Macroplastics were sampled by Oihana Latchere, Agnès-Feurtet-Mazel and Magalie Baudrimont. Micro and nanoplastics were generated and analysed by Julien Gigault. Chemical analysis of the micro and nanoplastic were conducted by Charlotte Catrouillet. Oihana Latchere, Hanane Perrein-Ettajani, Mohammed Mouloud, Didier Georges, Isabelle Métais, Amélie Châtel, participated in clam sampling and clam dissection after exposure. Oihana Latchere and Thybaud Audroin conducted the clam exposure. Biomarkers analysis were performed by Coraline Roman and Isabelle Métais. The first draft of the manuscript was written by Isabelle Métais and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript."

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References

1. Akcha F, Burgeot T, Budzinski H, Pfohl-Leszkowicz A, Narbonne JF (2000) Induction and elimination of bulky benzo[a]pyrene-related DNA adducts and 8-oxodGuo in mussels *Mytilus galloprovincialis* exposed *in vivo* to B[a]P-contaminated feed. Mar Ecol Prog Ser 205:195–206. https://doi.org/10.3354/meps205195
2. Amiard-Triquet C (2009) Behavioral disturbances: the missing link between suborganismal and supra-organismal responses to stress? Prospects based on aquatic research. Hum Ecol Risk Assess 15:87–110. https://doi.org/10.1080/10807030802615543
3. Andrady AL (2011) Microplastics in the marine environment. Mar Pollut Bull 62(8):1596–1605. https://doi.org/10.1016/j.marpolbul.2011.05.030
4. Andrady AL (2015) Persistence of plastic litter in the oceans. In: Bergmann M, Gutow L, Klages M (ed) Marine Anthropogenic Litter, pp57–72. https://doi.org/10.1007/978-3-319-16510-3_3

5. Arini A, Gigault J, Venel Z, Bertucci A, Baudrimont M (2022) The underestimated toxic effects of nanoplastics coming from marine sources: a demonstration on oysters (*Isognomon alatus*). Chemosphere 295:133824. https://doi.org/10.1016/j.chemosphere.2022.133824
6. Arthur C, Baker J, Bamford H (eds) (2008) Proceedings of the international research workshop on the occurrence, effects, and fate of microplastic marine debris. Sept 9–11. NOAA Technical Memorandum NOS-OR&R-30
7. Ateia M, Zheng T, Calace S, Tharayil N, Pilla S, Karanfil T (2020) Sorption behavior of real microplastics (MPs): insights for organic micropollutants adsorption on a large set of well-characterized MPs. Sci Total Environ 720:137634. https://doi.org/10.1016/j.scitotenv.2020.137634
8. Avio CG, 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 198:211–222. https://doi.org/10.1016/j.envpol.2014.12.021
9. Baroja E, Christoforou E, Lindström J, Spatharis S (2021) Effects of microplastics on bivalves: Are experimental settings reflecting conditions in the field? Mar Pollut Bull 171:112696. https://doi.org/10.1016/j.marpolbul.2021.112696
10. Barranger A, Akcha F, Rouxel J, Brizard R, Maurouard E, Pallud M, Menard D et al (2014) Study of genetic damage in the japanese oyster induced by an environmentally-relevant exposure to diuron: evidence of vertical transmission of DNA damage. Aquat Toxicol 146:93–104. https://doi.org/10.1016/j.aquatox.2013.10.032
11. Baudrimont M, Arini A, Guégan C, Venel Z, Gigault J, Pedrono B, Prunier J, Maurice L, Ter Halle A, Feurtet-Mazel A (2019) Ecotoxicity of polyethylene nanoplastics from the North Atlantic oceanic gyre on freshwater and marine organisms (microalgae and filter-feeding bivalves). Environ Sci Pollut Res 27(4):3746–3755. https://doi.org/10.1007/s11356-019-04668-3
12. Bergami E, Pugnalini S, Vannuccini ML, Manfra L, Faleri C, Savorelli F, Dawson KA, Corsi I (2017) Long-term toxicity of surface-charged polystyrene nanoplastics to marine planktonic species *Dunaliella tertiolecta* and *Artemia franciscana*. Aquat Toxicol 189:159–169. https://doi.org/10.1016/j.aquatox.2017.06.008
13. Besseling E, Wegner A, Foekema EM, van den Heuvel-Greve MJ, Koelmans AA (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
14. Besseling E, Wang B, Lürling M, Koelmans AA (2014) Nanoplastic affects growth of *S. obliquus* and reproduction of *D. magna*. Environ Sci Technol 48(20):12336–12343. https://doi.org/10.1021/es503001d
15. Blancho F, Davranche M, Fumagalli F, Ceccone G, Gigault J (2021) A reliable procedure to obtain environmentally relevant nanoplastic proxies. Environ Sci : Nano 8:3211–3219. https://doi.org/10.1039/D1EN00395J
16. Bonnard M, Roméo M, Amiard-Triquet C (2009) Effects of copper on the burrowing behavior of estuarine and coastal invertebrates, the polychaete *Nereis diversicolor* and the bivalve *Scrobicularia*

plana. Hum Ecol Risk Assess 15(1):11–26. https://doi.org/10.1080/10807030802614934

17. Bour A, Haarr A, Keiter S, Hylland K (2018) Environmentally relevant microplastic exposure affects sediment-dwelling bivalves. Environ Pollut 236:652–660. https://doi.org/10.1016/j.envpol.2018.02.006
18. Bradford M (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254. https://doi.org/10.1016/0003-2697(76)90527-3
19. Browne MA, Galloway T, Thompson R (2007) Microplastic-an emerging contaminant of potential concern? Integr Environ Assess Manage. 3:559–561. https://doi.org/10.1002/ieam.5630030412. 4
20. Browne MA, Dissanayake A, Galloway TS, Lowe DM, Thompson RC (2008) Ingested microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L.). Environ Sci Technol 42(13):5026–5031. https://doi.org/10.1021/es800249a
21. Browne MA, Crump P, Niven SJ, Teuten E, Tonkin A, Galloway T, Thompson R (2011) Accumulation of microplastic on shorelines worldwide: sources and sinks. Environ Sci Technol 45(21):9175–9179. https://doi.org/10.1021/es201811s
22. Buffet PE, Zalouk-Vergnoux A, Châtel A, Berthet B, Métais I, Perrein-Ettajani H, Poirier L et al (2014) A marine mesocosm study on the environmental fate of silver nanoparticles and toxicity effects on two endobenthic species: the ragworm *Hediste diversicolor* and the bivalve mollusc *Scrobicularia plana*. Sci Total Environ 470–471:1151–1159. https://doi.org/10.1016/j.scitotenv.2013.10.114
23. Chae Y, An YJ (2017) Effects of micro- and nanoplastics on aquatic ecosystems: current research trends and perspectives. Mar Poll Bul 124(2):624–632. https://doi.org/10.1016/j.marpolbul.2017.01.070
24. Châtel A, Bruneau M, Lièvre C, Goupil A, Mouneyrac C (2017) Spermatozoa: a relevant biological target for genotoxicity assessment of contaminants in the estuarine bivalve *Scrobicularia plana*. Mar Pollut Bull 116(1–2):488–490. https://doi.org/10.1016/j.marpolbul.2016.12.062
25. Cheng H, Feng Y, Duan Z, Duan X, Zhao S, Wang Y, Gong Z, Lei, Wang (2021) Toxicities of microplastic fibers and granules on the development of zebrafish embryos and their combined effects with cadmium. Chemosphere 269:128677. https://doi.org/10.1016/j.chemosphere.2020.128677
26. Claiborne A (1985) Catalase activity. In: Greenwald RA (ed) Handbook of methods for oxygen radical research. CRC Press, Boca Raton, pp 283–284
27. Cole M, Lindeque P, Halsband C, Galloway TS (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
28. Cole M, Lindeque P, Fileman E, Halsban C, Galloway TS (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

29. Cole M, Liddle C, Consolandi G, Drago C, Hird C, Lindeque PK, Galloway TS (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.marpolbul.2020.111552
30. Da Costa JP, Santos PSM, Duarte AC, Rocha-Santos T (2016) (Nano)plastics in the environment - Sources, Fates and Effects. Sci Total Environ 566–567:15–26. https://doi.org/10.1016/j.scitotenv.2016.05.041
31. Derraik JGB (2002) The pollution of the marine environment by plastic debris: a review. Mar Pollut Bull 44(9):842–852. https://doi.org/10.1016/S0025-326X(02)00220-5
32. de Souza Machado AA, Kloas W, Zarfl C, Hempel S, Rillig MC (2018) Microplastics as an Emerging Threat to Terrestrial Ecosystems. Global Change Biol 24(4):1405–1416. https://doi.org/10.1111/gcb.14020
33. Doyle MJ, Watson W, Bowlin NM, Sheavly SB (2011) Plastic particles in coastal pelagic ecosystems of the Northeast Pacific Ocean. Mar Environ Res 71(1):41–52. https://doi.org/10.1016/j.marenvres.2010.10.001
34. Fendall LS, Sewell MA (2009) Contributing to marine pollution by washing your face: microplastics in facial cleansers. Mar Pollut Bull 58(8):1225–1228. https://doi.org/10.1016/j.marpolbul.2009.04.025
35. Fernández B, Santos-Echeandía J, Rivera-Hernández JR, Garrido S, Albentosa M (2020) Mercury interactions with algal and plastic microparticles: Comparative role as vectors of metals for the mussel, *Mytilus galloprovincialis*. J Hazard Mater 396:122739. https://doi.org/10.1016/j.jhazmat.2020.122739
36. Gall SC, Thompson RC (2015) The impact of debris on marine life. Mar Pollut Bull 92(1–2):170–179. https://doi.org/10.1016/j.marpolbul.2014.12.041
37. Galloway TS, Cole M, Lewis C (2017) Interactions of microplastic debris throughout the marine ecosystem. Nat Ecol Evol 1(5):1–8. https://doi.org/10.1038/s41559-017-0116
38. Gardon T, Reisser C, Soyez C, Quillien V, Le Moullac G (2018) Microplastics affect energy balance and gametogenesis in the pearl oyster *Pinctada margaritifera*. Environ Sci Technol 52(9):5277–5286. https://doi.org/10.1021/acs.est.8b00168
39. Geyer R, Jambeck J, Law K (2017) Production, use, and fate of all plastics ever made. Sci Adv 3(7):25–29. https://doi.org/10.1126/sciadv.1700782
40. Gigault J, Pedrono B, Maxit B, Ter Halle A (2016) Marine plastic litter: the unanalyzed nano-fraction. Environ Sci Nano 3(2):346–350. https://doi.org/10.1039/c6en00008h
41. Gigault J, Ter Halle A, Baudrimont M, Pascal PY, Gauffre F, Phi TL, El Hadri H, Grassl B, Reynaud S (2018) Current opinion: what is a nanoplastic? Environ Pollut 235:1030–1034. https://doi.org/10.1016/j.envpol.2018.01.024
42. Godoy V, Blázquez G, Calero M, Quesada L, Martín-Lara MA (2019) The potential of microplastics as carriers of metals. Environ Pollut 255:113363. https://doi.org/10.1016/j.envpol.2019.113363
43. Goldstein MC, Titmus AJ, Ford M (2013) Scales of spatial heterogeneity of plastic 459 marine debris in the Northeast Pacific Ocean. PLoS ONE 8(11):1–11.

https://doi.org/10.1371/journal.pone.0080020

44. Gregory MR (1996) Plastic scrubbers' in hand cleansers: a further (and minor) source for marine pollution identified. Mar Pollut Bull 32(12):867–871. https://doi.org/10.1016/S0025-326X(96)00047-1
45. Habig WH, Pabst MJ, Jakoby WB (1974) Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 249(22):7130–7139 PMID: 4436300
46. Hahladakis JN, Velis CA, Weber R, Iacovidou E, Purnell P (2018) An overview of chemical additives present in plastics: migration, release, fate and environmental impact during their use, disposal and recycling. J Hazard Mater 344:179–199. https://doi.org/10.1016/j.jhazmat.2017.10.014
47. Hermabessiere L, Dehaut A, Paul-Pont I, Lacroix C, Jezequel R, Soudant P, Duflos G (2017) Occurrence and effects of plastic additives on marine environments and organisms: A review. Chemosphere 182:781–793. https://doi.org/10.1016/j.chemosphere.2017.05.096
48. Horton AA, Walton A, Spurgeon DJ, Lahive E, Svendsen C (2017) Microplastics in freshwater and terrestrial environments: evaluating the current understanding to identify the knowledge gaps and future research priorities. Sci Total Environ 586:127–141. https://doi.org/10.1016/j.scitotenv.2017.01.190
49. Huang W, Song B, Liang J, Niu Q, Zeng G, Shen M, Deng J, Luo Y, Wen X, Zhang Y (2021) Microplastics and associated contaminants in the aquatic environment: a review on their ecotoxicological effects, trophic transfer, and potential impacts to human health. J Hazard Mater 405:124187. https://doi.org/10.1016/j.jhazmat.2020.124187
50. Islam A, da Fonseca TG, Vilke J, Gonçalves JM, Pedro P, Keiter S, Cunha SC, Fernandes JO, Bebianno MJ (2021) Perfluorooctane sulfonic acid (PFOS) adsorbed to polyethylene microplastics: Accumulation and ecotoxicological effects in the clam *Scrobicularia plana*. Mar Environ Res 164:105249. https://doi.org/10.1016/j.marenvres.2020.105249
51. Jin Y, Xia J, Pan Z, Yang J, Wang W, Fu Z (2018) Polystyrene microplastics induce microbiota dysbiosis and inflammation in the gut of adult zebrafish. Environ Pollut 235:322–329. https://doi.org/10.1016/j.envpol.2017.12.088
52. Kale SK, Deshmukh AG, Dudhare MS, Patil VB (2015) Microbial degradation of plastic: a review. J Biochem Tech 6(2):952–961
53. Koelmans AA, Besseling E, Shim WJ (2015) Nanoplastics in the aquatic environment. Critical review. In: Bergmann M, Gutow L, Klages M (eds) Marine Anthropogenic Litter. Springer, Cham. https://doi-org.srvext.uco.fr/ 10.1007/978-3-319-16510-3_12
54. Lambert S, Wagner M (2016) Characterisation of nanoplastics during the degradation of polystyrene. Chemosphere 145:265–268. https://doi.org/10.1016/j.chemosphere.2015.11.078
55. Latchere O, Audroin T, Hétier J, Métais I, Châtel A (2021) The need to investigate continuums of plastic particle diversity, brackish environments and trophic transfer to assess the risk of micro and nanoplastics on aquatic organisms. Environ Pollut 6(273):116449. https://doi.org/10.1016/j.envpol.2021.116449

56. Law KL, Morét-Ferguson S, Maximenko NA, Proskurowski G, Peacock EE, Hafner J, Reddy CM (2010) Plastic accumulation in the North Atlantic subtropical gyre. Science 329(5996):1185–1188. https://doi.org/10.1126/science.1192321
57. Lebordais M, Venel Z, Gigault J, Langlois V, Baudrimont M (2021) Impacts of dietary exposure to nanoplastics combined or not with arsenic in the Guadeloupean mangrove oysters (*Isognomon alatus*). Nanomaterials 11(5):1151. https://doi.org/10.3390/nano11051151
58. Lei L, Wu S, Lu S, Liu M, Song Y, Fu Z, Shi H, Raley-Susman KM, He D (2018) Microplastic particles cause intestinal damage and other adverse effects in zebrafish *Danio rerio* and nematode *Caenorhabditis elegans*. Sci Total Environ 619–620:1–8. https://doi.org/10.1016/j.scitotenv.2017.11.103
59. Li Z, Feng C, Pang W, Tian C, Zhao Y (2021a) Nanoplastic-Induced Genotoxicity and Intestinal Damage in Freshwater Benthic Clams (*Corbicula fluminea*): Comparison with Microplastics. ACS Nano 15(6):9469–9481. https://doi.org/10.1021/acsnano.1c02407
60. Li Z, Chang X, Hu M, Fang JKH, Huang W, Xu EG, Wang Y (2021b) Is microplastic an oxidative stressor? Evidence from a meta-analysis on bivalves. J Hazard Mater. https://doi.org/10.1016/j.jhazmat.2021.127211
61. Liu S, Shi J, Wang J, Dai Y, Li H, Li J, Liu X, Chen X, Wang Z, Zhang P (2021) Interactions between microplastics and heavy metals in aquatic environments: a review. Front Microbiol 12:1–14. https://doi.org/10.3389/fmicb.2021.652520
62. Ludke JL, Hill EF, Dieter MP (1975) Cholinesterase (ChE) response and related mortality among birds fed ChE inhibitors. Arch Environ Contam Toxicol 3:1–21. https://doi-org.srvext.uco.fr/10.1007/BF02221128
63. Masiá P, Ardura A, García-Vázque E (2021) Virgin polystyrene microparticles exposure leads to changes in gills DNA and physical condition in the Mediterranean mussel *Mytilus galloprovincialis*. Animals 11(8):2317. https://doi.org/10.3390/ani11082317
64. Moore CJ (2008) Synthetic polymers in the marine environment: a rapidly increasing, long-term threat. Environ Res 108(2):131–139. https://doi.org/10.1016/j.envres.2008.07.025
65. Mouneyrac C, Lino ST, Amiard JC, Amiard-Triquet C, Métais I, Durou C, Minier C, Pellerin J (2008) biological indices, energy reserves, steroid hormones and sexual maturity in the infaunal bivalve *Scrobicularia plana* from three sites differing by their level of contamination. Gen Comp Endocrinol 157(2):133–141. https://doi.org/10.1016/j.ygcen.2008.04.010
66. Mouneyrac C, Buffet PE, Poirier L, Zalouk-Vergnoux A, Guibbolini M, Risso de Faverney C, Gilliland D et al (2014) Fate and effects of metal-based nanoparticles in two marine invertebrates, the bivalve mollusc *Scrobicularia plana* and the annelid polychaete *Hediste diversicolor*. Environ Sci Pollut Res 21(13):7899–7912. https://doi.org/10.1007/s11356-014-2745-7
67. Murphy M (2017) Microplastics expert worshop report. Trash free waters dialogue meeting.June28–29

68. O'Donovan S, Mestre NC, Abel S, Fonseca TG, Carteny CC, Cormier B, Keiter SH, Bebianno MJ (2018) Ecotoxicological effects of chemical contaminants adsorbed to microplastics in the clam *Scrobicularia plana*. Front Mar Sci 5:1–15. https://doi.org/10.3389/fmars.2018.00143
69. O'Donovan S, Mestre NC, Abel S, Fonseca TG, Carteny CC, Willems T, Prinsen E, Cormier B, Keiter SS, Bebianno MJ (2020) Effects of the UV filter, oxybenzone, adsorbed to microplastics in the clam *Scrobicularia plana*. Sci Total Environ 733:139102. https://doi.org/10.1016/j.scitotenv.2020.139102
70. Oliveira P, Barboza LGA, Branco V, Figueiredo N, Carvalho C, Guilhermino L (2018) Effects of microplastics and mercury in the freshwater bivalve *Corbicula fluminea* (Müller, 1774): Filtration rate, biochemical biomarkers and mercury bioconcentration. Ecotoxicol Environ Saf 164:155–163. https://doi.org/10.1016/j.ecoenv.2018.07.062
71. Oliveira M, Ribeiro A, Hylland K, Guilhermino L (2013) Single and combined effects of microplastics and pyrene on juveniles (0 + group) of the common goby *Pomatoschistus microps* (Teleostei, Gobiidae). Ecol Indic 34:641–647. https://doi.org/10.1016/j.ecolind.2013.06.019
72. Phuong NN, Zalouk-Vergnoux A, Poirier L, Kamari A, Châtel A, Mouneyrac C, Lagarde F (2016) Is there any consistency between the microplastics found in the field and those used in laboratory experiments? Environ Pollut 211:111–123. https://doi.org/10.1016/j.envpol.2015.12.035
73. Piccardo M, Renzi M, Terlizzi A (2020) Nanoplastics in the oceans: Theory, experimental evidence and real world. Mar Pollut Bull 157:111317. https://doi.org/10.1016/j.marpolbul.2020.111317
74. Regoli F, Giuliani ME (2014) Oxidative pathways of chemical toxicity and oxidative stress biomarkers in marine organisms. Mar Environ Res 93:106–117. https://doi.org/10.1016/j.marenvres.2013.07.006
75. Revel M, Lagarde F, Perrein-Ettajani H, Bruneau M, Akcha F, Sussarellu R, Rouxel J, Costil K, Decottignies P, Cognie B, Châtel A, Mouneyrac C (2019) Tissue-specific biomarker responses in the blue mussel *Mytilus* spp. exposed to a mixture of microplastics at environmentally relevant concentrations. Frontiers 7(33). https://doi.org/10.3389/fenvs.2019.00033
76. Revel M, Châtel A, Perrein-Ettajani H, Bruneau M, Akcha F, Sussarellu R, Rouxel J, Costil K, Decottignies P, Cognie B, Lagarde F, Mouneyrac C (2020) Realistic environmental exposure to microplastics does not induce biological effects in the Pacific oyster *Crassostrea gigas*. Mar Pollut Bull 150:110627. https://doi.org/10.1016/j.marpolbul.2019.110627
77. Ribeiro F, Garcia AR, Pereira BP, Fonseca M, Mestre NC, Fonseca TG, Ilharco LM, Bebianno MJ (2017) Microplastics effects *in Scrobicularia plana*. Mar Pollut Bull 122(1–2):379–391. https://doi.org/10.1016/j.marpolbul.2017.06.078
78. Rossi G, Monticelli L (2014) Modeling the effect of nano-sized polymer particles on the properties of lipid membranes. J Phys:Condens Matter 26(50):1–11. https://doi.org/10.1088/0953-8984/26/50/503101
79. Sadri SS, Thompson RC (2014) On the quantity and composition of floating plastic debris entering and leaving the Tamar estuary, Southwest England. Mar Pollut Bull 81(1):55–60. https://doi.org/10.1016/j.marpolbul.2014.02.020

80. Sardon H, Dove AP (2018) Plastics recycling with a difference. Science 360(6387):380–381. https://doi.org/10.1126/science.aat4997
81. Sendra M, Sparaventi E, Novoa B, Figueras A (2021) An overview of the internalization and effects of microplastics and nanoplastics as pollutants of emerging concern in bivalves. Sci Total Environ 753:142024. https://doi.org/10.1016/j.scitotenv.2020.142024
82. Silva MSS, Oliveira M, Valente P, Figueira E, Martins M, Pires A (2020a) Behavior and biochemical responses of the polychaeta *Hediste diversicolor* to polystyrene nanoplastics. Sci Total Environ 707:134434. https://doi.org/10.1016/j. scitotenv.2019.134434
83. Silva MSS, Pires A, Almeida M, Oliveira M (2020b) The use of *Hediste diversicolor* in the study of emerging contaminants. Mar Environ Res 159:105013. https://doi.org/10.1016/j.marenvres.2020.105013
84. Solé M, Kopecka-Pilarczyk J, Blasco J (2009) Pollution biomarkers in two estuarine invertebrates, *Nereis diversicolor* and *Scrobicularia plana*, from a marsh ecosystem in SW Spain. Environ Int 35(3):523–531. https://doi.org/10.1016/j.envint.2008.09.013
85. Sussarellu R, Suquet M, Thomas Y, Lambert C, Fabioux C, Pernet ME, Le Goïc N, Quillien V, Mingant C, Epelboin Y, Corporeau C, Guyomarch J, Robbens J, Paul-Pont I, Soudant P, Huvet A (2016) Oyster reproduction is affected by exposure to polystyrene microplastics. Proc Natl Acad Sci U S A 1(9):2430–2435. https://doi.org/10.1073/pnas.1519019113
86. Tang Y, Zhou W, Sun S, Du X, Han Y, Shi W, Liu G (2020) Immunotoxicity and neurotoxicity of bisphenol A and microplastics alone or in combination to a bivalve species, *Tegillarca granosa*. Environ Pollut 265:115115. https://doi.org/10.1016/j.envpol.2020.115115
87. Ter Halle A, Jeanneau L, Martignac M, Jardé E, Pedrono B, Brach L, Gigault J (2017) Nanoplastic in the north atlantic subtropical gyre. Environ Sci Technol 51(23):13689–13697. https://doi.org/10.1021/acs.est.7b03667
88. Thompson RC, Moore CJ, Votn Saal FS, Swan SH (2009) Plastics, the environment and human health: current consensus and future trends. Philos Trans R Soc Lond B 364(1526):2153–2166. https://doi.org/10.1098/rstb.2009.0053
89. Van Cauwenberghe L, Claessens M, Janssen CR (2013) Selective uptake of microplastics by a marine bivalve (*Mytilus edulis*). Commun Agric Appl Biol Sci 78(1):25–27 PMID: 23875293
90. Velzeboer I, Kwadijk CJAF, Koelmans AA (2014) Strong sorption of PCBs to nanoplastics, microplastics, carbon nanotubes, and fullerenes. Environ Sci Technol 48(9):4869–4876. https://doi.org/10.1021/es405721v
91. Vermeiren P, Muñoz CC, Ikejima K (2016) Sources and sinks of plastic debris in estuaries: a conceptual model integrating biological, physical and chemical distribution mechanisms. Mar Pollut Bull 113(1–2):7–16. https://doi.org/10.1016/j.marpolbul.2016.10.002
92. Von Moos N, Burkhardt-Holm P, Kohler A (2012) Uptake and effects of microplastics on cells and tissue of the blue mussel *Mytilus edulis* L. after an experimental exposure. Environ Sci Technol 46:11327–11335. https://doi.org/10.1021/es302332w

93. Walne R (1976) Experiments on the culture in the sea of the butterfish *Venerupis decussata* L. Aquaculture 8(4):371–381. https://doi.org/10.1016/0044-8486(76)90119-8
94. Wang J, Tan Z, Peng J, Qiu Q, Li M (2016) The behaviors of microplastics in the marine environment. Mar Environ Res 113:7–17. https://doi.org/10.1016/j.marenvres.2015.10.014
95. Ward JE, Rosa M, Shumway SE (2019) Capture, ingestion, and egestion of microplastics by suspension-feeding bivalves: a 40-year history. Anthropocene coasts 2(1):39–49. https://doi.org/10.1139/anc-2018-0027
96. Watts AJR, Urbina MA, Corr S, Lewis C, Galloway TS (2015) Ingestion of plastic microfibers by the crab *Carcinus maenas* and its effect on food consumption and energy balance. Environ Sci Technol 49(24):14597–14604. https://doi.org/10.1021/acs.est.5b04026
97. Wegner A, Besseling E, Foekema EM, Kamermans P, Koelmans. (2012) AA Effects of nanopolystyrene on the feeding behavior of the blue mussel (*Mytilus edulis* L.). Environ Toxicol Chem 31(11):2490–97. https://doi.org/10.1002/etc.1984
98. Worm B, Lotze HK, Jubinville I, Wilcox C, Jambeck J (2017) Plastic as a persistent marine pollutant. Annu Rev Environ Resour 42(1):1–26. https://doi.org/10.1146/annurev-environ-102016-060700
99. Wright SL, Thompson RC, Galloway TS (2013a) The physical impacts of microplastics on marine organisms: a review. Environ Pollut 178:483–492. https://doi.org/10.1016/j.envpol.2013.02.031
100. Wright SL, Rowe D, Thompson RC, Galloway TS (2013b) Microplastic ingestion decreases energy reserves in marine worms. Curr Biol 23(23):1031–1033. https://doi.org/10.1016/j.cub.2013.10.068
101. Zhang R, Wang M, Chen X, Yang C, Wu L (2020) Combined toxicity of microplastics and cadmium on the zebrafish embryos (*Danio rerio*). Sci Total Environ 743:140638. https://doi.org/10.1016/j.scitotenv.2020.140638
102. Zhou W, Tang Y, Du X, Han Y, Shi W, Sun S, Zhang W, Zheng H, Liu G (2021) Fine polystyrene microplastics render immune responses more vulnerable to two veterinary antibiotics in a bivalve species. Mar Pollut Bull 164:111995. https://doi.org/10.1016/j.marpolbul.2021.111995

Figures

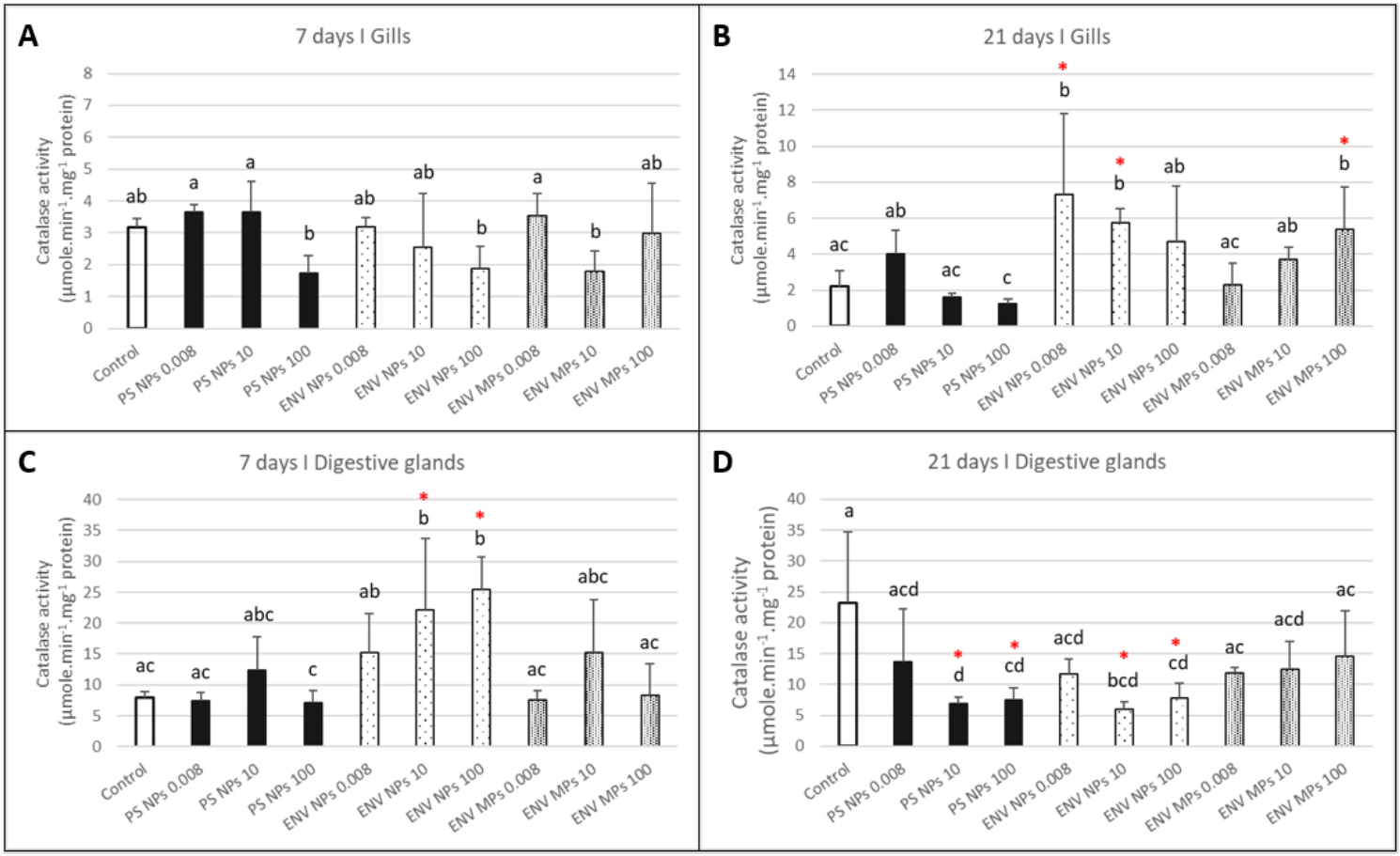

Figure 1

Enzymatic activity of CAT in gills (A, B) and digestive glands (C, D) of *Scrobicularia plana* after 7 (A, C) and 21 days (B, D) exposure to standard polystyrene nanoplastics (PS NPs) and environmental micro- (ENV MPs) and nanoplastics (ENV NPs) at 0.008, 10 and 100 µg.L^{-1} (mean ± SD; n=5; a, b, c, d showed significant differences between conditions; red star showed significant differences between each condition and control p<0.005)

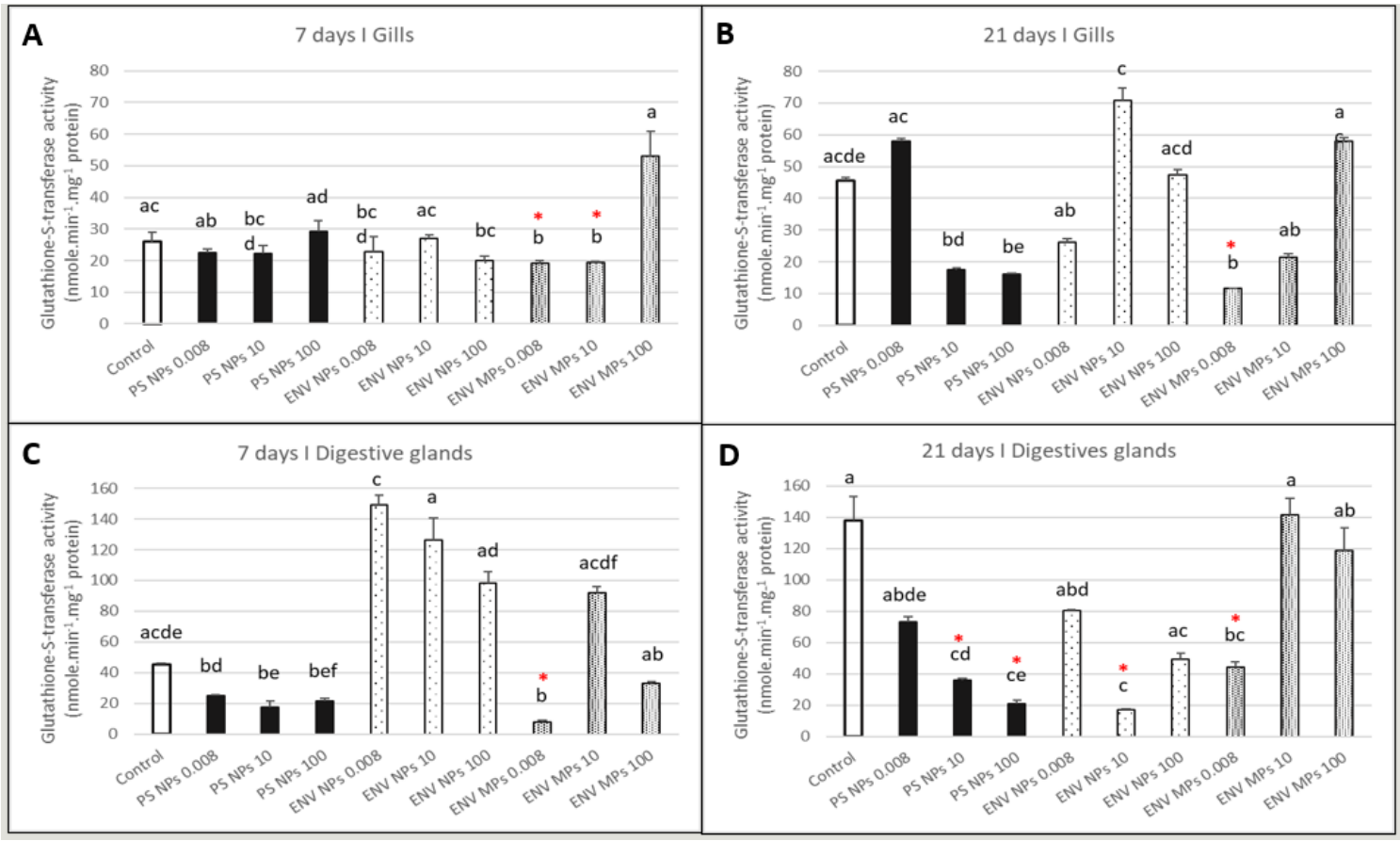

Figure 2

Enzymatic activity of GST in gills (A, B) and digestive glands (C, D) of *Scrobicularia plana* after 7 (A, C) and 21 days (B, D) exposure to standard polystyrene nanoplastics (PS NPs) and environmental micro- (ENV MPs) and nanoplastics (ENV NPs) at 0.008, 10 and 100 µg.L^{-1} (mean ± SD, N=5, letters a, b, c, d significantly differences between conditions and the red star significantly differences between conditions and *control* p<0.005)

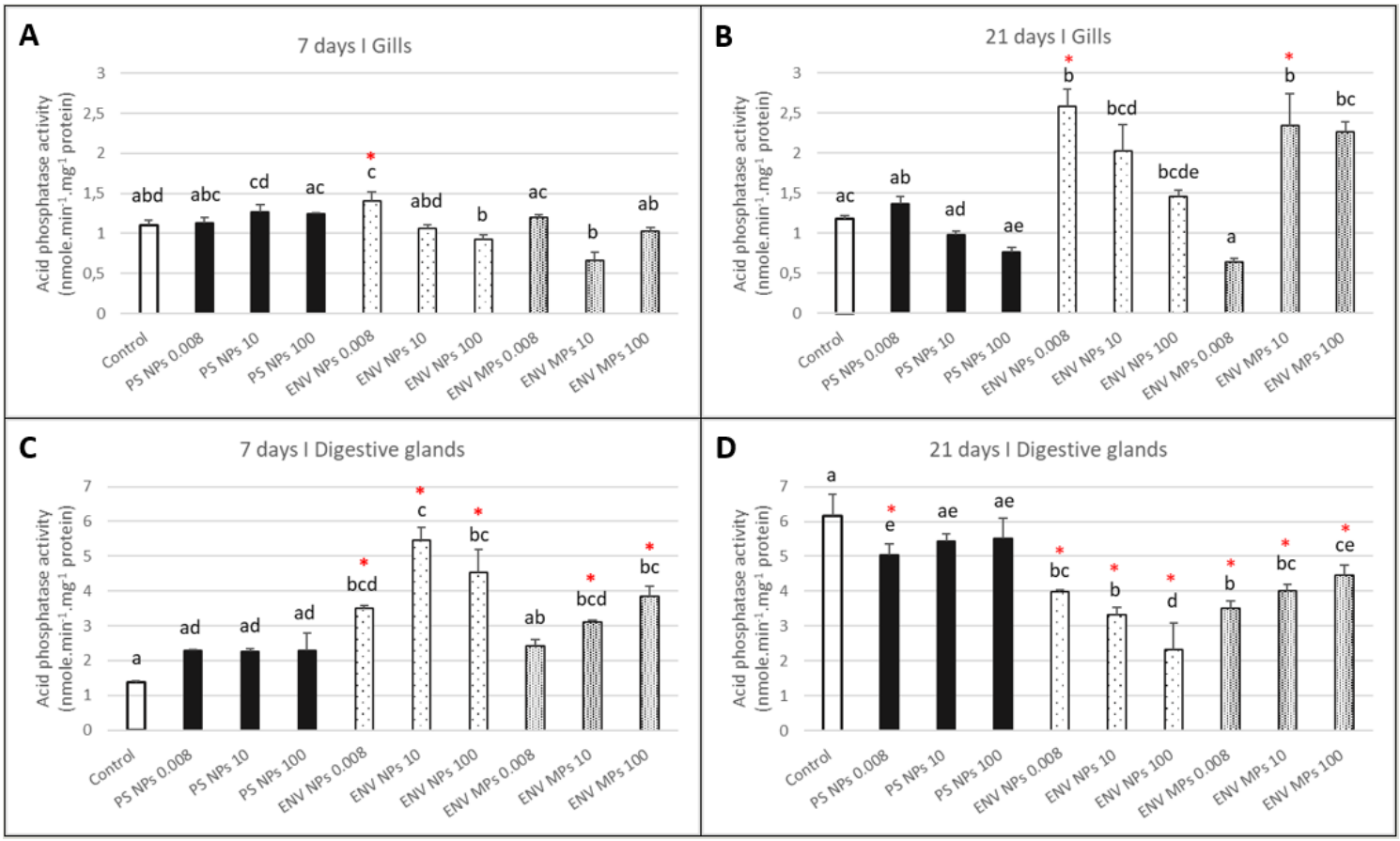

Figure 3

Enzymatic activity of AcP in gills (A, B) and digestive glands (C, D) of *Scrobicularia plana* after 7 (A, C) and 21 days (B, D) exposure to standard polystyrene nanoplastics (PS NPs) and environmental micro- (ENV MPs) and nanoplastics (ENV NPs) at 0.008, 10 and 100 µg.L^{-1} (mean ± SD, N=5, letters a, b, c, d, e significantly differences between conditions and the red star significantly differences between conditions and control p<0.005)

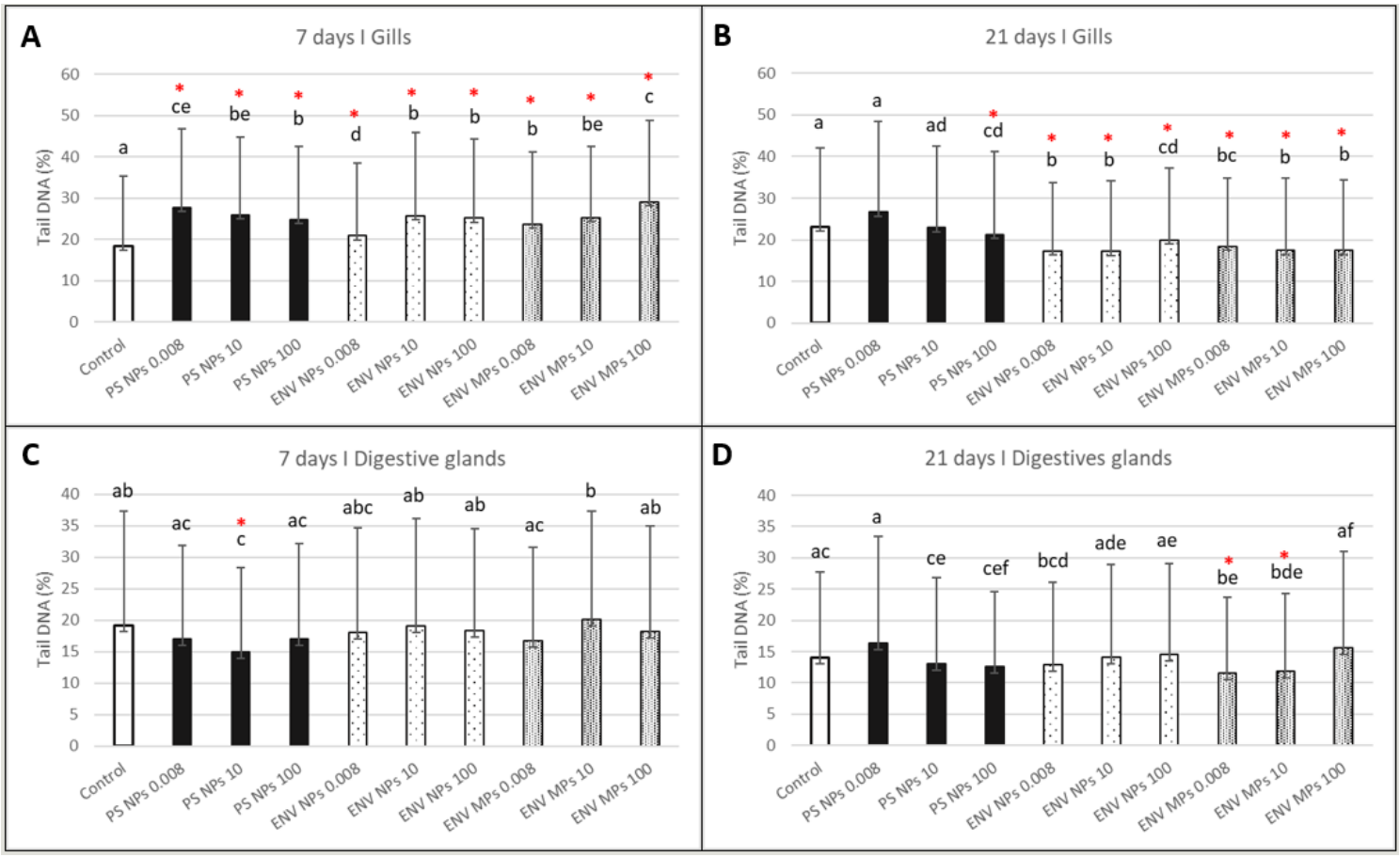

Figure 4

DNA integrity in gills (A, B) and digestive glands (C, D) of *Scrobicularia plana* after 7 (A, C) and 21 days (B, D) exposure to standard polystyrene nanoplastics (PS NPs) and environmental micro- (ENV MPs) and nanoplastics (ENV NPs) at 0.008, 10 and 100 µg.L^{-1} (mean ± SD, N=3, letters a, b, c, d, e significantly differences between conditions p<0.005 and the red star significantly differences between conditions and control p<0.005)

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