ISJ 19: 136-149, 2022 ISSN 1824-307X

REVIEW

Current research on the effects of plastics pollution in marine and freshwater aquatic invertebrates

V Pirillo, N Baranzini*

Department of Biotechnology and Life Sciences, University of Insubria, Via J. H. Dunant 3, 21100 Varese, Italy

 Accepted October 18, 2022

Abstract

Plastics pollution in the aquatic environments represents one of the most critical worldwide issue. Every year, million tons of waste products are reversed both in marine and freshwaters, persisting for long timings and determining serious effects to living organisms. Here, these synthetic materials are fragmented in small particles, known as micro- and nanoplastics, under the effects of both biotic and abiotic factors. Due to their characteristics, smaller fragments are easier accumulated inside animal tissues and organs, risking to enter in the trophic chain. To date, despite the current situation, only a small amount of research has been conducted, especially on aquatic invertebrates, which can represent a suitable model for better analyzing the possible plastics dangerous effects. For this reason, in the present review we aim to collect the recent information about micro and nanoplastics effects on both marine and freshwaters invertebrates. In particular, we do not only focus the attention on the obtained results, but also, we report the main experimental methods and particle types used. Regardless of the heterogeneity present in literature, the actual data result fundamental for setting up the future research.

Key Words: plastic pollution; invertebrates; microplastics; nanoplastics; plastics uptake

Introduction

Plastics are lightweight, versatile, resistant, and cheap materials. Thanks to their qualities, these polymers can be easily manufactured for a variety of civil and industrial applications. Since the middle of the last century, their peculiar physical and chemical characteristics made these synthetic materials hardly replaceable and, for this reason, employed in almost all industrial areas, such as food and textile industries, healthcare, transports, electronics, and telecommunications (Gourmelon, 2015). Moreover, due to their multiple use and the low costs, the total amount has significantly increased over the past 70 years, passing from 1,5 million tons in 1950 to 359 million tons in 2020 (Tournier *et al.*, 2020).

However, the wide distribution and the indiscriminate use, results into threatening consequences, making the environmental plastics pollution one of the most serious ecological problems at the global level (Guzzetti *et al.* 2018; Alimba and Faggio 2019; Prokić *et al.* 2019). In particular, most of the monomers that constitute these polymers derive from fossil hydrocarbons resulting not biodegradable and less than the 20 % are recycled, while most of them are burned, stored in landfill sites or environmental dispersed (Gourmelon, 2015). Furthermore, plastics not only are extremely resistant, persisting for a long time inside the ecosystems, but also result harmful for the living organisms with which interact. In this context, the aquatic environments are considered the most affected, in which waste products deriving from the human consumption or from the industrial processing rapidly spread transported by currents and winds (Barnes *et al.* 2009; Galgani, 2015; Jambeck *et al.* 2015). Of the 360 million tons produced, approximately between 4 to 12,7 million are reversed into oceans every year, constituting a significant part of the marine waste, even if this value is considered lower (Andrady, 2011; Tramoy *et al.,* 2020). Unfortunately, with this rate it is estimated that the total amount of dispersed plastics may soon exceed that of numerous species, leading to a great loss for many industrial sectors, not only in terms of biodiversity but also in the economic perspective (Letcher, 2020). Interestingly, plastics have been already recognized as a possible environmental problem for aquatic systems since 1960s, when the first studies have been conducted focused on their ecological impact on marine species. However, at the time any eventual considerations

Corresponding author:
Nicolò Baranzini
Department of Biotechnology and Life Sciences
University of Insubria
Via J. H. Dunant 3, 21100 Varese, Italy
E-mail: nicolo.baranzini@uninsubria.it

have been rejected, due to the inability to forecast whatever future trends (Letcher, 2020).

Larger plastics, known as macroplastics, induce different problems both in vertebrate and invertebrate aquatic species when ingested, blocking the digestive tract, damaging organs, and leading to a decrease in food intake (Taylor *et al.* 2016; Burgos-Aceves *et al.* 2018; Faggio *et al.* 2018; Aliko *et al.* 2022; Lombardo *et al.* 2022). Many organisms remain entangled in these debris that cause injuries and limit movements, also preventing the animal to feed on (Gregory, 2009). However, macroplastics represent only a small part of the plastics pollutants. In fact, when released in marine or freshwater environments, these products are subjected to biotic and abiotic processes, which lead to the formation of small particles. Microplastics (MPs) are in a range between 5 mm and 1 µm, while nanoplastics (NPs) show a lower diameters comprised between 1 µm and 1 nm and due to their size these particles are easier ingested or assimilated by animals, accumulating inside tissues and entering in the trophic chain (Arthur *et al.,* 2009; Xu *et al.*, 2020). Both types have been observed from sediment to the surface, differently floated into the water column (Van Cauwenberghe *et al.,* 2013; Lusher *et al.*, 2015; Mistri *et al.,* 2017; Honorato-Zimmer *et al.*, 2021). Furthermore, the different chemical properties and the diverse density make the comprehension of their fate highly complex (Haegerbaeumer *et al.,* 2019). To date, MPs and NPs not only have been found in drinking water and in 200 edible species, whose consume through the diet can expose humans to an inevitable absorption. Due to the ability in crossing the biological barriers and penetrating inside tissues, many studies showed as MPs and NPs are stored in biotic samples, leading to the activation of many different processes that tend to reduce vitality, promote metabolic disorders and decrease the reproductive fitness. Their assimilation is associated with many toxic responses that occur in organisms such as oxidative stress, activation of the immune system, inflammations and growth inhibition (Alimba *et al.* 2021; Burgos-Aceves *et al.* 2021a, b). Other research conducted in mammals reveal that the uptake of some MPs induces dysbiosis, hepatic and metabolic disorders in mice (Luo *et al.,* 2019). Moreover, another important aspect is determined by the fact that, although these materials should not be considered reactive for their biochemical structures, plastics particles can act as carrier for other pollutants, which are easier transported inside organisms producing further harmful effects.

At any rate, the plastic degradation increases the nature availability of these synthetic polymers with severe impacts on living organisms, especially in marine and freshwaters ecosystems (Tiwari *et al.,* 2020). Thus, it results essential to collect and extend the current knowledge to successfully understand their real impact and prevent the potential risks. For this reason, in this review we reported the most recent and relevant data available in literature on the MPs and NPs effects both in marine and freshwaters environments. In detail, we aim to underly how plastics are produced and used for the experimental applications and describe the effects both at organism and cellular level in aquatic invertebrate species. This work sets out to bring new insights and provide useful information for the future research.

Types of plastics

Despite, several emerging methods are developing for a more sustainable recycling of plastic (e.g., based on the enzymatic and microbial biodegradation (Tournier *et al.*, 2020; Lu *et al.,* 2022; Pirillo *et al.,* 2022; Sonnendecker *et al.,* 2022), there are a few critical steps for the removing process of high-density plastics from the environment and many bench-scale experiments are not enough for the application in large-scale process *(*Patil *et al.,* 2022). Indeed, due to their low biodegradability, the plastic materials are high recalcitrant in several environments and the deriving debris widespread vary in terms of chemical e physical properties (e.g., chemical composition and density).

Starting from their initial dimension, the engendering process that leads to formation of MPs and NPs can be classified in primary and secondary sources . With primary sources are identified all the discarded particles that derive either from the industrial production or that are generated by the fragmentation of common objects used in everyday life (e.g., fibers of synthetic textile products during washing, personal care products, or cleaning applications).

Instead, as secondary sources are considered the total amount of micro- and nanofragments that originate directly from a slow decomposition of the former, which result in a fragmentation in smaller size particles as effect of the environmental exposition to several both biotic (i.e., living organisms that influences its environment) and abiotic factors (i.e., UV rays, temperature, salinity, atmospheric events or ocean currents, and also microbial degradation), enhancing the persistence in natural ecosystems. Moreover, oxidation can be also the cause of plastics physical abrasion (Arthur *et al.,* 2009) and some polymers result highly inclined to this type of chemical process, which impairs the strong molecular bonds between chains (Tiwari *et al.*, 2020).

From packaging to the manufacture of single-used items, polyethylene terephthalate (PET), high-density (HDPE) and low density (LDPE) polyethylene, polyvinyl chloride (PVC), polypropylene (PP), and polystyrene (PS) are the polymers which possess a major industrial relevance and are the most common identified in the investigated marine environments (Table 1) (Auta *et al.,* 2017; Danso *et al.,* 2019). The particles found in nature are characterized by different both sizes and shapes (e.g., spheres, fiber, film, irregular) *(*Chubarenko *et al.,* 2020; Patil *et al.*, 2022), but also their density is an important aspect to be considered for determining their specific localization and their fate in the in the water column. In fact, debris with a density higher than seawater (1.020-1.029 g mL^1) (e.g., PET, PVC, and PS) would sink, on the contrary polymers less dense (e.g., HDPE, LDPE, and PP) would float on the surface (Table 1) (Brignac *et al.,* 2019). Considering all these aspects,

Table 1 The most diffused synthetic plastics in the aquatic environments. For each type, resin code, abbreviation, commonly use, decomposition rate for marine debris, density and toxicity levels are reported. [a]The reported chemical density referred to the pure plastic compounds in absence of additives (e.g., plasticizer, pigments, stabilizers) as reported by Brignac *et al.,* 2019

Polymer name	**POLYETHYLENE TEREPHTHALATE**	**HIGH-DENSITY POLYETHYLENE**	**POLYVINYL CHLORIDE**	**LOW-DENSITY POLYETHYLENE**	**POLYPROPYLENE**	**POLYSTYRENE**
Resin code	01	02	03	04	05	06
Abbreviation	**PET**	**HDPE**	**PVC**	**LDPE**	**PP**	**PS**
Use	Water bottles, medicine jars, clothing and carpet fiber	Detergent bottles, plastic bags, toys	Credit cards, window and door frames	Plastic wrap, bread bags, squeezable bottles	Bottle caps, potato chip bags, packing tape, drinking straws	Food boxes, watering cans, storage bins
Decomposition rates for marine debris	Up to 450 years	Up to 450 years	Up to 450 years	500-1000 years	20-30 years	900 years
Density ($g\ mL^{-1}$)[a]	1.37−1.41	0.94−0.98	1.38−1.45	0.89−0.93	0.85−0.92	1.04−1.06
Toxicity level	High	Low	High	Low	High	Low

it results necessary shed light on the presence of different types of MPs and NPs and their effects on living organisms in freshwater and marine ecosystems.

Overview on the MPs and NPs production and potential applications

In literature are reported several types of MPs and NPs for evaluating the *in vivo* effects that are based on a chemical-mechanical production or are commercially available (Fig. 1). Several techniques of synthesis are reported by Lee *et al.,* 2016 to produce MPs and NPs, that can be divided into four categories: (i) emulsion-based methods, (ii) precipitation-based methods, (iii) direct compositing methods, and (iv) new approaches including microfluidic technique. However, emulsion and precipitation are the most used to easily produce MPs and NPs. For examples, (Grillo *et al.*, 2021) reported a simple emulsion/solvent extraction synthesis method in chloroform (1 % m/V) and poly-vinyl alcohol (PVA, 0.025 % m/V), producing a range of PS microparticle sizes, where the 78 % present an average diameter < 5 µm and a spherical shape. Similarly, straightforward protocols are available to produce PET NPs based on a dissolution/precipitation method with water-soluble solvent hexafluoro-2-propanol (Pirillo *et al.*, 2021) or two step of trifluoroacetic acid solution (TFA, 90 % v/v and 20 % v/v) (Rodríguez-Hernández *et al.,* 2019; Pirillo *et al.,* 2021), starting from commercial PET (e.g., drink bottle, granulate, films, or fibers); then, thanks to a filtration-step, it is possible to obtain a particulate size that is usually in the 50- to 300-nm range. Moreover, the NPs so produced can be colored with different dye, such as the Nile red, which has been proposed by Maes *et al.*, 2017 for PET microparticles identification and exploited for cell internalization experiments and ecotoxicological studies (Rodríguez-Hernández *et al.,* 2019).

Beyond the chemical synthesis that allow to produce all spherical and in the same scale range particles, easier protocol for MPs and NPs production are also available based on a mechanical processing. As proposed by (Romero-Blanco *et al.,* 2021), MPs can be obtained by pulverization of PS tube-test, with a size ranged between 10 nm and 514 µm. Likewise, other reported protocols are based on the cutting and fragmentation of commercially plastics fibers or row pellets (Jemec *et al.,* 2016; Kim *et al.*, 2021). The major advantage of this method is to produce irregular fragments that are more similar to those found in nature, in order to better simulate the environmental conditions (Baranzini *et al.,* 2022 accepted).

Regarding commercial MPs and NPs, several modified particles exhibit fluorescence properties

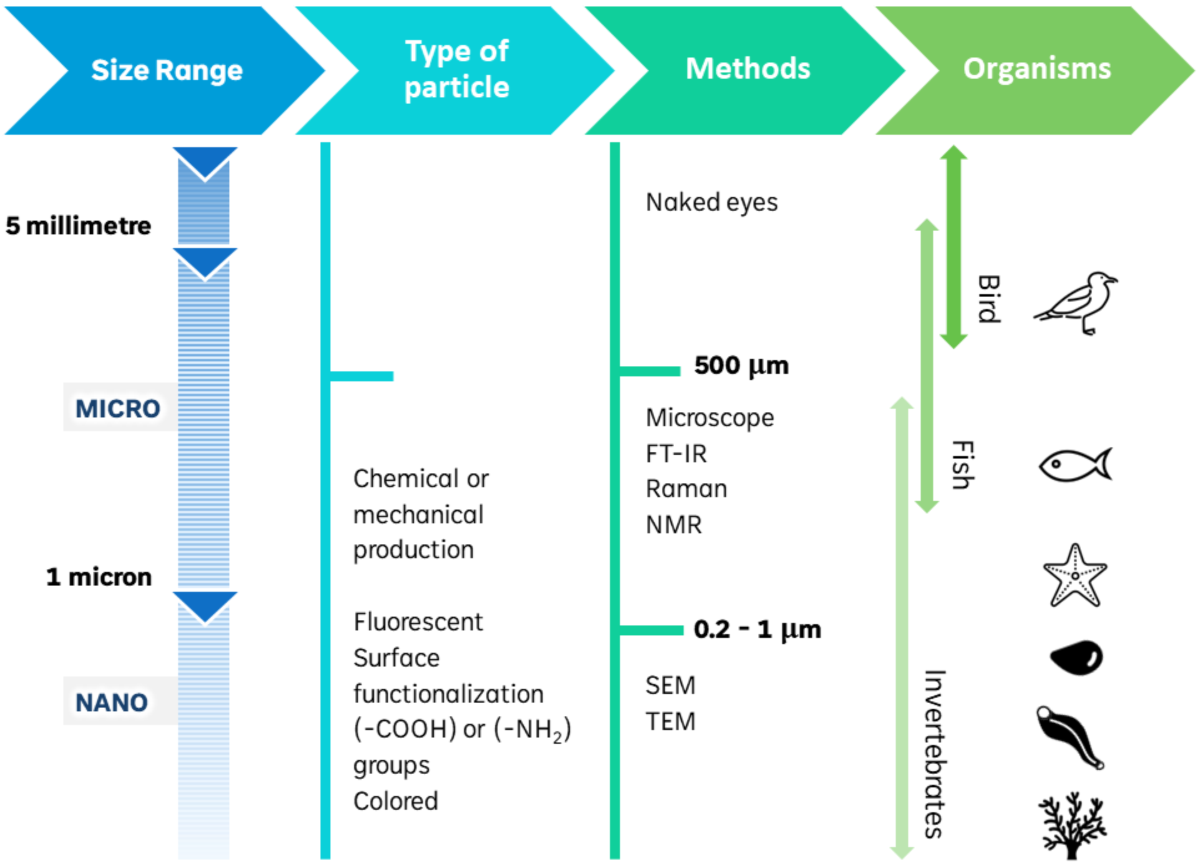

Fig. 1 Scheme representing environmental plastics dimensions, correlated to their production methodologies for *in vivo* studies research and example of affected organisms. FT-IR, Fourier-Transform Infrared spectroscopy; SEM: Scanning Electron Microscopy; TEM: Transmission Electron Microscopy

and can be functionalized with carboxyl (-COOH) or amino (-NH_2) groups on their surface. The most used fluorescent MPs and NPs are those of PS, in particular available as blue-dyed (345 nm excitation and 435 nm emission, Phosphorex), red-dye (552 nm excitation and 580 nm emission, Micromer®-redF), and yellow-green microspheres (441 nm excitation and 486 nm emission, Fluoresbrite® Plain YG) (Della Torre *et al.,* 2014; Canesi *et al.*, 2015; Bergami *et al.*, 2017; Gambardella *et al.*, 2017; Capolupo *et al.*, 2018; Liu *et al.*, 2019; Rist *et al.*, 2019; Cappello *et al.,* 2021; Gonçalves *et al.*, 2022). Thanks to their easy detection, fluorescently labeled and functionalized PS plastics are the most common used in ecotoxicological studies, toxicity bioassays and for the evaluation of the aquatic organisms uptake.

However, independently from how have been produced, MPs and NPs behavior is extremely variable and an in-depth characterization is required. A dynamic light scattering (DLS) analyses combined with a Zetasizer Nano Series software (ZS) should be necessary for determining several key parameters, such as Z-average (nm), polydispersity index (PDI, dimensionless) and zeta (ζ-) potential (mV), which describe MPs and NPs behavior in relation to the environmental media (Bergami *et al.*, 2017). In fact, salinity or pH can alter the plastics bioavailability and distribution in the water column, leading to polymers aggregation and changing many physicochemical properties (surface change or coating). Moreover, to evaluate the potential effects, other aspects must be taken into consideration. Concentration of MPs or NPs used in the experimental conditions do not always reflect the exposure scenario and the real environmental conditions (e.g., using extremely high concentrations). In this context, it is reported in literature the needed to mimic as close as possible the natural state, to obtain more accurate data on the real toxicity of plastics dispersion. Moreover, considering that the fate and the bioavailability of MPs and NPs depends on size, shape, and charge, it is important to have comparable methodologies and approaches, to obtain a deeper knowledge that considers all the physicochemical variables that can interfere with interactions between plastics and organisms (Oliveira and Almeida, 2019).

MPs and NPs effects on marine invertebrate organisms

Phylum of Cnidaria

Cnidarians are benthic invertebrates that were able to adapt to many different habitats. Living fixed on substrates in contact with sediments or floating in the water column, these animals represent one of the main targets for environmental pollutants. For this reason, many studies have been conducted on hydrozoan, scyphozoan and anthozoan marine models to evaluate toxic effects deriving from different compounds, such as chemicals or metals (Muñoz-Vera *et al.*, 2015; Lozano-Bilbao *et al.*, 2018). The presence of waste products can induce morphological changes, affect vitality and alter the expression levels of those gene related to metabolism and oxidative stress (Kalsom and Mehman, 2020). Due to their ability in the incorporation of various anthropogenic materials, both medusoid and polypoid forms are important bioindicators of MPs and NPs impact in marine ecosystems (Macali and Bergami, 2020). Moreover, representing one of the first step of the trophic chain, the comprehension about their ability to accumulate synthetic polymers is extremely relevant (Devereux *et al.*, 2021). In nature, the presence of plastic fragments has been detected in different cnidarians species, such as *Cyanea capillata, C. lamarckii* and *Aurelia aurita* (Schyphozona), *Cosmetira pilosella* (Hydrozoan) and *Coelogorgia palmosa* (Devereux *et al.*, 2021; Vencato *et al.,* 2021), confirming as these invertebrates are directly interested by MPs and NPs pollution.

In the scleractinia coral species *Porites porites*, PS MPs absorption was evaluated using chemically produced spherical particles presenting a diameter inferior to 5 µm, which were dispersed in seawater in a range between 1 and 1000 mg/L. The analyses conducted using both histological and enzymatic techniques, in which the levels of catalase have been evaluated, revealed that although the particles uptake occurred in every condition, no significant toxic effects on organisms behavior have been registered in short time (96 h) of exposition. Based on the initial concentration, PS microsphere have been stored in gastrovascular tissue, mesenterial filaments and coral tissue without affecting viability or inducing bleaching and stress response (Grillo *et al.*, 2021). Contrariwise, long-term treatments (17 days) with PS cause potential harmful effects both in polyps and ephyrae of the anthozoan jellyfish *Sanderia malayensis*. Independently from the dimensions, fluorescent FITC-conjugated microbeads were able to instantly interact (already after 24 h) with both epidermis tissue and digestive cavity, in which they were clearly detectable. Moreover, MPs can accumulate and persist until 52 days, altering feeding behavior and fitness. After 17 days, animals reproduction appeared reduced, revealing as prolonged exposures to plastics, although do not affect survival, can interfere with polyps budding *(*Eom *et al.,* 2022).

The effects of PE were evaluated in the coral *Stylophora pistillata*, analyzing the as MPs inhibit photosynthetic capacity of scleractinian zooxanthellae symbionts. After 4 weeks, chlorophyl reduced activity was analyzed by Pulse Amplitude Modulated (PAM) fluorometry method, indicating a condition of stress. Although this effect seemed to be due to a direct contact between algae and MPs, given the physical inability of this interaction, authors suggested that MPs produced a signaling interference between symbionts and hosts. Moreover, by means of Nuclear Magnetic Resonance (NMR) analyses, the metabolism of different important metabolites has been detected (Lanctôt *et al.,* 2020). PE MPs effects were also investigated in the *Aurelia sp.* ephyra stages using fluorescent particles at different concentrations (from 10 µg/L to 10 mg/L). By means of confocal and tomographic analyses, it was observed as MPs attached around mouth surface or were stored into the digestive tract. Moreover, already after 24 h from the initial exposition, in juveniles jellyfishes the survival rate, behavior and radial symmetry resulted significantly altered. Although after 72 h a total recovery is restored in absence of MPs, these data also confirmed a direct impact on larval immobility and frequency of pulsations (AFp) that impair animals viability (Costa *et al.*, 2020).

Coral reef cnidarians were also used to test the effects of PVC MPs. In the species *Zoanthus sociatus*, commercial MPs, possessing an irregular size, were dispersed in the aquarium under continuously moving water condition, in which two different PVC concentrations (1 and 10 mg/L) were assessed. Plastics immediately adhered to epidermis or gut cavity, stimulating stress response and inducing photosynthetic events. As regards the macroscopic effects, no variations in survival or behavior have been detected, also after long term exposure (Rocha *et al.,* 2020).

Phylum of Mollusca

Given their commercial and ecologically importance, many studies were conducted on several molluscs species, in which the effects of different plastic types have been tested with different aims. In particular benthic and sedentary organisms, such as bivalves, are the main models employed in toxicological research (Pagano *et al.* 2020; Stara *et al.* 2020, 2021; Curpan *et al.* 2022). Filtrating waters or directly feeding from the substrate, they are excellent candidates for investigating potential toxic effects, analyzing plastics bioaccumulation and morphological or behavioral changes (Rittschof and McClellan-Green, 2005; Jaeschke *et al.*, 2015).

Spherical and colored PS MPs, with a diameter of 3 µm, were administered to the marine mussel *Mytilus galloprovincialis* and the several metabolic parameters were analyzed by NMR-based metabolomics spectra. Filtered MPs deposit into the digestive gland and, especially after 48 h up to 72 h, alter the production of various metabolites, osmolytes and antioxidants. The levels of several amino acids, lactate, glycogen, taurine, hypotaurine and glutathione increased, suggesting a potential toxicity for PS MPs (Cappello *et al.,* 2021). Significant change in the expression levels of different genes was also observed after 3 µm-size spherical MPs uptake in *M. galloprovincialis* larval stages. Although plastics were assimilated and retained, bioaccumulating inside the digestive tract and risking persisting in the trophic chain, their presence did not cause visible morphological modifications during embryonic development up to 192 h. However, the upregulation of several genes related both to shell biogenesis and immune response or the downregulation of those involved in lysosomal activity were evident, highlighting as PS plastics mostly act at the cellular level (Capolupo *et al.*, 2018). A similar result was also observed in the *Mytilus edulis* larvae, in which although MPs were faster stored into embryo tissues after ingestion, the developmental stages were not altered, except under extreme conditions (high concentration and long exposition timings) (Rist *et al.*, 2019).

Interestingly, similar potentially dangerous effects were also detectable in mussels cells after NPs chemical modifications. Indeed, cationic NPs ($PS\text{-}NH_2$) affected cellular processes and influenced molecular intracellular pathways. Based on studies conducted in sea urchin embryos and mammalian cells, Canesi and colleagues demonstrated as the addition of positive charges not only impacted on the plastics uptake, but also on their final biological targets. Indeed, *M. galloprovincialis* hemocytes exposed to 50 nm $PS\text{-}NH_2$ particles showed both a reduced phagocytic activity and the increase of lysozyme in a dose-dependent manner, together with reactive oxygen species (ROS) and nitric oxide (NO) production. In parallel, the apoptotic rate and the loss of mitochondrial potential were evaluated by means of flow cytometry (Canesi *et al.*, 2015). The PS NPs ability in impairing immune system was also confirmed by electron microscopy (TEM) and molecular analyses. The presence of numerous laminar cytoplasmatic expansions and the diverse modification of the p38 MAPK and PKC phosphorylation state represented a symptom of cellular stress in mussels hemocytes. In addition, once entered inside tissues and cells, MPs and NPs can be also conditioned by the chemical interactions established with different proteins, known as corona-protein, that interfere with plastics behavior. The formation of a $PS\text{-}NH_2$-corona proteins complexes was investigated in the extracted hemolymph, combining electrophoresis method and HPLC-MS/MS techniques. Interestingly, the results showed a specific binding with a C1q domain protein, suggesting that although this interactions makes more difficult the comprehension of plastics fate, are essential to better assess their potential impact (Canesi *et al.,* 2016).

NPs toxic effects can be also calculated using the Integrated Biomarker Response (IBR) index that allows to compare and analyze the relations between biomarkers and pollutants levels. This tool has been used in *M. galloprovincialis* to better interpretate the obtained results, in which a concentration of 10 µg/L of PS particles caused a chronic response characterized by a tissue-specific genotoxicity. Among all the organs involved, gills resulted the main affected. PS NPs aggregate and interfere with the cell biological functions, damaging DNA and impacting on cell viability. Moreover, the antioxidant defenses, based on different enzymes, result ineffective and after 14 days from the beginning of exposure. The levels of ROS were so high that lipid peroxidation (LPO) occurred, leading to the disruption of cell membranes and causing a chronic response (Gonçalves *et al.*, 2022). Although the PS registered effects are different in diverse mussel species, the IBR index should represent a valuable tool to simplify and clarify data interpretation.

To date, although numerous investigations have been performed on PS particles, only few were conducted on the other plastic types. Among them, the potential role of HDPE was analyzed in the pacific oyster *Crassostrea gigas* larvae, in which develop, morphological modifications and swim ability were assessed. After 24 h from the initial exposition, the smallest HDPE microparticles, proposed at three different concentrations (100 µg/L, 1 and 10 mg/L), caused severe malformations and arrest larval growth. Moreover, although the speed during swim was not completely reduced, trajectories appeared significantly altered. In the 70 % of oyster embryo, the interaction with MPs produced more circular movements, decreasing rectilinear proceeding. All these consequences on their behavior not only interfere with the capacity of larvae to feed or escape from predators, but also with the possibility to naturally colonize new substrates (Bringer *et al.,* 2020).

Phylum of Arthropoda

Among all the invertebrate phyla, those of arthropods is certainly the most numerous. Indeed, it contains a great variety of species that, thanks to their characteristics, conquered different types of habitats and colonized quite all the ecological niches (Kremen *et al.*, 1993; Verma and Prakash, 2020). As for bivalve molluscs, the marine taxon of crustaceans possesses a large importance both in economic and biological terms and also recently acquired a certain relevance in scientific and ecotoxicological field (Anger, 2006). Therefore, it results fundamental to investigate the plastics toxic effects in these invertebrates, analyzing as MPs and NPs could bioaccumulate inside tissues, not only altering the development and life cycle, but also risking to consequently enter in the tropic chain. This the case of several classes such as Copepoda, Cladocera or Branchiopoda, composed by small crustaceans that form zooplankton and that represent the main prey of fishes and other invertebrates. Given that the chronic presence of plastics in these organisms can be extremely dangerous, numerous studies have been focused proper on these animals.

In the brine shrimp *Artemia salina*, chronic toxicity induced by PS MPs was determined using both labeled and not-labeled plastics particles at different concentrations (from 1 to 100 mg/L). Moreover, the employ of greenly fluorescent MPs better allowed to follow plastics fate and body localization. Although MPs accumulate inside tissues in all the developmental stages, organisms growth was considerably reduced only with the highest concentration. In this condition, a considerable modification of the midgut cell morphology was detected. In addition, to characterize the way by which plastics influence cells biology, transcriptome analyses have been conducted in combination with Gene Ontology (GO) analyses, confirming a change in the expression levels of those genes involved both in metabolic and catalytic activities, as already observed in other arthropods species (Suman *et al.,* 2020).

The PS plastics ingestion followed by harmful effects was detected also in another crustacean of the genus Artemia: *Artemia franciscana*. The exposure to various MPs concentrations and sizes revealed a variation in the activity of specific proteins and enzymes, such as Heat Shock Protein 70 (HSP70), catalase, superoxide dismutase and acetylcholinesterase, considered important markers of the oxidative stress. However, the most relevant data was represented by the increase in shrimps mortality after 30 days of treatment. MPs impaired survival rate in a dose-depending manner, by inducing chronic and acute responses at different biological levels (Eom *et al.*, 2020).

A. franciscana brine shrimps have been also treated in combination with the green microalgae *Dunaliella tertiolecta* to assess as charged PS NPs could induce diverse effects depending on the associated chemical group. Interestingly, if on one hand anionic carboxylate (PS-COOH) particles did not impact on organisms health until highest concentrations, although are easier internalized, on the contrary the anionic PS-NH_2 plastics induced both the inhibition of *D. tertiolecta* algae growth and the increase of *A. franciscana* shrimps mortality. All these results are extremely important to better understand the PS plastics role also in zooplankton organisms that occupy a relevant place in the trophic chain (Bergami *et al.,* 2017).

Phylum of Annelida

Thanks to the possibility to amputate body segments without compromise their survival, the class of marine polychaeta constitutes an important phylogenetic group to assess MPs and NPs effects on tissues regeneration. Moreover, being prey of many other animals and living directly on the sediments, these invertebrates are continuously exposed to environmental pollutants, representing one the main channels for the entrance of anthropogenic materials in the trophic chain (Pires *et al.*, 2022). However, despite their ecologic relevance, only few studies have been performed to determine how plastics could impact not only on polychaetes life cycle and behavior, but also interfere with the ability to regenerate tissues.

Two different species *Hediste diversicolor* and *Perinereis aibuhitensis* were treated with different concentrations of PS MPs and NPs, in which behavioral change were determined after plastics treatment. In detail, *H. diversicolor* worms exposed to different concentration of PS NPs showed a reduced burrowing ability. This parameter is probably connected with a decrease in the Cholinesterase activity, a fundamental enzyme that regulates muscular functions. Furthermore, in the 50 % of the samples exposed to a NPs concentration of 50 mg/L also the antioxidant defenses resulted significantly reduced. However, LPO levels remained lower than control groups, suggesting that in *H. diversicolor* mechanisms of membrane repair could be activated to cope with PS adverse effects (Silva *et al.,* 2020). Whereas Leung and Chan analyzed the impact PS MPs during regeneration in the polychaeta *P. aibuhitensis*. Authors demonstrated as the smallest microparticles (8-12 µm) increased mortality and affected the ability to regenerate removed segments (Leung and Chan, 2018). Moreover, these studies also revealed that, in particular smaller beads, aggregated and consequently compromised samples physiology and behavior, acting at the cellular level by influencing the expression of specific antioxidant enzymes.

H. diversicolor has been also used to investigate the potential effects of other environmental frequently found plastic types. PP and PE MPs were added at different concentrations into water (10 and 100 µg of MPs/L) and sediments compartments (10 and 50 mg of MPs/kg), demonstrating a different accumulation inside worms tissues after 96 h. By means of flow cytometry and enzymatic assays, the coelomocytes viability, the phagocytic activity and the levels of phenoloxidase (PO) and acid phosphatase (AcP) were analyzed. Both a slight reduction of both cells viability and PO and AcP were observed, suggesting as different plastic types can interfere with organisms integrity (Revel *et al.*, 2020). A similar result has been recently obtained mixing and using together more type of MPs. Indeed, a mixture of PE, PP, HDPE, LDPE, polyamide (PA) and polyethylene/ethylene combined with vinyl acetate copolymer (PEVA) was tested in *H. diversicolor*, revealing that plastics accumulate, affecting animals survival and growth rate at high concentration when exposed for long time (Missawi *et al.,* 2021).

Phylum of Echinodermata

Several studies have been also conducted in echinoderms, in which the attention has been mainly focused on the gametes formation or on the embryonic and larvae phases. Thanks to the higher sensitivity to pollutants then adults, juvenile stages represent a perfect model to test possible dangerous effects that can compromised the development (Nobre *et al*., 2015).

In both eggs and spermatozoa of the sand dollar *Scaphechinus mirabilis*, PS MPs induced a significant DNA damage, in which more than the 20 % of the sequences resulted shattered. By means of DNA comet assays, the genome loss was effectively observed already after 1 h after 10^5 particles/L exposure. However, despite this evident effect, spermatozoa did not lose their capacity to fertilize eggs (Mazur *et al.*, 2021).

PS toxicity was also evaluated in the embryonic stages of the sea urchin *Paracentrotus lividus*, in which, as observed for *A. franciscana* brine shrimps, positive and negative charged NPs showed different properties. The structures and dimensions were evaluated by means of TEM and Dynamic Light Scattering (DLS) analyses. Although fluorescently PS-COOH-labeled particles resulted more aggregated and were easily accumulated inside embryos gut, no particularly severe effects were observed. On the contrary, positive PS-NH_2 modified plastics, despite appearing more water dispersed, caused serious developmental deficiencies. This outcome is probably due to the activation of apoptotic pathways, given that after 24 h from exposure, NPs lead to a significant upregulation of the caspase 8 gene (Della Torre *et al.*, 2014). Interestingly, these data represent a further confirm of how any plastics chemical modification could not completely change the fate of these synthetic polymers, but also lead to diverse responses both at organism and cellular level.

MPs and NPs effects on freshwater invertebrate organisms

It is estimated that a considerable part of the plastics presents in seas and oceans arise from freshwaters, in which a large amount of waste products is reversed every year. Lakes and rivers very often are the main environments affected by plastics pollution, being in direct contact with urbans and rural locations (Strungaru *et al.,* 2019). Here, MPs and NPs float in the water column or settle on the sediments, entering in contact with living organisms. Notwithstanding it results necessary to better comprehend their impacts also in these ecosystems, nowadays only few studies have been performed compared to those conducted on marine species (Imhof and Laforsch, 2016). Moreover, freshwater benthic invertebrates are considered useful biomarkers, thank to their extremely vulnerability to pollutant, and can represent a fundamental instrument to easily decode the ecological conditions of a specific environment.

Phylum of Cnidaria

As marine cnidarians, also the freshwaters one are an important source of food for many aquatic species. Moreover, due to their anatomical and physiological characteristics, these invertebrates are extremely sensitive to chemical environmental pollutants, representing a valid indicator of the water quality (Beach and Pascoe, 1998). The presence of toxic substances could have severe effects on the body morphology and animals vitality. Among the major critical outcomes, hydrozoans are subjected to tentacles loss and impediment in food uptake. For these reasons, also the toxic effects of PS NPs have been tested on these invertebrate models, mainly focused the attention on regenerating processes (Auclair *et al.,* 2020). In detail, the freshwater *Hydra attenuata* was treated for 96 h with different concentrations of PS fluorescent particles (from 1.25 to 80 mg/L - 50 and 100 nm in size), whose diameter has been evaluated by DLS analyses. Despite NPs accumulation, depending on the administered dose, following 24 h of depuration severe loss of biological mass occurred. Moreover, an important sign of oxidative stress condition was determined by the fluorescent measuring of both LPO and lipid-like liquid crystal (LCs) formation inside cells cytoplasm. These data revealed as 100 nm particles were most effective then the smaller one and highlighted as lipids metabolism could play an important role in determining plastics toxicity in freshwater cnidarians *(Auclair et al.*, 2020).

Phylum of Mollusca

The effects of synthetic polymers have been assessed in the mud snail *Potamopyrgus antipodarum*, recreating the gastropods environmental conditions. In detail, freshwater was combined with river sands, in which MPs and NPs, presenting sizes between 10 nm and 514 μm and concentrations from 100 to 4000 mg/kg, have been added. After 31 days, no critical impacts on both mortality and reproduction rate were observed. However, adverse behavioral responses were detected analyzing animals immobilization, reaction time and distribution at different conditions. Interestingly, the highest concentrations of MPs did not cause negative effects compared to the control, while all the other treatments tent to increase the *P. antipodarum* reaction time. Although different hypotheses were developed, this factor is probably closely related to the plastics intake, which risks to block the digestive tract or to damage tissues. Nevertheless, these organisms seem to well tolerate plastics presence, independently from MPs and NPs concentrations (Romero-Blanco *et al.*, 2021).

Similar results were obtained by Imhof and Laforsch, which examined the potential plastics effects in *P. antipodarum* using a mixture of five different common non-buoyant polymers (PA, PET, PC, PS and PVC), directly added to food in two different doses. The main focus was to analyze possible morphological change in both adults and juvenile stages, but no significant modifications have been observed. Moreover, the shell formation was not affected by the presence of polymers also after 8 weeks at the higher concentration, suggesting that the juvenile development was not compromised. However, it must be considered that in the present work large particles have been used and the lack of effects should be due to plastics size and shape (Imhof and Laforsch, 2016).

Phylum of Arthropoda

Among freshwaters arthropods, *Daphnia spp.* are considered the main conventional model used in freshwater ecotoxicological studies, recognized at an international level (Taylor *et al.*, 2018). Filtering nutrients in a not-selective manner, these crustaceans come into direct contact with numerous contaminants, and, thanks to their simple body anatomy, they allow to easily detect any possible morphological or physiological modification. In *Daphnia pulex*, fluorescent PS NPs immediately entered inside digestive tract after 48 h from initial exposure, showing lethal effects at the concentration of 76.69 mg/L. Moreover, not only survival rate was significantly reduced, but also both the reproductive capacity and growth. The analyses of the expression levels of several gene involved in oxidative stress showed, as for other aquatic invertebrates, a significant increase depending on particles concentration. In particular, superoxide dismutase (SOD), glutathione transferase (GST) and HSP90 genes resulted more expressed than in control samples. Also after long-term treatments, a chronic response was visible, in which PS NPs influence the reproduction, hatching time and future offspring *(Liu et al.*, 2019b). A comparable result was also obtained in *Daphnia galeata*, in which 5 mg/L of PS NPs caused aberrant development and a fitness reduction after 5 days (Cui *et al.,* 2017). Plastics particles aggregated with lipid droplets located in the adult ovary, which play a fundamental role in regulating crustacean embryos development (Cai *et al.*, 2019). Although the NPs effects were also transient and did not impact on future offspring, by means of Nile Red staining assay, Cui and colleagues demonstrated that their presence led to a decrease in lipid storage (Cui *et al.*, 2017). These variations revealed as nanoplastics could modify metabolic pathways, becoming an effective barrier to embryonic development.

As regards other synthetic materials, the effects related to PET microfibers ingestion have been examined in *Daphnia magna* after 48 h of treatment and following 24 h of recovery. PET fibers were characterized by FTIR spectrometer in ATR mode comparing the results with spectral databases. Although plastics found in the digestive tract possessed a mean dimension of about 300 μm, also particles with large dimensions (1400 μm) were found in the gut. *D. magna* mortality appeared significantly increased in no prefeeding animals, while no effects were recorded in daphnids fed before treatment. Subsequent experiments, in which tissues were digested with H_2O_2 and the gut contents were analyzed by means of scanning electron microscopy (SEM), confirmed a considerable amount of PET fibers in *D. magna* tissues (Jemec *et al.,* 2016). Also PP particles accumulated in *D. magna* digestive tract, in a similar concentration, though these polymers caused a less response and minor lethal effects after 96 h after the initial exposure (Kim *et al.,* 2021).

Phylum of Annelida

Differently from all the other invertebrates, freshwater annelids that belong to the Oligochaeta and Hirudinea classes are considered extremely tolerant to waters contamination and very often their population benefit of these compounds compared with those of non-tolerant organisms (Sharma and Chowdhary, 2011). Indeed, especially oligochaete benthic species are generally the dominant in muddy sediments of lakes or in the swampy areas, able to exploit all the waste materials that decompose on the bottom (Abubakr, 2018). Despite the low number of studies, thank to their high resistance, these organisms should be considered extremely useful to understand how much plastics could be dangerous. In fact, any effect caused by these synthetic polymers represent a significant sign of their potential toxicity.

In the oligochaete aquatic worm *Allonais inaequalis*, the role of PE MPs was investigated. Particles possessed a size between 40 and 48 μm and were provided both in normal temperature (24 °C) and thermal stress (19 °C and 29 °C) conditions. Independently from temperatures, after 96 h MPs were ingested by worms without affecting animals survival. Moreover, also chronic response did not induce particular effects in terms of mortality and reproductivity. However, as suggested by the authors, although no specific effects were recorded, exposition time, particle size, age and plastic type could also produce different responses. Nevertheless, thanks to its resistance properties, *A. inaequalis* can represent a suitable model for deepening microplastics effects in freshwaters animals (Castro *et al*., 2020).

The resistance of oligochaete species to contaminants was also confirmed in *Tubifex spp.*, which inhabit areas in which the pollution levels are extremely high (Sharma and Chowdhary, 2011). It was demonstrated that in the species *Tubifex tubifex*, MPs ingestion did not affect life cycle and reproduction. As confirmed by FTIR analyses, a wide range of synthetic polymers were found, located in the digestive system and presenting different sizes and concentrations. Although no effects were registered, this study specifically suggests as the bioaccumulation of MPs into tissues and organs, make these oligochaetes a potential risk for the other organisms in relation to the trophic transfer (Hurley *et al.,* 2017).

As concern Hirudinea, leeches are considered a valid bioindicators, whose ability to accumulate pollutants is comparable only with that of oligochaetes. Is has been discovered that the concentrations of contaminants in different species of leeches were higher than in other analyzed organism, vertebrates included. For example, in *Erpobdella punctata* the levels of mirex, a chlorinated hydrocarbon used in the past as insecticide and nowadays banned due to its significant environmental impact, were higher than in other invertebrates and fishes. Whereas leeches of the Glossiphoniidae family collected in the Tahoe Lake (USA) presented a greater quantity of DDT than filter-feeding clams of the genus Pisidium (Metcalfe and Carey, 1984). In addition, leeches not only are considered a suitable model for studying innate immunity and regeneration (Grimaldi *et al.,* 2009, 2010; Baranzini *et al.,* 2020, 2021), but also they have been already used to investigate nanoparticles effects (Girardello *et al.,* 2015, 2017; Bodó *et al.,* 2020).

For all these reasons, the potential role of MPs and NPs PP has been analyzed in the freshwater leech *Hirudo verbana*, focused the attention on the possible inflammatory response activation. To better follow particles fate, mechanical-obtained fluorescent plastics were administered to water at the concentration of 400 mg/L and the effects were analyzed both after short and long exposure timings (1 h 6 h, 1 week, 1 month and 2 months). By means of microscopy and DLS analyses, the size and the fluorescent properties of manually created PP fragments have been evaluated. Morphological, histoenzymatic and molecular assays confirmed as these synthetic polymers not only entered inside tissues, passing the external cuticle, but also lead to the activation of angiogenetic processes and macrophage activation. Moreover, despite an initial protection by mucous cells, their presence caused an increase of the expression levels of important pro-inflammatory markers, such as *Hm*AIF-1 and *Hv*RNASET2 (Baranzini *et al*., 2019). Gene upregulation was also observed for SOD and GST antioxidant enzymes, suggesting as PP MPs and NPs induced oxidative stress and confirming their potential harmful effects, also at the cellular level (Fig. 2) (Baranzini *et al.,* 2022, accepted).

Concluding remarks

Plastic MPs and NPs, deriving from waste materials or directly released during industrial manufacturing, are widely present both in marine and

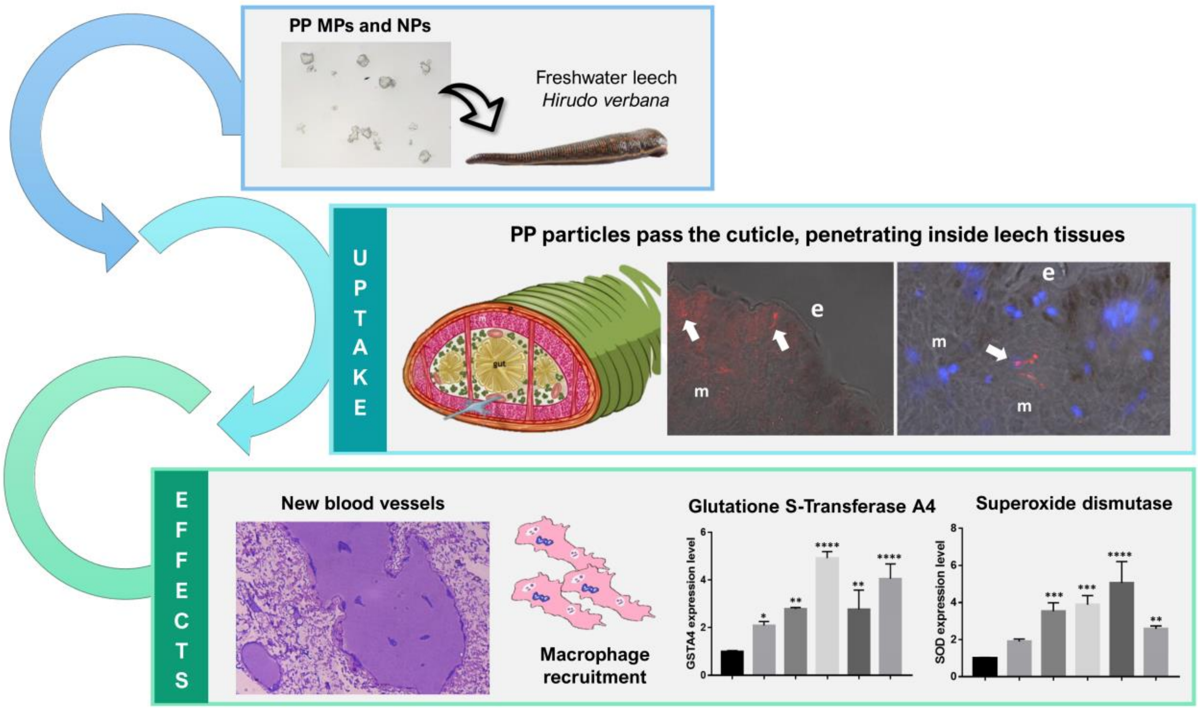

Fig. 2 Representation of PP plastics uptake and effects in the medicinal leech *H. verbana*. Leeches were exposed to water resuspended plastic fragments that are able to pass external cuticle and accumulate inside tissues. Once entered, the main effects immediately relevant induced by PP MPs and NPs involved angiogenesis, macrophage recruitment and activation, and oxidative stress

freshwater aquatic ecosystems. The consequent pollution represents a significant problem at the global level, due to the potential risks not only for organisms that inhabit a specific environment but also for human health. In this context, invertebrates are essential for better comprehend these aspects. Living in all the ecological niches, they are often the primary organisms that enter in contact with plastics particles, also representing an entrance for these synthetic materials in the trophic chain. However, compared with the actual situation, the number of studies present in literature is still low and investigating plastics effects must be considered crucial also in these models. Moreover, although the highest percentage of research is focused on PS MPs and NPs, less is known about other plastics and the different administering methods, particle types, timings and the diverse concentrations used make complicated to find uniformity in these results. Notwithstanding these critical issues, the current data are fundamental to lay the foundations to understand the environmental consequences caused by plastics presence and to direct the future research to analyze these effects both at organism and cellular level.

Acknowledgments

The authors would like to thanks the Biology Invertebrates Lab and The Protein Factory 2.0 for the support in the current publication.

References

Abubakr A. Macro-invertebrates (Annelida; Oligochaeta) as bio-indicator of water quality under temperate climatic conditions. Int. J. Pure Appl. Biosci. 6: 726-737, 2018.

Aliko V, Beqiraj EG, Qirjo M, Cani M, Rama A., Bego K, *et al*. Plastic invasion tolling: First evaluation of microplastics in water and two crab species from the nature reserve lagoonary complex of Kune-Vain, Albania. Sci. Total. Environ. 849: 157799, 2022.

Alimba CG, Faggio C. Microplastics in the marine environment: Current trends in environmental pollution and mechanisms of toxicological profile. Environ. Toxicol. Pharmacol. 68: 61-74, 2019.

Alimba CG, Faggio C, Sivanesan S, Ogunkanmi A, Krishnamurthi K. Micro(nano)-plastics in the environment and risk of carcinogenesis: Insight into possible mechanisms. J. Hazard. Mater. 416: 126143, 2021.

Andrady AL. Microplastics in the marine environment. Mar. Pollut. Bull. 62: 1596-1605, 2011.

Anger K. Contributions of larval biology to crustacean research: A review. Invertebr. Reprod Dev. 49: 175-205, 2006.

Arthur C, Baker J, Bamford H. Proceedings of the International Research Workshop on the Occurrence, Effects, and Fate of Microplastic Marine Debris. Group 530, 2009.

Auclair J, Quinn B, Peyrot C, Wilkinson KJ, Gagné F. Detection, biophysical effects, and toxicity of polystyrene nanoparticles to the cnidarian *Hydra attenuata*. Environ. Sci. Pollut. Res. 27: 11772-11781, 2020.

Auta HS, Emenike CU, Fauziah SH. Distribution and importance of microplastics in the marine environment. A review of the sources, fate, effects, and potential solutions. Environ. Int. 102: 165-176, 2017.

Baranzini N, Monti L, Vanotti M, Orlandi VT, Bolognese F, Scaldaferri D, *et al*. AIF-1 and RNASET2 play complementary roles in the innate immune response of medicinal leech. J. Innate Immun. 11: 150-167, 2019.

Baranzini N, Pulze L, Acquati F, Grimaldi A. *Hirudo verbana* as an alternative model to dissect the relationship between innate immunity and regeneration. Invertebr. Surviv. J. 17: 90-98, 2020.

Baranzini N, Pulze L, Tettamanti G, Acquati F, Grimaldi A. *Hv*RNASET2 Regulate connective tissue and collagen I remodeling during wound healing process. Front. Physiol. 12: 1-15, 2021.

Baranzini N, Pulze L, Bon C, Izzo L, Pragliola S, *et al*. *Hirudo verbana* as a freshwater invertebrate model to assess the effects of polypropylene micro and nanoplastics dispersion in freshwater. Fish Shellfish Immunol. 127: 492-507, 2022.

Barnes DKA, Galgani F, Thompson RC, Barlaz M. Accumulation and fragmentation of plastic debris in global environments. Philos. Trans. R. Soc. B. Biol. Sci. 364: 985-1998, 2009.

Beach MJ, Pascoe D. The role of hydra vulgaris (pallas) in assessing the toxicity of freshwater pollutants. Water Res. 32: 101-106, 1998.

Bergami E, Pugnalini S, Vannuccini ML, Manfra L, Faleri C, Savorelli F, *et al.* Long-term toxicity of surface-charged polystyrene nanoplastics to marine planktonic species *Dunaliella tertiolecta* and *Artemia franciscana*. Aquat. Toxicol. 189: 159-169, 2017.

Bodó K, Baranzini N, Girardello R, Kokhanyuc B, Németh P, Hayashi Y, *et al*. Nanomaterials and annelid immunity: A comparative survey to reveal the common stress and defense responses of two sentinel species to nanomaterials in the environment. Biology. 9: 1-22, 2020.

Brignac KC, Jung MR, King C, Royer S, Blickley L, Lamson MR, *et al*. Marine Debris Polymers on Main Hawaiian Island Beaches, Sea Surface, and Seafloor. Environ. Sci. Technol. 53: 12218–12226, 2019.

Bringer A, Thomas H, Prunier G, Dubillot E, Bossut N, Churlaud C, *et al*. High density polyethylene (HDPE) microplastics impair development and swimming activity of Pacific oyster D-larvae, *Crassostrea gigas*, depending on particle size. Environ. Pollut. 260, 2020.

Burgos-Aceves MA, Abo-Al-Ela HG, Faggio C. Impact of phthalates and bisphenols plasticizers on haemocyte immune function of aquatic invertebrates: A review on physiological, biochemical, and genomic aspects. J. Hazard. Mater. 419: 126426, 2021a.

Burgos-Aceves MA, Abo-Al-Ela HG, Faggio C. Physiological and metabolic approach of plastic additive effects: Immune cells responses. J. Hazard. Mater. 404: 124114, 2021b.

Burgos-Aceves MA, Cohen A, Smith Y, Faggio C. MicroRNAs and their role on fish oxidative stress during xenobiotic environmental exposures. Ecotoxicol. Environ. Saf. 148: 995-1000, 2018.

Cai M, Liu Z, Chen M, Huang Y, Zhang M, Yao Y, *et al*. Changes in ultrastructure of gonads and external morphology during aging in the parthenogenetic cladoceran *Daphnia pulex*. Micron. 122: 1-7, 2019.

Canesi L, Ciacci C, Bergami E, Monopoli MP, Dawson KA, Papa S, *et al*. Evidence for immunomodulation and apoptotic processes induced by cationic polystyrene nanoparticles in the hemocytes of the marine bivalve Mytilus. Mar. Environ. Res. 111: 34-40, 2015.

Canesi L, Ciacci C, Fabbri R, Balbi T, Salis A, Damonte G, *et al*. Interactions of cationic polystyrene nanoparticles with marine bivalve hemocytes in a physiological environment: Role of soluble hemolymph proteins. Environ. Res. 150: 73-81, 2016.

Capolupo M, Franzellitti S, Valbonesi P, Sanza Lanzas C, Fabbri E. Uptake and transcriptional effects of polystyrene microplastics in larval stages of the Mediterranean mussel *Mytilus galloprovincialis*. Environ. Pollut. 241: 1038-1047, 2018.

Cappello T, De Marco G, Oliveri Conti G, Giannetto A, Ferrante M, Mauceri M, *et al*. Time-dependent metabolic disorders induced by short-term exposure to polystyrene microplastics in the Mediterranean mussel *Mytilus galloprovincialis*. Ecotoxicol Environ Saf 209: 111780, 2021.

Castro GB, Bernegossi AC, Pinheiro FR, Felipe MC, Corbi JJ. Effects of Polyethylene Microplastics on Freshwater Oligochaeta *Allonais inaequalis* (Stephenson, 1911) Under Conventional and Stressful Exposures. Water Air Soil Pollut. 231, 2020.

Chubarenko I, Efimova I, Bagaeva M, Bagaev A, Isachenko I. On mechanical fragmentation of single-use plastics in the sea swash zone with different types of bottom sediments: Insights from laboratory experiments. Mar. Pollut. Bull. 150, 2020.

Costa E, Gambardella C, Piazza V, Vassalli M, Sbrana F, Lavorano S, *et al*. Microplastics ingestion in the ephyra stage of *Aurelia sp*. triggers acute and behavioral responses. Ecotoxicol. Environ. Saf. 189: 109983, 2020.

Cui R, Kim SW, An YJ. Polystyrene nanoplastics inhibit reproduction and induce abnormal embryonic development in the freshwater crustacean *Daphnia galeata*. Sci. Rep. 7: 1-10, 2017.

Curpan AS, Impellitteri F, Plavan G, Ciobica A, Faggio C. Review: *Mytilus galloprovincialis*: An essential, low-cost model organism for the impact of xenobiotics on oxidative stress and public health. Comp. Biochem. Physiol. Part - C Toxicol. Pharmacol. 256: 109302, 2022.

Danso D, Chow J, Streita WR. Plastics: Environmental and biotechnological perspectives on microbial degradation. Appl. Environ. Microbiol. 85, 2019.

Della Torre C, Bergami E, Salvati A, Faleri C, Cirino P, Dawson KA, *et al.* Accumulation and embryotoxicity of polystyrene nanoparticles at early stage of development of sea urchin embryos *Paracentrotus lividus.* Environ. Sci. Technol. 48: 12302-12311, 2014.

Devereux R, Hartl MGJ, Bell M, Capper A. The abundance of microplastics in cnidaria and ctenophora in the North Sea. Mar. Pollut. Bull. 173: 112992, 2021.

Eom HJ, Lee N, Yum S, Rhee JS. Effects of extremely high concentrations of polystyrene microplastics on asexual reproduction and nematocyst discharge in the jellyfish *Sanderia malayensis*. Sci. Total. Environ. 807: 150988, 2022.

Eom HJ, Nam SE, Rhee JS. Polystyrene microplastics induce mortality through acute cell stress and inhibition of cholinergic activity in a brine shrimp. Mol. Cell. Toxicol. 16: 233-243, 2020.

Faggio C, Tsarpali V, Dailianis S. Mussel digestive gland as a model tissue for assessing xenobiotics: An overview. Sci. Total. Environ. 636: 220-229, 2018.

Galgani F. Marine litter, future prospects for research. Front. Mar. Sci. 2:1–5, 2015.

Gambardella C, Morgana S, Ferrando S, Bramini M, Piazza V, Costa E, *et al*. Effects of polystyrene microbeads in marine planktonic crustaceans. Ecotoxicol. Environ. Saf. 145: 250-257, 2017.

Girardello R, Baranzini N, Tettamanti G, de Eguileor M, Grimaldi A. Cellular responses induced by multi-walled carbon nanotubes: In vivo and in vitro studies on the medicinal leech macrophages. Sci. Rep. 7: 1-12, 2017.

Girardello R, Tasselli S, Baranzini N, Valvassori R, de Eguileor M, Grimaldi A. Effects of Carbon Nanotube Environmental Dispersion on an Aquatic Invertebrate, *Hirudo medicinalis*. PLoS One 10:e0144361, 2015.

Gonçalves JM, Sousa VS, Teixeira MR, Bebianno MJ. Chronic toxicity of polystyrene nanoparticles in the marine mussel *Mytilus galloprovincialis*. Chemosphere 287, 2022.

Gourmelon G. Global Plastic Production Rises, Recycling Lags | Worldwatch Institute. WorldWatch Inst. 1-7, 2015.

Gregory MR. Environmental implications of plastic debris in marine settings- entanglement, ingestion, smothering, hangers-on, hitch-hiking and alien invasions. Philos. Trans. R. Soc. B. Biol. Sci. 364: 2013-2025, 2009.

Grillo JF, Sabino MA, Ramos R. Short-term ingestion and tissue incorporation of Polystyrene microplastic in the scleractinian coral *Porites porites*. Reg Stud. Mar. Sci. 43: 101697, 2021.

Grimaldi A, Banfi S, Bianchi C, Greco G, Tettamanti G, Noonan DM, *et al*. The Leech: A Novel Invertebrate Model for Studying Muscle Regeneration and Diseases. Curr Pharm Des 16: 968-977, 2010.

Grimaldi A, Banfi S, Gerosa L, Tettamanti G, Noonan DM, Valvassori R, *et al*. Identification, Isolation and Expansion of Myoendothelial Cells Involved in Leech Muscle Regeneration. PLoS One 4:e7652, 2009.

Guzzetti E, Sureda A, Tejada S, Faggio C. Microplastic in marine organism: Environmental and toxicological effects. Environ. Toxicol. Pharmacol. 64: 164-171, 2018.

Haegerbaeumer A, Mueller MT, Fueser H, Traunspurger W. Impacts of micro- and nano-sized plastic particles on benthic invertebrates: A literature review and gap analysis. Front. Environ. Sci. 7, 2019.

Honorato-Zimmer D, Kiessling T, Gatta-Rosemary M, Campódonico CK, Núñez-Faríasc P, Rech S, *et al.* Mountain streams flushing litter to the sea - Andean rivers as conduits for plastic pollution. Environ. Pollut. 291, 2021.

Hurley RR, Woodward JC, Rothwell JJ. Ingestion of Microplastics by Freshwater Tubifex Worms. Environ. Sci. Technol. 51: 12844-12851, 2017.

Imhof HK, Laforsch C. Hazardous or not - Are adult and juvenile individuals of *Potamopyrgus antipodarum* affected by non-buoyant microplastic particles? Environ. Pollut. 218: 383-391, 2016.

Jaeschke BC, Lind OC, Bradshaw C, Salbu B. Retention of radioactive particles and associated effects in the filter-feeding marine mollusc Mytilus edulis. Sci. Total Environ. 502: 1-7, 2015.

Jambeck JR, Geyer R, Wilcox C, Siegler TR, Perryman M, Andrady A, *et al*. Plastic waste inputs from land into the ocean. Sciences, 347: 768-771, 2015.

Jemec A, Horvat P, Kunej U, Belec M, Kržanb A. Uptake and effects of microplastic textile fibers on freshwater crustacean *Daphnia magna*. Environ. Pollut. 219: 201-209, 2016.

Kalsom M, Mehman B. The Environmental Effects of Sea Pollution on Microorganisms. 4: 161-168, 2020.

Kim D, Kim H, An YJ. Effects of synthetic and natural microfibers on *Daphnia magna*-Are they dependent on microfiber type? Aquat. Toxicol. 240: 105968, 2021.

Kremen C, Colwell RK, Erwin TL, Murphy DD, Noss RF, Sanjayan MA. Terrestrial Arthropod Assemblages: Their Use in Conservation Planning. Conserv Biol 7: 796-808, 1993.

Lanctôt CM, Bednarz VN, Melvin S, Jacoba H, Oberhaenslia F, Swarzenski PW, *et al*. Physiological stress response of the scleractinian coral *Stylophora pistillata* exposed to polyethylene microplastics. Environ. Pollut. 263, 2020.

Lee BK, Yun Y, Park K. PLA micro- and nano-particles. Adv Drug Deliv Rev 107: 176-191, 2016.

Letcher TM. Introduction to plastic waste and recycling. Elsevier Inc., 2020.

Leung J and Chan KYK. Microplastics reduced posterior segment regeneration rate of the polychaete Perinereis aibuhitensis. Mar. Pollut. Bull. 129: 782-786, 2018.

Liu Z, Yu P, Cai M, Zhang M, Chen M, Zhao Y, *et al*. Effects of microplastics on the innate immunity and intestinal microflora of juvenile *Eriocheir sinensis.* Sci. Total Environ. 685: 836-846, 2019a.

Liu Z, Yu P, Cai M, Huang Y, Zhao Y. Polystyrene nanoplastic exposure induces immobilization, reproduction, and stress defense in the freshwater cladoceran *Daphnia pulex*. Chemosphere. 215: 74-81, 2019b.

Lombardo J, Solomando A, Cohen-Sánchez A, Pinya S, Tejada S. Effects of Human Activity on Markers of Oxidative Stress in the Intestine of *Holothuria tubulosa*, with Special Reference to the Presence of Microplastics. Int. J. Mol. Sci. 23: 9018, 2022.

Lozano-Bilbao E, Alcázar-Treviño J, Fernández JJ. Determination of δ15N in Anemonia sulcata as a pollution bioindicator. Ecol. Indic. 90: 179-183, 2018.

Lu H, Diaz DJ, Czarnecki NJ, Zhu C, Kim W, Shroff R, *et al.* Machine learning-aided engineering of hydrolases for PET depolymerization. Nature 604: 662-667, 2022.

Luo T, Zhang Y, Wang C, Wang X, Zhou J, Shen M, *et al*. Maternal exposure to different sizes of polystyrene microplastics during gestation causes metabolic disorders in their offspring. Environ. Pollut. 255: 113122, 2019.

Lusher AL, Tirelli V, O'Connor I, Officer R. Microplastics in Arctic polar waters: The first reported values of particles in surface and sub-surface samples. Sci. Rep. 5: 1-9, 2015.

Macali A, Bergami E. Jellyfish as innovative bioindicator for plastic pollution. Ecol. Indic. 115, 2021.

Maes T, Jessop R, Wellner N, Haupt K, Mayes AG. A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile Red. Sci. Rep. 7: 1-10, 2017.

Mazur AA, Chelomin VP, Zhuravel EV, Kukla SP, Slobodskova VV, Dovzhenko NV. Genotoxicity of polystyrene (Ps) microspheres in short-term exposure to gametes of the sand dollar *Scaphechinus mirabilis* (agassiz, 1864) (echinodermata, echinoidea). J. Mar. Sci. Eng. 9, 2021.

Metcalfe and Carey. Aquatic leeches (Hirudinea) as bioindicators of organic chemical contaminants in freshwater ecosystems. Chemosphere. 13: 143-150, 1984.

Missawi O, Bousserrhine N, Zitouni N, Maisano M, Boughattas I, De Marco G, *et al.* Uptake, accumulation and associated cellular alterations of environmental samples of microplastics in the seaworm *Hediste diversicolor*. J. Hazard Mater. 406: 124287, 2021.

Mistri M, Infantini V, Scoponi M, Granata T, Moruzzi L, Massara F, *et al*. Small plastic debris in sediments from the Central Adriatic Sea: Types, occurrence and distribution. Mar. Pollut. Bull. 124: 435-440, 2017.

Muñoz-Vera A, García G, García-Sánchez A. Metal bioaccumulation pattern by *Cotylorhiza tuberculata* (Cnidaria, Scyphozoa) in the Mar Menor coastal lagoon (SE Spain). Environ. Sci. Pollut. Res. 22: 19157-19169, 2015.

Nobre CR, Santana MFM, Maluf A, Cortez FS, Cesar A, Pereira CDS, *et al.* Assessment of microplastic toxicity to embryonic development of the sea urchin *Lytechinus variegatus* (Echinodermata: Echinoidea). Mar. Pollut. Bull. 92: 99-104, 2015.

Oliveira M and Almeida M. The why and how of micro(nano)plastic research. TrAC - Trends. Anal. Chem. 114: 196-201, 2019.

Pagano M, Stara A, Aliko V, Faggio C. Impact of neonicotinoids to aquatic invertebrates-in vitro studies on *Mytilus galloprovincialis*: A review. J. Mar. Sci. Eng. 8: 1-14, 2020.

Patil SM, Rane NR, Bankole PO, Krishnaiah P, Ahn Y, Park Y-J, *et al*. An assessment of micro- and nanoplastics in the biosphere: A review of detection, monitoring, and remediation technology. Chem. Eng. J. 430: 132913, 2022.

Pires A, Figueira E, Silva MSS, Sà C, Marques PAAP. Effects of graphene oxide nanosheets in the polychaete *Hediste diversicolor*: Behavioural, physiological and biochemical responses. Environ. Pollut. 299: 118869, 2022.

Pirillo V, Orlando M, Tessaro D, Pollegioni L, Molla G. An efficient protein evolution workflow for the improvement of bacterial PET hydrolyzing enzymes. Int. J. Mol. Sci. 23, 2022.

Pirillo V, Pollegioni L, Molla G. Analytical methods for the investigation of enzyme-catalyzed degradation of polyethylene terephthalate. FEBS J. 288: 4730-4745, 2021.

Prokić MD, Radovanović TB, Gavrić JP, Faggio C. Ecotoxicological effects of microplastics: Examination of biomarkers, current state and future perspectives. TrAC - Trends Anal. Chem. 111: 37-46, 2019.

Revel M, Yakovenko N, Caley T, Guillet C, Châtel A, Mouneyrac C. Accumulation and immunotoxicity of microplastics in the estuarine worm *Hediste diversicolor* in environmentally relevant conditions of exposure. Environ. Sci. Pollut. Res. 27: 3574-3583, 2020.

Rist S, Baun A, Almeda R, Hartmann NB. Ingestion and effects of micro- and nanoplastics in blue mussel (*Mytilus edulis*) larvae. Mar. Pollut. Bull. 140: 423-430, 2019.

Rittschof D, McClellan-Green P. Molluscs as multidisciplinary models in environment toxicology. Mar. Pollut. Bull. 50: 369-373, 2005.

Rocha RJM, Rodrigues ACM, Campos D, Cicero LH, Costa APL, Silva DAM, *et al.* Do microplastics affect the zoanthid *Zoanthus sociatus*? Sci. Total. Environ. 713: 136659, 2020.

Rodríguez-Hernández AG, Muñoz-Tabares JA, Aguilar-Guzmán JC, Vazquez-Duhalt R. A novel and simple method for polyethylene terephthalate (PET) nanoparticle production. Environ. Sci. Nano. 6: 2031-2036, 2019.

Romero-Blanco A, Remón-Elola A, Alonso Á. Assessment of the Effects of Environmental Concentrations of Microplastics on the Aquatic Snail *Potamopyrgus antipodarum*. Water Air Soil Pollut. 232: 1-14, 2021.

Sharma KK and Chowdhary S. Macroinvertebrate assemblages as biological indicators of pollution in a Central Himalayan River, Tawi (J & K). Int. J. Biodivers. Conserv. 3: 167-174, 2021.

Silva MSS, Oliveira M, Valente P, Figueira E, Martins M, Pires A. Behavior and biochemical responses of the polychaeta *Hediste diversicolor* to polystyrene nanoplastics. Sci. Total. Environ. 707: 134434, 2020.

Sonnendecker C, Oeser J, Richter PK, Hille P, Zhao Z, Fischer C, *et al*. Low Carbon Footprint Recycling of Post-Consumer PET Plastic with a Metagenomic Polyester Hydrolase. ChemSusChem. 15, 2022.

Stara A, Pagano M, Albano M, Savoca S, Di Bella G, Albergamo A, *et al*. Effects of long-term exposure of *Mytilus galloprovincialis* to thiacloprid: A multibiomarker approach. Environ. Pollut. 289: 117892, 2021.

Stara A, Pagano M, Capillo G, Fabrello J, Sandova M, Vazzana I, *et al*. Assessing the effects of neonicotinoid insecticide on the bivalve mollusc *Mytilus galloprovincialis*. Sci. Total. Environ. 700: 134914, 2020.

Strungaru SA, Jijie R, Nicoara M, Plavan G, Faggio C. Micro- (nano) plastics in freshwater ecosystems: Abundance, toxicological impact and quantification methodology. TrAC - Trends Anal. Chem. 110: 116-128, 2019.

Suman TY, Jia PP, Li WG, Junaid M, Xin G-Y, Wang Y. Acute and chronic effects of polystyrene microplastics on brine shrimp: First evidence highlighting the molecular mechanism through transcriptome analysis. J. Hazard Mater. 400: 123220, 2020.

Taylor ML, Gwinnett C, Robinson LF, Woodall LC. Plastic microfibre ingestion by deep-sea organisms. Sci. Rep. 6: 1-9, 2016.

Taylor NS, Gavin A, Viant MR. Metabolomics discovers early-response metabolic biomarkers that can predict chronic reproductive fitness in individual D*aphnia magna*. Metabolites 8, 2018.

Tiwari N, Santhiya D, Sharma JG. Microbial remediation of micro-nano plastics: Current knowledge and future trends. Environ. Pollut. 265: 115044, 2020.

Tournier V, Topham CM, Gilles A, David B, Moya-Leclair E, Kamionka E, *et al.* An engineered PET depolymerase to break down and recycle plastic bottles. Nature 580: 216-219, 2020.

Tramoy R, Gasperi J, Colasse L, Tassin B. Transfer dynamic of macroplastics in estuaries - New insights from the Seine estuary: Part 1. Long term dynamic based on date-prints on stranded debris. Mar. Pollut. Bull. 152: 110894, 2020.

Van Cauwenberghe L, Vanreusel A, Mees J, Janssen CR. Microplastic pollution in deep-sea sediments. Environ. Pollut. 182: 495-499, 2013.

Vencato S, Isa V, Seveso D, Saliu F, Galli P, Lavorano S, *et al.* Soft corals and microplastics interaction: first evidence in the alcyonacean species *Coelogorgia palmosa*. Aquat Biol 30: 133-139, 2021.

Verma AK and Prakash S Status of Animal Phyla in Different Kingdom Systems of Biological Classification. Int. J. Biol. Innov. 02: 149-154, 2020.

Xu X, Wong CY, Tam NFY, Lo HS, Cheung SG. Microplastics in invertebrates on soft shores in Hong Kong: Influence of habitat, taxa and feeding mode. Sci. Total. Environ. 715:136999, 2020.