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Authors: Inês Ferreira, Cátia Venâncio, Isabel Lopes, Miguel Oliveira

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Nanoplastics and marine organisms: what has been studied?

Inês Ferreira[a], Cátia Venâncio[b], Isabel Lopes[b], Miguel Oliveira[b*]

[a] Department of Biology, Campus de Santiago, University of Aveiro, 3810-193 Aveiro.

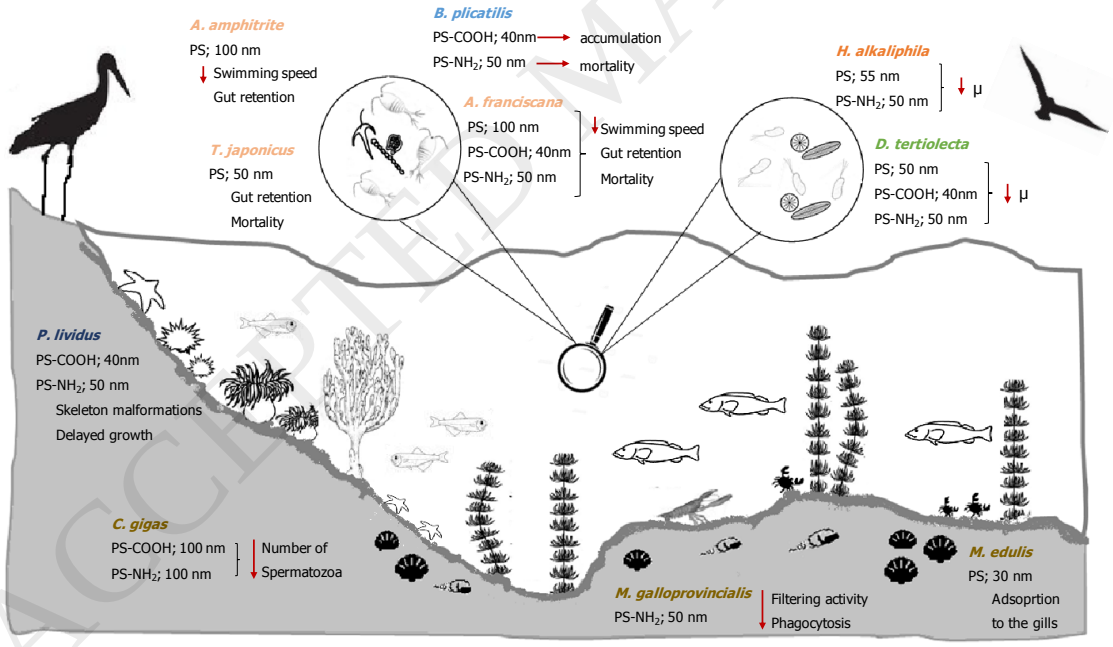
[b]Department of Biology & CESAM, Campus de Santiago, University of Aveiro, 3810-193 Aveiro.

*Corresponding author:

Miguel Oliveira

E-mail: migueloliveira@ua.pt

Graphical abstract



Abstract

Nowadays, there is an increased awareness on the threat that marine litter may pose to the marine environment. This review describes the major concerns related to plastic pollution, namely in terms of toxicity of different types and sizes of nanoplastics (particles smaller than 100 nm) to marine organisms, either producers or consumers. The available data show that nanoplastics may affect negatively organisms from different phyla with reported effects ranging from alterations in reproduction to lethality. Nevertheless, no information regarding marine vertebrates (e.g., fish) was found. Data show a high potential for bioaccumulation/biomagnification along marine food chains, since they can easily be retained inside organisms. The lack of standardized methodology for nanoplastics detection and the poor or inexistent legislation makes nanoplastics an environmental challenge.

Keywords: Ecological risks; nanoplastic; marine organisms; effects

1. Plastic in the marine environment

Marine litter consists of any persistent, manufactured or processed solid material that ends up in the sea. Its increment around the world is becoming a threat to the marine ecosystem. Among the different materials included in the marine litter category, microplastics and nanoplastics are recognized as emerging contaminants of concern. Plastics are defined as synthetic organic polymers that can be easily molded into different shapes and products (Worm et al., 2017), with high durability, light weight and cost effectiveness. These properties make plastics a support for a large variety of applications: from simple plastic bottles, containers for food products and consumer goods, up to the sectors of transport, construction, telecommunications and health care (Gourmelon et al., 2015). Their wide use increased their release into the environment, either deliberately (e.g., throw domestic and industrial effluents) or unintentionally (e.g., run-off) (Todd et al., 2010; Sá et al., 2018). Since the 1990s the annual plastic production increased from 1.7 to 335 million tones in 2016 (PlasticsEurope, 2017). Furthermore, it has been estimated that 4.8 to 12.7 million tons of plastic debris enter the ocean each year (Jambeck et al., 2015). According to the Plastic's Europe Market Research and Statistics (Plastics Europe, 2017), the most produced plastic polymers are polypropylene (PP), low-density polyethylene (LDPE), high-density polyethylene (HDPE), polyvinyl chloride (PVC), polyurethane (PUR) polyethylene terephthalate (PET) and polystyrene (PS), being employed in several manufacture industries, from electronics to health care, as illustrated in Figure 1. For instance, in a field study performed in the southern Adriatic sea, of a total of 120 samples (water and sediment), 80.6 % contained plastic debris, and 38.7% of the samples were composed of polystyrene plastics (Šilc et al., 2018).

One of the concerns associated with plastic pollution is the occurrence of particles smaller than 5 mm, particularly in the low micro and nano sizes. Although there is no established definition of nanoplastic, it has been assumed that they fall within the range of other types of nanoparticles i.e. a size range from 1 to 100 nm (Koelmans et al., 2015; Gigault et al., 2018). Microplastics and/or nanoplastics may be divided in primary or secondary. Primary micro(nano)plastics are those that enter the ecosystem in their originally small size associated with specific applications and consumer products, such as synthetic fibers, cosmetics, medicine and raw materials (Bessa et al., 2018; Tamminga et al., 2018; Wang et al., 2018). Their release to the

environment is frequently associated with inadequacy of the disposal infrastructures at wastewater treatment plants (WWTP). For example, in a study addressing this issue, a WWTP located in the Baltic Sea was able to reduce the burden of microplastics in wastewaters from hundreds to less than 10 microparticles per liter of wastewater (Talvitie et al., 2015). However, these values of particles per liter of wastewater were still 25 times higher than those reported for sea water samples (Talvitie et al., 2015). Alongside the disposal of primary micro(nano)plastics, their levels in the environment may increase as a result of the degradation of macroplastics, the so called secondary micro(nano)plastics (Andrady, 2011; Cole et al., 2011). This process of breakdown happens because once in the environment, polymers are susceptible to biological activity (such as the action of bacteria) and/or subjected to several abiotic processes (wind, rain, UV radiation, mechanical forces, photo-oxidation) (Andrady, 2003). Their action, solely or jointly, may promote a decrease in the size of the particles, first to micro and later to nanoplastics (Lambert & Wagner, 2016). The process of fragmentation/degradation has already been demonstrated to occur rapidly under laboratorial conditions. During a thermal cutting process of polystyrene foam (60 min of cutting and the following 10 min), Zhang et al. (2012) found that most of the particles emitted were of sizes between 22 and 220 nm. Using PS disposable coffee cup lids, Lambert & Wagner (2016) showed that 56 days were enough to reach a concentration of 1.26×10^8 particles/mL of particles with an average size of 224 nm. The time required to reach nano sized particles depends on the size of the initial plastic (Koelmans et al., 2015). The degradation process, one of the main problems associated with the presence of plastics in the environment, will drastically reduce the average molecular weight of the polymer, further increasing their susceptibility to breakdown but at the same time, making them more available to be ingested by the marine biota (Santos et al., 2009; Andrady, 2011). Thus, if not properly disposed, reused or recycled, plastics may become a serious threat to the aquatic environment. The presence of plastic particles in freshwater, estuarine and marine environments has been reported in several studies, as showed in Figure 2, with reports of up to thousands of particles/m^2 (Carvalho & Neto, 2016). Nevertheless, the estuarine/marine environment is of most concern as it constitutes the final recipient of these particles that reach this environment through rivers, water runoff, wastewater discharges and transportation through wind. Recreational activities at the beach and ship-generated litter dumped by commercial boats, cruises or private vessels or fishing

gear may also contribute to the discharge of microplastics to the marine/estuary compartment (Pruter, 1987; Sheavly & Register, 2007). Despite the efforts to establish effective analytical procedures, detection of nanoplastic in environmental matrices is not yet possible (Koelmans et al., 2017; Hurley & Nizzetto, 2018). Techniques commonly applied to identify microplastics techniques like FTIR and Raman still lack spatial resolution (1 µm of Raman *versus* 1-20 of FTIR). The isolation of plastics present in the environment, both in the water and biological matrices, requires the development of new procedures able to effectively isolate particles of sizes below 300 µm without compromising the biota present in water nor the integrity of the polymers.

There are three major problems related to plastics: a) toxicity towards biota caused directly by the plastics themselves; b) toxicity caused by additives used during the production process and c) their role as vectors for environmental contaminants and invasive/pathogenic organisms.

There is a huge concern about the additives used during plastics production. Additives are mainly used as plasticizers, stabilizers and brominated flame retardants (Hermabessiere et al., 2017). They may not only contribute to increase the time of degradation of plastic, enduring their permanence in the environment but also may leach into the marine environment, and become available to biota (Avio et al., 2017). The most commonly used additives are bisphenols [e.g., bisphenol A (BPA)] and phthalates [polybrominated diphenyl ethers (PBDE) and tetrabromobisphenol A (TBBPA)]. They have been shown toxic to biota and its use, for some items, is nowadays prohibited in some countries such as France that banned the use of Bisphenol A (BPA) from all food packaging (LOI n° 2010-729 du 30 Juin). For example, BPA has been reported to affect growth rate and sexual maturation, hormone levels in blood, reproductive organ function, immune function, enzyme activity and brain structure (vom Saal & Hughes, 2005).

The presence of micro and nanoplastics in the marine environment can affect biota and the environment through other pathways. Smaller plastics (both micro and nanoplastics) have a high surface area and adsorb hydrophobic substances from the marine environment, namely persistent organic pollutants (POPs), such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), polybrominated diphenyl ethers (PBDEs) and perfluorooctanoic acid (PFOA) as well as metals (Moore et al., 2007; Ashton et al., 2010; Frias et al., 2010; Andrady, 2011; Holmes et al., 2012; Velzeboer et al., 2014;

Li et al., 2018). Rochman et al. (2013) found that LDPE, HDPE and PP plastic debris from San Diego Bay had a great affinity for chemical pollutants such as PCBs and PAHs. This ability to adsorb contaminants and release additives highlights the possibility of micro(nano)plastics transferring these contaminants to biota.

The ubiquity of plastic particles and the recognition that macroplastics can be degraded to micro and nanoplastics and thus become more bioavailable to biota raises concerns on the molecular and physiological effects that these particles may cause. Effects at behavioral and reproductive levels, in addition to the well reported effects of physical damage and false satiation are only some of the examples of impacts that may be attributed to macroplastics (Lazar & Gračan, 2011) and can be transversal from marine invertebrates to mammals. The known effects of microplastics include alteration of hormone levels and enzyme activity, oxidative stress, growth inhibition, loss of energy and weight, retention on digestive tract as well as in immune and reproductive system and even mortality (Jin et al., 2018; Li et al., 2018; Naji et al., 2018; Xiong et al., 2018).

2. Nanoplastics

Besides being originated from plastic fragmentation, nanoplastics can also be produced to be included in products for coatings, biomedical purposes, drug delivery, medical diagnostics, electronics, magnetics and optoelectronics (Koelmans et al., 2015). Alongside the decrease in size and consequent increase in surface area, that promotes the adsorbance of other environmental contaminants, the particles may become more reactive. The nanoparticle formation changes the chemical and physical characteristics of the particle and, consequently, its availability and biological impact on aquatic organisms (Mattsson et al., 2015). Therefore, it is expected that at the nanoscale the characteristics of particles (e.g. strength, conductivity and reactivity) will differ substantially from macro and micro-sized ones (Klaine et al., 2012). The biological reactivity is also frequently increased with a size decrease. The nano size increases the ability of the particles to pass throw cellular boundaries and accumulate on organisms and the reactivity of the particles (Mattsson et al., 2015; Worm et al., 2017), with more atoms and molecules displayed on the surface which can lead to more reactive groups on it (Nel, 2006). Although an increasing number of studies are focusing on the effects of microplastics, the knowledge of the effects of nanoplastics

are still scarce, especially regarding marine biota. Considering the hypothesis that reactivity increases at the nanoscale and that the marine ecosystems are the final recipient, it is urgent to gather the available information to identify knowledge gaps and set priorities and lines of investigation that should be addressed. Therefore, the objective of this review was to summarize published data on the effects of nanoplastics on marine biota, focusing in types of plastic that are being used and organisms are being studied, from producers to consumers.

3. Effects of nanoplastic particles

A literature review (in Scopus database) revealed 1699 articles focusing on microplastics; however, when the search was narrowed to the keyword “nanoplastics” the number decreased to 80. There were 26 documents when the keywords “nanoplastic” and “marine” were combined and only 20 when “nanoplastic” and “marine” are combined with the keyword “effects”. It is evident that more information is needed and the knowledge concerning nanoplastics is increasing in the last 2 years. From those 20 results, 14 are from 2017 and 2018. Gathering the information on the effects of nanoplastics will broader perspective of what has already been achieved and to where should the science efforts on this matter be directed to fill knowledge gaps. Thus, a compilation of reported effects on marine organisms was included in Table 1. This review will focus mainly on the toxic effects that nanoplastics are cause on marine biota. Therefore, from those 20 results only the ones analyzing effects on marine organisms and particles with less or equal to 100 nm were considered. A brief analysis of Table 1 immediately shows that all of the studies used PS as a model particle. This fact may be explained by the easy synthesis of nanoplastics of this polymer when compared with others. Still, the toxic effects exerted by PS may not correspond to the toxic effects caused by other polymers, emphasizing the urgent need to further generate information on this topic.

3.1. Effects on bacteria

Bacteria constitute a large domain of prokaryotic microorganisms. *Halomonas alkaliphile* is a specie from the proteobacteria phylum. Sun et al. (2018) exposed, for two hours, this halophilic bacterium (bacteria that thrive in high salt concentrations) to 50 nm cationic amino (-NH_2) PS particles and 55 nm PS beads at 20, 40, 80, 160 and

320 µg/mL. For PS-NH_2, cell growth was significantly affected from 80 µg/mL onwards, with a maximum of inhibition (34%) found at 360 µg/mL. Similarly, PS beads decreased the cellular growth up to 32.7% at 360 µg/mL. A significant increase in the intracellular levels of reactive oxygen species (ROS) was detected after 0.5 and 2 h exposure to both types of plastics

3.2. Effects on algae

Algae are photosynthetic, unicellular or pluricellular organisms, that contain chlorophyll, with no tissue differentiation or vascular transport organs. These organisms are vital to the wellbeing of marine ecosystems as they are the base of food webs, source of oxygen production and other nutrients (Mao et al., 2018). Effects of nanoplastics have already been assessed in these organisms. PS-NH_2 particles (50 nm) caused a significant inhibition on the growth rates of the unicellular green microalgae *Dunaliella tertiolecta*, with an estimated 72 h EC_{50} of 12.97 ± 0.57 µg/mL, whereas no effect was found after 72 h exposure to anionic carboxylated (-COOH) PS particles (40 nm) (Bergami et al., 2017). The observed effect may be associated with a pernicious effect on photosynthesis and ROS formation. In the same line of evidence and for the same species, Sjollema et al. (2016) observed a clear reduction on the average cell density (about 45%), that was translated in a 57% effect on cellular growth, after exposure to 250 µg/mL of 50 nm PS beads. These results suggest that nanoplastics may impair algae growth rates. However, it is crucial to study other species.

3.3. Effects on echinoderms

The phylum Echinodermata englobes marine invertebrates such as sea stars, sea cucumbers and sea urchins. The available studies with these organisms reveal that they may accumulate nanoplastics. Della Torre et al. (2014) reported that PS-COOH (50 µg/mL) nanoplastics accumulated inside the digestive tract of sea urchin (*Paracentrotus lividus*) embryos, with no relevant malformations in the embryos. However, PS-NH_2 (10 µg/mL) nanoplastics induced a higher toxicity, though not accumulating as PS-COOH particles. Several larvae presented malformations within a period of 6 to 48 hours post fertilization (hpf). The reported malformations included thickening and abnormal proliferation of the ectodermal membrane (6 hpf), undeveloped embryos (24 hpf), incomplete or absent skeletal rods, fractured ectoderm

and reduced length of the arms (48 hpf). The EC_{50} computed for PS-NH_2 beads were of 3.82 µg/mL at 24 hpf and 2.61 µg/mL at 48 hpf. More recent studies with the same species revealed that, after exposure to 3 µg/mL of PS-NH_2 (50 nm) skeleton elongation was delayed and 4 µg/mL induced malformations on skeletal rods and arms (Pinsino et al., 2017).

3.4. Effects on rotifers

The Rotifera phylum include around 2200 described species, some of them from marine ecosystems. Manfra et al. (2017) exposed the marine rotifer *Brachionus plicatilis* to a concentration range of 0.5, 1, 5, 10, 25 and 50 µg/mL of PS-COOH (40 nm) and PS-NH_2 (50 nm) nanoplastics. For PS-COOH particles, although no mortality was found, gut retention was observed after 48 h of exposure. For PS-NH_2 particles, LC_{50s} of 13.04 ± 0.60 and 6.62 ± 0.87 µg/mL, were estimated after 24 and 48 h exposures.

3.5. Effects on mollusks

Mollusks are the largest marine phylum and contains the class Bivalvia where clams, oysters, cockles, mussels and scallops, organisms widely used in ecotoxicity studies are included (Brandts et al., 2018). *Crassostrea gigas* exposed to 0.1, 1, 10, 100 µg/mL of 100 nm PS-NH_2 and PS-COOH did not affect the percentage of viable cells in spermatozoa. However, 100 µg/mL of PS-COOH particles promoted the aggregation of spermatozoa, resulting in a decrease of 32% and 24% of single spermatozoa after 3 and 5 h of exposure, respectively. Spermatozoa exposed to 100 µg/mL of PS-COOH and PS-NH_2 showed an increase of 4–5 % in relative size after 1, 3 and 5 h exposure. Moreover, ROS levels were not significantly affected by PS-NH_2 but PS-COOH exposure resulted in an increased ROS production of 17.4 %, 59.4 % and 121 % after 1 h exposure to exposure 1, 10 and 100 µg/mL, respectively (González-Fernández et al., 2018). In the common, edible mussel, *Mytilus edulis*, exposure to 100, 200 and 300 µg/mL of 30 nm PS particles induced the production of pseudofeses, which increased with concentration increase (Wegner et al., 2012). This result suggests that PS particles are recognized as non or low nutritional food. A reduction in the filtration rate, dependent on the PS concentration was found. In *M. galloprovincialis*, reproduction fitness was affected by nanoplastics. Fertilized eggs of *M. galloprovincialis* exposed to PS-NH_2 (50 nm) particles presented a decrease in

lysosomal membrane stability (50% at 50 µg/mL) as well as cytochrome c reduction (Canesi et al., 2015). Thus, this nanoplastics may impair cell metabolism/nutrition, signaling and repairing (cellular functions in which the lysosome plays an important role), as well as inhibiting mitochondria activity. Canesi et al. (2015) reported a decreased by 50% in phagocytosis at a concentration of 50 µg/mL of PS-NH_2. More recently, Balbi et al., (2017) reported that 48 h exposure to 0.001 to 1 µg/mL of PS-NH_2 (50 nm) caused malformations of the D-larvae (early stage in the development of a veliger) of *M. galloprovincialis* and a delay in development at higher concentrations (2.5 to 10 µg/mL). An EC_{50} of 0.142 µg/mL was determined for larval development. A decrease in shell length of 20% to 30% was also observed in 48 hpf larvae at different concentrations (0.15, 1, 2.5 and 5 µg/mL).

3.6. Effects on arthropods

Phylum Arthropoda includes crustaceans and englobes crabs, lobsters, crayfish, shrimp and krill. In order to evaluate the lethal and sub-lethal effects of nanoplastics Gambardella et al. (2017) exposed two marine crustaceans (II stage nauplii of the barnacle *Amphibalanus amphitrite* and first instar larvae of the brine shrimp *Artemia franciscana*) to 0.001, 0.01, 0.1, 1 and 10 µg/mL of 100 nm PS particles. No significant effects on survival were found but PS nanoparticles affected swimming speed. In *A. amphitrite* there was a significant inhibition at 48 h in the highest concentrations (1 and 10 µg/mL) whereas in *A. franciscana* swimming speed was inhibited at 24 h but significantly increased at longer exposure periods and higher concentrations. Both species ingested the nanoparticles and accumulated them in the gut after 24 and 48 h exposure. The brine shrimp species was also studied in the same larval stage by Bergami et al. (2017) although exposed to PS-COOH (40 nm) and PS-NH_2 (50 nm) particles at 0.5, 1, 1.5, 2.5, 5 and 10 µg/mL, to understand effects of nanoplastics at the molecular level. There were no significantly differences on organisms exposed to PS-COOH. However, in organisms exposed to 1 µg/mL PS-NH_2, the expression of two genes (*clap* and *cstb*) connected to growth which includes molting, organogenesis and tissue remodeling in early larvae, was increased after 48 h exposure and related to an increase in the number of molts. After 14 days exposure to PS-NH_2 nanoparticles, high mortality rates were registered, with an LC_{50} computed around 0.83 µg/mL. Bergami et al. (2016) also studied the marine shrimp *A. franciscana* up to Instar III Nauplius. In this study, organisms were exposed to 5, 10,

25, 50, and 100 µg/mL of PS-COOH (40 nm) and PS-NH_2 (50 nm) with data showing that both nanoplastics may accumulate in biota, being retained inside the gut lumen. However, cationic particles were more harmful affecting brine shrimp larvae swimming (at 48 h), an effect that can limit their feeding ability. Furthermore, an increase of almost 50% in molts cycle was observed after 48 h exposure to PS-NH_2. Lee et al., (2013) exposed the marine copepod *Tigriopus japonicus* to 0.125, 1.25, 12.5 and 25 µg/mL of 50 nm PS particles and verified that particles could also accumulate in the gut lumen in this species. Survival started to be affected at concentrations of 1.25 µg/mL.

4. Final Considerations

The available studies with particles smaller than 100 nm were performed with PS. Thus, it becomes imperative to assess the effects of other types of plastics in a wide range of organisms. Particles that may cause severe damage in some organisms (e.g., PS-NH_2 to bacteria, algae or echinoderms larval stages (Della Torre et al., 2014; Bergami et al., 2017; Sun et al., 2018)), may present a lower threat to others (e.g., rotifers (Manfra et al. (2017)), making it difficult to accurately conclude on their toxicity. All studies involved exposure in laboratory conditions and the concentrations of the nanoplastics tested ranged between 1ng/mL and 320 µg/mL. However, even though studies have been performed to assess the amount of plastics in the marine environment, there is no information regarding the number of nanoplastics which makes difficult the evaluation of the relevance of the concentrations tested in laboratorial conditions. Thus, it is hard to predict the ecological risk of nanoplastics in the marine environment. The available data shows that these particles, alone, may be harmful to the marine ecosystem from producers to consumers. However, the available studies are scarce, particularly in what concerns to the effects on marine vertebrates like fish that in addition to their ecological importance, also present high commercial value. The lack regulatory frameworks regarding the emission of plastics into the environment and legislation concerning nanoplastics in food may justify the limited available studies. Furthermore, detection methodology limitations do not allow the establishment of cause/effect associations nor potential links to human and environmental health (EFSA, 2016). The analysis of the available studies showed that there is a lack of knowledge on generational and long-term effects of nanoplastics as well as their potential to be transferred along a marine food chain. In microplastics

food web transfer was already observed in several different marine species such as algae, zooplankton, mussels and crabs (Cole et al., 2013; Farrell & Nelson, 2013). The smaller microplastics have higher potential for accumulation in the tissues of organisms (Browne et al., 2008). Since nanoplastics are smaller particles, there is also a high probability for them to be incorporated in the diet of the organisms and, consequently, be transferred to other trophic levels. It is also imperative to study the interaction between nanoplastics and other contaminants because they may affect organisms differently.

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Figure Captions

Figure 1 – Representations of the percentages of the plastic polymers most produced in 2016 and example of products in which they are commonly employed. PP-polypropylene; LDPE-low-density polyethylene; HDPE-high-density polyethylene; PVC-polyvinyl chloride; PUR-polyurethane; PET-polyethylene terephthalate and PS-polystyrene. Data retrieved and adapted from Plastics Europe's Market Research and Statistics (Plastics Europe, 2017).

Figure 2 – World map summarizing field studies that report the presence of plastics in freshwater, estuarine or marine environments. Different shaped symbols (squares and circles) represent plastics concentration expressed in particles/m^2 and particles/m^3, respectively. References are listed as follows: [1.](Goldstein et al., 2013); [2.3.](Gray et al., 2018); [4.](Eriksen et al., 2013); [5.](Carvalho & Neto, 2016); [6.](Rayon-viña et al., 2018); [7.](Sadri & Thompson, 2014); [8.9.10.11.](Tamminga et al., 2018); [12.](Collignon et al., 2012); [13.](Imhof et al., 2013); [14.](Xiong et al., 2018); [15.](Lee et al., 2013); [16.17.](Zhao et al., 2014).

Table 1 – Effects of nanoplastic particles in marine organisms according to their type, size and concentration. Only studies about marine organisms and particles with less or equal to 100 nm were included. Abbreviations stand for: PS - polystyrene; PS-COOH - anionic carboxylated polystyrene; PS-NH_2 – cationic amino polystyrene; nsw – natural sea water; asw – artificial sea water; LC/EC_x – lethal or sublethal concentration causing x % of effect; ROS – reactive oxygen species; hpf – hours post fertilization; μ - growth rate; n.a. – not analyzed

Phylum/Order	**Organism**	**Type of plastic**	**Size** (nm)	**Concentration** (μg/mL)	**Detection in biological matrices**	**Effects**	**Reference**
Proteobacteria Oceanospirillales	*Halomonas alkaliphila*	PS	55	20, 40, 80, 160, 320	n.a.	Intracellular ROS levels ↑ μ ↓ by 32.7% at 320 μg/mL	(Sun et al., 2018)
		PS-NH_2	50		n.a.	Intracellular ROS levels ↑ μ ↓ by 34% at 320 μg/mL	
Chlorophyta Chlamydomonadales	*Dunaliella tertiolecta*	PS	50	25, 250	n.a.	μ ↓ by 57% at 250 μg/mL cell density ↓ by 45% at 250 μg/mL	(Sjollema et al., 2016)
		PS-COOH PS-NH_2	40 50	0.5, 1, 5, 10, 25, 50 in nsw	n.a.	EC_{50} for μ of 12.97 ± 0.57 μg/mL	(Bergami et al., 2017)
Echinodermata Camarodonta	*Paracentrotus lividus* (embryos)	PS-NH_2	50	3, 4 in nsw	n.a.	Delay in development; Deficient skeleton rods and arms	(Pinsino et al., 2017)
		PS-COOH	40	50 10 in nsw	n.a.	Larval malformations	(Della Torre et al., 2014)
		PS-NH_2	50		n.a.	Larval malformations $EC_{50\ 24\ hpf}$ of 3.82 μg/mL $EC_{50\ 48\ hpf}$ of 2.61 μg/mL	
Rotifera Ploimida	*Brachionus plicatilis*	PS-COOH	40	0.5, 1, 5, 10, 20, 50 in nsw	Accumulation in organisms	Not detected	(Manfra et al., 2017)
		PS-NH_2	50		n.a.	$LC_{50\ 24h}$ of 13.04 ± 0.60 μg/mL $LC_{50\ 48h}$ of 6.62 ± 0.87 μg/mL	
Mollusca Ostreoida	*Crassostrea gigas*	PS-COOH PS-NH_2	100	0.1, 1, 10, 100 In nsw	Aggregation attached to the cells	ROS levels significantly increased in PS-COOH; increase of 4–5 % in relative size in both plastics	(González-Fernández et al., 2018)

	Mytilus edulis	PS	30	0, 100, 200, 300 in asw	Particles adsorbed to the gills	Reduce filtering activity;	(Wegner et al., 2012)
	Mytilus galloprovincialis	PS-NH_2	50	1, 5, 50 in asw	n.a.	Cytochrome C ↓ ↓ in phagocytosis Strong lysosomal destabilization	(Canesi et al., 2015)
	Mytilus galloprovincialis (48hpf larvae)	PS-NH_2	50	0.001, 0.01, 0.05, 0.1, 0.25, 0.5, 1, 2.5, 5, 10, 20 in asw	n.a.	$EC_{50,\ growth}$ of 0.142 µg/mL; Embryos malformed and immature ↓ shell length by 20 to 30%	(Balbi et al., 2017)
Arthropoda Sessilia	*Amphibalanus amphitrite* (II stage)	PS	100	0.001, 0.01, 0.1, 1, 10 in nsw	Aggregation in the gut lumen	↓ swimming speed;	(Gambardella et al., 2017)
Arthropoda Anostraca	*Artemia franciscana* (1st instar larvae)	PS	100	0.001, 0.01, 0.1, 1, 10 in nsw	Aggregation in the gut lumen	↓ swimming speed	(Gambardella et al., 2017)
	Artemia franciscana (1st instar larvae)	PS-COOH	40	0.5, 1, 1.5, 2.5, 5, 10 in nsw	n.a.	Not detected	(Bergami et al., 2017)
		PS-NH_2	50		n.a.	Induction of *clap* and *cstb* genes; ↑ number of molts; $LC_{50,14days}$ of 0.83 µg/mL	
	Artemia franciscana (up to instar III Nauplius)	PS-COOH	40	5, 25, 50, 100 in nsw	Aggregation in the gut lumen	Not detected	(Bergami et al., 2016)
		PS-NH_2	50		Aggregation in the gut lumen	Difficulties in swimming; ↑ number of molts	
Arthropoda Harpacticoida	*Tigriopus japonicus*	PS	50	0.125, 1.25, 12.5, 25 in nsw	Gut retention	Survival affected at concentrations higher than 1.25 µg/mL.	(Lee et al., 2013)