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Nanoplastics impact on marine biota: A review☆

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ABSTRACT

Emerging contaminants, such as nanoplastics, are gaining a vast interest within the scientific community. Most of the plastic debris found in the marine environment originates from land-based sources, and once in the marine environment, plastic can be degraded into smaller fragments. Nanoplastics are considered to fall within the definition of other nanoparticles (1–100 nm in size) and may be divided into primary or secondary nanoplastics. Primary nanoplastics are those that enter the environment in their original small size associated with specific applications and consumer products, whilst secondary nanoplastics are a consequence of macro/microplastic degradation. The formation of nanoplastics changes the physical-chemical characteristics of the particle, thus at a nanoscale, it is expected that the strength, conductivity, and reactivity of the nanoparticles will differ substantially from macro/micro-sized particles. To date, the toxicity nanoplastics may pursue on marine biota is still scarce. Herein, a review of the available data on the effects of different polymer types of nanoplastics specific to marine biota is accounted for.

1. Introduction

An ongoing worldwide environmental concern is the plastic debris pollution, whereby in 2018 the global plastic production reached 359 million tons (PlasticsEurope, 2019). In Europe, plastics production almost reached 62 million tons, whilst China alone produced 30% of the world's plastics (Plastics Europe, 2019). Plastics are synthetic organic polymers with high durability, are lightweight, and can easily be moulded into any shape or product (Worm et al., 2017). Plastics chemical stability, persistence and bioaccumulation has plastic pollution becoming increasingly prominent (Shen et al., 2019; Hu et al., 2020). The wide use of plastic, in a variety of applications, increases their release into the marine environment, whereby most of the plastic debris found in the marine environment originates from land-based sources (Bouder and Friot, 2007; Jambeck et al., 2015). Plastics are quite resistant to decomposition, therefore large plastic litter will breakdown to meso-plastics (5–40 mm), microplastics (1–5000 μm) (Thompson et al., 2004), and nanoplastics (NPs) (1–100 nm) (Gigault et al., 2018) before decomposing completely. NPs are considered to fall within the definition of other nanoparticles (1–100 nm in size) (Koelmans et al., 2015; Gigault et al., 2018) and may be divided into primary or secondary NPs. Primary NPs are those that enter the environment in their original small size associated with specific applications and consumer products (e.g., cosmetics, clothing fibres, drug delivery, ink for 3D printers) (Bergami et al., 2016; Canesi et al., 2015; Bessa et al., 2018; Tamminga et al., 2018; Wang et al., 2018), whilst secondary NPs are a consequence of macro/microplastics degradation (Andrady, 2011; Cole et al., 2011). However, Koelmans et al. (2015) establish that the time required to reach nano-sized particles depends on the size of the initial plastic.

NPs formation leads to alterations in the physical-chemical characteristics of the particle, surface area and size, wherein, at a nanoscale, the strength, conductivity and reactivity will differ substantially from macro/micro-sized particles (Klaine et al., 2012; Mattsson et al., 2015, 2018). As the size of the plastic particle decreases, biological reactivity, on the other hand, increases, thus being crucial to comprehend the burden of nanoplastic availability and its biological impact on marine biota (Mattsson et al., 2018; Ferreira et al., 2019). The fate, mobility and resilience of NPs are highly dependent on their stability and feasibility in forming aggregates. The NP aggregation mechanism is critical as aggregates sediment or immobilize, whereas dispersed NPs are able to diffuse, be more mobile, more bioavailable and potentially more harmful (Ramirez et al., 2019). Natural organic matter (NOM), inorganic colloids, weathering, UV-radiation and biodegradation are all factors that affect the composition, stability, formation of NPs aggregates, and the particles nano-specific properties (Andrady, 2011;

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Oriekhova and Stoll, 2018; Shen et al., 2019). Weathering, for instance, increases crystallinity of plastic debris, acquiring carbonyl functionalities inclusive of negative surface charges as a result of oxidation (Fotopoulou and Karapanagioti, 2012; Rouillon et al., 2016). Likewise, NOM and inorganic colloids lead to aggregation of NPs (Oriekhova and Stoll, 2018), consequently becoming less bioavailable and less prone to enter biological membranes. Adsorbed NOM convey a negative charge and alters the surface characteristics of NPs by raising their absolute surface potential (Zhang et al., 2008), nonetheless, high NOM concentrations can reverse the surface charge of NPs, facilitating the stabilization of NPs (Ramirez et al., 2019). Therefore, the charge neutralization of the isoelectric point is a prerequisite for the creation of large aggregates composed of NPs, thus a crucial mechanism for managing environmental nanoplastics identification, as well as incrementing the importance of NPs surface charge neutralization (Oriekhova and Stoll, 2018).

Herein, another issue to be addressed are plastic polymers with adsorbed toxic compounds such as polycyclic aromatic hydrocarbons (PAHs), persistent organic pollutants (POPs), polychlorinated biphenyls (PCBs) and other chemical constituents (i.e., metals) (Rios et al., 2007; Teuten et al., 2009; Lee et al., 2014; Rochman et al., 2016; Davranche et al., 2019). NPs high surface area favours this sorption, by binding chemicals hydrophobically (Liu et al., 2016), which in turn also increases the potential toxicity of the nanoparticle towards marine biota, as a consequence of the possible release of these sorbates and constituent chemicals. This increments the importance of establishing the mode of action of NPs with sorption of pollutants present in the marine environment, how they behave as vectors, as well as the toxicity they may pursue on marine biota.

Plastic particle toxicity to marine organisms depends on particle size, concentration and exposure period, where the toxicity of NPs is also affected by pollutants, food availability, species and their developmental stage (Kögel et al., 2020). Decreased growth rate, energy and movement, stress, inflammation and malformations are associated to nanoplastic toxicity towards marine biota (Kögel et al., 2020). The smaller the particle size, the more prone marine organisms are to toxicity by these NPs as the surface area increases, and so does the possibility of passing through biological membranes. On the grounds of their nano-size, NPs have increased ability to pass through cellular boundaries and accumulate within organisms, easily entering the food web and possibly escalate trophic levels all the way to human beings (Mattsson et al., 2015, 2018; Worm et al., 2017; Peng et al., 2020). The knowledge of NPs effects regarding marine biota is still scarce. This review's purpose is to gather all information on nanoplastics toxicity available on marine biota to date.

2. Effects of nanoplastics in marine biota

According to the Plastics Europe Fact sheet (2019), the leading plastic polymers are low-density and high-density polyethylene (LDPE & HDPE) and polypropylene (PP), followed by polyvinyl chloride (PVC), polyurethane (PUR), polyethylene terephthalate (PET), and polystyrene (PS). These plastic polymers are employed in many industries, such as electronics, personal health care, food packaging, housing insulation, as well as in medical equipment and devices (PlasticsEurope, 2019).

Though nanoplastics have not yet been quantified in the marine environment, Halle et al. (2017) obtained a nanoplastic portion from the North Atlantic subtropical gyre, wherein PVC, PET, PS and polyethylene (PE) were the plastic polymers present at a nanoscale. Although the quantification of NPs in the marine environment has yet to be explored, Halle et al. (2017) provided evidence for the existence of plastic debris in the nano-fraction in the marine environment. Nevertheless, NPs effect on marine organisms at environmentally relevant concentrations are unknown due to the lack of quantification of NPs in the marine environment. Moreover, accurately measuring the concentration of NPs is a major challenge as several analytical methods are required, for various types of NPs polymers as well as for various complex matrices (soil, sediments, turbid waters and tissues) (Halle et al., 2017).

With regards to the evaluation of nanoplastic toxicity in marine biota, a summary of the data available in the literature for the species evaluated to date can be found in Fig. 1. Fig. 1 shows that the crustacean *A. fransiscana* and the mollusc *Mytilus galloprovincialis* are the species most evaluated in scientific literature on NPs exposure. With relation to the groups of marine biotas presented in Fig. 1, a wide array of species from primary producers to primary/secondary consumers have been studied, providing an insight into effects of NPs at various levels of the marine ecosystem, wherein, crustaceans and molluscs, again, are highly used in ecotoxicological assessments of NPs. Fig. 2 shows that PS NPs and poly(methyl methacrylate) (PMMA) were the only NPs frequently used to evaluate the effects in biota with either an amide-group or a carboxyl-group attached, focusing on how negatively charged and positively charged particles toxicity differ but PS-NH_2 was the type of PS NPs predominately used in comparison with other chemical groups attached to PS or to types of nanoplastic polymers. Moreover, on Fig. 3 the percentage of the different nanoparticle size for each polymer type is presented. As can be seen in Fig. 3 there are different size range percentage used for each NP polymer that made comparison of the effects difficult. Furthermore, in this review, the effects of nanoplastics on marine biota are characterized below, initiating with bacteria and ascending through the trophic levels. A summary of the effects of nanoplastics evaluated in marine biota, to date, are in Tables 1- 4.

2.1. Bacteria

Bacteria are an important component of marine ecosystems, and microbial community alterations due to the presence of environmental contaminants, such as nanoplastics, may have significant effects on biogeochemical cycling as well as other critical ecosystem services (Rotini et al., 2017). Bacteria exposed to several polymers of PS-NPs of different sizes and exposure times are summarised in Tables 1–3. Recently, Okshevsky et al. (2020) observed that marine bacteria formation of biofilm on PS NPs is impacted by both concentrations and surface functionalization of PS NPs, suggesting that species interaction along with surface properties and concentrations of plastic NPs are determining factors on how NPs impact marine bacteria biofilm formation. For instance, PS-NH_2 at the highest concentration (200 ppm; 20 nm; 5 days), lead to an increase in aggregation in all species studied, whilst generating a decrease on both growth rate and optical density (OD_{600}) (Okshevsky et al., 2020). PS-NH_2 also prompted a decrease in biofilm formation of all bacteria species with the exception of *Oceanobacter kriegii* (Okshevsky et al., 2020). On the other hand, negatively charged NPs (PS–COOH; 200 ppm; 20 nm; 5 days) promoted biofilm formation of some bacteria species (*Marinobacter adherens, Marinobacter algicola, Cobetia marina* and *O. kriegii*), whilst causing a decrease in others (*Phaeobacter inhibins*) (Okshevsky et al., 2020). PS-COOH additionally, increased growth rate, OD_{600} and aggregation of *C. marina*, as well as aggregation of *Pseudoalteromonas carrageenovora*, and a decrease in growth rate and OD_{600} of *O. kriegii* (Okshevsky et al., 2020). Undoubtably, surface properties of NPs have contrasting effects on a battery of bacteria, being imperative to understand the kinetics of toxicity of differently charged nanoplastics. In the marine bacterium

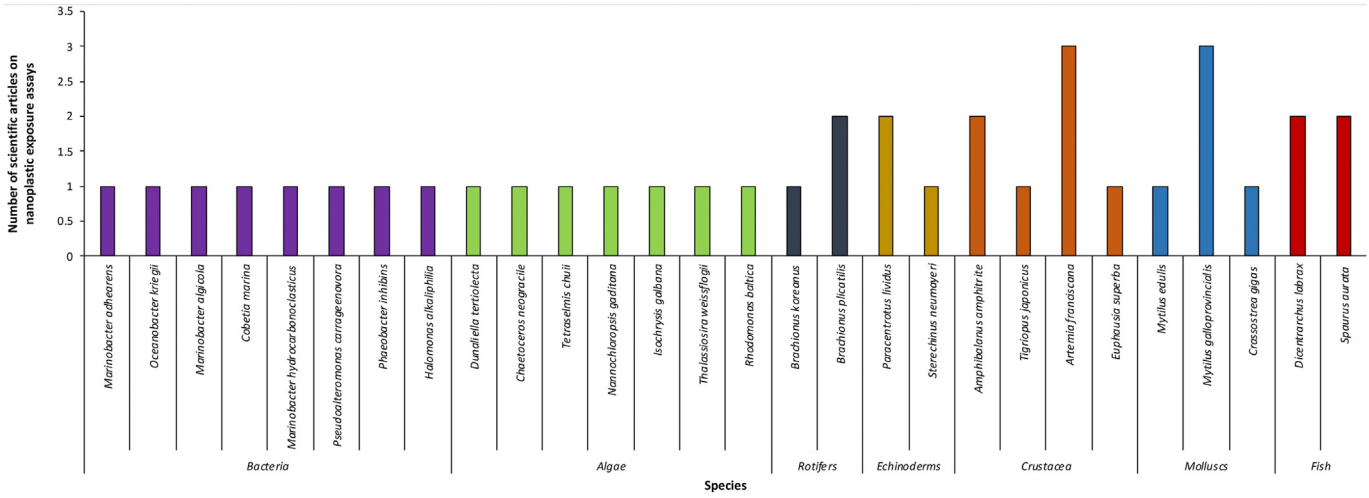

Fig. 1. Type of species evaluated for NPs exposure *vs* number of scientific publications.

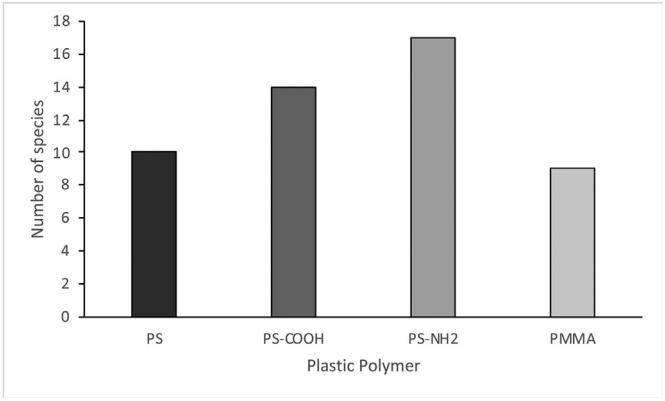

Fig. 2. Type of NP polymers used in ecotoxicological assessments *vs* number of species whereby the effects of NPs polymers have been assessed.

Halomonas alkaliphile, a bacterium which thrives in high salt concentrations, exposed to cationic amino PS nanoparticles (PS–NH_2; 50 nm) and to PS nano beads (PS-beads; 55 nm) for 2 h at different concentrations (20, 80, 160 and 320 μg/mL) affected cell growth, and influenced the chemical composition and ammonia conversion efficiencies at 80 μg/L (Sun et al., 2018). Furthermore, PS-NH_2 induce higher oxidative stress towards bacteria when compared to PS-beads, as these positively charged NPs adhere to the cell surface by the action of electrostatic activity, promoting higher toxicity by these NPs (Sun et al., 2018). Overall, in bacterial communities, NPs with an amide-group have more toxic effects when compared to NPs with carboxyl-group. Thereafter, the NPs toxicity is dependent on functionalized NPs surface charge and size.

2.2. Algae

Algae, as primary producers, are key organisms in sustaining a healthy aquatic environment, as they are the base of food webs, source of oxygen production as well as other nutrients (Mao et al., 2020). The use of algal assays for ecotoxicological assessment of emerging contaminants is mainly linked to their high-sensitivity response, enabling the detection of toxic effects at lower concentrations that are not detectable by other marine organisms (Palumo and Mingazzini, 2011). The ecotoxicological assessment of NPs on marine algae have been investigated in some species (Sjollema et al., 2016; Bergami et al., 2017; Venâncio et al., 2019; González-

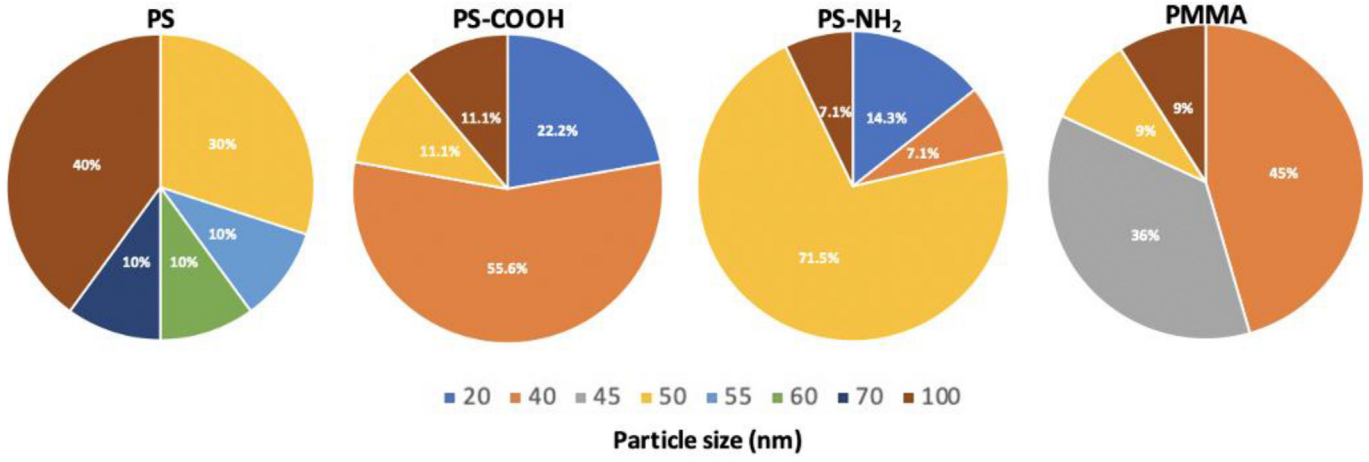

Fig. 3. Percentage of nanoparticle size (nm) for each type of polymer used. PS – polystyrene; PS-COOH – anionic carboxylated polystyrene; PS-NH_2 – cationic amino polystyrene; PMMA – poly(methyl methacrylate).

Table 1
Effects of polystyrene NPs (PS; ≤100 nm) on marine biota according to size, concentration and exposure period.

Phylum	Test species	Particle type	Particle size (nm)	Concentration (μg/mL)	Exposure time (h)	Effects	Reference
Bacteria	*Halomonas alkaliphilia*	PS (beads)	55	20–320	2	↓ growth rate; (32% at 320 μg/mL)	Sun et al. (2018)
Algae	*Dunaliella tertiolecta*		50	250	72	↓ 45% cell density; ↓ 57% growth rate	Sjollema et al. (2016)
Rotifers	*Brachionus koreanus*	PS	50	10	24	↑ mortality; NSW → EC_{50} = 6.62 ± 0.87 μg/mL; RSW → EC_{50} = 2.75 ± 0.67 μg/mL	Manfra et al. (2017)
Crustacea	*Amphibalanus amphitrite (II stage of nauplius)*		100	0.5–10	24 and 48	↑ expression of clap and cstb; ↑ nº of molts (48 h)	Bergami et al. (2017b)
	Artemia franciscana (1st instar larvae)			0.001–10	24 and 48	⊥ swimming speed (24 h); ⊥ swimming speed ↑ post 48 h (1 and 10 μg/mL); Ingested and accumulated PS in gut	Gambardella et al. (2017)
	Trigriopus japonicus		50	0.125–25	96	↓ fecundity; ↑ embryo malformations; ↑ mortality larvae; mortality of parents at 25 μg/mL	Lee et al. (2013)
Molluscs	*Mytilus edulis*		30	100, 200 and 300	8	⊤ production of pseudofaeces → ↑ proportionally to concentration	Wegner et al. (2012)
	Mytilus galloprovincialis	PS	106 ± 10	0.5–50	96	↓ enzymatic activity; ⊥ neurotransmission; ↑oxidative stress; ↑ LPO; ↑ genotoxicity; Δ gene expression	Brandts et al. (2018b)
		PS with carbamazepine (Cbz)		0.5 + 6.3 μg/L of Cbz		↑ genotoxicity; ↓gene expression	
Fish	*Dicentrarchus labrax* (DLB-1)	PS	100	0.001–10	24	↓ cell viability (66%) (0.001 μg/mL); minimum viability = 64% (1 μg/mL)	Almeida et al., 2019
	Spaurus aurata (SAF-1)			0.001–10	24	↓ cell viability (25%) (0.001 μg/mL); mild effects at higher concentrations	

Abbreviations stand for: ↑ - increased; ↓ - decreased; ⊥ - inhibited; ⊤ - induced; Δ- altered; ROS - Reactive oxygen species; LC/EC_x – Lethal or sublethal concentration causing x% of effect.

Fernández et al., 2020; Gomes et al., 2020) (*see* Tables 1–4). An inhibitory effect on growth and development of the microalgae *Dunaliella tertiolecta* exposed to PS beads (50 nm) at 250 μg/mL for 72 h (Sjollema et al., 2016) and exposed to PS-NH_2 (50 nm) and PS-COOH (40 nm) for the same time (Bergami et al., 2017) was observed, wherein PS-COOH showed no effects, though PS-COOH were adsorbed on microalgae (Bergami et al., 2017). PS-NH_2, on the other hand, inhibited cell growth (Bergami et al., 2017), and PS beads lead to a reduction of 45% on cell density and cellular growth was affected by 57% after exposure (Sjollema et al., 2016). There is a higher affinity of positively charged NPs (PS–NH_2) in comparison to negatively charged NPs (PS–COOH). This may be due to the electrostatic interactions between the positively charged particles and cellulose, the major component of cell walls in algae, as shown by Bhattacharya et al. (2010) in the freshwater single-celled algae *Chlorella* and multi-celled algae *Scenedesmus* (PS–COOH and PS-NH_2; 20 nm; 0.08–0.8 μg/mL; 2 h).

A battery of microalgae, *Tetraselmis chuii*, *Nannochloropsis gaditana*, *Isochrysis galbana*, and the marine diatom *Thalassiosira weissflogii*, after 96 h of exposure to PMMA (40 nm; 0–304.1 mg/L for microalgae and 0–293.0 mg/L for marine diatom) had significant impacts on growth rates of all microalgae (Venâncio et al., 2019). *T. chuii* presented the highest median effective concentration (EC_{50} = 132.5 mg/L), whereby *T. weissflogii* showed to be the most sensitive microalgae (EC_{50} = 83.4 mg/L) (Venâncio et al., 2019). In the marine diatom *Chaetoceros neogracile*, after 96 h of exposure to PS-NH2 (50 nm; 0.05 μg/mL and 5 μg/mL), pigment and lipid compositions of diatoms were affected (González-Fernández et al., 2020). Re-adjustment of lipid classes and fatty acids were noteworthy at both growth phases, whereby thylakoid membrane structure and cellular energy reserves of diatoms were observed after PS-NH_2 exposure. Highest concentrations of PS-NH_2 lead to impairment of long-chain fatty acids, particularly at exponential cultures (González-Fernández et al., 2020). In the red microalgae, *Rhodomonas baltica*, exposed to PMMA and PMMA-COOH (50 nm; 0.5–100 μg/mL; 72 h), both NPs were found to be toxic, wherein the interaction of NPs with microalgae were particle behaviour dependent (Gomes et al., 2020). A greater effect on cellular and physiological parameters was caused by PMMA exposure, meanwhile PMMA-COOH inhibition of microalgae growth was associated with cell cycle and cell viability (Gomes et al., 2020). A decrease in photosynthetic performance was also an outcome of both NPs, incrementing importance into comprehending the toxicity of NPs in algae, as its effects on photosystems of primary consumers consequently affects the entire ecosystem.

Contemplating herein information, the nanotoxicity of these particles leads to impacts on the cell wall and pigmentation of algae, possibly leading to inactivation of the photosystems, and therefore hindering photosynthesis. Nonetheless, the available data shows that PS-NH_2 have more pronounced effects than PS-COOH. Moreover, nanoplastics uptake by algae is dependent of particle charge and surface functionalization, still NPs appear to cause physiological distress once within the algal metabolism.

2.3. Rotifers

Rotifers are primary and secondary consumers of high importance and relevance as zooplankton members in several aquatic trophic webs (Wallace et al., 2006), playing an important role in marine ecosystems, as they transfer energy from the bottom of the food chain to species higher up (Jeong and Choi, 2019). The small size and sensitivity, as well as ease of culture and exponential growth, are favourable characteristics of rotifers as test models in ecotoxicological studies (Dahms et al., 2011). Data on the effects of NPs on rotifers are scarce (see Tables 1–4). The rotifer *Brachionus*

Table 2
Effects of anionic carboxylated polystyrene NPs (PS−COOH; ≤100 nm) on marine biota according to size, concentration and exposure period.

Phylum	Test species	Particle type	Particle size (nm)	Concentration (μg/mL)	Exposure time (h)	Effects	Reference
Bacteria	*Marinobacter adhearens*	PS-COOH	20	0−200	5[a]	<20 μg/mL → no impact on biofilm formation, growth rate, OD_{600} 200 μg/mL → no impact on living/dead ratio; ↑ biofilm formation	Okshevsky et al. (2020)
	Oceanobacter kriegii					<20 μg/mL → no impact on biofilm formation, growth rate, OD_{600} 200 μg/mL → no impact on living/dead ratio; ↑ biofilm formation; ↓ growth rate; ↓ OD_{600}	
	Cobetia marina					<20 μg/mL → no impact on biofilm formation, growth rate, OD_{600} 200 μg/mL → no impact on living/dead ratio; ↑ biofilm formation; ↑ growth rate; ↑ OD_{600}; ↑ aggregation	
	Marinobacter algicola					<20 μg/mL → no impact on biofilm formation, growth rate, OD_{600} 200 μg/mL → no impact on living/dead ratio; ↑ biofilm formation	
	Pseudoalteromonas carrageenovora					<20 μg/mL → no impact on biofilm formation, growth rate, OD_{600} 200 μg/mL → no impact on living/dead ratio; ↑ aggregation	
	Phaeobacter inhibins					<20 μg/mL → no impact on biofilm formation, growth rate, OD_{600} 200 μg/mL → no impact on living/dead ratio; ↓ biofilm formation	
	Marinobacter hydrocarbonoclasticus					<20 μg/mL → no impact on biofilm formation, growth rate, OD_{600} 200 μg/mL → no impact on living/dead ratio	
Algae	*Dunaliella tertiolecta*		50	250	72	no effects	Bergami et al. (2017a)
Rotifers	*Brachionus plicatilis*		40	0.5−50	24 and 48	no effects on mortality; ↑ gut retention of PS-COOH	Manfra et al. (2017)
Echinoderms	*Paracentrotus lividus*			2.5−50	6, 24, 48 hpf	Accumulated in embryos; no malformations	Della Torre et al. (2014)
	Sterechinus neumayeri			1 and 5	6 and 24	in vitro assay; ↑ antioxidant response; ↑ apoptosis; ↑ phagocytic capacity; ⊠ modulation of genes related to external challenges; ↑ inflammatory response	Bergami et al. (2019)
Crustacea	*Artemia franciscana*			0.5−10	24 and 48	no effects	Bergami et al. (2017b)
	- *upto instar III nauplius*			5−100	72 and 14[a]	Accumulated and retained in gut lumen	Bergami et al. (2016)
	Euphausia superba		60	2.5	48	No mortality; active swimming; waterbourne ingestion and egestion	Bergami et al. (2020)
Molluscs	*Crassostrea gigas (gametes)*		100	0.1−100	1 to 5	↑ cell size; ↑ spermatozoa complexity; ↓ nº spermatozoa; ↑ ROS (dose-response)	González-Fernández et al. (2018)

Abbreviations stand for: ↑ - increased; ↓ - decreased; ⊥ - inhibited; ⊠ - affected; ⊤ - induced; ROS - Reactive oxygen species; LC/EC_x − Lethal or sublethal concentration causing x% of effect; OD_{600} − optical density at 600 nm.
[a] Days **mins.

plicatilis exposed to PS-COOH and PS-NH_2 (40 and 50 nm, respectively) at concentrations between 0.5 and 50 mg/L for 24−48 h, PS-COOH had no acute toxicity, whilst PS-NH_2 nanoplastics, on the other hand, showed high mortality (Manfra et al., 2017). Also, in *B. plicatilis*, after exposure to PMMA (40 nm; 96 h; 4.7−75.0 mg/L), at 48 h into the exposure, survival rate was highly affected in both L type and S type rotifers (LC_{50} = 13.3 mg/L and LC_{50} = 37.6 mg/L, respectively) (Venâncio et al., 2019). In *Brachionus koreanus* exposed to two different sizes of PS microplastics (500 and 6000 nm, 10 μg/mL) and a PS nanoplastics (50 nm) for 24 h at a concentration of 10 μg/mL, the accumulation of NPs was higher than microplastics and associated with oxidative stress-induced damage to lipid membranes (Jeong and Choi, 2019). Rotifers survival is highly affected after exposure to NPs, wherein, NPs toxicity is dependent on both polymer type and size. Herein, though further studies are necessary, in particular those related to the effect of different NPs sizes, positively charged NPs present a more toxic effect on rotifers than negatively charged NPs as seen in algae and bacteria.

2.4. Echinoderms

Considering echinoderms, sea urchins are highly considered as excellent tools to assess the toxicity of many chemical compounds and environmental stressors, especially during embryonic life stages, as sea urchins have high sensitivity to low concentrations of contaminants (Sugni et al., 2007). The effects of exposure to rotifers are presented in Tables 1−4 *Paracentrotus lividus* embryos and larvae exposed to PS-COOH (40 nm; 50 μg/mL) and PS-NH_2 (50 nm; 10 μg/mL) for 6, 24 and 48 h lead to accumulation of both NPs, whereby PS-NH_2 induced higher toxicity that was unseen by PS-COOH (Della Torre et al., 2014). The half-maximum effective concentration (EC_{50}) of PS-NH_2 at 24 hpf and 48 hpf were 3.82 μg/mL and 2.61 μg/mL, respectively, wherein malformations of skeletal rods and arms, as well as undeveloped embryos, were some of the effects encountered after exposure to PS-NH_2 (Della Torre et al., 2014). Pinsino et al. (2017) also showed that *P. lividus* exposed to 3 and 4 μg/mL of PS-NH_2 (50 nm) for the same time, induced malformations of skeletal rods and arms at 4 μg/mL. Therefore, the impact of skeletal rods and arms can be a specific effect of NPs, but more data is necessary to confirm this. Moreover, in an *in vitro* assay of PS-COOH and PS-NH_2 exposure to the sea urchin *Sterechinus neumayeri* (40 and 50 nm, respectively; 6 and 24 h; 1 and 5 μg/mL) both NPs affected cellular phagocytosis, engendered inflammatory responses towards oxidative stress and provoked apoptosis at a molecular level (Bergami et al., 2019). Once more, Bergami et al. (2019) findings establish that different surface charged NPs is challenging for *S. neumayeri* immune cells. Comparatively, PS-NH_2 possess a more toxic effect towards echinoderms than PS-COOH, as seen in bacteria, algae and rotifers. Predominately associated with the lack of toxicity of PS-COOH is the electrostatic cell membrane repulsion (Bhattacharya et al., 2010), as well as the consequent loss of nanoscale properties and reactivity due to NP agglomeration in SW (Bergami et al., 2017). The toxicity of NPs towards echinoderms

Table 3
Effects of cationic amino polystyrene NPs (PS–NH_2; ≤100 nm) on marine biota according to size, concentration and exposure period.

Phylum	Test species	Particle type	Particle size (nm)	Concentration (μg/mL)	Exposure time (h)	Effects	Reference
Bacteria	*Marinobacter adhearens*	PS-NH2	20	0–200	5[a]	<20 μg/mL → no impact on biofilm formation, growth rate, OD_{600} 200 μg/mL → ↑ aggregation; ↓ growth rate; ↓ OD_{600}	Okshevsky et al. (2020)
	Oceanobacter kriegii					<20 μg/mL → no impact on biofilm formation, growth rate, OD_{600} 200 μg/mL → ↑ biofilm formation; ↑ aggregation; ↓ growth rate; ↓ OD_{600}	
	Cobetia marina					<20 μg/mL → no impact on biofilm formation, growth rate, OD_{600} 200 μg/mL → ↓ biofilm formation; ↑ aggregation; ↓ growth rate; ↓ OD_{600}	
	Marinobacter algicola					<20 μg/mL → no impact on biofilm formation, growth rate, OD_{600} 200 μg/mL → ↓ biofilm formation; ↑ aggregation; ↓ growth rate; ↓ OD_{600}	
	Pseudoalteromonas carrageenovora					<20 μg/mL → no impact on biofilm formation, growth rate, OD_{600} 200 μg/mL → ↓ biofilm formation; ↑ aggregation; ↓ growth rate; ↓ OD_{600}	
	Phaeobacter inhibins					<20 μg/mL → no impact on biofilm formation, growth rate, OD_{600} 200 μg/mL → ↓ biofilm formation; ↑ aggregation; ↓ growth rate; ↓ OD_{600}	
	Marinobacter hydrocarbonoclasticus					<20 μg/mL → no impact on biofilm formation, growth rate, OD_{600} 200 μg/mL → ↓ biofilm formation; ↑ aggregation; ↓ growth rate; ↓ OD_{600}	
	Halomonas alkaliphilia		50	20–320	2	⊥ growth rate; ↓ chlorophyll content; ↑ oxidative damage	Sun et al. (2018)
Algae	*Dunaliella tertiolecta*		40	250	72	⊥ growth rate (EC_{50} = 12.97 ± 0.57 μg/mL)	Bergami et al. (2017a)
	Chaetoceros neogracile		50	0.05 and 5	96	↓ cellular energy reserves; ↓ pigment composition; re-adjustment of galactolipids & triacylglycerol; ⊠ thylakoid membrane structures	González-Fernández et al. (2020)
Rotifers	*Brachionus plicatilis*			0.5–50	24 and 48	↑ mortality; NSW → EC_{50} = 6.62 ± 0.87 μg/mL; RSW → EC_{50} = 2.75 ± 0.67 μg/mL	Manfra et al. (2017)
Echinoderms	*Paracentrotus lividus*			1–50	6, 24, 48 hpf	⊤ malformations in larvae; 24 hpf → EC_{50} = 3.82 μg/mL; 48 hpf → EC_{50} = 2.61 μg/mL	Della Torre et al. (2014)
				3 and 4	24 and 48	⊥ skeletal elongation (3 μg/mL); ⊤ malformations of skeletal rods and arms	Pinsino et al. (2017)
	Sterechinus neumayeri			1 and 5	6 and 24	in vitro assay; ↓ phagocytic capacity; ↓ gene modulation; ↑ cellular debris at 5 μg/mL (24 h); ↑ inflammatory response; ↑ apoptosis	Bergami et al. (2019)
Crustacea	*Artemia franciscana*			0.5–10	24 and 48	↑ expression of clap and cstb; ↑ nº of molts (48 h)	Bergami et al. (2017b)
	- *upto instar III nauplius*			5–100	72 and 14[a]	Accumulated and retained in gut lumen; most toxic	Bergami et al. (2016)
	Euphausia superba			2.5	48	no mortality; ↑ exuviae production (12.6 ± 1.31); ↓ swimming activity	Bergami et al. (2020)
Molluscs	*Crassostrea gigas (gametes)*		100	0.1–100	1 to 5	↑ cell size; ↑ spermatozoa complexity; ↓ nº spermatozoa	González-Fernández et al. (2018)
	Mytilus galloprovincialis		50	0.0001–20	48	↑ malformations D-larvae; ↑ delay in development (2.5–10 μg/mL); ↓ 20–30% shell length (48 h); EC_{50} = 0.142 μg/mL	Balbi et al. (2017)
	- haemocytes			1–50	30[b]	↓ lysosomal membrane stability; ↑ ROS; ⊤ cellular damage	Canesi et al., 2016

Abbreviations stand for: ↑ - increased; ↓ - decreased; ⊥ - inhibited; ⊠ - affected; ⊤ - induced; ROS - Reactive oxygen species; LC/EC_x – Lethal or sublethal concentration causing x% of effect; OD_{600} – optical density at 600 nm.
[a] Days.
[b] mins.

are therefore particle size and type dependent.

2.5. Crustacea

Benthic and planktonic marine crustaceans are useful in ecotoxicological assays, as small crustaceans are a crucial link within the food web, as they play an important role as primary and sometimes also as secondary consumers (Luigi et al., 2012). They connect the energetic fluxes between primary producers (e.g., algae) and consumers found at higher levels (e.g., fish) and therefore are placed at a key level within the food web (Luigi et al., 2012). Thus, the effects of NPs present in Tables 1–4 are important to assess possible trophic transfer. In the brine shrimp *Artemia franciscana* exposed to PS-COOH (40 nm) and PS-NH_2 (50 nm) particles (0.5–10 μg/mL; 24–48 h), exposure to PS-NH_2 (1 μg/mL) lead to an increase in the expression of cathepsin L-associated protein (*clap*) and cathepsin B (*cstb*), two genes connected to growth. This increased after 48 h and was related to an increase in the number of molts (Bergami et al., 2017). Organisms, on the other hand, exposed to PS-COOH had no effects, however, did accumulate NPs (Bergami et al., 2017). In *A. franciscana* (up to instar III nauplis) exposed to the same NPs (5–100 μg/mL) a growth inhibition test (72 h) and a long-term sublethal test (14 d), showed NPs were accumulated and retained inside the gut lumen (Bergami et al., 2017). PS-NH_2 particles were more toxic than PS-COOH (Bergami et al., 2016). PS nanoparticles (100 nm; 0.001–10 μg/mL) exposed to the first instar larvae of *A. franciscana* and II stage of nauplii of the acorn barnacle *Amphibalanus amphitrite*, ingested and accumulated NPs in the gut

Table 4
The effects of poly(methyl methacrylate) NPs (PMMA; ≤100 nm) on marine biota according to size, concentration and exposure period.

Phylum	Test species	Particle type	Particle size (nm)	Concentration (μg/mL)	Exposure time (h)	Effects	Reference
Algae	*Tetraselmis chuii*	PMMA	40	0–304.1	96	↓ growth rates (>150 μg/mL); EC_{50} = 132.5 μg/mL; EC_{20} = 117.4 μg/mL	Venâncio et al. (2019)
	Nannochloropsis gaditana					↓ growth rates (>150 μg/mL)	
	Isochrysis galbana					↓ growth rates (≥213.6 μg/mL)	
	Thalassiosira weissflogii			0–293.0		⊥ growth rates (≥18.8 μg/mL); EC_{50} = 83.4 μg/mL; EC_{20} = 48.9 μg/mL	
	Rhodomonas baltica	PMMA	50	0.5–100	72	↓ cell viability, ↑ cell size & complexity; ↑ ROS; ↑ LPO; ↓ DNA content; ↓ photosynthetic capacity; hyperpolarization of mitocondrial membrane; membrane integrity loss; overproduction of pigments	Gomes et al. (2020)
		PMMA-COOH				↓ growth; ⊥ cell cycle; ↓ cell viability; ↓ metabolic activity; ↓ photosynthetic capacity	
Rotifers	*Brachionus plicatilis*	PMMA	40	4.7–75.0	96	☒ survival (48 h; L-type > S-type); L-type → EC_{50} = 13.3 μg/mL; S-type → EC_{50} = 37.6 μg/mL	Venâncio et al. (2019)
Crustacea	*Amphibalanus amphitrite*		45	25	24	↑ bioaccumulation	Bergami et al. (2017b)
Fish	*Spaurus aurata*			0–10	24 and 96	↑ antioxidant defences; ⊤ alterations in lipid metabolism pathways; ↑ genotoxicity in blood cells; ↑ cholesterol and triglycerides in plasma; ↑ Erythrocytic nuclear abnormalities	Brandts et al. (2021)
	Dicentrarchus labrax			0–20	96	↑ abundance of mRNA transcript; ⊥ fish immune system	Brandts et al. (2018)
			100	0.001–10	24	⊥ immune system; ↑ abundance of mRNA transcript; ☒ molecular signalling & pathways	Almeida et al., 2019

Abbreviations stand for: ↑ - increased; ↓ - decreased; ⊥ - inhibited; ☒ - affected; ⊤ - induced; ROS - Reactive oxygen species; LC/EC_x – Lethal or sublethal concentration causing x% of effect; OD_{600} – optical density at 600 nm.

after 24 and 48 h of exposure. PS particles affected organisms swimming speed, wherein *A. amphitrite* exposed to higher concentrations showed mobility inhibition after 48 h. On the other hand, *A. franciscana* swimming speed was inhibited at 24 h, and increased inhibition at higher concentrations (Gambardella et al., 2017). The acorn barnacle *A. amphitrite* after a chronic exposure test to PMMA (45 nm; 5–25 ppm; 24 h), bioaccumulation of NPs occurred in barnacles and persist in the body throughout the stages of growth and development (nauplius to juvenile barnacle) posing a potential long-term effect of NPs on invertebrate communities (Bhargava et al., 2018). The effects of PS (50 nm) in a marine copepod, *Tigriopus japonicus* exposed to NPs caused a significant decrease in fecundity, malformations of embryo development, and high mortality of larvae, wherein highest concentrations of PS nanoplastics lead to parent's death (Lee et al., 2013). More recently, the effects of NPs on Antarctic krill juveniles, *Euphausia superba*, was assessed (Bergami et al., 2020). Exposure to PS-COOH (48 h; 2.5 μg/L; 62 nm) had no adverse effects, in contrast PS-NH_2 (48 h; 2.5 μg/L; 50 nm) reduced swimming activity of krill and increased moulting (Bergami et al., 2020). Antarctic krill is a keystone species and plays a central role in the Antarctic food chains and carbon cycle, increasingly impacting the future effect of NPs on Antarctic pelagic ecosystems and biogeochemical cycle (Bergami et al., 2020).

In summary, crustaceans ingest and accumulate NPs, where at high concentrations mobility is affected as is growth and development, in all NPs assessed. A clear distinction between PS-COOH and PS-NH_2 toxicity is also noticeable, wherein PS-NH_2 has more severe effects on crustaceans, as shown in bacteria, algae, rotifers and echinoderms. Further investigation is necessary to understand the effects other types of NPs pursue on crustaceans such as copepods, brine shrimps, barnacles, and amphipods. Furthermore, within crustaceans, copepods are extremely valuable in ecotoxicological evaluations, whereby the use of a combination of whole-animal bioassays and gene expression studies indicate that copepods, such as *Tigriopus* spp., may serve as excellent tools to evaluate the impacts of marine pollution throughout coastal regions (Raisuddin et al., 2007).

2.6. Molluscs: Bivalvia

Bivalves, as sessile filter-feeding organisms have the ability to accumulate many NPs present in the surrounding environment, empowering measurements of stressors levels in their tissues. Their wide geographical distribution and presence at different latitudes are some of the characteristics that make bivalves excellent sentinel organisms for ecotoxicological evaluations. However, the effects of NPs exposure to bivalves is still scarce (see Table 1–4). In the blue mussel *Mytilus edulis*, PS nanoparticles (30 nm; 100–300 μg/mL) induced production of pseudofaeces, increasing with the increase in NPs concentration, wherein results suggested that PS particles were recognized as non or low nutritional food (Wegner et al., 2012). In the Mediterranean mussel, *Mytilus galloprovincialis*, haemocytes exposed to PS-NH_2 (50 nm; 1–50 mg/L), after 30 min, PS-NH_2 cause a decrease in lysosomal membrane stability and an increment in oxyradical generation, leading to rapid cellular harm such as membrane blebbing and misfortune of filopodia (Canesi et al., 2015). Additionally, the generation of the protein corona in haemolymph serum was observed with the existence of PS-NH_2 (Canesi et al., 2015). In early embryo development of the mussel *M. galloprovincialis*, after exposure to PS-NH_2 (50 nm; 0.001–20 mg/L), malformations of D-veligers were observed at concentrations more than or equal to 2.5 mg/L (Balbi et al., 2017). A dose-dependent delay concerning the development of larvae was also observed, with an increase in embryos

encountered at the pre-veliger stage, and a stable proportion found still at the trochophore stage (EC_{50} = 0.142 mg/L). The highest concentration (20 mg/L) of PS-NH_2 lead to complete inhibition of D-shaped veliger, whereby 90% of larvae remained at trochophore stage (Balbi et al., 2017). In gametes of the oyster *Crassostrea gigas*, exposed to PS-COOH and PS-NH_2 (100 nm; 0.1–100 mg/L) NPs caused an increase in relative cell size as well as the complexity of spermatozoa (González-Fernández et al., 2018). Moreover, a dose-response increase in reactive oxygen species production was observed in organisms exposed to PS-COOH, but not to PS-NH_2. Oocytes were found to be less related to different cell membranes (González-Fernández et al., 2018). In gametes and embryo-larval development of *C. gigas* exposed to PS beads, PS-COOH and PS-NH_2 (50 nm; 0.1–25 μg/mL), all NPs significantly impaired the fertilization yield in a dose-response manner at concentrations between 1 and 25 μg/mL, wherein PS-NH_2 exhibited the strongest toxicity, inducing a significant reduction in fertilization yield associated at an EC_{50} of 4.9 ± 0.9 μg/mL (Tallec et al., 2018). D-larval yield also decreased significantly after exposure to all NPs at 10 and 25 μg/mL, wherein the highest toxicity observed was also by PS-NH_2, presenting a decrease of 6.4% in D-larval yield at the lowest concentration (0.1 μg/mL). Embryo-larval development success was completely inhibited (100% reduction) at an EC_{50} of 0.15 ± 0.4 μg/mL (Tallec et al., 2018). Tallec et al. (2018) highlight that NPs exposures may have detrimental effects on planktonic stages of bivalves, seemingly interacting with biological membranes, leading to cytotoxicity and genotoxicity, potentially impairing reproductive success. Undoubtedly, NPs toxicity in bivalves is extremely concerning, as data have shown that NPs are recognized as low nutritional food and are uptaken by these filter-feeders. In addition, after exposure to NPs, the integrity of their immune response is compromised by decreasing the stability of the lysosomal membrane and in addition, bivalve gametes are also vulnerable to NP toxicity, as is embryo-larval development, thereby jeopardizing future populations and disrupting the surrounding environment. Therefore, it is important to access if other types of NPs induce the same type of effects.

2.7. Fish

Very few studies on the effects of NPs on marine fish have been encountered (see Tables 1 and 4). Most of the data available are focused on the effects in freshwater fish (Mattsson et al., 2015, 2017; Chen et al., 2017; Skjolding et al., 2017; Chae and An, 2017; Pitt et al., 2018; Lee et al., 2019). Recently, marine continuous fish cell lines have been developed to aid in the study of molecular and physiological responses to stressors (Langner et al., 2011; Villalba et al., 2017). Fish cell lines have become a crucial tool into understanding the effects of emerging contaminants (e.g., NPs) at a molecular level (Morcillo et al., 2017; Pannetier et al., 2018) as well as avoid the use of animals in experiments as recommended by the European Union (Directive 2010/63 EU).

In two fish cell lines of the seabream *Sparus aurata* and the seabass *Dicentrarchus labrax*, SAF − 1 and DLB − 1, respectively exposed to PS NPs (100 nm; 0.001–10 mg/L) for 24 h, in SAF − 1, though not significant, there was a slight decrease (25%), in cell viability at lowest concentrations (Table 1). However, at higher concentrations, mild effects were present on this cell line (Almeida et al., 2019). In DLB − 1, a decrease in cell viability was observed, whereby at 0.001 mg/L cell viability was 66%, showing oscillation at higher concentrations within minimum viability of 64% at 1 mg/L (Almeida et al., 2019).

More recently, a short-term exposure of PMMA to the seabream *S. aurata* (45 nm; 0–10 μg/mL; 24 and 96 h) resulted in upregulation of the mRNA levels of essential lipid metabolism-related genes, showing a global increase in plasma cholesterol and triglycerides (Brandts et al., 2021). Antioxidant enzymatic response increased throughout the exposure period, exceptionally with a decrease in total antioxidant capacity after 96 h. PMMA nanoparticles activated antioxidant defences, induced alterations in lipid metabolism pathways and genotoxicity in *S. aurata* blood cells (Brandts et al., 2021). Similarly, in the seabass *D. labrax* after exposure to the same NP and size (PMMA; 45 nm; 0–20 mg/L; 96 h) also led to an increase in abundance of mRNA transcript as well as the impairment of the fish's immune system (Brandts et al., 2018). In *D. labrax*, NPs change molecular signalling pathways and potentially interfere with lipid metabolism as seen in *S. aurata* (Brandts et al., 2018, 2021) (Table 4). Consequently, regarding all data available on fish exposed to NPs, mRNA transcript is highly affected, increasing the possibility of mutations, as well as cellular malfunctions.

Overall, NPs have the ability to disrupt the immune system of fish, induce oxidative stress, compromise lipid metabolism and cause cell viability to decline. However, more studies need to be carried out with other types of NPs to fully understand how the toxicity of NPs affects fish, great importance to evaluate different species, as well as understand how different NPs polymers can affect the quality of aquaculture, effects on fisheries, and consequent effects on human-beings.

Considering all the above, a summary of nanoplastics effects on marine biota, to date, is illustrated in Fig. 4. As can be seen, the information about the effects on marine organisms is still very limited and NPs along with their nano-specific properties are of major concern as they fundamentally differ from those of the same polymer type in bulk form and are size dependent (Klaine et al., 2012). Moreover, it is crucial to develop methods that accurately measure NPs concentrations in the different components of the marine environment and in biota. In addition, the concentrations used need to be environmentally relevant so that data can be harmonised, thus being possible to clearly compare the effects of NPs in marine biota.

3. Conclusions

Nanoplastics are found in the marine environment either as primary NPs or, more prone, as secondary nanoplastics. Their fate, at present, is still difficult to predict, contemplating that NPs are a recent emerging contaminant and that these NPs will interact with their surroundings. The effect of these interactions has not fully been enlightened, though, on the account of the available data, NPs have considerable effects on marine biota and marine ecosystems. Consequently, a wide interest is arising within the scientific community towards NPs and the effects they pursue within the marine environment, as marine organisms can interact and ingest this plastic debris due to their particle size and possibly lead to an impact on seafood production and human health.

Within the marine environment, few data exist on the effects of different types of virgin nanoplastics, having as a main focus the effects of PS NPs with a carboxyl group (-COOH) or amide group (-NH_2). Though not environmentally relevant, the surface properties of NPs with −COOH or −NH_2 toxicity are important model molecules to evaluate, as they provide information on the different forms of toxicity of negatively and positively charged NPs. The available data show that these NPs cause toxic effects on marine biota, within bacterial communities, primary producers, primary/secondary consumers, and top consumers. Furthermore, PS NPs with an amide group have more toxic effects than NPs with a carboxyl group, thus, suggesting that the charge of these NPs have an influential role in the nanotoxicity of these particles. Additionally, in certain marine organisms, NPs can accumulate in specific tissues of organisms leading to a more detrimental effect in these

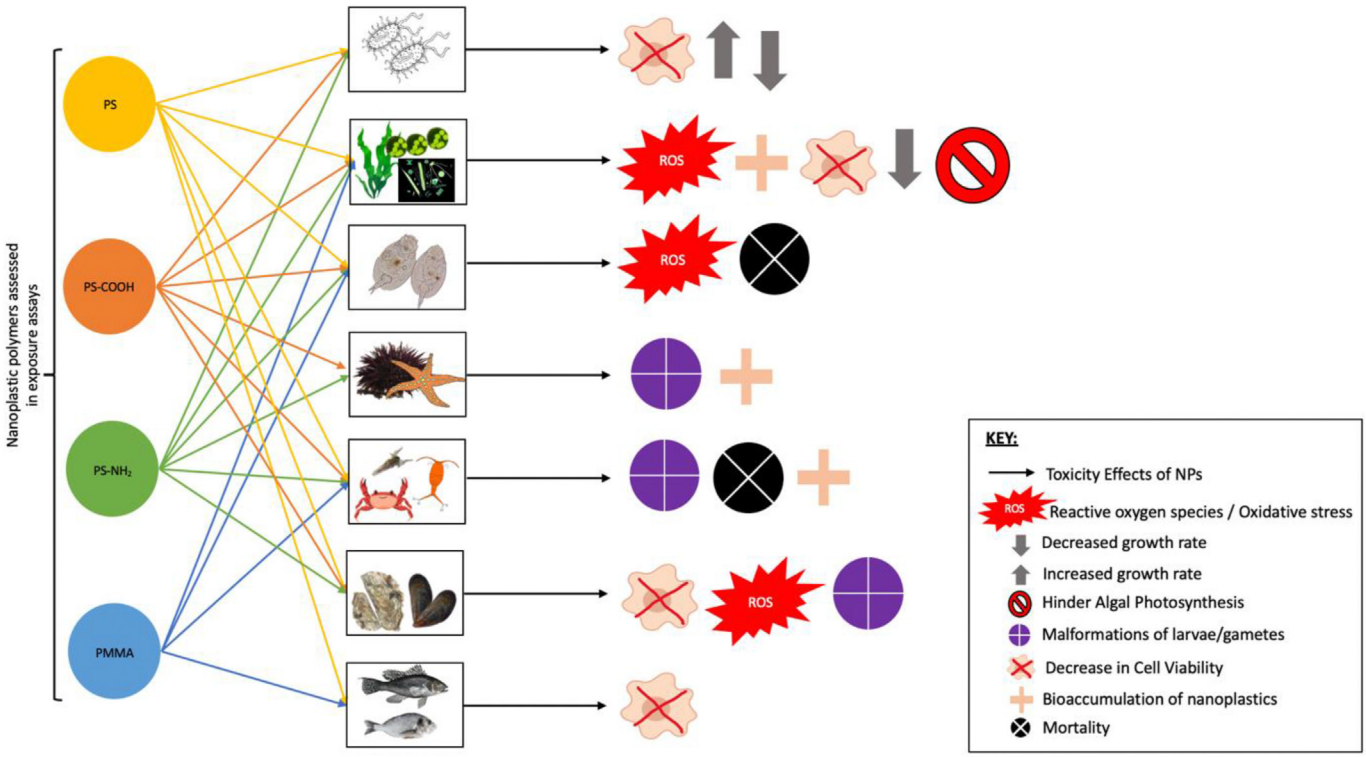

Fig. 4. Illustration of different NP polymers effects on marine biota.

organisms. Accordingly, as many organisms ingest these particles and accumulate them within tissues, highlights the need for more research regarding potential toxic effects of exposure to different types and sizes of virgin NPs, as well as different types and charged NPs.

In a more extensive context, the legion of data identifying ample effects of NPs should aid decision-makers in considering both production of nanomaterials as well as the breakdown of plastic consumer products as a potential environmental dilemma. Notwithstanding, the scientific obligation towards understanding the toxicity of NPs and the ecological effects, jointly with findings suggest that some types of NPs are less reactive than others, administering intuition into producing less potent nanomaterials.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Andrady, A.L., 2011. Microplastics in the marine environment. Mar. Pollut. Bull. 62 (8), 1596–1605. https://doi.org/10.1016/j.marpolbul.2011.05.030.

Balbi, T., Camisassi, G., Montagna, M., Fabbri, R., Franzellitti, S., Carbone, C., Dawson, K., Canesi, L., 2017. Impact of cationic polystyrene nanoparticles (PS-NH2) on early embryo development of *Mytilus galloprovincialis*: effects on shell formation. Chemosphere 186, 1–9. https://doi.org/10.1016/j.chemosphere.2017.07.120.

Bergami, E., Bocci, E., Vannuccini, M.L., Monopoli, M., Salvati, A., Dawson, K.A., Corsi, I., 2016. Nano-sized polystyrene affects feeding, behaviour and physiology of brine shrimp *Artemia franciscana* larvae. Ecotoxicol. Environ. Saf. 123, 18–25. https://doi.org/10.1016/j.ecoenv.2015.09.021.

Bergami, E., Krupinski Emerenciano, A., González-Aravena, M., Cárdenas, C.A., Hernández, P., Silva, J.R.M.C., Corsi, I., 2019. Polystyrene nanoparticles affect the innate immune system of the Antarctic sea urchin *Sterechinus neumayeri*. Polar Biol. 42 (4), 743–757. https://doi.org/10.1007/s00300-019-02468-6.

Bergami, E., Manno, C., Cappello, S., Vannuccini, M.L., Corsi, I., 2020. Nanoplastics affect moulting and faecal pellet sinking in Antarctic krill (*Euphausia superba*) juveniles. Environ. Int. 143 (July), 105999. https://doi.org/10.1016/j.envint.2020.105999.

Bergami, E., Pugnalini, S., Vannuccini, M.L., Manfra, L., Faleri, C., Savorelli, F., Dawson, K.A., Corsi, I., 2017. Long-term toxicity of surface-charged polystyrene nanoplastics to marine planktonic species *Dunaliella tertiolecta* and *Artemia franciscana*. Aquat. Toxicol. 189, 159–169. https://doi.org/10.1016/j.aquatox.2017.06.008.

Bessa, F., Barría, P., Neto, J.M., Frias, J.P.G.L., Otero, V., Sobral, P., Marques, J.C., 2018. Occurrence of microplastics in commercial fish from a natural estuarine environment. Mar. Pollut. Bull. 128, 575–584. https://doi.org/10.1016/j.marpolbul.2018.01.044.

Bhargava, S., Chen Lee, S.S., Min Ying, L.S., Neo, M.L., Lay-Ming Teo, S., Valiyaveettil, S., 2018. Fate of nanoplastics in marine larvae: a case study using barnacles, *Amphibalanus amphitrite*. ACS Sustain. Chem. Eng. 6 (5), 6932–6940. https://doi.org/10.1021/acssuschemeng.8b00766.

Bhattacharya, P., Lin, S., Turner, J.P., Ke, P.C., 2010. Physical adsorption of charged plastic nanoparticles affects algal photosynthesis. J. Phys. Chem. C 114 (39), 16556–16561. https://doi.org/10.1021/jp1054759.

Boucher, J., Friot, D., 2017. Primary microplastics in the oceans: a global evaluation of sources. In: Primary Microplastics in the Oceans: A Global Evaluation of Sources. https://doi.org/10.2305/iucn.ch.2017.01.en.

Brandts, I., Barría, C., Martins, M.A., Franco-Martínez, L., Barreto, A., Tvarijonaviciute, A., Tort, L., Oliveira, M., Teles, M., 2021. Waterborne exposure

of gilthead seabream (*Sparus aurata*) to polymethylmethacrylate nanoplastics causes effects at cellular and molecular levels. J. Hazard Mater. 403 (July 2020), 123590. https://doi.org/10.1016/j.jhazmat.2020.123590.
Brandts, I., Teles, M., Tvarijonaviciute, A., Pereira, M.L., Martins, M.A., Tort, L., Oliveira, M., 2018. Effects of polymethylmethacrylate nanoplastics on *Dicentrarchus labrax*. Genomics 110 (6), 435–441. https://doi.org/10.1016/j.ygeno.2018.10.006.
Canesi, L., Ciacci, C., Bergami, E., Monopoli, M.P., Dawson, K.A., Papa, S., Canonico, B., Corsi, I., 2015. Evidence for immunomodulation and apoptotic processes induced by cationic polystyrene nanoparticles in the haemocytes of the marine bivalve *Mytilus*. Mar. Environ. Res. 111, 34–40. https://doi.org/10.1016/j.marenvres.2015.06.008.
Chae, Y., An, Y.J., 2017. Effects of micro- and nanoplastics on aquatic ecosystems: current research trends and perspectives. Mar. Pollut. Bull. 124 (2), 624–632. https://doi.org/10.1016/j.marpolbul.2017.01.070.
Chen, Q., Yin, D., Jia, Y., Schiwy, S., Legradi, J., Yang, S., Hollert, H., 2017. Enhanced uptake of BPA in the presence of nanoplastics can lead to neurotoxic effects in adult zebrafish. Sci. Total Environ. 609, 1312–1321. https://doi.org/10.1016/j.scitotenv.2017.07.144.
Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: a review. Mar. Pollut. Bull. 62 (12), 2588–2597. https://doi.org/10.1016/j.marpolbul.2011.09.025.
Davranche, M., Veclin, C., Pierson-Wickmann, A.C., El Hadri, H., Grassl, B., Rowenczyk, L., Dia, A., Ter Halle, A., Blancho, F., Reynaud, S., Gigault, J., 2019. Are nanoplastics able to bind significant amount of metals? The lead example. Environ. Pollut. 249, 940–948. https://doi.org/10.1016/j.envpol.2019.03.087.
Della Torre, C., Bergami, E., Salvati, A., Faleri, C., Cirino, P., Dawson, K.A., Corsi, I., 2014. Accumulation and embryotoxicity of polystyrene nanoparticles at early stage of development of sea urchin embryos *Paracentrotus lividus*. Environ. Sci. Technol. 48 (20), 12302–12311. https://doi.org/10.1021/es502569w.
Ferreira, I., Venâncio, C., Lopes, I., Oliveira, M., 2019. Nanoplastics and marine organisms: what has been studied? Environ. Toxicol. Pharmacol. 67, 1–7. https://doi.org/10.1016/j.etap.2019.01.006.
Fotopoulou, K.N., Karapanagioti, H.K., 2012. Surface properties of beached plastic pellets. Mar. Environ. Res. 81, 70–77. https://doi.org/10.1016/j.marenvres.2012.08.010.
Gambardella, C., Morgana, S., Ferrando, S., Bramini, M., Piazza, V., Costa, E., Garaventa, F., Faimali, M., 2017. Effects of polystyrene microbeads in marine planktonic crustaceans. Ecotoxicol. Environ. Saf. 145, 250–257. https://doi.org/10.1016/j.ecoenv.2017.07.036.
Gigault, J., Halle, A. ter, Baudrimont, M., Pascal, P.Y., Gauffre, F., Phi, T.L., El Hadri, H., Grassl, B., Reynaud, S., 2018. Current opinion: what is a nanoplastic? Environ. Pollut. 235, 1030–1034. https://doi.org/10.1016/j.envpol.2018.01.024.
Gomes, T., Almeida, A.C., Georgantzopoulou, A., 2020. Characterization of cell responses in *Rhodomonas baltica* exposed to PMMA nanoplastics. Sci. Total Environ. 726, 138547. https://doi.org/10.1016/j.scitotenv.2020.138547.
González-Fernández, C., Le Grand, F., Bideau, A., Huvet, A., Paul-Pont, I., Soudant, P., 2020. Nanoplastics exposure modulate lipid and pigment compositions in diatoms. Environ. Pollut. 262 https://doi.org/10.1016/j.envpol.2020.114274.
González-Fernández, C., Tallec, K., Le Goïc, N., Lambert, C., Soudant, P., Huvet, A., Suquet, M., Berchel, M., Paul-Pont, I., 2018. Cellular responses of Pacific oyster (*Crassostrea gigas*) gametes exposed in vitro to polystyrene nanoparticles. Chemosphere 208, 764–772. https://doi.org/10.1016/j.chemosphere.2018.06.039.
Halle, A. Ter, Jeanneau, L., Martignac, M., Jardé, E., Pedrono, B., Brach, L., Gigault, J., 2017. Nanoplastic in the North atlantic subtropical gyre. Environ. Sci. Technol. 51 (23), 13689–13697. https://doi.org/10.1021/acs.est.7b03667.
Hu, E., Shang, S., Fu, Z., Zhao, X., Nan, X., Du, Y., Chen, X., 2020. Cotransport of naphthalene with polystyrene nanoplastics (PSNP) in saturated porous media: effects of PSNP/naphthalene ratio and ionic strength. Chemosphere 245, 125602. https://doi.org/10.1016/j.chemosphere.2019.125602.
Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Science 347 (6223), 768–771. https://doi.org/10.1126/science.1260352.
Jeong, J., Choi, J., 2019. Adverse outcome pathways potentially related to hazard identification of microplastics based on toxicity mechanisms. Chemosphere 231, 249–255. https://doi.org/10.1016/j.chemosphere.2019.05.003.
Klaine, S.J., Koelmans, A.A., Horne, N., Carley, S., Handy, R.D., Kapustka, L., Nowack, B., von der Kammer, F., 2012. Paradigms to assess the environmental impact of manufactured nanomaterials. Environ. Toxicol. Chem. 31 (1), 3–14. https://doi.org/10.1002/etc.733.
Koelmans, A.A., Besseling, E., Shim, W.J., 2015. Marine anthropogenic litter (Chapter 12). In: Bergmann, M., Gutow, L., Klages, M. (Eds.), Marine Anthropogenic Litter. https://doi.org/10.1007/978-3-319-16510-3, 2015.
Kögel, T., Bjorøy, Ø., Toto, B., Bienfait, A.M., Sanden, M., 2020. Micro- and nanoplastic toxicity on aquatic life: determining factors. Sci. Total Environ. 709 (5817), 136050. https://doi.org/10.1016/j.scitotenv.2019.136050.
Langner, S., Rakers, S., Ciba, P., Petschnik, A.E., Rapoport, D.H., Kruse, C., 2011. New cell line from adipopancreatic tissue of Atlantic herring *Clupea harengus*. Aquat. Biol. 11 (3), 271–278. https://doi.org/10.3354/ab00317.
Lee, H., Shim, W.J., Kwon, J.H., 2014. Sorption capacity of plastic debris for hydrophobic organic chemicals. Sci. Total Environ. 470–471, 1545–1552. https://doi.org/10.1016/j.scitotenv.2013.08.023.
Lee, K.W., Shim, W.J., Kwon, O.Y., Kang, J.H., 2013. Size-dependent effects of micro polystyrene particles in the marine copepod *Tigriopus japonicus*. Environ. Sci. Technol. 47 (19), 11278–11283. https://doi.org/10.1021/es401932b.
Lee, S., Gounley, J., Randles, A., Vetter, J.S., 2019. Performance portability study for massively parallel computational fluid dynamics application on scalable heterogeneous architectures. J. Parallel Distr. Comput. 129, 1–13. https://doi.org/10.1016/j.jpdc.2019.02.005.
Liu, L., Fokkink, R., Koelmans, A.A., 2016. Sorption of polycyclic aromatic hydrocarbons to polystyrene nanoplastic. Environ. Toxicol. Chem. 35 (7), 1650–1655. https://doi.org/10.1002/etc.3311.
Luigi, P., Chiara, A., Elisabetta, G., Alessandra, S., Luigi, M.G., 2012. Utilization of Marine Crustaceans as Study Models: a New Approach in Marine Ecotoxicology for European (REACH) Regulation. G. Begum, p. 91.
Manfra, L., Rotini, A., Bergami, E., Grassi, G., Faleri, C., Corsi, I., 2017. Comparative ecotoxicity of polystyrene nanoparticles in natural seawater and reconstituted seawater using the rotifer *Brachionus plicatilis*. Ecotoxicol. Environ. Saf. 145, 557–563. https://doi.org/10.1016/j.ecoenv.2017.07.068.
Mao, Y., Li, H., Huangfu, X., Liu, Y., He, Q., 2020. Nanoplastics display strong stability in aqueous environments: insights from aggregation behaviour and theoretical calculations. Environ. Pollut. 258, 113760. https://doi.org/10.1016/j.envpol.2019.113760.
Mattsson, K., Ekvall, M.T., Hansson, L.A., Linse, S., Malmendal, A., Cedervall, T., 2015. Altered behaviour, physiology, and metabolism in fish exposed to polystyrene nanoparticles. Environ. Sci. Technol. 49 (1), 553–561. https://doi.org/10.1021/es5053655.
Mattsson, K., Johnson, E.V., Malmendal, A., Linse, S., Hansson, L.A., Cedervall, T., 2017. Brain damage and behavioural disorders in fish induced by plastic nanoparticles delivered through the food chain. Sci. Rep. 7 (1), 1–7. https://doi.org/10.1038/s41598-017-10813-0.
Morcillo, P., Chaves-Pozo, E., Meseguer, J., Esteban, M.Á., Cuesta, A., 2017. Establishment of a new teleost brain cell line (DLB-1) from the European sea bass and its use to study metal toxicology. Toxicol. Vitro 38, 91–100. https://doi.org/10.1016/j.tiv.2016.10.005.
Okshevsky, M., Gautier, E., Farner, J.M., Schreiber, L., Tufenkji, N., 2020. Biofilm formation by marine bacteria is impacted by concentration and surface functionalization of polystyrene nanoparticles in a species-specific manner. Environmental Microbiology Reports 12 (2), 203–213. https://doi.org/10.1111/1758-2229.12824.
Oriekhova, O., Stoll, S., 2018. Heteroaggregation of nanoplastic particles in the presence of inorganic colloids and natural organic matter. Environ. Sci.: Nano 5 (3), 792–799. https://doi.org/10.1039/c7en01119a.
Palumbo, M.T., Mingazzini, M., 2011. Toxicity test with algae: evaluation of the sensitivity related to the use of biomass versus growth rate endpoint. In: 16th International MESAEP Symposium on Environmental Pollution.
Pannetier, P., Fuster, L., Clérandeau, C., Lacroix, C., Gourves, P.Y., Cachot, J., Morin, B., 2018. Usefulness of RTL-W1 and OLCAB-e3 fish cell lines and multiple endpoint measurements for toxicity evaluation of unknown or complex mixture of chemicals. Ecotoxicol. Environ. Saf. 150, 40–48. https://doi.org/10.1016/j.ecoenv.2017.12.027.
Peng, L., Fu, D., Qi, H., Lan, C.Q., Yu, H., Ge, C., 2020. Micro- and nano-plastics in marine environment: source, distribution and threats — a review. Sci. Total Environ. 698, 134254. https://doi.org/10.1016/j.scitotenv.2019.134254.
Pinsino, A., Bergami, E., Torre, C. Della, Vannuccini, M.L., Addis, P., Secci, M., Dawson, K.A., Matranga, V., Corsi, I., 2017. Amino-modified polystyrene nanoparticles affect signalling pathways of the sea urchin (*Paracentrotus lividus*) embryos. Nanotoxicology 11 (2), 201–209. https://doi.org/10.1080/17435390.2017.1279360.
Pitt, J.A., Trevisan, R., Massarsky, A., Kozal, J.S., Levin, E.D., Di Giulio, R.T., 2018. Maternal transfer of nanoplastics to offspring in zebrafish (*Danio rerio*): a case study with nanopolystyrene. Sci. Total Environ. 643, 324–334. https://doi.org/10.1016/j.scitotenv.2018.06.186.
PlasticsEurope, 2019. Plastics - the Facts 2019: an Analysis of European Plastics Production, Demand and Waste Data. https://www.plasticseurope.org/en/resources/market-data.
Raisuddin, S., Kwok, K.W.H., Leung, K.M.Y., Schlenk, D., Lee, J.S., 2007. The copepod Tigriopus: a promising marine model organism for ecotoxicology and environmental genomics. Aquat. Toxicol. 83 (3), 161–173. https://doi.org/10.1016/j.aquatox.2007.04.005.
Ramirez, L., Gentile, R.S., Zimmerman, S., Stoll, S., 2019. Drinking and Lake Geneva Waters. Impact of Water, Water.
Rios, L.M., Moore, C., Jones, P.R., 2007. Persistent organic pollutants carried by synthetic polymers in the ocean environment. Mar. Pollut. Bull. 54 (8), 1230–1237. https://doi.org/10.1016/j.marpolbul.2007.03.022.
Rotini, A., Tornambè, A., Cossi, R., Iamunno, F., Benvenuto, G., Berducci, M.T., Maggi, C., Thaller, M.C., Cicero, A.M., Manfra, L., Migliore, L., 2017. Salinity-based toxicity of CuO nanoparticles, CuO-bulk and Cu ion to *vibrio anguillarum*. Front. Microbiol. 8, 2076. https://doi.org/10.3389/fmicb.2017.02076.
Rouillon, C., Bussiere, P.O., Desnoux, E., Collin, S., Vial, C., Therias, S., Gardette, J.L., 2016. Is carbonyl index a quantitative probe to monitor polypropylene photodegradation? Polym. Degrad. Stabil. 128, 200–208. https://doi.org/10.1016/j.polymdegradstab.2015.12.011.
Shen, M., Zhang, Y., Zhu, Y., Song, B., Zeng, G., Hu, D., Wen, X., Ren, X., 2019. Recent advances in toxicological research of nanoplastics in the environment: a review. Environ. Pollut. 252, 511–521. https://doi.org/10.1016/j.envpol.2019.05.102.
Sjollema, S.B., Redondo-Hasselerharm, P., Leslie, H.A., Kraak, M.H.S., Vethaak, A.D., 2016. Do plastic particles affect microalgal photosynthesis and growth? Aquat. Toxicol. 170, 259–261. https://doi.org/10.1016/j.aquatox.2015.12.002.

Skjolding, L.M., Ašmonaitė, G., Jølck, R.I., Andresen, T.L., Selck, H., Baun, A., Sturve, J., 2017. An assessment of the importance of exposure routes to the uptake and internal localisation of fluorescent nanoparticles in zebrafish (*Danio rerio*), using light sheet microscopy. Nanotoxicology 11 (3), 351–359. https://doi.org/10.1080/17435390.2017.1306128.

Sugni, M., Mozzi, D., Barbaglio, A., Bonasoro, F., Candia Carnevali, M.D., 2007. Endocrine disrupting compounds and echinoderms: new ecotoxicological sentinels for the marine ecosystem. Ecotoxicology 16 (1), 95–108. https://doi.org/10.1007/s10646-006-0119-8.

Sun, X., Chen, B., Li, Q., Liu, N., Xia, B., Zhu, L., Qu, K., 2018. Toxicities of polystyrene nano- and microplastics toward marine bacterium *Halomonas alkaliphila*. Sci. Total Environ. 642, 1378–1385. https://doi.org/10.1016/j.scitotenv.2018.06.141.

Tallec, K., Huvet, A., Di Poi, C., González-Fernández, C., Lambert, C., Petton, B., Le Goïc, N., Berchel, M., Soudant, P., Paul-Pont, I., 2018. Nanoplastics impaired oyster free living stages, gametes and embryos. Environ. Pollut. 242, 1226–1235. https://doi.org/10.1016/j.envpol.2018.08.020.

Tamminga, M., Hengstmann, E., Fischer, E.K., 2018. Microplastic analysis in the South Funen Archipelago, Baltic Sea, implementing manta trawling and bulk sampling. Mar. Pollut. Bull. 128, 601–608. https://doi.org/10.1016/j.marpolbul.2018.01.066.

Teuten, E.L., Saquing, J.M., Knappe, D.R.U., Barlaz, M.A., Jonsson, S., Björn, A., Rowland, S.J., Thompson, R.C., Galloway, T.S., Yamashita, R., Ochi, D., Watanuki, Y., Moore, C., Viet, P.H., Tana, T.S., Prudente, M., Boonyatumanond, R., Zakaria, M.P., Akkhavong, K., Takada, H., 2009. Transport and release of chemicals from plastics to the environment and to wildlife. Phil. Trans. Biol. Sci. 364 (1526), 2027–2045. https://doi.org/10.1098/rstb.2008.0284.

Thompson, R.C., Olson, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D., Russell, A.E., 2004. Lost at sea: where is all the plastic? Science 304 (5672), 838. https://doi.org/10.1126/science.1094559.

Venâncio, C., Ferreira, I., Martins, M.A., Soares, A.M.V.M., Lopes, I., Oliveira, M., 2019. The effects of nanoplastics on marine plankton: a case study with polymethylmethacrylate. Ecotoxicol. Environ. Saf. 184, 109632. https://doi.org/10.1016/j.ecoenv.2019.109632.

Villalba, M., Pérez, V., Herrera, L., Stepke, C., Maldonado, N., Fredericksen, F., Yáñez, A., Olavarría, V.H., 2017. Infectious pancreatic necrosis virus infection of fish cell lines: preliminary analysis of gene expressions related to extracellular matrix remodeling and immunity. Vet. Immunol. Immunopathol. 193–194, 10–17. https://doi.org/10.1016/j.vetimm.2017.09.009.

Wallace, R.L., Snell, T.W., Ricci, C., Nogrady, T., 2006. Rotifera. Biology, ecology and systematics. In: Dumont, H.J.F. (Ed.), Guides to the Identification of the Microinvertebrates of the Continental Waters of the World. Kenobi Productions, Ghent, Belgium and. Backhuys Publishers, Leiden.

Wang, W., Yuan, W., Chen, Y., Wang, J., 2018. Microplastics in surface waters of dongting lake and hong lake, China. Sci. Total Environ. 633, 539–545. https://doi.org/10.1016/j.scitotenv.2018.03.211.

Wegner, A., Besseling, E., Foekema, E.M., Kamermans, P., Koelmans, A.A., 2012. Effects of nanopolystyrene on the feeding behavior of the blue mussel (*Mytilus edulis* L.). Environ. Toxicol. Chem. 31 (11), 2490–2497. https://doi.org/10.1002/etc.1984.

Worm, B., Lotze, H.K., Jubinville, I., Wilcox, C., Jambeck, J., 2017. Plastic as a persistent marine pollutant. Annu. Rev. Environ. Resour. 42 (1), 1–26. https://doi.org/10.1146/annurev-environ-102016-060700.

Zhang, Y., Chen, Y., Westerhoff, P., Hristovski, K., Crittenden, J.C., 2008. Stability of commercial metal oxide nanoparticles in water. Water Res. 42 (8–9), 2204–2212. https://doi.org/10.1016/j.watres.2007.11.036.