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Impact of polystyrene nanoplastics on early life stages of marine invertebrates: current knowledge and future research perspectives

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ABSTRACT

Nanoplastics (<1 μm) are emerging pollutants with potential adverse effects on marine organisms, particularly during sensitive early life stages such as embryos and larvae. Marine invertebrates are key targets of nanoparticles toxicity and suitable models for assessing developmental impacts, yet their embryonic and larval stages remain understudied. This review synthesizes current knowledge on the embryotoxic effects of nanoplastics in marine invertebrates, focusing on studies using polystyrene nanoparticles (PS NPs) across diverse taxa including Rotifera, Mollusca, Arthropoda, Echinodermata, and invertebrate Chordates. Toxicity of PS NPs depends on surface functionalization, with amino-modified PS-NH_2 generally more harmful than unmodified or carboxylated PS-COOH. Reported effects include oxidative stress, neurotoxicity, impaired fertilization in mollusks, skeletal defects in echinoderms, and altered behavior in crustaceans. Among tested models, ascidians emerge as particularly promising due to their phylogenetic proximity to vertebrates and compliance with EU legislation (Directive, 2010/63/EU), positioning them as ethically and scientifically valuable alternatives for developmental toxicity studies.

Despite growing awareness, the current body of literature is constrained by a limited range of particle types, simplified exposure scenarios, and a focus on a few model organisms. To improve ecological relevance, future research should prioritize the use of environmentally realistic concentrations, diversify polymer types beyond PS, and include early life stages of ecologically important but underrepresented marine invertebrates. This will be essential to better understand the real-world impact of nanoplastics on marine ecosystems and to support more effective environmental risk assessment and regulatory frameworks.

1. Introduction

Plastic pollution in the oceans has become one of the most significant ecological threats of the 21st century, affecting water columns, sediments and biota (Andrady, 2017; Lebreton and Andrady, 2019; Horton and Barnes, 2020). Human dependence on plastic has intensified and has been exacerbated by the COVID-19 pandemic and the marine environment has again been recognized as the ultimate sink (Aragaw, 2020; Prata et al., 2020). Annually, over 14 million tons of plastic waste end up in the oceans, representing approximately 80 % of all marine litter, with a wide range of spatial distribution from surface waters to deep sediments (Barrett et al., 2020). Due to physical, chemical and biological transformations such as waves, high salinity, and (micro)organisms, plastics are broken down into smaller fragments from micro to nanoscale (Enfrin et al., 2020; Llorca et al., 2021).

The smallest fraction named nanoplastic (<1 μm) (Hartmann et al., 2019) can also originate from primary sources during the production, usage and disposal of nanoplastics-enabled products. However, nanoplastics have yet to be quantified in environmental matrices, although some attempts have been recently made in marine waters (Oliveira and Almeida, 2019; Mitrano et al., 2021).

According to surface water circulation models and evidence from the literature, nanoplastics, similarly to other colloidal particles, tend to accumulate in estuarine and surface waters, where they are likely to be more bioavailable to planktonic organisms and to the early life stages (embryos and larvae) of both pelagic and benthic species (Della Torre et al., 2014; Gigault et al., 2018; Corsi et al., 2020). Furthermore, the entrapment of nanoplastics which occurs specifically within algal exudates (e.g., extracellular polymeric substances) can enhance their bioavailability and promote their trophic transfer across marine food

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webs (Bellingeri et al., 2020; Grassi et al., 2020; Corsi et al., 2021; Huang et al., 2021).

A comprehensive understanding of bio–nano interactions occurring during early developmental stages of marine species is essential for assessing ecological risks and anticipating potential cascade effects from individual organisms to populations, communities, and entire ecosystems (Corsi et al., 2023; Reilly et al., 2023).

During early life stages, from gametogenesis, fertilization, embryogenesis, and larval development up to metamorphosis, species are extremely vulnerable. Exposure to nanoplastics during this sensitive period can lead to adverse effects such as hypoxia, embryonic deformities and developmental disorders, potentially affecting growth and survival rates (Duan et al., 2020; Gonçalves and Bebianno, 2021).

Much attention has been paid to polymeric-engineered nanoparticles (NP) employed as a proxy for nanoplastics, due to their unique physicochemical properties, associated with their small size (often >100 nm) and high surface area, both allowing them to pass biological barriers, penetrate tissues, accumulate in organisms and strongly adsorb contaminants (Corsi et al., 2020; Zhang and Xu, 2022). Most studies have been carried out with commercial fluorescent or functionalized polystyrene nanoparticles (PS NPs), using spherical particles rather than irregular shapes like fragments, fibers, and films. This preference is because spherical particles are easier to produce, particularly in the nano-size range, compared to irregularly shaped particles (Burns and Boxall, 2018; Kooi and Koelmans, 2019; Phuong et al., 2016). As a result, most experimental research uses synthetically produced spherical PS NPs, due to their availability in various sizes and fluorescent labeling (Kik et al., 2020; Torres-Ruiz et al., 2021). Based on functionalization, PS NPs can be obtained as cationic, anionic, or neutral (unmodified), depending on the addition of specific chemical groups (-NH_2; -COOH), which affect their surface charge (Libralato et al., 2017; Corsi et al., 2021). Despite their widespread use, most PS NP exposures occur at concentrations several orders of magnitude higher than those predicted in the environment (Koelmans et al., 2017; Al-Sid-Cheikh et al., 2020), even though it is important to emphasize that Predicted Environmental Concentrations (PECs) are expected to increase as particle size decreases. Moreover, the distribution of nanoparticles (NPs) varies across spatial and temporal scales, with significantly higher concentrations observed in environmental compartments characterized by plastic particle release and accumulation (Lenz et al., 2016; Everaert et al., 2018). By using fluorescently labeled PS NPs to precisely assess uptake, biodistribution and accumulation of NPs in living organisms, studies have focused on early larval stages of marine species having both key ecological roles and high commercial value, such as the sea urchin *Paracentrotus lividus* (Della Torre et al., 2014; Pinsino et al., 2017), bivalves as *Mytilus* spp. (Balbi et al., 2017; Rist et al., 2019) and *Crassostrea gigas* (Cole and Galloway, 2015; Tallec et al., 2018). Overall, the species sensitivity to nanoplastics can be influenced by polymer characteristics as composition, size, shape, surface charge, and the presence of additives or adsorbed contaminants and their behavior in different media and environmental conditions (i.e., ionic strength, T°, pH) all driving exposure route as ingestion, dermal contact, and uptake through respiratory surfaces as well as intrinsic biological properties as species-specific physiology, life stage, feeding behavior, and overall health. Data collected so far indicate that amino-modified PS NPs (PS-NH_2), bearing a positive surface charge, lead to severe malformations in embryos of marine invertebrates, causing developmental delay and arrest. In contrast, carboxyl-modified PS NPs (PS-COOH) are mostly associated with limited or no effect, apart from ingestion/excretion, as shown for holoplankton (Bergami et al., 2016; Balbi et al., 2017; Eliso et al., 2020a; Eliso et al., 2023). Therefore, given the ecological sensitivity of early life stages, from fertilization to larval development and metamorphosis, it is essential to address existing knowledge gaps and deepen the scientific understanding of how nanoplastics can disrupt these developmental processes.

This review aims to provide a first synthesis of the current scientific understanding of nanoplastics' impact on early life stages of marine invertebrate species, the potential ecological risks nanoplastics pose, and outline future research directions.

2. Embryotoxicity of nanoplastics: studies on marine invertebrates

Nanoplastic ecological risk assessment, in terms of either bioaccumulation and biological response, has been investigated in embryos/larval stages of several marine species. A summary of the scientific publications available on Scopus and Google Scholar is reported in Fig. 1. The research was conducted between 2022 and 2024 with the following keywords: "polystyrene nanoparticles", "embryotoxicity", and "marine invertebrates". Fig. 1 shows a total of 27 scientific articles published between 2013 and 2024. The data indicate a marked increase in publications between 2016 and 2020, peaking in 2017 with five articles, followed by three articles per year from 2018 to 2020. A decline was observed in 2021 and 2022, with only one article published each year. However, this trend reversed in 2023, which saw an increase to five publications, followed by one article in 2024.

Fig. 2 shows the frequency distribution of concentration tested, ranging from a minimum of 0.0000002 μg/mL to a maximum of 100 μg/mL. Most of the studies investigate concentrations between 0.1 and 20 μg/mL. However, a significant portion of research focuses on concentrations below 0.1 μg/mL, addressing potential environmental levels and exploring the dose-response relationship across the spectrum of tested concentrations.

Fig. 3 shows the polymer type and sizes of nano- and micro-particles tested so far. PS, often functionalized with amine and/or carboxyl groups, is the most widely used polymer to evaluate embryotoxicity effects. Amino-modified PS NPs (PS-NH_2) are the most investigated compared to other PS NPs. Most of the studies selected here (41 %) investigate the toxicity of both amino and carboxyl groups in parallel, while 26 % investigate only PS-NH_2 and 30 % uncharged PS-NP. Only 1 study uses another plastic polymer, polymethyl methacrylate (PMMA).

Fig. 4 shows the number of studies per group investigated. Mollusks and Arthropods are the most investigated phyla, 10 articles out of 27 (37.03 %) and 9 out of 27 (33.3 %), respectively, followed by Chordates (11.11 %), Echinoderms (11.11 %) and Rotifer phyla (7.4 %). A summary of nanoplastics embryotoxicity effects evaluated in marine invertebrates, to date, is reported in Table 1, starting from rotifers and ascending through trophic levels.

2.1. Rotifera

Rotifers are organisms of microscopic size, with a common distribution in both freshwater and marine environments. As a component of zooplankton, these small organisms have an important role in aquatic ecosystems (Bakhtiyar et al., 2020). The genus *Brachionus* is the most used for ecotoxicological studies because of the sensitivity of the species to many pollutants, ease of culture, and exponential growth (Dahms et al., 2011). In the rotifer *Brachionus plicatilis,* exposure to PS-COOH and PS-NH_2 (40 and 50 nm, respectively) at concentrations between 0.5 and 50 μg/mL for 24 h and 48 h was made using PS NPs suspensions in standardized reconstituted seawater (RSW) and natural seawater (NSW) (Manfra et al., 2017). Anionic PS-COOH showed micro-aggregates and accumulation inside organisms with no acute toxicity, in the range of tested concentrations, while cationic PS-NH_2 showed a nano-aggregation state and high mortality, at concentrations ≥2.5 μg/mL. Furthermore, PS-NH_2 toxicity results are lower in NSW exposure media compared to RSW, suggesting that the compounds naturally present in NSW may affect ecotoxicological outcomes.

In *B. koreanus* the exposure to fluorescently labeled PS NPs (50 nm) for 24 h, at concentrations between 1 and 10 μg/mL, resulted in maternal transfer, as revealed by the detection of fluorescence in offspring embryos. The maternal transfer affected life-cycle parameters,

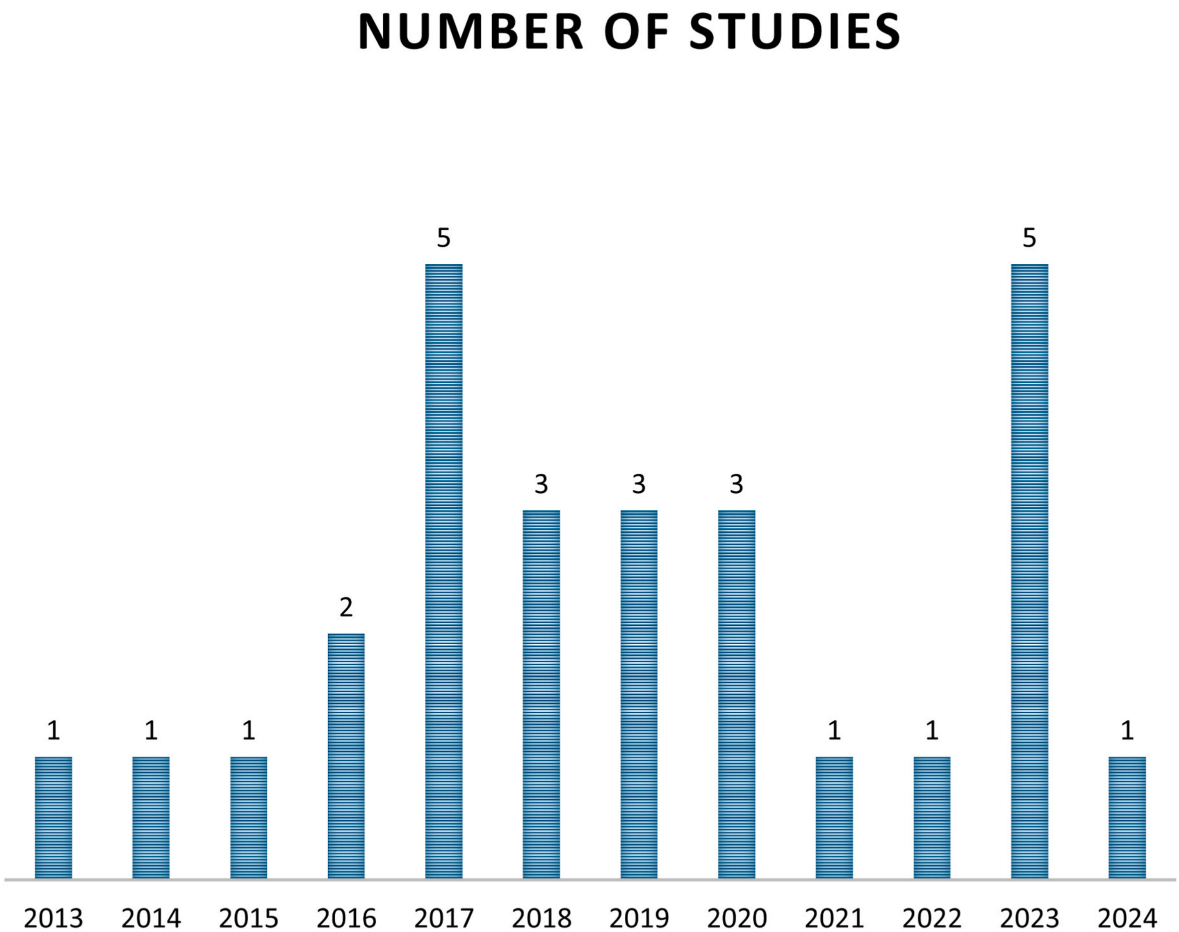

Fig. 1. Summary of the scientific publications available on Scopus until 2024.

including development and reproduction in offspring rotifers, and induced oxidative stress (Yeo et al., 2023). Thus, although more studies are needed, the data collected so far indicate that exposure to seawater media with different surface charges of PS NPs leads to different impacts on rotifer survival and the transgenerational effects should be considered when assessing the consequences of nanoplastics exposure.

2.2. Mollusca

The mollusk group includes several ecologically and commercially important filter feeders (e.g., mussels and oysters) that, due to their habitat and feeding behavior, are likely to encounter plastic particles of varying sizes (Gonçalves and Bebianno, 2021; Sendra et al., 2021). One of the first demonstrations of the adverse effects of nanoplastics on oysters' early-life stages is the study by Tallec and co-authors (2018). The exposure of gametes embryo- and larvae of *C. gigas* to PS NPs with different sizes (50 nm; 500 nm; 2 μm), functionalizations (plain 2-μm, 500-nm and 50-nm; COOH-50 nm and NH_2-50 nm) and in different concentrations (0.1–1–10 and 25 μg/mL) affected fertilization success and induced different malformations during embryo-larval development up to total developmental arrest. Here too, PS-NH_2 had the strongest toxicity to both gametes ($EC_{50} = 4.9$ μg/mL) and embryos ($EC_{50} = 0.15$ μg/mL), showing a functionalization-dependent effect. Focusing on spermiotoxicity, in 2020, Tallec and co-authors exposed oyster spermatozoa for 1 h to the same conditions (PS-COOH and PS-NH_2; 50 nm; 0.1–1–10 and 25 μg/mL). Microscopic observation revealed that the particles adhered but did not enter the cells. Nevertheless, PS-NH_2 beads at 10 μg/mL induced a decrease in the motility (−79 %) and velocity (−62 %) of spermatozoa, with an overall drop in embryogenesis success (−59 %); on the contrary PS-COOH, at the highest concentration, exerted only a transitory effect on oyster spermatozoa motility without affecting their reproductive success. Finally, Tallec et al. (2021a) exposed oyster embryos to 0.1 μg/mL PS-NH_2 (50 nm) to explore the consequences on *C. gigas* larval and adult performances over two generations, for 24 h. This study revealed that, besides a decrease in larval growth, no further effects were observed along the entire life cycle over two larval generations.

Also, González-Fernández et al., 2018 investigated the effects of PS-COOH and PS-NH_2 (100 nm; 0.1–100 μg/mL for 1, 3 and 5 h) on gametes of *C. gigas,* revealing a dose-response increase in reactive oxygen species (ROS) production in sperm cells exposed to 100 μg/mL PS-COOH, but not PS-NH_2. This suggests that the effects of NPs are related not only to the NP-associated functional groups but also to their interaction with the cell membranes, either directly or through interaction with specific biomolecules present in each seminal medium.

Concerning the consumption, in *C. gigas* larvae, the frequency and magnitude of plastic ingestion over 24 h varied with larval development, size and surface properties of PS NPs (70 nm–20 μm), with amino-modified particles ingested and retained more frequently (Cole and Galloway, 2015). Similarly, Rist and co-authors (2019) quantified ingestion and egestion of 100 nm and 2 μm PS NPs in blue mussel (*Mytilus edulis*) larvae after 4 h of exposure and 16 h of depuration, using different ratios between food and microplastics at 0.42, 28.2 and 282 μg/L within 15 days of exposure. On a mass basis, larvae ingested more 2 μm than 100 nm PS NPs and microplastics were retained in the larvae. Furthermore, although larval growth was normal, the number of abnormally developed larvae increased with increasing concentrations of PS beads and exposure time, and malformations were more pronounced for 100 nm beads. Seong et al., (2024) exposed d-shaped larvae of *C. gigas* to micro-sized polystyrene beads (MNPs) of three diameters

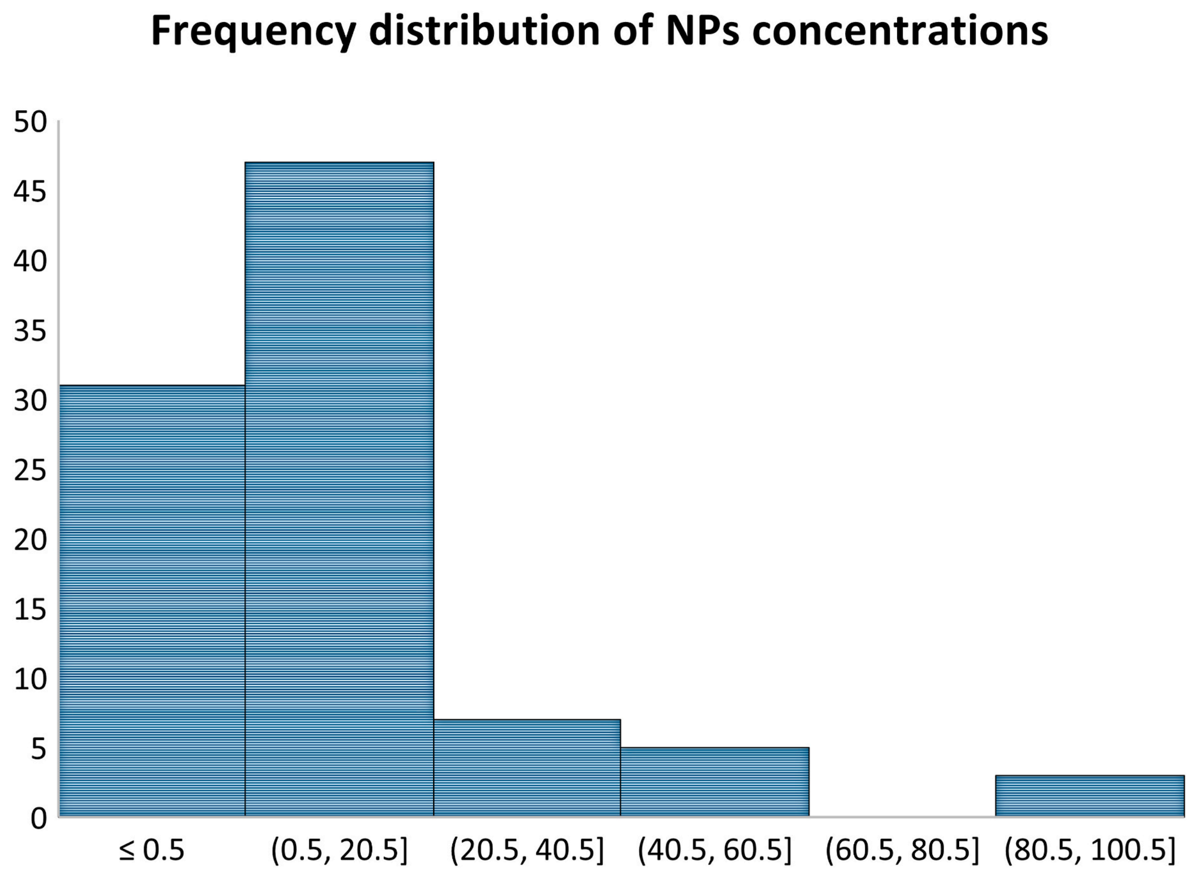

Fig. 2. The frequency distribution of concentration tested ranged from a minimum of 0.0000002 μg/mL to a maximum of 100 μg/mL.

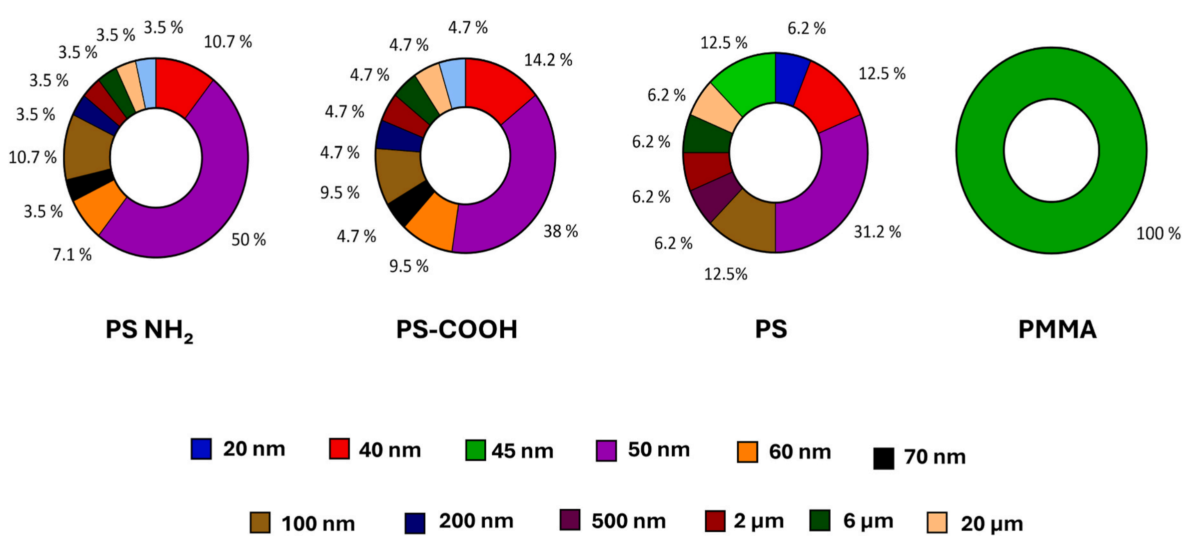

Fig. 3. Polymer type, functionalization, and sizes of nano- and micro-particles.

(0.55–3–6 μm) at five concentrations (0.1–1–10–20 μg/mL). Larval growth and survival were affected under all the exposure conditions and smaller particles at lower concentrations were found to have significant negative effects on growth parameters. In the mussel *M. galloprovincialis*, the exposure of fertilised eggs to PS-NH_2 (50 nm; 0.001–20 μg/mL), at lower concentrations, mainly induced malformations of the D-veligers (from 0.001 to 1 mg/L) (Balbi et al., 2017). At higher concentrations (from 2.5 mg/L to 10 mg/L), a progressive delay in larval development was detected with an increase in embryos at the pre-veliger stage or even at the trocophora stage. The highest concentrations (20 mg/L) resulted in approximately 90 % of larvae at the trocophora stage. In the clam *Meretrix* Luan et al. (2019), showed that PS-COOH (150 nm) and PS-NH_2 (100 nm) (0.02–0.2 - 0.5–1–2 μg/mL) treatment, at lower concentrations, significantly decreased the hatching rates by 5.79–39.5 % and developmental rates by 4.78–7.86 %. Furthermore, the toxicity of PS NPs induced stage-dependent toxic effects from the hatching stage to D-veliger larvae, with smaller PS-NH_2 (100 nm) exerting greater toxicity.

A recent study by Liu et al., (2023) examined for the first time the effects of PS NPs treatments in the golden cuttlefish *Sepia esculenta*

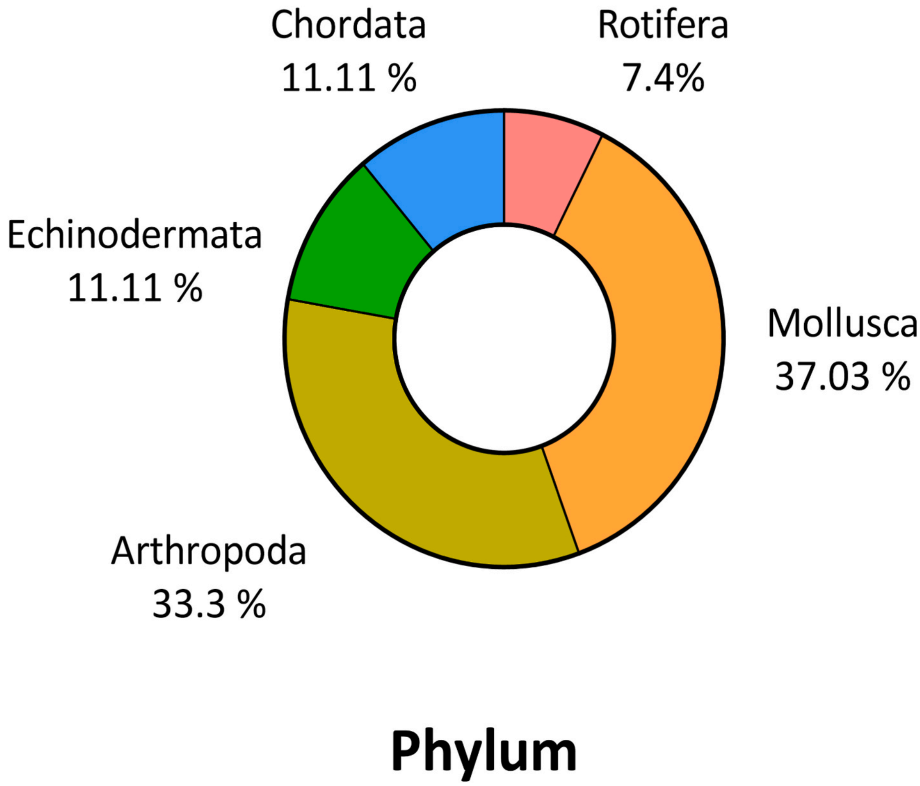

Fig. 4. The number of studies per group investigated.

larvae after short-term exposure (4 and 24 h) (50 nm, 50 μg/mL).

Transcriptome analysis identified 548 and 1990 differentially expressed genes at 4 and 24 h, respectively, highlighting immune-related genes involved in the response to PS NPs exposure.

The data collected so far in mollusks indicate that the toxicity of PS NPs is of great concern, with surface properties, size and concentration playing a crucial role in the onset of embryotoxicity. Smaller PS-NH_2 NPs exhibit the greatest effect on tested species, as shown by Balbi et al. (2017) in *M. galloprovincialis* and Tallec et al. (2018) in *C. gigas* embryos and gametes. Ingestion rates are higher with smaller NPs and dose-dependent (Rist et al., 2019). In contrast, PS-COOH NPs show less severe effects. Nevertheless, they increase the overall stress due to aggregation and adhesion on cell surfaces, as demonstrated by González-Fernández et al. (2018) and Tallec et al. (2021a) on *C. gigas* gametes. In the clam *M. meretrix,* amino and carboxy NPs affect hatching and development starting from 0.02 μg/mL (Luan et al., 2019), demonstrating that the early stages are more susceptible. High concentrations generally result in more pronounced effects, as observed by Balbi et al. (2017) in *M. galloprovincialis*, with complete developmental inhibition at 20 μg/mL of PS-NH_2. While some studies report no intergenerational effects, others highlight potential long-term impacts, emphasizing the need for further research to fully understand the chronic and multigenerational consequences of nanoplastics exposure on mollusc embryos.

2.3. Arthropoda

Benthic and planktonic marine crustaceans play a crucial role in the food web as primary and secondary consumers, facilitating energy transfer from primary producers, such as algae, to higher-level consumers, such as fish. This positions them at a vital level within the food web and as a significant target of increasing plastic pollution (Pisani et al., 2022). While many studies focused on marine species, model organisms such as *Artemia* spp. Are also frequently employed to investigate the effects of contaminants under controlled laboratory conditions.

To understand the effects of nanoplastics, it is important to assess potential trophic transfer and its implications on marine ecosystems. Here, studies investigate mainly planktonic crustaceans transferring energy from primary producers to high predators, such as the Antarctic krill (*Euphasia superba*) (Manno et al., 2020; Rowlands et al., 2023) and the brine shrimps *Artemia salina* and *Artemia franciscana,* largely used for acute and chronic toxicity testing as an aquatic model organism in ecotoxicological studies (Nunes et al., 2006; Libralato et al., 2017; Varó et al., 2019). Although both *E. superba* and *Artemia* spp. Are planktonic crustaceans, it should be noted that *Artemia* species inhabit hypersaline inland waters rather than marine environments and therefore are used as surrogate models rather than representative components of marine food webs. For instance, Bergami et al. (2016) observed no mortality of *A. franciscana* larvae after 48 h of exposure to PS-COOH (40 nm) and PS-NH_2 (50 nm) NPs, but several sub-lethal effects, such as gut lumen retention of PS-COOH (5–100 μg/mL), which limited food uptake. PS-NH_2 accumulated and adhered to the surface of antennules and appendages, hindering larvae' motility and inducing multiple molting events, thus suggesting a defense response. Another study by Bergami et al. (2017) investigated the long-term toxicity (72 h growth inhibition test; 14 d chronic exposure; 05–50 50 μg/mL) of both PS-COOH (40 nm) and PS-NH_2 (50 nm) on *A. franciscana* and the microalga *Dunaliella tertiolecta*, as a prey-predator experiment. PS-COOH did not affect the growth of microalgae or brine shrimp; however, they were adsorbed on microalgae and accumulated in brine shrimp, suggesting a potential trophic transfer. On the opposite, PS-NH_2 caused inhibition of algal growth (EC_{50} = 12.97 μg/mL) and mortality in *A. franciscana* at 14 d (LC_{50} = 0.83 μg/mL), significantly inducing physiological alterations, like the increase in molting.

Also, Gambardella et al. (2017) investigated lethal and sub-lethal responses in planktonic larvae of brine shrimp *A. franciscana* and *Amphibalanus amphitrite* exposed to non-functionalized PS NPs (100 nm; 0.001–10 μg/mL; 24–48 h), showing accumulation of NPs without mortality. However, neurotoxic and oxidative stress effects were induced by NPs treatment. In another study, Varó et al. (2019) observed

Table 1
Nanoplastics' embryotoxicity effects evaluated in marine invertebrates.

Phylum	Test species	Particle type	Particle size	Concentration (μg/ml)	Exposure time	Effects	Reference
Rotifera	*Brachionus plicatilis*	PS NP PS-NH_2 PS-COOH	40–50 nm	0.5–1 – 5–10 – 25–50	24 h–48 h	PS-COOH = No mortality; Gut retention PS-NH_2 = LC_{50} 2.75 ± 0.67 μg/mL; No retention/accumulation	Manfra et al. (2017)
	Brachionus koreanus	PS NP	50 nm	1.1–1 – 10	24 h	Maternal transfer in offspring; ↑ Malformed development ↑ Oxidative stress	Yeo et al. (2023)
Mollusca	*Crassostrea gigas*	PS-NH_2 PS-COOH	70 nm – 20 μm	2×10^{-7} 7×10^{-7} 2.30×10^{-6} 9×10^{-6} 3.6×10^{-4} 3.9×10^{-4} 1.25×10^{-3} 1.42×10^{-3} 3.42×10^{-3} 0.01 0.02 0.18 0.04 0.21 0.58 1.95 4.60	24 h–8 d	PS-NH_2 = retention/accumulation	Cole and Galloway (2015)
	Mitylus galloprovincialis	PS-NH_2	50 nm	0.001–20	48 h	↑ Malformed development (EC_{50} = 2.5 μg/mL)	Balbi et al. (2017)
	Crassostrea gigas	PS-NH_2 PS-COOH	100 nm	1–10 – 100	1 h–5 h	PS-COOH = ↑ Cell sizes ↑ ROS production (dose-response increase)	González-Fernández et al. (2018)
	Crassostrea gigas	PS-NH_2 PS-COOH	50 nm – 2 μm	1.1–10 – 25	36 h	↓ Fertilization success ↑ Malformed development PS-NH_2 (Gametes EC_{50} = 4.9 μg/mL Embryos EC_{50} = 0.15 μg/mL)	Tallec et al. (2018)
	Mytilus edulis	PS NP	100 nm – 2 μm	0.42–28.2 – 282	4 h–15 d	Retention/accumulation ↑ Malformed development	Rist et al. (2019)
	Meretrix	PS-NH_2 PS-COOH	100 – 200 nm	0.02–2	24 h – 7 d	↓ Hatching rates (PS-COOH = 5.79; PS-NH_2 = 39.5 %) ↓ Developmental rates (PS-COOH = 4.78; PS-NH_2 = 7.86 %)	Luan et al. (2019)
	Crassostrea gigas	PS-NH_2 PS-COOH	50 nm	1.1–25	1 h	↑Adhesion of particles to membranes PS-NH_2 ↓ Decrease spermatozoa motile and velocity at 10 μg/mL (−79 % and −62 %) ↓ Embryogenesis success (−59 %) PS-COOH ↓ Decrease spermatozoa motile and velocity at 25 μg/mL (−66 % and −38 %)	Tallec et al. (2021b)
	Crassostrea gigas	PS-NH_2	50 nm	0.1	24 h	↑ Cardiolipin content (+9.7 %) ↓ Larval growth (−24 %) No effect on adults	Tallec et al. (2021b)
	Sepia esculenta	PS NP	50 nm	50	4–24 h	↑ Immune-related genes	Liu et al. (2023)
	Crassostrea gigas	PS NP	0.55–3 – 6 μm	0.1–1 – 10–20	entire planktonic larval stages	↓ Larval growth and survival Pronounced effects with smaller particles and at lower concentrations	Seong et al. (2024)
	Tigriopus japonicus	PS NP	50 nm–500 nm - 6 μm	0.125–1.25 – 12.5–25	96 h	↓ Fecundity decreased ↑ Malformed development ↑ Mortality at concentrations grater then 12.5 μg/mL	Lee et al. (2013)
Arthropoda							
	Artemia franciscana	PS NP PS-NH_2 PS-COOH	40–50 nm	5–100	48 h	↑ Gut retention/accumulation ↑ Adhesion on surface ↓ Motility	Bergami et al. (2016)
	Artemia franciscana *Dunaliella tertiolecta*	PS-NH_2 PS-COOH	40–50 nm	0.5–50	72 h–14 d	↑ Adhesion on microalgae and uptake of PS-COOH PS-NH_2 ↑ Mortality (LC_{50} = 0.83 μg/mL) ↑ Inhibition of algal growth ↑ Increase molting and alteration of *clap* and *cstb* genes	Bergami et al. (2017)

(*continued on next page*)

Table 1 (*continued*)

Phylum	Test species	Particle type	Particle size	Concentration (µg/ml)	Exposure time	Effects	Reference
	Amphibalanus amphitrite *Artemia franciscana*	PS NP	100 nm	1.1–10	24–48 h	↑ Accumulation in *A.franciscana* ↑ Swimming activity ↓ Cholinesterase ↑ Oxidative stress	Gambardella et al. (2017)
	Amphibalanus amphitrite	PMMA	180 nm	5–10 – 25	24 h	↑ Uptake and accumulation	Bhargava et al. (2018)
	Artemia franciscana	PS-NH_2	50 nm	1.1–1 – 3–10	48 h	↓ Growth ↑ Malformed development ↑ Oxidative stress ↓ Cholinesterase	Varó et al. (2019)
	Euphausia superba	PS-NH_2 PS-COOH	60–50 nm	2.5	48 h	↑ Uptake ↑ Exuviae production (12.6 ± 1.3 %) ↓ Swimming	Bergami et al. (2020)
	Euphausia superba	PS-NH_2	50 nm	0.25–2.5	6 d	↑ Uptake and maternal transfer	Rowlands et al. (2023)
	Artemia salina	PS-NH_2	50–100 nm	10–20	48 h	↑ Gut retention ↑ Malformed development ↑ Apoptosis and oxidative stress	Contino et al. (2023)
Echinodermata	*Paracentrotus lividus*	PS-NH_2 PS-COOH	50 nm	1–2.5 – 3–5 – 10–25 – 50	6–24 – 48 h	PS-NH_2 ↑ Malformed development (EC$_{50}$ 3.85 µg/mL 24 hpf EC$_{50}$ 2.61 µg/mL 48 hpf) PS-COOH ↑ Accumulation ↑ Apoptosis	Della Torre et al. (2014)
	Paracentrotus lividus	PS-NH_2	50 nm	3–4	24 h–48 h	↑ Malformed development ↑ Gene alteration	Pinsino et al. (2017)
	Dunaliella tertioleta *Vibrio anguillarum* *Brachionus plicatis* *Paracentrotus lividus*	PS NP	100 nm	0.001–0.01 – 0.1–1 – 10	6 h–48 h	↑ Growth inhibition ↑ Mortality and swimming alteration ↑ Mobility alteration	Gambardella et al. (2018)
Chordata	*Ciona robusta*	PS-NH_2 PS-COOH	50–60 nm	2.5–5.25 – 7.5–10 – 15–100	22 h	PS-NH_2 ↑ Malformed development (EC$_{50}$ = 7.52 µg/mL) ↑ Impaired swimming (30 %) ↑ No development (15 µg/mL)	Eliso et al. (2020b)
	Ciona robusta	PS NP	20 nm	1.1–1	22 h	No effects	Ferrari et al. (2022)
	Ciona robusta	PS-NH_2	50 nm	10–15	22 h	↑ Malformed development ↑ Inhibition of hatching	Eliso et al. (2023)

a dose-dependent decrease in the growth of *A. franciscana* nauplii upon short term exposure (48 h) to PS NPs (PS-NH_2; 50 nm; 0.1–1–3–10 µg/mL), while long-term exposure (14 d) significantly impaired survival. Furthermore, both short- and long-term exposure increased oxidative stress and induced a decrease in cholinesterase (ChE) activity, indicating a potential neurotoxic effect. Recently, *A. salina* was used to investigate the effect of high concentrations of PS-NH_2 (50–100 nm; 10–20 µg/mL; 24–48 h) by Contino et al. (2023). Results reported several sub-lethal effects involving gut and body size malformations as well as the enhancement of apoptosis and oxidative stress with increased tested concentration and decreased NPs size.

Lee et al. (2013) investigated the acute and chronic effects of PS microbeads (0.05–0.5 µm and 6 µm) on the copepod *Tigriopus japonicus*, an omnivorous filter feeder widely exploited as an ecotoxicological model for marine coastal pollution monitoring. The study examined the copepods' survival, development, and fecundity, finding that, while the copepods ingested all sizes of PS NPs, without selective feeding, and survived in the acute test, chronic exposure to 50 nm and 500 nm beads reduced survival and fecundity across generations. Although 6-µm PS beads did not affect survival, they decreased fecundity at all tested concentrations. *A. amphitrite's* early life stages are also largely studied in the ecotoxicological field. Bhargava et al. (2018) investigated the effects of PMMA (Poly (methyl methacrylate) NPs (180 nm) at concentrations of 5, 10, and 25 µg/mL on these barnacles, using two microalgae species to assess feeding uptake. Acute exposure revealed that nanoplastics persist in the body through various growth stages, from nauplius to cyprid and juveniles (2–7 days). Chronic exposure showed bioaccumulation of nanoplastics even at low concentrations, indicating that NPs ingested during planktonic larval stages may persist into adulthood.

Finally, two studies explored the effects of PS NPs on the keystone species of Southern Ocean pelagic ecosystems, the Antarctic krill *Euphasia superba*. Firstly, Bergami et al., 2020 exposed krill juveniles to PS-COOH (50 nm) and PS-NH_2 (60 nm), and investigated lethal and sub-lethal endpoints after 48 h. No mortality in juveniles exposed to PS-NH_2 was observed, but there was an increase in exuviae production (12.6 ± 1.3 %) and a reduction in swimming activity. The fluorescent signal from fluorescently labeled PS-COOH NPs was localized, after 48 h, in krill fecal pellets, which resulted in an altered structure and sinking rate, indicating ingestion and egestion activity and potential impact on carbon pump due to sinking disruption Furthermore, Rowlands et al. (2023), investigated the intergenerational impact of exposing Antarctic krill females and their offspring to PS-NH_2 (50 nm) at varying concentrations, with and without the addition of a natural food (algae), by analyzing the lipid and fatty acid composition of embryos. Although nanoplastic exposure did not affect lipid metabolism, algae presence during maternal exposure improved levels of key fatty acids important for embryogenesis, underscoring the complex nanoplastic-fatty acid relationship and suggesting future research on long-term exposure and additional stressors.

Overall, these studies highlight the significant sub-lethal effects induced by nanoplastics on the early stage of development of marine crustaceans. From impaired growth and increased oxidative stress to neurotoxic impacts and alterations in feeding behavior, nanoplastics can disrupt vital physiological processes. Nanoplastics can also affect growth, survival, and reproductive success in a dose-dependent manner (Varó et al., 2019; Contino et al., 2023). Furthermore, chronic exposure can lead to bioaccumulation, persistent physiological alterations, and even transgenerational impacts, posing significant threats to population sustainability and ecosystem health (Rowlands et al., 2023).

2.4. Echinodermata

Another prominent group, the echinoderms, is commonly found in coral reefs and marine coastal ecosystems. Echinoderms, particularly sea urchins, are key species that play critical roles in stabilizing reef ecosystems. They control coral reef algae populations and provide shelter to various reef organisms, such as small fish, serving as both decomposers and primary consumers. Sea urchin embryos and gametes have been used in toxicity testing for plastic pollution, especially microplastics, and various complex mixtures. However, studies on the effects of nanoplastics remain limited (Gambardella et al., 2018; Zaki and Aris, 2022).

One of the first studies on nanoplastics embryotoxicity on sea urchins, by Della Torre et al. (2014), investigated Mediterranean sea urchin *P. lividus* embryos and larvae exposed to PS-COOH (40 nm; 2.5–5–10 - 25–50 μg/mL) and PS-NH_2 (50 nm; 1–2.5–3–5 - 10 and 50 μg/mL) for 6, 24 and 48 h. PS-COOH showed no embryotoxic effects at concentrations up to 50 μg/mL, whereas PS-NH_2 caused severe developmental defects, leading to an incorrect location of skeletal rods at the lowest concentration. The EC_{50} for PS-NH_2 was 3.85 μg/mL at 24 h post fertilization (hpf) and 2.61 μg/mL at 48 hpf. PS-COOH tended to accumulate inside the embryos' digestive tracts, whereas PS-NH_2 showed a more dispersed distribution. Gene expression analysis revealed that PS-COOH up-regulated the Abcb1 gene at 48 hpf, potentially related to detoxification mechanisms, while PS-NH_2 induced the cas8 gene at 24 hpf, indicating activation of apoptotic pathways. Pinsino et al. (2017) also showed that exposure of *P. lividus* embryos to 3 and 4 μg/mL of PS-NH_2 (50 nm) for the same time induced skeletal rod and arm malformations at 4 μg/mL. Another study investigated the lethal and sub-lethal response to a wide range of microplastic (MP) concentrations (100 nm; from 0.001 to 10 mg/L) (Gambardella et al., 2018) in *P. lividus* with changes in sea urchin larvae development. The results on echinoderms once again confirm the significant role of surface charges in driving the toxicity of PS NPs. Furthermore, these studies highlight the importance of investigating the sublethal responses and using a battery of marine organisms from the perspective of exploring the transfer of NPs through the food web.

2.5. Invertebrate chordates

Solitary ascidians, including species such as *Ciona intestinalis* and *Ciona robusta*, are regarded as optimal model organisms for ecotoxicological analyses. Their indirect development allows the effects of environmental stressors to be studied on three key ontogenetic stages: the non-feeding embryo-larvae, which possess a "simplified chordate ancestor" anatomy, the post-metamorphic, filter-feeding juvenile stages and the adult stage, both of which are directly in contact with the external environment. Thanks to several advantages, such as the phylogenetic position, a fully sequenced genome and available genomic tools (Dehal et al., 2002; Stolfi and Christiaen, 2012) ascidians have been used to evaluate the impact of pollutants, such as tributyltin, bisphenol A, drugs and oil dispersants, on survival and morphology (Cangialosi et al., 2013; Mizotani et al., 2015, Eliso et al., 2020a).

More recently, Eliso et al. (2020b) conducted the first research on the impact of 50 nm PS-NH_2 and 60 nm PS-COOH NPs on the development of C. robusta larvae. PS-COOH had no observable effect on larval phenotypes or development, whereas PS-NH_2 induced dose-dependent effects, with an EC_{50} of 7.52 μg/mL at 22 h, resulting in various malformations and impaired swimming. Exposure to 15 μg/mL of PS-NH_2 resulted in altered development in more than 70 % of larvae, most of which were unable to hatch. The *in vivo* phenotypic data were then coupled with transcriptome analyses (Eliso et al., 2023) for an integrative approach aimed at developing an Adverse Outcome Pathway (AOP) following acute exposure to amino-modified PS-NH_2 NPs (50 nm) during *C. robusta* embryogenesis. Transcriptomic data revealed significant impacts on genes associated with glutathione metabolism, the immune and nervous systems and aquaporin-mediated transport. This study highlighted as key initiating events the adhesion of PS-NH_2 to the chorion that affected transcription of specific genes and induced altered larval development, reduced metamorphosis and inhibition of hatching through oxidative stress.

On the other hand, according to our previous studies, short-term exposure to non-functionalized PS NPs did not result in significant embryotoxic effects in *C. robusta* larvae, and when co-exposed with the plastic additive Bisphenol A (BPA), no synergistic or additive toxicity was observed (Ferrari et al., 2022). While BPA alone induces specific developmental alterations, particularly affecting the pigmentation of larval sensory organs, the presence of PS NPs did not exacerbate these effects. The lack of interaction is likely attributable to the high ionic strength of seawater, which probably reduced the potential for PS NPs to adsorb and carry BPA, thus limiting their combined bioavailability and toxicity.

3. Current gaps and future research perspectives

As this review shows, there is still a limited number of studies addressing the impact of polystyrene nanoplastics on embryos and larvae of marine invertebrates. To date, fish embryos have been the most widely used experimental model in the field of ecotoxicology, with particular emphasis on zebrafish, which significantly contributed to our understanding of environmental contaminants' effects on vertebrate development and physiology, due to their similarities with other vertebrates, including humans (Elizalde-Velázquez and Herrera-Vázquez, 2023). However, in compliance with the current EU regulation on animal testing (Directive, 2010/63/EU) (European Parliament, 2010), which mandates the reduction, refinement, and replacement of animal use in scientific research, there is a growing emphasis on finding suitable alternative models. In this respect, marine invertebrates are excellent experimental models, especially ascidians, as they are an abundant component of marine mesozooplankton communities and represent an early and simplified model of chordate development at the larval stage. Moreover, as invertebrates, ascidians are not subject to the same regulatory restrictions as vertebrate models under Directive 2010/63/EU, allowing for more flexible and ethically responsible research.

Most of the reviewed studies rely on a single type of nanoplastic proxy, PS NPs, often functionalized and spherical. Among them, PS-NH_2, particularly those ≤50 nm, were consistently associated with the most severe toxic effects across taxa, likely due to increasing cellular uptake, higher surface area and reactivity. For instance, PS-NH_2 particles of 50 nm induced significant malformations in *P. lividus* (EC_{50} = 2.61 μg/mL at 48 hpf) and *C. robusta* (EC_{50} = 7.52 μg/mL), while larger particles (>500 nm) were typically associated with milder effects such as gut retention or accumulation. Exposure levels in studies selected here are often higher than those found in the environment but sometimes fall within environmental ranges (Fig. 1B). Despite the variability of concentrations tested, several studies reported significant toxic effects at concentrations lower than 5 μg/mL, especially with PS-NH_2 particles and in filter feeder's species and planktonic larvae. However, environmentally relevant concentrations for nanoplastics have yet to be quantified (Kögel et al., 2020), making it challenging to establish appropriate exposure ranges for different species. Indeed, it is crucial to encourage the test exposure concentrations that resemble those predicted for marine waters. Although high concentrations as those used in many studies are valuable for understanding toxicological outcomes and the mode of action, they are typically at least two orders of magnitude higher than those predicted for surface waters (i.e., >1 mg/L vs. 0.001–20 μg/L) (Lenz et al., 2016; Al-Sid-Cheikh et al., 2020).

Until recently, the lack of reliable field data has represented a major limitation in assessing the ecological relevance of nanoplastic exposure. A recent study by ten Hietbrink et al. (2025), quantified nanoplastics concentrations across the north Atlantic Ocean using thermal-desorption proton-transfer-reaction mass spectrometry

(TD-PTR-MS), reporting approximately values between 1.5 and 32 mg m^{-3} for PS, PET and PVC in the water column. In addition, a complementary advance has been made by Moon et al. (2024), who achieved the first direct visualisation of nanoplastics in ocean water using a novel shrinking-surface-bubble deposition (SSBD) technique. This study provides critical morphological and compositional evidence that environmental nanoplastics differ in shape and type from the idealised laboratory particles often employed in toxicity tests. Taken together, these data represent one of the first large-scale estimates of nanoplastics in the marine environment and provide an important reference for defining environmentally realistic exposure levels. Despite recent advances, the quantification of nanoplastics in marine environments remains technically challenging due to nanoscale dimensions, low concentrations and the lack of standardized analytical protocols. Available approaches, ranging from fractioning and particle characterization to spectroscopic and thermal degradation techniques, still face limitations in detecting nano-scale particles. Emerging indirect and *in situ* methods, like the ones cited above, together with the development of reference materials, are expected to enhance detection accuracy and enable more environmentally realistic exposure assessments.

The current body of research is constrained by its focus on certain developmental stages, typically preferring adult stages, which are easier to study and preferably freshwater model organisms such as zebrafish, but also *Daphnia* spp. This approach overlooks critical developmental periods when embryos and larvae might be more susceptible to nanoplastics exposure (Jimenez-Guri et al., 2024). Indeed, critical toxic responses were consistently observed within 24–48 h of exposure, during the early embryogenesis and larval stage. These timeframes correspond to high developmental sensitivity, including organogenesis and morphogenesis, where external perturbations can permanently impair growth and functionality. Studies on *C. gigas*, *M. meretrix* and *C. robusta* confirmed that short exposures to PS-NH_2 significantly reduced fertilization success, hatching and larval growth. While *E. superba* showed reduced swimming activity and evidence of maternal transfer after 6 days of exposure to PS-NH_2, highlighting that long-term effects could lead to behavioral alterations and more severe consequences. Ecotoxicological testing typically focuses on model organisms used in standard protocols, creating a significant knowledge gap regarding the effects of plastic particles on other species essential for ecosystem balance. Species at the highest risk of exposure due to their feeding strategies and water column positions, such as planktonic species not included in ISO and OECD guidelines, should be prioritized for ecotoxicity testing. The ecology of species and the time they spend in various environmental compartments are crucial factors in selecting suitable bioindicators. Additionally, since the marine environments are considered ultimate sinks for plastic particles and other contaminants, there is a need for increased testing with early life stages of suspension and deposit feeders (Alimi et al., 2022; Corsi et al., 2023). Moreover, experimental conditions often lack environmental realism. Laboratory tests commonly use spherical PS NPs that poorly represent the irregular, weathered particles found in a natural environment (Gigault et al., 2018). Additives like surfactants and fluorescent tracers may further influence particle behavior and toxicity (Pikuda et al., 2019; Catarino et al., 2019). While surface charge has been widely investigated, non-functionalized nanoplastics and those present in natural environments are more likely to carry oxidized, negatively charged functional groups, which are underrepresented in current testing strategies (Lehner et al., 2019; Corsi et al., 2023).

In conclusion, the available evidence clearly shows that early life stages of marine invertebrates are highly sensitive to nanoplastic exposure, particularly to dimensions below 50 nm and to positively charged particles. The most pronounced effects include malformations, developmental arrest, impaired motility and behavioral disruptions, often occurring within 48 h of exposure and with relatively low concentrations. Species such as *P. lividus*, *C. robusta* and *M. galloprovincialis* emerged as particularly vulnerable and should be prioritized in future studies. Moving forward, ecotoxicological research should expand beyond current model systems by incorporating a broader diversity of polymers and particle shapes, realistic exposure concentrations and underrepresented yet ecologically critical species. These steps are essential to improve ecological risk assessment and to better understand the consequences of nanoplastic pollution on marine biodiversity and ecosystem stability.

CRediT authorship contribution statement

E. Ferrari: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **A. Spagnuolo:** Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **I. Corsi:** Writing – review & editing, Writing – original draft, Investigation, Conceptualization.

Ethics approval consent to participate

No approval of research ethics committees was required to accomplish the goals of this study.

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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.

Data availability

No data was used for the research described in the article.

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