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Nano-Scale Plastic Pollution in the Marine Species: A Review

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ABSTRACT

The long-term properties of plastic have been causing persistent marine pollution for decades. The adverse impacts have been found in marine organisms worldwide. Currently, their degraded products-microplastics and nanoplastics-represent emerging plastic issues. Microplastic pollution has drawn attentions in many research fields and the general public. Many types of literature have documented their adverse impacts, distribution, and origins. Hence, many review studies have been conducted on microplastics rather than nanoplastics. Therefore, this review is focused on nanoplastic contamination in marine ecosystems, their origins, distributions, fate, and impacts on marine organisms. This review paper provides an overall picture of nanoplastic pollution on a global scale. The impacts of nanoplastic on marine organisms' gene expression at the cellular and tissue levels are evaluated. Moreover, the adverse effects of nanoplastics on the embryonic stages, growth, and mortality of marine species are also discussed. The present review also gathers information to generate future research perspectives, and aims to highlight the need for researching on nanoplastics in the aquatic environment while providing critical perspectives for setting future research objectives.

1. Introduction

Plastic pollution in the marine environment is a growing concern among current pollution issues [1]. In the 21st century, plastic waste management represents a challenge to the scientific community, as the exponential consumption of plastics since the 1950s has led to the significant release of plastic waste into the marine environment [2-4]. In 2010, there was approximately 13,200 to 34,800 tons of plastic litter released to into the marine environment, and this figure is expected to increase by an order by 2025 [5]. Legislation related to plastic worldwide has been implemented and showed positive outcomes [6]; however, plastic pollution has not been entirely solved by the implemented legislation. Although the plastic degradation could occur in the marine environment, the breakdown of larger debris into smaller plastic particles remains harmful to the environment [7]. The smaller plastic debris particles are generally classified as either microplastic (MP) or nanoplastic (NP) (Table 1).

Table 1 Size class definition of aquatic nanoplastic from current research studies

Prefix	Size Class	Size Range	Reference
Nano	Nanoplastic	<20 µm	[8]
		<0.1 µm	[9]
Micro	Microlitter	~0.06 – 0.5 mm	[10]
	Microplastic	<0.5 mm	[11-15]
	Microplastic	0.33 – 5.0 mm	[16]
	Micro debris	<2 mm	[17]
	Small microplastic	<1 mm	[18,19]
		0.2 – 1 mm	[19]
		>0.3 mm (<1 mm)	[20]
	Large microplastic	1-5 mm	[19, 20]

NP is defined as plastic of a particle size smaller than 20 µm, based on [8]. This review paper considers particles between 0.1 and 2.0 µm in size as the NP particle size-class in the marine environment, and evaluates NP studies with corresponding impacts. The origins, distribution, and concentrations of NPs in the marine environment were evaluated based on current research. Their adverse impacts to marine organisms are evaluated at the molecular, cellular, and physiological level, while also evaluating impacts on growth and reproductive output. Based on current survey studies and ecotoxicity, this paper provides improvement strategies and future perspectives for NP research in order to increase the coverage of the NP research field.

2. Distribution and Origins of Nanoplastic

2.1 Distribution of Plastic Pollution

The marine environment has been affected by plastic pollution for decades. Many surveys have been conducted on the concentration of plastic particles in the U.S.A., Belgium, France, Australia, and Russia (Table 2). The observed distribution suggests that plastic pollution affects marine ecosystems on a global scale. The highest plastic particle concentration was observed in the North Pacific Central Gyre (334,271 plastic fragments/km^2) (Table 2). Plastic particles distributed in the marine environment are strongly related to the climate system in this particular area, which has resulted in a high concentration of particles [21]. The survey conducted in the North Pacific Ocean indicated that climate conditions were the drivers for the results. The dominant clockwise gyral currents act as a retention mechanism that prevents plastic particles from moving toward coasts [21]. In addition, surface current modelling simulated that plastic particles in the North Pacific Ocean could remain in the area for at least 12 years [21]. Moreover, other climatic factors could include the natural eddy system concentrating plastic particles in the area [21]. While the accumulation of plastic materials in the marine environment is increasing over time, they will be degrading slowly into MP and NP in the North Pacific Ocean [1]. Hence, as new plastics were added, MP and NP formation could not exit the oceanic system once introduced. The hydrodynamic effects distributed this plastic debris around the Australian region. An Australian coastline survey on plastic pollution indicated that plastics travelled with a range of currents, including the Antarctic Circumpolar current [22]. Moreover, the South Equatorial current in the Pacific Ocean brought the plastics to Australia, Fiji, and New Caledonia [22, 23]. The East Australian current carried plastic debris from the Australian populated area (Brisbane and Sydney) to the east coast of Australia and the Tasman Sea [22, 24]. Furthermore, the Holloway, Leeuwin, South Australian, and Zeehan coastal current systems brought plastic from international areas to the North West Shelf [25-28]. In addition, the West Australia current brought plastics from the Indian Gyre

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to the North West Shelf and Perth area [28]. These oceanic hydrodynamics indicated that climate conditions could be associated with marine plastic pollution.

Table 2 Microplastic distributed in marine environment in worldwide

Country	Location	Concentration	Ref.
USA	North Pacific Central Gyre	334,271 plastic fragments/km^2	[21]
USA	North Atlantic Subtropical Gyre	20,328±2,324 (0~>20,000) pieces/km^2	[29]
USA	North Pacific Subtropical Gyre	0.092-8.649 particles/m^3	[30]
	Alaska	0.000-0.140 particles/m^3	
	California Current	0.000-0.228 particles/m^3	
	Eastern Tropical Pacific	0.000-0.034 particles/m^3	
	Total	0.000-4.696 particles/m^3	
Belgium and France	Northwestern Mediterranean Basin	0.116(0-0.892) particles/m^2	[31]
Australia	Australian vessels	4256.4±757.79 pieces/km^2	[32]
Russia	Kuril–Kamchatka Trencharea	60-2020 particles/m^2	[33]

2.2. Origins of Nanoplastics

Nanoplastics can be classified into two major categories: primary and secondary nanoplastic. The primary sources of NP are defined as primarily produced in the nano-scale size. NP products include plastics from medical applications and cosmetic products. The medical applications of NP are primarily used in drug delivery with biodegradable solid lipid properties [34]. Cosmetic products include plastic microbeads in skin cleansers for exfoliating scrubs [35]. The smallest size of nanoplastic cleansing beads was found to be 4 μm [35].

Secondary NPs are classified as products of the degradation of larger plastics or microplastics [1]. There are three major mechanisms forming secondary NP, which can be divided into physical degradation, phytodegradation, and biological breakdown. This degradation is caused by weak bonding between polymer chains. For example, expanded polystyrene (EP) beads were fragmented in a mechanical degradation experiment that accelerated mechanical abrasion using glass beads and sand to produce smaller plastics from larger plastic sources (EP beads) under experimental conditions [36]. While the conditions were not involved with other factors, the results indicated that natural secondary NP production could occur in beach and river systems. In fact, the realistic situations of sunlight UV exposure, temperature, and humidity could accelerate secondary NP production time. Photodegradation is another pathway for the breakdown of larger plastics into smaller plastics [37]. Furthermore, biological mechanisms can also break down larger plastics through the action of microorganisms and other marine organisms. For example, Harshvardhan et al. [38] determined that the marine microorganisms *Kocuria palustris*, *Bacillus pumilus*, and *Bacillus subtilis* were able to degrade plastics such as polyethylene. Overall PE mass loss was 1.75% higher following 30 days of exposure to microorganisms [38]. Recently, the biological breakdown of microplastic into nanoplastic was observed in a keystone species in the Antarctic ecosystem when Antarctic krill (*Euphausia superba*) was exposed to polypropylene (PE) microbeads (27-32 μm) alongside algal food resources [39]. The ingested PE was fragmented by the Antarctic krill into particles less than 1 μm (diameter) in size [40].

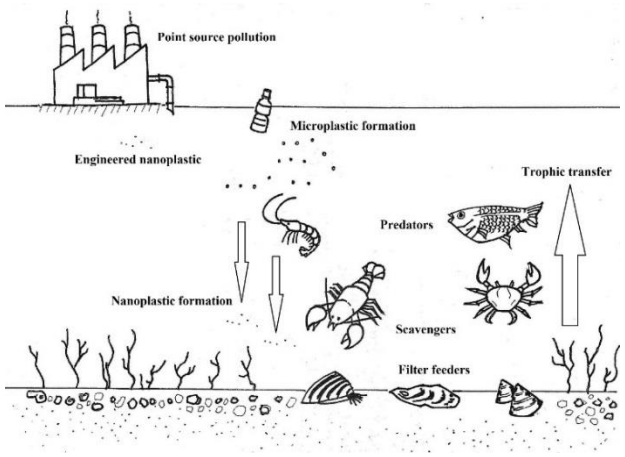

Fig. 1 Nanoplastic formation and its environmental fate in the marine ecosystem

3. Impacts to Organisms

The existence of NPs in the marine environment could lead to damage in organisms (Fig. 1 and Table 3). Numerous research studies have found that marine organisms were capable of NP uptake, which can cause molecular, cell, tissue, and embryo level damages. Moreover, the growth, biochemistry, and behaviour of marine species can also be negatively impacted. Herein, this section evaluates previous studies on such adverse effects on marine species.

3.1 Ingestion of Nanoplastics and Trophic Transfer

In one study, high-density polyethylene (HDPE) NPs were taken up by *Mytilus edulis L.* (blue mussel) cells and tissues, which caused significant adverse effects [41]. The HDPE particles were drawn into the stomach and transported into the digestive gland, and then eventually accumulated in the lysosomal system [41]. Histological changes, a strong inflammatory response (granulocytoma formation), and lysosomal membrane destabilisation were observed within blue mussels [41]. Another exposure experiment demonstrated that blue mussels ingested and accumulated plastic particles (3.0 or 9.6 μm) within their guts [42]. Hence, following ingestion, the plastic particles caused damage, as NP was translocated to the circulatory system from the digestive system in *Mytilus edulis* [42]. This translocation occurred within 3 days and persisted for over six weeks [42]. These studies suggest that NPs could affect entire physiological systems within marine organisms through translocation. For example, blue mussels exposed to both NP (30 nm) and their typical food resource (*Pavloca lutheri*) altered their filtering activity [43]. The exposure assessment was conducted with present of algae and NP indicated that filtering activity decreased, though NP concentration in water remained low [43]. This indicates that NP was ingested by the blue mussels under decreased filtering activity conditions. Moreover, the bivalves produced pseudofaeces while exposed to both NP and *Pavloca lutheri* (algae) (contaminated by NP) [43]. The energy consumed in the production of pseudofaeces and reduced filtering activity indicated that the ingestion of NP led to starvation [43]. In another study, the aggregation of polystyrene (PS) beads enhanced the uptake efficiency of studied marine species (*Mytilus edulis* and *Crassostrea virginica*) [44], as the NPs travelled into the digestive gland tubules and was taken up by digestive cells via endocytosis [44]. This suggests that bioaccumulation occurred within the organisms and potentially transferred to higher trophic levels of marine organisms. The retention of NP within blue mussels does not only impact the marine species that feed on them, but human consumption could also represent another serious issue. Furthermore, a trophic transfer study by [45] indicated that polystyrene (PS) spheres were transferred by blue mussel to *Carcinus maenas* (L.) (littoral crab) through predation. This translocation was observed within the crab through the discovery of PS spheres within the haemolymph and tissues [45]. Furthermore, the polypropylene (PE) plastic particles (smallest size: ~10μm) were found inside scleractinian corals (*Dipsastrea pallida*, classified as *Favia pallida*) [40]. Scleractinian corals took up plastic particles and accumulated them in mesenterial tissue within the gut cavity [40]. This implies that high concentrations of PE particles could induce health impairments in corals [40]. Moreover, the marine copepod *Calanus helgolandicus* ingested PS beads (20 μm), resulting in an eventual 40% decrease in carbon biomass [46]. Moreover, prolonged NP exposure caused a significant reduction in hatching success, resulting in the production of smaller eggs, as well as reduced reproductive output [46]. Furthermore, *Littorina littorea* (marine snails) could not distinguish between plastic particles, clean algae (*Fucus vesiculosus*, seaweed), and contaminated algae [47]. The plastics were found in the stomach and gut of snails following feeding with NP-contaminated algae.

Polystyrene nanoparticles (PS-NP) significantly increased *Amphora sp.* exopolymeric substances (EPS) assembly and eventually formed PS-EPS microscopic gel (approximately 4-6 μm) [48]. EPS is a polysaccharide-rich anionic colloid polymer released by microorganisms [48] and is the most important source of marine dissolved organic carbon and particulate organic carbon in the marine environment [49-52]. This disturbance could further affect the carbon cycle in the marine ecosystem [48]. However, *Amphibalanus amphitrite* (barnacles) ingested poly methyl methacrylate (PMMA) NP during planktonic larval stages, and the NP persisted into their adult stages [53]. This persistence of NP within the barnacles implies long-term impacts of NP within sessile invertebrate communities. Bioaccumulation even occurred at low PMMA NP concentrations, though some (but not all) PMMA particles were egested through molting and faecal excrement during acute exposure to PMMA NP [53]. Moreover, ingestion frequency and the magnitude of NP were determined by larval age, NP size, and NP surface properties [54]. For instance, the aminated surface of PS-NP was ingested and retained more frequently than the

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carboxylated surface of PS-NP [54]. This provided information regarding which surface property of PS-NP is more readily ingested by marine organisms. While PS-NP coated with aminated groups (PS-NH_2) was ingested by *Artemia franciscana* (brine shrimp), they absorbed it at the surface of sensorial antennules and appendages [55]. Moreover, the accumulation of PS-NP within brine shrimp indicated the potential for trophic transfer. However, brine shrimp larvae took up PS-NP particles coated with carboxylated groups (PS-COOH) and sequestrated them inside the gut lumen [55].

The brine shrimp excreted part of the PS-NP (but not all), which eventually caused starvation. Moreover, the plastic beads could act as a vector transfer polybrominated diphenyl ether (PBDEs) congener (e.g., BDE-28, -47, -99, -100, -153, -154, and -183) for assimilation into *Allorchestes compressa* (amphipod) [56]. These plastic beads were associated with a greater proportional uptake of higher-brominated congeners (BDE-154 and -153 compared to BDE-28 and -47) [56]. This implies that persistent organic pollutants (POPs) in the marine environment could transfer to organisms through the ingestion of NPs.

3.2 Impacts on the Gene Expression, Cell, and Tissue Level Biochemistry of Organisms

The ingestion of NP would affect gene expressions in several marine organisms. In one study, PS-NP and carbamazepine (Cbz) co-exposure induced downregulation in gene expression (e.g., *hsp*70) in *Mytilus galloprovincialis*, and lipid peroxidation indicated that a PS-NP concentration of 0.05 mg/L caused oxidative damage [57]. Moreover, total oxidant status showed that the mussels' digestive glands were affected by PS-NP concentration at 0.5 mg/L [57]. Furthermore, total antioxidant capacity and esterase activity in digestive glands and gills were induced by PS-NP at a concentration of 50 mg/L [57]. PS-NP also induced the inhibition of cholinesterase activity in haemolymph [57]. Furthermore, the gene expression of *Artemia franciscana* (brine shrimp) that ingested PS-NH_2 particles was also affected [58]. Additionally, the *clap* and *cstb* genes were significantly affected by physiological alterations (increased molting) in brine shrimp [58].

In another study, *Mytilus galloprovincialis* exhibited cellular damage in hemolymph serum following PS-NP ingestion [59]. These adverse effects were mediated by the abnormal regulation of p38 Mitogen Activated Protein Kinase (MAPK) signalling. MAPK is an important signal transduction pathway linking extracellular signals to intracellular processes, and is closely related to apoptosis and inflammation [60]. Notably, PS-NP coated with aminated groups could form eco-corona in *Mytilus galloprovincialis* hemolymph. The PS-NH_2 eco-corona was discovered in hemolymph serum, which was the initial evidence for the formation of eco-corona in marine organisms, which potentially impacts their health status. Furthermore, the high concentration of PS-NH_2 particles caused cytotoxicity in *Mytilus galloprovincialis*, while decreased phagocytic activity and increased lysozyme activity were dose dependent [61]. Additionally, PS-NH_2 particles increased extracellular Reactive oxygen species (ROS) production, nitric oxide (NO) production, and induced apoptosis in *Mytilus galloprovincialis* [61].

Therefore, co-exposure of NP and other contaminants could pose adverse effects on marine organisms. *Mytilus* spp. (mussel) exhibited down-regulation of a P-glycoprotein, causing impairment of filtration activity and the presence of PS particles in the gut compartment following exposure to PS particles and fluoranthene co-exposure [62]. Moreover, co-exposure to pollutants resulted in histopathological damage and higher anti-oxidant levels in mussels [62]. *Mytilus* spp. exposed to PS-NP alone induced hemocyte mortality, increased ROS production in hemocytes, and anti-oxidant and glutathione-related enzymes in tissues [62]. Furthermore, a significant decrease (<31%) in acetylcholinesterase (AChE) activity was observed in *Pomatoschistus microps* (common goby) following exposure to polyethylene (PE) spheres and the polycyclic aromatic hydrocarbon (PAH) pyrene. In addition, it also caused an increase of bile pyrene metabolites [63]. When PE spheres and pyrene were combined, the PE spheres delayed pyrene-induced common goby mortality, and the combined contaminants decreased the common goby's isocitrate dehydrogenase (IDH) activity [63]. The PE spheres also induced significant inhibition of AChE activity in common goby [63].

A study by Canesi et al. [61] observed a dose-dependent decrease in phagocytic activity and increased lysosome activity. However, the different surface properties of PS-NP (PS-COOH and PS-NH_2 NP) demonstrated varying adhesive ability for *Crassostrea gigas* (oyster) gametes, which caused various effects [64].

PS-COOH at a concentration of 100 mg/L increased ROS production (121% higher than normal) in oyster sperm cells, though no observable effects were caused by PS-NH_2 [64]. The increase of relative spermatozoa and oocyte cell size and complexity could increase NP adhesion effects [64].

Brachionus koreanus (monogonont rotifer) serves a role in transferring energy in the aquatic food chain, and also has the ability to carry contaminants to higher trophic levels via ingestion and accumulation [65-67]. Moreover, they experience adverse physiological responses following exposing to NP particles. The MAPKs and c-Jun N-terminal kinase (JNK) were phosphorylated in response to 0.05μm PS beads [66]. However, the negative correlation of ROS levels and NP size was observed [66]. ROS plays a major role in the activation of the MAPK pathway, and PS-NP-induced ROS is the major toxicity factor in response to NP exposure among rotifers [66].

3.3 Growth Inhibition, Mortality, and Behaviour Changes

Dunaliella tertiolecta (green microalgae) and *Artemia franciscana* (brine shrimp) ingested PS-NH_2 and caused growth inhibition (EC_{50}=12.97 μg/mL) in green microalgae as well as mortality (LC_{50}=0.83 μg/mL) in brine shrimp [58]. The exposure of *Brachionus koreanus* (monogonont rotifer) to PS beads resulted in a reduced growth rate, reduced fecundity, decreased lifespan, and longer reproduction period [66]. Furthermore, such reduced fecundity and lifespan could lead to decreasing rotifer populations.

Fecundity is associated with population growth, which can be negatively affected by the number of offspring being reduced due to ingestion of PS beads [66]. Moreover, a longer reproduction period was caused by a lower rotifer growth rate following PS bead ingestion [66]. In another study, uncharged PS particles (0.05 μm) reduced *Dunaliella tertiolecta* cell density by up to 45% at a concentration of 250 mg/L [68]. The *D. tertiolecta* growth rate was inhibited by 57% following exposure to PS particles [68]. In addition, decreasing NP particle sizes led to increased inhibition of microalgal growth [68].

NP exposure can also lead to mortality in marine organisms. PS particles increased hemocyte mortality in *Mytilus* spp. caused by a decrease in circulating granulocytes and total hemocyte concentration, which indicates the recruitment of active hemocytes for incursion in PS-exposed mussels [62]. This could modify the balance of live circulating hemocytes in the hemolymph [62].

Tigriopus japonicus (copepod) exposed to a PS bead concentration of 12.5 μg/mL caused mortality in nauplii and copepodites in the F_0 generation (69), while a PS bead concentration reaching 1.25 μg/mL caused F_1 generation mortality [69]. Also, PS-NH_2 exposure caused mortality (LC_{50}=0.83 μg/mL) in *Artemia franciscana* (brine shrimp) [55,58]. The surface properties of NP could be a key factor responsible for the behavioural and ecological interactions of marine species [58]. Notably, behavioural changes were observed following NP ingestion in *Pomatoschistus microps* (common goby). The predatory performance of common gobies exposed to chromium (VI) (Cr (VI)) and PE spheres decreased by nearly 67% [70]. In the long-term, it could reduce individual performance and eventually cause death with negative effects on the population fitness.

3.4 Embryo Impacts

The Tallec et al. [71] performed a PS-NP dose-response experiment on *Crassostrea gigas* (Pacific oyster) gametes. PS-NP decreased fertilisation success and embryo-larval development with various malformations and some cases of total developmental arrest [71]. The study determined that 50nm PS-NP exhibited the strongest toxicity to both gametes (EC_{50}=4.9 μg/mL) and embryos (EC_{50}=0.15 μg/mL). Furthermore, *Paracentrotus lividus* (sea urchin) embryos demonstrated a different response to various surface coatings of PS-NP (PS-COOH and PS-NH_2) [72].

While several development were defects brought on by PS-NH_2 at EC_{50} concentrations of 3.85 μg/mL (24 hpf) and 2.61 μg/mL (48 hpf), there was upregulation in the *Abcb1* gene at 48 hpf by PS-COOH, and the *cas8* gene was induced by PS-NH_2 at 24 hpf [72]. The two types of PS-NP were distributed differently within sea urchin embryos, where PS-COOH was distributed in the digestive tract and PS-NH_2 was dispersed [72]. Overall, PS-NH_2 NP impacted the development of the normal D-shaped larvae of *Mytilus galloprovincialis* at 48 hpf, and also affected shell formation (EC_{50}=0.142 mg/L) [73]. The high embryotoxicity/ developmental arrest in *Mytilus galloprovincialis* was observed at a PS-NH_2 NP concentration of 5-20 mg/L [73]. Moreover, PS-NH_2 NP induced dysregulation of gene transcription at the 24 and 48 hpf stage of shell formation (Chitin synthase, Carbonic anhydrase, extrapallial protein) in *Mytilus galloprovincialis* [73]. During the 48hps mussel embryo stage, mRNA levels of the ABC transporter p-glycoprotein-ABCB and lysozyme were decreased [73].

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Table 3 The adverse effects on aquatic organisms

Species	NP Type and Condition	NP Size	Exposure Concentration	Effect	Reference
Paracentrotus lividus (urchin embryos)	Carboxylated polystyrene	40 nm	50 µg/mL	No embryotoxicity. Accumulated inside embryo's digestive tract	[72]
			25 µg/mL	*Abcb1* gene resulted up-regulated at 48 hpf (apoptotic pathway)	
	Amine polystyrene	50 nm	EC_{50}: 3.85 µg/mL	24 hpf affected	[72]
			EC_{50} 2.61 µg/mL	48 hpf affected	
			3 µg/mL	*cas8* gene at 24 hpf (apoptotic pathway)	
Allorchestes compressa (amphipods)	Polyethylene sorbed with PBDEs	11–700 µm	20% of Nivea exfoliating face scrub soap	Greater proportional of brominated congeners such as BDE-154 and -153 compared to BDE-28 and -47 assimilated to tissues.	[56]
Crassostrea gigas (pacific oyster gametes)	Polystyrene (coated with carboxylic or amine group)	100 nm	10 mg/L	Higher relative cellular complexity was observed while exposed to PS-COOH and PS-NH_2.	[64]
			100 mg/L	Suggested a differential effect according to NP-associated functional group. Significant increase of Reactive Oxygen Species production were only observed in sperm cell while exposed to PS-COOH but not PS-NH_2.. Decreased in number of single spermatozoa while exposed to PS-COOH due to spermatozoa' aggregates for the NP condition. Spermatozoa exposed PS-COOH and PS-NH_2 showed 4-5% increase in its size. Higher cellular relative complexity was observed.	
Dunaliella tertiolecta, *Thalassiosira pseudonana* and *Chlorella vulgaris* (microalgae)	Polystyrene (negatively charged and uncharged)	0.05, 0.5 and 6 µm	250 mg/L	Microalgal growth was suppressed at 45% by uncharged NP. The relationship between microalgal growth and NP size was negatively related.	[68]
Pomatoschistus microps (goby)	Polyethylene (with Potassium dichromate (Cr(VI))	1-5 µm	Cr(VI)+NP: 5.6 mg/L+0.184 mg/L; 8.4 mg/L+ 0.184 mg/L; 12.6 mg/L+0.184 mg/L ;18.9 mg/L+0.184 mg/L; 28.4 mg/L+0.184 mg/L	Significant decrease of the predatory performance at ≤67% and significant inhibition of AChE activity at ≤31%. Significant increase of lipid peroxidantion levels.	[70]
Vibrio fischer (bacteria)	Polyethyleneimine polystyrene-silica (PS-PEI)	50 nm and 100 nm	3-1000 µg/mL	EC_{50}: >1000 µg/mL	[74]
Carcinus maenas (shore crab)	Polystyrene (coated with carboxylic group or amine group)	8 µm	10^6 and 10^8 spheres/L	Significant but transient effects on branchial function through gill chamber inhalation. Lowered oxygen consumption. Significant drop in the concentration of Na^+ ions within the hemolymph with increasing neutral plastic dose.	[75]
Amphora sp., *Ankistrodesmus angustus and Phaeodactylum tricornutum* (phytoplankton)	Polystyrene	23 nm		Engineered nanoplastic (EP) accelerated *Amphora* sp. exopolymeric substances (EPS) assembly and form microscopic gels of ~4-6 µm. EN only moderately accelerated *A. angustus* and *P. tricornutum* EPS assembly. The change of EPS assembly kinetics means marine carbon cycle could be disturbed.	[48]
Mytilus galloprovincialis (mussel)	Polystyrene (PS)	129 nm	0.05-50 mg/L (PS)	Total oxidant status increased in digestive glands after exposure to 0.5 mg/L PS. In digestive glands and gills, the total antioxidant capacity and esterase activity were increased at 50 mg/L PS. Inhibition of cholinesterase activity in haemolymph was observed. Genotoxicity was found in haemocytes after exposure. Lipid peroxidation (oxidative damage) was found while exposed to 0.05 mg/L of PS.	[57]
Mytilus galloprovincialis (mussel)	Polystyrene (PS) (with and carbamazepine (Cbz))	129 nm	0.05 mg/L(PS), 6.3 µg/L (Cbz)	Induced significant downregulation in gene expression (e.g., hsp70) when compared to individual exposure. Genotoxicity was found in haemocytes after exposure.	[57]
Mytilus edulis (blue mussel)	Polystyrene (PS) (coated with sulfate groups) and *Pavlova lutheri*	30 nm	0.1, 0.2, and 0.3 g/L	Produced pseudofeces. The total weight of the feces and pseudofeces increased along with increasing NP and algae concentration. Filtering activity was decreased but still remove the NP in the water.	[43]
Mytilus galloprovincialis	Polystyrene (coated with amine group)	50 nm	1, 5, 50 µg/mL	Decrease in phagocytic activity and increase in lysozyme activity were found. The NP stimulated increase in extracellular reactive oxygen species (ROS) and nitric oxide (NO) production, with maximal effects at 1 mg/mL of NP. The NP induced apoptotic process while at 50 µg/mL.	[61]
Littorina littorea	Polystyrene (adhered) on seaweed *Fucus vesiculosus*)	1–100 µm	/	Plastic found in the stomach and in the gut but not found in midgut gland.	[47]
Mytilus spp (mussels)	Polystyrene	2 and 6 µm	32 µg/L	An increase in hemocyte mortality and triggered substantial modulation of cellular oxidative balance. An increase in reactive oxygen species production in hemocytes and enhancement of anti-oxidant and glutathione-related enzymes in mussel tissues were detected.	[62]
	Polystyrene and fluoranthene	2 and 6 µm	32 µg/L (NP) and 30 µg/L (fluoranthene)	After depuration, a higher fluoranthene concentration was detected. Down regulation of a P-glycoprotein involved in pollutant excretion was found. Highest histopathological damages and levels of anti-oxidant markers were detected.	[62]
Amphibalanus Amphitrite (barnacle)	Poly(methyl methacrylate) (PMMA)	<0.2 µm	Stage II nauplii exposed to PMMA particles at	Acute exposure (occurred at 25ppm, 3hrs exposure) indicated that NP persist in the body throughout stages of growth and development (from nauplius to cyprid and juvenile barnacle,	[53]

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			concentrations of 5, 10, and 25 ppm;	continuous exposure). Egestion of NP was through moulting and fecal excrement. While chronic exposure (occurred at 1ppm) showed that bioaccumulation of the NP occurred at low concentration.	
Macoma balthica, Mytilus trossulus, Gammarus spp., Mysid shrimps, Monoporeia affinis, Marenzelleria spp.	Polystyrene	10 µm	5, 50 and 250 beads/mL	Rank of beads ingested: Bivalves (Mytilus trossulus>Macoma balthica) Free-swimming crustaceans (*Gammarus spp*. and Mysid shrimps) Benthic animals (Monoporeia affinis and Marenzelleria spp.)	[76]
Mytilus galloprovincialis (embryo)	Polystyrene (coated with amine groups)	50 nm	0.001-0.01-0.05-0.1-0.25-0.5-1-2.5-5-10-20 mg/L	NP affected the development of normal D-shaped larvae at 48hpf (EC_{50}=0.142 mg/L). At EC_{50}, the shell formation was affected. This concentration induced dysregulation of transcription of genes involved in early shell formation (Chitin synthase, Carbonic anhydrase, Extrapallial Protein) at both 24 and 48 hpf At 5-20 mg/L, it resulted in high embryotoxicity/developmental arrest.	[73]
Mytilus edulis (L.)	Polystyrene	2 µm, 4-16 µm	0.51 g/L for uptake experiment	Mussels uptake the plastics.	[42]
	Polystyrene	3 µm, 9.6 µm	~42 spheres/mL	Translocation occurred in the guts to the circulatory systems.	
(pacific oyster)	Polystyrene (coated with carboxylic groups)	70 nm–20 µm	1000 plastics/mL	The frequency and magnitude of NP ingestion varied by larval age and size of polystyrene particle	[54]
	Polystyrene (coated with amine groups)	70 nm–20 µm		NP ingested retained more frequently	
Mytilus galloprovincialis (mussel)	Polystyrene (coated with amine groups)	50 nm	50 µg/mL	PS-NH_2 increased cellular (lysosomal membrane stability, oxyradical production and phagocytosis) damage and Reactive Oxygen Species production in hemolymph serum compare to artificial seawater. The effects were mediated by dysregulation of p38 Mitogen Activated Protein Kinase signaling. The putative C1q domain containing protein (MgC1q6) which was the only component of the PS-NH_2 hard protein corona.	[59]
Dunaliella tertiolecta (green microalga)	Polystyrene (coated with carboxylic and amine groups)	40 nm	PS-COOH: 50 µg/mL, PS=NH_2: EC_{50}: 12.97 µg/mL	Long-term exposure (72 hours growth inhibition). PS-COOH affected growth. PS-NH_2 caused inhibition.	[58]
Artemia franciscana (brine shrimp)	Polystyrene (coated with carboxylic and amine groups)	50 nm	PS-COOH: 10 µg/mL, PS-NH_2: LC_{50}: 0.83 µg/mL, 1 µg/mL	Long-term exposure (14 days toxicity test). The expression of target genes (*clap* and *cstb*)in brine shrimp larval growth and molting were measured. PS-COOH affected growth. PS-NH_2 caused mortality. While PS-NH_2 at 1 µg/mL, it induced *clap* and *cstb* genes. It also increased molting and apoptotic pathway (triggered by cathepsin L-like protease).	[58]
Mytillus edulis (mussels)	Polystyrene	100 nm	$1.3x10^4$ particles/mL	Aggregated NP was ingested more amount than suspended NP. NP was transported to digestive gland.	[44]
Crassostrea virginica (oyster)	Polystyrene	100 nm	$1.3x10^4$ particles/mL	Aggregated NP was ingested more amount than suspended NP. NP was transported to digestive gland.	
Dipsastrea pallida/ Favia pallida (coral)	Polypropylene	10 µm-2 mm	0.395 g/L	Ingested plastics were found wrapped in mesenterial tissue within the coral gut cavity.	[40]
Brachionus koreanus (Monogonont Rotifer)	Polystyrene	0.05,0.5 and 6 µm	0.1,1,10 and 20 µg/mL	Increased oxidative stress, antioxidant enzymes and phosphorylation of c-Jun N- terminal kinase (p-JNK) and p38 (p-p38) and mitogen-activated protein kinases (MAPKs). Decreased growth rate, fecundity, lifespan, reproduction time and body size.	[66]
Crassostrea gigas (oyster)	Polystyrene (coated with carboxylic groups)	50 nm	0.1,1,10 and 25 µg/mL	NP induced significant decrease in fertilization success and embryo-larval development with numerous malformations up to total developmental arrest.	[71]
	Polystyrene (coated with amine groups)	50 nm	0.1,1,10 and 25 µg/mL	NP induced significant decrease in fertilization success and embryo-larval development with numerous malformations up to total developmental arrest. The strongest toxicity to gametes (EC_{50}=4.9 µg/mL) and embryos (EC_{50}=0.15 µg/mL). The result showed functionalization-dependent toxicity.	[71]
Artemia franciscana (brine shrimp larvae)	Polystyrene (coated with carboxylic groups)	40 nm	5-100 µg/mL	NP was massively sequestered inside the guy lumen of larvae (48h) and limited food intake.	[55]
	Polystyrene (coated with amine groups)	50 nm	5-100 µg/mL	NP accumulated in larvae (48 h) but also absorbed at the surface of sensorial antennules and appendages which possibly hampering larvae motility. It also processed multiple molting events during 48 h exposure time.	
Pomatoschistus microps (Teleostei, Gobiidae)	Polyethylene (presence with pyrene)	1-5 µm	Polyethylene: 0,18.4 and 184 µg/L; Pyrene: 20 and 200 µg/L	Polyethylene delayed pyrene-induced fish mortality and increased the concentration of bile pyrene metabolites. The acetylcholinesterase (AChE) activity was significantly reduced by the present of polyethylene alone or in combination with pyrene. Isocitrate dehydrofenase (IDH) was decreased while polyethylene and pyrene co-exist.	[63]
Tigriopus japonicus (copepod)	Polystyrene	0.05,0.5 and 6 µm	First experiment: (9.1×10^{11} particles/mL for 0.05-µmPS bead; 9.1×10^8 particles/mL for 0.5µm PS bead; 5.25×10^5 particles/mL for	No selective feeding when phytoplankton were added. While the concentration of 0.05 µm PS beads greater than 12.5 µg/mL caused mortality of nauplii and copepodites in the F_0 generation and triggered mortality at concentration of 1.25 µg/mL in the next generation. The concentration of 25 µg/mL of 0.5 µm PS beads induced a significant decrease in survival	[69]

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			6μm PS bead); Second experiment: 2.5×10^5 particles/mL	in the F_1 generation. The 0.5 and 6μm PS beads caused decrease in fecundity at all concentration.	
Calanus helgolandicus (copepod)	Polystyrene (with cultured algae)	20 μm	75 plastics/mL, 250 μg/cl	Copepod ingested 11% fewer algal cells and 40% less carbon biomass. There was a net downward shift in the average size of algal prey consumed, with a 3.6 folds increase in ingestion rate for the smallest size class of algal prey (11.6-12.6 μm), implying that postcapture or postingestion rejection. Prolonged exposure to polystyrene significantly decreased reproductive output.	[46]
Mytilus edulis (L.) to *Carcinus maenas* (L.)	Polystyrene	0.5 μm	411 million spheres	Trophic transfer study. The number of plastic spheres in the haemolymph of the crabs was highest at 24 hrs (around 0.04% of the amount to which the mussels were exposed) and then they were almost gone after 21 days. The plastic spheres were also discovered in the stomach, hepatopancreas, ovary and gills of the crabs.	[45,77]
Mytilus edulis (L.) (mussel)	Hight density polyethylene (HDPE)	0-80 μm	2.5 g HDPE-fluff	HDPE particles were uptake into the stomach and transported into digestive gland. They accumulated in the lysosomal system. Histological changes, strong inflammatory response indicated by the formation of granulocytomas and lysosomal membrane destabilization. HDPE caused tissue and cellular level damages.	[41]

4. Future Perspectives

Based on the aforementioned information, marine NP pollution has been extensively documented as harmful to marine species. As such, there is an urgent need for further NP studies to be conducted, as numerous knowledge gaps remain to be filled. The physical and chemical properties of NP demonstrate different behaviours in marine organisms and the environment. Suggested areas for future research on NP are summarised below.

While NP ingestion has been observed upon studying the digestive systems of many marine organisms, studies on the biological uptake and effects of ingested NP particles in the marine environment remain scarce. Notably, uptake facilitation mechanisms should be studied. Moreover, Carcinus maenas altered rope fibre size and shape following NP ingestion and egestion, which reduced plastic size and formed ball-shaped plastic through their gut [39,78]. Furthermore, Antarctic krill are capable of reduce MP to NP [39,78]. The physical changes occurring in plastic materials following ingestion and egestion by marine organisms requires further study due to these abilities being critical to studying how NP is distributed in the marine environment, as well as its environmental fate and origin.

Additionally, further research is required to support the potential of NP particles to adsorb organic/metal contaminants. Also, the combined toxicological interactions between NP and organic/metal contaminants also require further study. For instance, Manila clams (*Ruditapes philippinarum*) are a seafood resource for human consumption. The sorption of other contaminants onto NP surfaces may pose a synergistic effect in marine organisms or even humans. Short-term and long-term exposure to NP and its interaction with metal/organic pollutants should also be further researched. Notably, NP could act as a vector in transporting chemicals to marine organisms. Future studies should evaluate the transfer of adsorbed chemicals on NP through trophic levels. Importantly, the biomagnification of NP could accumulate pollutants and impact the health of marine animals, and ultimately humans. NP accumulates up through the trophic levels, among which humans are the end consumers. Hence, NP issues related to the seafood industry must be adequately monitored.

Furthermore, the adverse effects of NP on early life stages should be accounted for in the 'Adverse Outcome Pathway' scheme for marine organisms. This is because NP toxicity affecting early life stages could influence offspring viability and overall reproductive output [71]. Most current studies have focused on the effects of a few types of NP on limited types of marine organisms in a controlled environment, which is not sufficient to represent the overall impacts of marine NP pollution on marine organisms. As different marine species expose to different polymer types, sizes, shapes, and environmental conditions, they may demonstrate different responses. Therefore, additional research on a broader range of species, NP types, and chemicals remain vitally important.

5. Conclusion

With regards to occurrence and distribution studies, the current lack of available techniques for the accurate measurement of environmental concentrations of NP at smaller particle sizes should be improved. While NP concentrations in the environment are increasing overall due to larger plastic debris and MP degradation, industrial NP production is also increasing and resulting in an increased amount of NP in the environment. The enhancement, variation, and modification of current NP protocols should be improved in order to develop revised protocols for future NP research. The development of these methods is urgently required for research, monitoring, and risk assessment purposes. A full risk assessment of NP with different physical and chemical properties for different marine organisms is also required. Moreover, current NP research studies tend to investigate the effects of higher concentrations than those present in the natural marine environment. Environmentally relevant concentrations should thus be studied to evaluate realistic scenarios. The bioavailability of NP in the marine environment and its impact on keystone species and commercial species must also be investigated. Long-term studies on NP bioavailability and its effects on marine organisms are thus required. The characterisation and quantification of NP occurrence in various marine organisms is also needed.

Furthermore, biochemical molecular biological traits (e.g., variations in RNA content and gene expression patterns) related to stress and detoxification should also be studied. In addition, further investigations on the effect of different plastic types on the growth, hatching, and reproduction of various marine organisms are also needed. The toxic endpoints (maintenance costs, immune responses, detoxification, and oxidative balance regulation) of NP should also be studied further. Current research studies lack information on specific protective mechanisms and pathways against NP toxicity in marine species, which should be elucidated. Further details regarding NP properties and their stability in natural seawater are also needed in relation to their characteristics upon exposure to embryos, as different aggregation states may bring about different bioavailability and disposition routes to affected embryos. Many studies have focused on carboxylated and aminated groups of PS-NP, but have neglected the study of additional surface coatings. Moreover, many NP ecotoxicity studies have focused directly on NPs in relation to their impacts on marine organisms and the environment. However, the indirect influences of NPs were ignored. In fact, unknown indirect impacts could potentially be posing a greater environment threat than known direct impacts. For instance, the high concentration of NP interrupted the NH_4^+-N conversion efficiencies of *Halomonas alkaliphila* through enhanced ROS generation (77). This implies that NP could indirectly affect the marine nitrogen cycles. As such, further studies on the indirect impacts of NPs are needed.

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