



Review

Ecotoxicity of microplastics to freshwater biota: Considering exposure and hazard across trophic levels



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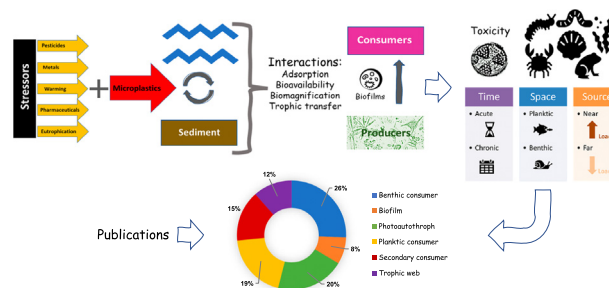
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HIGHLIGHTS

- Evidence shows microplastics (MPs) transfer in freshwater foodweb experiments.
- Up to 2021 just 12% of microplastics research addressed freshwater trophic transfer.
- MPs & multiple stressor hazards to freshwater biota studied in 14% of publications.
- Huge disparity in MPs toxicity studies of freshwater organisms: Daphnia alone 21%.
- Future research needed on freshwater biofilm associated MPs, macrophytes and Diatoms.

GRAPHICAL ABSTRACT

What has been done and which areas need further attention on the toxicity of microplastics in freshwater food webs?



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ABSTRACT

In contrast to marine ecosystems, the toxicity impact of microplastics in freshwater environments is poorly understood. This contribution reviews the literature on the range of effects of microplastics across and between trophic levels within the freshwater environment, including biofilms, macrophytes, phytoplankton, invertebrates, fish and amphibians. While there is supporting evidence for toxicity in some species e.g. growth reduction for photoautotrophs, increased mortality for some invertebrates, genetic changes in amphibians, and cell internalization of microplastics and nanoplastics in fish; other studies show that it is uncertain whether microplastics can have detrimental long-term impacts on ecosystems. Some taxa have yet to be studied e.g. benthic diatoms, while only 12% of publications on microplastics in freshwater, demonstrate trophic transfer in foodwebs. The fact that just 2% of publications focus on microplastics colonized by biofilms is hugely concerning given the cascading detrimental effects this could have on freshwater ecosystem function. Multiple additional stressors including environmental change (temperature rises and invasive species) and contaminants of anthropogenic origin (antibiotics, metals, pesticides and endocrine disruptors) will likely exacerbate negative interactions between microplastics and freshwater organisms, with potentially significant damaging consequences to freshwater ecosystems and foodwebs.

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1. Introduction

It has been demonstrated via a plethora of recent research that microplastics (size ≤ 5 mm) are ubiquitous in water, soil and air and available to a wide range of organisms (Brahney et al., 2020). Naqash et al. (2020) provides a comprehensive review on microplastic (MP) abundance on surface water and sediments globally, illustrating MPs global reach. Sewage effluent and urban drainage systems are the main source of river and stream MP pollution (Wu et al., 2019); in arable/agricultural regions MPs enter waterbodies from runoff using similar pathways to pesticides and fertilizers (Müller et al., 2020; Waldschläger et al., 2020; Zhang and Chen, 2020); or MPs may emanate from landfill or from the breakdown of in situ litter in rivers (Horton et al., 2017), illustrated by the prevalence ($70 \pm 19\%$) of water samples containing acrylates/polyurethane/varnish (APV) antifouling paint from the River Rhine (Mani and Burkhardt-Holm, 2020). Furthermore, hydrodynamic processes inherent to lentic and lotic water bodies as well as seasonal climatological conditions will affect MP sinking and re-suspension rates into and from sediment (Hurley et al., 2017; Rodrigues et al., 2018; Dahms et al., 2020; Zhang and Chen, 2020), ultimately determining concentrations in, and bioavailability to, planktonic and benthic freshwater organisms (Meng et al., 2020; Krause et al., 2021) and further adding to the complexity of understanding of their impacts on, and toxicity to, freshwater biota.

Microplastics are a versatile group of synthetic materials that can be defined by their origin: primary MPs are manufactured for a purpose, and secondary MPs result from successive fragmentation or damage (abrasion, delamination, weathered). Fragmentation or damage follow exposure to mechanical processes (e.g. sewage treatment or run off), natural elements (e.g. sunlight or by action of hydrodynamic processes), or biological processes (C. Li et al., 2020; Li et al., 2018; Waldschläger et al., 2020). Biological processes are common on aged or damaged MPs by direct microbial activity and bioassimilation or indirect processes by agglomeration of MPs and nanoplastic particles (nanoplastics or NPs) mediated by biofilms (a consortium of bacteria, cyanobacteria, algae and other protists embedded in exopolysaccharides, EPS) (Provencher et al., 2019). Microplastics can be classified according to their physico-chemical characteristics: size, shape (pellets, fibres, films, fragments, foams), or material used in manufacture. Microplastics are further subdivided into nanoplastics (NPs) as they disintegrate (Mattsson et al., 2015a). There is not yet a consensus on the size threshold classification between MPs or NPs (Gigault et al., 2018) with

suggestions that nanoplastics range from $<20 \mu\text{m}$ (Wagner et al., 2014), to $<1000 \text{ nm}$ (da Costa et al., 2016), and even $<100 \text{ nm}$, as is commonly used for engineered nanomaterials (Koelmans et al., 2015). The commonest MP shape found in rivers, lakes and sediments are fibres. The commonest size range is $>300 \mu\text{m}$ – $\leq 1 \text{ mm}$, for the Widawa River in Central Europe (Kuśmierek and Popiołek, 2020) and $>1 \text{ mm}$ in the River Thames, UK (Horton et al., 2018a). Li et al. (2018) reported MPs fibres $<1 \text{ mm}$ in 93.8% in surface water samples and 94.8% in sediment samples from 18 lakes along the Yangtze River, China. The highest reported prevalence and concentration of fibres ($>500 \mu\text{m}$) for any aquatic environment, including marine systems, is in fish from urban and agricultural reservoirs in the USA (Hurt et al., 2020). Examples of MPs materials are: acrylics (AC), nylon, polyamide (PA), polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), acrylamide (ACA) and polyvinyl chloride (PVC), including any combination with chemical additives: BPA (bisphenol), phthalates (added as plasticisers), PBDEs, TBBPA (bromine-containing compounds for fire retardation properties) and colorants (Bretas Alvim et al., 2020; Provencher et al., 2019).

The prevalence of microplastics in all aquatic environments globally has been well documented. For example, in an urban stream in Braamfontein Spruit, Johannesburg, mean MPs abundance of 705 items m^{-3} was recorded in water samples, 166.8 $\text{kg}^{-1} \text{ d/w}$ in sediment, while 53.4 MPs $\text{g}^{-1} \text{ w/w}$ was found in *Chironomus* sp. larvae sampled from the sediment highlighting the relationship between benthic organisms and ingestion of settled MPs (Dahms et al., 2020). Rodrigues et al. (2018) reported MPs seasonal variations from the Antuã River, Portugal, where the range of abundance in water was 58–193 items m^{-3} in March to 71–1265 items m^{-3} in October; while in sediment, the abundance ranged from 13.5–52.7 mg kg^{-1} in March to 2.6–71.4 mg kg^{-1} in October. Triebkorn et al. (2019) summarize their findings for a variety of global sites on MPs concentrations from effluents, surface water and sediments, varying with the type of waterbody (river or lake), size and closeness to urban areas, and population density. For MPs $>300 \mu\text{m}$ concentrations range from 0.012 to 0.027 MPs m^{-3} for Lake Khovsgo, Mongolia and Laurentian Great Lakes, Canada-USA border; compared to urban Lake Hangian and Wuhan rivers in China for MPs $>50 \mu\text{m}$ with a range of 1660–8925 MPs m^{-3} ; and for canals in Amsterdam huge concentrations of 48,000–187,000 MPs m^{-3} were found for MPs size $>10 \mu\text{m}$. Turner et al. (2019) studied sediment cores from a lake in London, UK, revealing that MPs contamination doubled since the 1960s, with MPs concentrations of 226 kg^{-1} to

(maximum) 539 particles kg^{-1} dry weight between 2005 and 2009 with fibres being the commonest MPs. The authors suggest that atmospheric deposition of fibres is an important source of MPs in isolated lakes without a direct wastewater effluent discharge, and that closed lake ecosystems in agricultural/urban landscapes may be particularly susceptible to atmospheric MPs contamination and sediment accumulation (Rochman and Hoellein, 2020).

A direct potential mechanism of toxicity by MPs and NPs is chemical leaching (Mohamed Nor and Koelmans, 2019). Bioavailability of particles and leaching of additives is potentially higher in NPs, due to their size and large surface-area to volume ratio (Alimi et al., 2018). The internalization of NPs and accumulation within sensitive tissues may therefore increase the risk and impact of endocrine disruptors e.g. phthalates (Yang et al., 2011) causing damage to aquatic organisms. From an environmental standpoint, changing pH, solute concentration, temperature, and chemical composition may influence the rate of leaching with the potential to bioaccumulate in certain tissues which could allow critical concentrations of leachates to impact negatively aquatic organisms (Yang et al., 2011). Yand and Nowack (2020) published an evaluation on MPs and NPs toxicity studies (until April 2020) using probabilistic species sensitivity distributions, but their analysis did not find supporting evidence that NPs can be more hazardous than MPs for aquatic organisms.

Microplastic surface area, and in particular the comparatively larger surface area of NPs, increases their propensity to adsorb chemicals and pathogens from the environment (Velzeboer et al., 2014). Changes in environmental pH could facilitate the enhanced liberation of metal ions, pathogens and other chemical adsorbents such as polyaromatic hydrocarbons (PAHs) from the particle surface into aquatic environments (Liu et al., 2016). This unpredictable behaviour of co-contaminants varies with the aquatic environmental conditions the NPs or MPs are found in and further increases the complexity of determining potential toxic impacts.

Importantly, inherent differences exist between freshwater and marine taxa: for example, water uptake by marine fish (by mouth) and freshwater fish (absorption through skin and gills) is likely to lead to ingestion of different amounts of MPs from the same concentration; some small organisms such as the diatoms *Nitzschia* and *Pseudonitzschia* have a wide range of salinity tolerances (Singer et al., 2021) but most organisms are adapted to either a marine or a freshwater existence. The knowledge acquired from organisms in marine environments may not, therefore, be applicable to freshwater environments (Rochman, 2018). It is worth noting that the volume of peer-reviewed research on MPs and NPs in freshwaters has appreciably increased since 2018 (see Fig. 1 in Section 3) although numbers of publications are low compared to marine publications and the research is limited to a few target freshwater species. This review, therefore, takes a whole trophic-web approach. We synthesise and compare research on the ecotoxicity of

MPs and NPs in organisms from a range of freshwater ecosystems with the aim of a) showcasing data on exposure of organisms at lower trophic levels, b) assessing biomagnification, c) evaluating the evidence for the cumulative effects of exposure and multiple stressors, and d) assessing impacts and potential hazards on primary to secondary consumers and energy flows.

2. Literature search methods

The literature review was carried out until 6th March 2021 using the following search engines: ScienceDirect (www.sciencedirect.com); Web of Science (www.webofknowledge.com) and Pubmed (<https://pubmed.ncbi.nlm.nih.gov/>). The search was defined as follows: 'microplastics' OR 'nanoplastics' AND 'freshwater' were kept constant in all searches with keywords incorporated with the Boolean connector AND. Keywords used in this search were: 'macrophytes', 'cyanobacteria', 'microalgae', 'phytoplankton', 'biofilm', 'protozoans', 'invertebrates', 'crustaceans', 'benthic consumers', 'fish', 'amphibians', 'trophic chain' and 'trophic web'. For example, 'microplastics' OR 'nanoplastics' AND 'freshwater' AND 'phytoplankton'. The search was broadened to research papers and reviews with no limits in years or subject area (no books, chapters or proceedings). Our objective, therefore, was to record relevant existing literature for freshwater organisms within the producers, first and secondary consumer trophic levels. Mammals were not considered. Following Blettler and Wantzen (2019), not all existing published papers on the topic could be included, but we obtained a representative sample after an exhaustive check of the results (paper by paper). This was crucial to avoid papers outside the terms of reference of the review, repetitions, or ensure scientific rigour e.g. use of experimental controls, replications, and statistical analysis. We found that ecotoxicological terms were not always used accurately creating confusion for the reader. To clarify the approach followed in this review a glossary of ecotoxicological terms used is presented in Appendix A (extracted from Nordberg et al., 2009) as supplementary information.

3. Freshwater biota exposure to microplastics

Our review yielded 153 publications in total, with 19 already published in 2021 (until 6th March). Fig. 1 shows a steady increase ($R^2 = 99\%$) since 2010, when the first publication that met the selected criteria was found; and demonstrating the rising interest of the scientific community on the ecotoxicity of MPs and NPs in freshwater ecosystems.

Fig. 2A shows the breakdown of main topics for this review: trophic web and trophic levels. Publications with research involving two or more trophic levels were grouped in "Trophic web", research on this topic is clearly underrepresented and featured in only 12% of the publications. Research that involved single species and MPs/NPs was grouped under their corresponding trophic level. Only photoautotrophs were considered as producers, and, therefore, organisms such as fungi or protozoa (e.g., ciliates) were not considered as producers despite their role in transforming decaying organic matter into energy for other organisms. Freshwater biofilms were considered in their interaction with microplastics and potential transfer role within the trophic web; with 8% of publications, it is clear this topic has been neglected.

Fig. 2B shows the prevalence of higher taxonomic groups (above genus or species). Four taxa dominate: Crustacea (31%), Fish (18%), Chlorophyte (11%), and Mollusca (10%). The number of Nematoda publications is small, in fact, there is one study by Fueser et al. (2019) who tested seven nematode species in the same experiment, inflating the proportion of studies that appear dedicated to this taxon (Table 4).

Even with the relevance that freshwater fish have for many societies 18% of the literature is dedicated to this group. Overall the most studied group of organisms is invertebrates (61%) with *Daphnia* being the single most studied genus (21%) of the 83 model organisms (genus and species) used for the assessment of microplastics toxicity in freshwater.

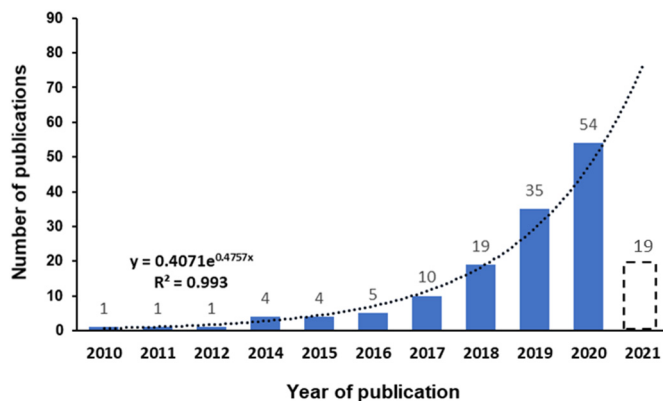


Fig. 1. Yearly publications on microplastics and nanoplastics in freshwater organisms. The total for 2021 is for the first two months. See Section 2 for the selection criteria.

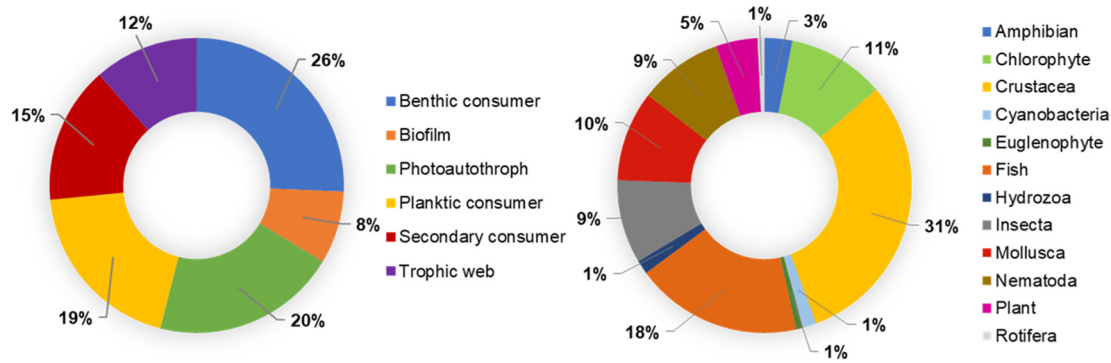


Fig. 2. A. Classification breakdown of topics selected in this review with attention to trophic levels. B. Freshwater taxonomic groups (%) currently used for the assessment of microplastics toxicity.

Microplastics and nanoplastics are considered stressors in combination with other pollutants in this review. Therefore, those publications which involved one or several species with MPs/NPs and in the presence of another stressor e.g. pesticides or metals were grouped under “Stressors” featured in 16 publications which corresponds to 14% of research (113) publications out of the 153 on freshwater MPs (Section 3.2, Table 2). This category includes research that aims to understand the synergistic or antagonistic interactions between MPs and known pollutants and their toxicity impact on freshwater organisms.

There are many discrepancies from the published data, either reflecting the lack of consistency in protocols, measuring and monitoring MPs or that research is scarce or non-existent for some groups (Barbosa et al., 2020) e.g. benthic diatoms. In addition, there may be differing effects from the type of freshwater ecosystem e.g. lotic, lentic, or from cumulative effects of environmental change including nutrient enrichment and/or increase in temperature. Toxicity effects of MPs/NPs may be greater at low trophic levels with potential reductions of biodiversity in freshwater ecosystems (Redondo-Hasselerharm et al., 2020; Prata et al., 2019) and disruption of ecosystem functioning (W. Huang et al., 2021; Y. Huang et al., 2021).

A number of publications (Caruso, 2019; Bretas Alvim et al., 2020; C. Li et al., 2020; S. Li et al., 2020; The Royal Society, 2019; Scherer et al., 2017) showed the impacts of MPs/NPs on freshwater organisms and ecosystems depend on several interacting factors difficult to categorize. The physico-chemical characteristics of MPs/NPs: the type of chemical, shape, size and age of these particles could increase their toxicity effect on freshwater organisms with aging particles leaking toxic chemicals; fibres and spheres being easier to ingest but fibres being more difficult to egest. The toxicity is also dependent on the length of exposure of organisms to MPs/NPs: acute or chronic, where chronic exposure is likely to affect several generations; but also depend on MPs/NPs habitat concentration, their ingestion or absorption e.g. tadpoles. The uptake of MPs/NPs differs on their size with larger particles being ignored or more difficult to uptake; and their uptake rate is dependent on feeding strategies: e.g. shredders or filter feeders, if the organisms are generalists or specialists or their morphological stage e.g. larvae or adult. The presence or absence of associated stressors e.g. metals or pesticides will also influence the toxicity of the ingested, translocated to other tissues or egested MPs/NPs on freshwater organisms (Fig. 3).

3.1. Microplastic biomagnification or trophic enrichment

A unique four trophic level experimental work by Chae et al. (2018) demonstrated PS-NPs are transferred throughout the foodweb, as well as NPs translocated to the intestinal cells of the fish using confocal laser scanning microscopy and fluorescent dye to identify the NPs (Table 1). These authors exposed *Chlamydomonas reinhardtii* to fluorescent PS-NPs, *Daphnia magna* was exposed to the microalgae, NPs were observed in the intestine of *D. magna* with some damage observed to

the intestinal microvilli when compared to controls. The secondary consumer, *Oryzias sinensis* was fed with *D. magna* and later to the top predator *Zacco temminckii*. Fluorescent NPs were found in the digestive system and internalized in the intestinal lumen of the fish. However, no direct toxicity (mortality) effects were reported for 72 and 48 h for the microalgae or Crustacean even though were exposed to very high PS-NPs concentrations, but changes in the biochemistry, liver, intestines, and behaviour were reported in both fish. In comparison, Elizalde-Velázquez et al. (2020) experimented with two trophic levels *D. magna* and a fish, flathead minnow (*Pimephales promelas*). *D. magna* was exposed to low and high concentrations (20 and 2000 parts ml^{-1}) of PS-MPs (6 μm) and later exposed to *P. promelas*. Less than 1% of MPs were transferred through the food chain (from *D. magna* to the fish) and MPs were only present in the gastrointestinal tract of the fish when the fish was fed only with MPs. Their view was that MPs presence in the digestive tract is an indicator of MPs ingestion time length. Earlier experiments exposed the *Scenedesmus obliquus* to different concentrations of NPs-PS of 70 nm size. Results demonstrated a slight reduction in growth of 2.5% at the very high 1 g L^{-1} of NPs, and a reduction in chlorophyll-*a* concentration as concentration in NPs increased (Besseling et al., 2014).

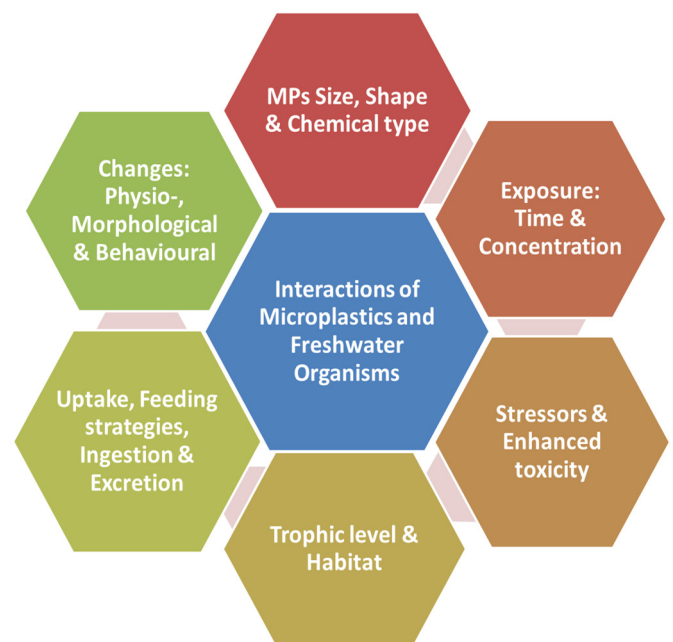


Fig. 3. Interactions of microplastics and freshwater organisms: physico-chemical characteristics of MPs; concentration and exposure of MPs; autoecology of aquatic organisms.

Table 1
Microplastics transfer through the foodweb. Trophic level category, species, research approach, type and size of MPs, exposure: time and concentration, toxicity effect and reference.

Category	Species	Research approach	MP or NPS material & size	Exposure to MPs	MP concentration	Effect	Reference
Photoautotroph Planktic consumer	<i>Scenedesmus obliquus</i> , <i>Daphnia magna</i>	Laboratory	PS-NPs	21 d	0.22–150 mg L ⁻¹	Reduction growth of microalgae and reproduction of the crustacean.	Besseling et al., 2014
Photoautotroph Planktic consumer Secondary consumer	<i>Scenedesmus</i> sp., <i>D. magna</i> , <i>Carassius carassius</i>	Laboratory	NPs (24–27 nm)	3 d 24 & 61 d	0.01% w/v or 9.3 × 10 ¹² NPs mL ⁻¹	Three level trophic web experiment that demonstrated trophic transfer. Severe effects on both behaviour and lipid metabolism in fish.	Cedervall et al., 2012; Mattsson et al., 2015b)
Photoautotroph Benthic consumer Secondary consumer	<i>Pavlova</i> sp., <i>Corbicula fluminea</i> , <i>Acipenser transmontanus</i>	Laboratory with PCB	PET-MPs, PVC-MPs, PS-MPs (12–704 nm)	28 d	0.0003% w/v or 2.8–4.2 mg L ⁻¹	Direct and indirect effects with different types of MPs and PCBs. Clams showed histopathological changes with MPs. * <i>Pavlova</i> sp. is a marine microalgae used in this freshwater experiment.	Rochman et al., 2017
Photoautotroph Primary consumer Secondary consumer Top predator	<i>Chlamydomonas reinhardtii</i> , <i>D. magna</i> , <i>Oryzias sinensis</i> , <i>Zacco temminckii</i>	Laboratory	PS-NPs (57.3–60.4 nm)	48 & 72 h 7 d	50 mg L ⁻¹	Four level trophic transfer and individual impact. No significant toxicity for the microalga or <i>D. magna</i> . Toxicity on liver tissue, lipid metabolism, embryos, and locomotive activities of fish (both species)	Chae et al., 2018
Benthic consumer Secondary consumer	<i>Caenorhabditis elegans</i> , <i>Danio rerio</i>	Laboratory	PA-MPs, PE-MPs, PS-MPs, PVC-MPs, PP-MPs, (70 µm)	10 d	0.001–10.0 mg L ⁻¹	Severe toxicity effects in the fish intestine highest lethality, bioaccumulation in nematodes was observed with 1 µm MPs independently of the type.	Lei et al., 2018
Photoautotroph Benthic consumer	<i>Lemna minor</i> , <i>Gammarus duebeni</i>	Laboratory	PE-MPs (10–45 µm)	24–48 h 7 d	7 PE-MPs mm ²	Bioaccumulation 1–2 PE-MPs were observed in the animal but not morbidity or mortality.	Mateos-Cárdenas et al., 2019
Planktic consumer Secondary consumer	<i>D. magna</i> , <i>Pimephales promelas</i>	Laboratory	PS-MPs (6 µm)	72–96 h	20 & 2000 MPs mL ⁻¹	<1% transfer from <i>D. magna</i> to the fish. MPs found in the fish with food absence. No adverse effect.	Elizalde-Velázquez et al., 2020
Leaf litter Benthic consumer	<i>Sericostoma pyrenaicum</i>	Laboratory	MPs (10 µm)	72 h	0–10 ³ MPs mL ⁻¹	Leaf litter decomposition was reduced with increasing MP concentrations.	López-Rojo et al., 2020
Benthic consumer Secondary consumer	<i>Poecilia reticulata</i> , <i>D. rerio</i> , <i>Geophagus brasiliensis</i>	Laboratory	PE-MPs (35.5 ± 18.2 µm)	48 h 10 d	60 mg L ⁻¹	Toxicity and trophic transfer of PE-MPs from clam to fish. MPs enter the fish' organs, behavioural changes and mutagenic and cytotoxic processes.	da Costa Araújo et al., 2020
Benthic consumer Planktic consumer Secondary consumer	<i>Chironomus riparius</i> , <i>D. magna</i> , <i>D. rerio</i>	Laboratory combined with PAH	PMMA- MPs (48 µm)	24 h 48 h	1 × 10 ⁶ MPs L ⁻¹ 0.05 g L ⁻¹	Two level trophic transfer and the stressor benzo(k)fluoranthene a PAH.	Hanslik et al., 2020
Photoautotroph Benthic consumer	<i>Chlorella pyrenoidosa</i> , <i>Cipangopaludina cathayensis</i>	Laboratory combined with amphetamine	PS-MPs (700 nm)	96 h	0.01–15 mg L ⁻¹ 20 mg L ⁻¹	Acute toxicity of methamphetamine significantly increased in the presence of MPs for the microalgae (EC50 shift from 0.77 to 0.32 mg L ⁻¹) and the snail LC50 shift from 4.15 to 1.48 mg L ⁻¹ . Oxidative damage and apoptosis of the algae increased, as well as the filtration rate of the snails.	Qu et al., 2020

Hour (h), days (d), year (y); polystyrenes (PS), microplastics (MPs), nanoplastics (NPs), polyethylene-terephthalate (PET), polypropylene (PP), polyethylene (PE), polyvinyl-chloride (PVC), polyamide (PA), polymethyl methacrylate (PMMA), polyaromatic hydrocarbons (PAH), polychlorinated biphenyl (PCB).

López-Rojo et al. (2020) conducted an experiment with MPs adhered to leaf litter and a detritivore feeding aquatic insect (*Sericostoma pyrenaicum*). They observed that leaf litter decomposition was reduced with increasing MP concentrations which was significant only in the presence of the detritivores, but microbially-mediated decomposition showed a similar trend. Although not conclusive for microbial decomposition, this experiment highlights the impact MPs may have on ecosystem functioning. Mateos-Cárdenas et al. (2019) exposed *Lemna minor* to MPs in a toxicity experiment with polyethylene MPs (MPs-PE) (10–45 µm) for 7 d. Results showed the adsorption of PE-MPs to *L. minor* with an abundance of 7 PE-MPs per mm², but not changes in photosynthetic efficiency or growth. *G. duebeni* was subsequently fed on MP-contaminated *L. minor* - during an acute toxicity experiment (24–48 h). It was confirmed that 1–2 PE-MPs were observed in the animal indicating transferability of MPs through ingestion.

Perhaps the acute toxicity experiment time of exposure was too short and longer exposure might reveal other toxicity effects on the invertebrate. A three-level trophic chain investigation was conducted by Cedervall et al. (2012), *Scenedesmus* sp. was exposed to PS-NPs (24 nm) at a concentration of 0.01% (w/v) which after 24 h was filtered, and 250 ml algal culture was fed to *Daphnia magna* (30 adults). After another 24 h the zooplankton was presented to *Carassius carassius* (four individuals per replicate tank), in total 16. The experiment was run for 30 days and every third day the food chain was restarted with the fish remaining the same. These authors demonstrated the transport from *Scenedesmus* sp. to *C. Carassius* with metabolic parameters changes in the fish: weight loss, the triglycerides: cholesterol ratio in serum, and its distribution between muscle and liver tissues; plus changes in behaviour.

Toxicity effects shown on Table 2 from the trophic combined experiments with other stressors (PCB, PAH, and methamphetamine) provide

Table 2

Combined MPs and/or NPs toxicity experiments with other stressors: pesticides, metals and/or temperature. Category, species, research approach, type and size of MPs, exposure: time and concentration, toxicity effect and reference.

Category	Species	Research approach	MP or NPS material & size	Exposure to MPs	MP concentration	Effect	Reference
Plant (Macrophyte)	<i>Vallisneria natans</i>	Mesocosm-combined with Cd	PVC-MPs (100 nm & 5 µm)	14 d	5 g at 1% sediment DW (500 g)	Reduced fresh weight regardless of Cd exposure	Wang et al., 2021
Phytoplankton	<i>Chlorella pyrenoidosa</i>	Mesocosm-with Nonylphenol.	PE-MPs, PA-MPs & PS-MPs (13, 100, 150 & 1000 µm)	96 h	10, 30, 50, 70 & 100 mg L ⁻¹	Single: Growth, PSII inhibition, increased ROS due to enzymatic algal activity: superoxide dismutase (SOD), malondialdehyde (MDA) and catalasa. Combined: pesticide-MPs had an antagonistic effect	Yang et al., 2020
Phytoplankton	<i>Chlamydomonas reinhardtii</i>	Mesocosm-with As (III)	PS-MPs PS-MPS-As (II)	72 h	10, 25, 50 & 100 mg L ⁻¹	Suppressed Rubisco activity, reduced photosynthesis & growth rates. PS-MP-As (III) triggered oxidative processes and damaged membrane cells.	Dong et al., 2021
Phytoplankton	<i>Chlorella pyrenoidosa</i>	Laboratory with Cu	PAN-MP (0.05–0.8 mm)	6 d	0, 50, 150, 250, & 500 mg L ⁻¹	Synergistic effects on growth, production and function of cellular pigments with decreasing chlorophyll <i>a</i> and <i>b</i> , increasing carotenoids and antioxidant enzymes.	Lin et al., 2020
Phytoplankton	<i>Raphidocelis subcapitata</i>	Mesocosm-with Cu	PS-NPs	72 h 7 d	0.5, 1, 2.5, 5, 10, 50 mg MPs L ⁻¹	Inhibition of growth rate morphological alterations, potential disturbances in the mitotic cycle	Bellingeri et al., 2019
Invertebrate	<i>Daphnia</i> sp.	Mesocosm-with two temperature	PS-MPs Beads (575 ± 18.9 nm)	n/a	1 mg L ⁻¹ or 1.46 × 10 ⁷ MPs L ⁻¹	Synergistic effect of MPs and temperature on phenotypical responses on clones. The exposure to MPs lasted two full <i>D. magna</i> life cycles	Sadler et al., 2019
Invertebrate	<i>C. riparius</i>	Mesocosm-with Imidacloprid	PVC-MPs	28 d	Concentrations exceeding environmental conditions	No evidence for enhanced toxicity of MPs	Scherer et al., 2020
Invertebrate	<i>D. magna</i>	Mesocosm-with deltamethrin (40 ng/L)	MPs (1–4 µm)	21 d	1 mg L ⁻¹	Synergistic effect of the pesticide and MPs was demonstrated with reductions of <i>D. magna</i> 51% offspring per surviving female and 46% in brood numbers.	Felten et al., 2020
Invertebrate	<i>D. magna</i>	Laboratory-with dimethoate and deltamethrin	PS-MPs Beads (1–1.4 µm)	72 h	0.29 µg mL ⁻¹ or 3 × 10 ⁵ MPs mL ⁻¹	Results did not support altered toxicity of either pesticide to <i>D. magna</i> in combination with MPs or act as a vector for increased uptake of pesticide.	Horton and Dixon, 2018
Invertebrate	<i>D. magna</i> <i>D. pulex</i> <i>Ceriodaphnia pulex</i>	Mesocosm-with three temperatures	Primary & secondary MPs (1–10 µm)	72–96 h	10 ³ , 10 ⁴ , 10 ⁵ , 10 ⁶ , 10 ⁷ MPs mL ⁻¹	Sensitivity to toxicity effects were found to be temperature and time exposure dependent.	Jaikumar et al., 2018
Invertebrate	<i>D. magna</i>	Laboratory with Bishphenol	PA-MPs (5–50 µm)	24–48 h	25–250 mg L ⁻¹ 200 mg L ⁻¹	The combination of BPA and MPs led to decreased immobilization, after ingestion by <i>D. magna</i> .	Rehse et al., 2018
Invertebrate	<i>D. magna</i>	Laboratory with Glyphosate	PE-MPs Beads PET/PA-MPs fibres	6 days 48 h	10–30 mL ⁻¹ 2.2 × 10 ⁶ mL ⁻¹	Synergistic toxicity effects of glyphosate acid and glyphosate-IPA salts on <i>D. magna</i> but glyphosate-IPA toxicity was reduced after 48 h when using PE beads. Suggesting sorption of the pesticide.	Zocchi and Sommaruga, 2019
Invertebrates	<i>Gammarus roeseli</i>	Laboratory with phenanthrene	MPs	24 h 48 h	471 µg L ⁻¹ 441 µg L ⁻¹	No synergistic effects were found in the presence of anthropogenic and natural particles contradicting findings from other authors.	Bartonitz et al., 2020
Invertebrate	<i>Gammarus pulex</i>	Microcosm-field (river) collected organisms combined with temperature	PMMA-MPs (40.2 µm)	24 h	0.52, 26.12 & 104.48 cm ⁻²	MPs adverse effects on metabolic rate were manifested at higher temperatures	Kratina et al., 2019
Invertebrate	<i>Lymnaea stagnalis</i>	Mesocosm-combined with PDBE	PA-MPs (13–19 µm)	96 h	≥20,000 times environmental conditions	No supporting evidence for PA-MPs toxicity or PBDE bioaccumulation in the presence of PA-MPs	Horton et al., 2020
Invertebrate	<i>Dreissena polymorpha</i>	Mesocosm-temperature	PS-MPs fragments (2–60 µm)	14 d	100,000 MPsmL ⁻¹	MPs have minor effects on a freshwater mussel compared to thermal stress, neither alone nor as interactive effect.	Weber et al., 2020
Fish	<i>Symphysodon aequifasciatus</i>	Mesocosm-temperature	PE-MPs PP-MPs (70–80 µm)	30 d	200 µg L ⁻¹	MPs affected predatory performance, digestion and energy production. No impact on juvenile fish	Wen et al., 2018

Hour (h), days (d), year (y); microplastics (MPs), nanoplastics (NPs), polyvinyl chloride (PVC), polyacrylonitrile (PAN), polyethylene (PE), polystyrenes (PS), polypropylene (PP), polyamide (PA), polymethyl methacrylate (PMMA).

evidence that their co-occurrence with MPs may increase the potential hazards throughout the foodweb (Rochman et al., 2017; Hanslik et al., 2020; Qu et al., 2020). Microplastics interactions with multi-stressors and impact on organisms (singly or several within the same trophic level) is developed in Section 3.2 and Table 2.

Trophic transfer is demonstrated in 12 of the 13 studies (Section 3.1 and Table 1), evidently the sample size is still small. Provencher et al. (2019) reviewed MPs transfer throughout the foodweb focusing on the imbalanced research efforts between field studies and laboratory experiments where the former continues scarce. Most field studies are

limited to collection from water or sediment of individuals (Yardy and Callaghan, 2020; Iannilli et al., 2020) or MPs (Binelli et al., 2020) which provide great value for the understanding of the interactions between species, habitat and proximity to the source of MPs pollution (Windsor et al., 2019). However, MPs contamination affect freshwater ecosystems in space and time, thus field research should reflect spatial and temporal variability of MPs in the environment and interactions with freshwater biota.

3.2. Multiple stressors: do microplastics increase the hazard?

The impact of MPs on freshwater biota linked to other anthropogenic stressors may be neutral, synergistic or antagonistic. Microplastics shape and size influences bioavailability, but the uptake will depend on ecological and physiological functionalities e.g. species feeding strategies or developmental stage. Weak evidence on combined toxicity between MPs/NPs and pesticides (Table 2), may be attributed to chemical dilution in the aqueous media, polymer type, chemical-MPs binding, material, experimental conditions, or exposure and age of the MPs (Luo et al., 2019a, 2020; Ma et al., 2020), but how species at various stages of development will respond, is still in need of further investigation. In fact, Gerdes et al. (2019) demonstrated that MPs can act as sink of organic contaminants by removing PCB from previously PCB-loaded *D. magna* individuals. Jackson et al. (2021) present a seminal theoretical framework to investigate temporal dynamics of multiple stressors (such as, invasions and temperature) and assess their impacts on ecosystems. They make a compelling case for the importance of considering sequence and overlap (in time) of past stressors in influencing future responses of individuals and ecosystems. This temporal approach should be applied to MPs and NPs research where stressor-response relationships are not consistent through time reflecting a) the varying pathways and fate of MPs/NNPs into freshwaters, b) the differing metabolic rates of target species, and c) the differing time scales over which individuals operate, including feeding and reproduction.

Pesticides and other organic pollutants are adsorbed onto MPs depending on their polymer type and binding affinities. Horton et al. (2018b) exposed *Daphnia magna* to two pesticides at six concentrations with low and high binding affinity (log Kow): dimethoate and deltamethrin; and PS-MPs beads (1–1.4 µm), in an acute toxicity experiment (72 h). Results did not support altered toxicity of pesticides to *D. magna* in combination with MPs, regardless of chemical binding affinity (log Kow). The authors stated that MPs are unlikely to act as vector for increased uptake of pesticides by aquatic organisms. By contrast, life history traits of *D. magna* were dramatically modified (51% offspring reduction) by exposure to a combination of the pesticide deltamethrin (40 ng/L) and MPs (1 mg L⁻¹, 1–4 µm) when compared to MPs or the pesticide exposure alone (Felten et al., 2020). Other arguments regarding the lack of conclusive findings on chemical leaching or additive toxicity might be attributed to bioavailability, weakness of chemical-MPs binding, material of MPs, time exposure of the MPs to the chemicals used in the laboratory and age/weathering of the MPs (Luo et al., 2020; Ma et al., 2020).

Testing of the combined toxicity of PVC-MPs and the pesticide imidacloprid in an extended-time period toxicity experiment (28 d) with *Chironomus riparius* failed to provide evidence for enhanced toxicity of MPs when compared to natural particles i.e. kaolin and the pesticide. Having said this, toxicity was observed, at concentrations not yet seen in the environment, the authors suggested that the results indicated a high tolerance of *C. riparius* to the effects of PVC-MPs (Scherer et al., 2020).

Horton et al. (2020) exposed *Lymnaea stagnalis* to polybrominated diphenyl ethers (PBDE) and PA-MPs (13–19 µm) to investigate potential bioaccumulation but found no supporting evidence for either toxicity of PA-MPs or enhanced bioaccumulation of PBDEs in the presence of PA-MPs. Information on organic pollutants adsorption onto MPs has been published (Fred-Ahmadu et al., 2020; Liu et al., 2019; Mao et al.,

2018), as well as research addressing the issue of inner body desorption/absorption of organic pollutants bound to MPs due to enzymatic processes i.e. pH changes under real (Bakir et al., 2014) or simulated physiological conditions (Lee et al., 2019; Mohamed Nor and Koelmans, 2019), however, gaps still exist here for other model organisms which might lead to understand if similar mechanisms could take place in humans.

Existing research on the combined impact of MPs and climate change or increased temperature, with MPs on freshwater ecosystem processes is scant (Table 2). One of the first studies exposed an Amazonian cichlid *Symphysodon aequifasciatus* to temperatures of 28–31 °C and concentrations of 200 µg L⁻¹ of PE-MPs (size 70–88 µm) for 30 days. Although microplastics (rather than temperature) affected the predatory performance, digestion and energy production of *S. aequifasciatus* adults, juvenile survival and growth were not significantly impacted (Wen et al., 2018). *Daphnia magna*, *D. pulex* and *Ceriodaphnia dubia* were exposed to primary and secondary MPs (1–10 µm) at three temperatures (18, 22 and 26 °C) during an acute toxicity experiment (72–96 h), and it was found that sensitivity was temperature and time dependent for *Daphnia* species (Jaikumar et al., 2018). The exposure of the benthic detritivore *Gammarus pulex* showed that the negative effects of MPs concentration on metabolic rate only manifested at higher temperatures, highlighting the potential for climate change or even seasonal fluctuations in environmental temperature to alter MPs effects on organismal physiology (Kratina et al., 2019). A similar scenario was found by Sadler et al. (2019) who demonstrated that phenotypical different responses to increased temperature in the presence of MPs were underpinned by *Daphnia* genetic variation, strongly suggesting the synergistic effects of MPs contamination and climate change on this primary consumer.

Not all studies on climate change and MPs point towards clear synergistic effects. In a recent study by Weber et al. (2020), *Dreissena polymorpha* was exposed (14 days) to a set of temperatures (14, 23 or 27 °C) combined with a maximum concentration of 100,000 particles/ml of polystyrene MPs fragments (2–60 µm). The results indicate that MPs have minor effects on the freshwater mussels used in the experiment compared to thermal stress, singly or combined. The limited MPs toxicity could respond to adaptive evolutionary processes to suspended solids as shown when compared to a natural suspended solid (diatomite), something similar was observed by Scherer et al. (2020) for *Chironomus riparius* exposed to kaolin. Where species or whole communities (e.g. vulnerable ecosystems in remote regions) are particularly sensitive to stressors, multi-stressor impacts as a result of microplastics exposure may be greater than in resilient communities that regularly encounter environmental change (Jackson et al., 2021).

Metals adsorption on plastic is facilitated by photo-oxidative weathering that increases surface polarity and charge with formation of oxygen containing functional groups such as ketones, alcohols, and aldehydes. However, not all MPs type are equally reactive, with high-density polyethylene (HDPE) with the lower metal adsorption capability (Naqash et al., 2020). All four studies investigating metals and MPs report synergistic toxicity effects (Table 2).

3.3. Biofilms

Biofilm formation and microbial attachment processes on the surface of MPs can result in density changes that in turn could influence their distribution, transport from the water column to the riverbed and bioavailability (Möhlenkamp et al., 2018; Blair et al., 2019; C. Li et al., 2020; S. Li et al., 2020). The residence times of MPs in the water column control the removal pathways either by incorporation in the food chain for planktic organisms or natural removal by sedimentation (Nguyen et al., 2020), thereby being either buried in the sediments or incorporated in the food chain of benthic organisms. Biofilm development on MPs will follow seasonal succession, hence abiotic factors such as water temperature, irradiance and hydrodynamics will affect

both growth and sinking rates (Chen et al., 2019). During the growth phase of the biofilm, minerals and/or NPs, can be trapped within the exopolysaccharide (EPS) produced by bacteria, cyanobacteria and microalgae modifying sinking rates of various types of MPs (Chen et al., 2019).

Microbial selective adhesion on MPs were reported by Miao et al. (2019a) and Mughini-Gras et al. (2021), where the microbial community composition clearly differed among MPs colonized biofilms compared to natural substrates. These authors collected natural microorganisms from a stream exposing them in the laboratory to PE-MPs, PP-MPs and natural substrates for 21 days. Di Pippo et al. (2020) reported that microbial biofilm composition from colonized MPs differed from the corresponding planktonic population and suggested that associated biofilms to MPs are microorganisms from generalist taxa but no evidence of selective microorganism attachment to the MPs was found. This was a field study where MPs from seven lakes were sampled, and associated biofilm communities analysed. In another laboratory experiment, Miao et al. (2019b) demonstrated that MPs size and surface modification may determine the impact of PS-MPs on biofilm communities. Toxicity effects from NPs (100 nm) to the biofilms were oxidative stress, and inhibition of enzymatic processes mediating carbon and nitrogen cycling which are essential for ecosystem functioning of lotic ecosystems.

Biofilm community composition can be modified by the type of MPs and biofilms can modify MPs/NPs surface by attaining a greater surface area, thus enhancing with time the accumulation of metals and anthropogenic hazardous contaminants (Naqash et al., 2020). Freshwater aggregates of various sizes, shapes, density and composition are formed by biofilms, detritus, EPS, minerals and other aggregates including MPs/NNPs (Zhang and Chen, 2020). Microplastics aggregates can be easily confused with food particles being ingested by consumers, directly or indirectly when adhered to other organisms or vegetative structures, and thus transferred into the foodweb (Arias-Andres et al., 2019; Roch et al., 2020). Some species, e.g. *Gammarus pulex* (a generalist) actively avoid ingesting fibres (Yardy and Callaghan, 2020) while other species have shown gender differences on their tendency to ingest more MPs (Horton et al., 2018a; Su et al., 2019). These examples appear to be the exception rather than the norm, with more studies on behavioural intake of MPs and biofilm colonized MPs needed for a better understanding of MPs pathways to the foodweb and their impact could be made.

These aggregates can also act as vectors of pathogen distribution and antimicrobial gene transfer (Wu et al., 2019; Mughini-Gras et al., 2021). Pathogens from the genus *Pseudomonas* were selectively hosted by PVC-MPs colonized biofilm, opportunistic to humans (*P. monteilii* and *P. mendocina*) and a plant pathogen (*P. syringae*). González-Pleiter et al. (2021), demonstrated that the sorption and desorption of antibiotics (azithromycin and clarithromycin) on/from MPs depended on the hydrophobicity of the antibiotic but was independent of the type of MP used. Toxicity was tested and observed against cyanobacterium *Anabaena* sp. Therefore, antibiotic loaded MPs act as a biocide reservoir which can influence the composition of biofilm microbial communities, increase antimicrobial resistance, and affect primary producers and the foodweb, ultimately altering freshwater ecosystems.

3.4. Photoautotrophs

3.4.1. Macrophytes

Aquatic macrophytes are defined as the vast group of emergent, submerged and floating phototrophs including vascular plants, mosses, liverworts and macro-algae responsible for much of the primary production of inland and coastal waters (Hughes, 2018). Macrophytes provide a habitat for a range of functional groups, including periphyton, zooplankton, invertebrates, fish and frogs (Bornette and Puijalon, 2009); and widely distributed in freshwater ecosystems. Therefore likely, given the widespread MPs contamination of these systems, that

macrophytes will interact with microplastics (Kalčíková, 2020). Despite their vital importance, a handful of studies have studied MPs ecotoxicological effects on macrophytes. van Weert et al. (2019) demonstrated that MPs and NPs affect the growth of sediment-rooted macrophytes *Myriophyllum spicatum* and *Elodea* sp. exposed to five doses of polystyrene (PS) NPs (PS-NPs) (50–190 nm) 3% sediment dry weight (sediment weight approx. 350 g) and four doses' PS-MPs (20–500 µm) 10% sediment dry weight (Table 3). Most effects were observed with NPs but shoot length was reduced for *M. spicatum* with increasing MPs concentration. However, the concentration of MPs and NPs in the sediment were too high, therefore not realistic to be considered an ecological risk (van Weert et al., 2019). *Lemna minor* roots were also shortened when exposed to PET-MPs (~100 µm, 60 mg L⁻¹ or ~435 mg kg⁻¹) in mesocosm experiments conducted for 36 days by Green et al. (2021). Polystyrene NPs of 100 nm size adsorbed on the spore surface of aquatic fern *Ceratopteris pteridoides*, inhibited spore size and germination, and entered the roots of gametophytes (Yuan et al., 2019).

3.4.2. Microalgae and cyanobacteria

Microalgae are the primary producers of aquatic ecosystems and alterations on their populations by MPs can further disrupt the balance of foodwebs in already imbalanced and stressed freshwater ecosystems (Lüring et al., 2016). Bhattacharya et al. (2010) provided the pioneering work of MPs direct impact on microalgae i.e. impaired photosynthesis by shading effect; increased EPS production as a response to MPs and decreased energetic budget for other processes (Table 3). To understand how MPs/NPs could bind to microalgae, Nolte et al. (2017) investigated the impact of charged carboxylate-modified polystyrene (PS) MPs/NPs PS-MPs (100–500 nm) and PS-NPs (20-nm) on a microalgae. Binding affinity was function of inner-wall and inter-particle interactions where positively or neutrally charged MPs/NPs adhered to *Pseudokirchneriella subcapitata* cell walls while negative charged particles did not. Adsorption onto the cell wall may be also influenced by the hardness of the media and particle concentration. Direct toxicity of MPs can alter the structure of photosynthetic complexes of microalgae (e.g. *Chlorella sorokiniana*) by changing the chloroplasts' fatty acids, potentially lessening food quality of microalgae in the foodweb. Moreover, alterations on the permeability of the lipidic cell membrane could weaken microalgae resistance to other stressors with further implications for foodweb sustainability (Guschina et al., 2020). Gao et al. (2021) provide a review of MPs and NPs on autotrophs in marine and freshwater ecosystems stating that only Prata et al. (2019) had reviewed the impact of MPs on microalgae before them. Gao et al. (2021) statistically reviewed the effects of exposure on five important responses of microalgae with most studies on growth. Twenty-seven publications were found on the MPs effect on the Photosystem II capacity as determined by the maximum quantum yield, Fv/Fm; but only five papers were found on reactive oxygen species (ROS) production, superoxide dismutase (SOD) levels (involved in detoxification of ROS), catalase (CAT) and levels of malondialdehyde (MDA, an oxidation product of ROS attack on lipids) for freshwater microalgae (Zheng et al., 2021; Xiao et al., 2020). Their general findings indicated that effects increased with high concentration of MPs or NPs while at lower concentrations microalgae can activate anti-stress mechanisms to revert adverse effects. Positively charged MPs or NNPs affected microalgae at low concentrations (<1 mg L⁻¹).

Other mechanisms, i.e. indirect toxicity, were described by Shen et al. (2020) where MPs may affect the biological and carbon pump sequestration (CO₂). Biological sequestration is the process by which phytoplankton transforms inorganic carbon into particulate organic carbon (POC) through photosynthesis, self-deposition and zooplankton feeding, and POC ultimately is transmitted to the deep waters. Hence, if photosynthesis in microalgae is impaired the whole biological and carbon sequestration process is compromised. Another example of indirect toxicity occurs when the consumption of phytoplankton (microalgae) by primary or secondary consumers (top-down mechanisms) is affected

Table 3

Relation of MPs and/or NPs toxicity experiments photoautotrophs (macrophytes, microalgae and cyanobacteria) as model organism; research approach (field or mesocosm); MPs and/or NPs shape, chemical type and size; exposure (time and concentration) and toxicity effects.

Category	Organism/species	Research approach	MP or NPS material & size	Exposure to MPs	MP concentration	Effect	Reference
Plant (Macrophyte)	<i>Myriophyllum spicatum</i> <i>Elodea</i> sp.	Mesocosm	PS-NPs (50–190 nm) & PS-MPs (20–500 µm)	21 d	0.1, 0.3, 1 and 3% sediment DW (~350 g) & 0.03, 0.1, 0.3, 1 and 10% sediment DW (350 g)	Shoot length reduced with NPs and high doses of MPs. Not ecological risk demonstrated.	van Weert et al., 2019
Plant (Macrophyte)	<i>Lemna minor</i>	Mesocosm	PET-MPs (~100 µm)	36 d	~435 mg kg ⁻¹	Root shortening was significant compared to controls.	Green et al., 2021
Phytoplankton	<i>Chlorella vulgaris</i>	Microcosms	PUF-MPs Foam 3 mm ³ (aged × 12 months)	48 h	0.7 g MPs	Photosynthetic efficiency decreased with increasing leachate concentrations. Leachate increased with increased pH and exposure time.	Luo et al., 2019b
Phytoplankton	<i>Pseudokirchneriella subcapitata</i>	Laboratory	MPs (20–500 nm) NPs (<1 µm)	2 h	10 mg L ⁻¹	Particles found adhered to microalgae cell wall, potential indirect toxicity effects: shading leaching effects.	Nolte et al., 2017
Phytoplankton		Mesocosm				Shading impaired photosynthesis; affected metabolic processes: increased exopolysaccharides production & hampered the energetic budget.	Bhattacharya et al., 2010
Phytoplankton	<i>Microcystis aeruginosa</i>	Laboratory	PVC-MPs PS-MPs PE-MPs (3 µm)	96 h	10, 25, 50, 100 & 200 mg L ⁻¹	All types of MPs affected growth, increased cyanotoxin production, affected cell membrane integrity and function of superoxide dismutase and catalase.	Zheng et al., 2021
Phytoplankton	<i>Scenedesmus quadricauda</i>	Laboratory	PS-MPs (1.0, 2.0, 3.0, 4.0 & 5.0 µm)	72 h	10 mg L ⁻¹	Internalization of MPs size 1 µm (43.3%) and 2 µm (15.3%) with significant reduction in the density of <i>S. quadricauda</i> affecting photosynthetic processes.	Y. Chen et al., 2020
Phytoplankton	<i>Euglena gracilis</i>	Laboratory	PS-MPs (0.1 & 5 µm)	24 h	1 mg L ⁻¹	Reduction of pigments, evidence of enzymatic oxidative stress and dysregulation of genes at molecular level.	Xiao et al., 2020
Phytoplankton	<i>Chlamydomonas reinhardtii</i>	Laboratory	PS-NPs	8 d	2 mg L ⁻¹	PS-NPs affected growth and carbohydrate biochemical composition of <i>C. reinhardtii</i> .	Déniel et al., 2020
Phytoplankton	<i>Chlorella sorokiniana</i>	Laboratory	PS-MPs (<70 µm)	4 w	60 mg L ⁻¹	Direct toxicity. MPs weakens membrane permeability, more vulnerable to stressors.	Guschina et al., 2020

Hour (h), days (d), week (w), year (y); microplastics (MPs), nanoplastics (NPs), polyvinyl chloride (PVC), polystyrenes (PS), polyethylene (PE), polyamide (PA).

as result of changes in the consumers' physiology or behaviour (Barbosa et al., 2020). Consequently, the trophic cascade might be broken at two levels: planktivorous fish population decreasing in the level-up and phytoplankton increasing in the level-down, with the potential formation of microalgae/cyanobacteria blooms and the well-known consequences for freshwater ecosystems (Foley et al., 2018; Yokota et al., 2017).

The majority of laboratory studies conducted to determine toxicity of NPs to various species involve nano-scale polystyrene particles (PS) due to its ease to obtain, low cost and high prevalence (Eerkes-Medrano et al., 2015; Déniel et al., 2020). Toxicity of aged versus pristine PCV-MPs/NPs was tested against *C. reinhardtii* and demonstrated by the increase in the enzymatic activity of superoxide dismutase (SOD) and malondialdehyde (MDA), as well as a reduction in chlorophyll content (Wang et al., 2020).

Research on macrophytes and planktic primary producers in relation to MPs impact is slowly building up. The impact of MPs on periphytic primary producers has been indirectly approached by Boyero et al. (2020) during their main investigation with amphibians. Benthic diatoms are important autotrophs for lotic systems, structural integrant of biofilm communities and producers for benthic consumers and are well-known bioindicators of nutrients and metal pollution. By contrast, freshwater benthic diatoms have not been assessed on the ecotoxicity of MPs/NPs.

3.5. Consumers - invertebrates

3.5.1. Planktic consumers

The impact MPs could have on primary consumers (e.g. ciliates – *Vorticella* sp.; rotifers – *Brachyonus calyciflorus*; crustaceans – *Daphnia* sp.) remains unclear with contradictory findings (Scherer et al., 2017; Xue

et al., 2021). Differences in primary consumer susceptibility to MPs lie in their feeding strategies (Fueser et al., 2019), on the polymer type, size and shape, and providing that there is enough exposure and retention time after ingestion (Jemec et al., 2016; Scherer et al., 2017; De Felice et al., 2019; Schrank et al., 2019). Evidence shows that fibres have greater retention time than beads in the digestive system of invertebrates to cause direct (i.e. digestive obstruction) or indirect impact (i.e. affecting food assimilation rates) (Blarer and Burkhardt-Holm, 2016; Foley et al., 2018). Physiological (i.e. larger body size) and behavioural changes (i.e. swimming activity and phototactic sensitivity) for *D. magna* were found after chronic exposure to MPs beads (10 µm) (De Felice et al., 2019). Further supporting evidence comes from Schrank et al. (2019) who observed *D. magna* body enlargement after exposure to polyvinyl chloride (PVC) MPs (PVC-MPs) fragments (Table 4). These results were controversial for the use of high MPs concentrations said to be unlikely found in the environment. However, Binelli et al. (2020) point out that research addressing quantification of MPs (<100 µm) in the environment is scarce and abundances of small MPs and NPs particles are likely to be proportionally higher than the concentrations of larger particles commonly reported.

Aljaibachi et al. (2020) conducted a long-term experiment (12 weeks) where *D. magna* was exposed to MPs (15 µm) in the field experimental mesocosm and laboratory. *D. magna* population during the first seven weeks of the experiment declined but recovered later with *Daphnia* offspring. The authors stated that the most relevant factor for *Daphnia* population growth and survival was the microalgae availability in the presence of natural competitors colonizing the mesocosm, rather than the presence of MPs. It should be noted that Aljaibachi et al. (2020) experiment showed the potential effects on a long-term population mimicking natural populations of *D. magna*. The population declined in the controls after eight weeks while in the treatment with MPs

Table 4
Single MPs or NPs toxicity experiments on planktic and benthic consumers.

Category	Organism	Research approach	MP or NPS material & size	Exposure to MPs	MP concentration	Effect	Reference
Planktic consumer	<i>Daphnia magna</i>	Laboratory	PS-NPS beads (70 nm)	21 d	0.22–103 mg L ⁻¹	Reduced body size, reproduction & malformation of neonates (≥ 30 mg L ⁻¹)	Besseling et al., 2014;
Benthic consumer	<i>Ceriodaphnia dubia</i>	Laboratory	PE-MPs-beads (1–4 μ m) PS-MPs- fibres	48 h 8 d	0.5–16 mg L ⁻¹ or 1.7×10^4 – 5.4×10^5 beads L ⁻¹ 0.125–4 mg L ⁻¹ or 1.1×10^3 – 3.4×10^4 fibres L ⁻¹	MPs fibres caused deformities. Effects were dose-dependent for both beads and fibres with 50% reduction brood with higher than reported environmental MPs concentrations. Fibres had consistently greater negative effects than beads.	Ziajehromi et al., 2017
Planktic consumer	<i>D. magna</i>	Laboratory	MPs-beads (1–10 μ m)	21 d	0.125, 1.25 & 12.5 mg L ⁻¹	Physiological (i.e. larger body size) and behavioural changes (i.e. swimming activity and phototactic sensitivity)	De Felice et al., 2019
Planktic consumer	<i>D. magna</i>	Laboratory	PVC-MPs fragments (12–274 μ m)	31 d	0.1–0.15 mg L ⁻¹	Body shrinkage and reduced brood. Body shrinkage was observed after 21 days exposure.	Schrank et al., 2019
Planktic/benthic consumer	<i>Chironomus riparius</i>	Laboratory	PS-MPs, PET-MPs, PVC-MPs, PA-MPs (20–100 μ m)	12 d	Environmental concentrations	Morphological deformities in larval mandibles, mentus and female wings development	Stanković et al., 2020
Planktic consumer	<i>D. galeata</i>	Laboratory	PS-NPs beads (52 nm)	5 d	5 mg L ⁻¹	Survival and reproduction were significantly decreased. Embryos showed abnormal development and a low hatching rate. Lipid storage was reduced in exposed adults, but not in pregnant individuals. Internalization of NPs was observed in adults only.	Cui et al., 2017
Planktic consumer	<i>D. magna</i>	Laboratory	PA-MPs, PET-MPs, PVC-MPs beads (~40 μ m)	48 h	1% of food ~33 \pm 22 particles	Effects of microplastics were seen in adults at molecular level with alterations in gene expression related to stress. Juvenile individuals showed small responses on the morphological traits (body length, width and tail spine length).	Imhof et al., 2017
Planktic consumer	<i>D. magna</i>	Laboratory	PS-NH ₂ -NPs (53 nm) PS-COOH-NPs (26–62 nm)	103 d	0.32 mg L ⁻¹ 3.2 & 0.32 mg L ⁻¹ 7.6, 3.2 & 0.32 mg L ⁻¹	<i>D. magna</i> showed an increased mortality with aminated NPs compared to controls at 0.32 mg L ⁻¹ . First study showing the lowest lethal concentration compared to previous 25 mg L ⁻¹ used in acute test with aminated NPs. Carboxylated NPs showed to increase toxicity with 26 nm and long-term exposure.	Kelpsiene et al., 2020
Planktic consumer	<i>D. magna</i>	Laboratory & mesocosm	PS-MPs beads (15 μ m)	12 w	0–800 MPs mL ⁻¹	Population declined during the first seven weeks but recovered later. MPs effect in a natural situation is unpredictable, environmental conditions and invertebrate communities may add additional stresses.	Aljaibachi et al., 2020
Planktic consumer	<i>D. magna</i>	Laboratory	PS-MPs beads (1–5 μ m)	21 d	10 ² to 10 ⁵ particles mL ⁻¹	Adult population decreased with a 21% reduction in total biomass compared to control. However, there were no clear pattern of effect for the juveniles and neonates.	Bosker et al., 2019
Planktic Benthic consumer	<i>D. magna</i> <i>C. riparius</i> <i>Physella acuta</i> <i>Gammarus pulex</i> <i>Lumbriculus variegatus</i>	Laboratory	PS-MPS beads (1, 10 & 90 μ m)	3–24 h	3–3000 particles mL ⁻¹	Pioneers at demonstrating that freshwater invertebrates have the capacity to ingest microplastics. Ingestion rates were optimized for each species according to their development age. However, the quantity of uptake depends on their feeding type and morphology as well as on the availability of microplastics. The presence of natural particles reduced the intake of microplastics.	Scherer et al., 2017
Planktic Benthic consumer	<i>Gammarus pulex</i> , <i>Hyalella azteca</i> <i>Asellus aquaticus</i> <i>Sphaerium corneum</i> <i>Tubifex</i> spp. <i>Lumbricus variegatus</i>	Laboratory organisms collected from various sources	PS-MPs beads (20–500 μ m)	28 d	211 g/w sediment 0, 0.1, 1, 5, 10, 20, 30 and 40% MPs w/sediment	No evidence to support toxicity. Organisms collected from various sources: brook, ditch, pond, Wageningen Environmental Research and a pet shop.	Redondo-Hasselerharm et al., 2018

Table 4 (continued)

Category	Organism	Research approach	MP or NPS material & size	Exposure to MPs	MP concentration	Effect	Reference
Planktic consumer	<i>D. magna</i>	Laboratory	PS-MPs beads ($\leq 100 \mu\text{m}$)	7 d	$8.4 \pm 0.5 \text{ mg L}^{-1}$	Hydrodynamic conditions play a role in the ingestion of MPs to food ratios. Calm conditions and length of exposure to MPs increases mortality in <i>D. magna</i> .	Colomer et al., 2019
Planktic consumer	<i>D. magna</i>	Laboratory	PS-NH ₂ -NPs PS-COOH-NPs	24 h	$0.1 \mu\text{g mL}^{-1}$ to 1 mg mL^{-1}	Demonstrated that the interactions between protein secretion by <i>D. magna</i> and modified PS-NPs leads to the formation of an eco-corona on PS-NPs, increasing their uptake and retention in the gut. Thus, affecting <i>D. magna</i> ability to feed over six hours.	Nasser and Lynch, 2016
Benthic consumer	<i>Gammarus fossarum</i>	Laboratory	PA-MPs-Fibres ($500 \times 20 \mu\text{m}$) PS-MPS-beads ($1.6 \mu\text{m}$)	0.5 32 h 28 d	100, 540, 2680, 13,380 fibres cm^{-2} 500, 2500, 12,500, 60,000 beads mL^{-1}	Ingestion and egestion of fibres and beads, but significantly reduced the assimilation efficiency of the animals.	Blarer and Burkhardt-Holm, 2016
Benthic consumer	<i>Chironomus tepperi</i>	Laboratory	PE-MPs beads (1–4, 10–27, 43–54 & 100–126 mm)	5–10 d	500 MPs Kg^{-1} sediment	Toxicity effects were strongly dependent on MPs size. The environmental relevant concentrations used in the experiment induced morphological changes e.g. small heads and antennae, reduced emergence of adults.	Ziajahromi et al., 2018
Benthic consumer	<i>Elliptio complanate</i>	Laboratory with field individuals	PS-NPs (50 nm)	24 h	0.1, 0.5, 1 & 5 mg L^{-1} 1 mg L^{-1} - 2.575×10^{10} particles L^{-1}	Results demonstrated that NPs could change the biophysical properties of the cytoplasm such as the fractal organization of the intracellular environment during the reaction.	Auclair and Gagné, 2020
Benthic consumer	<i>G. pulex</i>	Laboratory G. pulex from River	PA-MPs fibres (200–500 μm)	4 h	Not specified	No effect. <i>G. pulex</i> actively avoids ingesting MPs fibres	Yardy and Callaghan, 2020
Benthic consumers	<i>Cryptorhestia garbinii</i>	Field Lakes	MPs fragments $25 \mu\text{m W} \times 55 \mu\text{m L}$	n/a	n/a	Ingestion in natural conditions confirmed with findings of 1.8–5 MPs (various shapes) in <i>C. garbinii</i> individuals from three lakes.	Iannilli et al., 2020
Planktic/benthic consumer	<i>C. riparius</i>	Laboratory	PA-MPs beads 10–180 μm	28 d	100 mg PA kg^{-1} or 10,100 MPs kg^{-1} sediment.	Toxicity experiment carefully designed according to existing OECD standards for which did not support any evidence of adverse effects on any of the stages of <i>C. riparius</i> life cycle.	Khosrovyan and Kahru, 2020
Planktic/benthic consumer	<i>C. riparius</i>	Laboratory Microcosm	PE-MPs fragments	28 d	0.1% w/w	Showed the interference of microplastics on the cycling of nitrogen from sediments, thus their impact for biogeochemical cycling and ecosystem function.	W. Huang et al., 2021; Y. Huang et al., 2021
Benthic consumer	<i>Hydra viridissima</i>	Laboratory	PMMA-NPs (40 nm)	0, 24 & 96 h	1, 10 and 40 mg L^{-1} 1, 5, 10, 20, 40, 80, 160, 320, 640 mg L^{-1}	Toxicity for this species varied depending on exposure and NPs concentration. Mortality was observed after 72 h at 80 mg L^{-1} . Morphological changes were observed at lower concentrations (40 mg L^{-1}).	Venancio et al., 2021
Benthic consumer	<i>Aphylla williamsoni</i>	Laboratory	PS-NPs beads	48 h	34 $\mu\text{g L}^{-1}$	Evidence of bioaccumulation associated with REDOX imbalance reflected by the increase in the number of oxidative stress biomarkers and antioxidants. Adding to this, the authors suggested the neurotoxic effect by the reduced activity of acetylcholinesterase observed.	Guimarães et al., 2021
Benthic consumer	<i>Dreissena bugensis</i>	Laboratory	PE-MPs beads (10–45 μm)	24 h	0.0-to 0.8 g L^{-1}	Retention of MPs was shown after 24 h of ingestion but no effects on survival, reproduction or oxygen consumption rates were observed. Over time MPs can decrease filtration rates and potentially reducing overall fitness.	Pedersen et al., 2020
Benthic consumers	<i>Caenorhabditis elegans</i> <i>Panagrolaimus thienemanni</i> <i>regenfussi</i> <i>Plectus acuminatus</i> <i>Poikilolaimus regenfussi</i> <i>Acrobeloides</i>	Laboratory	PS-MPs beads (0.5, 1.0, 3.0 and 6.0 μm)	4, 24 and 72 h	3×10^6 - 10^7 MPs mL^{-1}	Ingestion of beads was nematode-buccal cavity size dependent and transported into the gastrointestinal tract if the average size of the buccal cavity was >1.3 times than the beads. Ingestion rates was time and concentration exposure dependent.	Fueser et al., 2019 Mueller et al., 2020

(continued on next page)

Table 4 (continued)

Category	Organism	Research approach	MP or NPS material & size	Exposure to MPs	MP concentration	Effect	Reference
Benthic consumers	<i>nanus</i> <i>Pristionchus pacificus</i> <i>Gyraulus albus</i> <i>Hippeutis complanatus</i> <i>Valvata</i> sp. <i>Naididae</i> <i>Orthocladinae</i>	Field Mesocosm	PS-NPs beads (96.3 ± 1.85 nm) PS-MPs fragments (20–516 µm)	15 m	0.0, 0.005, 0.05, 0.5 & 5% (0.1%–1 g Kg ⁻¹)	A long-term macroinvertebrate community experiment with environmentally realistic NPs/MPs concentrations (0.005 & 0.05%). Abundance was generally species specific. Significant differences for NPs or MPs treatment were found for <i>Valvata</i> and <i>G. albus</i> . Naididae species did not differ under NPs or MPs. Orthocladinae and <i>H. complanatus</i> had lower abundances	Redondo-Hasselerharm et al., 2020
Benthic consumer	<i>Dreissena polymorpha</i>	Laboratory	PS-MPs (1–10 µm)	6 d	5 × 10 ⁵ MPs L ⁻¹ & 2 × 10 ⁶ MPs L ⁻¹	Despite using primary MPs, results showed that even the low concentrations of MPs induced changes in the modulation of gill proteins involved in the response to oxidative stress,	Magni et al., 2019
Benthic consumer	<i>Dreissena polymorpha</i>	Field MPs Mesocosm	PS-MPs, PP-MPs fibres & fragments (15 µm–2.97 mm)	7 d		Direct toxicological effects on the mussels	Binelli et al., 2020

Hour (h), days (d), months (m), year (y); microplastics (MPs), nanoplastics (NPs), polystyrenes (PS), polyethylene (PE), polyethylene-terephthalate (PET), polymethylacrilate (PMMA), polypropylene (PP), polyvinyl-chloride (PVC), polyamide (PA).

declined after three weeks. This may suggest similar implications of long-term exposure for other aquatic organisms and should be investigated further for a better understanding of the risks and hazards from MPS/NPs exposure in the environment. Xue et al. (2021) demonstrated that PE-MPs (10–22 µm) induce behavioural (low algae ingestion) and metabolic changes: oxidative stress and cell membrane damages; these toxicity effects will affect the reproductive metabolism of the rotifer *Brachionus calyciflorus* in the long term.

One caveat of the majority of experimental ecotoxicity studies is that exposure concentrations are generally much higher than those currently reported within the environment (De Felice et al., 2019; Felten et al., 2020; Scherer et al., 2020; Schrank et al., 2019; Stanković et al., 2020). Nonetheless, many particles used in exposures are of a size range that cannot currently be easily measured within environmental samples, and thus we do not have a good handle on real environmental concentrations of these. Further, high-concentration exposures allow for the determination of toxicity thresholds for different species, which is important for determining current and future risk.

3.5.2. Benthic consumers

The ubiquity of MPs in benthic habitats is probably a greater determinant for ingestion by aquatic species than MPs suspended in the water, transferability of contaminants in the sediment to benthic invertebrates/detritivores and to the foodweb (Turner et al., 2019). Microplastics were found to adhere to leaf litter and be ingested by the detritivore *Sericostoma pyrenaicum*, an aquatic insect (López-Rojó et al., 2020); accumulating in digestive and reproductive systems of different trophic freshwater organisms such as *Hyalella azteca* (Au et al., 2015) and *Lumbricus variegatus* (Imhof et al., 2013), *Tubifex tubifex* (Hurley et al., 2017). However, despite this evidence of ingestion, Silva et al. (2021a) and previously Redondo-Hasselerharm et al. (2018) found no evidence to support toxicity for *L. variegatus*. The latter authors neither found evidence for *Gammarus pulex*, *H. azteca* (both shredders and also active swimmer), *Asellus aquaticus* (shredder and epibenthic), *Sphaerium corneum* (facultative filter-feeder and epibenthic), *Tubifex* spp. and *L. variegatus* (endobenthic) and MPs (20–500 µm) at environmentally relevant concentrations. Nematodes are the most abundant taxon in benthic habitats and as occupants of basal trophic levels their role on MPs trophic transferred has yet to be studied (Fueser et al.,

2019, 2020). These authors also demonstrated that the ingestion of MPs by nematodes depends on the size of their buccal cavity, time exposure and concentration of MPs in the media (Table 4).

while some evidence supports that the effect of MPs on crustaceans might be species-specific, other indicates that the shape of MPs is more relevant. *G. pulex* actively avoids ingesting MPs fibres (200–500 µm) (Yardy and Callaghan, 2020) but fibres were found to affect assimilation efficiency of *Gammarus fossarum* (Blarer and Burkhardt-Holm, 2016). A reduced reproductive output of *Ceriodaphnia dubia* was reported when exposed, but not ingested, microfibres (Ziajahromi et al., 2017). By contrast, microbeads did not affect *Gammarus duebeni* (Mateos-Cárdenas et al., 2019); and after exposure of *C. dubia* to MPs beads, Ziajahromi et al. (2017) did not find the deformities they observed when this organism was exposed to MPs fibres. Nevertheless, these findings suggest that further research should assess species sensitivity to MPs contamination and their contribution to biodiversity loss, which poses questions for their impact on the ecosystem processes balance.

Freshwater ecosystems receive a mixture of MPS/NPs shape and type with a very few studies exposing aquatic species to mixtures to be expected in natural environments. Stanković et al. (2020) conducted an experiment using a mixture of MPs, PS-MPs, polyethylene-terephthalate (PET), polyvinyl-chloride (PVC) and polyamide (PA) at environmental concentrations and size (20–100 µm) found morphological deformities in larval mandibles, mentus and female wings development of *C. riparius* larval stages (12 days). Silva et al. (2021b) tested *C. riparius* with PE-MPs (three size-classes within 32–500 µm) at concentrations reported from riverbanks in urbanized areas (1.25 and 5 g Kg⁻¹), and after 48 h exposure found adverse effects on cellular metabolism, redox status and antioxidant-detoxification defence mechanisms. These authors proposed that oxidative stress biomarkers and metabolic responses can be used as early warning indicators of acute stress to compare the sensitivity of difference species.

Windsor et al. (2019) studied two orders of insects, Ephemeroptera and Trichoptera in five sites in South Wales, UK within urban catchments receiving wastewater effluent discharge. Microplastics were identified in approximately 50% of macroinvertebrate samples collected from three families (n = 18): Baetidae, Heptageniidae (mayflies which feed upon periphyton) and Hydropsychidae (caddisflies which are generalist feeders) at concentrations up to 0.14 MPs mg tissue⁻¹ and they

occurred at all sites. Microplastics abundance was associated with macroinvertebrate biomass and taxonomic family, but MPs occurred independently of feeding strategy and biological traits such as habitat affinity and ecological niche. There is likelihood that MPs concentration and bioassimilation by these invertebrates might be related to high and low flows affecting pollutants dilution, thus low dilution of discharged effluents or urban drainage systems might increase MPs bioavailability and bioassimilation.

Microplastics collected from freshwater environments are rarely used in experimental work, the research conducted by Binelli et al. (2020) is unique. These researchers exposed *Dreissena polymorpha* (zebra mussel) to PS-MPs and polypropylene (PPE) MPs (PPE-MPs) fibres and fragments (15 μm – 2.97 mm) all collected from three lakes. The experiment was run in a laboratory mesocosm over 7 days. The outcome demonstrated the direct toxicological effects on biological pathways of the mussels but the caveat for the experiment was the high MPs concentration used that is considered to be approximately 20,000 higher than those found in real conditions.

3.6. Fish

Evidence for MPs ingestion and bioaccumulation by fish has been consolidated for a diversity of lotic, lentic, wetland and controlled environments. There are a number of factors influencing ingestion, bioavailability, and the degree of toxicity in fish which have been reviewed by Wang et al. (2019) and W. Wang et al. (2020). They highlight how the type of MP particle (e.g. colour, size and density) affects ingestion, together with habitat preference (pelagic, benthic or demersal). MP particles can cause blockages, toxic effects and malnutrition in laboratory fish, but the review cautions against inferring the same for fish in natural environments. The hydrophobicity of NPs can enable them to pass through embryo walls and bioaccumulate around lipids, as demonstrated by their presence in yolk sacks of juvenile fish (Chae et al., 2018). In this study it was also observed that direct exposure and ingestion of PS-NPs elicited changes in fish liver tissue. A proposed method of physical damage by NPs is by oxidative stress, though organic acids present in the environment may reduce potential damage (Liu et al., 2019).

Overall, ingestion of microplastics by freshwater fish is less widely studied than in the marine environment, but where studies exist, ingestion is widely observed. Ingestion of MPs is not necessarily correlated with high MPs pollution in rivers (Bosshart et al., 2020). These authors attributed the low detection in the round benthic goby, *Neogobius melanostomus* from the Rhine River to other factors, such as feeding behavior. Thirty-three percent of *Rutilus rutilus* sampled in the River Thames, UK, contained MPs, mainly fibres. The abundance of MPs in individuals strongly correlated with proximity to MPs source, body size and gender, with larger females ingesting more than smaller males (Horton et al., 2018a). Kuśmierk and Popiołek (2020) studied *R. rutilus* (n = 187) and *Gobio gobio* (n = 202) from the Widawa River in Central Europe, again confirming that fibres were the commonest MPs. Fibres ($\geq 500 \mu\text{m}$) were found in 50% of fish per species but did not support evidence for MPs uptake by sex.

Differentiation of MPs uptake based on habitat (lotic or lentic) has been observed for fish. For example, Hurt et al. (2020) collected fish specimens from two reservoirs in the USA (agricultural and urban) to study a filter feeder, *Dorosoma cepedianum* (n = 72), and a predator of *D. cepedianum*, *Micropterus salmoides* (n = 24). All fish studied contained MPs (20 particles per fish) which is the highest reported prevalence and concentration found in an organism for any aquatic environment, including marine systems. The results suggest that closed lake ecosystems in agricultural/urban landscapes may be particularly susceptible to MPs contamination and accumulation, findings that are reinforced by a study of urban lake sediments in North London, UK (Turner et al., 2019). An evaluation of the impact of MPs on fish was carried out from nine urban wetlands in Melbourne, Australia by Su et al.

(2019) who sampled eastern mosquitofish *Gambusia holbrooki* (n = 180). The results showed MPs uptake was just below 20%, with 0.6 MPs per individual, and uptake was proportional to size and weight of the fish. Furthermore, female individuals showed a tendency to ingest more MPs than males corroborating the findings of Horton et al. (2018a).

To consolidate understanding on feeding strategies and passive versus active uptake of MPs by fish, Roch et al. (2020) selected four fish species, wild and cultured, and with varying foraging style (visual vs. chemosensory): *Oncorhynchus mykiss*, *Tymallus thymallus*, *Cyprinus carpio*, and *Carassius carassius*. Fed and starved fish were exposed to a mixture of PP, PE, PS, PET and PVC MPs fragments (1–2 mm). All fish (n = 50 per species) were exposed to three environmentally relevant MPs concentrations (0.19, 1.9 and 9.1 MPs per litre). Starved fish were exposed to MPs for 2 h and fed fish for an hour. Factors linked to increased MPs uptake included: MPs concentration in the water, foraging behaviour, the availability of genuine food, and fish size. Wild fish discriminated between food and MPs better than cultured fish whereas chemosensory fish were better at discriminating inedible food, particularly when starved. This experiment highlights the complexity of factors interacting within the framework of understanding MPs and aquatic organisms in freshwater ecosystems.

Knowledge of MPs ingestion within wild-caught fish are crucial for understanding environmental exposure, however, no indication of potential hazards from the ingestion MPs chemical and physical toxicity. For a better understanding, laboratory experiments should investigate possible links and, cause-and-effect. Zebrafish (*Danio rerio*) is a model organism used by Lu et al. (2016) in toxicity testing experiments who found that zebrafish exposed to 5 μm PS-MPs accumulated the particles in the gills, gut and liver, while 20 μm PS-MPs only accumulated in the gills and gut. For 5 μm particles, resulting inflammation was observed in liver tissues. Oxidative stress was also observed, leading to alterations in metabolism. Ding et al. (2018) carried out a similar study using freshwater red tilapia (*Oreochromis niloticus*), although with smaller particles (0.1 μm PS). They saw similar accumulation in gills, gut and liver, and also within brain tissues. Although altered enzymatic activity was observed and the response varied over time, it was concluded that in this instance, the antioxidative enzymatic system prevented oxidative damage. These studies show that translocation of MPs and NPs within the body is particle size-dependent, and that toxicity may be either size or species dependent. Further, different polymer types were not considered, but are likely to cause significantly different effects.

One common criticism of MPs toxicity studies is that the (pristine) materials used are not fully representative of the (aged) materials that the organism will encounter within the environment. To this end, Pannetier et al. (2020) carried out a study specifically using environmentally-derived particles. They exposed Japanese medaka to concentrations of 0.01, 0.01 and 1% w/w MPs in food. While mortality was not observed, sublethal effects were seen including decreased growth and DNA damage. Interestingly, the most significant effects were observed at the lower concentrations. When subsequently comparing the toxicity of MPs from different environmental locations, significant differences in mortality were observed depending on the origin of the plastics collected. It is not clear if this is related to the particle size, polymer type or associated contaminants, as particle composition also varied. Nonetheless, this highlights the importance of the role that spatial variability can play in MPs exposure, and thus location-specific hazard.

Based on recent modelling efforts, sensitivity benchmark concentrations have been predicted for both zebrafish and red tilapia at ~ 1 and $\sim 119 \mu\text{g g}^{-1}$ body weight respectively (Chen et al., 2020). Based on predicted exposure to sources and sinks (Whitehead et al., 2021) and uptake (Hurt et al., 2020), there are concerns that the risks posed by microplastics may be unacceptably high in some geographical locations, for example in some regions of Asia where environmental contamination is high (Chen et al., 2020). Further research is also urgently

needed on the role that MPs play in transferring hazardous chemicals to fish, as well as pathogens. For example, the presence of MPs can modify the accumulation and toxicity of associated toxic chemicals to fish, *Oreochromis niloticus*, (compared to exposure to the chemical alone) (Zhang et al., 2019). However, these mechanisms are not yet well-understood.

3.7. Amphibians

Few studies on amphibian response to microplastics are published within our selection criteria. A recent review on the health risks that plastic particles represent for this group of vertebrates by da Costa Araújo et al. (2021) found 12 publications. da Costa Araújo et al. (2021) showed that a 57% of the research on MPs toxicity on amphibians is conducted in the laboratory addressing: feeding and elimination, morphological, histological, cytological and genetic changes; while field studies looked at behaviour and mortality; and uptake and accumulation was equally investigated in the field and laboratory. However, in contrast to the contradictory findings in other taxa (dependent on species and particle characteristics), almost all studies conducted on amphibians during their aquatic phase (tadpoles) support the toxic effect of MPs. Even with the caveat that the number of experiments is low, and with excessively high MPs concentrations yet to be found in the environment, most studies show high sensitivity of tadpoles of different species to MPs including the common midwife toad *Alytes obstetricans* (Boyero et al., 2020) and the South American frog species *Physalaemus cuvieri* (da Costa Araújo et al., 2020). An exception is that of the African clawed frog *Xenopus laevis*, for which bioaccumulation within the gut was observed, but toxic effects were not, even at high concentrations of $12.5 \mu\text{g mL}^{-1}$ PS-MPs in water (De Felice et al., 2019). The western clawed frog *X. tropicalis*, also accumulates microplastics in the gut under experimental conditions, although health effects were not measured (Hu et al., 2016).

When exposed to polyethylene fragments (mean size $35 \mu\text{m}$) at a concentration of 60 mg L^{-1} for seven days, tadpoles of *P. cuvieri* showed a significant number of biometric, morphological and cytological changes which is a clear indication of MPs/NPs translocation to tissues and incorporation into the cells with evident toxicity (da Costa Araújo et al., 2020). These researchers also confirmed MPs accumulation in the gills and digestive system seen in previous studies; and also translocation in the muscles of the tail and in the blood stream. Boyero et al. (2020) found that 1800 MPs/mL ($10 \mu\text{m}$ polyethylene) induced bioaccumulation in the gut and mortality of *A. obstetricans* tadpoles, a periphytic grazer amphibian. In their study they showed that the MPs adhered to the periphyton were ingested by tadpoles while feeding.

4. Research challenges

Freshwater ecosystems are complex with a diverse ecological niche, vast biodiversity and unknowns on the impact of MPs/NPs yet to be better understood. Working with living organisms is always going to be challenging, and more so if research is conducted with interacting organisms from various trophic levels. Nonetheless, adjustments for realistic assessments of MPs/NPs toxicity on freshwater biota e.g. MPs/NPs concentrations used and/or spatial and time scale, should be made to increase the body of literature on this fundamental topic, particularly on the impact of ecosystem functioning if toxicity leads to reduction of reproduction rates.

We synthesised and compared research on the ecotoxicity of MPs and NPs in organisms from a range of taxa and freshwater ecosystems. Data on exposure for organisms at lower trophic levels have increased since 2018 with most research focused on individual organisms from Crustacean, Fish Chlorophyte, Mollusca, and closely followed by Nematoda studies. *Daphnia magna* is the chosen toxicity model organism, accounting for 18% of the reviewed literature. While the use of model

organisms can be extremely useful in terms of the existence of agreed test guidelines and thus comparability between a large number of studies, the gap of knowledge on MPs/NPs toxicity studies for other organisms is particularly worrying because of the species-specific sensitivity to MPs/NPs toxicity demonstrated from various studies. Numerous key Taxa in ecosystem functioning are still understudied i.e. plants (macrophytes), benthic diatoms, cyanobacteria or protozoa to name a few.

While it is encouraging to see research efforts greater than 19% for planktic and benthic consumers, more attention is needed for the topics highlighted in this review and greatly understudied across all aspects of microplastics research (not just for freshwater) which are biofilms, trophic transfer, and multi-stressors mainly in the field but also in the laboratory. The nine studies on MP-associated biofilms point strongly to the potential hazards that colonized MPs/NPs represent to human and aquatic ecosystem health, as well freshwater ecosystem functioning. With only three biofilm-MP field study and six in the laboratory, there is an imminent need to increase the research effort. Another associated topic is the selectivity of MPs/NPs vs natural food; or palatability of the colonized MPs by various consumer taxa to further assess MPs/NPs trophic transfer. To fully assess the impacts of microplastics across the trophic web, understanding primary producer responses and interactions is key.

With only thirteen studies on trophic transfer, too many unknown variables remain to be studied to assess the hazards of MPs and NPs on foodwebs. While this review only considered photoautotrophs as producers, we found only two studies: da Costa Araújo et al. (2020) and De Felice et al. (2019); that tangentially mentioned leaf litter adsorbed MPs/NPs, but with no mention of decomposers. Clearly this is an area of research waiting to be developed. Given the toxicity effects shown by individual benthic organisms and the high concentration of microplastics in freshwaters, (water and sediment) globally, the potential detrimental cascading effect on the functioning of freshwater ecosystems cannot be ignored. Having only a limited number of existing studies on the subject does not reflect the acute need for research in freshwater ecosystems and we urgently call for more studies on trophic transfers across organisms with differing time scales of life histories and metabolic rate.

The evidence for the cumulative effects of exposure and multiple stressors is still scarce. Experimental studies on the neutral, synergistic or antagonistic effects of multiple stressors with MPs or NPs are in their infancy, where one can only glimpse the consequences if warming exacerbates MPs and NPs hazards for freshwater ecosystems (Jaikumara et al., 2018). New frameworks of investigation have been put forward by Jackson et al. (2021) and it is hoped that some of these hypotheses can be tested in freshwater ecosystems, foodwebs and individuals. Despite result discrepancies, MPs may contribute to environmental impacts of multiple anthropogenic stressors and given their ubiquity, should be considered part of multiple stressor studies to assess their synergistic, antagonist or neutral effects with co-occurrent stressors. NPs studies emphasise that particle size can allow interaction with porous membranes in numerous species, and that particle environment and therefore surface charge may have a highly significant effect on particle toxicity and concomitant pollutants. This ability to enter living cells raises concerns for bioaccumulation of NPs in living organisms, biomagnification in the food chain, and potential negative toxicity with implications for human health (Wang et al., 2019; Vethaak and Legler, 2021).

With so few studies on metal toxicity interactions with MPs/NPs, it is dangerous to speculate that there is a synergistic toxicity effect, but the outcome cannot be ignored. Certainly, further research should involve a wider range of taxonomic groups, more so when industrial and sewage effluents, as well as abandoned mines are known sources of metal pollution.

Microplastic contamination affects freshwater ecosystems in space and time, thus field research should reflect spatial and temporal

variability of MPs in the environment related, for example, to hydrodynamics, weather events and seasonality, and how these influence interactions with freshwater biota. While some high MPs concentrations tested in laboratory have been deemed unrealistic, more efforts should be addressed to identify in freshwater environments what concentration range should be accepted as realistic. While MPs occurrence in the environment have received some attention (Naqash et al., 2020), as shown in this review just a handful of studies have looked into natural populations and their interactions with MPs in the freshwater environment.

The accuracy of MPs detection and quantification from environmental and biological samples depends on sampling protocols and processing methods (Strungaru et al., 2019). Some limitations have been overcome for detection and quantification of MPs size >25 µm from water and sewage samples (Liu et al., 2019; Johnson et al., 2020; Horton et al., 2021) with a standardised approach to obtain repeated samples in time and space, making use of recent developments in analytical spectroscopy and software technology (Strungaru et al., 2019). Despite the progress, it is still difficult to compare findings and to assess MPs impact on organisms and tissues which extends to marine and terrestrial environments.

5. Conclusion

This review has highlighted the growing body of literature with respect to the ecological interactions and effects of microplastics in freshwater environments. The existing evidence shows microplastics to be widespread and easily transferred through trophic webs, regardless of whether microplastics are adhered to, or fully ingested by, lower trophic organisms. Biofilms on microplastics can comprise different species and altered community composition compared to those found on natural materials and may act as a vector for harmful or invasive species. Furthermore, they can increase the likelihood that microplastics will be ingested. Understanding the effects of microplastics on primary producers and subsequent consumers is essential to understand the potential cascading effects of microplastics through trophic webs. This paper demonstrates that lower trophic organisms can be adversely affected both under acute and chronic timescales, depending on the exposure conditions, and that microplastics can both bioaccumulate and biomagnify. However, the majority of single-organism toxicity testing has focussed on a limited number of model species (especially *Daphnia magna*). To be fully representative of organism responses, a greater range of species must be assessed, especially given the importance of species sensitivity in driving toxicity. Finally, multiple stressors can modify the effects of microplastics compared to exposure to microplastics alone and must be studied in greater detail given the relevance of these to real environmental scenarios.

CRedit authorship contribution statement

Ana Castro-Castellon: Conceptualization, Writing- Original draft preparation, Writing - Review and Editing; Writing - Submission edits. **Alice Horton:** Writing - Review and Editing, Resources, Writing - Submission edits. **Jocelyne Hughes:** Conceptualization, Resources, Writing - Original draft; Writing - Submission edits. **Cordelia Rampley:** Writing- Original draft for Nanoplastic section. **Paul Whitehead:** Conceptualization, Funding acquisition and Supervision, Editing; **Elizabeth Jeffers:** Funding acquisition and Original draft minor editing; **Giambattista Bussi** - Minor Editing original draft.

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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Appendix A. Glossary of terms used in ecotoxicology

Term	Definition
Absorption (in biology)	Penetration of a substance into an organism and its cells by various processes, some specialized, some involving expenditure of energy (active transport), some involving a carrier system, and others involving passive movement down an electrochemical gradient. Note: In mammals, absorption is usually through the respiratory tract, gastrointestinal tract, or skin into the circulatory system and from the circulation into organs, tissues, and cells.
Acute	Of short duration, in relation to exposure or effect. Aquatic toxicology exposure of the test organisms is continuous and of four days or less.
Bioaccessibility	Environmental availability. Able to come in contact with a living organism and perhaps interact with it with the possibility of absorption into the organism.
Bioaccumulation Factor	Ratio of tissue chemical residue to chemical concentration in an external environmental phase (e.g., sediment, water, soil, air, or food). BAF is measured at a steady state in situations where organisms are exposed to multiple sources (e.g., water, sediment, food), unless noted otherwise. Note 1: The concentration in the organism is typically expressed per unit body mass or per gram of lipid (bioaccumulation factor, lipid-based). Note 2: The concentration in sediment may be expressed per gram dry weight of sediment or per gram of organic carbon and may be referred to as the biota-sediment accumulation factor (BSAF). Note 3: The compound may have entered the organism by any available route and from any component of the water or sediment. Note 4: In relation to uptake from food, the concentration in the organism is typically expressed per unit body mass or per gram of lipid and the concentration in food is expressed per gram dry weight of food.
Bioaccumulation	Progressive increase in the amount of a substance in an organism or part of an organism that occurs because the rate of intake from all contributing sources and by all possible routes exceeds the organism's ability to eliminate the substance from its body.
Bioavailability	Potential for uptake of a substance by a living organism, usually expressed as a fraction of the total amount of the substance available in the matrix of exposure. Bioavailability, like bioaccessibility, is a function of both chemical speciation and biological properties. Even surface-bound substances may not be bioaccessible, and hence not bioavailable, to organisms which require substances to be in solution before they can interact with them.
Bioavailable	Able to be absorbed by living organisms.
Bioconcentration	Process leading to a higher concentration of a substance in an organism than in environmental media to which it is exposed.

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Term	Definition
Bioconcentration Factor	Usually applied to uptake by aquatic organisms directly from water Measure of the tendency for a substance in water to accumulate in aquatic organisms defined as the ratio of the concentration of the substance of concern in the organism to the concentration in water at equilibrium. Note 1: The equilibrium concentration of a substance in an aquatic organism can be estimated by multiplying its concentration in the surrounding water by its BCF in that organism. Note 2: This parameter is an important determinant for human intake of contaminants from water by ingestion of aquatic food.
Biological monitoring (ecotoxicology)	biological monitoring (in ecotoxicology) biomonitoring Regular systematic use of living organisms (indicator species, bioindicators, sentinel species) to evaluate changes in environmental quality, by repetitive measurements taken in a statistical design. Note: Biomonitoring may involve the study of individuals, species, populations, and communities to understand changes due to exposures over extended time periods. It may involve continuous or repeated, invasive or non-invasive measurement of behavioural parameters, physiological parameters, or other biomarkers, in captive animals or indigenous species at the individual or a lower organizational level and may contribute to the determination of biotic indices.
Biomagnification = Trophic enrichment = Ecological magnification	1. Sequence of processes by which higher concentrations of a substance are attained in organisms at higher trophic levels. 2. Result of these processes of bioconcentration and bioaccumulation by which tissue concentrations of bioaccumulated chemicals increase as the chemical passes up through two or more trophic levels. Note: Biomagnification occurs in a food chain as a consequence of efficient transfer of a substance from food to consumer accompanied by the lack of, or very slow, excretion or degradation of the substance.
Bottom-up ecotoxicological study	Approach to investigating ecotoxicological effects that starts with a determination of the presence and nature of any adverse effects via responses at the suborganismal (cellular and biochemical) levels of organization rather than via the community and (or) ecosystem levels of organization. See also top-down ecotoxicological study.
Chronic	Consequence that develops slowly and (or) has a long-lasting course: may be applied to an effect which develops rapidly and is long lasting.
Direct Toxicity	Toxicity that results from, and is readily attributable to, substances acting at the sites of toxic action in and (or) on the exposed organisms that are exhibiting the adverse biological response in question.
Ecotoxicology	Study of the toxic effects of chemical and physical agents on all living organisms, especially on populations and communities within defined ecosystems; it includes transfer pathways of these agents and their interactions with the environment.
Effect time	Time taken for a substance to produce a precisely defined effect. Note: ET50 is the median time it takes for a toxicant to produce a precisely defined effect in 50% of a population
Effective concentration	Concentration of a substance that causes a defined magnitude of response in a given system after a specified exposure time, e.g., concentration that affects x % of a test

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Term	Definition
Effective dose	population after a given time (ECx). Note: EC50 is the median concentration that causes 50% of maximal response Dose of a substance that causes a defined magnitude of response in a given system after a specified exposure time, e.g., dose that affects x % of a test population after a given time (EDx). Note: ED50 is the median dose that causes 50% of maximal response.
Environmental bioavailability	Ratio of uptake clearance to the rate at which an organism encounters a given contaminant in an environmental medium (e.g., soil, sediment, water, food) being processed by the organism. Note: This is a measure of an organism's extraction efficiency, via respiratory, dietary, and surface absorption processes, from the environmentally available (bioaccessible) portion of a material.
Environmental monitoring	Continuous or repeated measurement of agents in the environment to evaluate environmental exposure and possible damage by comparison with appropriate reference values based on knowledge of the probable relationship between ambient exposure and resultant adverse effects. Note: Measurements of substance, and (or) biological indicators, and (or) biomarkers may be repeated daily, weekly, monthly, or quarterly. Such measurements are recorded systematically and assessed in relation to location and time for any change in order to determine its possible significance.
Indirect toxicity	Adverse effects that result from agent(s) acting on and producing changes in the chemical, physical, and (or) biological environment external to the organisms under study (e.g., decrease in food for predatory species due to direct toxicity from a chemical to prey may produce adverse effects in the predator species due to starvation rather than inducing any direct chemical toxicity in predator organisms).
Pollutant	Any undesirable solid, liquid, or gaseous matter occurring, as a result of human activities, in a solid, liquid, or gaseous environmental medium and causing adverse effects. Note 1: "Undesirability", like toxicity, is concentration-dependent, low concentrations of most substances being tolerable or even essential in many cases. Note 2: A primary pollutant is one emitted into the atmosphere, water, sediments, or soil from an identifiable source. Note 3: A secondary pollutant is a pollutant formed by chemical reaction in the atmosphere, water, sediments, or soil.
Teratogenesis	Process resulting in permanent structural malformations or defects in the offspring of a parent exposed to a teratogen.
Top-down ecotoxicological study	Approach to investigating ecotoxicological effects that starts with a determination of the presence and nature of any adverse effects via responses at community and ecosystem levels of organization rather than the suborganismal levels of organization.
Toxicity	1. Capacity to cause injury to a living organism defined with reference to the quantity of substance administered or absorbed, the way in which the substance is administered and distributed in time (single or repeated doses), the type and severity of injury, the time needed to produce the injury, the nature of the organism(s) affected, and other relevant conditions. 2. Adverse effects of a substance on

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Term	Definition
	a living organism defined as in 1. 3. Measure of incompatibility of a substance with life: This quantity may be expressed as the reciprocal of the absolute value of median lethal dose (1/LD50) or median lethal concentration (1/LC50).
Trophic dilution	Decrease in contaminant concentration as trophic level increases; this results from a net balance of ingestion rate, uptake from food, internal transformation, and elimination processes favouring loss of contaminant that enters the organism via food.
Trophic transfer	Transfer of a substance from one trophic level to another.
Trophic transfer ratio	Ratio between the concentration of a compound in a predator and in its prey
Uptake	Entry of a substance into the body, into an organ, into a tissue, into a cell, or into the body fluids by passage through a membrane or by other means. Note: The term may also be applied to sorption of a substance onto the outside of an organism, e.g., the shell of a mollusc or the exoskeleton of an insect even without any entering the body or its cells.

Note: All definitions are from Nordberg et al. (2009).

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