

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

Ana T. Castro-Castellon<sup>1,\*</sup>, Alice A. Horton<sup>2</sup>, Jocelyne M.R. Hughes<sup>1</sup>, Cordelia Rampley<sup>3</sup>, Elizabeth S. Jeffers<sup>4</sup>, Gianbattista Bussi<sup>1</sup> and Paul Whitehead<sup>1</sup>

<sup>1</sup>School of Geography and the Environment, University of Oxford, Oxford, OX1 3QY, UK.

<sup>2</sup>National Oceanography Centre, European Way, Southampton, SO14 3ZH, UK.

<sup>3</sup>Oxford Molecular Biosensors, Centre for Innovation and Enterprise, Begbroke Science Park, Oxford OX5 1PF, UK.

<sup>4</sup>Department of Zoology, University of Oxford, Oxford, OX1 3SZ, UK.

\*Current: \*Industrial Phycology Limited, Unit 2 Axis, Hawkfield Business Park, Bristol, BS14 0BY, UK.

Email: [ana.castro-castellon@i-phyc.com](mailto:ana.castro-castellon@i-phyc.com)

## 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 11% of publications on microplastics in freshwater, demonstrate trophic transfer in foodwebs. The fact that just two per cent of publications focus on microplastics colonized by biofilms is hugely

concerning given the cascading detrimental effects this could have on freshwater ecosystem functioning. 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.

## Keywords

Water Pollution, Aquatic Toxicology, Food Web, Multiple Stressors, Nanoplastics

## Contents

<b>Abstract</b> .....	1
<b>1. Introduction</b> .....	2
<b>2. Literature search methods</b> .....	7
<b>3. Freshwater biota exposure to microplastics</b> .....	7
<b>3.1 Microplastic biomagnification or trophic enrichment</b> .....	12
<b>3.2 Multiple stressors: do microplastics increase the hazard?</b> .....	16
<b>3.3 Biofilms</b> .....	21
<b>3.4 Photoautotrophs</b> .....	22
<b>3.4.1 Macrophytes</b> .....	22
<b>3.4.2 Microalgae and Cyanobacteria</b> .....	23
<b>3.5 Consumers - Invertebrates</b> .....	27
<b>3.5.1 Planktic consumers</b> .....	27
<b>3.5.2 Benthic consumers</b> .....	31
<b>3.6 Fish</b> .....	33
<b>3.7 Amphibians</b> .....	36
<b>4. Research challenges</b> .....	37
<b>Acknowledgements</b> .....	Error! Bookmark not defined.
<b>References</b> .....	40
Appendix A.	

## 1. Introduction

It has been demonstrated via a plethora of recent research that microplastics (size  $\leq 5$  mm) are ubiquitous in water, soil and air and available to a wide range of organisms (Brahney *et al.*, 2020). Nagash *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 ±  
 19%) of water samples containing acrylates/polyurethane/varnish (APV) antifouling paint from the  
 River Rhine (Mani et al. 2019). Furthermore, hydrodynamic processes inherent to lentic and lotic  
 water bodies as well as seasonal climatological conditions will affect MP sinking and resuspension  
 rates into and from sediment (Rodrigues et al., 2018; Dahms et al., 2020; Zhang and Chen, 2020),  
 ultimately determining concentrations in, and bioavailability to, planktonic and benthic freshwater  
 organisms and further adding to the complexity of understanding of their impacts on 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 (S. Li et al., 2020; 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) (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., 2015). 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 µm (Wagner et al., 2014), to <1000 nm (da Costa et al., 2016; Ter Halle  
 et al., 2017), and even <100 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 > 300µm - ≤1mm, for the Widawa River in Central Europe (Kuśmierczak and Popiołek, 2020) and  
 >1mm in the River Thames, UK (Horton et al., 2018a). Li et al. (2019) reported MPs fibres <1mm 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 ( $>500\text{ }\mu\text{m}$ ) 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  $\text{m}^{-3}$  was recorded in water samples, 166.8  $\text{kg}^{-1}$  d/w in sediment, while 53.4 MPs  $\text{g}^{-1}$  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  $\text{m}^{-3}$  in March to 71–1265 items  $\text{m}^{-3}$  in October; while in sediment, the abundance ranged from 13.5–52.7  $\text{mg kg}^{-1}$  in March to 2.6–71.4  $\text{mg kg}^{-1}$  in October. Triebskorn et al. (2019) summarize for a variety of global sites their findings 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  $>300\text{ }\mu\text{m}$  concentrations range from 0.012 to 0.027 MPs  $\text{m}^{-3}$  for Lake Khovsgo, Mongolia and Laurentian Great Lakes, Canada-USA border; compared to urban Lake Hangian and Wuhan rivers in China for MPs  $>50\text{ }\mu\text{m}$  with a range of 1,660–8,925 MPs  $\text{m}^{-3}$ ; and for canals in Amsterdam huge concentrations of 48,000–187,00 MPs  $\text{m}^{-3}$  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  $\text{kg}^{-1}$  to (maximum) 539 particles  $\text{kg}^{-1}$  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.

A direct potential mechanism of toxicity by MPs and NPs is chemical leaching (Michałowicz, 2014). 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 internalisation 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 (Al-Thawadi, 2020)). 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 Figure 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.

Europe contributed 57.8 of the 370 million tonnes of plastics produced worldwide in 2019. Global primary plastic production saw a sharp decrease from an expected 420 million in 2020 due to COVID-19 (PlasticsEurope 2020). However, an average of 33% of the plastics produced in Europe is likely to end up in landfill, rivers and the ocean even after the introduction of the best management options (Lau et al., 2020). The ideal solution to the global plastics crisis would be to produce less plastic and to recycle it better; or at least prevent it from reaching aquatic ecosystems by establishing stronger environmental legislation and producer/consumer responsibilities (Blettell and Wantzen, 2019; Whitehead et al., 2021). The pollution of freshwater lentic and lotic ecosystems with plastic is evident and prevention of the release of plastic waste to river ecosystems is urgently needed. Understanding the pathways from source to rivers and the fate of aquatic plastics is paramount in assessing the ecological impact on freshwater biota and evaluating the effectiveness of environmental regulation and legislation (Whitehead et al., 2021).

This review 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.

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.

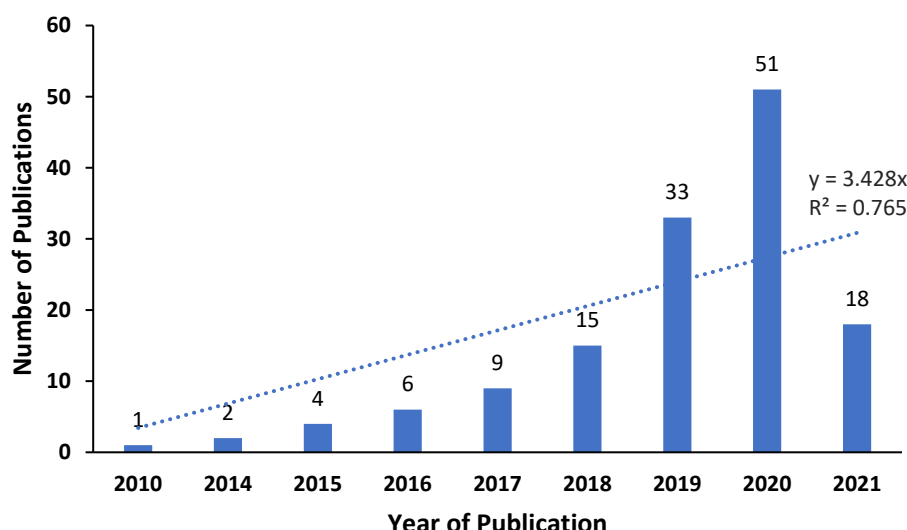
## 2. Literature search methods

The literature review was carried out until 6<sup>th</sup> March 2021 using the following search engines: ScienceDirect ([www.sciencedirect.com](http://www.sciencedirect.com)); Web of Science ([www.webofknowledge.com](http://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 Bletter and Wantzel (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*, 2019) as supplementary information.

## 3. Freshwater biota exposure to microplastics

Our review yielded 139 papers in total, with 18 papers already published in 2021 (until 6<sup>th</sup> March), representing a steady increase year on year since 2010 (Figure 1), when the first publication that meet

the selected criteria was found, and demonstrating that the scientific community is addressing a proliferation of research questions on the ecotoxicity of MPs and NPs in freshwater ecosystems.



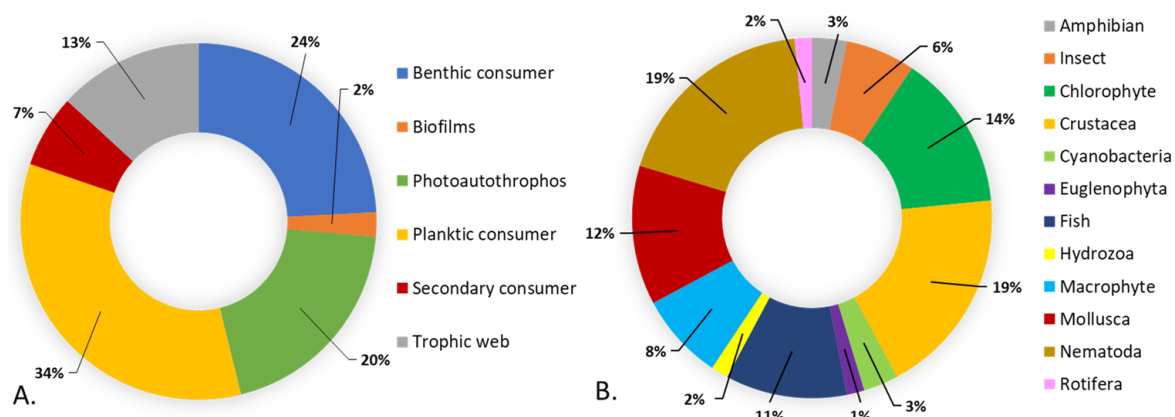
**Figure 1. Yearly publications on microplastics and nanoplastics in freshwater organisms. The total for 2021 is for the first two months. See section 2 for the selection criteria.**

Figure 2A shows the breakdown of topics for this review: trophic web, stressors, and trophic levels. Microplastics and nanoplastics are considered stressors in this review. 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 11% of the publications. Those publications which involved one or several species with MPs/NPs but in the presence of another stressor e.g. pesticides or metals were grouped under “Stressors” featured in 17% 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 only 2% of publications this topic has been neglected.



206

207



208 **Figure 2A. Classification breakdown of topics selected in this review with attention to trophic levels. 2B.**  
209 **Freshwater organisms (Taxa %) currently used for the assessment of microplastics toxicity.**  
210

211 Four taxa dominate: Crustacea (19%), Nematoda (19%), Chlorophyte (14%), and Mollusca (12%). The  
212 number of Nematoda publications is small, in fact, there is one study by Fueser et al. (2019) who tested  
213 seven nematode species in the same experiment, inflating the proportion of studies that appear  
214 dedicated to this taxon (Table 5).

215 Even with the relevance that freshwater fish have for many societies only 11% of the literature is  
216 dedicated to this group. Overall the most studied group of organisms is invertebrates (60%) with  
217 *Daphnia magna* being the single most studied species (40.6%) (Table 1). Sixty-four model organisms  
218 (genus and species) (Table 1) are used for the assessment of microplastics toxicity in freshwater. Note  
219 that taxa is included in this table for reference of the model organism and do not represent the data  
220 shown on Figure 2B.

221  
222

**Table 1. Model organism chosen (%) for MPs/NPs research in freshwater (n=64). Taxa is included for reference of the species chosen as model organism.**

Species	Taxa	Frequency (%)	Species	Taxa	Frequency (%)
<i>Daphnia magna</i>	Crustacea	40.6	<i>Cryptorchestia garbinii</i>	Crustacea	1.6
<i>Chironomus riparius</i>	Insect	10.9	<i>Daphnia galeata</i>	Crustacea	1.6
<i>Daphnia pulex</i>	Crustacea	10.9	<i>Dreissena bugensis</i>	Mollusca	1.6
<i>Chlamydomas reinhardtii</i>	Chlorophyte	7.8	<i>Elliptio complanata</i>	Mollusca	1.6
<i>Gammarus pulex</i>	Crustacea	7.8	<i>Elodea sp.</i>	Macrophyte	1.6
<i>Danio rerio</i>	Fish	4.7	<i>Ephemera danica</i>	Insect	1.6
<i>Lumbriculus variegatus</i>	Nematoda	4.7	<i>Euglena gracilis</i>	Euglenophyta	1.6
<i>Raphidocelis subcapitata</i>	Chlorophyte	4.7	<i>Gammarus roeseli</i>	Crustacea	1.6
<i>Anabaena sp.</i>	Cyanobacteria	3.1	<i>Hydra attenuata</i>	Hydrozoa	1.6
<i>Caenorhabditis elegans</i>	Nematoda	3.1	<i>Lemna minor</i>	Macrophyte	1.6
<i>Ceriodaphnia dubia</i>	Crustacea	3.1	<i>Lepidostoma basale</i>	Insect	1.6
<i>Chironomus sp.</i>	Insect	3.1	<i>Microcystis aeruginosa</i>	Cyanobacteria	1.6
<i>Chlorella vulgaris</i>	Chlorophyte	3.1	<i>Myriophyllum spicatum</i>	Macrophyte	1.6
<i>Dreissena polymorpha</i>	Mollusca	3.1	<i>Nematoda</i>	Nematoda	1.6
<i>Hyaella azteca</i>	Crustacea	3.1	<i>Neogobios melanostomus</i>	Fish	1.6
<i>Macrobrachium nipponense</i>	Mollusca	3.1	<i>Odontocorum albicorne</i>	Insect	1.6
<i>Physella acuta</i>	Mollusca	3.1	<i>Oryzias sinensis</i>	Fish	1.6
<i>Pimephales promelas</i>	Mollusca	3.1	<i>Panagrolaimus thienemanni</i>	Nematoda	1.6
<i>Platichthys flesus</i>	Nematode	3.1	<i>Plectus acuminatus</i>	Nematoda	1.6
<i>Tubifex tubifex</i>	Nematode	3.1	<i>Poecilia reticulata</i>	Fish	1.6
<i>Acipenser transmontanus</i>	Fish	1.6	<i>Poikilolaimus regenfussi</i>	Nematoda	1.6
<i>Acrobeloides nanus</i>	Nematoda	1.6	<i>Pristionchus pacificus</i>	Nematoda	1.6
<i>Alytes obstetricans</i>	Amphibian	1.6	<i>Rotifera</i>	Rotifera	1.6
<i>Aphelenchoides parietinus</i>	Nematoda	1.6	<i>Scenedemus subspicatus</i>	Chlorophyte	1.6
<i>Aphylla williamsoni</i>	Nematoda	1.6	<i>Scenedesmus obliquus</i>	Chlorophyte	1.6
<i>Asellus aquaticus</i>	Crustacea	1.6	<i>Scenedesmus sp.</i>	Chlorophyte	1.6
<i>Carassius carassius</i>	Fish	1.6	<i>Scenesmus quadricauda</i>	Chlorophyte	1.6
<i>Chlorella pyrenoidosa</i>	Chlorophyte	1.6	<i>Sericostoma pyrenaicum</i>	Crustacea	1.6
<i>Cipangopaludian cathayensis</i>	Mollusca	1.6	<i>Spaerium corneum</i>	Macrophyte	1.6
<i>Cladophora</i>	Chlorophyte	1.6	<i>Vallisneria natan</i>	Macrophyte	1.6
<i>Copepoda</i>	Crustacea	1.6	<i>Xenopus tropicalis</i>	Amphibian	1.6
<i>Corbicula fluminea</i>	Mollusca	1.6	<i>Zacco temminckii</i>	Fish	1.6

223

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 (Walkinshaw *et al.*, 2020; Prata *et al.*, 2019; Redondo-Hasselerham *et al.*, 2020) and disruption of ecosystem functioning (Huang *et al.*, 2021).

A number of publications (Bretas Alvim *et al.*, 2020; Caruso, 2019; Guo *et al.*, 2020; C. 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 a number of 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. (Figure 3).



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

### 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 2). 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 latipes* 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 48h 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 organisms at different 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<sup>-1</sup>) 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.

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.

Toxicity effects shown on Table 2 from the trophic combined experiments with other stressors (PCB, PAH, and methamphetamine) provide 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 is developed in section 3.2 and Table 3.

Trophic transfer is demonstrated in 10 of the 11 studies presented in Table 2, evidently the sample size 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 (Yardi and Callagham, 2020; Iannilli et al., 2021) 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., 2020). Microplastic 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.

288 **Table 2. Microplastics transfer through the foodweb. Trophic level category, species, research approach, type and size of MPs, exposure: time and**  
 289 **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 <sup>-1</sup>	Reduction growth of microalgae and reproduction of the crustacean.	<i>Besseling, et al., 2014</i>
Photoautotroph Planktic consumer Secondary consumer	<i>Scenedesmus sp.</i> <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 <sup>12</sup> NPs ml <sup>-1</sup>	Three level trophic web experiment. Severe effects on both behaviour and metabolism in fish.	<i>Mattson et al., 2015</i>
Photoautotroph Benthic consumer Secondary consumer	<i>Pavlova sp.*</i> <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 aver. 2.8-4.2 mg L <sup>-1</sup>	Direct and indirect effects with different types of MPs and PCBs. Clams showed histopathological changes with MPs. * <i>Pavlova sp.</i> is a marine microalgae used in this freshwater experiment.	<i>Rochman et al., 2017</i>
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 <sup>-1</sup>	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)	<i>Chae et al., 2018</i>
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 <sup>-1</sup>	Severe toxicity effects in the fish intestine highest lethality, bioaccumulation in nematodes was observed with 1 µm MPs independently of the type.	<i>Lei et al., 2018</i>
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 <sup>2</sup>	Bioaccumulation 1-2 PE-MPs were observed in the animal but not morbidity or mortality.	<i>Mateos-Cárdenas et al. 2019</i>
Planktic consumer Secondary consumer	<i>D. magna</i> & <i>Pimephales promelas</i>	Laboratory	PS-MPs (6 µm)	72-96 h	20 & 2000 MPs ml <sup>-1</sup>	<1% transfer from <i>D. magna</i> to the fish. MPs found in the fish with food absence. No adverse effect.	<i>Elizalde-Velasquez et al., 2020</i>
Leaf litter Benthic consumer	<i>Sericostoma pyrenaicum</i>	Laboratory	MPs (10 µm)	72 h	0-10 <sup>3</sup> MPs ml <sup>-1</sup>	Leaf litter decomposition was reduced with increasing MP concentrations.	<i>Lopez-Rojo et al, 2020</i>
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 <sup>-1</sup>	Toxicity and trophic transfer of PE-MPs from clam to fish. MPs enter the fish' organs, behavioural changes and mutagenic and cytotoxic processes.	<i>da Costa Araujo et al., 2020</i>

Benthic consumer	<i>Chironomus</i>	Laboratory	PMMA- MPs	24 h	$1 \times 10^6$ MPs L <sup>-1</sup>	Two level trophic transfer and the stressor benzo(k)fluoranthene a PAH.	<i>Hanslik, 2020</i>
Planktic consumer	<i>riparius</i>	combined	(48 µm)	48 h	0.05 g L <sup>-1</sup>		
Secondary consumer	<i>D. magna</i> <i>D. rerio</i>	with PAH					
Photoautotroph	<i>Chlorella</i>	Laboratory	PS- MPs	96 h	0.01 - 15 mg L <sup>-1</sup>	Acute toxicity of methamphetamine significantly increased in the presence of MPs for the microalgae (EC50 shift from 0.77 to 0.32 mg L <sup>-1</sup> ) and the snail LC50 shift from 4.15 to 1.48 mg L <sup>-1</sup> . Oxidative damage and apoptosis of the algae increased, as well as the filtration rate of the snails.	<i>Qu et al, 2020</i>
Benthic consumer	<i>pyrenoidosa</i>	combined	(700 nm)		20 mg L <sup>-1</sup>		
	<i>Cipangopaludian cathayensis</i>	with amphetamine					

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

### 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 3), 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., 2020, 2019a; Ma et al., 2020), but how species at various stages of development will respond, is still in need of further investigation. 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  $\mu\text{m}$ ), in an acute toxicity experiment (72 hr). 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, 1-4  $\mu\text{m}$ ) 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



318 to bioavailability, weakness of chemical-MPs binding, material of MPs, time exposure of the MPs to  
 319 the chemicals used in the laboratory and age/weathering of the MPs (Luo et al., 2020; Ma et al., 2020).  
 320 Testing of the combined toxicity of PVC-MPs and the pesticide imidacloprid in an extended-time  
 321 period toxicity experiment (28 d) with *Chironomus riparius* failed to provide evidence for enhanced  
 322 toxicity of MPs when compared to natural particles i.e. kaolin and the pesticide. Having said this,  
 323 toxicity was observed, at concentrations not yet seen in the environment, the authors suggested that  
 324 the results indicated a high tolerance of *C. riparius* to the effects of PVC-MPs (Scherer *et al.*, 2020)  
 325 Horton *et al.* (2020) exposed *Lymnaea stagnalis* to polybrominated diphenyl ethers (PBDE) and PA-  
 326 MPs (13-19 µm) to investigate potential bioaccumulation but found no supporting evidence for either  
 327 toxicity of PA-MPs or enhanced bioaccumulation of PBDEs in the presence of PA-MPs. Information on  
 328 organic pollutants adsorption onto MPs has been published (Fred-Ahmadu *et al.*, 2020; Liu *et al.*, 2019;  
 329 Mao *et al.*, 2018), as well as research addressing the issue of inner body desorption/absorption of  
 330 organic pollutants bound to MPs due to enzymatic processes i.e. pH changes under real (Bakir et al.,  
 331 2014) or simulated physiological conditions (Lee et al., 2019; Mohamed Nor and Koelmans, 2019),  
 332 however, gaps still exist here for other model organisms which might lead to understand if similar  
 333 mechanisms could take place in humans.  
 334 Existing research on the combined impact of MPs and climate change or increased temperature, with  
 335 MPs on freshwater ecosystem processes is scant (Table 3). One of the first studies exposed an  
 336 Amazonian cichlid *Symphysodon aequifasciatus* to temperatures of 28-31°C and concentrations of 200  
 337 µg/L of PE-MPs (size 70–88 µm) for 30 days. Microplastics (rather than temperature) affected the  
 338 predatory performance, digestion and energy production of *S. aequifasciatus*, however, juvenile  
 339 survival and growth were not significantly impacted (Wen et al., 2018). *Daphnia magna*, *D. pulex* and  
 340 *Ceriodaphnia dubia* were exposed to primary and secondary MPs (1-10 µm) at three temperatures  
 341 (18, 22 and 26 °C) during an acute toxicity experiment (72-96 h), and it was found that sensitivity was  
 342 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 3).

366 **Table 3. Combined MPs and/or NPs toxicity experiments with other stressors: pesticides, metals and/or temperature. Category, species, research**  
 367 **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
Macrophyte	<i>Vallisneria natan</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 <sup>-1</sup>	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 W. et al. 2020
Phytoplankton	<i>Chlamidomonas reinhardtii</i>	Mesocosm-with As (III)	PS-MPs PS-MPS-As (II)	72 h	10, 25, 50 & 100 mg L <sup>-1</sup>	Suppressed Rubisco activity, reduced photosynthesis & growth rates. PS-MP-As (III) triggered oxidative processes and damaged membrane cells.	Dong et al., 2021
Phytoplankton	<i>Raphidocellis subcapitata</i>	Mesocosm-with Cu	PS-NPs	72 h 7 d	0.5, 1, 2.5, 5, 10, 50 mg MPs L <sup>-1</sup>	Inhibition of growth rate morphological alterations, potential disturbances in the mitotic cycle	Bellingeri et al., 2019
Invertebrate	<i>Daphnia sp.</i>	Mesocosm-with two temperature	PS-MPs Beads (575 ± 18.9 nm)	n/a	1 mg L <sup>-1</sup> or 1.46 x10 <sup>7</sup> MPs L <sup>-1</sup>	Synergistic effect of MPs and temperature on phenotypical responses on clones. The exposure to MPs lasted two full D. magna 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 <sup>-1</sup>	Synergistic effect of the pesticide and MPs was demonstrated with reductions of D. magna 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 <sup>-1</sup> or 3 x10 <sup>5</sup> MPs ml <sup>-1</sup>	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 et al., 2018b
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 hr	10 <sup>3</sup> , 10 <sup>4</sup> , 10 <sup>5</sup> , 10 <sup>6</sup> , 10 <sup>7</sup> MPs ml <sup>-1</sup>	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 <sup>-1</sup> 200 mg L <sup>-1</sup>	The combination of BPA and MPs led to decreased immobilization, after ingestion by <i>D. magna</i> .	Rehse et al., 2018

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 <sup>-2</sup>	MPs adverse effects on metabolic rate were manifested at higher temperatures	<i>Kratina et al., 2019</i>
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	<i>Horton et al. 2020</i>
Invertebrate	<i>Dreissenia polymorpha</i>	Mesocosm-temperature	PS-MPs fragments (2-60 µm)	14 d	100,000 MPs/ ml	MPs have minor effects on a freshwater mussel compared to thermal stress, neither alone nor as interactive effect.	<i>Weber et al., 2020</i>
Fish	<i>Symphysodon aequifasciatus</i>	Mesocosm-	PE-MPs PP-MPs (70-80 µm)	30 d	200 µg L <sup>-1</sup>	MPs affected predatory performance, digestion and energy production. No impact on juvenile fish	<i>Wen et al., 2018</i>

---

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

### 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; 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 was reported by Miao *et al.* (2019a), 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 generalists) actively avoid ingesting fibres (Yardy and Callaghan, 2020) whilst other species have shown gender differences on their tendency to ingest more MPs (Horton et al., 2018b; Sue 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). Pathogens from the genera *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 (Fenchel 2017; 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 4). 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). 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). Mateos-Cárdenas et al. (2019) exposed *Lemna minor* to MPs in a toxicity experiment with polyethylene (PE) MPs (MPs-PE) (10–45 µm) during 7 d. Results showed the adsorption of PE-MPs to *L. minor* with an abundance of 7 PE-MPs per mm<sup>2</sup>, 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 hr). It was confirmed that 1-2 PE-MPs were observed in the animal indicating transferability of MPs through ingestion, despite no adverse impact seen on the plants. Perhaps the acute toxicity experiment time of exposure was too short and longer exposure might reveal other toxicity effects on the invertebrate.

### 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 (Lürling 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 4). 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 whilst 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) and levels of malondialdehyde (MDA, an oxidation product of ROS attack on lipids) for freshwater microalgae. Their general findings indicated that effects increased with high concentration of MPs or NPs whilst at lower concentrations microalgae can activate anti-stress mechanisms to revert adverse effects. Positively charged MPs or NNPs affected microalgae at low concentrations (< 1mg/L). Other mechanisms, i.e. indirect toxicity, were described by Shen *et al.*, (2020) where MPs may affect the biological and carbon pump sequestration (CO<sub>2</sub>). 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 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). Table 4 shows for example, that freshwater green algae *Scenedesmus obliquus* was exposed to different concentrations of NPs-PS of 70nm size. Results demonstrated a slight reduction in growth of 2.5% at the very high 1g/L of NPs, and a reduction in chlorophyll a concentration as concentration in NPs increased (Besseling *et al.*, 2014).

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/NNPs.

492 **Table 4. Relation of MPs and/or NPs toxicity experiments photoautotrophs (macrophytes, microalgae and cyanobacteria); research approach (field or mesocosm); MPs and/or NPs shape,**  
493 **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
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.	<i>Van Weert et al., 2019</i>
Macrophyte	<i>Lemna minor</i>	Mesocosm	PE-MPs (10–45 µm)	7 d	7 PE-MPs per mm <sup>2</sup>	Adsorption. No photosynthetic or growth changes.	<i>Mateos-Cárdenas et al. 2019</i>
Phytoplankton	<i>Chlorella vulgaris</i>	Microcosms	PUF-MPs Foam 3 mm <sup>3</sup> (aged x 12 months)	48 h	0.7 g MPs	Photosynthetic efficiency decreased with increasing leachate concentrations. Leachate increased with increased pH and exposure time.	<i>Luo et al., 2019</i>
Phytoplankton	<i>Pseudokirchneriella subcapitata</i>	Laboratory	MPs (20-500 nm) NPs (<1µm)	2 hr	10 mg L <sup>-1</sup>	Particles found adhered to microalgae cell wall, potential indirect toxicity effects: shading leaching effects	<i>Nolte et al., 2017</i>
Phytoplankton	<i>Scenedesmus obliquus</i>	Laboratory	PS-NPS beads (70 nm)	72 h	44–1100 mg L <sup>-1</sup>	Hampered growth and reduced chlorophyll concentrations	<i>Besseling et al., 2014</i>
Phytoplankton		Mesocosm				Shading impaired photosynthesis; affected metabolic processes: increased exopolysaccharides production & hampered the energetic budget	<i>Bhattacharya et al., 2010</i>
Phytoplankton	<i>Chlorella sorokiniana</i>	Laboratory	PS-MPs (< 70 µm)	4 w	60 mg L <sup>-1</sup>	Direct toxicity. MPs weakens membrane permeability, more vulnerable to stressors.	<i>Guschina et al., 2020</i>
Phytoplankton	<i>Chlorella pyrenoidosa</i>	Mesocosm - Single & combined with Nonylphenol.	PE-MPs, PA-MPs & PS-MPs 13, 100, 150 & 1000 µm	96 h	10, 30, 50, 70 & 100 mg L <sup>-1</sup>	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	<i>Yang W. et al. 2020</i>

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

## 3.5 Consumers - Invertebrates

### 3.5.1 Planktic consumers

The impact MPs could have on primary consumers (e.g. ciliates –*Vorticella* sp.; rotifers – *Anuraeopsis fissa*; crustaceans – *Daphnia* sp.) remains unclear with contradictory findings. (Scherer et al., 2017). Differences in primary consumer susceptibility to MPs lie in their feeding strategies (Scherer et al., 2017; Fueser et al., 2019), on the polymer type, size and shape, and providing that there is enough exposure and retention time after ingestion (De Felice et al., 2019; Jemec et al., 2016; Scherer et al., 2017; 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 5). 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 whilst in the treatment with MPs declined after three weeks. This may

521 suggest similar implications of long-term exposure for other aquatic organisms and should be  
522 investigated further for a better understanding of the risks and hazards from MPS/NPs exposure in  
523 the environment.

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

**Table 5. 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 mgL <sup>-1</sup>	Reduced body size, reproduction & malformation of neonates ( $\geq 30$ mg/L)	<i>Besseling et al., 2014;</i>
Benthic consumer	<i>Ceriodaphnia dubia</i>	Laboratory	PE-MPs-beads (1-4µm) PS-MPs- fibres	48 h 8 d	0.5–16 mg/L or $1.7 \times 10^4$ – $5.4 \times 10^5$ beads L <sup>-1</sup> 0.125–4 mg/L or $1.1 \times 10^3$ – $3.4 \times 10^4$ fibres L <sup>-1</sup>	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.	<i>Ziajahromi et al. 2017</i>
Planktic consumer	<i>D. magna</i>	Laboratory	MPs- beads (1-10 µm)	21 d	0.125, 1.25 and 12.5 mgL <sup>-1</sup>	Physiological (i.e. larger body size) and behavioural changes (i.e. swimming activity and phototactic sensitivity)	<i>De Felice et al., 2019</i>
Planktic consumer	<i>D. magna</i>	Laboratory	PVC-MPs fragments (12-274 µm)	31 d	0.1 – 0.15 mgL <sup>-1</sup>	Body shrinkage and reduced brood. Body shrinkage was observed after 21 days exposure.	<i>Schrank et al. 2019</i>
Planktic/ benthic consumer	<i>Chironomus riparius</i>	Laboratory	PS-MPs, PET-MPs, PVC-MPs, PA-MPs (20-100 µm)	12 d	Environmental concentrations	Morphological deformities in larval mandibles, mentus and female wings development	<i>Stanković et al., 2020</i>
Planktic consumer	<i>D. magna</i>	Laboratory & mesocosm	PS-MPs 15 µm)	12 w	0 - 800 MPs mL <sup>-1</sup>	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.	<i>Aljaibachi et al. 2020</i>
Planktic benthic consumers	<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	MPs beads (20-500 µm)	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.	<i>Redondo-Hasselerharm et al. 2018</i>
Benthic consumer	<i>Gammarus fossarum</i>		PA-MPs-Fibres (500 x 20 µm) PS-MPs- beads (1.6 µm)	0.5 32 h 28 d	100, 540, 2,680, 13,380 fibres cm <sup>-2</sup> 500, 2500, 12,500, 60,000 beads ml <sup>-1</sup>	Ingestion and egestion of fibres and beads, but significantly reduced the assimilation efficiency of the animals.	<i>Blarer and Burkhardt-Holm, 2016</i>
Benthic consumer	<i>Chironomus tepperi</i>	Laboratory	PE-MPs beads (1-4, 10-27, 43-54 & 100-126 µm)	5-10 d	500 MPs Kg <sup>-1</sup> 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. .	<i>Ziajahromi et al. 2017</i>
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	<i>Yardy and Callaghan, 2020</i>
Benthic consumers	<i>Cryptorhestia garbinii</i>	Field Lakes	MPs (25 µm W x 55 µm 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.	<i>Iannilli et al, 2021</i>

Planktic/ benthic consumer	<i>C. riparius</i>	Laboratory	PA-MPs beads (10-180 µm)	28 d	100 mg PA kg <sup>-1</sup> or 10,100 MPs kg <sup>-1</sup> 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.</i> <i>riparius</i> life cycle.	<i>Khosrovyan and Kahru, 2020</i>
Benthic consumers	<i>Caenorhabditis elegans</i> <i>Panagrolaimus thienemanni</i> <i>regenfussi</i> <i>Plectus acuminatus</i> <i>Poikilolaimus regenfussi</i> <i>Acrobeloides nanus</i> <i>Pristionchus pacificus</i>	Laboratory	PS-MPs Beads (0.5,1.0, 3.0 and 6.0 µm)	4, 24 and 72 h	3x10 <sup>6</sup> - 10 <sup>7</sup> MPs ml <sup>-1</sup>	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.	<i>Fueser et al., 2019</i>
Benthic consumer	<i>Dreissenia polymorpha</i>	Field MPs Mesocosm	PS-MPs, PP-MPs fibres & fragments (15 µm – 2.97 mm)	7 d		Direct toxicological effects on the mussels	<i>Binelli et al. 2020</i>

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

532

### 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 (Walkinshaw *et al.*, 2020, Turner *et al.*, 2019). Microplastics were found to adhere to leaf litter and be ingested by the detritivore *Sericostoma pyrenaicum*, an aquatic insect (López-Rojo *et al.*, 2020); accumulating in digestive and reproductive systems of different trophic freshwater organisms such as *Hyaella azteca* (Au *et al.*, 2015) and *Lumbricus variegatus* (Imhof *et al.*, 2013). However, despite this evidence of ingestion, Redondo-Hasselerharm *et al.* (2018) found no evidence to support toxicity for the same species as well as for other species: *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). Fueser *et al.* (2019) 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 5).

Whilst 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 found 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.

559 Freshwater ecosystems receive a mixture of MPS/NPs shape and type with a very few studies exposing  
560 aquatic species to mixtures to be expected in natural environments. Stanković *et al.* (2020) conducted  
561 an experiment using a mixture of MPs, PS-MPs, polyethylene-terephthalate (PET), polyvinyl-chloride  
562 (PVC) and polyamide (PA) at environmental concentrations and size (20-100  $\mu\text{m}$ ) found morphological  
563 deformities in larval mandibles, mentus and female wings development of *C. riparius* larval stages (12  
564 days).

565 Windsor et al.(2019) studied two orders of insects, Ephemeroptera and Trichoptera in five sites in  
566 South Wales, UK within urban catchments receiving wastewater effluent discharge. Microplastics  
567 were identified in approximately 50% of macroinvertebrate samples collected from three families  
568 (n=18): Baetidae, Heptageniidae (mayflies which feed upon periphyton) and Hydropsychidae  
569 (caddisflies which are generalist feeders) at concentrations up to 0.14 MPs mg tissue<sup>-1</sup> and they  
570 occurred at all sites. Microplastics abundance was associated with macroinvertebrate biomass and  
571 taxonomic family, but MPs occurred independently of feeding strategy and biological traits such as  
572 habitat affinity and ecological niche. There is likelihood that MPs concentration and bioassimilation  
573 by these invertebrates might be related with flow dilution, thus low dilution of effluents or urban  
574 drainage systems might increase MPs concentration and bioassimilation.

575 Microplastics collected from freshwater environments are rarely used in experimental work, the  
576 research conducted by Binelli et al. (2020) is unique. These researchers exposed *Dreissena*  
577 *polymorpha* (zebra mussel) to PS-MPs and polypropylene (PPE) MPs (PPE-MPs) fibres and fragments  
578 (15  $\mu\text{m}$  – 2.97 mm) all collected from three lakes. The experiment was run in a laboratory mesocosm  
579 over 7 days. The outcome demonstrated the direct toxicological effects on biological pathways of the  
580 mussels but the caveat for the experiment was the high MPs concentration used that is considered to  
581 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.* 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 (Jeong *et al.*, 2018; 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. 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). Kuśmierek 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 (100%) 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 two hours 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 interactions and ingestion within wild-caught fish are crucial for understanding environmental exposure, however this does not give an indication of the likely hazard posed by ingested microplastics as a result of physical or chemical toxicity. To better understand this, laboratory experiments must be undertaken to pinpoint possible cause-and-effect. Zebrafish (*Danio rerio*) are a model freshwater fish used for toxicity testing. A study by Lu *et al.* (2016) found that zebrafish exposed

to 5  $\mu\text{m}$  PS-MPs accumulated the particles in the gills, gut and liver, while 20  $\mu\text{m}$  PS-MPs only accumulated in the gills and gut. For 5  $\mu\text{m}$  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  $\mu\text{m}$  PS). They saw similar accumulation in gills, gut and liver, and also within brain tissues. Altered enzymatic activity was observed and the response varied over time, however 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  $\sim 1$  and  $\sim 119 \mu\text{g g}^{-1}$  body weight respectively. Based on predicted exposure and uptake, 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. 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 (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 exist. However, in contrast to the contradictory findings in other taxa (dependent on species and particle characteristics), almost all studies that have been conducted on amphibians during their aquatic phase (tadpoles) seem to support the toxic effect of MPs. Even with the caveat that the number of experiments is low, and that in some of them the environmental MPs concentrations are 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*, has also been shown to accumulate 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  $60 \text{ mg/L}$  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  $1800 \text{ MPs/mL}$  ( $10 \mu\text{m}$  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, Nematoda, Chlorophyte, Mollusca, and closely followed by fish studies. *Daphnia magna* is the chosen toxicity model organism, accounting for 40.6% 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 20% for planktic and benthic consumers, more attention is needed for producers and particularly for secondary consumers (6%). Topics greatly understudied across all aspects of microplastics research (not just for freshwater) are biofilms, trophic transfer, and multi-stressors mainly in the field but also in the laboratory. The four 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 one biofilm-MP field study and three 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 eleven 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-Araujo et al. (2020) and De Felice et al. (2020); 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 synergistic, neutral 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 (Jaikumar 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 (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. Whilst 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. Whilst 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. Some limitations have been overcome for detection and quantification of MPs size >25 µm from water and sewage samples (Liu et al., 2019; Ball et al., 2020; 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. 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.

## 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.

## Acknowledgements

This research was funded by Research England's Strategic Priority Fund (SPF) QR allocation. We thank the UK Government Department DEFRA for excellent feedback. However, the views in this paper represent those of the authors.

## References

Al-Thawadi, S., 2020. Microplastics and Nanoplastics in Aquatic Environments: Challenges and



781 Threats to Aquatic Organisms. Arab. J. Sci. Eng. <https://doi.org/10.1007/s13369-020-04402-z>

782 Alimi, O.S., Farner Budarz, J., Hernandez, L.M., Tufenkji, N., 2018. Microplastics and Nanoplastics in  
 783 Aquatic Environments: Aggregation, Deposition, and Enhanced Contaminant Transport.  
 784 Environ. Sci. Technol. <https://doi.org/10.1021/acs.est.7b05559>

785 Aljaibachi, R., Laird, W.B., Stevens, F., Callaghan, A., 2020. Impacts of polystyrene microplastics on  
 786 *Daphnia magna*: A laboratory and a mesocosm study. Sci. Total Environ. 705, 135800.  
 787 <https://doi.org/10.1016/j.scitotenv.2019.135800>

788 Arias-Andres, M., Rojas-Jimenez, K., Grossart, H.P., 2019. Collateral effects of microplastic pollution  
 789 on aquatic microorganisms: An ecological perspective. TrAC - Trends Anal. Chem.  
 790 <https://doi.org/10.1016/j.trac.2018.11.041>

791 Au, S.Y., Bruce, T.F., Bridges, W.C., Klaine, S.J., 2015. Responses of *Hyaella azteca* to acute and  
 792 chronic microplastic exposures. Environ. Toxicol. Chem. 34. <https://doi.org/10.1002/etc.3093>

793 Azevedo, L.S., Pestana, I.A., Almeida, M.G., Ferreira da Costa Nery, A., Bastos, W.R., Magalhães  
 794 Souza, C.M., 2021. Mercury biomagnification in an ichthyic food chain of an amazon floodplain  
 795 lake (Puruzinho Lake): Influence of seasonality and food chain modeling. Ecotoxicol. Environ.  
 796 Saf. 207, 111249. <https://doi.org/10.1016/j.ecoenv.2020.111249>

797 Bakir, A., Rowland, S.J., Thompson, R.C., 2014. Enhanced desorption of persistent organic pollutants  
 798 from microplastics under simulated physiological conditions. Environ. Pollut. 185.  
 799 <https://doi.org/10.1016/j.envpol.2013.10.007>

800 Ball, H., Cross, R., Grove, E., Horton, A., Johnson, A., Jürgens, M., Read, D., Svendsen, C., 2020. Sink  
 801 to river – river to tap. a review of potential risks from nanoparticles and microplastics. Report  
 802 Ref. No. 19/EQ/01/18. UKWIR.

803 Barbosa, F., Adeyemi, J.A., Bocato, M.Z., Comas, A., Campiglia, A., 2020. A critical viewpoint on  
 804 current issues, limitations, and future research needs on micro- and nanoplastic studies: From

805 the detection to the toxicological assessment. *Environ. Res.* 182, 109089.  
806 <https://doi.org/10.1016/j.envres.2019.109089>

807 Besseling, E., Wang, B., Lürling, M., Koelmans, A.A., 2014. Correction to Nanoplastic Affects Growth  
808 of *S. obliquus* and Reproduction of *D. magna*. *Environ. Sci. Technol.* 48, 14065–14065.  
809 <https://doi.org/10.1021/es5052028>

810 Bhattacharya, P., Lin, S., Turner, J.P., Ke, P.C., 2010. Physical adsorption of charged plastic  
811 nanoparticles affects algal photosynthesis. *J. Phys. Chem. C* 114.  
812 <https://doi.org/10.1021/jp1054759>

813 Binelli, A., Pietrelli, L., Di Vito, S., Coscia, L., Sighicelli, M., Torre, C. Della, Parenti, C.C., Magni, S.,  
814 2020. Hazard evaluation of plastic mixtures from four Italian subalpine great lakes on the basis  
815 of laboratory exposures of zebra mussels. *Sci. Total Environ.* 699, 134366.  
816 <https://doi.org/10.1016/j.scitotenv.2019.134366>

817 Blair, R.M., Waldron, S., Phoenix, V.R., Gauchotte-Lindsay, C., 2019. Microscopy and elemental  
818 analysis characterisation of microplastics in sediment of a freshwater urban river in Scotland,  
819 UK. *Environ. Sci. Pollut. Res.* 26, 12491–12504. <https://doi.org/10.1007/s11356-019-04678-1>

820 Blarer, P., Burkhardt-Holm, P., 2016. Microplastics affect assimilation efficiency in the freshwater  
821 amphipod *Gammarus fossarum*. *Environ. Sci. Pollut. Res.* 23, 23522–23532.  
822 <https://doi.org/10.1007/s11356-016-7584-2>

823 Blettler, M.C. and Wantzen, K.M., 2019. Threats underestimated in freshwater plastic pollution:  
824 mini-review. *Water, Air, & Soil Pollution*, 230(7), pp.1-11.

825 Bonefeld-Jørgensen, E.C., Long, M., Hofmeister, M. V., Vinggaard, A.M., 2007. Endocrine-Disrupting  
826 Potential of Bisphenol A, Bisphenol A Dimethacrylate, 4- n -Nonylphenol, and 4- n -Octylphenol  
827 in Vitro : New Data and a Brief Review. *Environ. Health Perspect.* 115, 69–76.  
828 <https://doi.org/10.1289/ehp.9368>

829 Boyero, L., López-Rojó, N., Bosch, J., Alonso, A., Correa-Araneda, F., Pérez, J., 2020. Microplastics  
830 impair amphibian survival, body condition and function. *Chemosphere* 244.  
831 <https://doi.org/10.1016/j.chemosphere.2019.125500>

832 Brahney, J., Hallerud, M., Heim, E., Hahnenberger, M., Sukumaran, S., 2020. Plastic rain in protected  
833 areas of the United States. *Science* (80-. ). 368, 1257–1260.  
834 <https://doi.org/10.1126/science.aaz5819>

835 Bretas Alvim, C., Mendoza-Roca, J.A., Bes-Piá, A., 2020. Wastewater treatment plant as microplastics  
836 release source – Quantification and identification techniques. *J. Environ. Manage.*  
837 <https://doi.org/10.1016/j.jenvman.2019.109739>

838 Browne, M.A., Dissanayake, A., Galloway, T.S., Lowe, D.M., Thompson, R.C., 2008. Ingested  
839 microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L.).  
840 *Environ. Sci. Technol.* 42. <https://doi.org/10.1021/es800249a>

841 Cedervall, T., Hansson, L.A., Lard, M., Frohm, B., Linse, S., 2012. Food chain transport of  
842 nanoparticles affects behaviour and fat metabolism in fish. *PLoS One* 7.  
843 <https://doi.org/10.1371/journal.pone.0032254>

844 Chae, Y., Kim, D., Kim, S.W., An, Y.J., 2018. Trophic transfer and individual impact of nano-sized  
845 polystyrene in a four-species freshwater food chain. *Sci. Rep.* 8.  
846 <https://doi.org/10.1038/s41598-017-18849-y>

847 Chen, C.Y., Lu, T.H., Yang, Y.F. and Liao, C.M., 2020. Toxicokinetic/toxicodynamic-based risk  
848 assessment of freshwater fish health posed by microplastics at environmentally relevant  
849 concentrations. *Science of The Total Environment*, 756, p.144013.

850 Chen, X., Xiong, X., Jiang, X., Shi, H. and Wu, C., 2019. Sinking of floating plastic debris caused by  
851 biofilm development in a freshwater lake. *Chemosphere*, 222, pp.856-864.

852 D’Souza, J.M., Windsor, F.M., Santillo, D., Ormerod, S.J., 2020. Food web transfer of plastics to an

853 apex riverine predator. Glob. Chang. Biol. 26. <https://doi.org/10.1111/gcb.15139>

854 da Costa Araújo, A.P., de Melo, N.F.S., de Oliveira Junior, A.G., Rodrigues, F.P., Fernandes, T., de  
855 Andrade Vieira, J.E., Rocha, T.L., Malafaia, G., 2020. How much are microplastics harmful to the  
856 health of amphibians? A study with pristine polyethylene microplastics and *Physalaemus*  
857 *cuvieri*. J. Hazard. Mater. 382. <https://doi.org/10.1016/j.jhazmat.2019.121066>

858 da Costa, J.P., Santos, P.S.M., Duarte, A.C., Rocha-Santos, T., 2016. (Nano)plastics in the environment  
859 - Sources, fates and effects. Sci. Total Environ. <https://doi.org/10.1016/j.scitotenv.2016.05.041>

860 Di Pippo, F., Venezia, C., Sighicelli, M., Pietrelli, L., Di Vito, S., Nuglio, S. and Rossetti, S., 2020.  
861 Microplastic-associated biofilms in lentic Italian ecosystems. *Water Research*, 187, p.116429.

862 Dahms, H.T., van Rensburg, G.J. and Greenfield, R., 2020. The microplastic profile of an urban African  
863 stream. *Science of The Total Environment*, 731, p.138893.

864 De Felice, B., Sabatini, V., Antenucci, S., Gattoni, G., Santo, N., Bacchetta, R., Ortenzi, M.A., Parolini,  
865 M., 2019. Polystyrene microplastics ingestion induced behavioral effects to the cladoceran  
866 *Daphnia magna*. Chemosphere 231. <https://doi.org/10.1016/j.chemosphere.2019.05.115>

867 Ding, J., Zhang, S., Razanajatovo, R.M., Zou, H. and Zhu, W., 2018. Accumulation, tissue distribution,  
868 and biochemical effects of polystyrene microplastics in the freshwater fish red tilapia  
869 (*Oreochromis niloticus*). *Environmental pollution*, 238, pp.1-9.

870 Dunn, C., Owens, J., Fears, L., Nunnerley, L., Kirby, J., Armstrong, O.L., Thomas, P.J., Aberg, D., Gilder,  
871 W., Green, D., Antwis, R., Freeman, C., 2019. An affordable methodology for quantifying  
872 waterborne microplastics - an emerging contaminant in inland-waters. J. Limnol.  
873 <https://doi.org/10.4081/jlimnol.2019.1943>

874 Eerkes-Medrano, D., Thompson, R.C., Aldridge, D.C., 2015. Microplastics in freshwater systems: A  
875 review of the emerging threats, identification of knowledge gaps and prioritisation of research  
876 needs. Water Res. <https://doi.org/10.1016/j.watres.2015.02.012>

877 Elizalde-Velázquez, A., Carcano, A.M., Crago, J., Green, M.J., Shah, S.A., Cañas-Carrell, J.E., 2020.  
878 Translocation, trophic transfer, accumulation and depuration of polystyrene microplastics in  
879 *Daphnia magna* and *Pimephales promelas*. *Environ. Pollut.* 259.  
880 <https://doi.org/10.1016/j.envpol.2020.113937>

881 Fahrenfeld, N.L., Arbuckle-Keil, G., Beni, N.N. and Bartelt-Hunt, S.L., 2019. Source tracking  
882 microplastics in the freshwater environment. *TrAC Trends in Analytical Chemistry*, 112, pp.248-  
883 254.

884 Felten, V., Toumi, H., Masfaraud, J.F., Billoir, E., Camara, B.I., Féraud, J.F., 2020. Microplastics  
885 enhance *Daphnia magna* sensitivity to the pyrethroid insecticide deltamethrin: Effects on life  
886 history traits. *Sci. Total Environ.* 714. <https://doi.org/10.1016/j.scitotenv.2020.136567>

887 Fenchel, T., 2017. Role of Microorganisms (microbes). *Reference Module in Life Sciences*.  
888 *Encyclopedia of Biodiversity*, 2013, pp. 299-308. [https://doi.org/10.1016/B978-0-12-809633-](https://doi.org/10.1016/B978-0-12-809633-8.02277-9)  
889 [8.02277-9](https://doi.org/10.1016/B978-0-12-809633-8.02277-9)

890 Foley, C.J., Feiner, Z.S., Malinich, T.D., Höök, T.O., 2018. A meta-analysis of the effects of exposure to  
891 microplastics on fish and aquatic invertebrates. *Sci. Total Environ.*  
892 <https://doi.org/10.1016/j.scitotenv.2018.03.046>

893 Fred-Ahmadu, O.H., Bhagwat, G., Oluyoye, I., Benson, N.U., Ayejuyo, O.O., Palanisami, T., 2020.  
894 Interaction of chemical contaminants with microplastics: Principles and perspectives. *Sci. Total*  
895 *Environ.* <https://doi.org/10.1016/j.scitotenv.2019.135978>

896 Fu, D., Zhang, Q., Fan, Z., Qi, H., Wang, Z., Peng, L., 2019. Aged microplastics polyvinyl chloride  
897 interact with copper and cause oxidative stress towards microalgae *Chlorella vulgaris*. *Aquat.*  
898 *Toxicol.* 216. <https://doi.org/10.1016/j.aquatox.2019.105319>

899 Fueser, H., Mueller, M.T., Weiss, L., Höss, S. and Traunspurger, W., 2019. Ingestion of microplastics  
900 by nematodes depends on feeding strategy and buccal cavity size. *Environmental*

901        *Pollution*, 255, p.113227.

902        Gao, G., Zhao, X., Jin, P., Gao, K. and Beardall, J., 2021. Current understanding and challenges for  
903        aquatic primary producers in a world with rising micro-and nano-plastic levels. *Journal of*  
904        *Hazardous Materials*, 406, p.124685. <https://doi.org/10.1016/j.jhazmat.2020.124685>

905        Gigault, J., Halle, A. ter, Baudrimont, M., Pascal, P.Y., Gauffre, F., Phi, T.L., El Hadri, H., Grassl, B.,  
906        Reynaud, S., 2018. Current opinion: What is a nanoplastic? *Environ. Pollut.*  
907        <https://doi.org/10.1016/j.envpol.2018.01.024>

908        González-Pleiter, M., Pedrouzo-Rodríguez, A., Verdú, I., Leganés, F., Marco, E., Rosal, R. and  
909        Fernández-Piñas, F., 2021. Microplastics as vectors of the antibiotics azithromycin and  
910        clarithromycin: Effects towards freshwater microalgae. *Chemosphere*, 268, p.128824.

911        Guo, J.J., Huang, X.P., Xiang, L., Wang, Y.Z., Li, Y.W., Li, H., Cai, Q.Y., Mo, C.H., Wong, M.H., 2020.  
912        Source, migration and toxicology of microplastics in soil. *Environ. Int.*  
913        <https://doi.org/10.1016/j.envint.2019.105263>

914        Guschina, I.A., Hayes, A.J., Ormerod, S.J., 2020. Polystyrene microplastics decrease accumulation of  
915        essential fatty acids in common freshwater algae. *Environ. Pollut.* 263, 114425.  
916        <https://doi.org/10.1016/j.envpol.2020.114425>

917        Hanslik, L., Sommer, C., Huppertsberg, S., Dittmar, S., Knepper, T.P. and Braunbeck, T., 2020.  
918        Microplastic-associated trophic transfer of benzo (k) fluoranthene in a limnic food web: Effects  
919        in two freshwater invertebrates (*Daphnia magna*, *Chironomus riparius*) and zebrafish (*Danio*  
920        *rerio*). *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 237,  
921        p.108849.

922        Harush-Frenkel, O., Bivas-Benita, M., Nassar, T., Springer, C., Sherman, Y., Avital, A., Altschuler, Y.,  
923        Borlak, J., Benita, S., 2010. A safety and tolerability study of differently-charged nanoparticles  
924        for local pulmonary drug delivery. *Toxicol. Appl. Pharmacol.* 246.

925 <https://doi.org/10.1016/j.taap.2010.04.011>

926 Heddegard, F.E., Møller, P., 2020. Hazard assessment of small-size plastic particles: is the  
 927 conceptual framework of particle toxicology useful? *Food Chem. Toxicol.*  
 928 <https://doi.org/10.1016/j.fct.2019.111106>

929 Horton, A.A., Svendsen, C., Williams, R.J., Spurgeon, D.J., Lahive, E., 2017. Large microplastic  
 930 particles in sediments of tributaries of the River Thames, UK – Abundance, sources and  
 931 methods for effective quantification. *Mar. Pollut. Bull.* 114, 218–226.  
 932 <https://doi.org/10.1016/j.marpolbul.2016.09.004>

933 Horton, A.A., Dixon, S.J., 2018. Microplastics: An introduction to environmental transport processes.  
 934 Wiley Interdiscip. Rev. Water 5. <https://doi.org/10.1002/wat2.1268>

935 Horton, A.A., Jürgens, M.D., Lahive, E., van Bodegom, P.M. and Vijver, M.G., 2018a. The influence of  
 936 exposure and physiology on microplastic ingestion by the freshwater fish *Rutilus rutilus* (roach)  
 937 in the River Thames, UK. *Environmental Pollution*, 236, pp.188-194.

938 Horton, A.A., Vijver, M.G., Lahive, E., Spurgeon, D.J., Svendsen, C., Heutink, R., van Bodegom, P.M.,  
 939 Baas, J., 2018b. Acute toxicity of organic pesticides to *Daphnia magna* is unchanged by co-  
 940 exposure to polystyrene microplastics. *Ecotoxicol. Environ. Saf.* 166.  
 941 <https://doi.org/10.1016/j.ecoenv.2018.09.052>

942 Horton, A.A., Newbold, L.K., Palacio-Cortés, A.M., Spurgeon, D.J., Pereira, M.G., Carter, H., Gweon,  
 943 H.S., Vijver, M.G., van Bodegom, P.M., Navarro da Silva, M.A., Lahive, E., 2020. Accumulation of  
 944 polybrominated diphenyl ethers and microbiome response in the great pond snail *Lymnaea*  
 945 *stagnalis* with exposure to nylon (polyamide) microplastics. *Ecotoxicol. Environ. Saf.* 188.  
 946 <https://doi.org/10.1016/j.ecoenv.2019.109882>

947 Horton, A.A., Cross, R.K., Read, D.S., Jürgens, M.D., Ball, H.L., Svendsen, C., Vollertsen, J. and  
 948 Johnson, A.C., 2021. Semi-automated analysis of microplastics in complex wastewater

949 samples. *Environmental Pollution*, 268, p.115841.

950 <https://doi.org/10.1016/j.envpol.2020.115841>

951 Hu, L., Su, L., Xue, Y., Mu, J., Zhu, J., Xu, J. and Shi, H., 2016. Uptake, accumulation and elimination of

952 polystyrene microspheres in tadpoles of *Xenopus tropicalis*. *Chemosphere*, 164, pp.611-617.

953 Hughes, J. ed., 2018. *Freshwater ecology and conservation: approaches and techniques*. Oxford

954 University Press.

955 Hurt, R., O'Reilly, C.M. and Perry, W.L., 2020. Microplastic prevalence in two fish species in two US

956 reservoirs. *Limnology and Oceanography Letters*, 5(1), pp.147-153.

957 Iannilli, V., Corami, F., Grasso, P., Lecce, F., Buttinelli, M. and Setini, A., 2020. Plastic abundance and

958 seasonal variation on the shorelines of three volcanic lakes in Central Italy: can amphipods help

959 detect contamination?. *Environmental Science and Pollution Research*, pp.1-12.

960 Imhof, H.K., Ivleva, N.P., Schmid, J., Niessner, R., Laforsch, C., 2013. Contamination of beach

961 sediments of a subalpine lake with microplastic particles. *Curr. Biol.*

962 <https://doi.org/10.1016/j.cub.2013.09.001>

963 Jackson, M.C., Pawar, S. and Woodward, G. 2021. The temporal dynamics of multiple stressor

964 effects: from individuals to ecosystems. *Trends in Ecology and Evolution*

965 DOI:<https://doi.org/10.1016/j.tree.2021.01.005>

966 Jaikumar, G., Baas, J., Brun, N.R., Vijver, M.G. and Bosker, T., 2018. Acute sensitivity of three

967 Cladoceran species to different types of microplastics in combination with thermal

968 stress. *Environmental Pollution*, 239, pp.733-740.

969 Jemec, A., Horvat, P., Kunej, U., Bele, M., Kržan, A., 2016. Uptake and effects of microplastic textile

970 fibers on freshwater crustacean *Daphnia magna*. *Environ. Pollut.* 219.

971 <https://doi.org/10.1016/j.envpol.2016.10.037>

972 Jeong, C.B., Kang, H.M., Lee, Y.H., Kim, M.S., Lee, Jin Sol, Seo, J.S., Wang, M., Lee, Jae Seong, 2018.



973 Nanoplastic Ingestion Enhances Toxicity of Persistent Organic Pollutants (POPs) in the  
 974 Monogonont Rotifer *Brachionus koreanus* via Multixenobiotic Resistance (MXR) Disruption.  
 975 *Environ. Sci. Technol.* 52. <https://doi.org/10.1021/acs.est.8b03211>  
 976 Johnson, A.C., Ball, H., Cross, R., Horton, A.A., Jürgens, M.D., Read, D.S., Vollertsen, J. and Svendsen,  
 977 C., 2020. Identification and quantification of microplastics in potable water and their sources  
 978 within water treatment works in England and Wales. *Environmental Science &*  
 979 *Technology*, 54(19), pp.12326-12334. <https://dx.doi.org/10.1021/acs.est.0c03211>  
 980 Kalčíková, G., 2020. Aquatic vascular plants – A forgotten piece of nature in microplastic research.  
 981 *Environ. Pollut.* 262, 114354. <https://doi.org/10.1016/j.envpol.2020.114354>  
 982 Khosrovyan, A. and Kahru, A., 2020. Evaluation of the hazard of irregularly shaped co-polyamide  
 983 microplastics on the freshwater non-biting midge *Chironomus riparius* through its life  
 984 cycle. *Chemosphere*, 244, p.125487.  
 985 Koch, H.M., Calafat, A.M., 2009. Human body burdens of chemicals used in plastic manufacture.  
 986 *Philos. Trans. R. Soc. B Biol. Sci.* <https://doi.org/10.1098/rstb.2008.0208>  
 987 Koelmans, A.A., Besseling, E., Shim, W.J., 2015. Nanoplastics in the Aquatic Environment. Critical  
 988 Review, in: *Marine Anthropogenic Litter*. Springer International Publishing, Cham, pp. 325–340.  
 989 [https://doi.org/10.1007/978-3-319-16510-3\\_12](https://doi.org/10.1007/978-3-319-16510-3_12)  
 990 Kratina, P., Watts, T.J., Green, D.S., Kordas, R.L., O’Gorman, E.J., 2019. Interactive effects of warming  
 991 and microplastics on metabolism but not feeding rates of a key freshwater detritivore. *Environ.*  
 992 *Pollut.* 255. <https://doi.org/10.1016/j.envpol.2019.113259>  
 993 Kuśmierek, N. and Popiołek, M., 2020. Microplastics in freshwater fish from Central European  
 994 lowland river (Widawa R., SW Poland). *Environmental Science and Pollution Research*, pp.1-5.  
 995 Lau, W.W., Shiran, Y., Bailey, R.M., Cook, E., Stuchtey, M.R., Koskella, J., Velis, C.A., Godfrey, L.,  
 996 Boucher, J., Murphy, M.B. and Thompson, R.C., 2020. Evaluating scenarios toward zero plastic

997 pollution. *Science*, 369 (6510), pp.1455-1461.

998 Lee, H., Lee, H.J., Kwon, J.H., 2019. Estimating microplastic-bound intake of hydrophobic organic  
 999 chemicals by fish using measured desorption rates to artificial gut fluid. *Sci. Total Environ.* 651.  
 1000 <https://doi.org/10.1016/j.scitotenv.2018.09.068>

1001 Lehner, R., Weder, C., Petri-Fink, A., Rothen-Rutishauser, B., 2019. Emergence of Nanoplastic in the  
 1002 Environment and Possible Impact on Human Health. *Environ. Sci. Technol.*  
 1003 <https://doi.org/10.1021/acs.est.8b05512>

1004 Li, C., Busquets, R., Campos, L.C., 2020. Assessment of microplastics in freshwater systems: A review.  
 1005 *Sci. Total Environ.* <https://doi.org/10.1016/j.scitotenv.2019.135578>

1006 Li, J., Liu, H. and Chen, J.P., 2018. Microplastics in freshwater systems: A review on occurrence,  
 1007 environmental effects, and methods for microplastics detection. *Water research*, 137, pp.362-  
 1008 374.

1009 Li, S., Wang, P., Zhang, C., Zhou, X., Yin, Z., Hu, T., Hu, D., Liu, C., Zhu, L., 2020. Influence of  
 1010 polystyrene microplastics on the growth, photosynthetic efficiency and aggregation of  
 1011 freshwater microalgae *Chlamydomonas reinhardtii*. *Sci. Total Environ.* 714.  
 1012 <https://doi.org/10.1016/j.scitotenv.2020.136767>

1013 Liu, L., Fokkink, R., Koelmans, A.A., 2016. Sorption of polycyclic aromatic hydrocarbons to  
 1014 polystyrene nanoplastic. *Environ. Toxicol. Chem.* 35. <https://doi.org/10.1002/etc.3311>

1015 Liu, Y., Wang, Z., Wang, S., Fang, H., Ye, N., Wang, D., 2019. Ecotoxicological effects on *Scenedesmus*  
 1016 *obliquus* and *Danio rerio* Co-exposed to polystyrene nano-plastic particles and natural acidic  
 1017 organic polymer. *Environ. Toxicol. Pharmacol.* 67. <https://doi.org/10.1016/j.etap.2019.01.007>

1018 López-Rojo, N., Pérez, J., Alonso, A., Correa-Araneda, F., Boyero, L., 2020. Microplastics have lethal  
 1019 and sublethal effects on stream invertebrates and affect stream ecosystem functioning.  
 1020 *Environ. Pollut.* 259. <https://doi.org/10.1016/j.envpol.2019.113898>

1021 Lu, Y., Zhang, Y., Deng, Y., Jiang, W., Zhao, Y., Geng, J., Ding, L. and Ren, H., 2016. Uptake and  
 1022 accumulation of polystyrene microplastics in zebrafish (*Danio rerio*) and toxic effects in  
 1023 liver. *Environmental science & technology*, 50(7), pp.4054-4060.

1024 Luo, H., Li, Y., Zhao, Y., Xiang, Y., He, D., Pan, X., 2019a. Effects of accelerated aging on  
 1025 characteristics, leaching, and toxicity of commercial lead chromate pigmented microplastics.  
 1026 *Environ. Pollut.* <https://doi.org/10.1016/j.envpol.2019.113475>

1027 Luo, H., Xiang, Y., He, D., Li, Y., Zhao, Y., Wang, S., Pan, X., 2019b. Leaching behavior of fluorescent  
 1028 additives from microplastics and the toxicity of leachate to *Chlorella vulgaris*. *Sci. Total Environ.*  
 1029 678. <https://doi.org/10.1016/j.scitotenv.2019.04.401>

1030 Luo, H., Zhao, Y., Li, Y., Xiang, Y., He, D., Pan, X., 2020. Aging of microplastics affects their surface  
 1031 properties, thermal decomposition, additives leaching and interactions in simulated fluids. *Sci.*  
 1032 *Total Environ.* 714. <https://doi.org/10.1016/j.scitotenv.2020.136862>

1033 Lürling, M., Mackay, E., Reitzel, K., Spears, B.M., 2016. Editorial – A critical perspective on geo-  
 1034 engineering for eutrophication management in lakes. *Water Res.*  
 1035 <https://doi.org/10.1016/j.watres.2016.03.035>

1036 Ma, H., Pu, S., Liu, S., Bai, Y., Mandal, S., Xing, B., 2020. Microplastics in aquatic environments:  
 1037 Toxicity to trigger ecological consequences. *Environ. Pollut.*  
 1038 <https://doi.org/10.1016/j.envpol.2020.114089>

1039 Mao, Y., Ai, H., Chen, Y., Zhang, Z., Zeng, P., Kang, L., Li, W., Gu, W., He, Q., Li, H., 2018.  
 1040 Phytoplankton response to polystyrene microplastics: Perspective from an entire growth  
 1041 period. *Chemosphere* 208. <https://doi.org/10.1016/j.chemosphere.2018.05.170>

1042 Miao, L., Wang, P., Hou, J., Yao, Y., Liu, Z., Liu, S. and Li, T., 2019a. Distinct community structure and  
 1043 microbial functions of biofilms colonizing microplastics. *Science of the Total Environment*, 650,  
 1044 pp.2395-2402.

1045 Miao, L., Hou, J., You, G., Liu, Z., Liu, S., Li, T., Mo, Y., Guo, S. and Qu, H., 2019b. Acute effects of  
 1046 nanoplastics and microplastics on periphytic biofilms depending on particle size, concentration  
 1047 and surface modification. *Environmental Pollution*, 255, p.113300.

1048 Mateos-Cárdenas, A., Scott, D.T., Seitmaganbetova, G., van, van P., John, O.H., Marcel A.K., J., 2019.  
 1049 Polyethylene microplastics adhere to *Lemna minor* (L.), yet have no effects on plant growth or  
 1050 feeding by *Gammarus duebeni* (Lillj.). *Sci. Total Environ.* 689.  
 1051 <https://doi.org/10.1016/j.scitotenv.2019.06.359>

1052 Mattsson, K., Ekvall, M.T., Hansson, L.A., Linse, S., Malmendal, A., Cedervall, T., 2015a. Altered  
 1053 behavior, physiology, and metabolism in fish exposed to polystyrene nanoparticles. *Environ.*  
 1054 *Sci. Technol.* 49. <https://doi.org/10.1021/es5053655>

1055 Mattsson, K., Hansson, L.A., Cedervall, T., 2015b. Nano-plastics in the aquatic environment. *Environ.*  
 1056 *Sci. Process. Impacts.* <https://doi.org/10.1039/c5em00227c>

1057 Miao, L., Wang, P., Hou, J., Yao, Y., Liu, Z., Liu, S. and Li, T., 2019. Distinct community structure and  
 1058 microbial functions of biofilms colonizing microplastics. *Science of the Total Environment*, 650,  
 1059 pp.2395-2402.

1060 Michałowicz, J., 2014. Bisphenol A - Sources, toxicity and biotransformation. *Environ. Toxicol.*  
 1061 *Pharmacol.* <https://doi.org/10.1016/j.etap.2014.02.003>

1062 Mohamed Nor, N.H., Koelmans, A.A., 2019. Transfer of PCBs from Microplastics under Simulated Gut  
 1063 Fluid Conditions Is Biphasic and Reversible. *Environ. Sci. Technol.*  
 1064 <https://doi.org/10.1021/acs.est.8b05143>

1065 Möhlenkamp, P., Purser, A., Thomsen, L., 2018. Plastic microbeads from cosmetic products: An  
 1066 experimental study of their hydrodynamic behaviour, vertical transport and resuspension in  
 1067 phytoplankton and sediment aggregates. *Elementa* 6. <https://doi.org/10.1525/elementa.317>

1068 Müller, A., Österlund, H., Marsalek, J., Viklander, M., 2020. The pollution conveyed by urban runoff:

1069 A review of sources. *Sci. Total Environ.* <https://doi.org/10.1016/j.scitotenv.2019.136125>

1070 Naqash, N., Prakash, S., Kapoor, D. and Singh, R., 2020. Interaction of freshwater microplastics with  
1071 biota and heavy metals: a review. *Environmental Chemistry Letters*, pp.1-12.

1072 Nasser, F., Lynch, I., 2016. Secreted protein eco-corona mediates uptake and impacts of polystyrene  
1073 nanoparticles on *Daphnia magna*. *J. Proteomics* 137.  
1074 <https://doi.org/10.1016/j.jprot.2015.09.005>

1075 Nguyen, T.H., Tang, F.H.M., Maggi, F., 2020. Sinking of microbial-associated microplastics in natural  
1076 waters. *PLoS One* 15. <https://doi.org/10.1371/journal.pone.0228209>

1077 Nolte, T.M., Hartmann, N.B., Kleijn, J.M., Garnæs, J., van de Meent, D., Jan Hendriks, A., Baun, A.,  
1078 2017. The toxicity of plastic nanoparticles to green algae as influenced by surface modification,  
1079 medium hardness and cellular adsorption. *Aquat. Toxicol.* 183.  
1080 <https://doi.org/10.1016/j.aquatox.2016.12.005>

1081 Nordberg, M., Templeton, D.M., Andersen, O., Duffus, J.H., 2009. Glossary of terms used in  
1082 ecotoxicology (IUPAC Recommendations 2009). *Pure Appl. Chem.* 81, 829–970.  
1083 <https://doi.org/10.1351/PAC-REC-08-07-09>

1084 Oriekhova, O., Stoll, S., 2018. Heteroaggregation of nanoplastic particles in the presence of inorganic  
1085 colloids and natural organic matter. *Environ. Sci. Nano* 5. <https://doi.org/10.1039/c7en01119a>

1086 Pannetier, P., Morin, B., Le Bihanic, F., Dubreil, L., Clérandeau, C., Chouvellon, F., Van Arkel, K.,  
1087 Danion, M. and Cachot, J., 2020. Environmental samples of microplastics induce significant  
1088 toxic effects in fish larvae. *Environment international*, 134, p.105047.

1089 PlasticsEurope, 2020. Plastics – the Facts 2020 . An analysis of European plastics production, demand  
1090 and waste data  
1091 [https://www.plasticseurope.org/application/files/8016/1125/2189/AF\\_Plastics\\_the\\_facts-](https://www.plasticseurope.org/application/files/8016/1125/2189/AF_Plastics_the_facts-)  
1092 [WEB-2020-ING\\_FINAL.pdf](https://www.plasticseurope.org/application/files/8016/1125/2189/AF_Plastics_the_facts-WEB-2020-ING_FINAL.pdf)

1093 Pinto da Costa, J., Reis, V., Paço, A., Costa, M., Duarte, A.C., Rocha-Santos, T., 2019.

1094 Micro(nano)plastics – Analytical challenges towards risk evaluation. *TrAC - Trends Anal. Chem.*

1095 <https://doi.org/10.1016/j.trac.2018.12.013>

1096 Prata, J.C., da Costa, J.P., Lopes, I., Duarte, A.C., Rocha-Santos, T., 2019. Effects of microplastics on

1097 microalgae populations: A critical review. *Sci. Total Environ.*

1098 <https://doi.org/10.1016/j.scitotenv.2019.02.132>

1099 Provencher, J.F., Ammendolia, J., Rochman, C.M., Mallory, M.L., 2019. Assessing plastic debris in

1100 aquatic food webs: what we know and don't know about uptake and trophic transfer. *Environ.*

1101 *Rev.* 27, 304–317. <https://doi.org/10.1139/er-2018-0079>

1102 Qu, H., Ma, R., Barrett, H., Wang, B., Han, J., Wang, F., Chen, P., Wang, W., Peng, G. and Yu, G., 2020.

1103 How microplastics affect chiral illicit drug methamphetamine in aquatic food chain? From

1104 green alga (*Chlorella pyrenoidosa*) to freshwater snail (*Cipangopaludina*

1105 *cathayensis*). *Environment international*, 136, p.105480.

1106 Redondo-Hasselerharm, P.E., Falahudin, D., Peeters, E.T.H.M., Koelmans, A.A., 2018. Microplastic

1107 Effect Thresholds for Freshwater Benthic Macroinvertebrates. *Environ. Sci. Technol.* 52.

1108 <https://doi.org/10.1021/acs.est.7b05367>

1109 Rehse, S., Kloas, W. and Zarfl, C., 2018. Microplastics reduce short-term effects of environmental

1110 contaminants. Part I: effects of bisphenol A on freshwater zooplankton are lower in presence of

1111 polyamide particles. *International journal of environmental research and public health*, 15(2),

1112 p.280.

1113 Rodrigues, M.O., Abrantes, N., Gonçalves, F.J.M., Nogueira, H., Marques, J.C. and Gonçalves, A.M.M.,

1114 2018. Spatial and temporal distribution of microplastics in water and sediments of a freshwater

1115 system (Antuã River, Portugal). *Science of the total environment*, 633, pp.1549-1559.

1116 Roch, S., Friedrich, C., Brinker, A., 2020. Uptake routes of microplastics in fishes: practical and

1117 theoretical approaches to test existing theories. *Sci. Rep.* 10. <https://doi.org/10.1038/s41598->  
1118 020-60630-1

1119 Rochman, C.M., 2018. Microplastics research—from sink to source. *Science* (80-. ). 360, 28–29.  
1120 <https://doi.org/10.1126/science.aar7734>

1121 Rochman, C.M., Hoellein, T., 2020. The global odyssey of plastic pollution. *Science* (80-. ). 368, 1184–  
1122 1185. <https://doi.org/10.1126/science.abc4428>

1123 Sadler, D.E., Brunner, F.S., Plaistow, S.J., 2019. Temperature and clone-dependent effects of  
1124 microplastics on immunity and life history in *Daphnia magna*. *Environ. Pollut.* 255.  
1125 <https://doi.org/10.1016/j.envpol.2019.113178>

1126 Salvati, A., Åberg, C., dos Santos, T., Varela, J., Pinto, P., Lynch, I., Dawson, K.A., 2011. Experimental  
1127 and theoretical comparison of intracellular import of polymeric nanoparticles and small  
1128 molecules: Toward models of uptake kinetics. *Nanomedicine Nanotechnology, Biol. Med.* 7.  
1129 <https://doi.org/10.1016/j.nano.2011.03.005>

1130 Scherer, C., Brennholt, N., Reifferscheid, G., Wagner, M., 2017. Feeding type and development drive  
1131 the ingestion of microplastics by freshwater invertebrates. *Sci. Rep.* 7.  
1132 <https://doi.org/10.1038/s41598-017-17191-7>

1133 Scherer, C., Wolf, R., Völker, J., Stock, F., Brennholt, N., Reifferscheid, G., Wagner, M., 2020. Toxicity  
1134 of microplastics and natural particles in the freshwater dipteran *Chironomus riparius*: Same  
1135 same but different? *Sci. Total Environ.* 711. <https://doi.org/10.1016/j.scitotenv.2019.134604>

1136 Schrank, I., Trotter, B., Dummert, J., Scholz-Böttcher, B.M., Löder, M.G.J., Laforsch, C., 2019. Effects  
1137 of microplastic particles and leaching additive on the life history and morphology of *Daphnia*  
1138 *magna*. *Environ. Pollut.* 255. <https://doi.org/10.1016/j.envpol.2019.113233>

1139 Sendra, M., Saco, A., Yeste, M.P., Romero, A., Novoa, B., Figueras, A., 2020. Nanoplastics: From  
1140 tissue accumulation to cell translocation into *Mytilus galloprovincialis* hemocytes. resilience of

1141 immune cells exposed to nanoplastics and nanoplastics plus *Vibrio splendidus* combination. J.

1142 Hazard. Mater. 388. <https://doi.org/10.1016/j.jhazmat.2019.121788>

1143 Singer, D., Seppey, C.V., Lentendu, G., Dunthorn, M., Bass, D., Belbahri, L., Blandenier, Q., Debroas,

1144 D., de Groot, G.A., De Vargas, C. and Domaizon, I., 2021. Protist taxonomic and functional

1145 diversity in soil, freshwater and marine ecosystems. *Environment International*, 146, p.106262.

1146 Stanković, J., Milošević, D., Savić-Zdraković, D., Yalçın, G., Yildiz, D., Beklioğlu, M., Jovanović, B., 2020.

1147 Exposure to a microplastic mixture is altering the life traits and is causing deformities in the

1148 non-biting midge *Chironomus riparius* Meigen (1804). *Environ. Pollut.* 262.

1149 <https://doi.org/10.1016/j.envpol.2020.114248>

1150 Su, L., Nan, B., Hassell, K.L., Craig, N.J. and Pettigrove, V., 2019. Microplastics biomonitoring in

1151 Australian urban wetlands using a common noxious fish (*Gambusia*

1152 *holbrooki*). *Chemosphere*, 228, pp.65-74.

1153 Ter Halle, A., Jeanneau, L., Martignac, M., Jardé, E., Pedrono, B., Brach, L., Gigault, J., 2017.

1154 Nanoplastic in the North Atlantic Subtropical Gyre. *Environ. Sci. Technol.* 51.

1155 <https://doi.org/10.1021/acs.est.7b03667>

1156 The Royal Society, 2019. Microplastics in freshwater and soil. An evidence synthesis.

1157 Triebkorn, R., Braunbeck, T., Grummt, T., Hanslik, L., Huppertsberg, S., Jekel, M., Knepper, T.P.,

1158 Krais, S., Müller, Y.K., Pittroff, M., Ruhl, A.S., Schmieg, H., Schür, C., Strobel, C., Wagner, M.,

1159 Zumbülte, N., Köhler, H.-R., 2019. Relevance of nano- and microplastics for freshwater

1160 ecosystems: A critical review. *TrAC Trends Anal. Chem.* 110, 375–392.

1161 <https://doi.org/10.1016/j.trac.2018.11.023>

1162 Turner, S., Horton, A.A., Rose, N.L., Hall, C., 2019. A temporal sediment record of microplastics in an

1163 urban lake, London, UK. *J. Paleolimnol.* 61. <https://doi.org/10.1007/s10933-019-00071-7>

1164 van Weert, S., Redondo-Hasselerharm, P.E., Diepens, N.J., Koelmans, A.A., 2019. Effects of



1165 nanoplastics and microplastics on the growth of sediment-rooted macrophytes. *Sci. Total*  
 1166 *Environ.* 654. <https://doi.org/10.1016/j.scitotenv.2018.11.183>  
 1167 Velzeboer, I., Kwadijk, C.J.A.F., Koelmans, A.A., 2014. Strong sorption of PCBs to nanoplastics,  
 1168 microplastics, carbon nanotubes, and fullerenes. *Environ. Sci. Technol.* 48.  
 1169 <https://doi.org/10.1021/es405721v>  
 1170 Vethaak, A.D. and Legler, J. 2021. Microplastics and human health. *Science* 371, 672-674. DOI:  
 1171 10.1126/science.abe5041  
 1172 Wagner, M., Scherer, C., Alvarez-Muñoz, D., Brennholt, N., Bourrain, X., Buchinger, S., Fries, E.,  
 1173 Grosbois, C., Klasmeier, J., Marti, T., Rodriguez-Mozaz, S., Urbatzka, R., Vethaak, A.D., Winther-  
 1174 Nielsen, M., Reifferscheid, G., 2014. Microplastics in freshwater ecosystems: what we know  
 1175 and what we need to know. *Environ. Sci. Eur.* 26. <https://doi.org/10.1186/s12302-014-0012-7>  
 1176 Waldschläger, K., Lechthaler, S., Stauch, G., Schüttrumpf, H., 2020. The way of microplastic through  
 1177 the environment – Application of the source-pathway-receptor model (review). *Sci. Total*  
 1178 *Environ.* <https://doi.org/10.1016/j.scitotenv.2020.136584>  
 1179 Walkinshaw, C., Lindeque, P.K., Thompson, R., Tolhurst, T., Cole, M., 2020. Microplastics and  
 1180 seafood: lower trophic organisms at highest risk of contamination. *Ecotoxicol. Environ. Saf.*  
 1181 190. <https://doi.org/10.1016/j.ecoenv.2019.110066>  
 1182 Wang, W., Gao, H., Jin, S., Li, R. and Na, G., 2019. The ecotoxicological effects of microplastics on  
 1183 aquatic food web, from primary producer to human: A review. *Ecotoxicology and*  
 1184 *environmental safety*, 173, pp.110-117. <https://doi.org/10.1016/j.ecoenv.2019.01.113>  
 1185 Wang, W., Ge, J., and Yu, X. 2020. Bioavailability and toxicity of microplastics to fish species: A  
 1186 review. *Ecotoxicology and Environmental Safety*, 189, 109913.  
 1187 Weber, A., Jeckel, N. and Wagner, M., 2020. Combined effects of polystyrene microplastics and  
 1188 thermal stress on the freshwater mussel *Dreissena polymorpha*. *Science of The Total*

1189 *Environment*, 718, p.137253.

1190 Whitehead, Paul G.; Bussi, Gianbattista; Hughes, Jocelyne M.R.; Castro-Castellon, Ana T.; Norling,  
 1191 Magnus D.; Jeffers, Elizabeth S.; Rampley, Cordelia P.N.; Read, Daniel S.; Horton, Alice A. 2021.  
 1192 "Modelling Microplastics in the River Thames: Sources, Sinks and Policy Implications" *Water* 13,  
 1193 no. 6: 861. <https://doi.org/10.3390/w13060861>

1194 Windsor, F.M., Tilley, R.M., Tyler, C.R., Ormerod, S.J., 2019. Microplastic ingestion by riverine  
 1195 macroinvertebrates. *Sci. Total Environ.* 646. <https://doi.org/10.1016/j.scitotenv.2018.07.271>

1196 Wu, P., Huang, J., Zheng, Y., Yang, Y., Zhang, Y., He, F., Chen, H., Quan, G., Yan, J., Li, T., Gao, B., 2019.  
 1197 Environmental occurrences, fate, and impacts of microplastics. *Ecotoxicol. Environ. Saf.*  
 1198 <https://doi.org/10.1016/j.ecoenv.2019.109612>

1199 Xu, S., Ma, J., Ji, R., Pan, K., Miao, A.J., 2020. Microplastics in aquatic environments: Occurrence,  
 1200 accumulation, and biological effects. *Sci. Total Environ.*  
 1201 <https://doi.org/10.1016/j.scitotenv.2019.134699>

1202 Yang, C.Z., Yaniger, S.I., Jordan, V.C., Klein, D.J., Bittner, G.D., 2011. Most plastic products release  
 1203 estrogenic chemicals: A potential health problem that can be solved. *Environ. Health Perspect.*  
 1204 119. <https://doi.org/10.1289/ehp.1003220>

1205 Yang, W., Gao, X., Wu, Y., Wan, L., Tan, L., Yuan, S., Ding, H. and Zhang, W., 2020. The combined  
 1206 toxicity influence of microplastics and nonylphenol on microalgae *Chlorella*  
 1207 *pyrenoidosa*. *Ecotoxicology and environmental safety*, 195, p.110484.  
 1208 <https://doi.org/10.1016/j.ecoenv.2020.110484>

1209 Yardy, L., Callaghan, A., 2020. What the fluff is this? - *Gammarus pulex* prefer food sources without  
 1210 plastic microfibers. *Sci. Total Environ.* 715. <https://doi.org/10.1016/j.scitotenv.2020.136815>

1211 Yokota, K., Waterfield, H., Hastings, C., Davidson, E., Kwietniewski, E., Wells, B., 2017. Finding the  
 1212 missing piece of the aquatic plastic pollution puzzle: Interaction between primary producers

1213 and microplastics. *Limnol. Oceanogr. Lett.* 2. <https://doi.org/10.1002/lol2.10040>

1214 Yuan, W., Zhou, Y., Liu, X., Wang, J., 2019. New Perspective on the Nanoplastics Disrupting the  
1215 Reproduction of an Endangered Fern in Artificial Freshwater. *Environ. Sci. Technol.* 53.  
1216 <https://doi.org/10.1021/acs.est.9b02882>

1217 Zhang, Z., Chen, Y., 2020. Effects of microplastics on wastewater and sewage sludge treatment and  
1218 their removal: A review. *Chem. Eng. J.* <https://doi.org/10.1016/j.cej.2019.122955>

1219 Zhang, S., Ding, J., Razanajatovo, R.M., Jiang, H., Zou, H. and Zhu, W., 2019. Interactive effects of  
1220 polystyrene microplastics and roxithromycin on bioaccumulation and biochemical status in the  
1221 freshwater fish red tilapia (*Oreochromis niloticus*). *Science of the total environment*, 648,  
1222 pp.1431-1439.

1223 Ziajahromi, S., Kumar, A., Neale, P.A., Leusch, F.D.L., 2017. Impact of Microplastic Beads and Fibers  
1224 on Waterflea ( *Ceriodaphnia dubia* ) Survival, Growth, and Reproduction: Implications of Single  
1225 and Mixture Exposures. *Environ. Sci. Technol.* 51, 13397–13406.  
1226 <https://doi.org/10.1021/acs.est.7b03574>

1227 Ziajahromi, S., Kumar, A., Neale, P.A. and Leusch, F.D., 2018. Environmentally relevant  
1228 concentrations of polyethylene microplastics negatively impact the survival, growth and  
1229 emergence of sediment-dwelling invertebrates. *Environmental Pollution*, 236, pp.425-431.

1230 **Appendix A. Glossary of terms used in Ecotoxicology.**

Term	Definition
<b>Absorption (in biology)</b>	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.
<b>Acute</b>	Of short duration, in relation to exposure or effect. Aquatic toxicology exposure of the test organisms is continuous and of four days or less.
<b>Bioaccessibility</b>	Environmental availability. Able to come in contact with a living organism and perhaps interact with it with the possibility of absorption into the organism.
<b>Bioaccumulation Factor</b>	<p>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.</p> <p>Note 1: The concentration in the organism is typically expressed per unit body mass or per gram of lipid (bioaccumulation factor, lipid-based).</p> <p>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).</p> <p>Note 3: The compound may have entered the organism by any available route and from any component of the water or sediment.</p> <p>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.</p>
<b>Bioaccumulation</b>	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.
<b>Bioavailability</b>	<p>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.</p> <p>Bioavailability, like bioaccessibility, is a function of both chemical speciation and biological properties. Even surface-bound substances may not be bioaccessible, and</p>

	hence not bioavailable, to organisms which require substances to be in solution before they can interact with them.
<b>Bioavailable</b>	Able to be absorbed by living organisms.
<b>Bioconcentration</b>	Process leading to a higher concentration of a substance in an organism than in environmental media to which it is exposed. Usually applied to uptake by aquatic organisms directly from water
<b>Bioconcentration Factor</b>	<p>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.</p> <p>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.</p> <p>Note 2: This parameter is an important determinant for human intake of contaminants from water by ingestion of aquatic food.</p>
<b>Biological monitoring (ecotoxicology)</b>	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.
<b>Biomagnification = Trophic enrichment</b>	1. Sequence of processes by which higher concentrations of a substance are attained in organisms at higher trophic levels.

<b>= Ecological magnification</b>	<p>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.</p> <p>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.</p>
<b>Bottom-up ecotoxicological study</b>	<p>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.</p>
<b>Chronic</b>	<p>Consequence that develops slowly and (or) has a long-lasting course: may be applied to an effect which develops rapidly and is long lasting.</p>
<b>Direct Toxicity</b>	<p>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.</p>
<b>Ecotoxicology</b>	<p>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.</p>
<b>Effect time</b>	<p>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</p>
<b>Effective concentration</b>	<p>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 population after a given time (EC<sub>x</sub>). Note: EC50 is the median concentration that causes 50 % of maximal response</p>
<b>Effective dose</b>	<p>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</p>

	<p>given time (ED<sub>x</sub>). Note: ED<sub>50</sub> is the median dose that causes 50 % of maximal response.</p>
<b>Environmental bioavailability</b>	<p>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.</p> <p>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.</p>
<b>Environmental monitoring</b>	<p>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.</p> <p>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.</p>
<b>Indirect toxicity</b>	<p>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).</p>
<b>Pollutant</b>	<p>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.</p> <p>Note 1: "Undesirability", like toxicity, is concentration-dependent, low concentrations of most substances being tolerable or even essential in many cases.</p> <p>Note 2: A primary pollutant is one emitted into the atmosphere, water, sediments, or soil from an identifiable source.</p>

	Note 3: A secondary pollutant is a pollutant formed by chemical reaction in the atmosphere, water, sediments, or soil.
<b>Teratogenesis</b>	Process resulting in permanent structural malformations or defects in the offspring of a parent exposed to a teratogen.
<b>Top-down ecotoxicological study</b>	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.
<b>Toxicity</b>	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 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).
<b>Trophic dilution</b>	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.
<b>Trophic transpher</b>	Transfer of a substance from one trophic level to another.
<b>Trophic transpher ratio</b>	Ratio between the concentration of a compound in a predator and in its prey
<b>Uptake</b>	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.



1231 Note: All definitions are from Nordberg et al. (2009).