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Review article

Nano and microplastic interactions with freshwater biota – Current knowledge, challenges and future solutions

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ABSTRACT

Current understanding of nano- and microplastic movement, propagation and potential effects on biota in freshwater environments is developing rapidly. Still, there are significant disconnects in the integration of knowledge derived from laboratory and field studies. This review synthesises the current understanding of nano- and microplastic impacts on freshwater biota from field studies and combines it with the more mechanistic insights derived from laboratory studies. Several discrepancies between the field and laboratory studies, impacting progress in process understanding, were identified including that the most prevalent plastic morphologies found in the field (fibres) are not those used in most of the laboratory studies (particles). Solutions to overcome these disparities are proposed to aid comparability of future studies. For example, environmental sampling and separation of biota into its constituents is encouraged when conducting field studies to map microplastic uptake preferences. In laboratory studies, recommendations include performing toxicity studies to systematically test possible factors affecting toxicity of nano- and microplastics, including morphology, chemical makeup (e.g., additives) and effects of plastic size. Consideration should be given to environmentally relevant exposure factors in laboratory studies, such as realistic exposure medium and effects of plastic ageing. Furthermore, based on this comprehensive review recommendations of principal toxicity endpoints for each of the main trophic levels (microbes, primary producers, primary consumers and secondary consumers) that should be reported to make toxicity studies more comparable in the future are given.

1. Introduction

Plastic pollution in the world's oceans is a widely reported issue (Cózar et al., 2014; Geyer et al., 2017; Jambeck et al., 2015). Besides the visible macroplastic, there is also concern surrounding smaller plastics, such as microplastics (dimension less than 5 mm) (Arthur et al., 2009; GESAMP, 2016; Thompson et al., 2004), and nanoplastics (despite some ongoing debate in the literature generally considered less than 1000 nm). These micro and nanoscale plastics are referred to hereafter as MnP, unless specifically only micro or nanoplastics are discussed. MnP can be either directly designed and produced for this specific size-range (primary), or result from breakdown of macroscale plastic into smaller pieces (secondary) due to physical, photochemical and/or biological degradation (Corcoran et al., 2009; Dawson et al., 2018; Mateos-Cárdenas et al., 2020; O'Brine and Thompson, 2010; Zbyszewski et al., 2014).

To date, a vast majority of plastic research has focused on marine and coastal environments (Desforges et al., 2014; Kanhai et al., 2017; Lusher et al., 2015; Munari et al., 2017; Van Cauwenberghe et al., 2015; Woodall et al., 2014) with the transport, fate and impact of plastics in freshwater systems only recently gaining attention (Li et al., 2018; Krause et al., 2021). This is likely driven by the increasing realisation that lakes and rivers are not merely conduits transporting MnP from terrestrial sources to the marine environment, but also have the potential to act as temporary/long-term sinks (Hurley et al., 2018; Luo et al., 2019b). There is, thus, a risk that MnP may cause detrimental effects to freshwater ecosystems.

Microplastics have been found in the surface waters and sediments of both rivers and lakes (Eerkes-Medrano et al., 2015; Li et al., 2018; Tibbetts et al., 2018). They can enter freshwater systems via various pathways, such as outfall from waste water treatment plants (WWTP) (Murphy et al., 2016), or through surface runoff (Corradini et al., 2019;

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Kole et al., 2017; Liu et al., 2019a). Microplastics can also find their way into freshwater systems through transport via air and accumulate as dry or wet deposits (Allen et al., 2019; Dris et al., 2017, 2016). Although the extent of our knowledge is not the same for nanoplastics, due to extraction (sampling) and detection limitations, it is suspected that nanoplastics are present everywhere that microplastics are, and may follow similar transport and fate pathways to those of microplastics (Wang et al., 2021).

MnP interaction and/or ingestion and their effects have been documented for a variety of freshwater biota, such as algae, zooplankton, fish and birds (Faure et al., 2015; Sadler et al., 2019; Wu et al., 2019) (Fig. 1).

There is some initial evidence of pollutant transfer from MnP to organisms (Coffin et al., 2019; Rochman et al., 2013; Wardrop et al., 2016). However, it is still debated whether MnP can be considered an environmentally relevant pathway for sorbed pollutants (Hartmann et al., 2017; Koelmans, 2015; Koelmans et al., 2016). Nevertheless, MnP could be toxic to biota in their own right through physical effects, such as slowed egestion times (Au et al., 2015; Shang et al., 2020), that could lead to clogging or damage of intestinal tracts (Lei et al., 2018). This, together with the realisation that MnPs are omnipresent in freshwater systems, has led to an expansion of interaction and toxicity studies in freshwater organisms. However, this research field is still in its infancy and potential entry points, uptake mechanisms, and fate for MnP in biota and consequently into food-webs have not yet been clearly established (Krause et al., 2021; Wong et al., 2020). In particular, analytical methods to quantify MnP burden are still being optimised for environmental matrices (O'Connor et al., 2020) and biological tissues (Wagner et al., 2017). There are also questions regarding how well laboratory studies reflect field data and vice versa. This review aims to provide a pathway forward whereby harmonisation between field and laboratory studies is discussed. This extensive review of over 76 field and 164 laboratory studies also aims to find commonalities amongst published literature that may support future studies by providing a solid baseline against which to design and implement future experimental and sampling campaigns, thereby maximising their increased comparability and utility for risk assessment. Therefore, a comprehensive synthesis of current research on MnP interactions with freshwater biota was performed to:

1. Identify freshwater species shown to be capable of interacting with MnP in the field and the laboratory at the different trophic levels;
2. Establish potential patterns in the freshwater MnP literature including types of plastics found/used, geographical context and the physical characteristics of the MnP assessed;
3. Identify key areas for increased alignment of laboratory and field research, to facilitate risk assessment, and the priority challenges for future research, for which we propose solutions.

2. Methods

A comprehensive literature review was carried out using Web of Science with the following topic search equation:

TS=(((microplastic OR nanoplastic*) AND (freshwater OR lake OR river)) AND (ingestion OR abundance OR occurrence OR content OR quantity OR feeding OR contamination OR gut OR intestines OR gastrointestinal OR bioaccumulation OR bioavailability OR uptake OR organism*))*

Publications for the period 2011 and 2020 have been included in this review. Studies were analysed using the following four criteria: 1) Original study (reviews were excluded), 2) Focus on freshwater ecosystems (salinity less than 0.05% or 0.5 ppt), 3) Report results showing an interaction between MnP and freshwater organisms, and 4) Quantifies or uses plastics less than 5 mm in diameter (Arthur et al., 2009; GESAMP, 2016; Thompson et al., 2004). The initial search yielded 878 matches from which 656 articles were excluded due to not conforming with the criteria set above. An additional 18 articles met the criteria for inclusion after citation analysis was carried out for the studies included from the first step, bringing the total number of journal articles reviewed for this synthesis to 240 (Table S1).

The studies were divided into field or laboratory based research. Studies were also grouped into: algae, vascular plants, microbes, cladocerans (separated from the rest of crustaceans, due to high occurrence in the literature), crustaceans, gastropods, dipterans, bivalves, annelids, amphibians, fish, birds, and others. These biota groups were then organised into the following four trophic levels:

1. Microbes
2. Primary producers (algae, vascular plants)
3. Primary consumers (cladocerans, crustaceans, gastropods, dipterans, bivalves, annelids and others)
4. Secondary consumers (amphibians, fish, and birds).

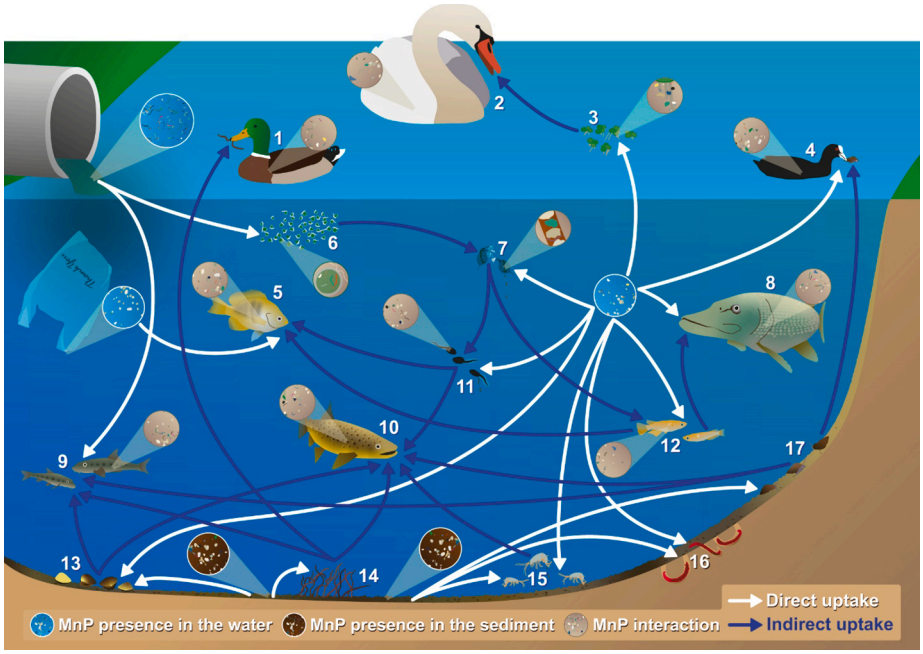

Fig. 1. Conceptual model for MnP movements in the freshwater food web. Numbers (1–16) indicate that MnP interaction with this biotic group has been confirmed (Table S1). Species in the figure: 1) *Anas platyrhynchos*, 2) *Cygnus olor*, 3) *Lemna minor*, 4) *Fulica atra*, 5) *Lepomis macrochirus*, 6) *Chlorella vulgaris*, 7) *Daphnia magna*, 8) *Esox lucius*, 9) *Gobio gobio*, 10) *Squalius cephalus*, 11) *Microhyla ornate*, 12) *Alburnus alburnus*, 13) *Dreissena polymorpha*, 14) *Tubifex tubifex*, 15) *Gammarus pulex* and 16) *Chironomus* spp.

From each of these trophic levels the following were identified; 1) species studied, 2) physical (i.e., size, morphology, colour) and chemical (i.e., plastic type) characteristics of the MnP identified (field) or used (laboratory) and 3) methodologies (characterisation and toxicity endpoints) used. Toxic effects were included in the context of identifying potential endpoints that could be used for baseline studies, but were not extensively reviewed, as this topic has been covered previously (e.g., Anbumani and Kakkar, 2018; Prokić et al., 2019; Triebskorn et al., 2019) and was considered to be out of the scope of this study.

Size was divided into two separate categories; microplastics (1–5000 μm) and nanoplastics (<1000 nm). Morphology of plastics was assigned into three main categories; a) fibres, b) particles (that encapsulated both spheres and granules due to lack of morphology characterisation in several studies) and c) fragments.

To identify any regional hotspots of MnP research, the origin of laboratory studies was assessed according to the locations of the first and last author's facilities/addresses, while field studies had their respective sampling location(s) recorded independently of the author's respective locations.

3. Results

3.1. General results

From the 240 studies, 76 (~32%) field studies and 164 (~68%) laboratory studies were identified (Table S1).

3.2. Types of plastics

Polyethylene (PE) was the most common plastic type found in field studies, followed by polypropylene (PP), polyethylene terephthalate (PET), polyamide (PA) and polystyrene (PS), as shown in Fig. 2. 'Not given' was the largest group in field studies, highlighting the lack of chemical verification tools used, with forty-two percent of studies not including polymer identification. In laboratory studies, the most frequently used plastic was PS, followed by PE, 'others', PP and PET. Here also, several papers used a commercial microplastic for which no compositional information was available (grouped as "others"), reducing the utility of the data for risk assessment.

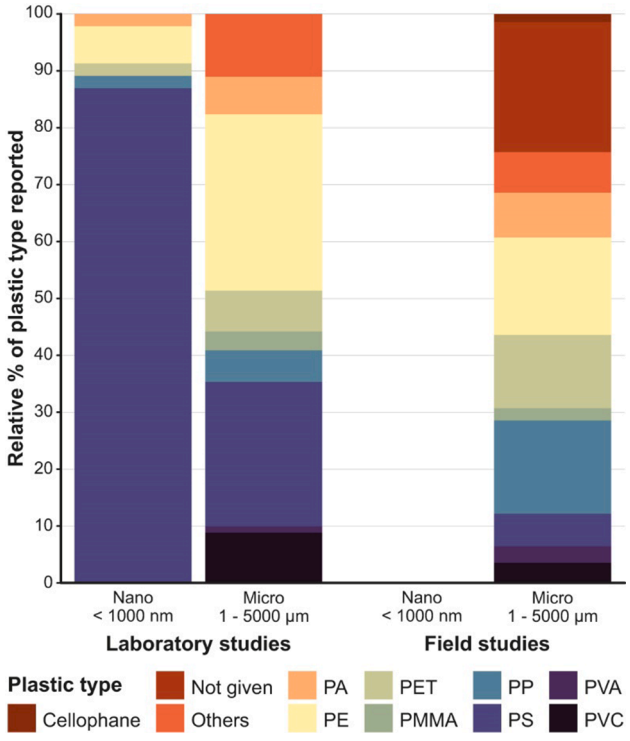

Fig. 2. Most common MnP plastic types reported across laboratory and field studies.

3.3. Sizes and morphology of plastics

Most of the studies fell into the size range of microplastics, with reports on nanoplastics being absent from field studies. Twenty-six percent of field studies did not report the respective size of the observed plastics (Fig. 3), while laboratory studies included size by stating the commercial size range and in some instances provided additional size characterization information, via techniques such as dynamic light scattering (DLS) and/or scanning electron microscopy (SEM).

The most prevalent morphology reported in field studies, where this information was available, was fibres (76.5%). However, particles were the most abundant morphology used (76.2%) in laboratory settings, with fibres used in eight studies (4.8%).

3.4. Geographical spread

Analysis of the spatial distribution identified some clear geographical hotspots for MnP research. The majority of laboratory studies originated from China (n = 43), North America (n = 20) and Europe (Germany (n = 22), Italy (n = 11) and UK (n = 11)) as shown in Fig. 4. This spread in laboratory studies is matched by patterns identified in the sampling locations of field studies, that were clustered in Europe (n = 22), North America (n = 14) and China (n = 15).

3.5. Trophic levels and biota groups

From the four trophic levels including thirteen biota groups defined above, fish were the most studied (n = 90) making up ~59% of field studies and ~27% of laboratory studies. This was followed by cladocerans (n = 43), which were all laboratory studies (Fig. 5).

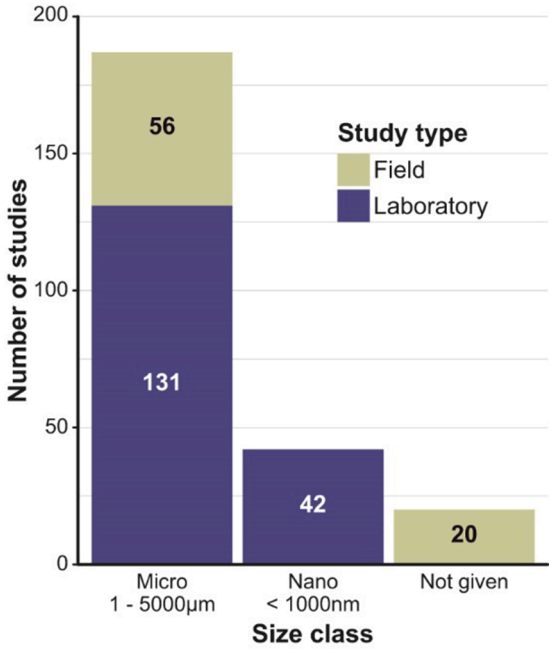

Fig. 3. Relative abundance of studies per MnP size class. Note that studies that included MnP from different size classes were counted as separate studies for the purpose of this figure.

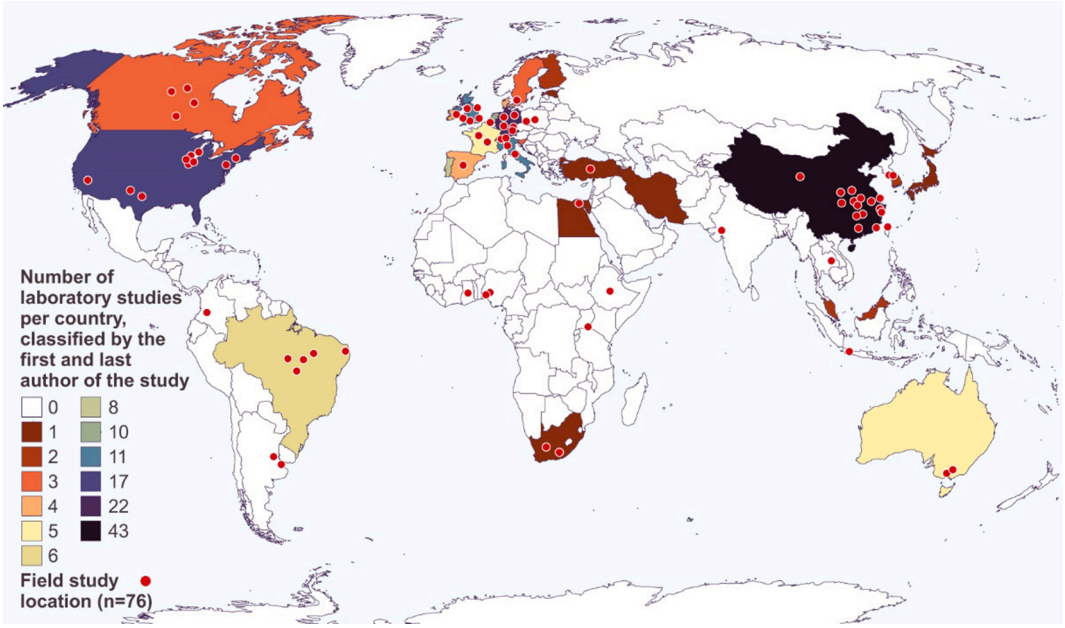

Fig. 4. Number of laboratory studies per country, grouped via first and last authors of the studies and locations of the field sampling sites.

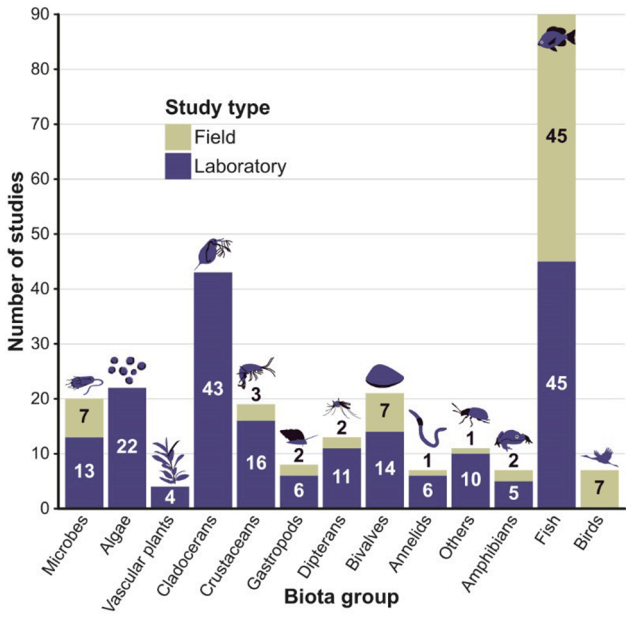

Fig. 5. Number of published MnP interaction studies in freshwater biota groups. Note that one field study and some laboratory studies included more than one biota group and each species is counted separately for this figure.

4. Discussion

4.1. General discussion

The major plastic types used in laboratory settings and found in biota loosely follow the trends in global plastic production. According to PlasticsEurope (2020), the major plastics manufactured in 2019 were PE (29.8%), PP (19.4%), polyvinyl chloride (PVC) (10%), polyurethane (PUR) (7.9%), PET (7.9%) and PS (6.2%). A similar trend was observed in field studies (Fig. 2), with PE and PP being the most common plastic types identified from the field samples, while the most common plastics used in laboratory studies were PS, followed by PE, PP and PET. Although most common plastic types are well represented in laboratory studies, it is recommended to extend laboratory effect testing to a more diverse range of plastic types. Special consideration should be given to those identified in biota from field studies, such as PA and cellophane, as the current trend favours certain plastic types and makes results difficult to compare

Regional hotspots for laboratory and field research were found to largely overlap (Fig. 4). Critically, Asian (apart from China) and African systems appear under-represented despite many of these regions, such as South and Southeast Asia, having been identified as being amongst the most plastic polluted areas globally (Jambeck et al., 2015; Lebreton et al., 2017). The identified lack of field studies in these areas is therefore of concern, as this impedes our holistic understanding regarding global MnP pollution and its impacts on biota. Furthermore, the total number of field studies is still relatively low (n = 76), when compared to laboratory studies. More observations are thus required, especially in highly polluted and under-represented regions, such as parts of Southeast Asia and Africa (Nel et al., 2021b).

There was a clear predisposition towards laboratory studies in all biotic groups, with the exception of fish and birds (Fig. 5). Studying higher trophic level organisms in laboratory settings can pose ethical challenges, as well as being time consuming, which can partly explain this trend. From all biotic groups, fish were the most studied. Fish represent one of the largest classes of Animalia in freshwater systems and can stretch over several trophic levels and feeding guilds. As they are generally higher up in the food chain and have commercial importance and health implications for humans via consumption, it is logical that studies on MnP ingestion have focused on this group. However, more studies are required across the different biotic groups to map out the occurrence of MnPs in the field and to identify their potential ecosystem-level effects. In addition to more field studies, we identified three key challenges that freshwater MnP research is currently facing, described below, along with proposed solutions to overcome them, which have also been summarised into Tables 1 and 2.

4.2. Challenges for future research

4.2.1. Challenge 1: The mismatch between field and laboratory studies

In order to predict effects of MnP on biota and freshwater ecosystems, exposure scenarios used in the laboratory should reflect real environmental conditions as closely as possible. Here, we explore the

Table 1
Recommendations for field sampling that aims to understand the interplay between ingested MnP and environment.

Recommendations	
Collection of field samples inconjunction with biota samples	Sediment and water samples (background concentrations needed for assessment of potential biomagnification)
Separation of organism into itsconstituents	Stomach, muscle, liver and brain when applicable (i.e. fish) and quantification of MnP load in each
Usage of relevant digestion method	KOH, NaOH or similar. Match to that used for environmental samples
Identification methods	Tagging methods, such as fluorescent dyes, Chemical identification, such as FT-IR, Raman spectroscopy or GC-MS (50% of putative MnP)
Recording physical characteristics of MnP	Colour (where possible), morphology and size
Reporting	Lowest detection limit and internalised dose, as follows; number mg^{-1} tissue, mg mg^{-1} tissue and items $individual^{-1}$
Method development	To enable nanoplastic identification from the field samples

Table 2
Recommendations for laboratory studies that aim to systematically understand the drivers and toxicological effects of MnP exposure.

Recommendations	
Morphology	Carry out exposures with similar parent plastic but different morphology (i.e. fragments, fibres and spheres)
Chemical composition	1) Identify chemical fingerprint: e.g., colourants, plasticisers 2) Wash a sub-set of parent plastic for toxicity assessment of leachates 3) Avoid usage of proprietary polymers whose composition is unknown
Size	1) Systematic testing of similar parent plastic in different sizes e.g., PS 1 μm, 5 μm and 10 μm, match exposure dose by surface area, mass and MnP number
Exposure	1) Environmental considerations i.e., always provide food, consider the presence of organic matter, natural medium and mode of exposure etc. 2) Aging of the plastic i.e., Pristine vs aged (natural or laboratory aged) 3) Abundance i.e., Inclusion of lower concentrations with life-time exposure 3) Duration i.e., Extended monitoring periods and consider depuration steps
Endpoints	1) Microbes i) Principal endpoints: taxon richness ii) secondary endpoints: species specific preference for plastic type 2) Primary producers i) Principal endpoints: photosynthetic activity and growth ii) Secondary endpoints: gene regulations and stress responses 3) Primary consumers i) Principal endpoints: growth and mortality ii) Secondary endpoints: reproduction and multigenerational studies 4) Secondary consumers i) Principal endpoints: growth, mortality and reproduction ii) Secondary endpoints: behavioural changes, AChE and oxidative stress

mismatch between field and laboratory studies and suggest solutions to close this gap.

Fibres were the most prominent microplastic morphology identified across taxa in field studies. This trend potentially reflects the relative abundance of fibres in the environment, as fibres are commonly identified in freshwater studies (e.g., Dris et al., 2015; Luo et al., 2019b; Wang et al., 2017). There appears to be a relationship between microplastic load within sediment and water, and microplastic burden within biota (Akindele et al., 2019; Li et al., 2020a; Lv et al., 2019; Nel et al., 2018; Su et al., 2018, 2016; Yan et al., 2019). For example, Lv et al. (2019) found that the microplastic composition was similar between the crayfish *Procambarus clarkii* and surrounding sediments, with fibres prevalent in both. Furthermore, Su et al. (2018) found that the microplastic load in bivalves at the Yangtze River basin was significantly dependent on both water and sediment microplastic contamination. In contrast, Su et al. (2016) reported that the microplastic load in bivalves was negatively correlated with the contamination in surrounding sediments. However, with only a handful of studies collecting data on MnP contamination from all compartments, this relationship remains inconclusive.

The prevalence of fibres could also be an indication of preferential consumption by certain biotic groups, especially in fish (Collard et al., 2019). There is evidence from laboratory studies that visually orientated fish actively forage on MnP resembling their prey items (Roch et al., 2020). Indeed, Yuan et al. (2019) noted that the proportion of white fibres in goldfish from Poyang Lake, China, was higher than that found in the surrounding environment, which could indicate confusion with prey species, such as mosquito larvae or zooplankton. This is further supported by McNeish et al. (2018) where microplastic colours varied in surface water collected within the tributaries of Lake Michigan, (USA), which was not reflected in the fish who preferentially ingested blue and transparent fibres. Hurley et al. (2017) reported an absence of microbeads in the freshwater worm *Tubifex tubifex*, even though they were found in the surrounding sediment. Similarly, Schessl et al. (2019) reported an absence of microbeads in bivalves *Dreissena polymorpha* and *D. bugensis* despite their presence in the environment. Drivers of this apparent preferential uptake remain unknown; possibilities include size, morphology, biofouling and/or plastic type. Laboratory studies have begun focusing on this question, with Li et al. (2019) stating that uptake of plastic fibres in bivalves was related to the elastic modulus, i.e., "softness" of the plastic type. It is essential to test these possible drivers in laboratory settings.

An alternative explanation may be that fibres are accumulating in the gastrointestinal (GI) tracts of biota due to their elongated shape. There is some initial support for this hypothesis; the only two field studies that separated the individual fish into different parts (i.e., stomach, muscle, gills and liver), reported different microplastic morphologies present in the various regions. For example, Collard et al. (2018) indicated that in field conditions PS and PE fragments ranging from 147 to 567 μm appeared to translocate into the livers of European chubs *Squalius cephalus* after ingestion, while fibres were prevalent in the stomach. Similarly, Garcia et al. (2020) reported microplastic films being more prevalent in gut tissues of *Prochilodus magdalenae* and *Pimelodus grosskopfii* whereas fragments were more prevalent in muscle and gills. Microfibre retention in the gut was also observed by Au et al. (2015) in a laboratory study, where PP fibres remained in the gut of the crustacean *Hyalella azteca* for longer than natural food and/or PE particles. This offers some initial support for the hypothesis that fibres could be retained in the gut for a longer duration. However, more research is needed targeting gut retention times of fibres.

The apparent dominance of fibres in field samples could also be a result of overestimation due to methodological bias in the applied analytical approach. For example, 31 out of 69 field studies (excluding studies on microbes) did not use any chemical verification tools and relied solely on visual identification and/or a hot needle test to confirm the presence of plastics. It is plausible that during the visual identification, the larger, less ambiguous fibres are more readily identified compared to other morphologies. The lack of chemical verification means that the identified fibres could also be natural or cotton fibres. Moreover, only six of the analysed papers stated clearly the lower size detection limit (Domogalla-Urbansky et al., 2019; Park et al., 2020; Roch et al., 2019; Winkler et al., 2020; Xiong et al., 2018; Yuan et al., 2019). This raises questions about the 'non-detected' fraction, and underestimation of smaller plastics could lead to overestimation of the

pervasiveness of more easily identified fibres.

In order for the research community to address these challenges, there is a need to improve the extraction of MnP from field biotic samples: Firstly, relying on visual identification solely should be minimised, as this can discriminate against smaller MnP which are more difficult to identify. The use of tagging methods, such as fluorescent dyes, for example, Nile Red (Erni-Cassola et al., 2017; Maes et al., 2017; Nel et al., 2021a; Shim et al., 2016) that can reduce visual bias, should be explored (Table 1). Researchers are encouraged to refer to the reporting guideline checklist and quality criteria published by Cowger et al. (2020), Collard et al. (2019) and Hermsen et al. (2018). Secondly, the lower detection limit (where putative MnP can be confidently identified) should always be reported in order to facilitate comparability between studies (Table 1).

Thirdly, where applicable (e.g., fish), it is important to separate the biota samples into different parts, such as brain, muscle, liver and gut (Table 1), which can give more specific information on where MnP are found and any preferential distributions (Collard et al., 2018; Garcia et al., 2020). More studies including this step are required, as there is contrasting evidence on tissue translocation (Collard et al., 2017; Elizalde-Velázquez et al., 2020; Jovanović et al., 2018; Zeytin et al., 2020; Zitouni et al., 2020) and disagreement exists particularly regarding the size fractions that are capable of translocating into tissues (as reviewed by Triebskorn et al. 2019). In the field of nanomedicine, it is generally accepted that endocytosis pathways are limited to particle sizes up to 5 μm (Kou et al., 2013; Zhang et al., 2009), raising questions regarding any potential mechanisms for translocation of larger items. It is plausible that bigger fragments (>20 μm) could be methodological artefacts or contamination from processing of the samples, if not enough rigor is applied. However, granuloma formation has been proposed as a potential entry pathway for MnP into muscle tissue (Zeytin et al., 2020).

Digestion should be carried out for each separate body part, with the same digestive method, such as KOH (Karami et al., 2017a; Thiele et al., 2019), followed by the extraction step and chemical analysis (such as $ZnCl_2$ separation followed by Fourier-transform infrared (FT-IR) or Raman spectroscopy), and where possible 50% of putative MnP should be analysed (Hermsen et al., 2018) (Table 1). Extra care should be taken to reduce any false-positives, such as dislocation or introduction of particles and/or fragments during dissection and rigorous control measures should be in place.

Fourthly, to fully address the possibility of the selective uptake of MnPs and to examine which factors in the immediate environment drive their ingestion, environmental samples (water column and sediment) should always be collected and analysed to characterise the sizes, morphologies, colours and types of MnPs present (Table 1). The digestion, extraction and chemical analysis methods used for the environmental samples should match as closely as possible to those used for biota, in order to reduce any methodological bias. This would inform on the possible drivers of fibre uptake and would aid in the prioritisation of plastics for laboratory exposure experiments.

Despite fibres being the most common morphology reported in the field, this is not reflected in laboratory studies, which most commonly employed particles. Only 8 out of 164 laboratory studies used fibres in MnP interaction studies (Table S1), and only six studied their effects, from which all reported negative effects on the test species. For example, decreased growth was seen in the crustacean *H. azteca* and planarian *Dugesia japonica* exposed to 20–75 μm PP fibres (Au et al., 2015) and ~30 μm PET fibres (Gambino et al., 2020) and the freshwater flea *Daphnia magna* showed increased mortality rates when exposed to 60–1400 μm PET fibres (Jemec et al., 2016).

The type and origin of fibres used in these studies also varied. For example, Au et al. (2015) used aged PP marine rope. Jemec et al. (2016) used milled PET fabrics and Gambino et al. (2020) produced PET fibres in their laboratory. Evidence suggests that additives may influence toxicity (Boyle et al., 2020; Capolupo et al., 2020), therefore the same plastic type from different commercial sources/products may further confound results, especially if information about chemical composition and additive content is not given. However, all fibres studied to date, regardless of the source, have shown negative effects on biota. It is clear, however, that there are not enough studies focusing on the effects of fibres and their leachates to build a full picture of their deleterious effects, and more systematic testing of different plastic fibre types (such as PET fibres in different lengths and colours) on multiple biota groups in needed.

The respective size classes found/used in field and laboratory studies also differed (Fig. 3). The absence of nanoplastics in field data can be attributed to the current challenges in sample collection, extraction and quantification methods (Nguyen et al., 2019; Schwaferts et al., 2019). These smaller plastics should, however, be the focus of future research, as it is predicted that smaller plastics are more numeric, both in the environment and in biota (Roch et al., 2019; Triebskorn et al., 2019).

4.2.2. Challenge 2: Baseline studies comparing physical and chemical characteristics of MnPs and their effects on toxicity

To fully understand and map the effects of different MnP, a shift towards systematic reporting of MnP morphology, chemical makeup and size under specific exposure conditions is needed to allow correlation of properties with potential toxic effects. Here, we introduce four major factors that could affect the MnP toxicity, each of which needs to be separately and systematically tested. Special attention should be given to realistic environmental exposure scenarios in terms of exposure medium and the presence/absence of natural compounds, as well as the impact of aging of the MnP compared to effects of pristine MnP.

i. Morphology

Plastic fibres have been reported to be more harmful than other tested morphologies (i.e., particles). For example, Au et al. (2015) reported that acute exposure to PP fibres (20–75 μm at 45 fibres mL^{-1}) reduced the growth rate of crustacean *H. azteca* in a dose dependent manner whereas exposure to PE particles (10–27 μm at 1×10^5 particles mL^{-1}) did not. In this case it is difficult to determine whether the morphology or the chemical characteristics were causing the deleterious effect of PP as no controls with similar morphologies were carried out. Ziajahromi et al. (2017) reported a lower LC_{50} (lethal concentration required to kill 50% of test population) value in *Ceriodaphnia dubia* for PET fibres (280 ± 50 μm at 5×10^{-4} mg mL^{-1} or 13 fibres mL^{-1}) than for PE particles (1–4 μm at 1×10^{-3} or 74 particles mL^{-1}). This, however, does not translate directly into fibrous MnP being more deleterious, as morphology along with any additives and plasticisers, total surface area and particle number differences may have confounded these results. It is also plausible that mode of exposure, i.e., how biota are exposed to MnP, could affect their harmfulness. For example, exposure of *C. dubia* to PET fibres resulted in more severe effects on body size and neonate numbers than exposure to PE particles despite having no fibres in their guts (Ziajahromi et al., 2017), suggesting that fibres caused physical harm but not via ingestion. Thus, the mode of exposure can play an important role in determining the effects of MnPs and should be taken into consideration in the study design (see iv. Environmental factors).

In order to fully address to what degree the plastic morphology may impact toxicity, tests using the same/similar parent plastic with different morphologies must be carried out (Table 2), and the observed effects must be differentiated by physical versus chemical damage where possible (direct and indirect effects). Use of computational image descriptors for shape, such as circulatory, convexity and main elongation, which can be determined with transmission electron microscopy (Varsou et al., 2020), may provide an useful approach to normalise between different morphologies and enable direct comparison of non-spherical morphologies.

ii. Chemical composition

MnPs found in nature consist of a complex mixture of monomers, plasticisers, colourants and other additives (OECD, 2014a). Therefore, to fully understand the effects of MnP, it is not sufficient to report and

identify the different parent plastic types only (Fig. 2), but also their chemical make-up, including additives, such as plasticisers, flame-retardants and colourants should be documented. Different MnP leachates have shown different degrees of effect on the freshwater algae *Raphidocelis subcapitata* (Capolupo et al., 2020), while the leachate from PVC was more toxic to zebrafish *Danio rerio* larvae than the plastic fragments themselves (Boyle et al., 2020). Similar leaching of additives (such as Bisphenol A) has been demonstrated in simulated gut conditions of marine biota (Coffin et al., 2019). As this leaching of additives could affect the observed toxicity, there is a need to differentiate between the toxicity of parent plastic and any specific additives. For example, routine washing of a sub-set of the parent plastic should be carried out to remove leachable additives, followed by parallel testing of the pristine and washed MnP and the leachate (Table 2), as this may shed important light into the exact drivers of any observed toxicity.

The use of commercial polymers, where the composition is unknown should be discouraged in toxicity testing, and complete chemical mapping should be carried out for plastics used in the experiments. If this is not possible, the composition should be identified as a minimum to the level of parent polymer/copolymer. Typical equipment used to identify parent plastic type includes FT-IR, Raman spectroscopy or similar. Identification of the additives could be carried out using equipment like gas chromatography–mass spectrometry (GC-MS), which would also be useful to extend emerging databases on plastic additives and spectra (Rochman, 2020) and to correlate specific plastic sources with specific combination of additives that may prove diagnostic.

iii. MnP size

It has been shown in marine settings that plastic size can play an important role in MnP toxicity (Jeong et al., 2016; Lee et al., 2013). Similar size-dependant effects have been observed in freshwater species, for example, Lei et al. (2018) found that mortality of nematodes was related to the size of PS MnP (1.0 μm were less lethal than 0.1 or 5.0 μm). Similarly, the effect of PE MnP in dipterans *Chironomus riparius* and *C. tepperi* was more pronounced with decreasing particle sizes (Silva et al., 2019; Ziajahromi et al., 2018).

It has also been observed that nanoplastics had a higher toxicity than microplastics in the freshwater flea *D. magna* (Ma et al., 2016). This higher toxicity could be related to nanoplastics ability to cross cell-walls and accumulate in sites such as the egg-yolk sacs of water flea, the gills, gut and liver of zebrafish, and the digestive gland of the bivalve *Elliptio complanata* (Auclair et al., 2020; Brandts et al., 2020; Chae et al., 2018; Lu et al., 2016; Parenti et al., 2019). Indeed, size dependent toxicity needs to be systematically tested using similar parent plastic type (Table 2).

iv. Environmental exposure considerations

To fully address the drivers behind MnP toxicity, all potential contributing factors i.e., morphology, composition and size, need to be addressed in a proper environmentally relevant context (Table 2). Here, much can be learned from the nanomaterials eco-toxicological field, where formation of an eco-corona, biofouling and ageing of the materials are increasingly recognised as being essential to realistic exposure and hazard assessment studies (Ellis et al., 2020; Nasser et al., 2020). It is important to realise that aging of plastics and other mechanisms such as adsoprtion of organic matter may affect the physical properties of the plastics. For example, growth inhibition of freshwater algae *Scenedemus subspicatus* and *Chlamydomonas reinhardtii* were found to be greater when exposed to aged or weathered rather than pristine plastics (Baudrimont et al., 2020; Wang et al., 2020b). Furthermore, Liu et al. (2019b) found that when zebrafish were co-exposed to1 μg L^{-1} and 1 mg L^{-1} of nano-sized PS (50–100 nm) and fulvic and humic acid, the reactive oxygen species (ROS) levels were stimulated synergistically. In contrast, the presence of these natural acidic organic polymers reduced the ROS level in freshwater algae *Scenedesmus obliquus*, suggesting that the presence of natural compounds in the medium affects MnP toxicity (Liu et al., 2019b). Furthermore, Ziajahromi et al. (2018) reported that PE particles (10–27 μm) reduced the toxicity of the insecticide bifenthrin to Diptera *C. tepperi* in synthetic water but not in river water, indicating that consideration should be given to more realistic exposure media and their effects on toxicity. As discussed previously, mode of exposure might also play a key role; for example *C. tepperi* exposed to PE in sediment (500 particles Kg^{-1}) showed increased mortality (Ziajahromi et al., 2018), while exposure in water (1600 particles L^{-1}) showed no effect (Ziajahromi et al., 2019 Table S1), indicating that how biota is exposed needs to be accounted for when planning experiments, and consideration should be given to whether this reflects environmentally realistic modes of exposure.

It is also important to understand how laboratory exposure concentrations compare with those observed in the environment. However, this can be problematic, as reported units for laboratory and field studies varied. Exposure concentrations in laboratory studies were often mass per volume (mg L^{-1}) but reporting also MnP number and surface area might be more informative. Conversely, field studies reported mg individual^{-1}, or more often item individual^{-1}, making it difficult to compare the results between the two. In laboratory studies, it is common to use concentrations that span the expected effect threshold to ensure that an effect will be observed. Indeed, it is not uncommon to see arguably unrealistic exposure concentrations (e.g., De Felice et al., 2019; Li et al., 2020c) in laboratory studies. It is difficult, however, to predict what can be considered as 'environmentally relevant' concentrations, as there is uncertainty regarding the abundance of smaller MnP in the environment and occurrence of bigger microplastics varies with location (Li et al., 2018). Furthermore, the units used to report environmental concentrations are often item m^{-3} (water) and item Kg^{-1} (sediment), making it hard to relate directly to laboratory exposures which tend to report units as mass volume^{-1}. Regardless, it is imperative for the scientific community to expand the toxicity testing into lower exposure doses (such as MnP concentrations of 100 items L^{-1}) (Triebskorn et al., 2019) with extended exposure time scales to cover life-time and/or multiple generations (Table 2). For example, Kelpsiene et al. (2020) showed that when *D. magna* was exposed to 0.32 mg L^{-1} of 26 or 62 nm PS over its full life-time, increased mortality became obvious, which was not previously reported in acute 24 h exposures to 400 mg L^{-1} (Mattsson et al., 2017).

4.2.3. Challenge 3: Harmonisation of endpoints reported for each trophic level

To facilitate inter-comparability of MnP toxicity data, common principal endpoints need to be identified for each trophic level, which should be included in each study as minimum. Here, potential principal endpoints for the four trophic levels identified in this paper (Fig. 6, Table 2) are introduced, including justification for why these endpoints should be included in future studies.

i. Microbes

For microbes, the taxon richness and its possible changes when in contact with different plastic types is the most important reporting requirement. There is evidence that MnP can act as a favourable substrate for specific microbial communities (Hoellein et al., 2017; Kettner et al., 2019; Li et al., 2020b; McCormick et al., 2014, 2016). For example, McCormick et al. (2014), McCormick et al. (2016) and Kettner et al. (2019) found a lower species richness on microplastic surfaces when compared to the surrounding environment. This is further supported by Parrish & Fahrenfeld (2019) and by Kelly et al. (2020) who demonstrated that PE and PS particles (125–500 μm) and fragments (0.25–0.5 mm) exhibited distinct microbial communities irrespective of the source water. Common human pathogens, such as arcobacters (Gong et al., 2019; Kettner et al., 2019; McCormick et al., 2014, 2016) frequently colonise plastics, and certain antibiotic resistant genes can be aligned with the presence of microplastics (Ram and Kumar, 2020; Wang et al., 2020a), thus the likelihood of MnP acting as a transport medium cannot be negated. Hoellein et al. (2017) suggested that this preference was enhanced closer to a WWTP and diluted further downstream. Studying taxon richness and specifying any potential

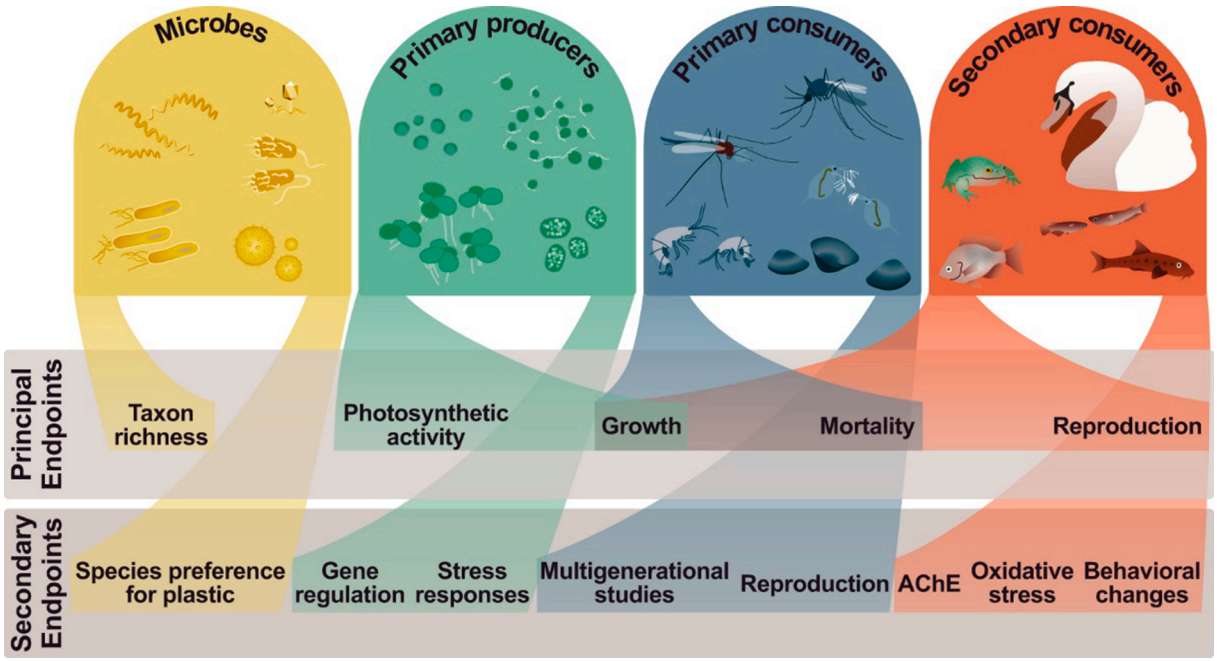

Fig. 6. Potential principal and secondary toxicity endpoints for the four trophic levels.

preferential colonisation needs to be tested with different plastic types in order to fully address these interactions and their consequences.

ii. Primary producers

Photosynthetic activity was one of the most common endpoints measured when assessing effects on primary producers (19 out of 26 studies). 3 out of 4 studies on the vascular-plant duckweed included this endpoint. From these, all reported no significant effect, even at high exposure concentrations, such as 5 x10^4 PE particles mL^{-1} (Mateos-Cárdenas et al., 2019). Photosynthetic activity was measured as chlorophyll-a (Chl-α) content with fluorometry (Mateos-Cárdenas et al., 2019) and as chlorophyll pigment content with UV-VIS spectroscopy (Dovidat et al., 2020; Kalčíková et al., 2017). Contrary to this, the majority of studies showed a significant effect of MnP on algal photosynthetic activity. Sjollema et al. (2016) measured the photosynthetic activity in *C. vulgaris* with Pulse Amplitude Modulation (PAM) fluorometer after exposure to high concentrations of PS particles (500 nm at 2.5 × 10^{-2}, mg mL^{-1}) and found no conclusive impact. Interestingly, four other studies on cyanobacteria and freshwater algae *Microcystis aeruginosa*, *S. obliquus* and *C. reinhardtii* exposed to high PS particle concentrations in similar size-ranges (50 nm at 3.4 × 10^{-3} mg mL^{-1}, 200 nm at 0.01 mg and 0.02 mg mL^{-1}, 70 nm at 0.1 mg mL^{-1} and 300–600 nm at 0.1 mg mL^{-1}) showed decreased Chl-α content when measured with UV-VIS spectroscopy (Besseling et al., 2014; Feng et al., 2020; Zhang et al., 2018) and with a portable plant efficiency analyser (Li et al., 2020c). Conversion between mass and particle numbers was not possible in these cases and it is possible that the observed effects are related to the MnP sizes and concentrations, or are species specific. The difference in method should not affect the outcome parameter (efficiency of photosynthetic activity), as UV -VIS Spectroscopy (Lichtenthaler, 1987) and fluorometry can both be used to estimate the chlorophyll content of plants (Maxwell and Johnson, 2000). In order to obtain baseline data, experiments with different plastic types with specific sizes and concentration ranges should be repeated on different species of algae. Photosynthetic activity appears to be a universally used endpoint for MnP toxicity tests and should continue being used to aid future comparability of studies.

Another common toxicity endpoint included for primary producers is growth (i.e., OECD, 2006, 2011, 2014b) which is relatively easy to measure. Only 2 out of 4 vascular-plant based studies reported an inhibitory effect on root growth of *Lemna minor* or *Spirodela polyrhiza* duckweeds. Although all MnP used in these studies adhered to the roots and leaves, it was only the larger primary PE particles (71.30 ± 34.29 μm, 96.00 ± 69.99 μm and 180.5 ± 118.7 μm) extracted from facewash that showed a statistically significant inhibitory effect on the root length of *L. minor* (Kalčíková et al., 2017, Kalčíková et al., 2020). Leachate effects were accounted for, suggesting that the microplastics themselves caused some type of physical inhibitory effect at all tested concentrations (0.01, 0.05, and 0.1 mg mL^{-1}) (Kalčíková et al., 2017). This effect could be due to the MnP size or morphology, as the other MnP tested, i.e., PE (10–45 μm at 5 × 10^4 particles mL^{-1}) (Mateos-Cárdenas et al., 2019) and PS (50 and 500 nm at 1 × 10^6 particles mL^{-1}) (Dovidat et al., 2020), had more regular morphology, whereas the PE extracted from facewash was highly irregular. More studies are required with growth as a unifying endpoint, so that the effect of morphology and plastic type can be explored and clear comparisons made to establish what MnP features affect growth.

8 out of 15 studies on freshwater algae measuring growth reported a clear inhibitory effect after exposure to MnP. For instance, effects were induced by PS particles (70 nm at 1 mg mL^{-1} and 500 nm at 0.05 mg mL^{-1}) on *S. obliquus* and *C. vulgaris*, (Besseling et al., 2014; Tunali et al., 2020), by a mix of polymethyl methacrylate (PMMA) and PS fragments (<250 μm at 1.25 × 10^{-2} mg and 0.125 mg mL^{-1}) on *Scenedesmus sp* and *Microcystis panniformis* (Cunha et al., 2019) and by polyurethane (PUF) foam fragments (3 mm at 1.6 mg mL^{-1}) and its leachates on *C. vulgaris* (Luo et al., 2019a).

Lagarde et al. (2016) reported decreased growth of the algae *C. reinhardtii* after exposure to PP fragments (400 < 1000 μm at 100 mg *no volume reported*), but only after 78 days. This, in conjunction with results from Mao et al. (2018), who reported that initial growth inhibition of *Chlorella pyrenoidosa* exposed to PS particles (0.1 μm at 0.01, 0.05 and 0.1 mg mL^{-1}), was countered after 22 days with biomass showing recovery, indicating some degree of organism adaptability. This strongly suggests that longer exposure times are required when assessing the impacts of MnP on algal communities, as negative effects seen at short exposure times might decrease in intensity over time or vice versa.

It is thus suggested that growth measured as length of roots and leaves for plants and as algae biomass and/or cell densities should be

included as a principal endpoint. Other endpoints such as stress responses and gene regulation should be included, where feasible, as explorative variables. This minimum toxicity endpoint reporting will help to ensure that meta-analysis can be carried out in the future, leading to deeper understanding of MnP toxicity and the characteristics responsible.

iii. Primary consumers

Mortality is a common endpoint, determined often as the LC_{50} (lethal concentration required to kill 50% of the test population) or LD_{50} (lethal dose required to kill 50% of the test population). Most studies reported mortality if it occurred, or used alternative indirect mortality endpoints, such as immobilisation for cladecorans to estimate EC_{50} (half maximal effective concentration, which is halfway between baseline and maximum observed effect) (OECD, 2004). Where mortality was reported, higher exposures seemed to be more lethal. For example, some studies reported little to no mortality in *D. magna.* when exposed to PS particles (51 nm at 0.1 mg mL^{-1} and 1 μm at 1.25 × 10^{-4} mg mL^{-1}) (Chae et al., 2018; De Felice et al., 2019), whereas, some PS exposures with similar size ranges did induce mortality (52 nm at 50 mg mL^{-1} and 100 nm at 0.0863 mg mL^{-1}) (Mattsson et al., 2017; Reynolds et al., 2019). Another discrepancy was observed in the case of the crustacean *H. azteca*, whereby Panko et al. (2013) reported no mortality after exposure to tyre and road wear fragments (<150 μm at 1 × 10^{4} mg Kg^{-1}) over 41 days, while Khan et al. (2019) observed mortality of *H. azteca* when exposed to tyre rubber alone (<500 μm at 3426 fragments mL^{-1}) over 21 days. Here, the differences between mode of exposure, the rubber types, associated additives and/or the road mixture could affect toxicity. It is noted, that in order to better understand the toxicity mechanisms, food should always be provided, so that effects of starvation would not be confused with those arising from the exposure (Aljaibachi et al., 2020). Furthermore, mortality should always be recorded and if no mortality is observed, this should be clearly stated.

Growth as an endpoint can reveal differences in toxicity of plastic types and aid comparisons between biotic groups. For example, the crustacean *Gammarus pulex* showed a significant reduction in growth when exposed to PS fragments (20–500 μm at 10% of sediment) despite feeding activity not being affected (Redondo-Hasselerharm et al., 2018). Reduction in growth was also observed in *G. fossarum*, by Straub et al. (2017), when exposed to PMMA and polyhydroxybutyrate (PHB) fragments (32–63 μm, 63–125 μm, and 125–250 μm at 1 × 10^{5} fragments $individual^{-1}$), despite no effects on feeding rates. However, *G. pulex* did not exhibit growth inhibition when exposed to car tyre rubber of similar size and concentration (66 μm at 10% of sediment), despite retention times higher than for PS particles (Redondo-Hasselerharm et al., 2018). Similarly Panko et al. (2013) reported only slight non-statistically significant inhibition in growth of Diptera *Chironomus dilutus* exposed to tyre and road wear fragments < 150 μm at 1 × 10^{4} mg Kg^{-1} while *C. riparius* and *C. tepperi*, were effected when exposed to PE (32–63 μm at 1259 mg Kg^{-1} 63–250 μm and 125–500 μm at 1 × 10^{4} mg Kg^{-1}) (Silva et al., 2019) and PE (1–4, 10–27, 43–54 and 100–126 μm each at 500 particles Kg^{-1}) (Ziajahromi et al., 2018). However, whether the car tyre and road wear fragments are less toxic than other MnP, such as PS, PE, PMMA and PHB remains inconclusive. For example, Panko et al. (2013) reported no effect on growth of the crustacean *H. azteca* exposed to tyre and road wear fragments of size < 150 μm at 1 × 10^{4} mg Kg^{-1} while Khan et al. (2019) observed negative effects on growth in *H. azteca* when exposed to tyre rubber of size < 500 μm at 3.426 × 10^{3} fragments mL^{-1}. The difference observed could be driven by either the road pavement, rubber types and associated specific mixture of additives and/or size, dose and/or mode of exposure, suggesting that more systematic studies are needed to determine the specific drivers of toxicity. To ensure comparability of effects across plastic types and species, growth, measured as weight and length or biomass should be included as an endpoint for primary consumers. This will aid further meta-analysis and species sensitivity analysis in the future.

Besides inclusion of the primary endpoints mentioned above, it is crucial that the exposure times of primary producers will be extended. For example, the duration of exposure in *D. magna* and other cladocerans species is usually 48 h for acute, and 21 days for chronic toxicity tests, according to OECD guidelines. However, it has been reported that some acute toxic effects (such as immobilisation) of *D. magna* and *D. pulex* are not always obvious after 48 h (Jaikumar et al., 2018; Rehse et al., 2016), and life-time exposures have revealed higher toxicity than that at 24 h exposure (Kelpsiene et al., 2020), suggesting that acute exposure should be extended to at least 72 h. Indeed, the OECD toxicity test guidelines are currently being revised for use with nanoparticles (Nasser and Lynch, 2019).

A secondary endpoint for primary consumers should be reproduction and where possible, multigenerational studies should be carried out. This is required to improve our understanding on population level effects. For example, three studies using non-disclosed proprietary type polymer from Cospheric LLC (1–5 μm), which measured reproduction capacity in cladocerans *Daphnia* spp., all reported negative effects (Jaikumar et al., 2019; Ogonowski et al., 2016; Pacheco et al., 2018). In addition, Felten et al. (2020), showed that PE 1–4 μm at 1 × 10^{-3} mg mL^{-1} had a negative effect on reproduction of *D. magna* while a transgenerational study by Martins and Guilhermino (2018) showed that *D. magna* went extinct in just two generations after exposure to Cospheric LLC (1–5 μm). It is clear that these specific MnP can have population level effects on cladocerans, and this type of systematic testing is required on other types of plastics and species. Furthermore, multigenerational studies should be a considered for primary consumers, as otherwise the population level effects may be overlooked (Martins and Guilhermino, 2018). Most primary producers have a short life-cycle, making them ideal for this type of work.

Including these two principal toxicity parameters (growth and mortality) into each primary consumer MnP study is imperative. Considerations should be given to inclusion of secondary endpoints (reproduction and multigenerational studies). Other, especially sub-lethal parameters, such as enzyme activities for oxidative stress or immune response (e.g., Li et al., 2020d; Scopetani et al., 2020), should also be explored, to expand our understanding of MnP toxicity and their modes of action.

iv. Secondary consumers

For secondary consumers, similar principal endpoints as for primary consumers should be included (growth, mortality and reproduction). However, as secondary consumers develop much slower than primary consumers, it is not always possible to include all three principal endpoints in one study, and therefore a combination of any two principal endpoints is acceptable. Still, as only one study from the reviewed literature reported increased mortality of adult zebrafish exposed to PP particles (~70 μm at 0.01 mg mL^{-1}) (Lei et al., 2018), further sub-lethal endpoints should be included. Effects observed in secondary consumers include changes in histopathology, gene expression and behaviour (e.g., Chae et al., 2018; Lei et al., 2018; Limonta et al., 2019; Oliveira et al., 2013; Rochman et al., 2017; Wen et al., 2018). Therefore, for secondary endpoints we recommend assessment of behavioural changes (e.g., predatory performance or distance swam), acetylcholinesterase (AChE) activity, which is an important enzyme that catalyses the breakdown of acetylcholine (neurotransmitter), or measures of oxidative stress, namely ROS or CAT (catalase gene) and SOD (superoxide dismutase). One or more of these endpoints are encouraged to be included in future studies, to increase inter-comparability.

Behavioural changes can be used as a measure of sub-lethal effect in biota. This can be recorded using video combined with imaging software such as ImageJ (Chae et al., 2018; Critchell and Hoogenboom, 2018; da Costa Araújo et al., 2020) or more sophisticated tools and programs that have been designed for behavioural tracking of fish, such as DanioVision and EthoLog (Chen et al., 2017; Ottoni, 2000; Pedersen et al., 2020). For example, Chae et al. (2018) found that locomotive activity of two fish species, rice fish *Oryzias sinensis* and dark chub *Zacco temminckii*, were affected by PS particles (51 nm at 5 × 10^{-3} mg mL^{-1}). It was also

reported that the livers of *Z. temminckii* showed abnormal histological patterns. Wen et al. (2018) similarly found that predatory performance of discus fish *Symphysodon aequifasciatus* declined following exposure to PE particles (70–88 μm at 2×10^{-4} mg mL^{-1}). This study also reported sub-lethal effects, such as a decrease in activity of enzymes AChE and Trypsin. It should be noted that these types of behavioural parameters (such as predatory performance or distance swam) cannot explain the underlying mode of action, however, they can offer important insight into MnP effects and are encouraged to be monitored where possible.

Neurotransmitter AChE decreased in 5 out of 6 studies assessed in this review, indicating neurotoxicity for the tested plastic types (PE and PS). PE was found to affect juvenile discus fish *S. aequifasciatus* (PE 70–88 μm at 2×10^{-4} mg mL^{-1}) and streaked prochilod *Prochilodus lineatus* (PE 10–90 μm at 2×10^{-5} mg mL^{-1}) (Roda et al., 2020; Wen et al., 2018). Two separate studies on juveniles of the common goby *Pomatoschistus microps* reported similar effects on AChE when exposed to PE (1–5 μm at 1.84×10^{-5} mg mL^{-1} and 1.84×10^{-4} mg mL^{-1}) (Fonte et al., 2016; Oliveira et al., 2013). PS 0.1 μm also decreased AChE in red tilapia *Oreochromis niloticus* at exposure concentrations of 1×10^{-6}, 1×10^{-5} and 1×10^{-4} mg mL^{-1} (Ding et al., 2018). Only LDPE (~10.9 μm) at exposure concentrations of 5×10^{-6}, 5×10^{-5}, and 5×10^{-4} mg mL^{-1} showed no effect on AChE mRNA levels of zebrafish larvae (Karami et al., 2017b). The duration of exposure is unlikely to explain these differences, as the studies that showed effects on AChE ranged from 96 h to 31 days and the study of Karami et al. (2017b) exposed the larvae for 20 days, which falls within the range above. Whether the observed effect on AChE levels was species specific, related to the age of exposed fish, and/or plastic type is unclear. Inclusion of this endpoint where possible will facilitate assessment of possible neurotoxicity of different plastic types in different species.

Another important sub-lethal endpoint for secondary consumers is oxidative stress, which can lead to tissue damage and disrupt cellular functions (Valavanidis et al., 2006). There is already evidence that some MnP can induce oxidative stress in secondary consumers. For example, CAT gene, that is related to the presence of oxidative stress, was upregulated in zebrafish after exposure to PS particles (1 μm, 5 μm and 70 nm) at 1×10^{-3} mg mL^{-1} (Qiang and Cheng, 2019), 2×10^{-4} or 2×10^{-3} mg mL^{-1} (Lu et al., 2016). However, we recommend that more than one parameter for oxidative stress should be measured, as this can give an indication of whether biota can induce protective action against ROS. For example, Parenti et al. (2019) showed that while PS (0.5 μm at 1×10^{-3} mg mL^{-1}) induced activity of SOD it did not induce ROS. This could indicate that high SOD activity in zebrafish could protect it from excessive ROS. This is supported by a study on red tilapia *O. niloticus* exposed to 1×10^{-6}, 1×10^{-5} and 1×10^{-4} mg mL^{-1} PS particles (0.1 μm), where particles induced SOD but did not affect malondialdehyde (MDA), suggesting a similar protective mechanism against oxidative damage (Ding et al., 2018). Furthermore, 5×10^{-6}, 5×10^{-5}, or 5×10^{-4} mg mL^{-1} LDPE (~10.9 μm) did not increase CAT in zebrafish, but decreased its concentration over the exposure period (Karami et al., 2017b). More studies capturing this endpoint in the future will enable further inferences on the effects of MnP type, morphology and size. It is also noteworthy that when zebrafish were co-exposed to 1×10^{-6} mg mL^{-1} and 1×10^{-3} mg mL^{-1} nano-sized PS (50–100 nm) and fulvic and humic acid, the ROS levels were synergistically affected (Liu et al., 2019b) indicating that natural conditions, such as organic matter should be considered further when conducting toxicity tests.

5. Conclusion

The synthesis of the reviewed research revealed evidence of a significant mismatch between field and laboratory studies, highlighting a significant gap in our knowledge on how the most common morphology of MnP might affect freshwater species. Further, there is lack of systematic testing on different factors that may affect MnP toxicity, such as different morphologies, plastic types and sizes, including their leachates. This review has outlined some of the key recommendations and reporting guidelines for field and laboratory studies, with the goal of increasing inter-comparability across studies and trophic levels. It is imperative that baseline studies on the parameters suggested in this review are systematically undertaken. Only by gathering this type of data, can a full meta-analysis and comprehension of the effects of MnP on freshwater ecosystems be produced.

Credit authorship contribution statement

Anna Kukkola: Conceptualization, Investigation, Methodology, Formal analysis, Visualization, Writing - original draft, Reviewing and editing. **Stefan Krause:** Conceptualization, Funding acquisition, Writing - reviewing and editing, Supervision. **Iseult Lynch:** Conceptualization, Funding acquisition, Writing - reviewing and editing, Supervision. **Gregory H. Sambrook Smith:** Conceptualization, Funding acquisition, Writing - reviewing and editing, Supervision. **Holly Nel:** Conceptualization, Visualization, Writing - reviewing and editing, Supervision.

Declaration of Competing Interest

The author declare that there is no conflict of interest.

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Appendix A. Supplementary material

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