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# Current understanding and challenges for aquatic primary producers in a world with rising micro- and nano-plastic levels

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ABSTRACT

The impacts of micro- and nanoplastics (MNPs) on aquatic animals have been intensively studied; however, the extent and magnitude of potential effects of MNPs on aquatic primary producers are poorly understood. In this study, we quantitatively analyzed the published literature to examine the impacts of MNPs on growth, photosynthesis, pigments, and metabolism of aquatic microalgae. MNPs negatively affected growth of microalgae but usually had a high $EC_{50}$ (> 25 mg/L). However, positively charged MNPs had a much lower $EC_{50}$ (< 1 mg/L). MNPs lowered maximum photochemical efficiency of photosystem II ($F_v/F_m$) with the effect increasing with concentration of MNPs but diminishing with exposure time, and also reduced chlorophyll *a* content to enhanced extent with increased MNPs concentration. MNPs induced relatively higher changes in superoxide dismutase (SOD) and malondialdehyde (MDA) levels in marine algae than in freshwater algae. Reactive oxygen species (ROS) levels increased with MNPs concentration and exposure time while SOD levels first increased and then decreased with increasing MNPs concentration. Macrophytes were found to be able to trap MNPs via multiple mechanisms. Future work should focus on the mechanisms behind MNPs impacts on primary productivity and global carbon cycle, and the combined effects of MNPs with other environmental factors.

## 1. Introduction

### 1.1. MNPs pollution and trends

Due to their ease of processing, high stability and low price, plastics have been increasingly used in industrial production, agriculture and our daily life since the 1950s (Barnes et al., 2009). The annual production of plastics in the world increased from 1.7 million tons in 1950 to an exceeded estimate of 359 million tons in 2018 (PlasticsEurope, 2019). Oceans are deemed to be the endpoint of plastic fluxes from hydrological catchments (Lebreton et al., 2017). It has been reported that 4.8–12.7 million tons of plastics have entered the ocean, accounting for 60–80% of marine litter (Jambeck et al., 2015). Among plastics, those particles between 5 mm and 1 μm in size are defined as microplastics (MPs) and those in the range from 1 to 1000 nm as nanoplastics (NPs) (Gigault et al., 2018). Micro- and nanoplastics (MNPs) have been found in both marine systems, from the equator to polar regions (Law and Thompson, 2014; Auta et al., 2017), and freshwater systems, including rivers and lakes (Eriksen et al., 2013; Rodrigues et al., 2018).

Furthermore, MNPs levels in aquatic environments are predicted to further increase in the future (Isobe et al., 2019). For instance, by 2060 concentrations of pelagic MPs are predicted to increase approximately fourfold from the level in 2016 (Isobe et al., 2019). This trend has led to a rising concern that we need to understand more about their toxic effects. Compared to large plastics, MNPs may have a wider impact on aquatic ecosystems since they are more easily dispersed throughout the water bodies due to their smaller particle size. It has been shown that MNPs can affect feeding, growth, reproduction, and survival of fish and aquatic invertebrates via diverse mechanisms, although the effects of exposure to microplastics are highly variable across taxa (Foley et al., 2018). Impacts on primary producers are, however, less well understood.

### 1.2. Impacts of MNPs on primary producers

As primary producers, autotrophic organisms provide materials and energy for higher trophic levels, supporting food webs. Thus small disruptions to autotrophic organisms may lead to noticeable repercussions

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on the whole ecosystem. In addition, autotrophic organisms play an essential role in the global carbon cycle and alleviating climate change (Sahoo et al., 2012; Gao et al., 2019). However, compared to heterotrophic organisms (Wang et al., 2019), autotrophs have received relatively less attention in terms of the impacts of MNPs, which may be due to the fact that most algae cannot ingest MNPs. Nonetheless, some studies have reported the negative effects of MNPs on photosynthesis of microalgae (Bhattacharya et al., 2010) and there has been a growing trend for studies on autotrophic organisms in recent years. There is, to date, one paper reviewing the impacts of microplastics on microalgae (Prata et al., 2019), but this did not systematically analyze the available data for general links with taxon, shape, size ratio of algal cells to MNPs, or exposure-specific trends in microplastic effects, or include data on macrophytes. Therefore, our understanding of the impacts of MNPs on primary producers is still very scarce. In the present study, we conducted quantitative analyses of data from the published literature to examine the effects of exposure to microplastics on five important responses of microalgae: (a) growth, (b) Photosystem II capacity as determined by the maximum quantum yield, $F_v/F_m$, (c) reactive oxygen species (ROS) production, (d) superoxide dismutase (SOD) levels (involved in detoxification of ROS) and (e) levels of malondialdehyde (MDA, an oxidation product of ROS attack on lipids). For each of the five response categories, we examined whether the reported effects of MNPs exposure were positive, negative, or neutral. We further assessed the relationship between these five responses and MNPs concentration, exposure period, or size ratio of algal cells to MNPs. We also reviewed the limited information on macrophytes, but quantitative analyses were not conducted on these due to the low availability of experimental data.

## 2. Materials and methods

### 2.1. Data compilation

To conduct our meta-analysis, we selected target studies through a search of

ISI Web of Science and Scholar Google on June 15, 2020, using the term "Microplastic OR nanoplastic AND microalgae", and thus all literature published before June 15th 2020 was screened. The database of ISI Web of Science is commonly used for microplastic meta-analysis papers (Foley et al., 2018; Erni-Cassola et al., 2019; Miller et al., 2020) and here we used Scholar Google to crosscheck. There might be still some literature omitted but we believe our search covers works published in specialized journals with considerable impact. The initial search yielded 114 papers. Of these, we retained 32 studies in English for our analyses according to the following criteria: (1) the paper was an original research study rather than a review, (2) the paper included a "no microplastics" control treatment; (3) the study reported mean, sample size, and measure of variance for controls and treatments. We extracted the following information from each study: the species, the type, shape, size, concentration and exposure period of MNPs used and the mean value of each parameter for both control (i.e., no microplastic exposure) and treatment(s). Means were extracted from tables when possible. If the data were presented in figures, we asked for the original data from the corresponding authors. For those data that could not be obtained through the approaches above, we used the analysis software of GetData Graph Digitizer to physically measure the values from published graphs. A full list of information extracted is included in Table 1.

When there were multiple treatments examined for a particular study or species, e.g., a researcher assessed the response over more than

**Table 1**
The studies included in quantitative analyses of the effects of MNPs on growth, photosynthesis ($F_v/F_m$), pigment and metabolism (ROS, SOD, MDA) in microalgae. ROS, reactive oxygen species; SOD, superoxide dismutase, and MDA, malondialdehyde.

| Study | Study organism | Number of records included in analyses | | | |
|---|---|---|---|---|---|
| | | Growth | $F_v/F_m$ | Pigment | Metabolism (ROS, SOD, MDA) |
| Bhattacharya et al. (2010) | *Chlorella* sp. | | | | 12 |
| Bhattacharya et al. (2010) | *Scenedesmus* sp. | | | | 12 |
| Besseling et al. (2014) | *Scenedesmus obliquus* | 5 | | 5 | |
| Davarpanah and Guilhermino (2015) | *Tetraselmis chuii* | 6 | | | |
| Lagarde et al. (2016) | *Chlamydomonas reinhardtii* | 10 | | | |
| Sjollema et al. (2016) | *Dunaliella tertiolecta* | 6 | | | |
| Bergami et al. (2017) | *Dunaliella tertiolecta* | 8 | | | |
| Bergami et al. (2017) | *Scenedesmus obliquus* | 5 | | 5 | |
| Long et al. (2017) | *Chaetoceros neogracile* | 1 | | 3 | |
| Long et al. (2017) | *Heterocapsa triquetra* | 1 | | 3 | |
| Long et al. (2017) | *Tisochrysis lutea* | 1 | | 3 | |
| Lyakurwa. (2017) | *Rhodomonas baltica* | 24 | | | |
| Nolte et al. (2017) | *Pseudokirchneriella subcapitata* | 6 | | | |
| Yokota et al. (2017) | *Dolichospermum flos-aqua* | 5 | | | |
| Yokota et al. (2017) | *Microcystis aeruginosa* | 5 | | | |
| Zhang et al. (2017) | *Skeletonema costatum* | 32 | | 10 | |
| Canniff and Hoang. (2018) | *Raphidocelis subcapitata* | 1 | | | |
| Chae et al. (2018) | *Chlamydomonas reinhardtii* | 5 | | | |
| Mao et al. (2018) | *Chlorella pyrenoidosa* | 90 | 90 | | |
| Prata et al. (2018) | *Tetraselmis chuii* | 7 | | 7 | |
| Zhu et al. (2018) | *Skeletonema costatum* | 64 | | | 32 |
| Chae et al. (2019) | *Dunaliella salina* | | 7 | 7 | |
| Garrido et al. (2019) | *Isochrysis galbana* | 4 | | | |
| Wu et al. (2019) | *Chlorella pyrenoidosa* | | 72 | 72 | |
| Wu et al. (2019) | *Microcystis flos-aquae* | | 78 | 78 | |
| Feng et al. (2020a, 2020b) | *Microcystis aeruginosa* | 18 | | 4 | 12 |
| Guo et al. (2020) | *Phaeodactylum tricornutum* | 12 | | | |
| Hazeem et al. (2020) | *Chlorella vulgaris* | 36 | | 24 | 6 |
| Liu et al. (2020) | *Scenedesmus obliquus* | 55 | 5 | | 5 |
| Song et al. (2020) | *Chlorella* sp. | 34 | | 40 | 8 |
| Song et al. (2020) | *Phaeodactylum tricornutum* | 35 | | 20 | 8 |
| Xiao et al. (2020) | *Euglena gracilis* | 18 | | 6 | 4 |
| Zhang et al. (2020) | *Chlorella pyrenoidosa* | 19 | | | 24 |
| Zhao et al. (2019) | *Karenia Mikimotoi* | 20 | 12 | 25 | |

one concentration, size, type, and exposure period of MNPs, we included an individual record for each treatment that was compared to a control. When there are other variables besides MNPs, only the information for MNPs treatment was extracted.

### *2.2. Data analysis*

The wide range of response traits assessed in the literature were classified into the categories described by Jin et al. (2017) including: (1) Growth, (2) photosynthetic parameters (e.g., maximum photochemical efficiency of photosystem II, $F_v/F_m$), (3) pigment (e.g. chlorophyll *a*, chlorophyll *b*, and carotenoids), and (4) metabolism including ROS, SOD and MDA. The response traits were standardized to allow comparisons across studies. Responses across experiments assessing different traits were compared through the logarithm-effect size (log effect size), calculated as the logarithm of the ratio of the response value in the experimental treatment to the control. A logarithm effect size greater than 0 signaled adverse effects on the organisms and a logarithm effect size less than 0 signaled improved organism performance (Jin et al., 2017).

We tested whether the marine algae assessed in these papers respond differentially to MNPs compared to those from freshwater habitats and whether sensitivities are related to the size ratio of algae: MNPs. If the microalgae cell volume was not reported in the original paper, we derived the cell volume of the species from other published reports. The taxonomic classification followed AlgaeBase (www.algaebase.org). We also examined the relationships between sensitivities and MP exposure time or MP concentrations. The MP exposure time or MP concentrations were recorded as per to the original study. Linear or quadratic regressions were used to quantify the relationship between log effect size and exposure time or MP concentrations or log size ratio. The related data could be considered to conform to a normal distribution by the method of frequency distribution (Fig. S1, Ghasemi and Zahediasl, 2012). We used one-way analysis of variance (ANOVA) to test for significant differences ($p = 0.05$) in the Log effect size for $F_v/F_m$ and MDA between two comparisons (e.g., freshwater microalgae vs. marine microalgae); the related data could be considered to conform to a normal distribution by the method of frequency distribution (Fig. S1, Ghasemi and Zahediasl, 2012) and the variances could be considered equal (Levene's test, $p > 0.05$). For those data (growth, pigment, ROS, and SOD) whose variances were not equal, a Mann–Whitney *U* test was used. If the comparison levels were more than 2, all-pair comparisons were conducted with Tukey–Kramer HSD.

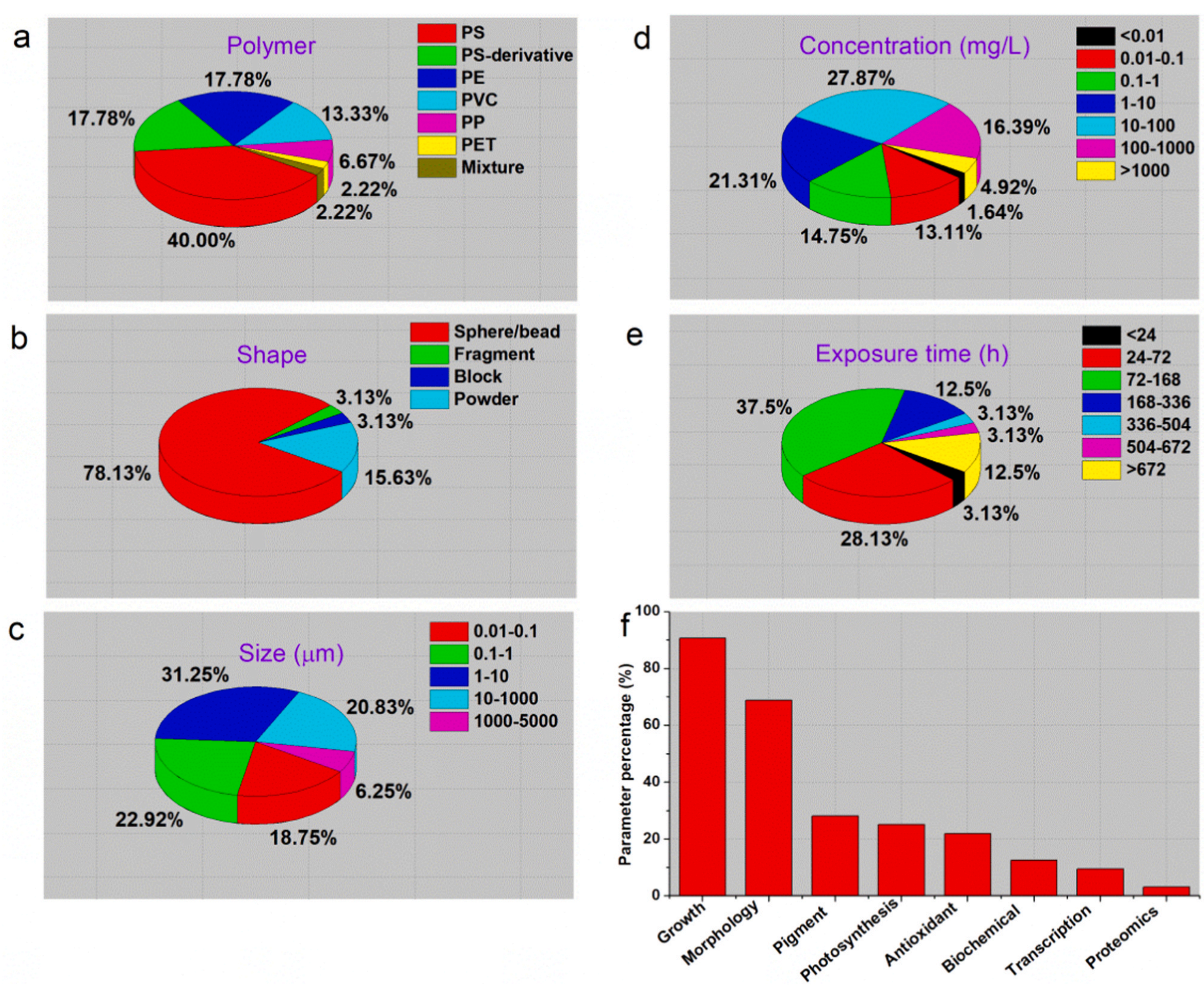


**Fig. 1.** Features of MNP studies on microalgae, involving the percentage of polymer, shape, size, concentration, exposure period of MNPs and the parameters investigated. PS, polystyrene; PS-derivative, polystyrene-derivative; PE, polyethylene; PVC, polyvinylchloride; PP, polypropylene; PET, Polyethylene terephthalate.

## 3. Results and discussion

### 3.1. Effect of MNPs on microalgae

Six materials were used in MNPs studies (Fig. 1a). Most studies used polystyrene (PS) or PS-derivatives, the sum of which accounts for 56.52% of polymer materials. Except for PS, polyethylene (PE) and polyvinylchloride (PVC) were the two other main materials in MNPs studies. The reason that PS is the most studied polymer could be attributed to its high commercialization and availability. In fact, PS is not the most abundant plastic type found in aquatic environments; rather PE (23%) and the group PP&A (20%; polyesters, PEST; polyamide, PA; and acrylics) are the dominant types, followed by PP (13%) (Erni-Cassola et al., 2019). Therefore, future studies may need to focus more on these more abundant plastic types.

Three specific shapes of MNPs, sphere/bead, fragment, and block, were reported in the studies though the sphere/bead form (78.79%) was absolutely dominant (Fig. 1b). There was a proportion (15.15%) of studies using tiny particles with irregular shapes termed powders. This pattern does not fit the real environment very well as fibers and fragments are usually the dominant types in seawater (Shim et al., 2018; Feng et al., 2019; Uurasjärvi et al., 2020). The reason for this is the high commercialization and availability of sphere or bead MNPs. In future studies, MNPs with other forms should be used more.

The size of MNPs ranged from 0.02 to 5000 μm (Fig. 1c). The smallest PS plastic (0.02 μm) was investigated in *Chlorella vulgaris* (Hazeem et al., 2020) while the largest PP and PVC (5000 μm) was used with *Phaeodactylum tricornutum* (Guo et al., 2020). The range of 1–10 μm was the most used (31.25%), followed by 0.1–1 μm (22.92%). Nearly one fifth of studies used plastics 0.01–0.1 μm or 10–1000 μm while larger MPs (1000–5000 μm) were the least used. Smaller sizes of plastics are usually found in higher proportions in aquatic environment (Chaturvedi et al., 2020; Feng et al., 2020a). Although NPs have been not quantified well yet, it has been speculated that particle concentrations of NPs are $> 10^{14}$ times higher than MPs concentrations measured to date (Besseling et al., 2019) if aggregation is not considered. Based on the analysis in the present study, only 41.67% of studies used NPs for microalgal studies. Therefore, more studies using NPs should be carried out in future.

There was a huge range for MNPs concentrations used in previous studies (Fig. 1d); the lowest concentration (0.00396 mg/L) of MNPs was used in a prymnesiophyte, *Tisochrysis lutea*, a dinoflagellate, *Heterocapsa triquetra*, and a diatom, *Chaetoceros neogracile* (Long et al., 2017) while the highest concentration (5000 mg/L) was used in *Phaeodactylum tricornutum* (Guo et al., 2020). The range of 10–100 mg/L (28.57%) leads the concentration list, followed by 1–10 (20.63%) and 100–1000 mg/L (17.46%). The lower concentrations ($<$ 0.01 mg/L) only account for 1.59% of all the studies. This pattern contradicts the real environmental concentrations; as summarized by Lenz et al. (2016), the environmentally realistic concentrations for MNPs fall in the range of 0.001–1 μg/L. Therefore, most of previous studies used orders-of-magnitude higher experimental concentrations than those reported from field studies, although some were designed to obtain $EC_{50}$ values.

The exposure time also showed a big range (Fig. 1e), with the shortest exposure of 3 h used for MNPs adsorption on *Chlorella* and *Scenedesmus* (Bhattacharya et al., 2010) and the longest exposure tested being 1872 h for *Chlamydomonas reinhardtii* (Lagarde et al., 2016). Nearly two fifths of the studies exposed algae to MNPs in the range of 72–168 h, followed by studies of 24–72 h (27.27%). Therefore, most investigations are based on short-term experiments of less than one week and more studies are needed to investigate the long-term effects of MNPs on microalgae.

In terms of study content (Fig. 1f), growth was the most focused-on parameter, involved in 90.63% of studies. Morphology, including changes of cell structure, MNPs adsorption, and homo-, hetero-aggregation, is the second most commonly reported parameter (68.75%). Approximately one quarter of studies investigated the effects of MNPs on pigments, photosynthesis and antioxidants (primarily ROS, SOD and catalase). In addition, 12.50% of studies referred to the production of biochemical materials, including exopolymeric substances, lipids and microcystin subsequent to MNPs exposure. Three papers (9.38%) studied the impacts of MNPs on gene transcription, with only one (Xiao et al., 2020) conducting comparative transcriptome analysis. Feng et al. (2020c) is the only study that employs comparative proteomic analysis to explore the mechanism of NPs effects on microalgae. Clearly, more studies are needed to reveal the molecular mechanisms of MNPs action(s) on algae via omics analysis.

#### 3.1.1. Effects of MNPs on microalgae growth

The growth of both marine and freshwater algae was negatively affected by MNPs, with the difference between marine and freshwater algae being statistically insignificant (Fig. 2a, Marine: $-0.06 \pm 0.02$ sem; Freshwater: $-0.13 \pm 0.01$ sem; Mann–Whitney U, $Z = 1.796$, $p = 0.0726$). It is worth noting that although the effect size in freshwater algae was more than two times bigger than marine algae it does not suggest a higher sensitivity to MNPs for freshwater algae because the larger negative effects were caused by PS with positively charged amino groups which was used for the freshwater algae *Scenedesmus obliquus* (Liu et al., 2020) but not used with any of the studies with marine algae. In addition, positive effects of MNPs were found in several studies, although the mean was below zero. There are two possible reasons to explain the stimulating effects. One is due to leaching of additives found in the MNPs. The low levels of microplastic additives may slightly stimulate algal growth, a process known as the "Hormesis" phenomenon (Chae et al., 2019; Song et al., 2020) despite the doubt on its universality (Axelrod et al., 2004). The other is that larger MNPs could serve as physical substrates for growth of microalgae (Canniff and Hoang, 2018; Song et al., 2020).

The differences across phyla were not significant (Fig. 2b; Tukey–Kramer HSD, all $p > 0.05$). Shape did not impose significant influence on growth (Fig. 2c; Tukey–Kramer HSD, all $p > 0.05$). On the other hand, it has been reported that irregular MNPs could lead to more harm to cells of zooplankton and humans compared to beads/spherical MNPs (Frydkjær et al., 2017). Considering the low proportion of studies involving fragment and block MNPs, the conclusion in the present study needs to be examined through more experiments. In terms of polymer type, all types had a negative effect (Fig. 2d). The modification of PS made a difference. Thus, compared to the unmodified PS, charged PS, particularly positively charged PS-$NH_2$ ($-0.29 \pm 0.47$ sem) and PS-COOH ($-0.26 \pm 0.33$ sem), had larger negative effects (Fig. 2d, Tukey–Kramer HSD, both $p < 0.0001$). Furthermore, it has been commonly reported that positively charged PS could result in high inhibition of microalgal growth compared to negatively charged PS (Bhattacharya et al., 2010; Bergami et al., 2017; Liu et al., 2020). This could be related to the adsorption of PS particles by cells. Bhattacharya et al. (2010) showed that there were more positively charged MPs adhering to *Chlorella* and *Scenedesmus* compared to negatively charged MPs. Nolte et al. (2017) also showed that adsorption of neutral and positively charged plastic nanoparticles onto the cell wall of *Pseudokirchneriella subcapitata* was stronger than that of negatively charged MPs. These findings indicate that positively charged PS particles possess a higher binding affinity for the microalgae than negatively charged ones, which could be caused by electrostatic attraction between the MPs and the cell walls. Cell walls of microalgae usually have a negative Zeta potential (Bhattacharya et al., 2010; Ozkan and Berberoglu, 2013; Nolte et al., 2017), which more easily bonded to bond positively charged MPs. Based on this argument, negatively charged MPs should adhere less to cell surfaces and do less harm to cells than neutral MPs. The reason that negatively charged PS had a larger effect on growth could be due to the smaller size (nano) used for these compared to uncharged PS particles.

There was no relationship between log effect size of growth and exposure period for each phylum (Fig. S2). There was a negative relation

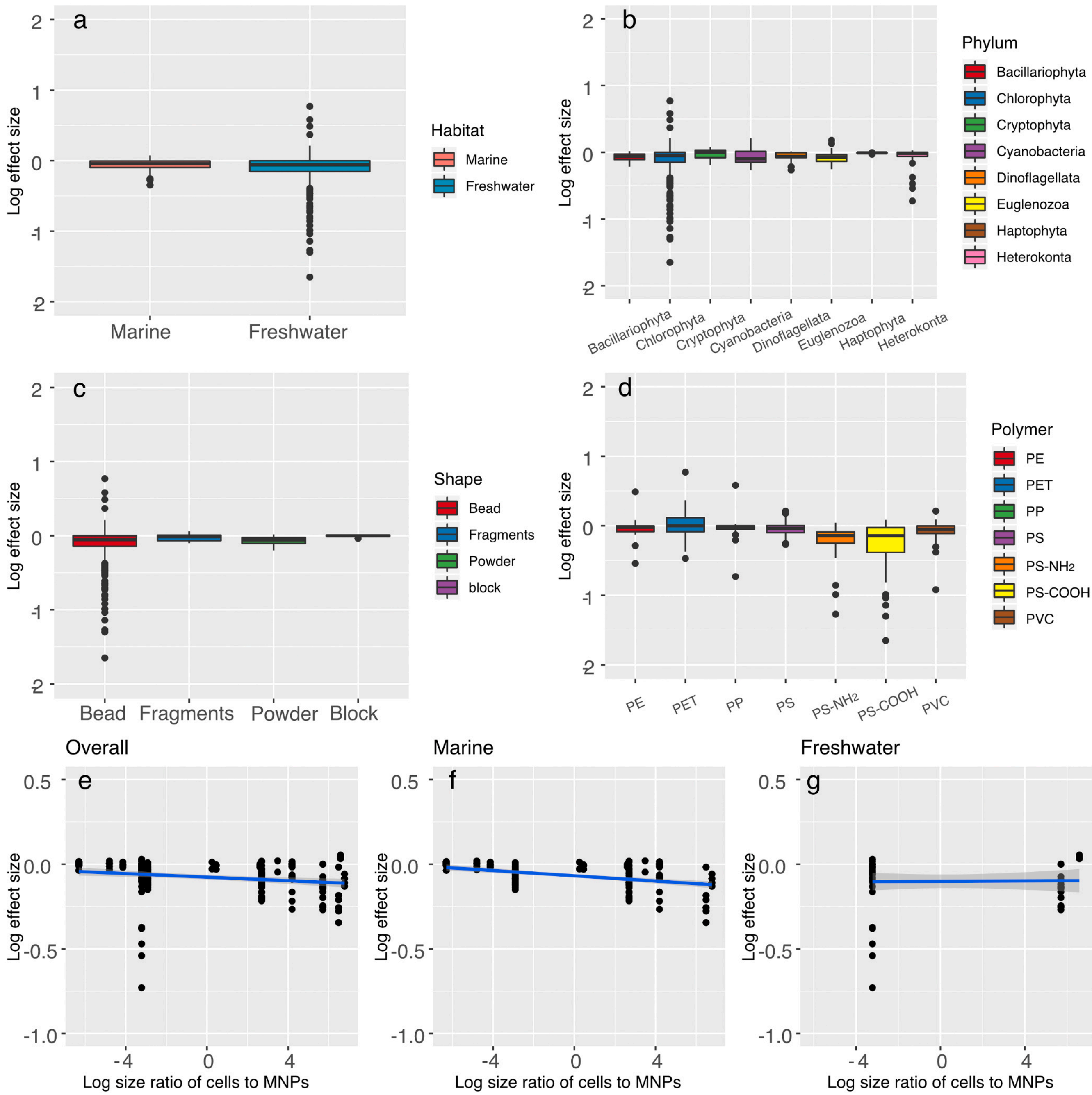


**Fig. 2.** Log effect size of microalgae growth for different habitats (a), taxonomic groups (b), MNP shape (c) and polymer type (d) size ratio of algal cells to MNPs (e–g). PE, polyethylene; PET, Polyethylene terephthalate; PP, polypropylene; PS, polystyrene; PS-$NH_2$, polystyrene-$NH_2$; PS-COOH, polystyrene-COOH; PVC, polyvinylchloride.

between log effect size and size ratio for marine algae (adjusted $R^2 = 0.194$, $p < 0.0001$); however this relationship was not detected either for algae overall or for freshwater algae (Fig. 2e–g). Chae et al. (2019) found, after analyzing the data of freshwater and marine microalgae from 12 studies, that the effect of MNPs could change from positive to negative when the size ratio increased. Taken together, it indicates that a higher size ratio of algal cells to MNPs could result in a larger negative effect on microalgal growth. Some positively charged MNPs were used for freshwater microalgae but not for marine algae, which could be the reason that, in the present study, this negative relationship could not be found across all algae or for freshwater algae.

Different phyla showed differential response to MNPs concentrations in terms of growth (Fig. 3). Log effect size of Bacillariophyta and Cyanobacteria increased with MNP concentration (from negative to null effects) (Linear regression, Bacillariophyta: adjusted $R^2 = 0.126$, $p = 0.0002$; cyanobacteria: adjusted $R^2 = 0.511$, $p < 0.0001$) (Fig. 3b, f). Chlorophyta, Heterokonta, Euglenozoa, or Haptophyta did not show changes with MNP concentration (Fig. 3a, c, d, g). While log effect size for Dinoflagellates and Cryptophyta decreased with MNPs concentrations (from null to negative) (Linear regression, Dinoflagellate: adjusted $R^2 = 0.323$, $p = 0.0042$; Cryptophyta: adjusted $R^2 = 0.690$, $p < 0.0001$) (Fig. 3e, h). Generally, inhibition increases with MNPs concentrations (Bergami et al., 2017; Mao et al., 2018; Liu et al., 2020; Xiao et al., 2020); the case of the Bacillariophyta and cyanobacteria is

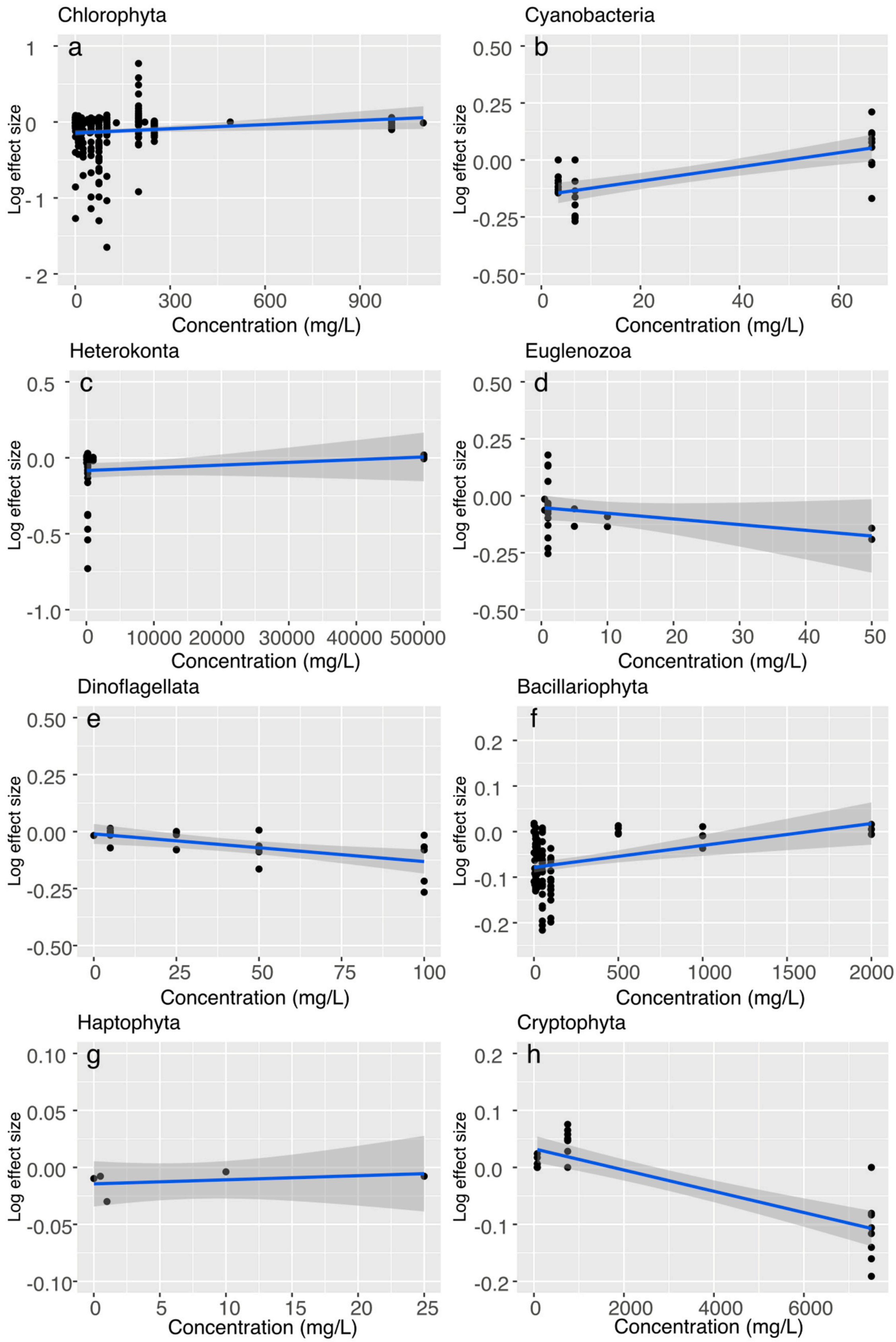


**Fig. 3.** Log effect size of microalgae growth versus MNP concentration broken down by taxonomic group (a–h).

probably because smaller size MNPs were used for studies using lower concentrations. MNPs with smaller size usually lead to higher inhibition on growth (Sjollema et al., 2016; Yokota et al., 2017). It is worth noting that MNPs concentrations could change during the exposure experiments owing to deposition, aggregation and other phenomena. Therefore, future studies are encouraged to monitor the real MNPs concentrations over time to better explain the results. The MNP concentration that induced 50% algal growth inhibition ($EC_{50}$) and the lowest observed adverse effects concentration (LOEC) for some microalgae are summarized in Table 2. For uncharged MNPs, the $EC_{50}$ and LOEC are usually above 25 mg/L, while modified MNPs, particularly for those with positive charge, could have an $EC_{50}$ below 1 mg/L. It is easier for cationic MNPs to interact with the cell membrane due to their similar molecular structure to proteins. In addition, they may induce the formation of nanoscale holes in the lipid bilayer and thus increase permeabilization of the cell membrane (Hong et al., 2006). Once internalized, they could do more harm to cells.

### 3.1.2. Effects of MNPs on microalgal photosynthesis

Maximum photochemical efficiency of photosystem ($F_v/F_m$) is commonly used to represent the physiological state of photosystem II (Warner et al., 1999; Gao et al., 2018a). Freshwater algae showed significantly lower log effect size of $F_v/F_m$ than marine algae when exposed to MNPs (Fig. 4a, Marine: 0.009 ± 0.009 sem; Freshwater: − 0.018 ± 0.003 sem; one-way ANOVA, $F_{1,\ 262} = 7.956$, $p = 0.005$). There was no significant difference across phyla (Fig. 4b; Tukey–Kramer HSD, all $p > 0.05$). Both beads and powder negatively affected $F_v/F_m$, with larger effects from powder (Log effect size: − 0.027 ± 0.035 sem) (Fig. 4c). Based on 27 studies (Table 1), it seems that $F_v/F_m$ is more sensitive to irregular shapes compared to growth. Different polymer types affected $F_v/F_m$ differentially (Fig. 4d). PS affected $F_v/F_m$ positively; PS-$NH_2$ had no effect, while PP, PVC and PS-COOH reduced it, with PS-COOH having the largest effect. This pattern seems different from that for growth, for which PS-$NH_2$ had the largest negative effect. However, it is not unusual that growth and photosynthesis are decoupled because growth is a comprehensive embodiment of all physiological activities (Gao et al., 2009, 2018b; Xu et al., 2017). Furthermore, the result for PS-$NH_2$ comes from only one data set and apparently more experimental data are needed to assess the effect of PS-$NH_2$ on $F_v/F_m$.

Log effect size of $F_v/F_m$ decreased with MNP concentration (from zero to negative) (Fig. 4e). Log effect size of $F_v/F_m$ slightly increased with exposure period for marine algae (from minus to zero) (Fig. 4f), indicating a decline in sensitivity with exposure period. This trend is mainly supported by data from the Chlorophyta and suggests an acclimation process of algal photosynthesis to MNPs stress. It has been shown that the negative effect of MNPs on the diatom *Skeletonema costatum* was reduced with culture time because *S. costatum* could respond to MNPs by cell wall thickening and cellular homo-aggregation (Mao et al., 2018).

### 3.1.3. Effects of MNPs on microalgal pigments

MNPs reduced the pigment content of all algae examined, based on 16 studies (Table 1), but with no significant differences between the responses of marine and freshwater species (Mann–Whitney U, $Z = 1.837$, $p = 0.0662$) (Fig. 5a). Pigment content of the Dinoflagellata and Heterokonta were the least affected while the Chlorophyta (− 0.124 ± 0.193 sem) was most sensitive to MNPs (Fig. 5b). MNPs shape did not affect pigments significantly (Tukey–Kramer HSD, all $p > 0.05$) (Fig. 5c). All polymer types affected pigments negatively, with the smallest effects from PET and PE and the largest from PS-$NH_2$ (Fig. 5d).

Log effect size of Chl *a* decreased with MNP concentration (from plus to minus), indicating that the effect changed from positive to negative (linear regression, adjusted $R^2 = 0.032$, $p = 0.002$) (Fig. 5e). This pattern was found in freshwater algae, particularly the Bacillariophyta, but not in marine algae (Fig. S3). There are two probably reasons to explain the negative effect of MNPs on photosynthetic pigments. Firstly, intracellular ROS caused by MNPs can damage the structure of photosynthetic pigments or inhibit their synthesis (Geoffroy et al., 2003);

**Table 2**
Summary of the MNP concentrations that induced 50% inhibition of algal growth ($EC_{50}$) and the lowest observed adverse effects concentration (LOEC) for freshwater and marine algae. The literature was listed in order of $EC_{50}$ or LOEC from high to low.

| Species | Polymer | Size (μm) | Shape | Duration (h) | $EC_{50}$[a]/LOEC[b] | Reference |
|---|---|---|---|---|---|---|
| *Skeletonema costatum* | PE | 74 | Particle[c] | 96 | > 100[a] | Zhu et al. (2018) |
| *Skeletonema costatum* | PS | 74 | Particle | 96 | > 100[a] | Zhu et al. (2018) |
| *Skeletonema costatum* | PVC | 74 | Particle | 96 | > 100[a] | Zhu et al. (2018) |
| *Skeletonema costatum* | PVC | 1 | Particle | 96 | > 100[a] | Zhu et al. (2018) |
| *Scenedesmus obliquus* | n-plain-PS | 0.1 | Bead | – | 61[a] | Liu et al. (2020) |
| *Chlorella pyrenoidosa* | PS | 5 | Spherical | 96 | > 60[a] | Yi et al. (2019) |
| *Chlorella pyrenoidosa* | PS(Fe)-COOH | 1 | Spherical | 72 | > 50[a] | Zhang et al. (2020) |
| *Chlorella pyrenoidosa* | PS-$NH_2$ | 1 | Spherical | 72 | > 50[a] | Zhang et al. (2020) |
| *Chlorella pyrenoidosa* | PS-COOH | 1 | Spherical | 72 | > 50[a] | Zhang et al. (2020) |
| *Scenedesmus obliquus* | n-plain-PS | 1 | Bead | – | 33[a] | Liu et al. (2020) |
| *Phaeodactylum tricornutum* | PE | 1–4 | Bead | 72 | > 25[a] | Gambardella et al. (2019) |
| *Phaeodactylum tricornutum* | PE | 4–6 | Bead | 72 | > 25[a] | Gambardella et al. (2019) |
| *Phaeodactylum tricornutum* | PE | 11–13 | Bead | 72 | > 25[a] | Gambardella et al. (2019) |
| *Phaeodactylum tricornutum* | PE | 20–25 | Bead | 72 | > 25[a] | Gambardella et al. (2019) |
| *Scenedesmus obliquus* | PS-$NH_2$ | 0.1 | Bead | – | 24[a] | Liu et al. (2020) |
| *Scenedesmus obliquus* | n-plain-PS | 2 | Bead | – | 22[a] | Liu et al. (2020) |
| *Chlorella pyrenoidosa* | PS | 0.55 | Spherical | 96 | 9.1[a] | Yi et al. (2019) |
| *Scenedesmus obliquus* | n-plain-PS | 0.5 | Bead | – | 7.5[a] | Liu et al. (2020) |
| *Pseudokirchneriella subcapitata* | PS-PEI | 0.055 | Bead | 72 | 0.58[a] | Casado et al. (2013) |
| *Pseudokirchneriella subcapitata* | PS-PEI | 0.11 | Bead | 72 | 0.54[a] | Casado et al. (2013) |
| *Chlorella pyrenoidosa* | PS(Fe)-$NH_2$ | 1 | Spherical | 72 | 0.35[a] | Zhang et al. (2020) |
| *Phaeodactylum tricornutum* | PE | 1–4 | Bead | 72 | > 25[b] | Gambardella et al. (2019) |
| *Phaeodactylum tricornutum* | PE | 4–6 | Bead | 72 | > 25[b] | Gambardella et al. (2019) |
| *Phaeodactylum tricornutum* | PE | 11–13 | Bead | 72 | > 25[b] | Gambardella et al. (2019) |
| *Phaeodactylum tricornutum* | PE | 20–25 | Bead | 72 | > 25[b] | Gambardella et al. (2019) |
| *Ulva prolifera* | HDPE | 1.45–52.48 | Fragment | 168 | > 25[b] | Feng et al. (2020a) |

[a] Refers to $EC_{50}$.
[b] Refers to LOEC; - represents no mention in the literature.
[c] Refers to powder with irregular shape; PE, polyethylene; PS, polystyrene; PVC, polyvinylchloride; n-plain-PS, negatively charged-plain-polystyrene; PS(Fe)-COOH, polystyrene(Fe)-COOH, PS-$NH_2$, polystyrene-$NH_2$; PS-COOH, polystyrene-COOH; PS-PEI; polystyrene-polyethyleneimine; PS(Fe)- $NH_2$, polystyrene(Fe)- $NH_2$; HDPE, High Density Polyethylene.

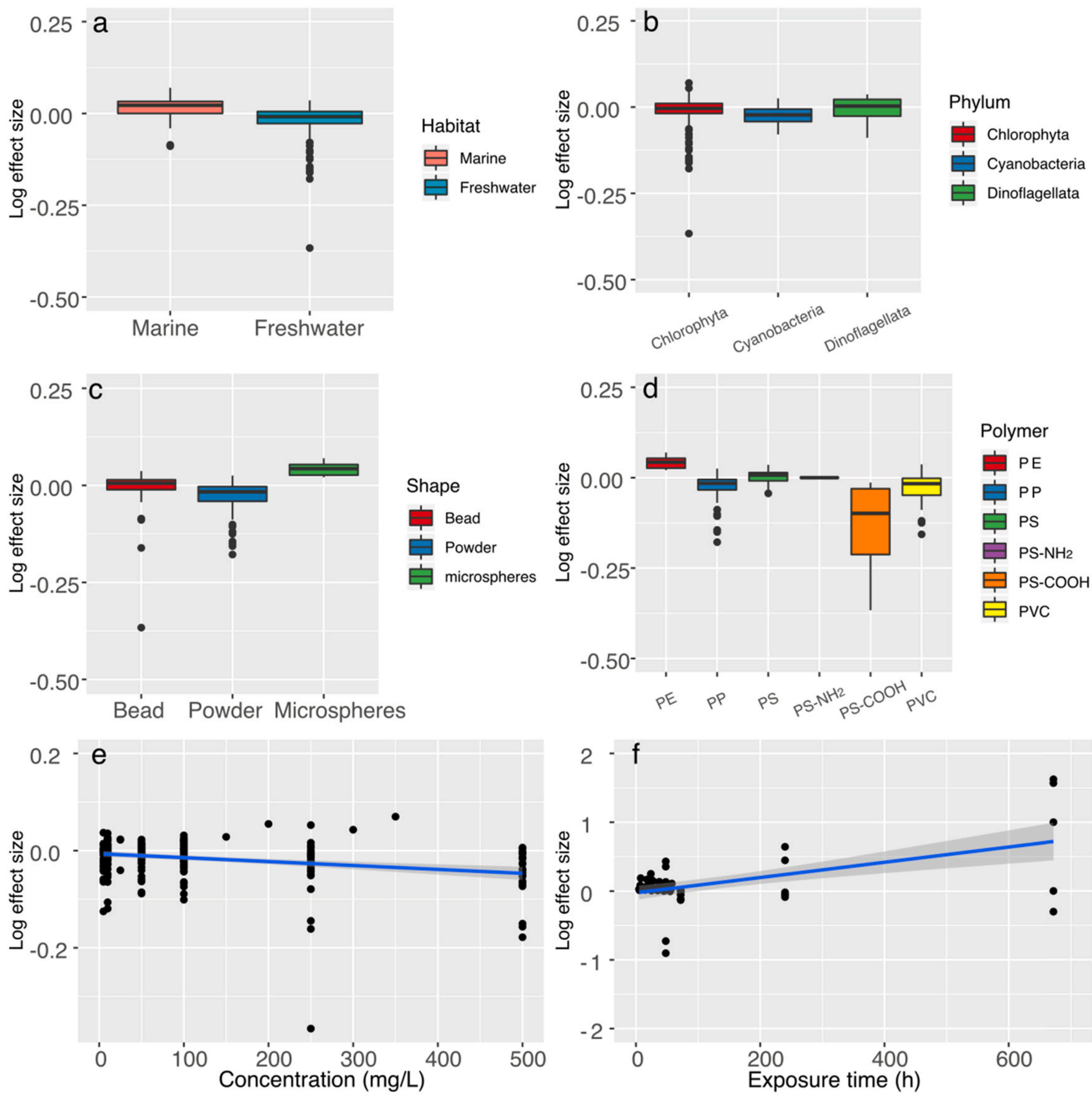


**Fig. 4.** Log effect size of microalgae $F_v/F_m$ for different habitats (a), taxonomic groups (b), MNP shape (c), polymer type (d), concentration (e), and exposure time (f). PE, polyethylene; PP, polypropylene; PS, polystyrene; PS-$NH_2$, polystyrene-$NH_2$; PS-COOH, polystyrene-COOH; PVC, polyvinylchloride.

secondly, microplastics could adhere to the surface of algae or form hetero-aggregates with algae (Lagarde et al., 2016; Long et al., 2017), which hinder light and nutrients entering cells, particularly when MNPs levels are high. Limited energy and materials input would also reduce synthesis of pigments. Log effect size of Chl *a* decreased with exposure time (from plus to minus) and this pattern occurred in both marine and freshwater algae (linear regression, overall: adjusted $R^2 = 0.172$, $p < 0.0001$; marine: adjusted $R^2 = 0.170$, $p = 0.0001$; freshwater: adjusted $R^2 = 0.178$, $p < 0.0001$) (Fig. 5f, Fig. S4). No clear pattern was found between the log effect size of Chl *a* and log size ratio (linear regression, overall: adjusted $R^2 = 0.018$, $p = 0.153$) (Fig. S5).

### 3.1.4. Effects of MNPs on microalgal antioxidative metabolism

It seems that MNPs induced more ROS in marine than in freshwater algae (Log effect size, Marine: $0.980 \pm 0.936$ sem, Freshwater: $0.042 \pm 0.044$ sem; Fig. 6a) based on five studies (Table 1) although statistical analysis shows that the difference was not significant (Mann–Whitney U, $Z = 1.706$, $p = 0.088$). It is highly recommended to conduct more studies to validate this conclusion as only one study among the five used marine algae. While phylum or polymer did not affect log effect size of ROS (Fig. 6b, c), log effect size of ROS increased with MNP concentration (linear regression, adjusted $R^2 = 0.404$, $p < 0.0001$) and exposure time (linear regression, adjusted $R^2 = 0.371$, $p < 0.0001$) for algae as a whole (Fig. 6d, e). ROS can be generated at multiple sites in microalgae, such as chloroplasts, mitochondria, and peroxisomes, when the cells are exposed to environmental stresses. MNPs, as an environmental stressor, have been shown to induce ROS in many microalgae (Bhattacharya et al., 2010; Liu et al., 2020; Hazeem et al., 2020). The potential mechanisms involved remain unclear, though probably involve physical damage or release of toxic chemicals leading to transfer of electrons to oxygen rather than the usual acceptors.

Marine algae also had a higher log effect size of SOD compared to freshwater algae (Log effect size, Marine: $0.393 \pm 0.065$ sem, Freshwater: $-0.044 \pm 0.053$ sem, Mann–Whitney U, $Z = 4.017$, $p < 0.001$) (Fig. 7a). MNPs induced higher SOD in the Heterokonta, Bacillariophyta and Euglenozoa but reduced it in the Chlorophyta (Fig. 7b) based on five studies. The decline of SOD in chlorophytes could be due to the use of charged MNPs in the studies involved because it has been shown that PS-COOH and PS-$NH_2$ could inhibit SOD while PET, PE, PVC, PS and PP induced higher SOD (Fig. 7c). Log effect size of SOD first increased and then decreased with MNP concentration (quadratic regression, adjusted

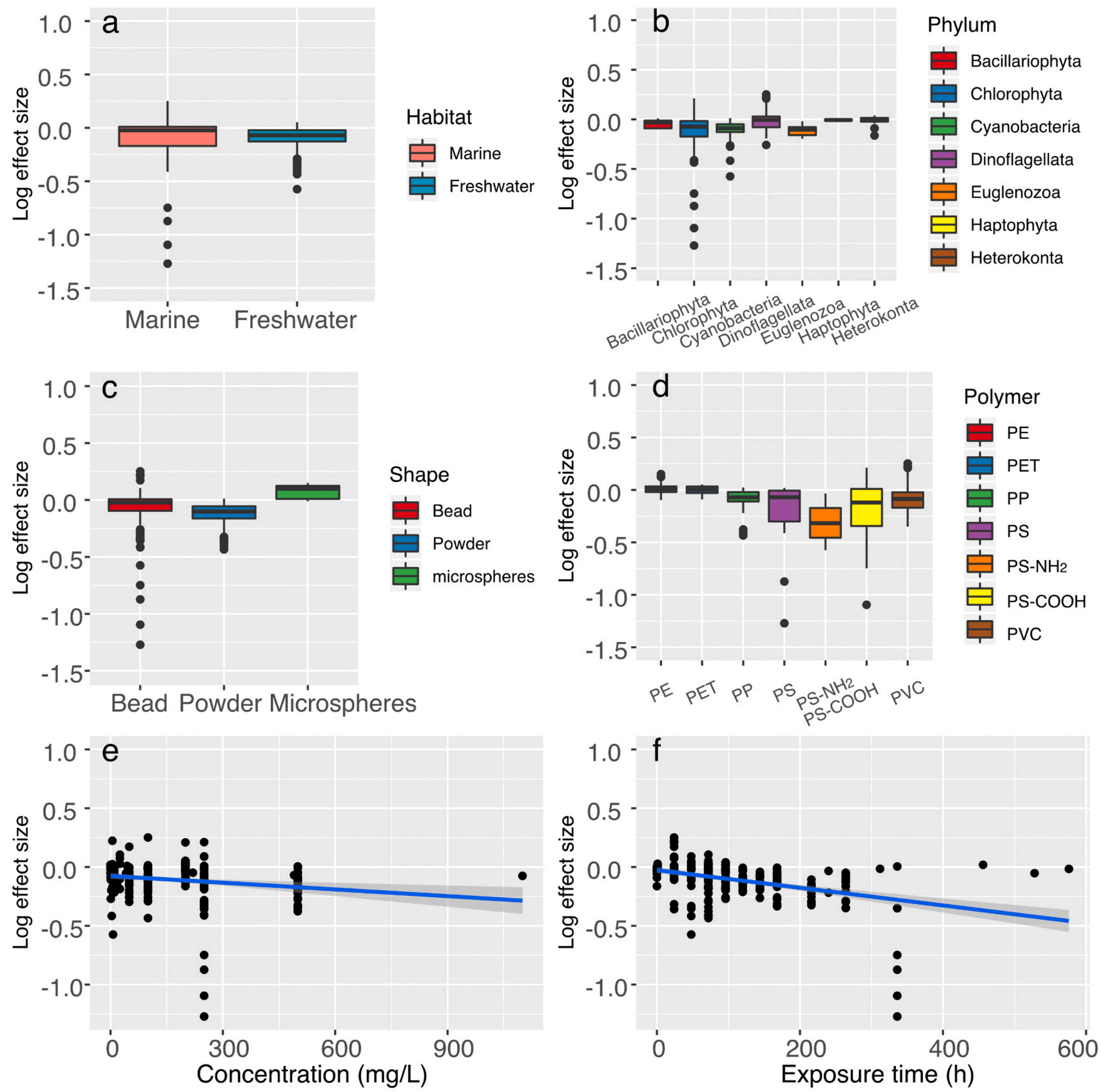


**Fig. 5.** Log effect size of microalgae pigments for different habitats (a), taxonomic groups (b), MNP shape (c), polymer type (d), concentration (e), and exposure time (f). PE, polyethylene; PET, Polyethylene terephthalate; PP, polypropylene; PS, polystyrene; PS-$NH_2$, polystyrene-$NH_2$; PS-COOH, polystyrene-COOH; PVC, polyvinylchloride.

$R^2 = 0.430$, $p < 0.0001$) (Fig. 7d). ROS are harmful to cells and thus microalgae have evolved an antioxidant network to scavenge ROS. SOD can convert the superoxide radical to $H_2O_2$, which is subsequently neutralized to $H_2O$ by catalase (CAT) (Smerilli et al., 2017; Gao et al., 2018d). The increase of log effect size for SOD with MNP concentration indicates that cells initiate antioxidant systems to deal with the increased ROS. The subsequent decrease of SOD with MNP concentration may be due to the inhibition of SOD synthesis at high MNPs concentrations. Log effect size of SOD increased with exposure time (from minus to plus), indicating that cells would synthesize more SOD with time (linear regression, adjusted $R^2 = 0.736$, $p < 0.0001$) (Fig. 7e).

MNPs increased MDA in the algae as a whole, with a larger effect in marine algae (Log effect size, Marine: 0.933 ± 0.041 sem, Freshwater: 0.109 ± 0.119 sem, one-way ANOVA, $F_{1,22} = 134.5297$, $p < 0.001$) (Fig. 8a). In terms of phylum, MNPs induced a higher MDA level in the Bacillariophyta compared to the Chlorophyta and Heterokonta (Tukey–Kramer HSD, both $p < 0.0001$; Fig. 8b). Powder MNPs induced higher MDA compared to beads (one-way ANOVA, $F_{1,22} = 134.5297$, $p < 0.001$) (Fig. 8c), while polymer type did not impose different effects on MDA (Tukey–Kramer HSD, all $p > 0.05$) (Fig. 8d). The log effect size of MDA initially increased with MNP concentration and then decreased rapidly beyond a maximum (quadratic regression, adjusted $R^2 = 0.885$, $p < 0.0001$) (Fig. 8e). The decrease was essentially attributable to data from the study by Song et al. (2020), in which a larger size of MP (74 μm) was used and larger MPs usually lead to less harm (Mao et al., 2018). This study also showed that log effect size of MDA also increased with size ratio (linear regression, adjusted $R^2 = 0.166$, $p = 0.042$) (Fig. 8f). MDA is indicative of lipid peroxidation and reflects damage to cell membranes. Excessive ROS can enhance cell membrane lipid peroxidation and is commonly indicated by an increase in MDA content (Hong et al., 2006).

### 3.2. MNP pollution and macroalgae

Macroalgae, the most productive marine macrophytes on a global scale, play an essential role in the coastal carbon cycle. They contribute about 10% of marine primary productivity and macroalgae could sequester about 173 TgC $year^{-1}$ (Israel et al., 2010; Krause-Jensen and Duarte, 2016). In addition, macroalgae provide important habitats for epifauna and infauna (Reed et al., 2016; Xu et al., 2017) and serve as animal feed and human food as well (Gao et al., 2018c), implying that MNPs in or on macroalgae could be transmitted to animal and humans

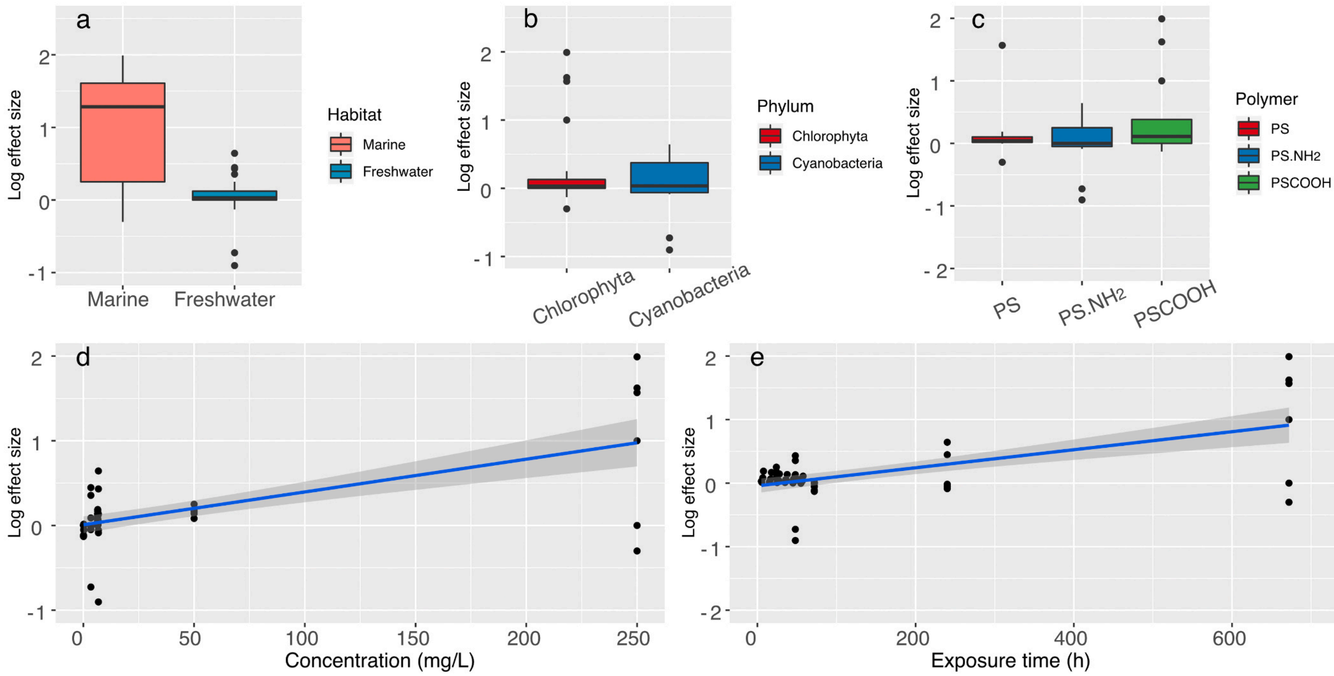


**Fig. 6.** Log effect size of microalgae ROS for different habitats (a), taxonomic groups (b), MNP polymer type (c), concentration (d), and exposure time (e). PS, polystyrene; PS-$NH_2$, polystyrene-$NH_2$; PS-COOH, polystyrene-COOH.

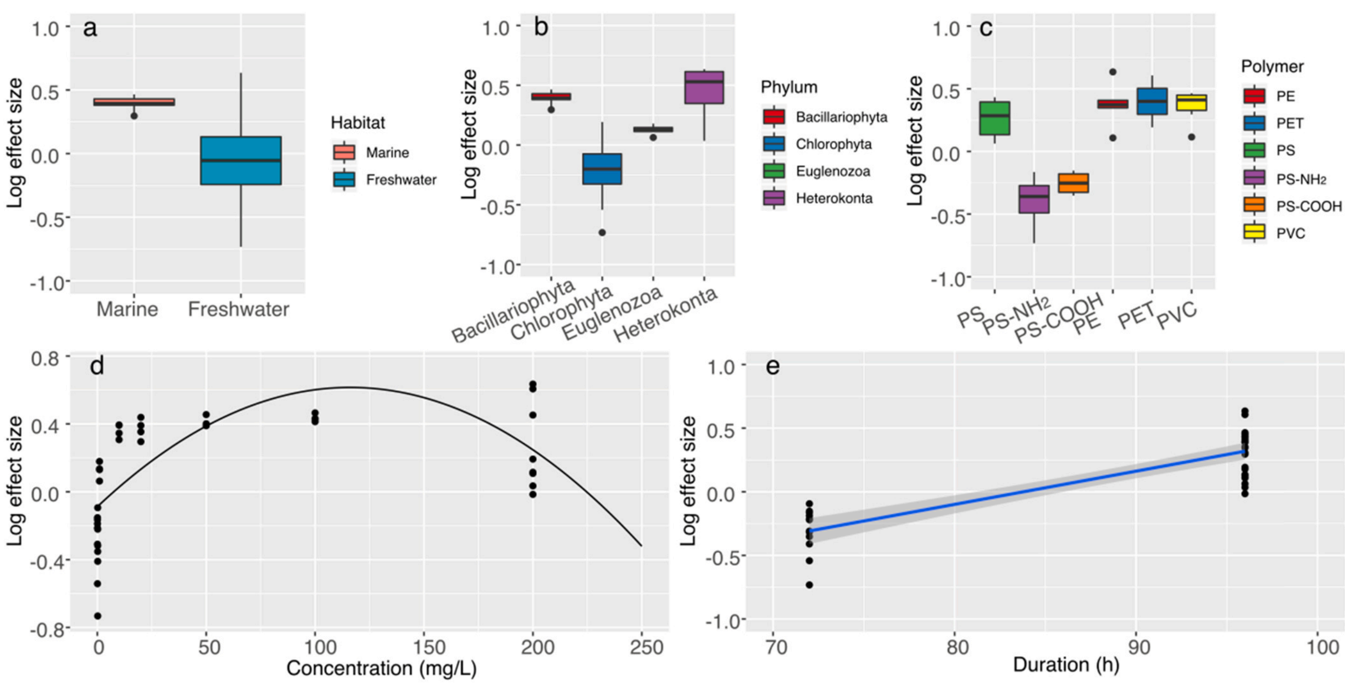


**Fig. 7.** Log effect size of microalgae SOD for different habitats (a), taxonomic groups (b), MNP polymer type (c), concentration (d), and exposure time (e). PE, polyethylene; PET, Polyethylene terephthalate; PS, polystyrene; PS-$NH_2$, polystyrene-$NH_2$; PS-COOH, polystyrene-COOH; PVC, polyvinylchloride.

via food webs.

It has reported that macroalgae can trap MNPs via diverse mechanisms, including twining, attachment, embedment, and wrapping (Feng et al., 2020a, 2020b). The effect of washing on trapped MNPs in macroalgae has also been investigated. Sundbæk et al. (2018) found washing could take away 94.5% of trapped MNPs in *Fucus vesiculosus*. On the other hand, Li et al. (2020) found that washing did not significantly affect MNPs adhered to *Pyropia yezoensis*. These different results could be due to the washing procedures used. In Sundbæk et al. (2018), seaweed samples were washed in the laboratory by agitation in closed bottles whereas in the study by Li et al. (2020) washing was conducted in nori processing factories where the levels of environmental MNPs are high.

Compared to microalgae, less information about the effects of MNPs on macrophytes is currently available (Table 3). Only one paper refers to the physiological performance of seaweeds upon exposure to MNPs. MNPs did not affect growth rate, effective photochemical efficiency of photosystem II (PSII), or saturating irradiance of *U. prolifera* until

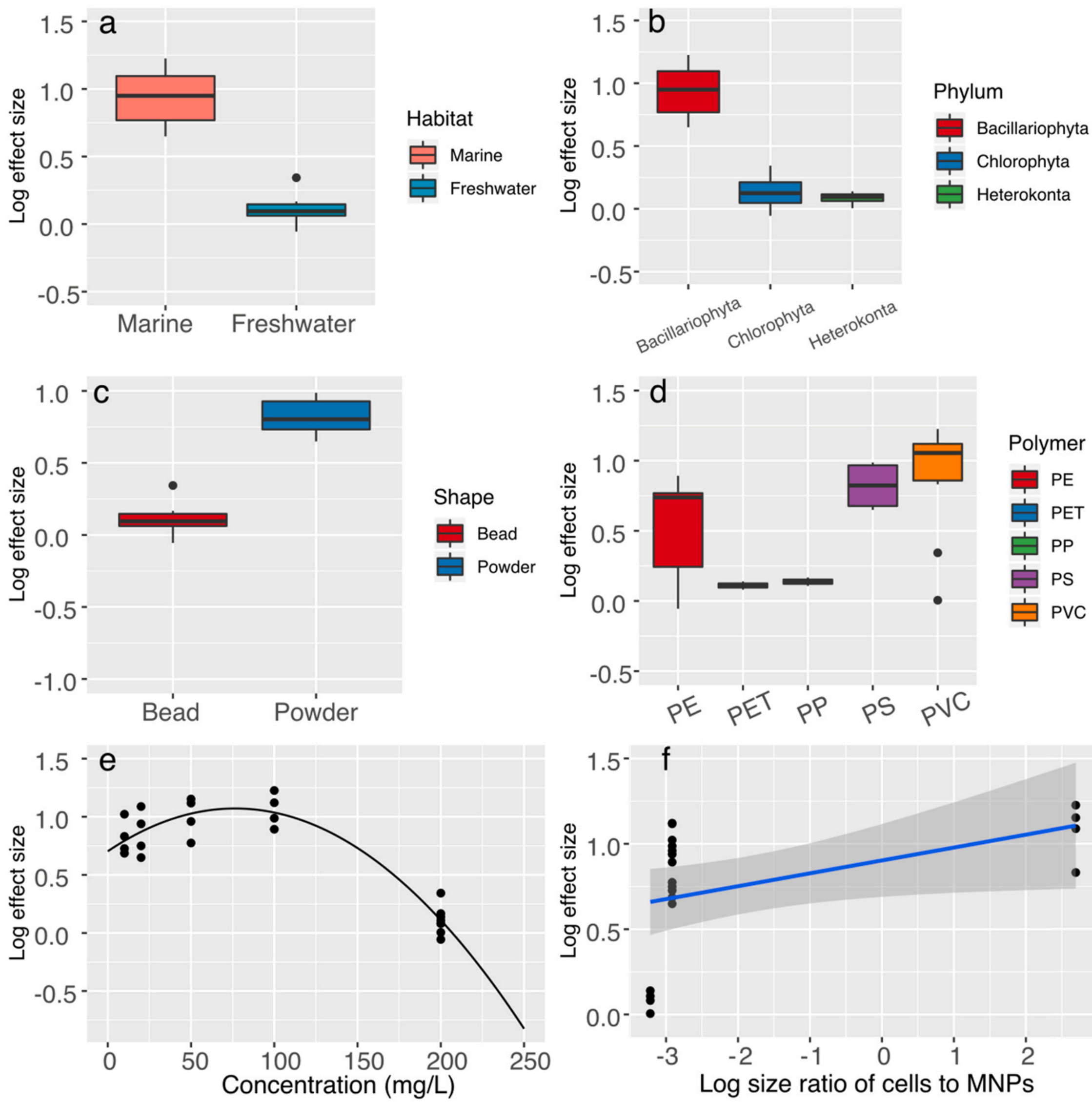


**Fig. 8.** Log effect size of microalgae MDA for different habitats (a), taxonomic groups (b), MNP shape (c), polymer type (d), concentration (e), and size ratio of algal cells to MNPs (f). PE, polyethylene; PET, Polyethylene terephthalate; PP, polypropylene; PS, polystyrene; PVC, polyvinylchloride.

reaching an extremely high concentration (100 mg/L) (Feng et al., 2020a). Gutow et al. (2016) demonstrated that the common periwinkle *Littorina littorea* did not distinguish between seaweeds with adherent microplastics and clean seaweeds without microplastics, suggesting that seaweeds could serve as a vector for MNPs into marine food webs.

### 3.3. MNPs pollution in seagrasses and freshwater macrophytes

MNPs were also found on blades of the seagrasses *Zostera marina* (Jones et al., 2020) and *Cymodocea rotundata* (Sora Datu et al., 2019). The MNPs on the surface of seagrass blades can be ingested by marine animals, and thus enter the food chain (Goss et al., 2018; Priscilla et al., 2019). MNPs abundance in sediments where seagrass is growing was shown to be more than 2 times higher than bare sites in Xincun Bay and Li'an Bay, Hainan, China and hence seagrass beds can act as a trap of microplastics (Huang et al., 2020). Macroplastics accumulated in all vegetated habitat but not in nearby unvegetated areas in the Ria Formosa lagoon, Portugal (Cozzolino et al., 2020). In that study, the capacity of sediments and the seagrass canopy to trap microplastics was higher for subtidal than for intertidal vegetated habitats (Cozzolino et al., 2020). Potential mechanisms for microplastic accumulation by seagrass include entrapment by epibionts, or attachment via biofilms (Goss et al., 2018).

Only one paper investigated the effect of MNPs on the physiological performance of seagrasses and freshwater macrophytes (Mateos-Cárdenas et al., 2019, Table 3). PE MPs at a concentration of 50, 000 items/mL did not affect photosynthetic parameters (maximum quantum yield of PSII, effective quantum yield of PSII, coefficient of photochemical quenching and coefficient of non-photochemical quenching) or growth of the duckweed species *Lemna minor* after a seven-day exposure. A longer exposure period (30 days) had a tendency to reduce the dry weight of L. *minor* although the decline was not statistically significant (Mateos-Cárdenas et al., 2019).

### 3.4. Other interactions between MNPs and algae

As described above, high concentrations of MNPs can affect photosynthesis and growth of algae and thus marine primary productivity and the carbon cycle. Low concentrations of MNPs may not affect carbon fixation of algae but can adhere to the surface of the algae, which may affect sinking of microalgae. Specifically, lower density of MNPs could reduce algal sinking and a higher density of MNPs could increase the

**Table 3**
Summary of effects of MNPs on macrophytes in field and laboratory studies.

| Species | Location | Polymer | Size | Shape | Abundance[a] | Duration /time | Parameter | Effect/conclusion | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Seaweeds | | | | | | | | | |
| *Ulva prolifera* | Laboratory | HDPE | 1.45–52.48 μm | Fragment | 0–100 mg/L | 168 h | Growth, $rETR_{max}$, effective photochemical efficiency, electron transport efficiency, NPQ, saturating irradiance | MPs did not affect these parameters until reaching 100 mg/L but induced NPQ at concentrations > 2.5 mg/L | Feng et al. (2020a) |
| | Yellow Sea, China | PE, PS, PP, PA, PET, Rayon, PEU | 13.5–4991.1 μm | Microbead, film, fragment, foam and fiber | 0.20–1.48 items/L | April 16 to July 23 in 2018 | Features of MPs in thalli | *U. prolifera* could trap a large amount of plastics via diverse mechanisms. | |
| *Pyropia yezoensis*, *Ulva prolifera*, *Sargassum horneri*, *Cladophora* sp., *Undaria pinnatifida*, *Ulva pertusa*, | Haizhou Bay, Yellow Sea, China | PE, Rayon, PP, PS, PET, PE-PP, CP, Nylon | 60.74–4993.15 μm | Fiber, foam, film, fragment | 0.07–0.29 items/L | February and June 2019 | Features of MPs in thalli | Macroalgae could be ideal biomonitors for MPs pollution in seawater due to their unbiased trapping and immovability | Feng et al. (2020b) |
| *Fucus vesiculosus* | Laboratory | PS | 10 μm | Microbead | 1.39–55.65 items/mL | 2 h | Density of MPs on thalli | *Fucus vesiculosus* retained suspended microplastics on its surface and represented an efficient pathway for microplastics into marine food webs | Gutow et al. (2016) |
| | | PS | 1–100 μm | Fragment | 1.10–56.95 items/mL | 2 h | | | |
| | | Polyacrylic | 90–2200 μm | Fiber | 0.004–0.027 mg/L | 2 h | | | |
| *Pyropia yezoensis* | Laboratory (alive) | PS | 100–2000 μm. | Fiber | 0– 10,000 items/L | 2 h | Adherence of MPs to nori | The abundance and composition of MPs in nori's final commercial products and the intermediate products were related to microplastic concentration and type in their ambient environments | Li et al. (2020) |
| | Laboratory (nori product) | PET, PP, PE-PP, PE-PP-D, rayon, resin, CP, PAN, PA, PVC, PS-PAE, PMMA | 110–4970 μm | Pellet, film, fragment, fiber | 0.9–3.0 items/g DW | January and February 2019 | Features of MPs on macroalgae | | |
| | Yellow Sea, China | PET, PE, PP, PE-PP, PE-PP-D, rayon, CP, PAN, PVC, PS | 70–4740 μm | Pellet, film, fragment, fiber | 1.0–2.8 items/g DW | February 2019 | Features of MPs on macroalgae | | |
| *Fucus vesiculosus* | Laboratory | PS | 20 μm | No mention | 2.65 mg/L (corresponding to 597 particles per mL) | 2 h | Sorption of MPs to thalli | Sorption of PS microplastic particles to *F. vesiculosus* and a significant reduction of 94.5% by washing was found | Sundbæk et al. (2018) |
| Seagrasses | | | | | | | | | |
| *Thalassia testudinum* | Calabash Patch Reef, Belize | Not mention | Not mention | Fiber, bead, chip | 4.56 items/blade | December 19th, 2017 | MPs abundance in thalli | Potential mechanisms for microplastic accumulation included entrapment by epibionts, or attachment via biofilms | Goss et al. (2018) |
| *Cymodocea rotundata* | Barrang Caddi Island, Indonesia | PS, Nylon | 1.053–4.081 mm | Fiber, fragment | 0.271–1.139 MP/$cm^2$ | August 3th 2019 | MPs number on thalli | MPs were found in the blades, with the dominant form of microfiber | Datu et al. (2019) |
| *Cymodocea rotundata* | Pramuka Island, Indonesia | Not mention | Not mention | Fiber, film, fragment | 185 items/$cm^{-2}$ | April 14th 2018 | MPs densities on thalli | MPs adhered to the surface of thalli and could enter the marine food chain through a marine biota | Priscilla et al. (2019) |
| *Zostera noltei*, *Cymodocea nodosa*, *Zostera marina* | Ria Formosa lagoon, Portugal | Not mention | 0.162–3.396 mm | Fiber | 0.002–0.056 items/$cm^2$ | Between November 2018 and June 2019 | MPs abundance in thalli | Trapping effect of coastal vegetated areas may be highly variable and depend on the plastic size, habitat and tidal position | Cozzolino et al. (2020) |
| *Zostera marina* | Deerness Sound, Scotland | PE, PP, PA, PEU, PET, PS, PTM | 0.04– 3.95 mm | Fiber, flake, fragment | 1.53–8.43/blade | June 13th 2018 | MPs number on thalli | MPs adhered to seagrass blades and could enter food chain | Jones et al. (2020) |
| | | Not mention | | | | | MPs densities on thalli | | Seng et al. (2020) |

(*continued on next page*)

**Table 3** (*continued*)

| Species | Location | Polymer | Size | Shape | Abundance[a] | Duration /time | Parameter | Effect/conclusion | Reference |
|---|---|---|---|---|---|---|---|---|---|
| *Cymodocea rotundata, Cymodocea serrulata, Thalassia hemprichii, Padina* sp., *Sargassum ilicifolium,* | Coastal water around Singapore | | 151–7807 µm for fiber | Fiber and fragment | 0.007–0.060 items/ $cm^2$ | September 2018 | | Higher microplastic densities on seagrasses than on macroalgae | |
| Fresh macrophytes | | | | | | | | | |
| *Lemna minor* | Laboratory | PE | 10–45 µm | Microsphere | 50,000 items /mL | 168 h | MPs adhesion, growth, photosynthetic efficiency | MPs could strongly adsorb to surfaces of the blades but photosynthetic efficiency or growth was not affected by MPs | Mateos-Cárdenas et al. (2019) |

[a] It refers to MPs abundance in media for laboratory work and in thalli for field work. PET, Polyethylene terephthalate; PE, polyethylene; PP, polypropylene; PE-PP, poly (ethylene-propylene); PE-PP-D, poly (ethylene-propylene-diene); CP, cellophane; PAN, polyacrylonitrile; PA, polyamide; PVC, polyvinylchloride; PS, polystyrene; PS-PAE, poly (styrene-acrylate ester); PMMA, poly (methyl methacrylate); PEU, polyether urethane; PTM, poly (trimellitic).

sinking. In addition, MNPs on/ in algae can be transferred to higher trophic levels since consumers cannot distinguish between algae with MNPs from those without MNPs (Gutow et al., 2016).

On the hand other, algae can also affect the transport and destination of MNPs. Phytoplankton can affect the sinking of MNPs when they adsorb or ingest (as with dinoflagellates) them. MNPs trapped in drifting macroalgae can be transported with thalli (Feng et al., 2020a). MNPs are kept in the surface waters when macroalgae are living and settle to the sediment or are released into the seawater when the macroalgae sink and decompose. Meanwhile, some heavy MNPs in the deeper layers of the water column or sea floor in the intertidal zone may be transported upward when normally attached macroalgae become detached and drifting due to tidal action and/or biotic disturbances. Therefore, the drifting macroalgae can affect the distribution of plastics both spatially and temporally via their trap and release (Feng et al., 2020a). Seagrasses can usually transport MNPs from seawater to the seabed as the MNPs become attached and then enter sediments as the seagrasses decay.

## 4. Conclusions and future research needs

### 4.1. Conclusions

Although there are fewer studies compared to aquatic animals, the increasing concern about the impacts of MNPs on primary producers is reflected in the greater number of studies seen since the pioneering work of Bhattacharya et al. (2010). For microalgae, almost all studies investigated the effects of MNPs on growth. Although positive effects were found in several studies, most studies showed a negative effect of MNPs. MNPs usually have an $EC_{50}$ above 25 mg/L for microalgae, while positively charged MNPs have a much lower $EC_{50}$, below 1 mg/L, suggesting a larger toxic effect. The negative effect increased with size ratio of cells to MNPs, indicating that larger size ratios can lead to larger negative effects.

$F_v/F_m$ of both marine and freshwater microalgae was reduced by MNPs. The decline was amplified with increasing MNP concentration but diminished with exposure time, suggesting an acclimation of algal photosynthetic physiology to MNP stress. MNPs could induce relatively higher SOD and MDA levels in marine algae than in freshwater algae. MNPs also reduced pigment content in all the microalgae investigated, with a larger effect on marine microalgae. ROS levels increased with MNP concentration and exposure time while SOD levels initially increased and then decreased with MNP concentration. Taken together, MNPs did not affect the growth of microalgae until reaching a very high concentration. Although MNPs can adhere to the surface of cells and induce ROS, microalgae could deal with MNP induced stress through morphological changes and activating antioxidant systems. When microalgae are exposed to very high MNP concentrations, these could damage the antioxidant system and cell structure and thus inhibit the growth of the algae.

Macrophytes appear to have a very high tolerance to MNPs although more studies are needed to confirm this. They can trap MNPs via multiple mechanisms, such as twining, attachment, embedment, and wrapping. These MNPs can them be transferred to the seabed and higher trophic levels.

### 4.2. Research needs

(1) Experimental design and data presentation

As mentioned above, most studies on MNPs ecotoxicology used commercial and spherical PS with much higher concentrations than those in real environments. Therefore, our understanding on the toxicity of "environmental" MPs is still very scarce. Shape, polymer type and aging may affect ecotoxicological effects of MNPs on primary producers. Accordingly, environmentally real MNPs are needed to be used in future studies. Those MNPs could be collected from the field or made manually. Furthermore, field experiments are highly encouraged to assess the impacts of MNPs on primary producers. In terms of data presentation, the mass unit, i.e. mg/L, is commonly used for ecotoxicological experiments while the number unit, i.e. particles /$m^3$ is usually presented for environmental MNPs investigations. We recommend that both units should be reported in future studies to facilitate the comparison among studies.

(2) Quantifying nanoplastics

Based on the previous studies, the effect of environmentally realistic MPs on algae is very limited and the published $EC_{50}$ values for algae are higher than the environmentally realistic concentrations (Table 2). On the other hand, NPs show a more significant effect on algae than MPs (Bergami et al., 2017; Feng et al., 2020c). It is easier for NPs to be adsorbed on the surface of microalgae, which may reduce the flow of substances and energy between microalgae cells and the environment. However, the studies of NPs on algae are very scarce and the main reason is that quantification of NPs in aquatic environments is still very difficult and thus the environmentally realistic NPs concentrations remain unknown yet. To better understand the impacts of NPs on algae, it is urgent to develop reliable techniques to quantify NPs in aquatic environments.

(3) The mechanisms of impact of MNPs

High levels of MNPs could affect growth, photosynthesis and other metabolic activities of algae. However, the potential mechanisms are still unclear. Some studies presume that these negative effects were caused by the physical adsorption of plastics and the following reduction in available light and nutrient conditions input (Bhattacharya et al., 2010). On the other hand, Zhang et al. (2017) verified that shading and light limitation was not a reason for the toxicity of microplastics to *Skeletonema costatum*. In addition, some studies presume that the toxicity may be related to the leaching of additives from MNPs (Luo et al., 2019; Song et al., 2020). However, there is no solid evidence to justify which effect is dominant. For NPs, it has been reported that both negatively and positively charged NPs ($<$ 200 nm) can enter cells of the terrestrial plant *Arabidopsis thaliana*. Furthermore, Li et al. (2020) found that submicrometre- (0.2 μm) and micrometer-sized (2 μm) polystyrene and polymethylmethacrylate particles could penetrate the stele of the vascular plants *Triticum aestivum* and *Lactuca sativa* using the crack-entry mode at sites of lateral root emergence. However, the internalization of NPs and MPs in algae has not been documented. If NPs can enter algal cells, they would result in more significant effects.

(4) Combined effects with other factors (e.g., heavy metals and organic pollutants)

The aquatic environment is very complex and many variables commonly interact with each other in their effects on organisms. For instance, Garrido et al. (2019) found that the toxicity of the pesticide chlorpyrifos (CPF) on *Isochrysis galbana* was reduced in the presence of MP because CPF could be adsorbed onto MP surfaces leading to lower bio-availability to the algal cells. Liu et el. (2020) also demonstrated that the addition of humic acid (HA) significantly alleviated the toxicity of smaller size (0.1 μm) MNPs on *Scenedesmus obliquus* because HA could form a corona on the surface of MNPs, lessening their adhesion to the microalgae and thus the adverse effect. Furthermore, no interaction between MP and copper was found in *Tetraselmis chuii* (Davarpanah and Guilhermino, 2015). Additionally, the presence of PS enhanced the toxicity of triphenyltin chloride (TPTCl) on *Chlorella pyrenoidosa* although PS reduced the bioavailability of TPTCI. This might be due to increased uptake of TPTCl by this green alga after damage to cellular structures caused by PS (Yi et al., 2019). Based on the studies above, the interaction between MNPs and other variables are complicated and further studies are needed.

(5) Impacts on DOC release, cell sinking and the carbon cycle

Algae convert inorganic carbon ($CO_2$) to organic carbon via photosynthesis. Fixed organic carbon is partially used for growth and metabolism and partially excreted, actively or passively, outside the cells. The dissolved organic carbon (DOC) excreted by phytoplankton can account for 2–50% of fixed carbon by photosynthesis, with the mean for field collected values being in the range of 10–20% (Maranón et al., 2004, 2005; Thornton, 2014). Most studies about MNPs focus on the growth and POC production and little is known about the effects of MNPs on excreted DOC although algal adsorption of MNPs may affect their excretion of DOC. Therefore, our understanding on MNPs on the carbon cycle is limited and fragmentary. It has been reported that MNPs can lead to homo-aggregations of algae or hetero-aggregations between algae and MNPs. However, little is known about how these aggregates would affect the sinking of algae and MNPs, which is vital for the mineralization of organic carbon. In addition, all such studies on microalgae were conducted in the laboratory at species level. Field or outdoors work using natural phytoplankton communities should be carried out in the future to better understand the impacts of MNPs on the global carbon cycle.

(6) Trophic transfer of micro- and nanoplastics

Trophic transfer of MNPs in aquatic food chains attracts extensive attention and some studies have been conducted using aquatic animals (Carbery et al., 2018). Although it has been showed that MPs can be transferred from macrophytes to animals (Gutow et al., 2016; Jones et al., 2020), the bioaccumulation and magnification effects of MNPs and associated contaminants remain unclear. Furthermore, studies on the transfer of MNPs and associated contaminants (e.g., persistent organic pollutants, heavy metals, plastic additives and harmful bacteria and virus) from aquatic primary producers to human beings and the resulting implications for human health are strongly required, as MNPs on aquatic primary producers could be transferred to human beings not only through long food chains but also directly via food consumption.

(7) Using algae to treat MNPs

MNP pollution is becoming a global concern, but little is known on how to deal with the MNPs that already exist in aquatic environments. Based on previous studies, the $EC_{50}$ and even the lowest observed adverse effects concentration (LOEC) for algae is far above the current environmental MNP concentrations (Table 2), indicating that algae have a high tolerance to MNPs. In addition, MNPs, particularly those with positive charge, have a strong affinity to algae. Therefore, algae may possibly be used to treat MNP-polluted water, as a bioremediation approach. This is particularly feasible for macrophytes because they are easy to collect and can trap all kinds of MNPs (Feng et al., 2020a). Furthermore, collecting floating macroalgae can be a way to reduce MNP abundance in seawater as well as hinder macroalgal blooms. Although the collected seaweeds may not qualify for use as food or animal feeds, they can be used as biofuel materials (Gao et al., 2018d).

## CRediT authorship contribution statement

**Guang Gao**: Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - reviewing & editing, Supervision, Funding acquisition. **Xin Zhao**: Methodology, Visualization, Writing - reviewing & editing. **Peng Jin**: Methodology, Formal analysis, Visualization, Writing - reviewing & editing. **Kunshan Gao**: Writing - reviewing & editing, Funding acquisition. **John Beardall**: Writing - original draft, Writing - reviewing & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2020.124685.

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