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Environmentally relevant concentrations and sizes of microplastic do not impede marine diatom growth

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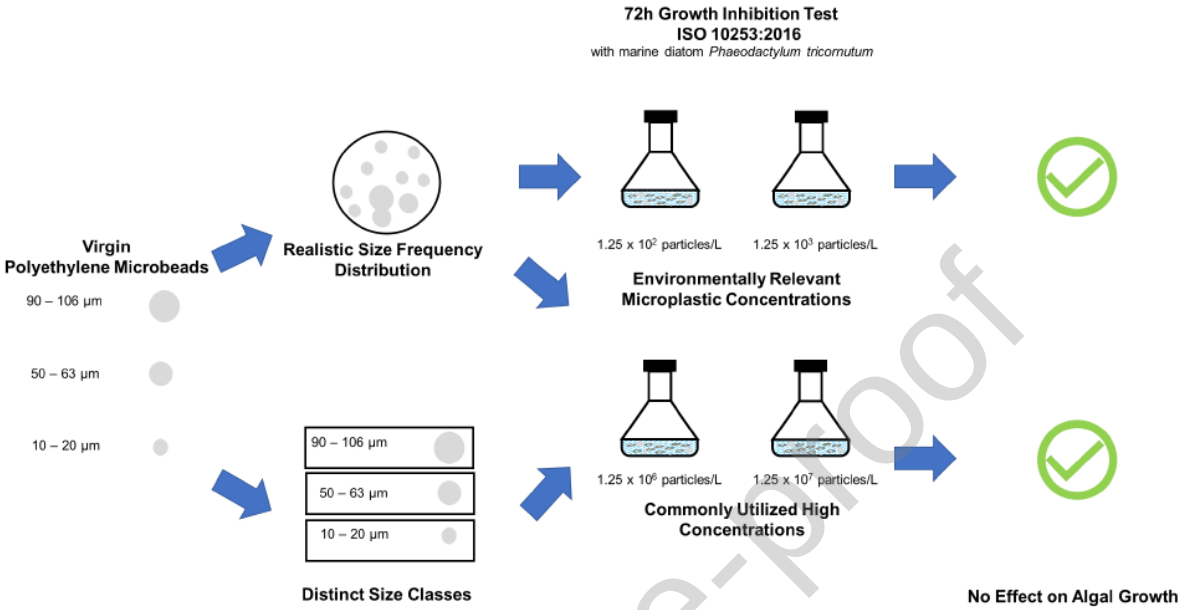
Abstract

The current knowledge about the ecological effects of microplastic (MP) remains limited, and to-date ecotoxicity tests often utilize standard microplastic with one or two distinct size classes and expose the organisms to unrealistically high MP concentrations. We exposed the marine diatom *Phaeodactylum tricornutum* to microplastic particles of a mimicked realistic size frequency distribution complemented with serial experiments with distinct size classes. To do so, we exposed this diatom to a concentration series of different sized polyethylene (PE) microbeads (sizes: 10 – 106 µm; 1.25 x 10^2 - 1.25 x 10^7 particles / L) in a 72-hour growth inhibition test. No effect on the growth of *P. tricornutum* by virgin PE microbeads up to 1.25 x 10^7 particles / L (or 499 mg / L), indicating environmentally relevant concentrations and sizes of MP does not alter the growth of marine diatoms. Results of smaller sized MPs (10 - 20 µm) did not differ from those obtained with larger MPs (90. – 106 µm) and mix sized MPs (10-106 µm), i.e. no impact on the microalgae growth. As a pioneer work, our results contribute with high quality dose-response data to an improved risk assessment of microplastic under realistic present and future marine MP pollution.

Graphical abstract



Keywords:

Microplastic, growth inhibition, marine diatom, environmentally relevant, size-effect

1. Introduction

Microplastic (MP), commonly known as plastic debris smaller than 5 mm in diameter (Arthur et al., 2009; Thompson et al., 2004), are widely distributed in the global ocean (Eriksen et al., 2014; Van Sebille et al., 2015). Given their wide distribution in the marine environment and their high availability to marine biota, concerns have been raised about their adverse effects for marine organisms (Galloway et al., 2017). In particular, the growth of marine diatoms, key organisms at the base of marine food webs (Harris, 2012; Kaiser et al., 2011), may be impacted by MP contamination (Casado et al., 2013). In recent years, a number of studies have focused on the effect of MP on the growth of marine microalgae (Table S1). For example, significant growth inhibition of the diatom *Skeletonema costatum* has been observed when exposed to 6.82×10^{10} particles / L polyvinyl chloride (PVC) particles (Zhang et al., 2017). At a lower MP dose, the effects on the growth of microalgae vary, and most studies showed no effect (e.g. Davarpanah and Guilhermino, 2015; Gambardella et al., 2019). Studies that showed inhibitory-effects are for example those of Fu et al. (2019) and Zhu et al. (2019), while growth stimulation after microplastic exposure has been reported by Guo et al. (2020). Each of these studies increase our knowledge into the possible effects of microplastic on primary producers, which is currently limited compared to other trophic levels.

However, MP exposures tested in most studies using diatoms (and other aquatic organisms) do not represent environmentally relevant concentrations nor conditions (e.g. using a single size range instead of a mixture of ranges). The current mismatch between (high) MP concentrations at which species are being exposed in ecotoxicity work versus the ambient (low) MP concentrations is a crucial element to be solved, and is key to perform better risk assessment and management in future (Everaert et al., 2020). To date, field studies have reported background waterborne MP concentrations ranging from 10^{-3} to 1 particles / L for open and coastal waters (e.g. Lorenz et al., 2019; Song et al., 2018). Even one of the highest

reported waterborne MP concentrations at global scale (i.e. 88 particles / L in Jinhae Bay, Song et al., 2015) is two to four orders of magnitude beneath most of the commonly applied test concentrations. Furthermore, smaller sized plastic is considered to be more harmful than larger particles, but so far, our knowledge remains limited (Liu et al., 2016; "Nanoplastic should be better understood," 2019; SAPEA, 2019) In most studies, microalgae have been exposed to standard microplastic with one distinct size class instead of diverse size MPs. A few studies have compared the growth of microalgae exposed to different sized MP (e.g. Zhang et al., 2017; Zhu et al., 2019), however, the MP particle dose of each size class MP in varies, leading to a different range of response-patterns. For example, Zhang et al. (2017) reported that 1 µm polyvinyl chloride (PVC) particles altered the growth of *Skeletonema costatum* while 1,000 µm PVC particles did not. Even though the authors used the same MP mass concentration (50 mg / L), the MP particle dose (particles / L) of 1 µm PVC MP was nine orders of magnitude higher, indicating that the number of particles may be play a role in growth inhibition effects.

In this study, we assessed (1) the effect of MP on a marine diatom exposed to environmentally relevant MP concentrations and sizes, using polyethylene (PE) microbeads; and (2) investigated whether effects were size-dependent. To do so, we exposed *Phaeodactylum tricornutum* (Bohlin, 1897) to a range of MP concentrations, from environmentally relevant to high concentrations, following the standardised ISO 10253 protocol (ISO, 2016). We performed an ecotoxicity study by exposing the marine diatom to a mimicked mixture of particle sizes using a realistic MP size frequency distribution (Kooi and Koelmans, 2019). To identify potential effects of single size classes, we further exposed *P. tricornutum* to three well defined size classes of microbeads.

2. Materials and Methods

2.1. Preparation of MP suspension

Ultra-violet (UV) fluorescent polyethylene (PE) microbeads (clear in daylight; Yellow-Green in UV Light), with density of 0.97-0.98 g / mL and three nominal particle sizes (10 - 20 µm; 50-63 µm; 90 - 106 µm; Cospheric, USA). The PE microbeads of these three size classes were pre-weighted and added in 0.2 µm filtered artificial seawater (salinity ≈ 33 PSU, prepared according to protocol ISO 10253 (ISO, 2016)) To do so, we made use of a MP stock suspensions of 1.25 x 10^9 particles / L. Particles were dispersed in the test vials by adding 0.1 % Tween80 (Cospheric, USA) followed by five minutes vertexing, prior to exposure. Further dilution series were prepared in 0.2 µm pre-filtered artificial seawater (1.25 x 10^4 particles / L - 1.25 x 10^8 particles / L). To prepare the size-mixture of PE microbeads, we mimicked a realistic MP size frequency distribution (10 - 106 µm), with the three used distinct size classes, following the equation derived in Kooi and Koelmans (2019) with a power exponent (α) of 1.6 ± 0.5. According to this exponential size frequency distribution, 83% of the number of particles was between 10 µm and 20 µm, 11 % was between 50 µm and 63 µm, and 6 % was between 90 µm and 106 µm (details of the calculation are in SI, final concentrations in section 2.3 below). The size-mixture exposures were made by mixing according to the above-mentioned percentages. As a complement to the effects of the MP mixture, we also performed ecotoxicity experiments with the distinct size classes (see further).

2.2. Test species

The microalgae *Phaeodactylum tricornutum* strain 1052/1A obtained from the Culture Collection of Algae and Protozoa (Oban, United Kingdom) was cultured in the Marine Station Ostend of the Flanders Marine Institute (VLIZ, Ostend, Belgium). According to protocol ISO 10253 (ISO, 2016), the microalgae growth medium was prepared in 0.2 µm filtered artificial seawater. The diatoms, *P. tricornutum*, were cultured in 250 mL Erlenmeyer

flasks (volume of culture = 100 mL) at 18 ± 2 °C under a continuous white light (> 2,500 lux), and manually shaken twice a day. Glassware was pre-cleaned and sterilized in an oven at 170 °C for over two hours.

2.3. Growth inhibition test

In the study design (Table 1), we used a realistic size-mixture of PE microbeads (10 - 106 µm) at two environmentally relevant concentrations (1.25 x 10^2 - 1.25 x 10^3 particles / L) and two commonly used high concentrations (1.25 x 10^6 - 1.25 x 10^7 particles / L). This experiment was complemented with treatments with three distinct size classes of PE microbeads (10 - 20 µm; 50 - 63 µm; 90 - 106 µm) at two commonly-used high concentrations (1.25 x 10^6 - 1.25 x 10^7 particles / L). We prepared extra control treatments for fluorescence dye leachates and Tween solution by pre-incubating and filtrating MP suspension of 90 - 106 µm at 1.25 x 10^7 particles / L (i.e. 5975 mg / L, the highest MP mass concentration; details of preparation are demonstrated in Paragraph. S1), to discriminate potential effects induced by Tween80 and fluorescence dyes from PE microbeads.

The 72 h microalgae growth inhibition test was performed according to ISO 10253 (ISO, 2016) with attention to avoid MP contamination. All glassware was pre-soaked with 0.15 g / L Sodium dodecyl sulphate (SDS, VWR) in Milli-Q water (Millipore Corporation) for two hours followed by rinsing with Milli-Q water. Next, the glassware was sterilised in an oven at 170 °C for another two hours. Four days prior to the test, a pre-culture was incubated at 20 °C with a light regime of 10,000 lux to obtain exponentially growing *P. tricornutum*. Treatments (three replicates each) and controls (six replicates) were prepared with an initial algal cell density of ~3 x 10^4 cells / mL. The MP suspensions of each treatment were made by adding 1 mL of prepared stock solutions (see section 2.1) to 100 mL microalgae growth medium, to obtain final treatment concentrations. To validate the final size distribution and concentration of PE microbeads (Table 2), triplicated MP-suspension aliquots (1 mL) were transferred to a

Sedgewick Rafter counting chamber, photographed using fluorescence microscopy (DM1000 connected with a separate beam path, Leica) and analysed using the ImageJ software. All flasks were incubated in the above conditions for 72 h and their positions were randomly switched every day. The cell density in all flasks were quantified every 24 h ± 2 h for the duration of incubation by a haemocytometer counting chamber under a light microscope (DM1000, Leica) with 10x objective lens.

2.4. Data analysis

According to ISO 10253 (ISO, 2016), the average specific growth rate μ (d^{-1}) for each flask was calculated as:

$$\mu = \frac{\ln N_e - \ln N_0}{t_e - t_0} \#[Eq.1]$$

where N_e and N_0 were the cell density (cells / mL) at time t_e (time of test termination) and t_0 (time of test start). The specific growth rate μ (d^{-1}) inferred was expressed as mean ± standard error. Analysis of variance (ANOVA) was performed to check if the specific growth rate was dependent on treatments (e.g. MP concentration, MP particle sizes, and dye and Tween control, CI 95%). Homogeneity of variances and normality were tested a priori and when ANOVA assumptions were not met, data was exponentially transformed. The Akaike information criterion (AIC) of each model was calculated to select the best-fit model. Then, Tukey's honestly significant difference (Tukey's HSD) test was applied to compare the variance in specific growth rate among groups. All statistics were performed in R v3.6.1 (R Core Team, 2019).

3. Results

3.1. Particle characterisation

Instead of using nominal concentrations, we quantified the actual MP exposure concentrations and sizes in our treatments. For PE microbeads of distinct size classes (i.e. 10

- 20 µm, 50 - 63 µm, and 90 - 106µm; quantified at 1.25 x 10^6 particles / L), the measured sizes were homogenous, meaning that the mean particles sizes were close to the nominal size range (Table 2). The standard deviation ranged from 11% for the biggest size class (i.e. 90 - 106 µm) to a maximum of 28% for the smallest size class (i.e. 10 - 20 µm). The actual measured concentrations in all size classes were lower than nominal concentrations (Table 2). The mean measured concentration for the smallest size class (i.e. 10 - 20 µm) was two times lower than the nominal concentration, while the mean measured concentration for the biggest size class (i.e. 90 - 106 µm) was five times lower. For PE microbeads of environmentally realistic sizes and concentrations (i.e.10 - 106 µm, 1.25 x 10^2 to 1.25 x 10^3 particles / L), the average figures detected in two nominal concentrations were close to the figure theoretically expected (i.e. maximum deviation of 46%, see Table 2). The size frequency distribution of two nominal concentrations were also quantified (Table S2, Fig. S1) and the measured frequencies of each size class were closed to the nominal values. The actual measured concentrations in each of the treatments have been quantified and all calculations have been performed with the final measured results, but for clarity we will keep referring to the nominal concentrations in the text.

3.2. Ecotoxicity results

No inhibitory effect was observed on the growth of *P. tricornutum* by PE microbeads at none of the MP treatments, nor in the "dye and Tween controls" (Fig. 1, Fig. 2 and Fig. 3). The average specific growth rates (d^{-1}) in 72 h for "control", "dye and Tween control", and MP exposure are 1.03 ± 0.07 d^{-1}, 1.05 ± 0.02 d^{-1}, and 1.01 ± 0.06 d^{-1}, respectively. Within the sizes tested and up to 1.25 x 10^7 particles / L (or 499 mg / L), the specific growth rate (d^{-1}) was independent from MP concentrations ($p = 0.99$). No deviation between environmentally relevant concentrations and high concentrations were found ($p = 0.88$, Fig. 1). Within the PE microbeads of various sizes (including three distinctive size classes and realistic sizes),

neither the particles sizes, nor MP concentrations had an effect on the specific growth rate ($p = 0.14$, Fig. 2) or differed from "control" (adjusted $p > 0.50$). The specific growth rate in "dye and Tween control" (1.05 ± 0.02 d^{-1}) did not differ from the "control" treatment (1.03 ± 0.07 d^{-1}, $p = 0.84$) nor from the treatment of the highest MP mass concentration (90 -106 µm, 1.25×10^7 particles / L or 499 mg / L, $p = 0.35$), indicating no adverse effects on the algae growth induced by addition of Tween80 and/or potential leachates from fluorescent dyes.

4. Discussion

In the present research, virgin polyethylene (PE) microbeads at concentrations up to 1.25×10^3 particles / L (or 0.05 mg / L) did not alter the growth of *Phaeodactylum tricornutum* (Fig.1), suggesting that virgin PE microplastic (MP) have no acute effect on the growth of diatoms at environmentally relevant MP concentrations and size distributions. These results are in line with Gambardella et al. (2019) and Guo et al. (2020) who found no inhibitory effect on the growth of *P. tricornutum* exposed to 11 - 13 µm and 20 - 25 µm PE MP, and 250 µm polyvinyl chloride (PVC) MP at MP concentrations similar to the present work (up to 7.59×10^3 particles / L). Also, Zhang et al. (2017) confirmed that exposure to 1,000 µm PVC particles up to 2.73×10^3 particles / L did not modify the growth of *Skeletonema costatum*. The MP exposure concentrations used in the present research are at the higher end of most of the reported ambient concentrations, but are still relevant to some estuaries (e.g. 30.8 particles / L in Winyah Bay, Gray et al., 2018; 88 particles / L in Jinhae Bay, Song et al., 2015) and current and future conditions in the marine environment (i.e. 52.2 particles / L in Mediterranean Sea in the year 2100, Everaert et al., 2020).

The non-toxicity of MP to microalgae growth by environmentally relevant MP concentrations are expected as their size is too large to penetrate the cytomembrane, unlike nano-sized plastic (NP) particles, which could be expected to have a higher potential of inducing adverse effects. For example, according to Besseling et al. (2014) and Sjollema et al. (2016), NP can

alter microalgae growth. In what concerns MP assessments, Zhang et al. (2017) exposed *Skeletonema costatum* to PVC MP at 6.82 x 10^{10} particles / L for 96 h, and suggested that particles may block nutrient uptake via adsorption and aggregation with microalgae, leading to growth inhibition. In this context, more obvious growth suppression might be expected under long-term exposure or higher exposure concentrations. Few studies have, however, exposed microalgae to environmentally relevant concentrations of MP at longer term (168 - 240 h), but still no effect on the growth of microalgae was observed at MP concentrations up to 7.5 x 10^{3} particles / L (e.g. Fu et al., 2019; Guo et al., 2020; Lyakurwa, 2017). In a broader context, MP/NP pollution might not affect the population dynamics of a single marine microalgae, but their effects on the composition of larger communities is not well known. For example, recently Redondo-Hasselerharm et al. (2020) reported a long-term biodiversity effect on the population and community composition of macroinvertebrates by NP and MP in a 15-month freshwater mesocosm experiment. Above all, the current ecotoxicological data of environmentally realistic concentrations and sizes of MP is still quite limited. Future studies should further explore the effect of environmentally realistic concentrations and sizes of MP on microalgae growth in both short-term and long-term exposure, to provide high quality data for risk assessments.

In our study, we mimicked the size frequency distribution of MP (Kooi and Koelmans, 2019) using PE microbeads three size ranges (10-20 μm; 50-63 μm; 90-106 μm). Together with treatments using a mixture of size ranges, we further exposed diatoms to the maximum concentration of particles in separate size classes. We found that MP particle sizes had no effect on the microalgae growth at MP concentration up to 1.25 x 10^{7} particles / L (Fig.2). Within the particle size range of 10 - 106 μm, Gambardella et al. (2019) showed that 11 - 13 μm and 20 - 25 μm PE MP up to 1.90 x 10^{7} particles / L did not alter the growth of *P. tricornutum*, consistent with the results of the present study. We acknowledge that by

selecting the 10 - 106 µm fraction we did not cover the entire MP size range (1 - 1,000 µm), especially the smallest sizes (1 - 10 µm) which are considered as the most abundant in the environment (Lindeque et al., 2020). Zhu et al. (2019) and Zhang et al. (2017) reported that 1 µm PVC MP induced higher growth inhibition of *Skeletonema costatum* than 74 µm and 1,000 µm particles. However, due to the difference in surface to volume ratio the particle concentration of 1 µm MP can be six to nine orders of magnitude above the particle concentration of larger-sized MP under the same mass concentrations. Future studies should further explore the effect of MP particle size on the microalgae growth with full size range of MP under the same MP particle concentrations. Based on our findings we could not confirm our hypothesis that smaller particles (i.e. 10 - 20 µm) are potentially more harmful than larger plastic particles (i.e. 90 - 106 µm).

Future research should focus not only on multiple sizes, but also perform experiments with (naturally) weathered microplastic of different shapes. As Kooi & Koelmans. (2019) suggested, environmentally relevant microplastics is a complex mixture of plastic debris which could be approached as being assemblages of continuous probability distributions for particles with different size, shape, and density. In a recent study, Kühn et al. (2018) proposed a practical way of generating highly environmentally relevant MP for ecotoxicological tests by cryo-milling MP harvested from the environmental. Applied similar methods, Jaikumar et al. (2019) exposed to three *cladocerans* to grinded PE microbeads (proxy of secondary MP) together with virgin seraphic MP, and found less chronic effect by mimicked secondary MP. We applied spherical microbeads in our experiment as this enabled us to quantify the actual exposure concentrations in a time-efficient manner. However, as technology is quickly evolving, future studies are encouraged to utilize (naturally) weathered grinded MP for the further effect assessment of MP on marine microalgae (and other species).

In this study, we quantified the actual measured concentrations which were closed to nominal values at environmentally relevant concentrations (i.e. 1.25 x 10^2 particles / L - 1.25 x 10^3 particles / L) but up to five times lower at high concentrations (i.e. 1.25 x 10^6 particles / L). The latter is likely to be related to particle aggregation (Davarpanah and Guilhermino, 2019; Zhao et al., 2019). Although we have added Tween80 to prevent particle aggregation, particle aggregations were visually observed (Fig. S2) but only at high concentrations (1.25 x 10^6 particles / L -1.25 x 10^7 particles / L). Similar to the present study, Sjollema et al. (2016) found their highest nominal concentration (250 mg / L or 2.11 x 10^9 particles / L) was up to nine times higher than the actual value. We quantified actual MP concentrations, assessed prior to exposure. Even though concentrations can potentially be altered throughout exposures (e.g. 13 - 39% decay in 96 h, Davarpanah and Guilhermino (2019)), concentrations of particles in each treatment would still be in the same order of magnitude of the nominal values. We recommend that studies clearly assess and report the real concentrations of exposure (de Ruijter et al., 2020; Reichelt and Gorokhova, 2020), as more transparent and realistic tests enable for improved data for future risk assessments.

We observed that the maximal amount of Tween80 together with the maximum potential leachates of the fluorescent microbeads used in our study did not induce any growth inhibition in our exposures. Together with MP treatments, we exposed organisms to pre-incubated and filtered MP suspensions of 90 -106 µm at 1.25 x 10^7 particles / L (5975 mg / L, i.e. the highest mass concentration), to identify potential procedure artifacts of dispersant and fluorescent leachates. Auto-fluorescent MP and NP are commonly applied in ecotoxicity studies, but few of them have assessed the possibility of leaching-out of fluoresce dyes. Catarino et al. (2019) and Schür et al. (2019) detected the presence of leachates of fluorescence dyes in the tissues of zebrafish and Daphnia magna respectively, indicating that potential reported effects of fluorescent nano- and microparticles can be confound with the

effects of leaching dyes. Taking into account the hetero-aggregation of microalgae and MP/NP particles (Liu et al., 2020; Zhang et al., 2017), we consider that it is highly necessary that studies include controls for fluorescent dyes and dispersants to distinguish particle and leachate or other effects.

5. Conclusion

We assessed the effect of MP on marine microalgae at environmentally relevant MP concentrations and sizes. A 72 h growth inhibition test was performed with *Phaeodactylum tricornutum* exposed to virgin PE microbeads of three distinct size classes and a realistic size mixture. Instead of only reporting nominal MP concentrations, the actual concentrations and sizes were verified. We revealed that the MP with size from 10 µm to 106 µm had no effect on *P. tricornutum* growth at environmentally relevant MP concentrations (up to 1.25 x 10^3 particles / L or 0.05 mg / L). MP particle size had no effect on their impact to microalgae growth. As a pioneer work, our results contribute to an improved risk assessment of microplastic under realistic present and future marine MP pollution.

Acknowledgement

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Figure Captions

Figure 1. Boxplot of growth rate, with the specific growth rate (d^{-1}) versus the MP concentrations (particles / L) of a realistic size mixture of PE microbeads. The legends represent the concentration groups: at environmentally relevant concentrations (i.e. 1.25 x 10^2 particles / L – 1.25 x 10^3 particles / L) and commonly-applied high concentrations (i.e. 1.25 x 10^6 particles / L – 1.25 x 10^7 particles / L). The specific growth rate (d^{-1}) was calculated by [Eq.1] using cell density (cells / mL). The average specific growth rate of controls in all panels is 1.03 ± 0.07 d^{-1}.

Figure 2. Boxplot of growth rate, with the specific growth rate (d^{-1}) versus the MP concentrations (particles / L) of three distinct size classes together with a realistic size-mixture of PE microbeads at commonly-applied high concentrations (i.e. 1.25 x 10^6 particles / L – 1.25 x 10^7 particles / L). The legends represent the particle size (µm) and the dots represent distribution of each individual flask. The specific growth rate (d^{-1}) was calculated by [Eq.1] using cell density (cells / mL). The average specific growth rate of controls in all panels is 1.03 ± 0.07 d^{-1}.

Figure 3. Boxplot of growth rate, with the specific growth rate (d^{-1}) versus the applied treatments of "control", "dye and Tween control" and MP suspension of the highest mass concentration (i.e. 90 - 106 µm at 1.25 x 10^7 particles / L, or 5975 mg / L, see Table 1). The dots represent the distribution of each individual flask. The specific growth rate (d^{-1}) was calculated by [Eq.1] using cell density (cells / mL). The average specific growth rate of "control" is 1.03 ± 0.07 d^{-1}.

Tables

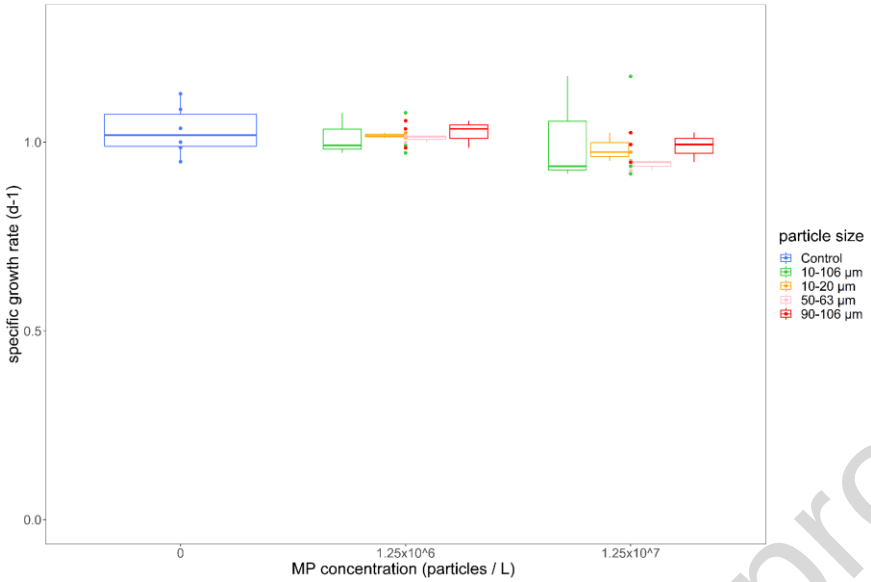
Table 1 Summary of growth inhibition experimental design and terms involved, including treatments and number of replicates in the growth inhibition test. Nominal MP concentrations are expressed both as particle concentration and mass concentration.

Treatment type	Particle Sizes (µm)	Nominal MP concentration (particles / L)	Nominal MP concentration (mg / L)	Replicates
MP exposure	10-20	1.25×10^7	21.426	3
		1.25×10^6	2.143	
	50-63	1.25×10^7	1114.889	
		1.25×10^6	111.489	
	90-106	1.25×10^7	5975.108	
		1.25×10^6	597.511	
	10-106	1.25×10^7	498.928	
		1.25×10^6	49.893	
		1.25×10^3	0.050	
		1.25×10^2	0.005	
Negative control				6
Dye and Tween control				3

Table 2 Nominal and final measured MP concentrations and sizes, based on triplicated quantification of measured concentrations. Measured number of particles and size of particles are expressed as mean ± standard deviation. For further information concerning the calculations on mass-number concentration, and size frequency distribution, please see SI.

Size class	Measured sizes	Nominal MP concentration	Nominal MP concentration	Nominal Number of Particles in 100 mL	Measured Number of Particles in 100 mL	Measured MP concentration
µm	µm	Particles / L	mg / L			Particles / L
10 - 20	23.67 ± 6.65	1.25×10^6	2.143	1.25×10^5	$6.12 \pm 0.95 \times 10^4$	$6.12 \pm 0.95 \times 10^5$
53 - 63	63.48 ± 14.26	1.25×10^6	111.489	1.25×10^5	$2.63 \pm 0.52 \times 10^4$	$2.63 \pm 0.52 \times 10^5$
90 - 106	109.76 ± 11.77	1.25×10^6	597.511	1.25×10^5	$2.14 \pm 0.95 \times 10^4$	$2.14 \pm 0.95 \times 10^5$
10 - 106		1.25×10^3	0.050	125	137 ± 8	$1.37 \pm 0.08 \times 10^3$
		1.25×10^2	0.005	13	19 ± 5	$1.90 \pm 0.50 \times 10^2$

Figure 1



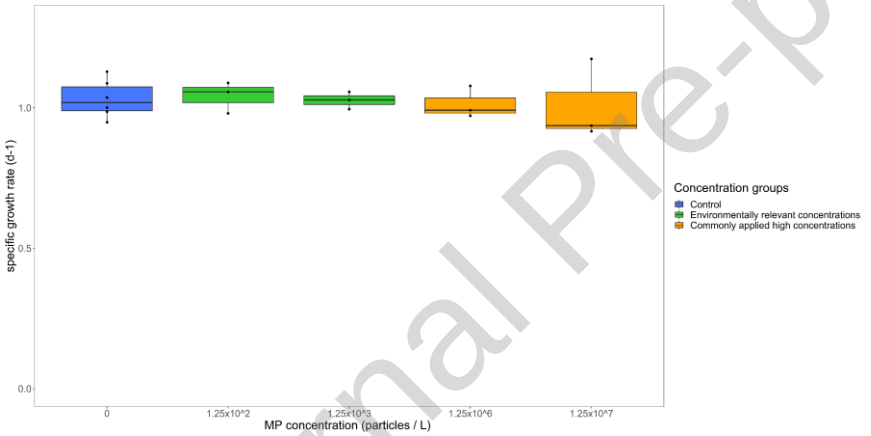


Figure 2

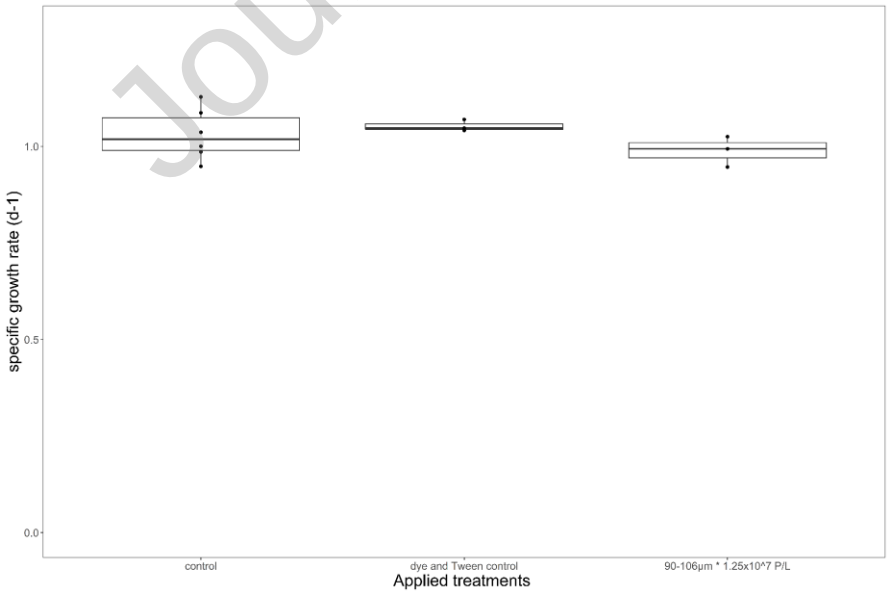


Figure 3

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Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights:

- Effect of MP on a marine diatom's growth at realistic concentrations was examined;
- *P. tricornutum* was exposed to a realistic size mixture of PE granules (10-106 µm);
- No growth inhibition to *P.tricornutum* by PE granules up to 1.25 x 10^7 particles/L;
- Particle size of PE microbeads had no effect on microalgae growth;
- Actual sizes and concentrations of PE MP was verified.