Oecologia (2025) 207:81
https://doi.org/10.1007/s00442-025-05723-2

ORIGINAL RESEARCH

Effects of microplastics and natural particles on the aquatic invertebrate *Daphnia magna* under different dietary quality scenarios

Guang-Jie Zhou[1,2] · **Eeva-Riikka Vehniäinen**[2,3] · **Minna Hiltunen**[2] · **Cyril Rigaud**[2] · **Sami Taipale**[2]

Received: 9 January 2025 / Accepted: 30 April 2025 / Published online: 14 May 2025

Abstract

Natural and synthetic particles co-occur in the aquatic environment. However, little information is available about the effects of natural particles on freshwater animals and how these effects differ from those of synthetic particles, especially under the scenarios of decreasing dietary quality and increasing cyanobacteria in the aquatic environment. Therefore, this study evaluated apical and molecular effects of polypropylene (PP) microplastics (MPs) and three natural non-food particles (i.e., kaolin, peat, and sediment) on the freshwater invertebrate *Daphnia magna* fed either a green alga or a mixture of green alga and cyanobacterium. After the 21-d chronic exposure of 10 mg/L PP when using the green alga *Acutodesmus* sp. as diet, the size of *D. magna* was significantly reduced, and the molting time was significantly extended compared with the control. However, the chronic effects of PP were masked when the cyanobacterium *Pseudanabaena* sp. was added to their diet. The natural particles kaolin, peat, and sediment posed insignificant effects on *D. magna* regardless of dietary quality. The expression of molting-related genes (e.g., *ecr-a*) and oxidative stress-related genes (e.g., *sod2*) was significantly upregulated in *D. magna* with the exposure of both natural and synthetic particles. The predicted no-effect concentration of PP was derived as 0.025 mg/L, raising concerns relating to their toxicity and risks in the contaminated aquatic environment. This study will improve our understanding of the effects and risks of natural and synthetic particles in freshwater environments, as well as facilitate ecoenvironmental authorities to make informed decisions on the appropriate management of MPs.

Keywords Synthetic particles · Natural particles · Food quality · Invertebrate · Aquatic environments

Introduction

Microplastics (MPs; plastic particles smaller than 5 mm) can be found in all habitats worldwide. MPs in aquatic ecosystems are a source of concern since they may be hazardous to aquatic organisms and can be transferred to higher trophic level consumers such as humans via food webs (de Sá et al. 2018; Castro-Castellon et al. 2022). Although their adverse effects have been explored, the research has primarily focused on marine organisms (e.g., Sun et al. 2021; Lai et al. 2022). For example, MPs released from incorrectly disposed surgical masks reduced the fertility of the marine copepod *Tigriopus japonicus* significantly (Sun et al. 2021). Given that the transport of MPs from freshwater to the ocean has been identified as one of the largest contributors of MPs into the oceans (Besseling et al. 2017; Lebreton et al. 2017), the exposure of MPs can also adversely affect aquatic organisms in natural freshwater environments (Aljaibachi et al. 2020; Bhardwaj et al. 2024). Thus, more experiments relating to effects of MPs on freshwater organisms are urgently needed for a deeper understanding of their toxicity and a reliable ecological risk assessment.

Aquatic species have been exposed to particles of natural origin even before plastics were manufactured. Natural particles, such as inorganic minerals (e.g., kaolin clay) and organic polymers derived from living organisms, usually have higher concentrations than MPs in the aquatic environment (Motiei et al. 2021; Doyle et al. 2022). Because many natural particles and MPs have comparable sizes and

Communicated by Ulrich Sommer.

✉ Guang-Jie Zhou
zhougj01@gmail.com; zhougj@jnu.edu.cn

[1] Department of Ecology and Institute of Hydrobiology, Jinan University, Guangzhou 510632, China

[2] Department of Biological and Environmental Science, University of Jyväskylä, 40014 Jyväskylä, Finland

[3] Department of Biology, University of Turku, 20014 Turku, Finland

other qualities such as shapes, aggregation behaviors and surface characteristics (e.g., Doyle et al. 2022), they may also have similar physical impacts. It is also possible that—given their long exposure to particles—aquatic organisms have adapted to their presence in the natural environment over time. Although there are already some studies about the effects of natural particles on freshwater life, these work are mostly focused on natural kaolin particles (Schür et al. 2020; Zimmermann et al. 2020; Motiei et al. 2021). To have a meaningful estimate on the influence of natural and synthetic particles on the performance of aquatic consumers, the effect comparison of MPs and various natural polymers (e.g., kaolin, peat and sediment) is necessary using aquatic invertebrates that directly feed on these particles.

Herbivorous cladocerans, e.g., *Daphnia*, perform an important function in connecting primary producers with higher trophic level consumers and moving energy and essential biomolecules up the food chain in freshwater systems (Kainz et al. 2004; Bownik 2020; Juan-García et al. 2023). They live in a variety of freshwater environments and eat a variety of food sources, such as bacteria, phytoplankton, protozoans, and microscopic particles in the water (Tkaczyk et al. 2021). MPs and natural particles may disrupt the filtering process of these filter-feeding *Daphnia*, thus affecting their growth and the flow of energy in the ecosystem. Existing toxicity research on *Daphnia* has usually focused on short-term acute toxicity effects at high MP doses (Kokalj et al. 2022; Samadi et al. 2022), and less information is available about their chronic toxicity effects (Hiltunen et al. 2021; Samadi et al. 2022). Besides, the nutritional quality of the diet determines the growth and reproduction of *Daphnia* (Peltomaa et al. 2017; Laine et al. 2024), and eutrophication and browning caused by anthropogenic activity and climate change are lowering the quality of filter-feeder diets (Taipale et al. 2019a; Strandberg et al. 2023) by favoring cyanobacteria over other phytoplankton species (Paerl and Paul 2012). It has recently been reported that eutrophication-driven decrease in food quality is more important in determining *Daphnia* fitness than the exposure to MPs (Hiltunen et al. 2021). As a result, it is critical to further compare the long-term effects of MPs and various natural polymers on *Daphnia*'s growth and reproduction under the scenario with poorer quality food present—as this is what the *Daphnia* will encounter in nature.

In recent years, pollution of polypropylene (PP) MPs has raised serious concerns because PP is the main component of face masks, other textiles and fabrics, consumer products, construction materials, and other medical supplies (Auta et al. 2018; Jeyavani et al. 2023). After the disposal of face masks into the natural environment, they undergo weathering processes, releasing their additives, and are eventually fragmented into microplastics and nanoplastics (Kokalj et al. 2022). It is important and timely to investigate PP toxicity, to derive its predicted no-effect concentration (PNEC), and assess its ecological risk in the aquatic ecosystem. Therefore, this study aims to (1) compare acute and chronic toxicities of PP and natural particles (e.g., kaolin, peat, and sediment) on *Daphnia magna*; (2) investigate the effects of a lower nutritional quality of diet with cyanobacteria on the potential toxicity of PP and natural polymers; (3) demonstrate the toxicity mechanisms by measuring genes relating to molting, reproduction, oxidative stress and xenobiotic metabolism; and (4) derive the PNEC of PP for ecological risk assessment through integrating the literature and the results originating from this study. The findings of this study will improve our understanding of MP toxicity and risks in freshwater ecosystems, as well as facilitate ecoenvironmental authorities to make informed decisions on the appropriate management of MPs.

Materials and methods

Preparation and characterization of particles

The polypropylene (PP, Cas no. 9003-07-0, purity 99%) was purchased from Nanochemazone (Alberta, Canada), and cosmetics-grade kaolin clay was provided by Limepop (Helsinki, Finland). For peat particles, we used non-fertilized, natural peat for gardening from Kekkilä (Kekkilä BVB, Finland). The sediment was collected from Lake Jyväsjärvi next to the University of Jyväskylä. The peat or collected sediment was dispersed in the ADaM medium, filtered by a 50-µm sieve, and then diluted to a stock concentration of 320 mg/L. PP or kaolin was dispersed in the ADaM medium at 320 mg/L. The stock suspensions of the four particles were incubated for 7 days prior to experimentation and their actual concentrations were measured as dry weight, and they were within 93–104% of nominal concentrations. The concentrations, diameters, and size distributions of the four particle stocks were then analyzed with the CASY cell counter (Cambridge Bioscience, UK) using a 60-µm capillary (for size range 1.4–40 µm). The 320 mg/L of particle stocks ranged from 3.55×10^6 to 7.33×10^6 counts/mL, and their mean diameters ranged from 2.83 to 3.43 µm (Fig. S1).

Acute immobilization test

The freshwater *D. magna* was maintained and acclimated in the ADaM medium at 20 °C in the laboratory of the Department of Biological and Environmental Science, University of Jyväskylä. The animals were fed with the green microalga *Acutodesmus* sp., and the medium was renewed regularly. The 48-h acute immobilization test was performed according to the OECD 202 guideline (OECD 2004). Briefly, five neonates hatched within 24-h were exposed to each of the control and six treatments including 1, 3.2, 10, 32, 100 and 288 mg/L (40 mL in ADaM each) for each of the four particles. There were five replicates for each treatment or control. The immobilization test lasted for 48-h at 20 °C without food provided during the test. Mortality and immobilization were recorded daily, and there was no renewal of test solutions during the exposure.

Chronic reproduction test

The 21-d chronic reproduction test followed the OECD 211 guideline (OECD, 2012). One neonate (<24 h old) was exposed at 20 °C to a 40 mL test solution of each particle type. There was one control without particle exposure (i.e., ADaM medium) and two treatments (i.e., 1 mg/L and 10 mg/L in ADaM medium) for each of the four particles, with 16 replicates for each treatment or control. It has been reported that MP concentrations ranged from 0.67 to 5.60 mg/L in a freshwater environment receiving treated wastewater effluent (Lasee et al., 2017). Thus, the concentrations of MPs used (i.e., 1 mg/L and 10 mg/L) in this study represented the worst-case scenarios with high environmental relevance in freshwater environments. To maintain particle concentrations, test solutions were renewed every 2–3 days and the food was provided by the ration level of 0.1 mg carbon (100% green alga *Acutodesmus* sp., or 25% green alga *Acutodesmus* sp. + 75% cyanobacterium *Pseudanabaena* sp.) per individual per day. The mortality, time to each brood, and reproduction were observed daily throughout the 21 days of the experiment, and *Daphnia* size was measured at the end of the experiment.

Quantification of gene expressions

For the chronic reproduction test, the expression of genes related to *D. magna* molting, reproduction, oxidative stress, and xenobiotic metabolism was also examined at the end of the experiment. The target genes included (1) molting-related genes: ecdysone receptor a (*ecr-a*), ecdysone receptor b (*ecr-b*), ultraspiracle (*usp*), cytochrome P450 314 family (*cyp314*); (2) reproduction-related genes: vitellogenin 1 (*vtg1*), vitellogenin 2 (*vtg2*), vitellogenin-superoxide dismutase (*vtg-sod*), juvenile hormone esterase (*jhe*); (3) oxidative stress-related genes: Cu/Zn-superoxide dismutase (*sod1*), Mn-superoxide dismutase (*sod2*), catalase (*cat*); (4) xenobiotic metabolism-related genes: monooxygenase (*mox*), glutathione-s-transferase (*gst*), p-Glycoprotein (ABCB/md) (*abcb1*) (Table S1). In brief, total RNA was extracted from the pooled *Daphnia* samples (two individuals for each of three replicates for each control or treatment) using TRI reagent (Molecular Research Center, Cincinnati, OH, USA) following the manufacturer's instructions. RNA quantity and purity was measured using a NanoDrop™ device (Thermo Fisher Scientific, Wal- tham, MA, USA). RNA quality was verified using a TapeStation 2200 (Agilent Technologies, Santa Clara, CA, USA). The RNA samples were then treated with DNase (Thermo Fisher Scientific), and an aliquot of 1 µg was reverse transcribed to cDNA (iScript cDNA Synthesis Kit, Bio-Rad, Hercules, CA, USA). The cDNA samples were diluted (1:10) and stored at –20 °C until further analyses.

Gene expression was measured by quantitative real-time PCR (qPCR). Primer sequences and parameters of the target genes are presented in Table S1. Cyclophilin and ubiquitin-conjugating enzymes were both used as reference genes as they showed the highest stability in the conditions of our experiments (data not shown). Each qPCR reaction was done in a final volume of 25 µL: 2 µL of the diluted cDNA, 0.75 µL of each of the forward and reverse primers (300 nM concentration), 9 µL of sterile water and 12.5 µL of iQ SYBR Green Supermix (Bio-Rad). No-template controls with sterile water instead of cDNA were run on each plate for each gene, as well as a positive control for inter-run calibration. The qPCR was run on a CFX96 Real-Time PCR cycler (Bio-Rad): the protocol was 3 min at 95 °C, 40 cycles of 10 s at 95 °C, 10 s at 58 °C and 30 s at 72 °C, followed by 10 s at 95 °C and melt curve from 65 to 95 °C. A single melting temperature peak was observed in the dissociation curves for each target gene. For each sample, the expression of each target gene (efficiency corrected) was calculated using the CFX Maestro™ software (Bio-Rad) according to the methods described by Pfaffl (2001) and Vandesompele et al. (2002).

Derivation of PNEC of PP for ecological risk assessment

Toxicity data of PP to aquatic organisms, including no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC), were extracted from peer-reviewed literature (Table S2) and this study. The taxonomic groups included algae and cyanobacteria, crustaceans, fish, insects, and mollusks. Chronic toxicity endpoints were obtained from their corresponding acute endpoints by applying an acute-to-chronic ratio of 10, and LOEC values could be divided by an assessment factor (AF) of 2.5 to convert to NOECs. Geometric mean was applied when there were multiple data available for the same species (European Commission 2003). The species sensitivity distribution (SSD) analysis was performed on the chronic NOEC data by use of the USEPA SSD generator. The hazardous concentration corresponding to 5% hazardous concentration (HC_5) and its respective corresponding 95% confidence interval (95% CI) derived from the SSD curve was then divided by an AF of 1 (European Commission 2003) to determine the PNEC of PP.

Data analyses

The median effective concentration (EC_{50}) at 48-h for the acute immobilization test was calculated using sigmoidal dose–response (variable slope) non-linear regression under constraints between 0 and 100% effect in the software Prism (version 8.0, Graphpad Inc., USA). Data of size, reproduction, time to each brood, and gene expression were checked for homogeneity of variances by use of Levene's test. Differences in endpoints between control and individual treatments were identified using one-way analysis of variances (ANOVA) followed by Dunnett's test. For datasets that failed the assumption of homogeneity of variances, a non-parametric Kruskal–Wallis test was conducted. All statistical analyses were carried out using IBM SPSS Statistics 23.0 package, and the significant level was set as 0.05.

Results

Acute immobilization test

D. magna was acutely affected by the exposure to PP, with the 48 h-EC_{50} of 199.9 mg/L (95% CI: 64.8–335.4 mg/L), while the natural particles kaolin, peat, and sediment posed an insignificant acute effect (Fig. 1).

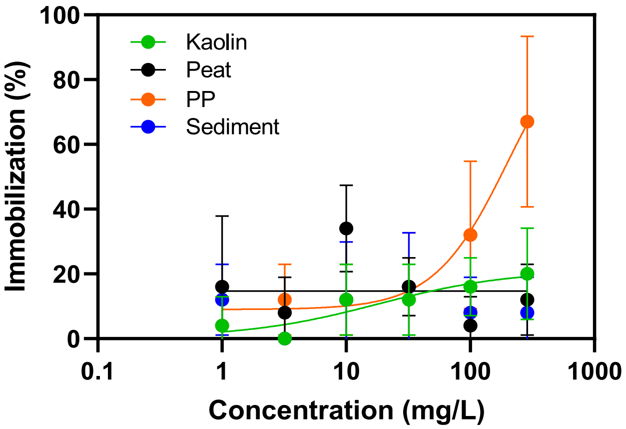


Fig. 1 Acute toxic effects of four particles (i.e., kaolin, peat, PP, and sediment) in terms of concentration–response relationships for the immobilization (mean ± SD, $n = 5$) of the freshwater invertebrate *D. magna*

Chronic reproduction test

When the green alga *Acutodesmus* sp. was used as their diet for the 21-day exposure, the natural particles (i.e., kaolin, peat and sediment) did not significantly affect the size of *D. magna*, while the presence of PP at 10 mg/L significantly decreased their size ($p < 0.001$; Fig. 2A1). Also, a significant correlation was found between the concentration of PP and the size of *D. magna* ($r^2 = 0.6161$, $p < 0.001$; Fig. S2). Apart from 10% of *D. magna* that had three broods at the exposure of peat at 10 mg/L, all the individuals of *D. magna* with the exposure of other particles had 4–5 broods (Fig. 2A2). The time to 4th brood was significantly longer in the treatment of 10 mg/L PP than in the control ($p < 0.01$; Fig. S3 A). There was no significant difference in the number of neonates between the control and two treatments (i.e., 1 mg/L and 10 mg/L) for the four particles (Fig. 2A3), and their neonate numbers ranged from 45 to 109, except for one individual that had only 8 neonates in the 10 mg/L sediment treatment (Fig. 2A4).

When the mixture of 25% green alga *Acutodesmus* sp. + 75% cyanobacterium *Pseudanabaena* sp. was used as their diet, all four particles, including PP, had no significant effect on the size of *D. magna* (Fig. 2B1). Although there was no significant difference in the number of neonates for the four particles (Fig. 2B3), the neonate numbers were reduced compared to those in the treatments with green alga as their diet. 44–92% of individuals fed the mixture of green alga and cyanobacterium had < 45 neonates, which was far lower than the individuals with only the green alga as their diet (Fig. 2B4). Furthermore, 45–93% of individuals only had 0–3 broods in the four particle treatments (Fig. 2B2), which was lower than the individuals with only the green alga as diet (Fig. 2A2).

Gene expressions

When the green alga was used as their diet, the molting-related genes (*ecr-a*, *ecr-b*, *usp*, and *cyp 314*) in *D. magna* exhibited differential expressions after the exposure to the four particles. The expression of *ecr-a* was significantly upregulated in *D. magna* for all treatments except for 1 mg/L of peat when compared with the control without the exposure of particles ($p < 0.05$, $p < 0.01$ or $p < 0.001$; Fig. 3A1). The expression of *ecr-b* and *cyp 314* was only significantly upregulated in the treatment of 1 mg/L kaolin (both $p < 0.05$; Fig. 3A2 and A4), while the expression of *usp* was not significantly different between any of the treatments of four particles and control (all $p > 0.05$; Fig. 3A3). When the mixture of green alga and cyanobacterium was used as diet, the expression of *ecr-a* was only upregulated in the treatments of 10 mg/L kaolin and 10 mg/L sediment (Fig. 3B1), while the expression of *usp* was induced in the

Fig. 2 Chronic effects of four particles (i.e., kaolin, peat, PP and sediment) on (1) size (min–max, $n=7$–10), (2) fractions of each brood, (3) neonate number (min–max, $n=9$–14), and (4) distribution of neonate number of *D. magna* when their diet was **A** 100% green alga *Acutodesmus* sp., or **B** the mixture of 25% green alga *Acutodesmus* sp. + 75% cyanobacterium *Pseudanabaena* sp. $***p < 0.001$

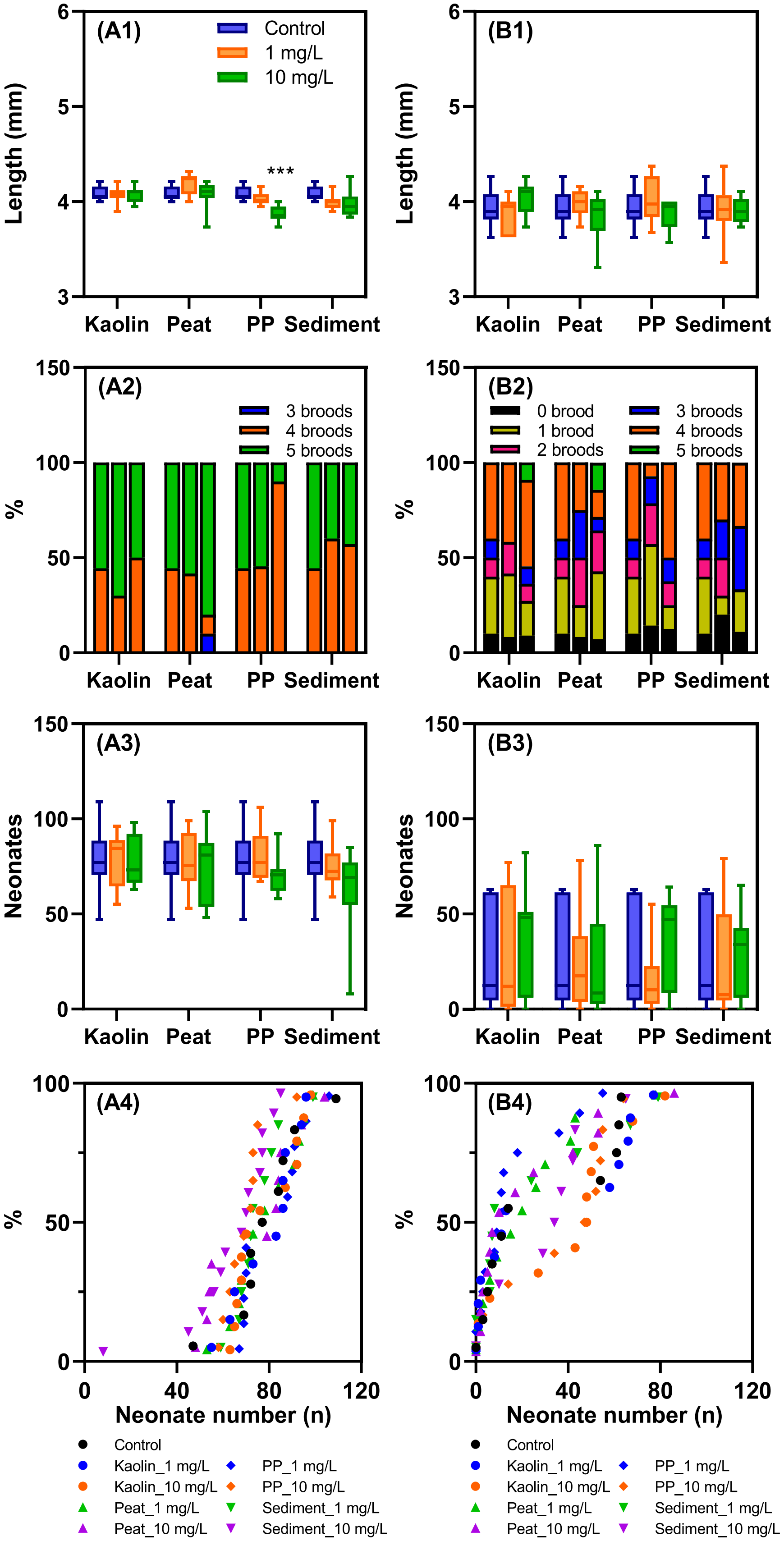
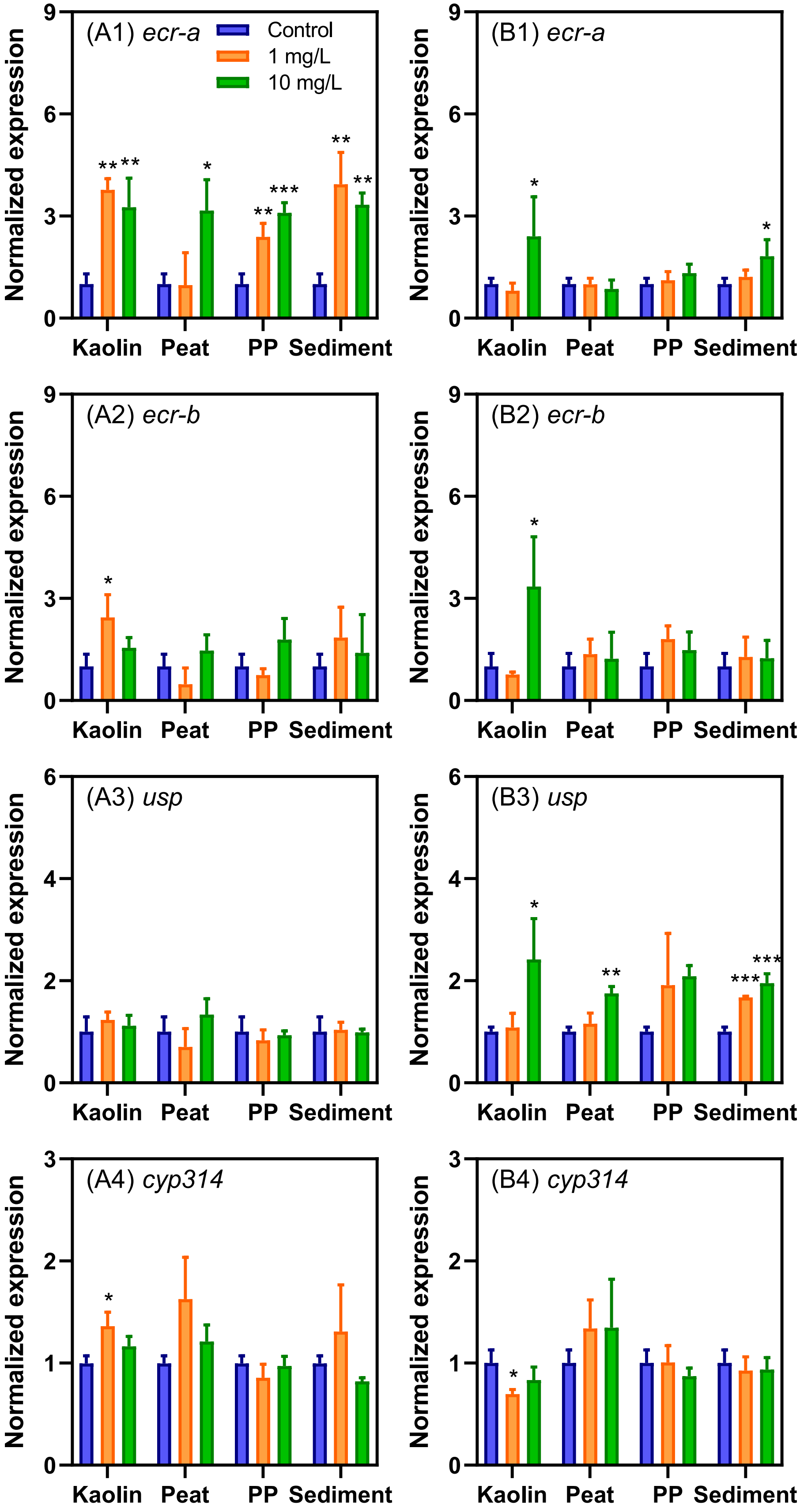


Fig. 3 Normalized expression of mRNA for the molting-related genes of (1) *ecr-a*, (2) *ecr-b*, (3) *usp*, and (4) *cyp314* in *D. magna* after exposure to four particles (i.e., kaolin, peat, PP and sediment) for 21 days when their diet was **A** 100% green alga *Acutodesmus* sp., or **B** the mixture of 25% green alga *Acutodesmus* sp. + 75% cyanobacterium *Pseudanabaena* sp. Cyclophilin and ubiquitin conjugating enzyme were both used as reference genes to normalize the expression. Error bar: + 1 SD ($n = 3$). *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$



treatments of kaolin, peat, and sediment compared with control (Fig. 3B3).

Regardless of particle types and concentrations, the expression of reproduction-related genes (i.e., *vtg1*, *vtg2*, *vtg-sod*, and *jhe*) in *D. magna* seemed random (Fig. S4), indicating that the presence of particles did not affect the production of *D. magna* under the two diet scenarios. When fed by a green alga, the presence of particles could increase the expression of some oxidative stress-related genes (i.e., *sod1*, *sod2*, and *cat*; Fig. S5) and xenobiotic metabolism-related genes (e.g., *abcb1*; Fig. S6) in *D. magna*, while these differences were mostly masked when the mixture of green alga and cyanobacterium was used as diet (Fig. S5 and S6).

Species sensitivity distribution

Based on the estimated chronic NOEC toxicity values of PP to aquatic organisms obtained from the peer-reviewed literature and the present study, a SSD curve was constructed (Fig. 4). The aquatic species used for the SSD analysis included 19 species, which were nine algae and cyanobacteria, five crustaceans, three fish, one insect, and one mollusk (Fig. 4; Table S2). Overall, aquatic animals were relatively sensitive to the PP exposure, while algal and cyanobacterial species were relatively tolerant (Fig. 4). The HC_5 value derived from the SSD was 0.025 mg/L (95% confidence interval: 0.002–0.320 mg/L), which was also considered the PNEC value of PP.

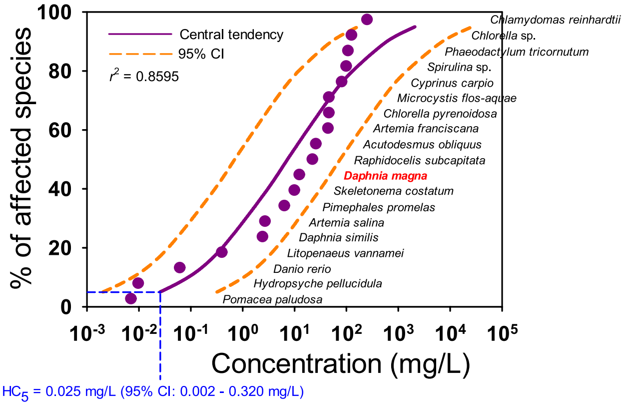


Fig. 4 Species sensitivity distribution (SSD) for polypropylene based on the toxicity data (Table S2). The *Daphnia magna* (red) is in the middle of the SSD curve

Discussion

Acute and chronic toxicity with high dietary quality

This study demonstrates that the exposure of PP with relatively high doses resulted in an acute immobilization effect on the freshwater *D. magna*. At the same time, the chronic effects of PP on their size and molting time were also observed when dietary quality was high (a green alga). These findings are supported by previous studies which found that various MPs including PP could produce adverse effects on aquatic organisms (de Sá et al. 2018; Castro-Castellon et al. 2022). Because *D. magna* is a filter feeder that forages on microscopic organisms and particles (Bownik 2020; Tkaczyk et al. 2021; Samadi et al. 2022), the ingestion of PP could disrupt their filtering process and result in growth disruption, reflected by the decreased size and extended molting time in our study. Such effects were further confirmed by the over-expression of the molting-related genes (e.g., *ecr-a*) and oxidative stress-related genes (e.g., *cat*). The expression of *ecr* gene is significantly suppressed after the exposure to ecdysteroids, including 20-hydroxyecdysone (20E; Hannas et al. 2011), while the exposure of contaminants like emamectin benzoate and PP can significantly upregulate *ecr* expression (Song et al. 2016; this study), which is probably attributable to the competitive binding of these contaminants with the EcR ligand 20E.

Although the molting-related genes (e.g., *ecr-a*) and the oxidative stress-related genes (e.g., *sod2*) were also significantly regulated by the exposure of the three natural particles (i.e., kaolin, peat and sediment), the effects on size and molting time were not significant compared to the control without particle exposure. These results are supported by previous studies relating to less negative effects of natural particles than MPs (Schür et al. 2020; Zimmermann et al. 2020; Doyle et al. 2022). In this study, the higher particle density (as particles per mL; Fig. S1) of PP even though the concentration (as mg/L) of the suspensions were the same, suggests that the three natural non-food particles were more dense than the PP. Thus, PP had a greater propensity for staying in the suspension than the three natural particles, potentially making it more bioavailable and toxic. The potential harmful effects of plastic additives cannot be ruled out either, though the 7-day incubation of particles prior to exposure may have diminished the additive concentrations due to microbial degradation. Our previous study with the toxicity of PE revealed a negative impact on survival and growth of *Daphnia* only when natural microbes were not present, thus emphasizing the role of natural microbes in detoxifying plastics (Taipale et al. 2019b). Altogether, the natural particles in the aquatic environment are unlikely to trigger adverse effects on *D. magna* even though their concentrations far exceed the synthetic particles. Furthermore, in the natural environment, *D. magna* individuals may have adapted to their presence, and thus they may avoid eating these non-food natural particles after recognition (Hartmann and Kunkel 1991).

Chronic effects with decreasing dietary quality

The exposure to natural and synthetic particles did not impact reproduction, reflected by the comparable neonate numbers after exposure to the different particles and concentrations. The availability of essential biomolecules is a crucial factor influencing *Daphnia* growth and reproduction (Peltomaa et al. 2017; Laine et al. 2024). Moreover, food quality has been proven to have a greater impact on *Daphnia* growth and reproduction than a small temperature increase or MPs (Hiltunen et al. 2021). The present study showed a decrease in hatched neonates with the addition of cyanobacterium (i.e., *Pseudanabaena* sp.) in their diet, which is most likely a result of a lack of sterols and eicosapentaenoic acid in the diet (Peltomaa et al. 2017; Hiltunen et al. 2021). This phenomenon would probably be further aggravated by the frequent occurrence of eutrophication and cyanobacterial blooms (Paerl and Paul 2012) when the nutritional value of phytoplankton as a diet for zooplankton decreases (Taipale et al. 2019a, 2019c).

The effects of PP on the size and molting time of *D. magna* were masked with the addition of cyanobacterium in their diet, probably due to the drastic decrease in food quality (Hiltunen et al. 2021). Similarly, it was previously reported that poor elemental food quality reduced the toxicity of the pharmaceutical fluoxetine on *D. magna*, probably due to alteration of physiological responses with different diets (Hansen et al. 2008). Because the PNEC values for chemicals used for ecological risk assessment are mainly derived from standardized toxicity tests without considering food quality, more studies on the relationship of chemical toxicity and food quality would be needed. More specifically, it would be important to estimate different scenarios of primary environmental stresses in the aquatic environment; how eutrophication, climate change, plastic pollution, and cyanotoxins together impact the functioning of aquatic consumers, e.g., herbivorous zooplankton.

The expression of *usp* gene, also called retinoid x receptor gene in vertebrates, was significantly upregulated by the exposure of the three natural particles when the mixture of green alga and cyanobacterium served as diet. It was reported that cyanobacteria could produce retinoic acids (Wu et al. 2012; Zhou et al. 2021), which may induce the *usp* gene over-expression when *D. magna* individuals were subjected to the pressure originated from natural particles in the scenario with cyanobacteria in the diet. Future studies should be directed towards monitoring gene responses in a time course to obtain robust results, considering that gene expressions vary with growth stages (Hannas et al. 2011).

Ecological risk assessment and future studies

It is challenging to assess the ecological risk of MPs in the aquatic environment due to the different units and types of MPs used in laboratory ecotoxicological studies and field studies. Approximately 70% of laboratory ecotoxicological studies reported amounts of MPs as mass per unit volume or area, while 97% of field studies reported MP concentrations as particles per unit volume or area (Ockenden et al. 2021). Besides, although field studies usually detected total MP concentrations without reporting their type and compositions, the laboratory toxicity tests were mainly focused on single MP types (de Sá et al. 2018; Castro-Castellon et al. 2022). In this study, we assess the ecological risks of MPs through comparing their environmental concentrations with the PNEC value (i.e., 0.025 mg/L) of PP that we derived. We acknowledge, that we are not accounting for the fact that different types of MPs may not exhibit similar behavior and toxicities. Nevertheless, considering the levels of MPs in the aquatic environment are mostly at the ng/L level (e.g., Li et al. 2018), it is unlikely for MPs to trigger toxicity effects on aquatic organisms or give rise to ecological risks to aquatic ecosystems. However, the mg/L level of MPs has also been detected in the aquatic environment receiving high loads of treated wastewater effluent (Lasee et al. 2017), raising concerns about their toxicity and risk to aquatic organisms living in the contaminated environment, at least as worst-case scenarios.

It is worth noting that previous studies derived the PNEC for MPs as 0.07 μg/L in the freshwater environment (Adam et al. 2019) and 0.5 μg/L in the marine environment (Adam et al. 2021), which is far lower than the one (i.e., 0.025 mg/L) derived in the present study. Such difference should be mainly due to the use of toxicity data points of several MP types for the derivation of PNEC in Adam et al. (2019, 2021) but only the use of PP toxicity data points (mostly 2019 or later after the outbreak of COVID-19 pandemic) in this study. Other possible reasons include different endpoints and assessment factors used during the data conversion from other endpoints to NOEC for the SSD construction and the PNEC derivation.

After the outbreak of COVID-19 pandemic, the face masks have widely been applied for self-protection worldwide (Sun et al. 2021; Kokalj et al. 2022). PP is one of the main components of face masks and also other PP materials (e.g., textiles and fabrics, consumer products, construction materials, and other medical supplies; Auta et al. 2018; Jeyavani et al. 2023) containing organic additives like dyes, plasticizers, and flame retardants. The release of such additives and the sorption of metals and organic contaminants by masks and MPs probably led to the alteration of their behavior and bioavailability to aquatic organisms and further affected their toxicity and risks in the aquatic environment (de Sá et al. 2018; Castro-Castellon et al. 2022; Kokalj et al. 2022). Further studies are warranted to investigate the interactive toxic effects of MPs and other contaminants on aquatic organisms at different trophic levels in the aquatic environment.

Conclusions

This study demonstrated that *D. magna* was acutely affected by the PP exposure, while natural particles (i.e., kaolin, peat, and sediment) did not trigger acute toxicity to *D. magna*. Although all four natural and synthetic particles upregulated the expression of molting-related genes and oxidative stress-related genes during a 21-day chronic exposure experiment, only the PP exposure significantly decreased the size of *D. magna*, and extended their molting time when using a green algal species as a diet. However, when adding a cyanobacterial species in their diet, these effects originating from PP were masked, probably due to the lowered nutritional food quality from cyanobacterial cells. The PNEC value of PP was derived as 0.025 mg/L from a species sensitivity distribution.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00442-025-05723-2.

Acknowledgements The authors thank the anonymous reviewers for their valuable comments on this manuscript.

Author contribution statement ST, ERV and GJZ conceived and designed the study. GJZ, MH, CR, ERV and ST performed the experiments. GJZ drafted the main manuscript text, and all authors improved the contents and organization of the manuscript.

Funding This research was funded by the Visiting Fellow Programme Grant (Registry number 1886/13.00.05.00/2021) of the University of Jyväskylä, Research Council of Finland grant (315163) and LIFE21-IPE-FI-PlastLIFE/101069513. The PlastLIFE project is co-funded by the European Union. Views and opinions expressed are however those of the authors only and do not necessarily reflect those of the European Union or CINEA. Neither the European Union nor the granting authority can be held responsible for them.

Availability of data and materials The data are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

Adam V, Yang T, Nowack B (2019) Toward an ecotoxicological risk assessment of microplastics: comparison of available hazard and exposure data in freshwaters. Environ Toxicol Chem 38:436–447

Adam V, von Wyl A, Nowack B (2021) Probabilistic environmental risk assessment of microplastics in marine habitats. Aquat Toxicol 230:105689

Aljaibachi R, Laird WB, Stevens F, Callaghan A (2020) Impacts of polystyrene microplastics on *Daphnia magna*: a laboratory and a mesocosm study. Sci Total Environ 705:135800

Auta HS, Emenike CU, Jayanthi B, Fauziah SH (2018) Growth kinetics and biodeterioration of polypropylene microplastics by *Bacillus* sp. and *Rhodococcus* sp. isolated from mangrove sediment. Mar Pollut Bull 127:15–21

Besseling E, Quik JTK, Sun M, Koelmans AA (2017) Fate of nano- and microplastic in freshwater systems: a modeling study. Environ Pollut 220:540–548

Bhardwaj LK, Rath P, Yadav P, Gupta U (2024) Microplastic contamination, an emerging threat to the freshwater environment: a systematic review. Environ Syst Res 13:8

Bownik A (2020) Physiological endpoints in daphnid acute toxicity tests. Sci Total Environ 700:134400

Castro-Castellon AT, Horton AA, Hughes JMR, Rampley C, Jeffers ES, Bussi G, Whitehead P (2022) Ecotoxicity of microplastics to freshwater biota: considering exposure and hazard across trophic levels. Sci Total Environ 816:151638

de Sá LC, Oliveira M, Ribeiro F, Rocha TL, Futter MN (2018) Studies of the effects of microplastics on aquatic organisms: what do we know and where should we focus our efforts in the future? Sci Total Environ 645:1029–1039

Doyle D, Sundh H, Almroth BC (2022) Microplastic exposure in aquatic invertebrates can cause significant negative effects compared to natural particles—a meta-analysis. Environ Pollut 315:120434

European Commission (2003) Technical guidance document on risk assessment in support of commission directive 93/67/EEC on risk assessment for new notified substances and commission regulation (EC) No. 1488/94 on risk assessment for existing substances and Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market. Part II. Official publications of the European Communities. pp 1–337

Hannas BR, Wang YH, Thomson S, Kwon G, Li H, LeBlanc GA (2011) Regulation and dysregulation of vitellogenin mRNA accumulation in daphnids (*Daphnia magna*). Aquat Toxicol 101:351–357

Hansen LK, Frost PC, Larson JH, Metcalfe CD (2008) Poor elemental food quality reduces the toxicity of fluoxetine on *Daphnia magna*. Aquat Toxicol 86:99–103

Hartmann HJ, Kunkel DD (1991) Mechanisms of food selection in *Daphnia*. Hdrobiologia 225:129–154

Hiltunen M, Vehniäinen ER, Kukkonen JVK (2021) Interacting effects of simulated eutrophication, temperature increase, and microplastic exposure on *Daphnia*. Environ Res 192:110304

Jeyavani J, Sibiya A, Gopi N, Mahboob S, AI-Ghanim KA, AI-Misned F, Ahmed Z, Riaz MN, Palaniappan B, Govindarajan M, Vaseeharan B (2023) Ingestion and impacts of water-borne polypropylene microplastics on *Daphnia similis*. Environ Sci Pollut Res. 30:13483–13494

Juan-García A, Pakkanen H, Juan C, Vehniäinen ER (2023) Alterations in *Daphnia magna* exposed to enniatin B and beauvericin provide additional value as environmental indicators. Ecotoxicol Environ Saf 249:114427

Kainz M, Arts MT, Mazumder A (2004) Essential fatty acids in the planktonic food web and their ecological role for higher trophic levels. Limnol Oceanogr 49:1784–1793

Kokalj AJ, Dolar A, Drobne D, Marinšek M, Dolenec M, Škrlep L, Strmljan G, Mušič B, Škapin AS (2022) Environmental hazard of polypropylene microplastics from disposable medical masks: acute toxicity towards *Daphnia magna* and current knowledge on other polypropylene microplastics. Microplast Nanoplast 2:1–15

Lai RWS, Zhou GJ, Kang HM, Jeong CB, Djurišić AB, Lee JS, Leung KMY (2022) Contrasting toxicity of polystyrene nanoplastics to the rotifer *Brachionus koreanus* in the presence of zinc oxide nanoparticles and zinc ions. Aquat Toxicol 253:106332

Laine MB, Martin-Creuzburg D, Litmanen JJ, Taipale SJ (2024) Sterol limitation of *Daphnia* on eukaryotic phytoplankton: a combined supplementation and compound-specific stable isotope labeling approach. Oikos 2024:e10359

Lasee S, Mauricio J, Thompson WA, Karnjanapiboonwong A, Kasumba J, Subbiah S, Morse AN, Anderson TA (2017) Microplastics in a freshwater environment receiving treated wastewater effluent. Integr Environ Assess Manag 13:528–532

Lebreton LCM, van der Zwet J, Damsteeg JW, Slat B, Andrady A, Reisser J (2017) River plastic emissions to the world's oceans. Nat Commun 8:15611

Li J, Liu H, Chen JP (2018) Microplastics in freshwater systems: a review on occurrence, environmental effects, and methods for microplastics detection. Water Res 137:362–374

Motiei A, Ogonowski M, Reichelt S, Gorokhova E (2021) Ecotoxicological assessment of suspended solids: the importance of biofilm and particle aggregation. Environ Pollut 280:116888

Ockenden A, Tremblay LA, Dikareva N, Simon KS (2021) Towards more ecologically relevant investigations of the impacts of microplastic pollution in freshwater ecosystems. Sci Total Environ 792:148507

OECD (2004) *Daphnia* sp. acute immobilization test (OECD 202)

OECD (2012) *Daphnia magna* reproduction test (OECD 211)

Paerl HW, Paul VJ (2012) Climate change: links to global expansion of harmful cyanobacteria. Water Res 46:1349–1363

Peltomaa ET, Aalto SL, Vuorio KM, Taipale SJ (2017) The importance of phytoplankton biomolecule availability for secondary production. Front Ecol Evol 5:128

Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT–PCR. Nucleic Acids Res 29:e45

Samadi A, Kim Y, Lee SA, Kim YJ, Esterhuizen M (2022) Review on the ecotoxicological impacts of plastic pollution on the freshwater invertebrate Daphnia. Environ Toxicol 37:2615–2638

Schür C, Zipp S, Thalau T, Wagner M (2020) Microplastics but not natural particles induce multigenerational effects in *Daphnia magna*. Environ Pollut 260:113904

Song Y, Rundberget JT, Evenseth LM, Xie L, Gomes T, Høgåsen T, Iguchi T, Tollefsen KE (2016) Whole-organism transcriptomic analysis provides mechanistic insight into the acute toxicity of emamectin benzoate in *Daphnia magna*. Environ Sci Technol 50:11994–12003

Strandberg U, Hiltunen M, Creed IF, Arts MT, Kankaala P (2023) Browning-induced changes in trophic functioning of planktonic food webs in temperate and boreal lakes: insights from fatty acids. Oecologia 201:183–197

Sun J, Yang S, Zhou GJ, Zhang K, Lu Y, Jin Q, Lam PKS, Leung KMY, He Y (2021) Release of microplastics from discarded surgical masks and their adverse impacts on the marine copepod *Tigriopus japonicus*. Environ Sci Technol Lett 8:1065–1070

Taipale SJ, Aalto SL, Galloway AWE, Kuoppamäki K, Nzobeuh P, Peltomaa E (2019a) Eutrophication and browning influence *Daphnia* nutritional ecology. Inland Waters 9:374–394

Taipale SJ, Peltomaa E, Kukkonen JVK, Kainz MJ, Kautonen P, Tiirola M (2019b) Tracing the fate of microplastic carbon in the aquatic food web by compound-specific isotope analysis. Sci Rep 9:19894

Taipale SJ, Vuorio K, Aalto SL, Peltomaa E, Tiirola M (2019c) Eutrophication reduces the nutritional value of phytoplankton in boreal lakes. Environ Res 179:108836

Tkaczyk A, Bownik A, Dudka J, Kowal K, Ślaska B (2021) *Daphnia magna* model in the toxicity assessment of pharmaceuticals: a review. Sci Total Environ 763:143038

Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F (2002) Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol 3(research0034):1

Wu X, Jiang J, Wan Y, Giesy JP, Hu J (2012) Cyanobacteria blooms produce teratogenic retinoic acids. Proc Natl Acad Sci USA 109:9477–9482

Zhou GJ, Ho KKY, Ip JCH, Liu S, Hu J, Giesy JP, Leung KMY (2021) Insights into the influence of natural retinoic acids on imposex induction in female marine gastropods in the coastal environment. Environ Sci Technol Lett 8:1002–1008

Zimmermann L, Göttlich S, Oehlmann J, Wagner M, Völker C (2020) What are the drivers of microplastic toxicity? Comparing the toxicity of plastic chemicals and particles to *Daphnia magna*. Environ Pollut 267:115392