Ecotoxicology and Environmental Safety 228 (2021) 113001

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Vertical distribution of microplastics in water bodies causes sublethal effects and changes in *Daphnia magna* swimming behaviour

Sergi Magester, Aina Barcelona, Jordi Colomer, Teresa Serra *

Department of Physics, University of Girona, 17003 Girona, Spain

A R T I C L E I N F O

Edited by Dr. Caterina Faggio

Keywords:
Daphnia magna
Microplastic
Transport mechanisms
Mortality
Toxicity
Body growth rate

A B S T R A C T

Plastic debris has been found to be ubiquitous in many aquatic ecosystems and is constantly accumulating, not only because more and more plastic is being rapidly released into the environment, but also because its slow degradation means it persists in the water. Some more buoyant plastics accumulate in the water column, whereas other heavier types sink to the bottom. Consequently, the presence of microplastics can threaten organisms living in the water column as well as those found in the benthic zone. In this study, the filter feeder *Daphnia* has been found to ingest microplastics as the particle diameter (< 30 μm) is within their edible particle size range and they are unable to differentiate between particles of different natures. Four different treatments were considered: food only; only microplastic particles; 50% food and 50% microplastic particles; neither food nor microplastics. Sinking microplastics have been found to decrease *Daphnia magna* individuals' swimming velocity during vertical or cruising swimming trajectories, therefore demonstrating the sublethal effects microplastics have on this organism. In addition, microplastics decreased their body growth and survival rates. In cases with the presence of only microplastics, the swimming trajectories of *Daphnia* indicated the most serious stress experienced as individuals reversed vertical or cruising swimming trajectories to hopping and sinking movements. Therefore, *Daphnia* individuals in freshwater systems polluted by microplastics might take on the role of ingesting them and later on transporting them to deeper layer water column. In this way microplastics that would remain in the water column for a long time due to their buoyancy, might accumulate at the bottom of the water column.

1. Introduction

There is growing concern about the impact plastic disposal is having on the environment, given that plastic can now be found in almost all ecosystems, especially in aquatic environments (Thompson et al., 2009). Plastics end up in aquatic systems mainly via direct emission or discharges from rivers where they continue to degrade, breaking down into small particles or fragments and dispersing polymers called microplastics (MP), i.e., any piece of plastic smaller than 5 mm in diameter (Lambert et al., 2014). Plastic fragments and MP have also been shown to contain organic contaminants on their surfaces due to manufacturing processes or by absorbing pollutants present in the environment (Teuten et al., 2009).

MP can be ingested by aquatic organisms and, although partially egested, MP accumulation can pose a serious problem for their development. For instance, zooplankton are known to ingest and accumulate MP inside their gut, thus making MP easily transferrable to a higher trophic level when, for example, fish feed on zooplankton individuals (Nelms et al., 2018). Because *D. magna* individuals ingest MP, and since egestion does not occur within 24 h (Rist et al., 2017), MP can accumulate in their bodies. The MP accumulated in the *D. magna* body can subsequently be transferred to other animals that can then accumulate the MP themselves. Consequently, MP can cause a severe environmental impact to the ecosystem (Provencher et al., 2018). That said, Elizalde-Velázquez et al. (2020) has demonstrated that *D. magna* showed rapid depuration rates and null translocation of MP between 72 and 96 h after a 5-d exposure to MP.

D. magna is a common zooplanktonic organism found in freshwater ecosystems. They are a model organism which is highly studied, especially in toxicological analysis (Guilhermino et al., 2000). Being easy to cultivate and manipulate, coupled with a high birth rate (Seda and Petrusek, 2011), makes them one of the most important invertebrate models available. *Daphnia* are known to ingest particles with diameters below 30 μm (Pau et al., 2013) and because they cannot distinguish between particles of different natures (DeMott, 1986), they will ingest particles when their size overlaps the size of edible particles. In general,

---

* Corresponding author.
E-mail address: teresa.serra@udg.edu (T. Serra).

https://doi.org/10.1016/j.ecoenv.2021.113001
Received 1 October 2021; Received in revised form 10 November 2021; Accepted 14 November 2021

these ingestions do not necessary imply fatal effects, but rather may produce chronic effects that could cause a number of problems such as oxidative stress, starvation etc., (Wright et al., 2013). While even low concentration levels of MP (25% of MP and 75% of food) have been shown to decrease *D. magna* filtration capacity, high concentration levels (e.g., 50% MP and 50% food, or higher MP to food ratios) produce more than a 50% reduction in their filtering capacity after seven days of exposure (Colomer et al., 2019).

D. magna migrate vertically, depending on whether it is day – to prevent predation, or night – for food requirements (Williamson et al., 2011). The velocity of *D. magna* individuals depends on their body size, following an allometric relationship between their swimming velocity and their body length (Serra et al., 2019a,b). In addition, the swimming behaviour of *Daphnia* is generally accepted as a biomarker that determines the toxicity produced by various groups of chemicals or stressors (Bownik, 2017). Therefore, the behaviour of *Daphnia* movements may indicate that the individuals are being affected by lethal or sublethal effects. A sublethal effect would mean that under negative environmental conditions *Daphnia* become stressed and will die after long-term exposure. Diel vertical migration may even be altered by sublethal concentrations of a persistent insecticide (Gutierrez et al., 2012). The presence of chemical additives in MP particles has been shown to increase their toxicity on *D. magna* (Zimmermann et al., 2020). Furthermore, irregular plastic particles (fragments), as opposed to regular spheric microplastic particles, have been found to have a higher negative impact on *D. magna* (Na et al., 2021). Likewise, *D. magna* have been shown to be critically affected by the combination of different stressors; MP being one of them (Serra et al., 2020). Some authors have shown that vertical swimming represents an additional energy cost because individuals must overcome both viscous drag and gravity when they swim upward (Wallace and Estephan, 2004), thus requiring a certain level of fitness to be able to swim. The presence of MP may modify the fitness levels of *D. magna* individuals, which would indicate that they are under stress or in stressful conditions. Hence, in this study the swimming behaviour of *D. magna* is analysed to determine whether the presence of MP do in fact represent a stressor to *D. magna*.

Normal concentrations of MP in urban rivers are, at most, 0.13 μg L^{-1} (He et al., 2020). On beaches, although more difficult to determine, their concentration range has been reported to be between 12 and 1300 particles per m^2 (de Carvalho and Neto, 2016). Furthermore, water reaching a sanitation system can have MP concentrations of up 10,044 particles L^{-1} which, after wastewater treatment, can be reduced to 450 particles L^{-1} (Sun et al., 2019). Finally, some toxicology studies have used MP concentrations of 0.1 mg L^{-1} (Rist et al., 2017).

Although several studies have focused on the presence of MP in water ecosystems and how they affect organisms, it is not entirely clear how MP affect *D. magna* mobility and swimming behaviour. In addition, the impact MP have on *D. magna* distribution in the water column is still unknown. This study aims to determine how MP affect the movement of *D. magna* individuals, influence their survival rates as well as the potential impact they might have on their migration and distribution in the water column. In addition, it is expected that *D. magna* individuals under adverse conditions will present lower body growth rates compared to optimal conditions. Finally, this study is also aimed at establishing the capacity of *D. magna* to vertically transport MP particles, therefore identifying their role in driving the vertical distribution of MP in the water column of aquatic ecosystems. The swimming behaviour of *D. magna* under conditions with MP will also be studied and compared with conditions without MP. Different experimental treatments will be considered: a control treatment with food only, a treatment without neither food nor MP, and two other treatments with different MP to food percentages (100% MP and 0% food, 50% MP and 50% food).

2. Materials and methods

2.1. *Daphnia magna*

All animals were selected from three laboratory cultures kept in the laboratory at 20.0 ± 0.5 °C, over a natural daylight photoperiod (8 h light and 16 h darkness) and with a continuous air supply to avoid anoxia. These laboratory cultures have been maintained for three years in the laboratory at the University of Girona (Spain). Individuals were fed three times a week (Monday, Wednesday, and Friday) with a mixture of dry *Chlorella* powder (100% *Chlorella* platensis, KeyPharm, Belgium) and Baker's yeast (*Saccharomyces cerevisiae*, Mondeléz International, Spain). One third of the water from the culture was renewed once every two weeks (Müller et al., 2018; Serra et al., 2019a,b, 2018). Mineral water rich in calcium (constant value of 93.8 mg L^{-1}) was used to avoid calcium depletion (Riessen et al., 2012). The mineral water used also contained the following components: 298 mg L^{-1} of HCO_3^-, 1.6 mg L^{-1} of SO_4^{2-}, 3.6 mg L^{-1} of Cl^-, 3.4 mg L^{-1} of Mg^{2+} and 1.8 mg L^{-1} of Na^+.

All tests, protocols and analyses with *D. magna* were carried out in accordance with the international OECD/OCDE Guidelines for the Testing (OECD, 2018) and the Protocol for the sampling and laboratory testing of in-vertebrates code ML-L-I-2013 (ML-L, 2013) from the Ministerio de Agricultura, Alimentación y Medio Ambiente of the Spanish Government.

2.2. Food and MP characteristics

The food used to feed the *D. magna* was dry chlorella powder (*Chlorella* sp., M. Torras Rafi, Spain). To prepare the *Chlorella* suspension, 1 g of *Chlorella* powder was diluted in 1 L of bottled mineral water, mixed for 60 s at 100 rpm and left for 1 h to let any large *Chlorella* particles settle. The supernatant was used as the *Chlorella* suspension for the experiments.

For experiments with MP, Polystyrene microspheres (Alpha Nanotech, Vancouver, Canada) were used. MP particle sizes were analysed with a particle size analyser (Laser In-Situ Scattering and Transmissometry LISST-100X, Sequoia Scientific, Inc, Bellevue, WA), classifying measurements into 32 class sizes logarithmically distributed over a range of 2.5–500 µm rings. To measure the diameter of the MP, a suspension with an initial concentration of 16 μL L^{-1} of each type of MP was prepared. The main peak for particle size distribution of MP was for particles in the range of 8.65 µm and 23.4 µm (Fig. SM1 of the Supplementary material). Furthermore, the particle size distribution of food (*Chlorella platensis*) was in the same particle range as the MP. MP had a median diameter of 15.65 µm, whereas *Chlorella platensis* particles had a median of 13.76 µm, i.e., all within the *D. magna* edible range size (Pau et al., 2013) (Fig. SM1).

Different treatments were considered depending on the presence of MP, and/or food and their proportions. Control experiments without MP or food were also carried out. In some experiments, *Chlorella* was added with the concentration required to reach the same total (MP+*Chlorella*) initial volume concentration of 16 μL L^{-1} of particles in all experiments. MP were applied using four different ratios (MP-food) for four days. The conditions were as follows: 1) 0% MP + 100% Food, 2) 50% MP + 50% Food, 3) 0% MP + 0% Food, 4) 100% MP + 0% Food.

2.3. Experimental procedure

On the first day of the experiment, three hundred *Daphnia* individuals were gently collected from the aquarium with a 0.5 mm mesh and carefully rinsed with bottled water (to avoid any particles adhered from their respective tanks) and kept for 24 h with water and 16 μL L^{-1}

of food. After this time, the *Daphnia* were gently rinsed again and then one hundred individuals transferred to each of the three beakers at 20 °C with the conditions of the treatment (i.e., three replicates of each treatment). The working height for all experiments was 15.6 cm.

To stimulate the vertical swimming of the *Daphnia* individuals, beakers were covered with a box for 30 min to ensure complete darkness. During these 30 min, all *Daphnia* individuals swam down and remained at the bottom of the beaker. After 30 min, the box was removed and illuminated with a lamp (230 V, 60 W and incandescent bulb) situated 50 cm above the water's surface. *Daphnia* swimming patterns were recorded and later analysed (as detailed in Methods).

When the beakers were uncovered, the animals were affected not only by the light from the lamp but also by the natural light. The quantity of light intensity was measured every day with a luxometer (PCE-L335, PCE Group Iberica S.L., Spain). A chi squared analysis was performed and no significant differences were found between the light conditions applied on the different days ($X^2_{df:5} = 5.37$, p-value > 0.05).

The same process was repeated every day for each treatment, i.e., four videos were recorded at 30 frames per second/per treatment. Over the four days that the experiments lasted, the water was renewed daily, once the videos had been recorded, to avoid anoxia and ensure that the conditions remained constant and consistent with no depletion of food and MP in the cases where they were present. The *D. magna* individuals were gently rinsed daily to avoid an excess of particles in the new proportions.

2.4. Swimming velocity and growth of D. magna individuals

The swimming patterns of the *D. magna* individuals were characterised by vertical swimming trajectories, cruising, and hopping and sinking and were differentiated based on the net displacement (Fig. 1). During a vertical swimming trajectory, the individuals swam in quasi-straight vertical trajectories with an overall angle greater than 45° with the horizontal axis (Fig. 1, left panel). During cruising, there was a near-straight trajectory in directions with an angle below 45° to the horizontal axis (Fig. 1, central panel), while during hopping and sinking, the individuals moved in successive ascending and descending short pathways (Fig. 1, right panel). From this range of movements, we chose vertical swimming trajectory and cruising, since these were the most frequent movements observed (Serra et al., 2019a,b) and were produced by the light stimulation.

The *D. magna* velocity analysis was carried out by videotaping the movements of the individuals. The camera recorded 30 frames per second for 120 s. These frames were then analysed and the swimming velocity was calculated with ImageJ software (version 1.49 g, Wayne Rasband, National Institutes of Health, USA) using an mTrack plug-in (Moison et al., 2012). The movements were classified following the three types of movement indicated in Fig. 1. The mean size of the *D. magna* was also obtained from ImageJ software by video recording, when possible, at least 20 individuals that showed mobility during each day in every treatment (Colomer et al., 2019). The mean vertical swimming trajectory and the cruising velocity were determined from the measurements.

2.5. D. magna vertical distribution

The distribution of the *Daphnia* in the system was determined daily throughout the duration of each experiment. Each beaker was divided into four vertical compartments or layers of 3.9 cm of thickness each, and the number of individuals per compartment and time in the compartment were counted for a time interval of 30 s until the end of the recording (2 min).

2.6. D. magna survival

The number of live *Daphnia* was calculated at the end of each day for all the treatments studied. Every day, after the video had been recorded, every individual was transferred into a new beaker with the initial proportions of MP or food. When the animals were transferred, they were counted, and any dead ones discarded.

2.7. Statistical analysis

Data for *Daphnia* body length and velocity in Day 3 was checked for normality by using the Shapiro-Wilk test with the RStudio software package (version 1.4.1106). In the case of non-normally distributed data, a data transformation was first applied following Sokal and Rohlf (1995) before performing a 2-way ANOVA on the data.

3. Results

3.1. D. magna survival

The ratio (N/N_0) between the number of live *D. magna* (N) versus the initial *D. magna* individuals (N_0) decreased with time for all the experiments carried out (Supplementary material, Fig. SM2). The temporal decrease of N/N_0 with time depended on the treatment, with the greatest being found for experiments without food and 100% of MP. In this case, after four days of exposure to such conditions, the survival rate had dropped to 10% of the initial value. This case was followed by the case with neither food nor MP, which exhibited a 40% survival rate after four days of exposure to these conditions. The cases with the presence of MP and food produced a decrease in N/N_0 lower than all the above-mentioned treatments (only MP or neither food nor MP), with a reduction to 84% of its initial value. On the last day of the exposure (Day 4), the treatments that produced a decrease in the percentage of *D. magna* survival below 50% were the control experiment with neither MP nor food, and the case without food but with MP. The other treatments had

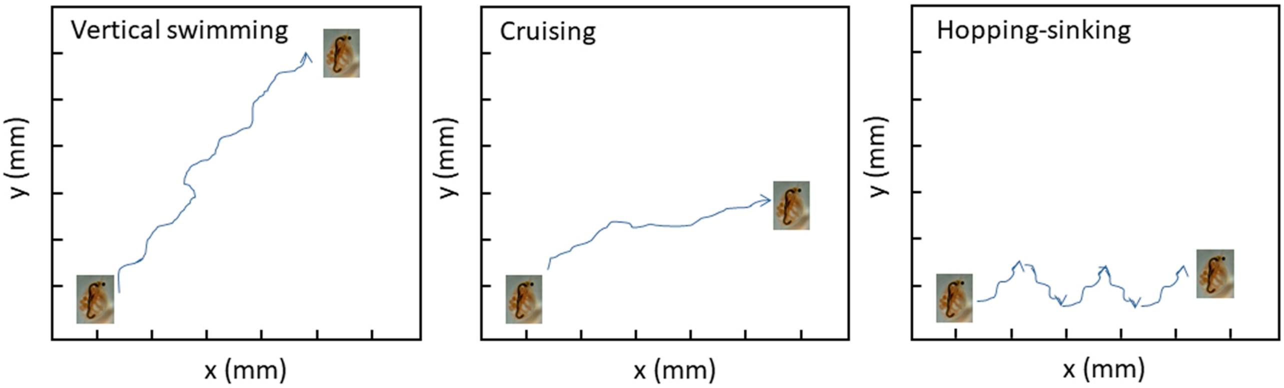

Fig. 1. Sketched swimming patterns of *D. magna*. Vertical swimming trajectory (left panel), cruising (central panel) hopping and sinking (right panel).

survival rates above 50% (Supplementary material, Fig. SM3).

3.2. *D. magna growth rate*

The *Daphnia* body length presented different temporal evolutions depending on the treatment administered (Fig. 2). The greatest increase was observed for the treatments 0% MP + 100% Food and 50% MP + 50% Food. The daily growth rate for all the cases was calculated and is presented in Table 1. The statistical analysis performed indicated that no significant differences were found for the *Daphnia* growth between experiments provided food was present (i.e., 0% MP+100% Food and 50% MP+50% Food). The *Daphnia* body length was found to remain constant for the case without food or MP (Fig. 2), with a zero-growth length for these days and this treatment (Table 1). For the treatment with only MP, there was a decrease in *Daphnia* body length with time (Fig. 2). In this case, the reduction in body length is presented as negative values in Table 1. In the control treatment with neither MP nor food, individuals grew on the first day which meant the value was positive (0.03 for four days). The *Daphnia* body length on the fourth day of treatment decreased with the presence of MPs (Fig. 2). Treatments without food and with 100% MP presented a decrease in the mean *Daphnia* body length, the smaller corresponding to the case of PE MP (Fig. 2).

3.3. *D. magna swimming velocity*

The *Daphnia* vertical velocities for the food-only experiment were greater than for the other treatments (Fig. 3) and with significant differences ($p < 0.05$). Therefore, the vertical velocity decreased if individuals were exposed to MP (i.e., 50% MP+50% Food, and 100% MP+0% Food) or if they had no food (0% MP+0% Food), whereas it increased with time (days) for the case 0% MP+ 100% Food (only food in the system). However, for the other treatments, the vertical velocity decreased with time.

The mean cruising velocities of *Daphnia* were greater than the vertical velocities in all the experiments. Contrary to what was found for the *Daphnia* vertical velocity, the cruising velocity remained constant with time for the case of 0% MP+ 100% Food (only food) with a mean value of 1.67 ± 0.03 cm/s (Fig. 3). The same happened for the case 50% MP+ 50% Food. No significant differences were found between the experiment 0% MP+ 100% Food (only food) and 0% MP+ 0% Food (without food or MPs). However, the trend for the systems where no food was provided, was to decrease while in systems with food the cruising velocity remained constant. On Day 4, for treatments with only MP and without food, cruising and vertical trajectory velocities equal to zero corresponded to cases where *Daphnia* movements were not observed. In such treatments and after four days of exposure, *Daphnia* remained immobilised at the bottom or performed hopping and sinking movements (Fig. 3). Therefore, when individuals were exposed to MP or if they had no food (0% MP+0% Food), their swimming velocities (both cruising and vertical trajectories) decreased compared to the first day of the treatment (Fig. 4). Contrary to this, when individuals had only food (0%MP+100% Food), their cruising and vertical trajectory velocities increased.

Table 1
Table with the mean *Daphnia* body growth rate (in mm day^{-1}). Negative values indicate the presence of fewer large individuals, resulting in mean body lengths smaller than the previous days.

Treatment	Mean *Daphnia* body growth rate (mm d^{-1})
0% MP-100% Food	0.04
50% MP-50% Food	0.03
0% MP-0% Food	0
100% MP-0% Food	-0.01

3.4. *Distribution of D. magna in the water column*

On Day 1, more than 50% of the *Daphnia* were to be found in the upper layer (Fig. 5), albeit except for the case of 0% MP-0% Food, with 41% of *Daphnia* encountered in this layer. In each of the other layers, the percentage remained below 20%. On Day 4, and for the treatments with food, *Daphnia* remained mainly in the upper layers (Fig. 6). For the case of 100% Food, the percentage of *Daphnia* individuals in the upper layer was the greatest compared to those cases with the presence of MP. For the treatments without food, more than 70% of *Daphnia* were found in the lower layer, especially for the treatments with only MP that presented the greatest percentages in the lower layer. Note that: On all days the percentage of *Daphnia* in the middle water layers for any treatment remained lower than the percentage in the upper and lower water layers.

4. Discussion

4.1. *D. magna survival and growth rate*

The mortality rate for *D. magna* thriving in the experiment with food only, remained very low; only two individuals died over the four days of experiments. However, when MP were present in the system, the mortality presented the greatest increase with time, indicating the lethal effect MP have on *D. magna*. The mortality for the treatment with 50% of

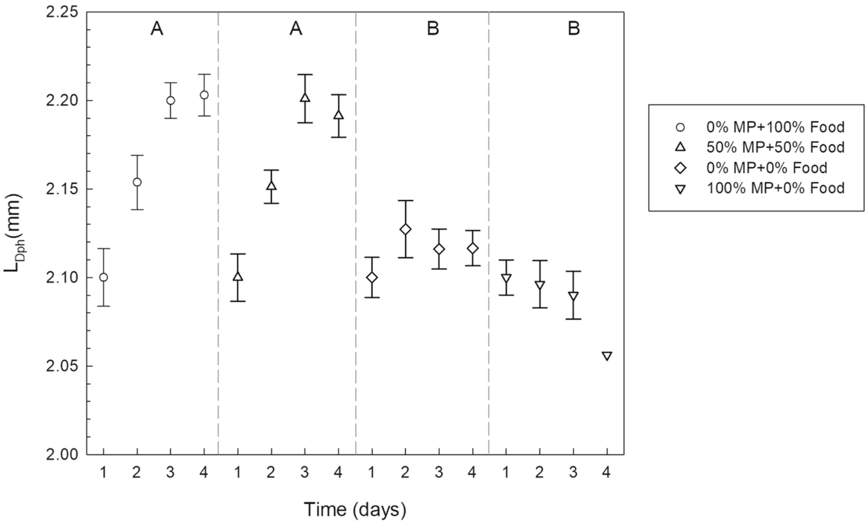

Fig. 2. Temporal evolution of the *Daphnia* body length for the different treatments considered. Areas shaded in different colours represent the results for the *Daphnia* body length for each treatment; green refers to treatments with food, white with neither food nor MP and grey corresponds to the treatment with only MP. Letters at the top are classified as statistical equal groups that ANOVA for Day 3 has shown with a p-value less than 0.05. For normality purposes data was transformed as $(x + 1)^2$, where x is the data being considered for the ANOVA analysis. Error bars represent standard errors of the mean (SE). The number of measurements considered for the calculation of SE are given in Table SM1 of the Supplementary material.

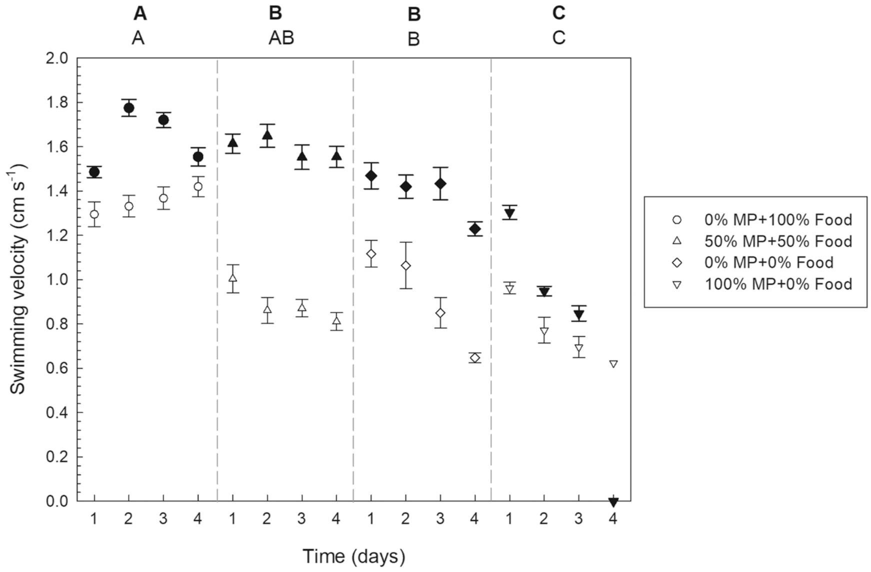

Fig. 3. *Daphnia* cruising (solid symbols) and vertical velocities (open symbols) for each day and treatment. Values of cruising in 100%MP + 0%Food have been represented as 0 when no *Daphnia* were performing such movements in the system. Likewise, the final value of vertical velocity in 100% MP + 0% Food has no error bars because only one individual was performing this movement. Letters at the top are classified as statistical equal groups that ANOVA for Day 3 has shown with a p-value less than 0.05. Letters in bold font correspond to the results for the cruising velocity while normal font corresponds to the vertical velocity. To fulfil normality data vertical velocity was transformed by a root function. Data for cruising velocity did not need any transformation because the distribution was already normal. Error bars represent standard errors of the mean (SE). The number of measurements considered for calculating SE are included in Table SM2 of the Supplementary material.

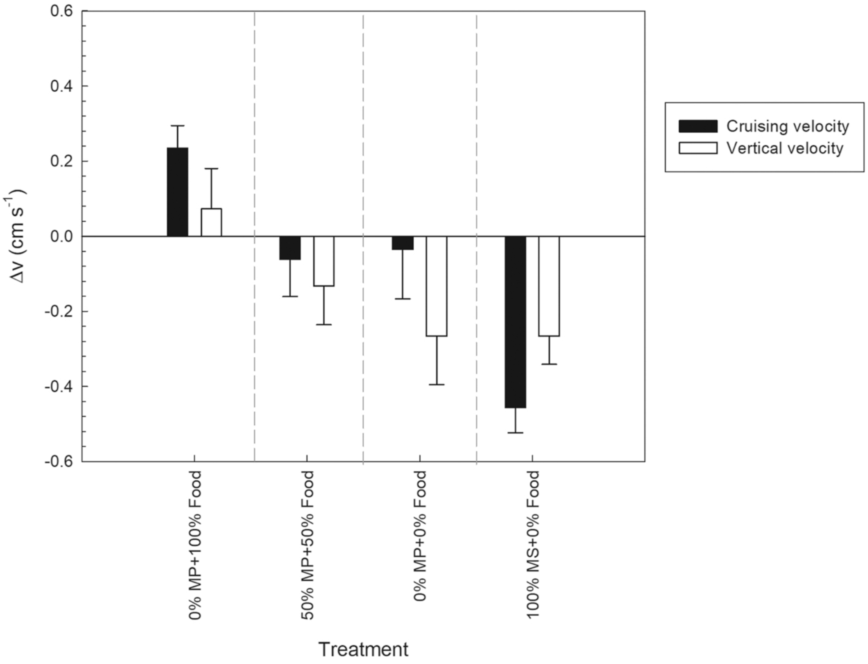

Fig. 4. Change in the swimming velocity between Day 3 and Day 1 for the different treatments. Negative values mean that velocity decreased at Day 3 with respect to Day 1. Error bars indicate the standard error of the mean from days 1–3 (i.e. error=$SE_{day1}+SE_{day3}$).

MP, was lower (16% after four days of treatment) than the treatments with 100% MP or with no food. Therefore, provided there is food in the system, animal body burdens of particles are sharply reduced (Rist et al., 2017; Canniff and Hoang, 2018). The highest mortality was attained for the treatment with 100% MP+ 0% Food, with a reduction of up to 10% of the initial number of *D.magna* individuals after four days of exposure. The observed impact MP had on *D. magna* mortality could be due to the possible blocking in the *D. magna* gut caused by the MP (Scherer et al., 2017). The decrease in the *D. magna* survival, indicates that in the presence of MP, *D. magna* individuals were stressed with time, even though food was available. In this case, an accumulation of MP in the individuals caused the low survival rates after 4 d of treatment. Survival decreased during the first two days but then remained nearly constant afterwards. However, in the treatments without MP and food or only MP, the high *D. magna* mortality indicates that they could not cope with the stressful conditions. Indeed, the concentration of MP has been found to be a critical parameter, with increasing MP concentration adding a stress factor for *D. magna* survival and growth rate (Yuan et al., 2020). For other species, like the detritivore *Sericostoma pyrenaicum*, the mortality increased 9-fold for MP, but did not affect their growth (López-Rojo et al., 2020).

D. magna individuals grew about 0.04 ± 0.01 mm per day in the control experiment of 0% MP+ 100% Food, which is comparable to the experiments by Wickramarathna et al. (2014). When the media presented 50% MP and 50% Food, the growth rate was lower, while for the cases of only MPs the growth rate of individuals was negative, indicating

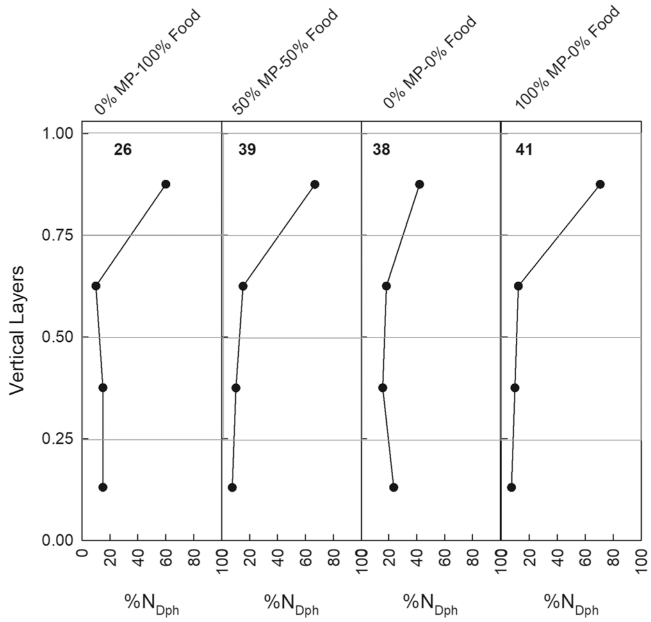

Fig. 5. Percentage of animals found in each vertical layer for each treatment on Day 1. The treatment applied is indicated at the top of each subplot. The top figures indicate the number of *Daphnia* counted in every case. For instance, the figure 26 for treatment with only food (0%MP+100%Food) on Day 1 means that 26 individuals were counted. This value is related to survival when the number of *Daphnia* counted is low.

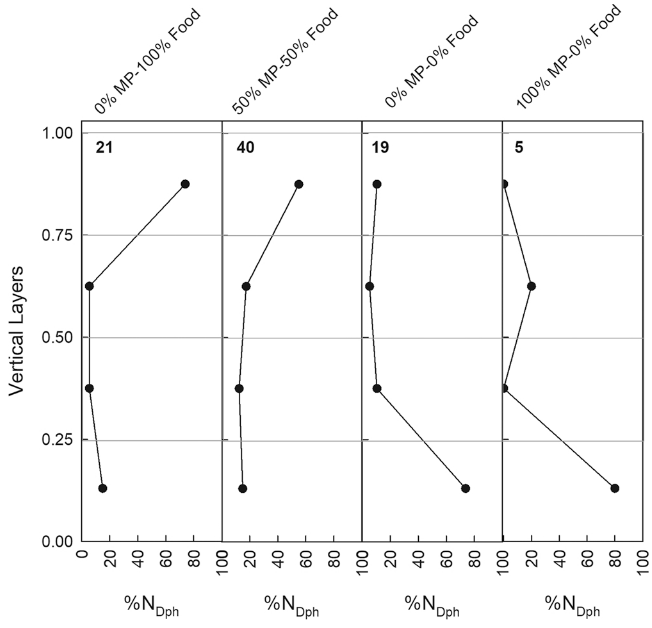

Fig. 6. Percentage of animals found in each vertical layer for each treatment on Day 4. The treatment applied is indicated at the top of each subplot. The top figures indicate the number of *Daphnia* counted in every case.

that the biggest individuals were more affected by MP, and only the smallest ones with lower food requirements remained (Besseling et al., 2014).

4.2. D. magna swimming velocity

Wickramarathna et al. (2014) found that *D.magna* swimming velocity could be correlated with body length, i.e., bigger *Daphnia* individuals swim faster, according to the optimal growth observed for the food-only treatment. Serra et al. (2019a,b) found that the *Daphnia* swimming velocity increased with body length following an allometric equation v ~ $L_{Dph}^{-0.31}$. In this current study, the *D. magna* swimming velocity was constant for the treatment with 50% MP+ 50% Food and, decreased in the treatments with neither food nor MP. The variation of the *Daphnia* swimming velocity after three days of exposure to the treatment compared with the velocity in the first day was positive only for the control treatment with only food, and negative for all the other treatments studied. The temporal change in the swimming velocities presented greater negative values for treatments where there were only MP or no food nor MP. This indicates that MP produced sublethal effects on *D. magna* individuals in all the treatments except for the treatment with food only. It must be pointed out that sublethal effects were also observed in cases of 50% of MP+ 50% Food, concurrent with a reduction of swimming velocities despite the *Daphnia* body length having increased or despite showing a small reduction in their survival after four days of treatment. Therefore, the analysis of their swimming velocity provided information on the sublethal effects to *D. magna* when individuals were under adverse environmental conditions. This result is in accordance with Bownik (2017) and Pan et al. (2017) who used *D. magna* swimming velocities to discern the sublethal effects the presence of pollutants have on *D. magna*.

Even though the differences between the cruising and vertical velocities were not found to be statistically significant, cruising velocities were found to be slightly greater than vertical velocities. Most likely, the *D. magna* energy requirements to follow vertical trajectories in order to overcome the pressure gradient exerted by water are greater than those required for cruising trajectories. So, this movement could be only sustained when the individuals were in favourable conditions (i.e., systems with food and/or without the presence of MP). During cruising, individuals do not need to overcome a high vertical pressure gradient and this movement might be sustained even under adverse conditions. In addition, for high MP concentration conditions, and after a certain exposure time, *D. magna* individuals predominantly exhibited hopping and sinking movements, indicating sublethal conditions. This may have an important implication for the ecosystem, because if *D. magna* individuals cannot swim up due to the stress imposed by the presence of MP, they will not be able to transfer MP across the water column and, therefore, organisms that predate on them will not be affected.

4.3. Distribution of D. magna in the water column

At the beginning of the experiments (Day 1), for all the treatments considered (with and without MP), *D. magna* individual concentrations were higher in the upper layer of the water column as a result of the phototactic behaviour of the individuals under the imposed light conditions. However, with time (as in Day 4), in the treatment with only MP or no food nor MP, most *D. magna* individuals remained at the bottom of the water column. In contrast, for treatments with the presence of food, the percentage of *Daphnia* remained high in the upper layer of the water column. These results can be attributed to the fact that in the experiments with only MP or without food, *Daphnia* did not have enough energy to swim up and therefore remained at the bottom with their movements reduced to hopping and sinking. Moreover, it has to be pointed out that for all the treatments only a few individuals were found in the middle layers of the water column. This is explained by the fact that under optimal conditions *D. magna* individuals are stimulated to swim to the top, which is possible providing they have the energy required to do so. However, under adverse conditions, *D. magna* individuals were not able to swim and remained in the lower layer of the water column. Therefore, there was no stimulae for *Daphnia* to remain in suspension in the middle layers. Furthermore, the presence of *Daphnia* in treatments with only MP was far below that for with neither food nor MP. Therefore, only a few individuals were able to withstand the adverse conditions in the MP only treatments, and all of them remained at the bottom of the water column. Consequently, the presence of MP determined the vertical distribution of *Daphnia* in the water column.

The density of MP might also be modified by biofouling growing on the surface of the MP (Chubarenko et al., 2016), making the MP more buoyant and increasing even more the residence time in the water column. However, the presence of *Daphnia* provides a mechanism for buoyant MP to be transported from the water surface downwards to the bottom. Therefore, filter feeders like *Daphnia* might be also a sink of MP that have to be accounted for in the balance of MP in the water column. In this study, the vertical distribution of MP was modified depending on the *D. magna* organisms thriving in the water column; just as Long et al. (2015) found that phytoplankton aggregates are a mechanism of transporting MP to the seabed. All these mechanisms have to be considered in order to understand the flow of MP to the bottom of the water column. In fact, Hidalgo-Ruz et al. (2012) stated that the concentration of MP in the bottom sediments is greater than in the water surface. The high concentration of MP in bed sediments is expected to impact benthic communities. While Urban-Malinga et al. (2021) did not find a negative effect of MP on the survival of the cockle *Cerastoderma glancum* or the baltic clam *Limecola* some alterations in their behaviour were found. Redondo-Hasslerharm et al. (2018) found that MP did not impact on the survival of several benthic organisms but some of them experienced a lower growth rate.

5. Conclusions

Microplastics have been found to cause sublethal and lethal effects to *D. magna* depending on the concentration levels in the environment. Survival is an indicator of the lethal effects MP have on *D. magna*, while velocity patterns can serve as a sublethal indicator. High MP-to-food ratios produced lethal effects on *Daphnia magna* by reducing their survival after a short exposure time under such conditions. After four days of exposure to MP *D. magna* remained mainly at the bottom of the water column performing hopping and sinking movements. Therefore, hopping and sinking patterns in *D. magna* movements are indicators of stressful conditions. MP negatively affected *D. magna*, especially in the case of the larger individuals that need more food to fulfil their fitness requirements. Low MP-to-food ratios caused sub-lethal effects on *Daphnia*, reducing *D. magna* body growth rate, decreasing swimming velocity and producing a heterogeneous vertical distribution of *D. magna* in the system. Provided there was food in the system, exposure time to MP below two days produced slight changes in *D. magna* swimming velocity compared to the food-only experiments. In such conditions, *D. magna* can transfer MP though the different layers of the water column. Nevertheless, as the exposure time increases, MP negatively impacted *D. magna* mobility and forced individuals to remain in the bottom layer as they were unable to swim up, even when given an external stimulus. In such cases, the transference of MP in the water column because of *D. magna* migration is unlikely to happen.

CRediT authorship contribution statement

Sergi Magester: Methodology, Formal analysis, Investigation, Data curation, Writing – original draft. **Aina Barcelona:** Methodology, Investigation. **Jordi Colomer:** Methodology, Writing – original draft, Writing – review & editing. **Teresa Serra:** Methodology, Writing – review & editing, Data curation, Supervision.

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.

Data availability

Data will be provided under upon request.

Acknowledgements

This research was supported by the Grant 2017SGR1118 from the Catalan Government. Sergi Magester was funded by the Spanish Government through the scholarship 19CO1/011321.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2021.113001.

References

Besseling, E., Wang, B., Lürling, M., Koelmans, A.A., 2014. Nanoplastic affects growth of S. obliquus and reproduction of D. magna. Environ. Sci. Technol. 48, 12336–12343.

Bownik, A., 2017. *Daphnia* swimming behaviour as a biomarker in toxicity assessment: a review. Sci. Total Environ. 601, 194–205.

Canniff, P.M., Hoang, T.C., 2018. Microplastic ingestion by *Daphnia magna* and its enhancement on algal growth. Sci. Total Environ. 633, 500–507.

de Carvalho, D., Neto, J., 2016. Microplastic pollution of the beaches of Guanabara Bay, Southeast Brazil. Ocean Coast. Manag. 128, 10–17.

Chubarenko, I., Bagaev, A., Zobkov, M., Esiukova, E., 2016. On some physical and dynamical properties of microplastic particles in marine environment. Mar. Pollut. Bull. 108, 105–112.

Colomer, J., Müller, M.F., Barcelona, A., Serra, T., 2019. Mediated food and hydrodynamics on the ingestion of microplastics by *Daphnia magna*. Environ. Pollut. 251, 434–441.

DeMott, W.R., 1986. The role of taste in food selection by freshwater zooplankton. Oecologia 69 (3), 334–340.

Elizalde-Velázquez, A., Carcano, A.M., Crago, J., Green, M.J., Shah, S.A., Cañas-Carrell, J.E., 2020. Translocation, trophic transfer, accumulation and depuration of polystyrene mi, plastics in *Daphnia magna* and Pimephales promelas. Environ. Pollut. 259. Article 113937.

Guilhermino, L., Diamantino, T., Silva, M.C., Soares, A.M.V.M., 2000. Acute toxicity test with *Daphnia magna*: an alternative to mammals in the prescreening of chemical toxicity? Ecotoxicol. Environ. Saf. 46 (3), 357–362.

Gutierrez, M.F., Gagneten, A.M., Paggi, J.C., 2012. Exposure to sublethal chromium and endosulfan alter the diel vertical migration (DVM) in freshwater zooplankton crustaceans. Ecotoxicology 21 (1), 37–47.

He, B., Wijesiri, B., Ayoko, G.A., Egodawatta, P., Rintoul, L., Goonetilleke, A., 2020. Influential factors on microplastics occurrence in river sediments. Sci. Total Environ. 738, 139901.

Hidalgo-Ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2012. Microplastics in the marine environment: a review of the methods used for identification and quantification. Environ. Sci. Technol. 46, 3060–3075.

Lambert, S., Sinclair, C., Boxall, A., 2014. Occurrence, degradation, and effect of polymer-based materials in the environment. Rev. Environ. Contam. Toxicol. 227, 1–53.

Long, M., Moriceau, B., Gallinari, M., Lambert, C., Huvet, A., Raffray, J., Soudant, P., 2015. Interactions between microplastics and phytoplankton aggregates: impact on their respective fates. Mar. Chem. 175, 39–46.

López-Rojo, N., Pérez, J., Alonso, A., Correa-Araneda, F., Boyero, L., 2020. Microplastics have lethal and sublethal effects on stream invertebrates and affect stream ecosystem functioning. Environ. Pollut. 259, 113898.

ML-L, 2013. Protocolo de muestreo y laboratorio de invertebrados bentonicos en lagos.

Moison, M., Schmitt, F.G., Souissi, S., 2012. Effect of temperature on Temora longicornis swimming behaviour: illustration of seasonal effects in a temperate ecosystem. Aquat. Biol. 16 (2), 149–162.

Müller, M.F., Colomer, J., Serra, T., 2018. Temperature-driven response reversibility and short-term quasi-acclimation of *Daphnia magna*. PLOS One 13 (12), e0209705.

Na, J., Song, J., Achar, J.C., Jung, J., 2021. Synergistic effect of microplastic fragments and benzophenone-3 additives on lethal and sublethal *Daphnia magna* toxicity. J. Hazard. Mater. 407, 123845.

Nelms, S.E., Galloway, T.S., Godley, B.J., Jarvis, D.S., Lindeque, P.K., 2018. Investigating microplastic trophic transfer in marine top predators. Environ. Pollut. 238, 999–1007.

OECD, 2018. Implementing the OECD Principles on Water Governance: Indicator Framework and Evolving Practices. OCDE, Paris, France.

Pan, Y., Yan, S., Li, R., Hu, Y., Chang, X., 2017. Lethal/sublethal responses of *Daphnia magna* to acute norfloxacin contamination and changes in phytoplankton-zooplankton interactions induced by this antibiotic. Sci. Rep. 7, 40385.

Pau, C., Serra, T., Colomer, J., Casamitjana, X., Sala, L., Kampf, R., 2013. Filtering capacity of *Daphnia magna* on sludge particles in treated wastewater. Water Res. 47 (1), 181–186.

Provencher, J.F., Ammendolia, J., Rochman, C.M., Mallory, M.L., 2018. Assessing plastic debris in aquatic food webs: what we know and don't know about uptake and trophic transfer. Environ. Rev. 27 (3), 304–317.

Redondo-Hasslerharm, P.E., Falahudin, D., Peeters, E.T.H.M., Koelmans, A.A., 2018. Microplastic effect thresholds for freshwater benthic macroinvertebrates. Environ. Sci. Technol. 52 (4), 2278–2286.

Riessen, H.P., Linley, R.D., Altshuler, I., Rabus, M., Sollradl, T., Clausen-Schaumann, H., Laforsch, C., Yan, N.D., 2012. Changes in water chemistry can disable plankton prey defences. Proc. Natl. Acad. Sci. Unit. States Am. 109, 15377–15382. https://doi.org/10.1038/s41598-019-40777-2.

Rist, S., Baun, A., Hartmann, N.B., 2017. Ingestion of micro-and nanoplastics *in Daphnia magna*–quantification of body burdens and assessment of feeding rates and reproduction. Environ. Pollut. 228, 398–407.

Scherer, C., Brennholt, N., Reifferscheid, G., Wagner, M., 2017. Feeding type and development drive the ingestion of microplastics by freshwater invertebrates. Sci. Rep. 7 (1), 1–9.

Seda, J., Petrusek, A., 2011. *Daphnia* as a model organism in limnology and aquatic biology: introductory remarks. J. Limnol. 70 (2), 337–344.

Serra, T., Barcelona, A., Soler, M., Colomer, J., 2018. *Daphnia magna* filtration efficiency and mobility in laminar to turbulent flows. Sci. Total Environ. 621, 626–633.

Serra, T., Müller, M.F., Colomer, J., 2019a. Functional responses of *Daphnia magna* to zero-mean flow turbulence. Sci. Rep. 9 (1), 1–11.

Serra, T., Soler, M., Pous, N., Colomer, J., 2019b. *Daphnia magna* filtration, swimming and mortality under ammonium, nitrite, nitrate and phosphate. Sci. Total Environ. 656, 331–337.

Serra, T., Barcelona, A., Pous, N., Salvadó, V., Colomer, J., 2020. Synergistic effects of water temperature, microplastics andammonium as second and third order stressors on *Daphnia magna*. Environ. Pollut. 267, 115439.

Sokal, R.R., Rohlf, F.J., 1995. Biometry. WH Freeman, New York, NY.

Sun, J., Dai, X., Wang, Q., van Loosdrecht, M.C., Ni, B.J., 2019. Microplastics in wastewater treatment plants: detection, occurrence and removal. Water Res. 152, 21–37.

Teuten, E.L., Saquing, J.M., Knappe, D.R., Barlaz, M.A., Jonsson, S., Björn, A., Rowland, S.J., Thompson, R.C., Galloway, T.S., Yamashita, R., Ochi, D., Watanuki, Y., Moore, C., Viet, P.H., Tana, T.S., Prudente, M., Boonyatumanod, R., Zakaria, M.P., Akkhavong, K., Ogata, Y., Hirai, H., Iwasa, S., Mizukawa, K., Hagino, Y., Imamura, A., Saha, M., Takada, H., 2009. Transport and release of chemicals from plastics to the environment and to wildlife. Philos. Trans. R. Soc. Lond. Ser. B 364 (1526), 2027–2045.

Thompson, R.C., Moore, C.J., Vom Saal, F.S., Swan, S.H., 2009. Plastics, the environment and human health: current consensus and future trends. Philos. Trans. R. Soc. Lond. Ser. B 364 (1526), 2153–2166.

Urban-Malinga, B., Jakubowska, M., Białowas, M., 2021. Response of sediment-dwelling bivalves to microplastics and its potential implications for benthic processes. Sci. Total Environ. 769, 144302.

Wallace, W.G., Estephan, A., 2004. Differential susceptibility of horizontal and vertical swimming activity to cadmium exposure in a gammaridean amphipod (Gammarus lawrencianus). Aquat. Toxicol. 69 (3), 289–297.

Wickramarathna, L.N., Noss, C., Lorke, A., 2014. Hydrodynamic trails produced by *Daphnia*: size and energetics. PLOS One 9 (3), e92383.

Williamson, C.E., Fischer, J.M., Bollens, S.M., Overholt, E.P., Breckenridge, J.K., 2011. Toward a more comprehensive theory of zooplankton diel vertical migration: integrating ultraviolet radiation and water transparency into the biotic paradigm. Limnol. Oceanogr. 56 (5), 1603–1623.

Wright, S.L., Rowe, D., Thompson, R.C., Galloway, T.S., 2013. Microplastic ingestion decreases energy reserves in marine worms. Curr. Biol. 23 (23), R1031–R1033.

Yuan, W., Zhou, Y., Chen, Y., Liu, X., Wang, J., 2020. Toxicological effects of microplastics and heavy metals on the *Daphnia magna*. Sci. Total Environ. 746, 141254.

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