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Mediated food and hydrodynamics on the ingestion of microplastics by *Daphnia magna**

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Abstract

There is consensus on the need to study the potential impact microplastics (MP) have on freshwater planktonic organisms. It is not yet fully understood how MP enter the aquatic food web or the effect they have on all the trophic levels. As a result of the potential for MP to accumulate throughout food webs, there is increasing interest in evaluating their fate in a variety of environmental conditions. This study investigated the variability in the ingestion of MP to food ratios and the exposed time of MP to *Daphnia magna* in non-sheared and sheared conditions. The sheared environment provided *Daphnia magna* with the conditions for optimal filtering capacity. Regardless of the ratios of MP concentration to food concentration (MP:Food), the filtration capacity of the *Daphnia magna* was enhanced in the sheared experiments. In both the sheared and non-sheared experiments, filtration capacity decreased when the ratios of MP to food concentration and the exposure times to MP were increased. Mortality was mainly enhanced in the non-sheared conditions at higher MP concentrations and exposure times to MP. No mortality was found in the sheared conditions for the exposure times studied. Therefore, in aquatic systems that undergo constant low sheared conditions, *Daphnia magna* can survive longer when exposed to MP than in calm conditions, provided food concentrations do not limit their capacity to filter.

Keywords: Microplastics, Ingestion, Hydrodynamics, Filtration Capacity, *Daphnia magna*

Declaration of interest: none.

Highlights:

1) The filtration capacity of *D. magna* is enhanced in sheared flows.
2) High MP concentrations lead to a reduction in D. *magna* filtration capacity.
3) The amount of exposure time to MP reduces the filtration capacity of *D. magna*.

1. Introduction

Plastic has become virtually indispensable in our daily lives. In 2016, a total of 335 million tons of plastic was produced worldwide (Eerkes-Medrano et al., 2015; Horton et al., 2017; Phuong et al., 2016; Plastics Europe, 2018), leading to an ever-increasing quantity of plastic waste, much of which ends up in marine and freshwater environments (Andrady et al., 2003). Microplastics, MP, are globally recognized as an emerging environmental contaminant (Hurley et al., 2018). Although large plastic items initially attracted most attention, microscopic plastic fragments have also been proven to contaminate sediments in beaches, and estuarine and subtidal areas (Thompson et al., 2004). Based on current trends, a future scenario predicts that forty billion tons of plastic will be produced by 2050 (Zalasiewicz et al., 2016). Many authors have studied the effect MP (<5mm in size) have on marine organisms (Andrady et al., 2011; Beiras et al., 2018; de Sá et al., 2018; Van Cauwenberghe et al., 2015; Wright et al., 2013) and, to a lesser extent, on freshwater organisms (Eerkes-Medrano et al., 2015, Rist et al., 2017; Scherer et al., 2017; Wagner et al., 2014). Microplastics often contain chemicals that were incorporated during manufacture (Fries et al., 2013) or absorbed from the environment (Velzeboer et al., 2014) and which may be toxic to organisms, including humans (Rist el al., 2018), if ingested (Rochman et al., 2013; Tanaka et al., 2013). Moreover, MP ingestion may have physical consequences such as obstructing the gut of the organism, or disrupting feeding and digestion (Canniff and Hoang, 2018; Cole et al., 2013; Setälä et al., 2014) and thus affecting the fitness of the organism, i.e., the reproduction of organisms can be altered by decreasing the oocyte number or sperm velocity and also increasing haemocyte mortality (Besseling et al., 2014; Paul-Pont et al., 2016; Sussarellu et al., 2016).

Even though the great majority of plastic debris ends up in the oceans (Rochman et al. 2013), freshwater ecosystems are often more exposed to MP because of their greater proximity to densely human-populated areas, along with receiving effluent from wastewater treatment plants (Eerkes-Medrano et al., 2015). However, even sites with low flow or situated further down river have also been found to have high amounts of MP as, although these sites have a low population density, they are exposed to high agricultural use (Kapp and Yeatman, 2018). The low density, shape and size of MP enable them to travel extremely easily through water bodies, often remaining suspended in the pelagic zone.

One mounting concern about MP is because of their small size. They can enter the aquatic food web at very low levels (e.g. plankton) and later affect higher trophic levels (Besseling et al., 2014; Setälä et al., 2014; Wright et al., 2013). As such, species that play important roles in aquatic environments are of particular concern. For example, *D. magna* is a filter feeder key zooplanktonic species present in many freshwater ecosystems and plays an important role in transferring energy through the different levels of the food webs (Elser et al., 2000). Moreover, as *D. magna* also perform diel vertical migration (Lampert et al., 1993), they potentially contribute to transporting MP further to predators occupying different depths of the water column (Wright et al., 2013).

As *D. magna* have been reported to ingest particles ranging from nanometres to micrometres in size (Burns, 1968; Pau et al., 2013; Serra et al., 2014; Serra et al., 2018), they are likely to ingest MP. Indeed, in laboratory conditions, *D. magna* have been proven to ingest MP (Canniff and Hoang, 2018; Ogonowski et al., 2016; Rist el al., 2017; Scherer et al., 2017, Wagner et al., 2014). However, MP ingestion has been observed to significantly decrease in the presence of additional particles of natural matter, such as algae or sand (Ogonowski et al., 2016; Rist el al., 2017; Scherer et al., 2017), thus reducing exposure to potentially toxic MP.

To assess the potential effects MP have on *D. magna*, the present study aims to evaluate the mediation in the MP:food ratio using two new parameters. First, by investigating the role hydrodynamic conditions play on the *D. magna* ingestion rate of MP, and second, by exploring *D. magna* filtration efficiency under differing exposure times to MP. The hypotheses were as follows: 1) the filtration capacity of *D. magna* is higher in sheared conditions compared to quiescent flows, 2) the presence of high MP concentrations leads to a low filtration capacity of *D. magna* and 3) increasing exposed time to MP reduces filtration capacity of *D. magna*.

2. Methods

2.1. *Daphnia magna*.

The age of the individuals was chosen based on optimizing the filtering efficiency as a function of the characteristic body length of the Daphnia individuals which, in turn, is optimal around a week of life (Wickramarathna et al. 2014).

D. magna were collected from three laboratory cultures that were maintained in 40 L containers at a temperature of 20.0 ± 0.5 °C, and at a natural daylight photoperiod and continuous air supply (to avoid anoxia) for two years at the University of Girona (Spain). Individuals were fed three times a week (Monday, Wednesday and Friday) with a mixture of dry spirulina powder (100% *Spirulina 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., 2018; Serra et al., 2019a; Serra et al., 2019b). Mineral water rich in calcium (constant value of 35.7 mg/L) was used to avoid calcium depletion (Riessen et al., 2012). The individuals used in the experiments were seven days old. The age of the individuals was chosen based on optimizing the filtering efficiency as a function of the characteristic body length of the *Daphnia* individuals (Serra et al., 2018) which, in turn, is maximized in the first two weeks of life (Wickramarathna et al. 2014). To control the age of the individuals, ephippia eggs from the laboratory cultures were hatched and left in a dark box for ten days and then continuously lighted afterwards. The *Daphnia* new-borns were fed with spirulina daily until day 7. By analysing a video recording of 10 *D. magna* with the ImageJ software, the mean body length of the individuals was determined at the start of the experiments (2.0 ± 0.2 mm) and during the evolution of the experiments. During the experiments in both non-sheared, (shear rate of $G=0\ s^{-1}$ i.e., calm conditions), and sheared, shear rate of $G=2.1\ s^{-1}$ (Serra et al., 2018), and especially for those with high concentrations of MP, the mean body length of the individuals did not show any significant increase (Table 1). All tests, protocols and analyses with *D. magna* were carried out in accordance with the international 'OECD/OCDE Guidelines for the Testing' (OECD/OCDE, 1998) and the `Protocol for the sampling and laboratory testing of invertebrates' code ML-L-I-2013 (ML-L, 2013) of the Ministerio de Agricultura, Alimentación y Medio Ambiente of the Spanish Government.

2.2. *Microplastics and feeding particles.*

Standard spherical polystyrene microparticles with a density of 1.05 $g{\cdot}mL^{-1}$ were provided by SonTek/Xylem Inc (San Diego, USA) and were used for the experiments. A spirulina suspension was used to feed the *Daphnia*. To make the spirulina suspension, 1 g of dry spirulina powder (with a density of 1.153 $g{\cdot}mL^{-1}$) was mixed with 1 L of bottled mineral water for 1 min and left for 1 h so that the bigger spirulina particles settled. The supernatant was used as the spirulina suspension for the experiments at a concentration of 50 mL spirulina suspension per litre of bottled mineral water, for which the final food had a particle volume concentration of 8.0 ± 0.5

μL L^{-1} (mass concentration of 8.4 ± 0.5 mg L^{-1}). Therefore, the initial volume concentration for the 0 % MP:100 % food concentration was 8 ± 0.5 μL L^{-1} and, consequently, the initial volume concentration for the 100% MP:0 % food concentration was chosen to be the same, that is, 8.0 ± 0.1 μL L^{-1} (mass concentration of 9.2 ± 0.1 mg L^{-1}) of MP at their maximum concentration. The particle concentration of spirulina and MP in the desired particle size range was calculated by integrating the concentration of the particles within the range. Since *D. magna* primarily feed on particles <30 μm in diameter, the volume concentration of particles within the range of 2.5 to 30 μm was calculated and used as a proxy to evaluate particle removal (Serra et al., 2018). Cladocera ingest particles when their size overlaps those of the organic particles they feed on (Arruda et al., 1983; Gliwcz, 1990). This implies that *D. magna* feed on both MP and algae since *D. magna* individuals lack the ability to differentiate food quality.

The initial particle volume distributions of both the spirulina and MP presented particles in the *Daphnia* ingestible range (< 30 μm) (Fig 1). Spirulina also presented larger particles of diameters > 100 μm and MP presented very small particles diameters < 5 μm. Both types of particles presented similar particle distributions in the range between 5 and 100 μm (Fig. 1). The volume concentration was used as the key parameter to characterize the concentration of food and MP and has been used extensively in laboratory experiments (Müller et al., 2018; Serra et al., 2018; Serra et al., 2019a and Serra et al., 2019b). The water in both the sheared and non-sheared experiments was changed daily throughout the experiments (i.e., on days 2, 3, 4, 5, 6 and 7). The experiments were conducted at a temperature of 20 ± 1ºC and a photoperiod of 12h:12h (light:dark).

2.3. *Experimental procedures.*

The sheared experiments were carried out in a plexiglass container filled with 600 mL of water, while the non-sheared experiments, where the underlying mechanism of particle transport is the sedimentation of particles, were conducted in glass containers filled with 2 L of water and maintained at a constant temperature of 20 $\pm$ 0.2 °C. Five different MP:algae ratios were fixed: 100% MP:0% food concentration (hereafter 100:0 MP), 75% MP:25% food suspension (hereafter 75:25 MP), 50% MP:50% food suspension (hereafter 50:50 MP), 25% MP:75% food suspension (hereafter 25:75 MP) and 0% MP:100% food suspension (hereafter 0:100 MP). The concentrations of polystyrene MP varied between 2.1 mg L^{-1} (25:75 MP) and 8.4 mg L^{-1} (100:0 MP), or between 258 particles mL^{-1} (25:75 MP) and 1031 particles mL^{-1} (100:0 MP), thus aligned with the MP particle concentration of 3-3000 particles mL^{-1} used by Scherer et al., (2017), and mass MP concentrations of 0.1 to 1 mg L^{-1} employed by Rist et al. (2017). The ratios were achieved by mixing the spirulina suspension with the MP suspension at the corresponding proportions, achieving final volumes of 0.6 L (sheared experiments) and 2 L (non-sheared experiments). Once the corresponding ratios had been achieved, 30 *D. magna* were carefully added to the sheared experiment and 100 *D. magna* to the non-sheared experiment, thus achieving a final *D. magna* concentration of 50 ind L^{-1}.

For each MP:algae ratio, three replicates were submitted to a sheared environment characterized by a fluid velocity of 3.13 cm s^{-1}, a shear rate of $G = 2.1\ s^{-1}$ and a turbulent kinetic energy dissipation of $4.4\ 10^{-6}\ m^2 \cdot s^{-3}$. Shear was chosen to operate within a low shear flow regime in which filtration was enhanced up to 2.6 times that of a steady flow (Serra et al., 2018). This sheared environment was made possible by using a Couette flow device; as described in Serra et al. (2018). Three replicates were carried out for the non-sheared experiments, in which sedimentation took place. Replicates of the non-sheared conditions were made concurrently, while the three replicates of the sheared experiments were carried out consecutively. All sheared and non-sheared experiments began with the *D. magna* being exposed to the food concentration for 24 h, and then continued for seven days at the five fixed MP:algae ratios.

For both hydrodynamic conditions (sheared and non-sheared experiments), three replicate control experiments, i.e., experiments without *D. magna*, were performed to quantify the particle sedimentation in the five MP:algae ratio experiments. The experiments lasted for either 12 h of measurements or until the steady state was achieved.

2.4. *Filtration capacity.*

To quantify particle removal by the *D. magna*, the volume concentration of particles, C_t, removed from the suspension~~,~~ within their feeding range (2.5 – 30 μm) (Müller et al., 2018; Serra et al., 2018; Serra et al., 2019a; Serra et al., 2019b) was calculated and its ratio over the initial particle volume concentration, C_0, was analysed. This ratio was calculated with the following expression:

$$\frac{C_t}{C_o},$$

where C_t is the volume concentration in suspension at time t, and C_o is the initial volume concentration.

The particle volume concentration was measured with a laser particle size analyser (Lisst-100x, Sequoia Inc.) at working particle diameters between 2.5 and 500 μm (Serra et al., 2001). Pau et al. (2013) showed that spirulina particle ingestion by *D. magna* caused an exponential decrease in suspended particle concentrations in non-sheared experiments carried out in the presence of *D. magna*~~,~~ so that, after 4 h, the initial concentration of spirulina decreased by e^{-1} = 0.37.

Henceforth, they fixed 4 h as the characteristic time for analysing the ingestion rate of *D. magna*. Given that Serra and Colomer (2016) and Serra et al. (2018) showed that an optimal particle concentration for *D. magna* development was 0.16 μl/L per individual per day, that same concentration rate was used for the 0:100 food experiments as well as for the 100:0 MP experiments. Therefore, for the 25:75 MP experiments, a volume concentration of 0.04 μl/L of MP per individual per day was used. For the 100:0 MP experiments, the particle number concentration was calculated assuming a particle median diameter of 19.8 μm, which resulted in being 1031 particles mL^{-1}, (i.e., within the range used by other authors (Scherer et al., 2017)), and consequently the number of particles for the 25:75 MP experiments was 258 particles mL^{-1}.

Since the suspended particle volume concentration C_t, decreased exponentially with time, it can be expressed as follows:

$$C_t = C_0 e^{-kt}$$

where C_o is the suspended particle volume concentration at the beginning of the experiment, t is the exposed time and k is the rate of particle removal by both sedimentation (k_s) and *D. magna* filtration (k_{Dph}), i.e., $\mathrm{k} = \mathrm{k_s} + \mathrm{k_{Dph}}$. Therefore, it is possible to write k as

$$k = -\frac{1}{t}\ln\left(\frac{C_t}{C_0}\right)$$

and k_s can be calculated from the control experiment without *D. magna* (where $k_{Dph} = 0$), so that k_{Dph} can be determined for the rest of the experiments. The rate of particle removal by *D. magna* filtration depends on the filtering rate of each *D. magna* individual (F, in mL ind^{-1} L^{-1}) and the *D. magna* concentration (C_{Dph}) according to $k_{Dph} = F\ x\ C_{Dph}$, (Pau et al., 2013), thus the filtration capacity can be obtained from

$$F = \frac{k_{Dph}}{C_{Dph}}$$

Therefore, the feeding rates of the *D. magna* were investigated for three parameters: sheared and non-sheared conditions, dependence on ~~the ratios of~~ MP to food concentration ratios, and time of exposure to MP.

3. Results

In the control experiments without *D. magna* and for both the non-sheared and the sheared experiments, an exponential decrease in C_t/C_o was observed with time regardless of the MP:food ratio (Fig. 2). The C_t/C_0 decreased faster as the percentage of MP increased. After 12 h of experiment, the final ratio of C_t/C_0 was greater for the sheared conditions (Fig. 2b) than for the non-sheared (Fig. 2a). The non-sheared 100:0 MP experiment proved full sedimentation of MP at 6 h (Fig. 2a).

D. magna feed on both food and MP at a rate ~~that was~~ dependent on both the MP:food concentrations and hydrodynamic conditions. As for the control experiments, both the non-sheared (Fig. 3a) and sheared experiments (Fig. 3b) showed that in the presence of *D. magna*~~,~~ the C_t/C_o was lower than in the experiments without *D. magna* (Fig. 2), proving the individuals ingested ~~the ingestion of~~ both algae and MP by the individuals. For both 0:100 MP and 100:0 MP, the C_t/C_o for sheared experiments was greater than that in the non-sheared conditions.

Daphnia had higher filtration capacity (F) in the sheared than in the non-sheared experiments (Fig. 4a and 4b), and in both the non-sheared and sheared experiments~~,~~ *Daphnia* exposed to higher concentrations of MP reduced their filtration capacity. In addition, this effect was intensified at longer exposed times to the MP (Fig. 4c and 4d). In the non-sheared experiments, during the first week of exposure to MP, the 100:0 MP at day 5 and the 75:25 MP and 100:0 MP at day 7 could be considered lethal because the filtration $F_{MP:FOOD}/F_{FOOD}$ could not be measured as all *Daphnia* were found dead. For days 1 to 4, the $F_{MP:FOOD}/F_{FOOD}$ decreased with increasing the MP:Food ratio. In the first week of the non-sheared experiments, the $F_{MP:FOOD}/F_{FOOD}$ decreased with increasing the MP:Food ratio but no lethal MP:Food ratio was found since the $F_{MP:FOOD}/F_{FOOD}$, was greater than zero. For the non-sheared and sheared experiments and at the 0% MP, the filtration capacity was constant during the seven days of the experiment (Fig. 4c and 4d). For the non-sheared experiments with the presence of MP, the filtration remained nearly constant during the first three days of exposure (Fig. 4c) then decreased afterward for longer exposure times (Fig. 4c). The decrease was greater as the percentage of MP increased (Fig. 4c). Similar results were found for the sheared experiments. However, the filtration remained nearly constant for a longer period, i.e., four days (Fig. 4d).

4. Discussion

4.1. *Filtration in sheared and non-sheared conditions.*

The hydrodynamic flow regime was found to be a crucial parameter in determining the performance of *D. magna* because it modified their filtering capacity on a mixture of food and MP. As described by Rist et al. (2017), *Daphnia* ingested both MP and food thus proving their lack of ability to differentiate food quality. A reduction of the particle sedimentation was found in the sheared experiments therefore proving that MP could remain in suspension for longer times. In comparison to stagnant conditions (no shear), the low shear dominated flow regime enhanced filtration efficiency for all the exposure times and MP concentrations.

Rist et al. (2017) found that when daphniids were exposed to 100 nm and 2 μm polystyrene particles, their ingestion was significantly lower than in the algae-only experiment with the reduction being 20.5 % and 6.8 % for a 1 d exposure time. Our results showed that in the non-sheared conditions, for an exposed time to MP of 4 h (first day), the reduction in filtration varied from 2.2 % to 8.2 % for the ratio concentrations of 25:75 MP and 100:0 MP, respectively. In the sheared conditions with the same particle range, the reduction in filtration was lower and varied from 1.9 % to 2.6 % for the ratio concentrations 25:75 MP and 100:0 MP, respectively. ~~However~~Nevertheless, the ingestion of MP does not imply a toxic effect for the organism (Beiras et al., 2018). Canniff and Hoang (2018) found that the ingestion of polyethylene microbeads by *D. magna* does not endanger their survival and reproduction. However, the combination of MP with pollutants like heavy metals (e.g., Cu, Zn, Ni) increases metal accumulation and toxicity to aquatic organisms (Kim et al., 2017; Brennecke et al., 2016). For example, Khan et al. (2015) found that exposing zebrafish (*Danio rerio*) to Ag-incubated MP reduced the uptake of Ag, together with higher proportions of Ag in the intestines of the zebrafish.

D. magna usually feed on organic (bacteria and algae) and inorganic (sludge) particles that lie within the particle-size range of 1-50 μm (Ebert, 2005; Serra et al., 2018). Nevertheless, they can also ingest MP of different types and sizes. In the gut of daphniids, authors have reported the presence of ingested polystyrene beads of 20 nm and 1 μm (Rosenkranz et al., 2009) and of 100 nm and 2 μm (Rist el al., 2017), polystyrene spheres of 1 μm and 10 μm (Scherer et al., 2017), and also fluorescent MP like polymethyl methacrylate of 29.5 μm (Imhof et al., 2013) or polyethylene of 6-75 μm (Canniff and Hoang, 2018). Recently, Jemec et al. (2016) proved that *D. magna* can ingest elongated microplastic textile fibres of 300 μm~~, and also~~ as well as some very large twisted MP fibres around 1400 μm. Those larger microplastic fibres did not produce any daphnid mortality after ~~2~~ two days of treatment in experiments where daphniids were pre-fed with algae. Our results show that daphniids present an inability to differentiate MP from food, as was also found by Imhof et al. (2013), indicating a higher risk of MP accumulation in zooplankton feeders thriving in environments presenting low shear mixing. Indeed, on day 1, after 4 h of exposure to MP, the filtration capacity of *D. magna* in the low sheared environments was 3.48 to 1.94 times the filtration capacity in the non-sheared environment for the ratio concentrations of 100:0 MP and 25:75 MP, respectively. In terms of filtration efficiency, on day 1 and ~~so on~~there after, filtration deteriorated more significantly upon increasing the MP concentrations in the non-sheared environment, in comparison to the sheared environment.

The increased ingestion of MP by daphniids in low shear environments reveals a phenomenon never described before. Low shear flows increase the contact frequency between daphniids and suspended particles, thus enhancing *Daphnia magna* ingestion. That is, since the MP are ubiquitously present in the aquatic ecosystems (Scherer et al., 2017), persistent ingestion of MP by the geographically widespread zooplankter *D. magna* in sheared environments might affect organisms in higher trophic levels since the capture of daphniids is promoted in sheared environments (Kiørboe and Saiz, 1995; Romero et al., 2012). In addition, for the same concentration of MP in sheared environments, filtration capacity is higher since shear promotes the interaction between the daphniids and the MP and food particles. Shear enhances daphnid mobility (Serra et al., 2018) which, in turn, results in optimal fitness, higher survival rates, and better conditions for ingestion. Finally, ~~we~~ it can be speculated that during the diel vertical migration in the water column of freshwater lakes, schooling of *D. magna* (they migrate up and down in groups), can favour the ingestion of suspended MP by each individual. During the movement of the schooling, the mean turbulent energy dissipation has been estimated to vary between 3.4 10^{-6} and 1.8 10^{-6} m^2/s^3, depending on the size of the individuals (Wickramarathna et al., 2014) which coincides with the 4.4 10^{-6} m^2/s^3 imposed turbulent energy dissipation in the sheared experiments in which ingestion was promoted. The results of the present study indicate that the daphniids thriving in the sheared environment present higher filtering efficiencies for all days of exposure and for all concentrations of MP, therefore, corroborating the first hypothesis of this study: that the filtration capacity of *D. magna* is higher in sheared conditions compared to quiescent flows.

4.2. *Filtration in the presence of variable MP to food concentrations.*

Regardless of the sheared conditions and exposure times, the higher the concentration of MP, the lower the capacity of *D. magna* to filtrate. In terms of ingested mass, in the sheared conditions and during the first 4 h exposure, the ingestion of MP by *D. magna* in the higher 100:0 MP mass concentration was 3.42 times higher than that of the 25:75 MP mass concentration. As pointed out by Scherer et al. (2017) and Rist et al. (2017), and presuming non-selective feeding, a high concentration of MP leads to a higher encounter rate, which results in an increased feeding rate. This process is exacerbated under sheared conditions. Indeed, for the first day of exposure, after 4 h of exposure to MP, the filtering efficiency of the daphniids in the experiments with MP was only 49% in the quiescent environment and 86~~ ~~% in the sheared.

Overall, the relationship between the efficiency of filtering ~~capacity~~ capacities and the MP concentrations is quadratic and in accordance with the literature (DeMott, 1982; Porter et al. 1982; Scherer et al., 2017; Serra et al., 2014), indicating that given the inability to distinguish between food and MP, the reduction in food ingested has a significant impact on *D. magna* feeding. Contrary to the findings of Aljaibachi and Callaghan (2017) who reported a significant negative impact of MP uptake in the presence of algae, ~~our~~ the results of this study show that filtration was severely reduced in the presence of high MP concentrations, even when food was present. The difference may be attributed to the MP uptake, since Aljaibachi and Callaghan (2017) fed 24-h starved individuals mass concentrations 8.3 10^{-5} to 5.3 10^{-3} times lower than those used in ~~our~~ the experiments in the present study. In addition, when MP were added in suspension, Rist et al. (2017) found that after 24 h of exposure the feeding rates for 100 nm and 2 μm Polystyrene plastics were 80% and 93% lower than those for experiments with algae only. In ~~our~~ the experiments in the present study, when MP were added and after 28 h of exposure (day 2), filtration rates for the experiments 75:25 MP, 50:50 MP and 25:75 MP were 0.88, 0.93 and 0.98 lower than those for the experiments with algae only, thus proving that the presence of MP concentrations leads to lower filtration capacities of *D. magna* than filtrations capacities for food only.

4.3. *Filtration on exposure times to MP.*

Only a few studies report the effects of MP ingestion by zooplankton for time exposures greater than one day. Jemec et al. (2016) proved that *D. magna* can ingest 300 μm and 1400 μm microplastic textile fibres without producing any mortality after ~~2~~ two days of treatment. ~~Also~~Likewise, Canniff and Hoang, (2018) found *D. magna* can ingest 63-75 µm plastic microbeads that accumulated in their gut but did not affect mortality. Time exposures of 72 h for *Allorchestes Compressa* to polyethylene MP proved no mortality of individuals along with ingestion of MP in the first 12 h of exposure (Chua et al., 2014). Adult *Daphnia* exposed to MP for 21 d showed mortality after 7 d of exposure in treatments where the concentrations of MP were varied (Aljaibachi and Callaghan, 2018), and Rist et al. (2017) showed that after 21 d of exposure to MP, *D. magna* body burdens of particles (mass/animal) of 100 nm or 2 μm increased as MP concentrations increased (from 0.1 mgL^{-1} to 1 mgL^{-1}). The results of the present study support the third hypothesis of increasing exposed time of MP reduces the filtration capacity of *D. magna*. The filtration efficiency of daphniids decreased with increasing exposure times for all MP concentrations. In addition, exposure to MP in non-sheared conditions ~~results~~ resulted in the mortality of daphniids after 5 d at MP to food ratios larger than 75:25 MP, while after 7 d the critical ratio ~~is~~ was 50:50 MP. No mortality was found when daphniids were exposed to sheared conditions, although at the 100:0 MP ratio, i.e., in an environment with full MP presence, the filtering capacity of daphniids on the 7th day of exposure was reduced to 16% of that for the ratio 0:100 MP, i.e., in an environment without MP. Furthermore, for the 75:25 MP it was reduced to 51% for ~~that of~~ the ratio 0:100 MP. Lower reductions in the filtering efficiencies over time were found for the sheared conditions due to the favourable hydrodynamic conditions *Daphnia* had to filter, given that shear enhances the contact between *Daphnia* and MP.

5. Conclusions

Under different controlled characteristics in laboratory tests, the significant phenomena of polystyrene microplastics being ingested by the freshwater zooplanktonic *D. magna* was evaluated. The findings support three hypotheses: that *D. magna* filtration capacity is higher in low shear environments than in quiescent flows, that higher MP concentrations lead to a reduction in *D. magna* filtration capacity, and that at higher exposure times to MP *D. magna* ~~their~~ filtration capacity is reduced. This was especially so for exposure times greater than three days in a quiescent environment with high concentrations of microplastics, and more than four days in sheared environments. Lethal conditions were observed in quiescent flows after 5 d of exposure to MP:food ratios larger than 75:25 MP, while after 7 d the critical ratio was 50:50 MP. Overall, it can be concluded that in the freshwater ecosystems ~~at~~ in which MP and *D. magna* are present, the shear produced by mixing can increase the filtration capacity of the individuals, which might affect organisms at higher trophic levels.

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Captions to Figures.

Figure 1. Particle distribution of the initial concentration of spirulina (black dots) and MP (white dots). The limit of *Daphnia magna* particle diameter ingestion range is expressed with a discontinuous line at 30 µm.

Figure 2. a) Temporal evolution of the ratio C_t/C_o for the experiments in the non-sheared conditions, for 12 h of experiment. Data corresponds to the control experiments in non-sheared conditions, without *D. magna*, for the experimental MP:food concentrations of 0:100 MP, 25:75 MP, 50:50 MP, 75:25 MP and 100:0 MP. The mean and standard deviations for the three replicates are included. b) Temporal evolution of the ratio C_t/C_o for the experiments in the sheared conditions, for 12 h of experiment. Data corresponds to the control experiments in the sheared condition, without *D. magna*, for the experimental MP:food concentrations of 0:100 MP, 25:75 MP, 50:50 MP, 75:25 MP and 100:0 MP. The mean and standard deviations for the three replicates are included.

Figure 3. a) Temporal evolution of the ratio C_t/C_o for the experiments in the non-sheared conditions, for 12 h of experiment, for algae only and algae+Daphniids and also for 100:0 MP (microplastics only) and 100:0 MP+ Daphniids. b) Temporal evolution of the ratio C_t/C_o for the experiments in the sheared conditions, for 12 h of experiment, for algae only and algae+ Daphniids and also for 100:0 MP (microplastics only) and 100:0 MP+ Daphniids. The mean and standard deviations for the three replicates (non-sheared experiment) and for the three replicates (sheared experiment) are shown.

Figure 4. Filtration capacity of daphniids versus concentrations of MP, for different MP:Food ratios, for the times of exposure (days 1, 2, 3, 4, 5 and 7), for both the non-sheared (a) and sheared experiments (b). Filtration capacity efficiency, $F_{MP:FOOD}/F_{FOOD}$, as the ratio between the measured filtration capacity of daphniids in the presence of variable concentrations of MP, and the measured filtration capacity in the presence of food, i.e., experiments without the presence of MP, for different MP:Food ratios, for the times of exposure for both the non-sheared (c) and

sheared experiments (d). The mean and standard deviations for the three replicates of both (non-sheared experiment) and (sheared experiment) are shown. Results had significant statistical differences (ANOVA test) for experiments with different exposure times (99% confidence) but not within replicas.

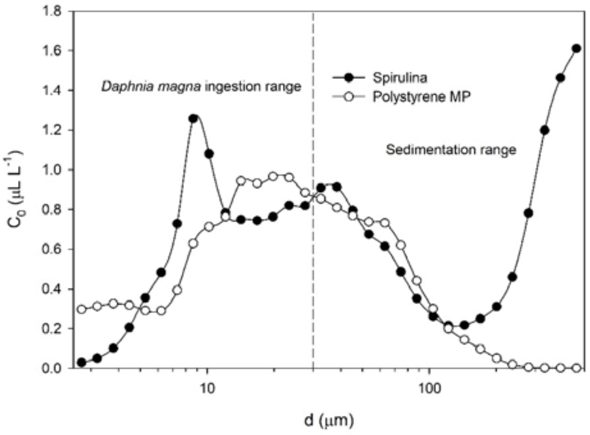

Figure 1

Figure 1. Particle distribution of the initial concentration of spirulina (filled circles) and MP (open circles). The limit of *Daphnia magna* particle diameter ingestion range is expressed with a discontinuous line at 30 µm.

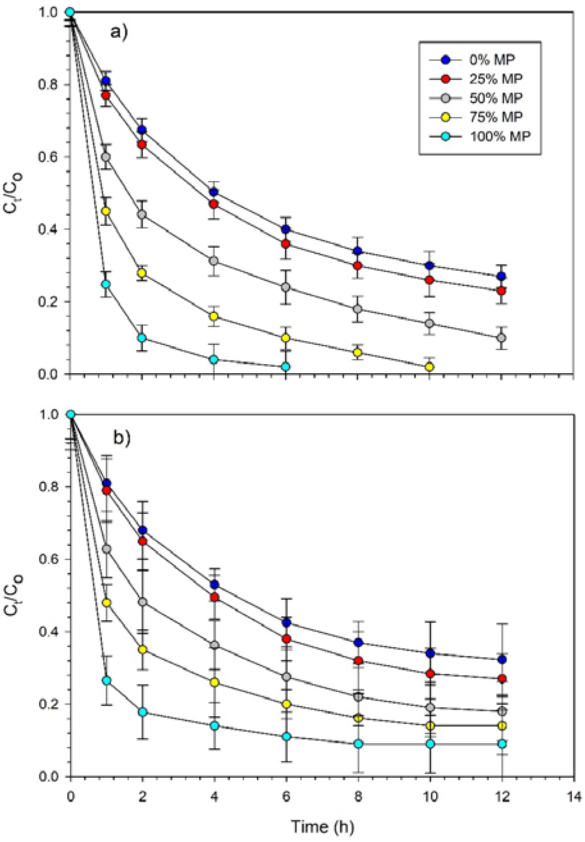

Figure 2

Figure 2. a) Temporal evolution of the ratio C_t/C_o for the experiments in the non-sheared conditions, for 12 h of experiment. Data corresponds to the control experiments in non-sheared conditions, without *D. magna*, for the experimental MP:food concentrations of 0:100 MP, 25:75 MP, 50:50 MP, 75:25 MP and 100:0 MP. The mean and standard deviations for the three replicates are included. b) Temporal evolution of the ratio C_t/C_o for the experiments in the

sheared conditions, for 12 h of experiment. Data corresponds to the control experiments in the sheared condition, without *D. magna*, for the experimental MP:food concentrations of 0:100 MP, 25:75 MP, 50:50 MP, 75:25 MP and 100:0 MP. The mean and standard deviations for the three replicates are included.

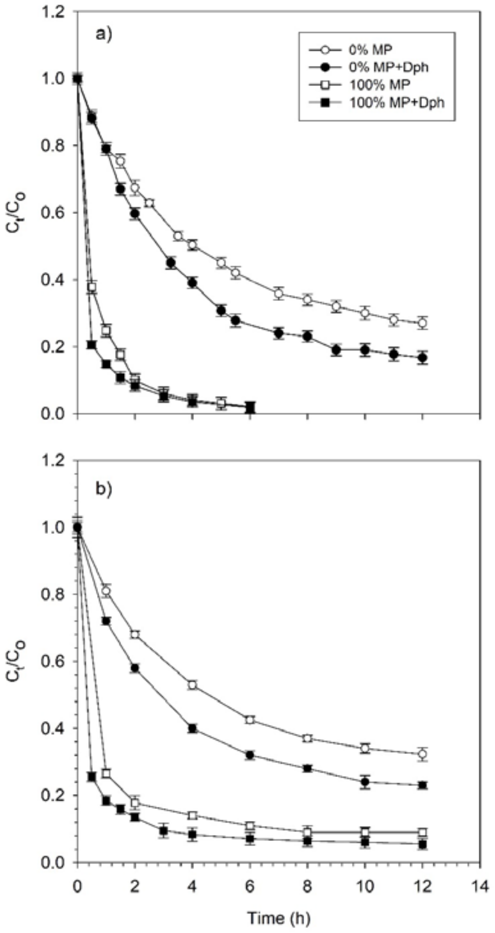

Figure 3

Figure 3. a) Temporal evolution of the ratio C_t/C_o for the experiments in the non-sheared conditions, for 12 h of experiment, for algae only and algae+Daphniids and also for 100:0 MP (microplastics only) and 100:0 MP+ Daphniids. b) Temporal evolution of the ratio C_t/C_o for the experiments in the sheared conditions, for 12 h of experiment, for algae only and algae+ Daphniids and also for 100:0 MP (microplastics only) and 100:0 MP+ Daphniids. The mean and

standard deviations for the three replicates (non-sheared experiment) and for the three replicates (sheared experiment) are shown.

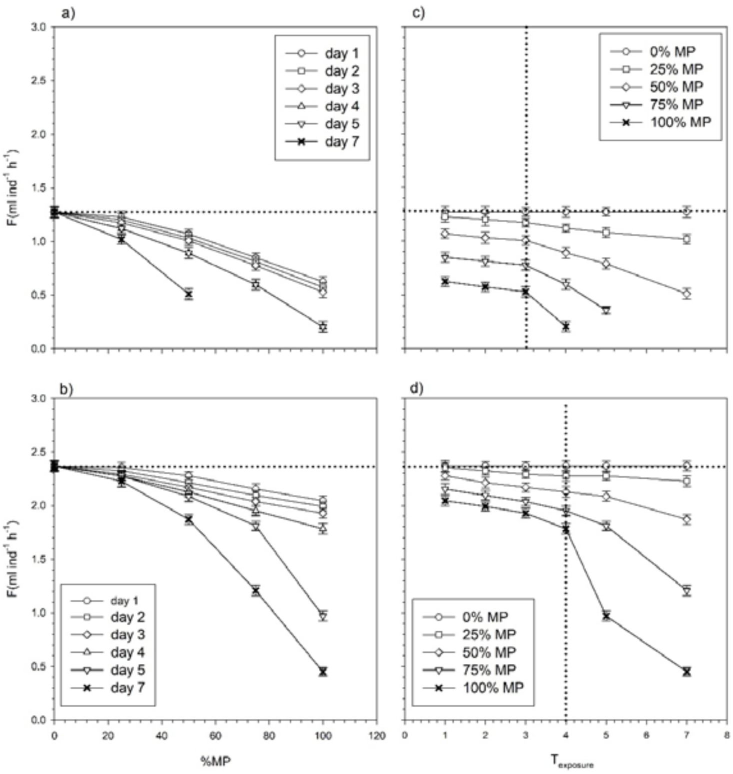

Figure 4

Figure 4. Filtration capacity of daphniids versus concentrations of MP, for different MP:Food ratios, for the times of exposure (days 1, 2, 3, 4, 5 and 7), for both the non-sheared (a) and sheared experiments (b). Filtration capacity efficiency, $F_{MP:FOOD}/F_{FOOD}$, as the ratio between the measured filtration capacity of daphniids in the presence of variable concentrations of MP, and

the measured filtration capacity in the presence of food, i.e., experiments without the presence of MP, for different MP:Food ratios, for the times of exposure for both the non-sheared (c) and sheared experiments (d). The mean and standard deviations for the three replicates of both (non-sheared experiment) and (sheared experiment) are shown. Results had significant statistical differences (ANOVA test) for experiments with different exposure times (99% confidence) but not within replicas.

Table 1. Sizes of *D. magna* at the end of the 7 d of experiments, in which individuals were exposed to MP at the experimental MP:food ratio concentrations of 0:100 MP, 25:75 MP, 50:50 MP, 75:25 MP and 100:0 MP, for both sheared and non-sheared conditions. The body sizes of 10 *D. magna* were analysed with the ImageJ software. The mean body length of the individuals, which was of 2.0 ± 0.2 mm, was determined at the start of the experiments.

MP:food	*0:100 MP*	*25:75 MP*	*50:50 MP*	*75:25 MP*	*100:0 MP*
Non-sheared	2.4 ± 0.2 mm	2.2 ± 0.3 mm	2.0 ± 0.2 mm	2.0 ± 0.2 mm	2.0 ± 0.2 mm
Sheared	2.4 ± 0.2 mm	2.3 ± 0.2 mm	2.0 ± 0.2 mm	2.0 ± 0.2 mm	2.0 ± 0.2 mm

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