




8-2018

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### Recommended Citation

Canniff, Patrick M. and Hoang, Tham C.. Microplastic Ingestion by *Daphnia Magna* and its Enhancement on Algal Growth. *Science of the Total Environment*, 633, : 500-507, 2018. Retrieved from Loyola eCommons, School of Environmental Sustainability: Faculty Publications and Other Works, <http://dx.doi.org/10.1016/j.scitotenv.2018.03.176>

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# Microplastic ingestion by *Daphnia magna* and its enhancement on algal growth

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Editor: D. Barcelo

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## Abstract

The rapid increase in plastic use over the last few decades has resulted in plastic pollution in freshwater and marine ecosystems. However, more attention has been paid to plastic pollution in marine ecosystems than to freshwater ecosystems. This research determined microplastic ingestion by *Daphnia magna* and the potential effect of microplastics on the organism's survival and reproduction. The study also examined the potential of microplastics to enhance algal growth in support of understanding effects of microplastic ingestion on the organism. When exposed to 25, 50, and 100 mg/L fluorescent green polyethylene microbeads at size of 63–75  $\mu\text{m}$ , *D. magna* ingested significant amount of plastic microbeads. The number of ingested beads increased with increasing particle concentration and exposure time. However, no significant effect on survival and reproduction was observed although the gut of *D. magna* was filled with plastic microbeads. In the algal experiment, *Raphidocelis subcapitata* grew more in the exposure media with the present of plastic microbeads than without plastic microbeads. This result suggests that plastic microbeads could serve as substrates for *R. subcapitata* to grow. *Raphidocelis subcapitata* then could be transferred to the organism's gut and provided energy for survival and reproduction. Results of the present study add to the literature of microplastic ingestion by aquatic organisms. Caution should be taken when interpreting hazards of microplastics based on ingestion, such as the measurement unit and the presence of algae in the environment.

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**Keywords:** Microplastics; [Fluorescent green polyethylene microbeads](#); [Microplastic ingestion](#); *Daphnia magna*; *Selenastrum capricornutum*; *Raphidocelis subcapitata*

## 1.1 Introduction

Plastics have a variety of use and applications in commercial and consumer goods. The use of plastic materials has been increasing dramatically during the last six decades. The global plastic production in 1950 was 1.7 million tons ([Plastics Europe, 2015](#)). In 2013, it was estimated to be 299 million tons ([Van Wezel et al., 2016](#)). Most of plastic materials will enter the environment at the end of their life cycle. It has been estimated that between 4.8 and 12.7 million tons of mismanaged plastic waste entered the ocean directly and indirectly via freshwater ways in 2010 ([Jambeck et al., 2015](#)). In the US, approximately, 29.9 million tons of plastic waste were generated in 2013 ([US EPA, 2015](#)). There are different types, shapes, and sizes of plastics that are produced, depending on the application. For example, up to 80% of plastic demand are polyethylene, polypropylene, polyvinyl chloride, polystyrene, polyurethane, and polyethylene terephthalate ([Plastics Europe, 2014](#)). Upon entering the environment, large plastic materials can be broken into small pieces due to heating, photochemical reaction, oxidation processes, etc. and can exist for long period of time. Plastics with size [greater than](#)  $\geq 5\text{mm}$  are defined as macroplastics. Microplastics are plastic particles of [less than](#)  $\leq 5\text{mm}$  or 1 mm in size, depending on the study ([Rochman et al., 2016](#)). Nanoplastics are plastic particles with size ranging from 1  $\mu\text{m}$  to 100 nm ([Rochman et al., 2016](#)).

According to [Gouin et al. \(2011\)](#), approximately, 263 tons of polyethylene microbeads were purchased in liquid soap products in the US in 2009. Approximately 4,073 tons of polyethylene microbeads were used in cosmetic products in European Union countries, Norway, and Switzerland in 2012 ([Gouin et al., 2015](#)). Recent research demonstrated that large quantity of plastic originates from consumer products then proceeds to wastewater treatment plants and finally into freshwater systems. Microplastics at concentrations of 20–150 particles/L or 0.2–66  $\mu\text{g/L}$  have been found in sewage treatment plant effluent in the Netherlands ([Van Wezel et al., 2016](#)). In the US, the daily release of microbeads from cosmetic and personal care products into waterways via wastewater treatment facilities has been estimated to be between 3 and 23 billion particles per day ([Mason et al., 2016](#)). A study by [Eriksen et al. \(2013\)](#) found microplastics in the Great Lakes at concentrations from 43,000 to 466,000 particles/ $\text{km}^2$ . [Moore et al. \(2011\)](#) reported plastic concentrations of 4,999 items/ $\text{m}^3$ , 51,603 items/ $\text{m}^3$ , and 1,146,418 items/ $\text{m}^3$  in Coyote Creek, San Gabriel River, and Los Angeles River, respectively. The persistence of plastics, especially micro- and nano-plastics in the natural environment poses potential risks to living organisms.

The concern about the fate and effects of microplastics in the environment has been growing recently. Much research looking at exposure and the potential effect of microplastics has been conducted with marine organisms (Van Franeker et al., 2011; Besseling et al., 2014; Von Moos et al., 2012; Wegner et al., 2012; Cole et al., 2013; Farrell and Nelson, 2013; Wright et al., 2013; Baulch and Perry, 2014; Chua et al., 2014; Watts et al., 2014; Avio et al., 2015; Van Cauwenberghe et al., 2015). Additionally, adverse effects of plastic pollution have been reported for ~~more than~~ >600 marine species (Secretariat of the Convention on Biological Diversity, 2012). Since plastics are commonly found in natural aquatic ecosystems, it is important to determine the potential effect of microplastics on freshwater organisms. Some previous studies showed that freshwater organisms ingested microplastics and adverse effects to the organisms were subsequently observed (Rosenkranz et al., 2009; Ramos et al., 2012; Rochman et al., 2013; Sanchez et al., 2014; Au et al., 2015; Booth et al., 2016; Greven et al., 2016; Jemec et al., 2016; Ogonowski et al., 2016). Ingestion of plastic particles by *D. magna* is dependent on the particle type, size, and shape. *Daphnia magna* has been found to ingest microplastic fibers at sizes up to 1400 µm in length and 528 µm in width (Jemec et al., 2016) or 106 µm microplastic beads (Frydkjær et al., 2017). Effects of plastic particles are dependent on both ingestion and egestion capability of the organisms. Research demonstrated that spiky particles (e.g., fragments, fibers) had greater effect potential than do smooth particles (i.e., spherical beads) because egestion of spiky particles is more difficult than egestion of smooth particles (Frydkjær et al., 2017).

A number of studies on microplastic ingestion and egestion by *D. magna* (Rosenkranz et al., 2009; Jemec et al., 2016; Frydkjær et al., 2017) and copepod *Centropages typicus* (Cole et al., 2013) have been conducted using short-term exposure methods with ~~less than~~ ≤48 h of exposure and depuration. In the natural environment, organisms usually experience long-term exposure. Therefore, to look at the potential effect of plastic particles in the natural environment, the long term exposure method is more relevant. For zooplankton, such as *D. magna*, the main diet is green algae, which can colonize and grow on the surface of plastic materials (Gross et al., 2016; Kumar et al., 2017). Therefore, the presence of plastic materials can inhibit food intake of the organism due to occupying the gut space when ingesting plastics or benefit the organism, such as enhancing algal growth in the environment. A study by Ogonowski et al. (2016) showed that at low algal concentrations, negative effects of microplastics on reproduction of *D. magna* was found but positive effects on reproduction were observed at high algal concentrations. Interference of plastic particles on food intake by *D. magna* is not fully understood.

The aims of the present study are 1) to determine microplastic ingestion by *D. magna* and its potential effect on the organisms' survival and reproduction and 2) to determine whether microplastics enhance algal growth. The later study aim is to support understanding the potential effect of microplastic ingestion on survival and growth of *D. magna* in chronic exposure conditions at which, green algae is added to the exposure media as food for the organism.

## 2.2 Materials and Methods

### 2.1.2.1 Organisms

*Daphnia magna* used in the present study was from the Institute of Environmental Sustainability (IES) at Loyola University Chicago. *Daphnia magna* cultures were maintained in 1 L glass beakers (30 per beaker). Beakers were filled with 800 mL reconstituted moderately hard water (MHW) made from 16–18 MΩ MilliQ water and laboratory grade chemicals (CaSO<sub>4</sub>·2H<sub>2</sub>O, MgSO<sub>4</sub>, NaHCO<sub>3</sub>, and KCl) based on the U.S. EPA method for chronic toxicity testing (US EPA, 2002). The water quality of the culture media were as follows: hardness ranged from 80 to 84 mg/L as CaCO<sub>3</sub>, alkalinity ranged from 55 to 61 mg/L as CaCO<sub>3</sub>, pH ranged from 7.19 to 7.89, and dissolved oxygen (DO) concentrations ranged from 6.87 to 8.32 mg/L. Cultures were maintained at a temperature ranging from 21.2 to 25.6 °C and at a light:dark photoperiod of 16 h:8 h. Dissolved oxygen, pH, and temperature were recorded following subsequent water changes. Dissolved oxygen and temperature were measured with a DO meter: model YSI 550A, and pH was measured with a Fisher Scientific accumet portable laboratory meter: model AP110. *Daphnia. magna* were fed 6 mL of an algae suspension, *Raphidocelis subcapitata* (formerly known as *Selenastrum capricornutum*) at a concentration of 3 × 10<sup>7</sup> cells/ml, and 3 mL yeast, cereal leaves, and trout chow (YCT). The algae and YCT were cultured and prepared in the Loyola IES based on the U.S. EPA Method (US EPA, 2002).

### 2.2.2.2 Experimental design and procedures

To determine ingestion of microplastics and the potential effect of ingested microplastics on survival of *D. magna*, *D. magna* (7-day-old) were exposed to microplastics for 21 days in MHW. *Daphnia magna* at this life stage were used for study by Rosenkranz et al. (2009), Ogonowski et al. (2016) and Rist et al. (2017). The MHW was prepared by the US EPA Method as described above. Microplastics used in the present study were green fluorescent polyethylene microbeads at a size range of 63–75 µm and particle density of 0.99–1.01 g/cm<sup>3</sup>. The microbeads (item # UVPMS-BG, excitation wavelength of 470 nm) were purchased from Cospheric Inc. (Santa Barbara, CA) and verified by a Thermo Scientific FTIR spectrometer model Nicolet IS10 in the Loyola IES. Plastic microbeads at this size range are commonly used in personal care products, such as toothpaste, face wash, and soaps and end up in the environment as wastewater treatment facilities do not effectively filter them (Browne et al., 2011; Eriksen et al., 2013; McCormick et al., 2014; Carr et al., 2016; Mason et al., 2016; Van Wezel et al., 2016). Microplastics released from cosmetic and personal care products and enter the US waterways via wastewater treatment facility has been estimated to be between 3 and 23 billion (average of 13 billion) particles per day (Mason et al., 2016). In addition, our screening study showed that 7-day-old *D. magna* consumed plastic microbeads at this size range within 6 hours of exposure (unpublished). Au et al. (2015) used these sizes of plastic microbeads for their research with *Hyalella azteca*. A control (no microplastics) and three concentrations of microplastics (25, 50, and 100 mg/L or 1,905,357, 3,810,714, 7,621,429 particles/L) with four replicates each were designed for this study aim. These concentrations were chosen based on the results our

screening study (not published) and within the concentrations used by Au et al. (2015). The replicate chambers were 473 mL Mason glass jars containing 400 mL MHW and 10 7-day-old *D. magna*. Two sets of test chambers were set up. One of them was used for determining ingestion of microplastics and was terminated after 5 days of exposure at which first brood of neonate *D. magna* was observed. The other set was for measuring the potential effects of microplastic on survival and reproduction of *D. magna* and was run for 21 days. Ingestion of microplastics was also determined for this experiment at the experiment termination. The experiments were conducted at laboratory conditions of  $23 \pm 1^\circ\text{C}$  and light:dark = 16h:8h in the Ecotoxicology and Risk Assessment Laboratory of the Loyola IES.

Microplastic treatments were prepared by weighting an equivalent amount of plastic microbeads and mixed with 300 mL MHW separately in each treatment replicate chamber. The microplastic solutions were mixed with a glass rod for one minute followed by four minutes setting in laboratory conditions. The mixing-setting procedure was repeated three times with a total duration of 15 minutes. A separate microplastic solution was prepared (1.5 mg/mL) based on the procedure published by Au et al. (2015) for quantification of the number of microplastic particles (MPs) per mg microplastics. Quantification of MPs was conducted on an Olympus SZX7 microscope equipped with an Infinity 2-1RC camera in the Loyola IES. Ten measurements were carried out and an average concentration of 76,214 MPs/mg microplastics with a standard deviation of 9,110 was determined. This plastic concentration was used to calculate the treatment concentrations based on the used amount of microplastics for each treatment. The mass ingestion by *D. magna* was also calculated based on the number of ingested particles per organism and number of MPs per mg microplastics (76,214). Two adult *D. magna* were randomly selected and transferred to the treatment chambers at a time to ensure unbiased distribution of organisms in the exposure chambers. This step was repeated five times until each treatment chamber received 10 organisms. Treatment test chambers were randomly placed on a wire shelving in the testing room of Loyola IES described above. Transferring organisms into exposure chambers initiated the experiment.

Organisms were fed daily with algae (*Raphidocelis subcapitata*) suspension at a concentration of  $3 \times 10^7$  cells/mL and YTC at a rate of 0.1 mL each per organism. Water of the control (MHW) and exposure treatments (MHW with microplastics) were renewed every other day. To reduce microplastics carrying over during water renewal, *D. magna* in each replicate were first transferred into a temporary holding container (Mason glass jar filled with MHW) using a pipette. The carried over test water was minimized as much as possible. The organisms were then again transferred into another replicate test chamber containing new exposure media. Mortality and number of neonates produced were counted and recorded daily. An individual was considered dead if it was immobilized, and if no organ movements were observed after examination with a 6X Bausch and Lomb magnifier. After counting, the neonates were removed from the test chambers.

Verification and quantification of microplastic ingestion by *D. magna* were conducted for both the 5-d and 21-d exposure experiments. For the 5-d exposure experiment, at the experiment termination, two *D. magna* were randomly chosen and verified for microplastic ingestion on an Olympus SZX7 microscope equipped with an Infinity 2-1RC camera. After verification for microplastic ingestion, *D. magna* were returned to their exposure chambers and the organisms were collected by treatment replicate and frozen at  $0^\circ\text{C}$  prior to further analysis. Quantifying ingested microplastics was conducted based on a Draft Method for Analysis of Microplastics in the Marine Environment developed by Masura et al. (2015) with a modification to the present study. *Daphnia magna* were digested with 50% nitric acid at  $50^\circ\text{C}$  for approximately 3 hours. This acid concentration and temperature chosen were based on our preliminary experiments to ensure that only *D. magna* tissue was dissolved at this digested conditions but not the microplastics. The digested solution was then diluted with deionized water and filtered through a 47 mm Whatman glass microfiber filter. A Welch 2511 dry vacuum pump/compressor was used for the filtration. After filtration, the filters were allowed to air dry at room temperature for an hour and then placed on the microscope station to quantify the number of MPs. Similar procedure was used for quantifying microplastics in *D. magna* from 21-d exposure experiment.

To determine whether microplastics enhance algal growth, green fluorescent polyethylene microspheres and *Raphidocelis subcapitata* were used for the experiment. The experiment included a control (no microplastics) and a treatment of 130 mg/L microplastics with three replicates each. One mL of algae at concentration of  $3 \times 10^7$  cells/mL was added to each treatment chamber to start the experiment. The experiment was conducted for 5 days in the same size Mason glass chambers and in the same testing room as for the microplastic ingestion experiment. However in this experiment, *D. magna* was not used. The experimental chambers were placed on stirring places. The lowest stirring speed was set for the experimental chambers to simulate agitation by swimming of *D. magna*. At the termination of the experiment, a water sample from each control and treatment replicate was collected for counting algal cells. After collecting the samples, the stirring speed was increased to dissociate algae from microplastics for 15 minutes and a water sample was collected from each chamber for counting algae cells. Microplastics are considered to enhance algal growth if the algal concentrations in the later samples are significantly higher than those in the earlier samples. Counting algal cells was conducted using hemocytometer and Olympus SZX7 microscope in the Loyola IES.

### 2.3.2.3 Data analysis

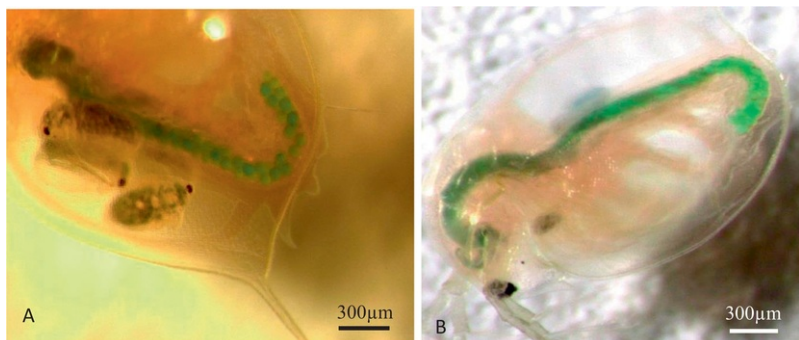
Average number of ingested MPs per *D. magna* was determined for each treatment replicate by dividing the total number of particles counted by the number of organisms. Average number of ingested particles per organisms was calculated for each treatment and was compared for significant difference within the treatments. Mass ingestion was also calculated based on the number of ingested particles per organism and the number of particles per mg plastics determined in the present study (76,214 MPs/mg). Statistical analysis was also performed for the mass ingestion results.

Average mortality of *D. magna* for each treatment was calculated and compared for statistically significant difference. A reproduction rate per organisms per day for a treatment replicate was calculated by dividing the total number of neonates produced in a treatment replicate on a day by the number of living adult *D. magna* in the same treatment replicate on the same day. Average 21-d cumulative number of neonates produced by a living adult and average reproductive rate per day for each treatment were calculated and compared for significantly difference between the treatments.

Average number of algal cells per treatment in the algal experiment was calculated for the treatment and control. Statistically significant differences between the treatment average and control average were compared using the Tukey test method in the R-studio program (Version 3.1.1). A comparison with a  $p$ -value  $\leq 0.05$  was considered significantly different. The arsine squared root transformation method was used to transform mortality data to satisfy the assumption of homogeneous variance and normal distribution. Other data met the assumption of homogenous variance and normal distribution, therefore, no transformation was needed.

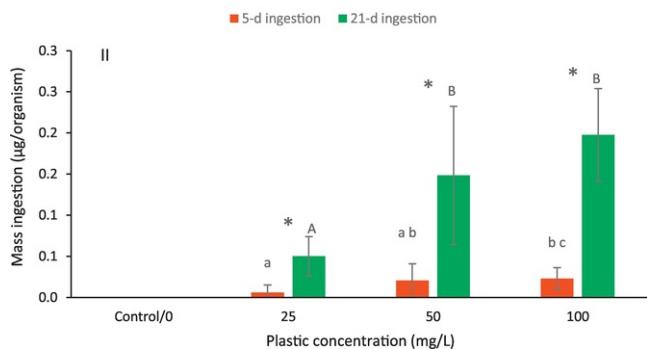
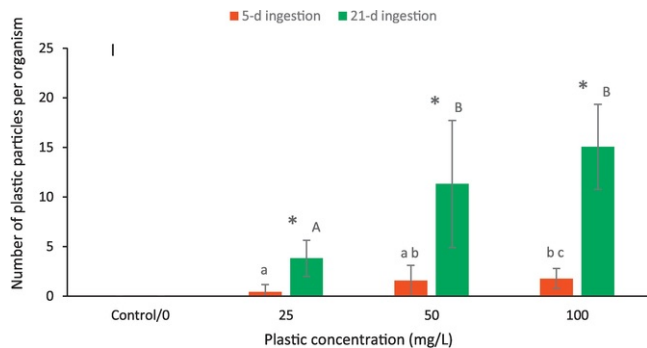
### 3.3 Results

The present study showed that *D. magna* ingested microplastics during the 5-d and 21-d exposure experiments (Fig. 1). In general, the number of ingested microplastic particles increased with increasing microplastic concentration in water. The average number of microplastic particles ingested by an adult *D. magna* after 5 days of exposure to 25, 50, and 100 mg/L microplastics or 1,905,357, 3,810,714, 7,621,429 MPs/L was 0.44, 1.56, and 1.75 MPs/organism, respectively (Fig. 2 I). There was no microplastics in control *D. magna*. The number of ingested microplastic particles was significantly higher in treatment with 100 mg/L than 25 mg/L microplastics. The number of ingested microplastic particles in the 21-d exposure experiment increased with increasing concentration of microplastics in the water. The average concentrations of ingested microplastics per adult *D. magna* were 3.81, 11.31, and 15.06 MPs/organism when exposed to 25, 50, and 100 mg/L microplastics or 1,905,357, 3,810,714, 7,621,429 MPs/L, respectively. These concentrations were significantly higher compared to those at the same water concentration of microplastics in the 5-d exposure experiment (Fig. 2I). These results indicate that longer exposure time allowed the organisms to ingest and accumulate more plastic particles. The ingestion in 50 and 100 mg/L microplastics treatments were significantly higher than the ingestion in 25 mg/L microplastics treatment. There was no significant difference between the ingestion in 50 and 100 mg/L microplastics (Fig. 2I).



**Fig. 1.** Ingestion of microplastics by *Daphnia magna* at an exposure concentration of 100 mg/L (A: 5-d exposure, B: 21-d exposure, round green shapes in the organism's gut are microplastic particles). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

alt-text: Fig. 1

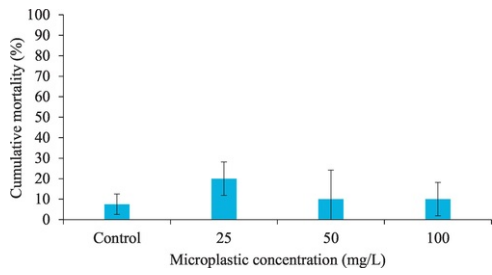


**Fig. 2; Fig. 2** Plastic ingestion by *Daphnia magna* (I: ingestion measured in number of particles/organism; II: mass ingestion measured in µg/organism; treatments with the same small or capital letters are not significantly different; treatments with different small or capital letters are significantly different; asterisks indicate significantly different between initial and final ingestions; error bars represent standard deviations; particle concentrations at 25, 50, and 100 mg/L are 1,905,357, 3,810,714, 7,621,429 MPs/L, respectively).

alt-text: Fig. 2

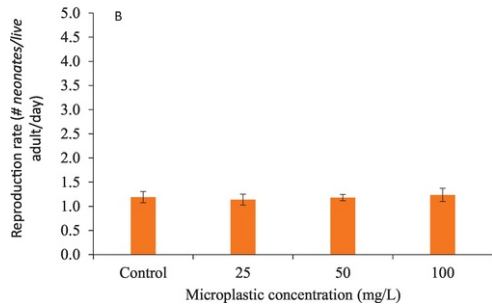
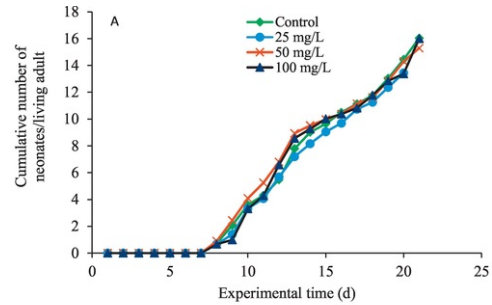
When expressing the ingestion of microplastics in mass units, the amount of ingested microplastics by *D. magna* after 5 days of exposure to 25, 50, and 100 mg/L microplastics or 1,905,357, 3,810,714, 7,621,429 MPs/L was 0.006, 0.021, and 0.023 µg/organism, respectively (Fig. 2II). The ingestion after 21 days of exposure was 0.050, 0.148, and 0.198 µg/organism, respectively (Fig. 2II). Results of statistical comparisons for significant differences in mass ingestion between and within the treatments were similar to the ingestion in particles per organism.

Microplastics did not significantly affect survival and reproduction of *D. magna*. The average percent mortality of *D. magna* in 25, 50, and 100 mg/L microplastics treatments were 20, 10, and 10%, respectively (Fig. 3). These percent mortalities were not significantly different from the percent mortality of the control (7.5%). The average 21-d cumulative reproduction of an adult *D. magna* was 16, 15, 15, and 16 neonates/living adult for control, 25, 50, and 100 mg/L microplastics or 1,905,357, 3,810,714, 7,621,429 MPs/L, respectively (Fig. 4A). The reproductive rate of *D. magna* was at 1.2 neonates/day for the control and treatments (Fig. 4B). There was no statistically significant differences between the reproductive measurements of the control and exposure treatments.



**Fig. 3.** Fig. 3 21-d cumulative mortality of *Daphnia magna* when exposed to microplastics (error bars represent standard deviations; particle concentrations at 25, 50, and 100 mg/L are 1,905,357, 3,810,714, 7,621,429 MPs/L, respectively).

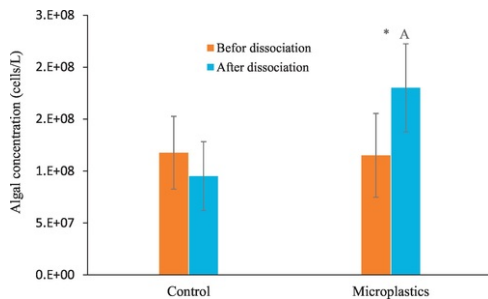
alt-text: Fig. 3



**Fig. 4.** Fig. 4 Cumulative reproduction (A) and reproduction rate (B) of *Daphnia magna* when exposed to microplastics (error bars represent standard deviations; particle concentrations at 25, 50, and 100 mg/L are 1,905,357, 3,810,714, 7,621,429 MPs/L, respectively).

alt-text: Fig. 4

Microplastics did enhance algal growth in the exposure media. The average concentrations of algae in the control media before and after dissociation were  $118 \times 10^6$  and  $95 \times 10^6$  cells/L, respectively (Fig. 5). There was no statistically significant difference between these concentrations. However, the algal concentration in the exposure media with the presence of microplastics before dissociation ( $115 \times 10^6$  cells/L) was significantly lower than that after dissociation ( $180 \times 10^6$  cells/L) (Fig. 5). The algal concentration after dissociation in exposure media with microplastics was significantly higher than that in the control media regardless dissociation. These results suggest that microplastics could potentially serve as substrates for algae to grow.



**Fig. 5.** Enhancement of microplastics on algal growth (A indicates significantly higher than the control regardless of dissociation; asterisk indicates significant difference between before and after dissociation; error bars represent standard deviations)

alt-text: Fig. 5

## 4.4 Discussion

### 4.1.4.1 Microplastic ingestion: particle size dependence and importance of measurement unit

The present study demonstrated that *D. magna* ingested microplastics at a size range of 63–75 μm. This result is within the results published by other studies on zooplankton in marine environment (Cole et al., 2013; Desforges et al., 2015) and in freshwater ecosystems (Rosenkranz et al., 2009; Besseling et al., 2014; Au et al., 2015; Jemec et al., 2016; Ogonowski et al., 2016; Frydkjær et al., 2017; Rist et al., 2017). Ingestion of microplastics and effects are dependent on various factors, such as the particle type, size, concentration, organism size, exposure duration, etc. Measurement unit is also important when interpreting the ingestion and effects of microplastics. According to Frydkjær et al. (2017), *D. magna* ingested microbeads and resulted in body concentrations of 30–50 MPs/organism. These body concentrations are higher than the body concentrations found in the present study (0.44 to 15.06 MPs/organism). The wider range of the particle size (10–106 μm) and exposure concentration (10–5000 mg/L) used by Frydkjær et al. (2017) might explain the difference in ingestion. The number of ingested plastic microbeads by *D. magna* in the present study was approximately 10<sup>5</sup>- and 10<sup>7</sup>-fold lower than the number of ingested plastic nanobeads founded by Rist et al. (2017) for 2 μm- and 100 nm particles, respectively (1.24 × 10<sup>5</sup> and 5.29 × 10<sup>7</sup> particles/organism). This could be due to the higher exposure concentrations used by Rist et al. (2017) (3.1 × 10<sup>10</sup>–3.1 × 10<sup>11</sup> particles/L for 100 nm particles and 1.4 × 10<sup>7</sup>–7 × 10<sup>7</sup> particles/L for the 2 μm particles) than in the present study (0.191 × 10<sup>7</sup>–0.762 × 10<sup>7</sup> particles/L). When expressing the ingestion in mass, the amount of ingested plastic particles in the present study (0.006–0.198 μg/organism) and the study by Rist et al. (2017) (0.17–0.89 μg/organism) are within 30-fold difference. The least difference in mass ingestion between the two studies could be due to the similar size of the organisms. The organisms used in the two studies were at the same age at the start of the study (7-day-old). The study duration was also the same (21 days). Organisms at the same age could have similar body and gut size to contain similar amount of plastic particles. These results suggest that when mass ingestion is concerned, particle size seems to be less important, as long as they are within the consuming range. Caution should be taken when evaluating the hazards of microplastics to aquatic organisms. Plastic ingestion by *D. magna* also appeared to be dependent on exposure time. The present study found more plastic particles in *D. magna* after 21 days of exposure than 5 days of exposure regardless depuration. Rist et al. (2017) also reported that the amount of ingested particles increased with increasing exposure time. These results indicate that organisms would ingest more plastic particles through their lifespan in the natural environment than in short term laboratory exposure conditions. However, it is also important to mention that plastic concentrations used in laboratory research are usually higher than the environmental concentrations (Lenz et al., 2016).

The ingestion of microplastics in the present study appeared to be higher than those ingested by *Hyalella azeteca* (e.g., 15.06 MPs/*D. magna*) at a water concentration of 7.621 × 10<sup>6</sup> MPs/L and 21-d exposure compared to 1.5–2 MPs/*H. azeteca* at water concentrations between 5 × 10<sup>6</sup> and 20 × 10<sup>6</sup> MPs/L and 28-d exposure (Au et al., 2015). The difference in microplastic consumption between the two species could be due to the difference in feeding habit and life stage. *Daphnia magna* is a filter feeder species. The main feeding strategy of *D. magna* is filtration of suspended particles to extract nutrients, such as green algae. *Hyalella azeteca* is a deposit feeder species and feed on detritus (Strong, 1972; Geoffrey, 1991). In the present study, *D. magna* fed on green algae. Our results suggest that microplastics served as substrates for algae to grow. Algae has been found to grow and developed biofilms on plastic substrates (Gross et al., 2016; Kumar et al., 2017). Therefore, consuming microplastic particles could allow *D. magna* to extract algae. In addition, during this 21-d study period, *D. magna* was in its most reproductive life stage and would require more food whereas *H. azeteca* was at an earlier reproductive life stage in the study by Au et al. (2015). This might explain the higher microplastic consumption in the present study compared to the study by Au et al. (2015).

### 4.2.4.2 Reproduction: role of algal growth on plastics and particle shape and excretion

Although *D. magna* ingested a significant amount of plastic microbeads, there was no significant effect on survival and reproduction after 21 days of exposure to the microplastics. These results are in agreement with the results published by Rist et al. (2017) but appear to be in contrast with the results published by Ogonowski et al. (2017) at which, effects of microplastic particles on survival and reproduction of *D. magna* were demonstrated. Besseling et al.



(2014) also reported negative effects of nanoplastic consumption on reproduction of *D. magna*. Au et al. (2015) found that survival and reproduction of *H. azteca* were affected by microplastic exposure. Other recent studies demonstrated toxic effects of microplastics on bivalve *Corbicula fluminea* (Guilhermino et al., 2018) and damage on the gut of zebra fish when exposed to common microplastics (Lei et al., 2018). The no significant effect on survival and reproduction found in the present study suggests that the organisms obtained sufficient energy for survival and reproduction.

While the gut was filled with microplastic beads (Fig. 1), the organisms must have a mechanism to obtain food. We believe that the organisms digested algae from the surface of microplastic beads after ingestion. Once the algae was digested, microplastic beads could be excreted and thereby create room for the ingestion of new beads with their associated algae. Our microscopy observations revealed that *D. magna* excreted microplastic beads during exposure. Excretion of plastic particles by *D. magna* has been reported by previous research (Rosenkranz et al., 2009; Jemec et al., 2016; Ogonowski et al., 2016; Rist et al., 2017). Our results suggest that the process of ingestion and egestion of microplastic beads could likely happen during the exposure and helped the organisms obtain sufficient food from the microplastic beads for their survival and reproduction, resulting in no significant effect on survival and reproduction after 21 days of exposure.

It is important to mention that the results of our laboratory study might not totally represent the natural environment. Plastic particles in the natural ecosystem exist in different shapes, sizes, and types (Cole et al., 2011; Eerkes-Medrano et al., 2015; Syberg et al., 2015). Spiky particles (e.g., fragments, fibers) may not be easily egested (Eerkes-Medrano et al., 2015) as smooth and round shape beads used in the present study. A recent study by Frydkjær et al., 2017 reported that *D. magna* egested microplastic beads at a higher rate than microplastic fibers. Microplastic fibers have been found to twist in the gut of dead *D. magna* (Jemec et al., 2016). Murray and Cowie (2011) reported the formation of filament balls of plastic fibers in the stomach of the decapod crustacean *Nephrops norvegicus*. Slow or no egestion of microplastic particles would lead to decrease ingestion rate and hence reduce food intake. This can cause starving and adverse effects (Cole et al., 2011; Ramos et al., 2012; Wright et al., 2013; Cole et al., 2015; Jemec et al., 2016). According to Jemec et al. (2016), no significant mortality of *D. magna* was found after 48 h of exposure to microplastic fibers when the organisms were fed prior to conducting the experiment. However, significant mortality was found when the organisms were not fed before the experiment. Dead *D. magna* were found to contain full or half gut of fibers with no or little algae while surviving *D. magna* completely depurated the fibers and contained algae (Jemec et al., 2016). Wright et al. (2013) reported a decrease in nutrient intake and energy reserves in marine lugworms *Arenicola marina* after consumption of microplastics. Another study suggested a reduction in feeding in field collected fish (*Eugerres brasiliensis*) because of ingestion of plastic fragments (Ramos et al., 2012).

In the natural environment, hydrophobic organic contaminants adhere to the surface of microplastics and would be transferred into organisms in the same manner as algae on microplastics are when consumed. This can increase chemical exposure and hazard potential to the organisms (Teuten et al., 2007; Engler, 2012; Chua et al., 2014; Eerkes-Medrano et al., 2015; Koelmans et al., 2016; Ziccardi et al., 2016). Another recent research showed that microplastic fibers adsorbed phenanthrene and inhibited mobility of *D. magna* (Frydkjær et al., 2017).

## 5.5 Conclusions and suggestions

*Daphnia magna* ingested microplastic beads at a size range of 63–75 µm. Microplastics enhanced algal growth during the 21 days of experiment. No significant effect on survival and reproduction of *D. magna* was found although the organism's gut was filled with microplastic beads. Organisms likely obtained sufficient food that adhered to the surfaces of microplastic beads when consume the beads. Caution should be taken when interpreting the hazardous effects of microplastics based on ingestion, such as the measurement unit and especially when algae is present in the exposure media. More studies should be conducted to determine the potential transfer of hydrophobic organic contaminants that adhere to the surface of plastic particles and to ascertain their effects on bioaccumulation, survival, and reproductive effect potential to aquatic organisms.

## Acknowledgement

The authors are grateful to Madeline Wrey for her assistance with the experiment. Financial support for this study was from a Carbon Fellowship of Loyola University Chicago.

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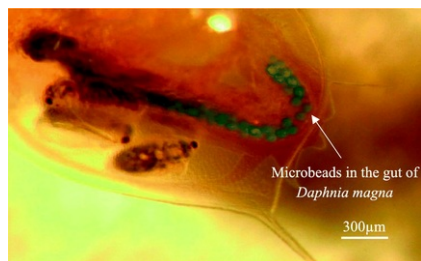
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## Graphical abstract



alt-text: Unlabelled Image

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## Highlights

- *Daphnia magna* ingested plastic microbeads at size of 63–75 μm.
- Body concentrations of microplastics increased with exposure time.
- No significant effect on survival and reproduction of *D. magna* was found.
- Microplastics enhanced algae growth.
- A procedure for quantifying microplastics in *D. magna*'s gut was presented.

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