Study on the Microplastics' Effect on the Life History of *Daphnia magna*

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Abstract. Microplastics (MPs), a type of new pollutant, have shown negative impacts on the aquatic environment. To understand how MPs impact zooplanktons, we studied the life history of *Daphnia magna*. *D. magna* those fed with fluorescent microspheres at two different concentrations (0.1mg/L and 1mg/L) and particle sizes respectively (0.1μm and 5μm). The results showed that MPs negatively affected the growth and reproduction of *D. magna* significantly. Specifically, the impact of higher concentration of 5μm microspheres > high concentration of 0.1μm microspheres > low concentration of 0.1μm microspheres > low concentration of 5μm microspheres. In addition, prolonged feeding time in large-particle-size microspheres (5μm) enhanced the ingestion of microspheres by *D. magna*, resulting in higher impact on life history. In conclusion, small-particle-size microspheres (0.1μm) are more harmful at low concentrations. However, microplastic pollution shows impacts only on the life history (growth and reproduction), but not the survival rate of *D. magna* in our study.

1 Introduction

Microplastics (MPs) pollution in the aquatic environment has been widely concerned by all sectors of society. Compared to large-particle size plastics, MPs have higher residual concentrations in the environment and are more likely to be swallowed by organisms, enter the food chain, and accumulate in consumers, endangering the biological health and the ecological environment ultimately. MPs pollution has both direct and indirect hazards. Direct hazards include as followed: MPs can adsorb on the surface of plant roots and then affect plant respiration, accumulate in the intestinal tract of some organisms and then hinder biological digestion and absorption, enter biological tissues and then induce serious and sustained inflammatory responses in organisms [1]. Indirect hazards include as followed: MPs can concentrate organic pollutants to form contaminant complexes, which have synergistic effects on aquatic organisms; the richness of MPs in sediments interferes with the reproductive ability and offspring development of marine benthos such as oysters; MPs can also affect the structure of intestinal flora and the ecological environment of the organism, and then affect the health of the organism [2]. Organisms at low trophic level were more likely to ingest MPs than those at high trophic level, and trigger cumulative effects by transmitted through the food chain [3]. Zooplanktons, as primary consumers, cannot distinguish plastic particles from food effectively [4], when MPs smaller than 100μm are mixed in available food. Therefore, they are one of the aquatic organisms threatened by MPs mostly [5]. Zooplankton is also the prey of higher trophic level organisms and plays a pivotal role in the food chain of aquatic ecosystem [6]. Therefore, it is of importance to understand the influences of MPs on zooplanktons.

Daphnia magna, a crustacean zooplankton in freshwater, has a short life cycle, asexual reproduction, and high responsiveness to environmental stress. It provides a unique opportunity to investigate the influences of microplastic pollution on the zooplankton [7]. As known, MPs could reduce water filtration and feeding rate of *D. magna*, and then result in underfeeding, malnutrition, slow growth and even death [8]. In addition, MPs could increase the content of MDA in *D. magna*, which cause oxidative damage [8]. However, how MPs affect the development and reproduction of *D. magna* remains unclear. To understand how MPs affect the life history of *D. magna*, we fed *D. magna* with MPs at different particle sizes and concentrations.

2 Methods and materials

2.1 Experimental organisms and their cultivation

Two particle sizes of green fluorescent polystyrene microplastics -- 0.1μm and 5μm were purchased.

The crustacean *Daphnia magna* from freshwater was cultured in ADaM medium. Every day the researchers fed *D. magna* with the green alga *Scenedesmus quadricauda* (1×10^6 cell/mL) daily in 250-mL glass beakers which contained 100mL of ADaM medium at 20 °C and 1200 lx with a 14 : 10 h light : dark cycle.

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S. quadricauda were mass cultured in 1-L conical flask containing BG11 medium under continuous aeration at 25 °C, and with a 14 : 10 h light : dark cycle. The cultures were shaken more than three times every day.

2.2 Experimental design and sample collection

The algal solution (*S. quadricauda*) was collected at the exponential growth phase. It was centrifuged at 4500 rpm for 15 min, and then the supernatant was removed. The algal solution was centrifuged again with sterile water to remove the supernatant and stored in dark at 4 °C.

Ten neonates of *D. magna* were cultured in 250-mL glass beaker with ADaM medium containing three different densities (0, 0.1 and 1 mg/L) of MPs with two particle sizes (0.1 and 5 μm) at 20℃ and 1200 lx with a 14 : 10 h light : dark cycle. *D. magna* was fed by *S. quadricauda* daily at the concentration of 1×10^6 cells /mL. Each treatment was set as more than five replicates. The experimental groups were as followed: CK group (blank control group, no MPs), group A (0.1 μm MPs, 0.1mg/L), group B (0.1 μm MPs, 1mg/L), group C (5 μm MPs, 0.1mg/L), and group D (5 μm MPs, 1mg/L) (Table 1).

Table 1. Group information for the experimental design.

Group	Particle size of MPs	Concentration of MPs
ck (Control check)	/	0
A	0.1 μm	0.1mg/L
B	0.1 μm	1mg/L
C	5 μm	0.1mg/L
D	5 μm	1mg/L

Body length of *D. magna* was surveyed by ordinary microscope and the medium was refreshed every day. Four index including time to first brood, number of neonates produced at first brood, total number of broods and neonates produced were recorded for seven weeks. The survival rate of *D. magna* (10 individuals) under different MPs treatments was surveyed for 49 days. In addition, the fluorescence intensities of *D. magna* at 0, 2, 7, 14, 21, 28, 35, and 49 days was observed by fluorescence microscope.

3 Results and discussion

3.1 MPs ingested by *D. magna* during 49 days

The fluorescence intensity of *D. magna* in microspheres was analyzed by fluorescence microscope at 0, 2, 7, 14, 21, 28, 35 and 49 days (Fig. 1), and there was a significant difference of the fluorescence intensities among groups ($P < 0.001$). Fig. 2 showed that the order of fluorescence intensity was D > C > B > A. The nonlinear fitting of each experimental group showed that only group D formed a regular change trend ($P = 0.00621$), and the fluorescence intensity showed an increasing trend with the passage of microplastic exposure time, while the other groups did not form a significant change trend, and the fluorescence intensity did not change much during the whole experiment. The CK group did not add microplastics, and all the fluorescence intensity was 0, so it is not represented in Fig. 2. Thus, the consumption of 1 mg/L 5 μm MPs in *D. magna* increased throughout the experiment, while the consumption of 0.1 μm MPs (both concentrations) and 0.1mg/L 5 μm MPs did not differ significantly.

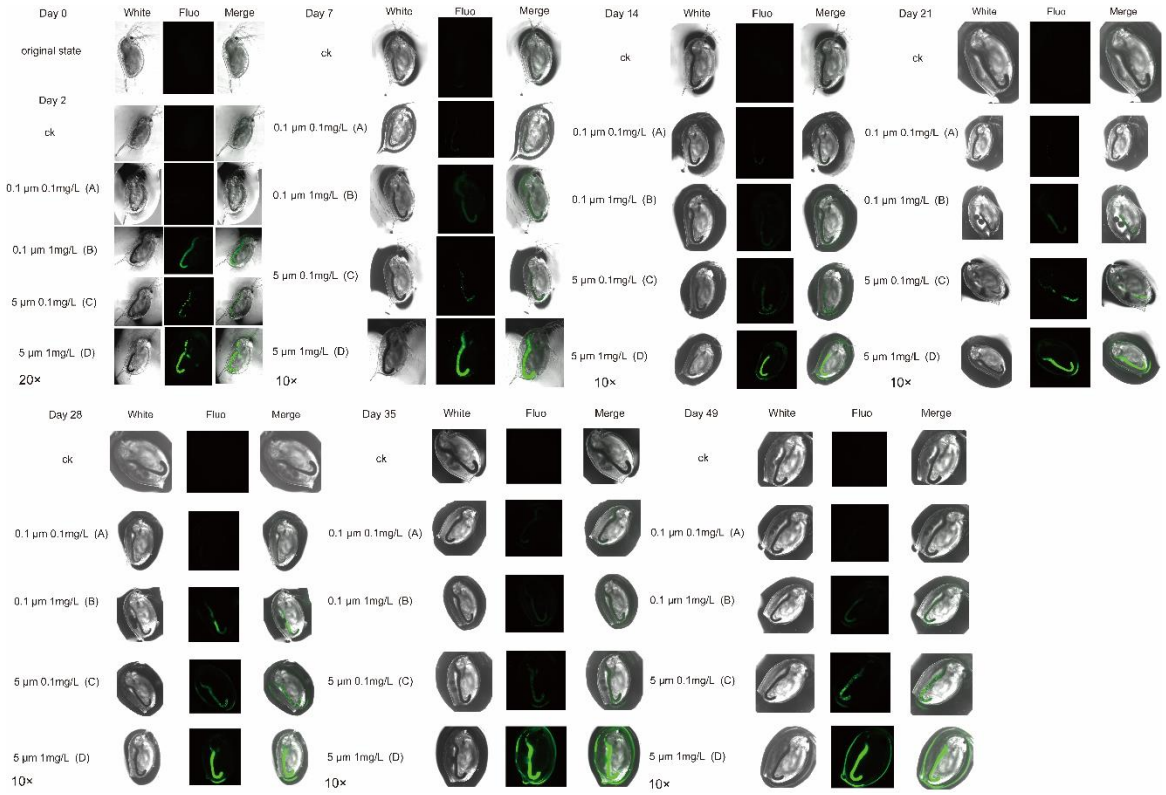

Fig. 1. Changes of *D. magna* ingested fluorescent microspheres.

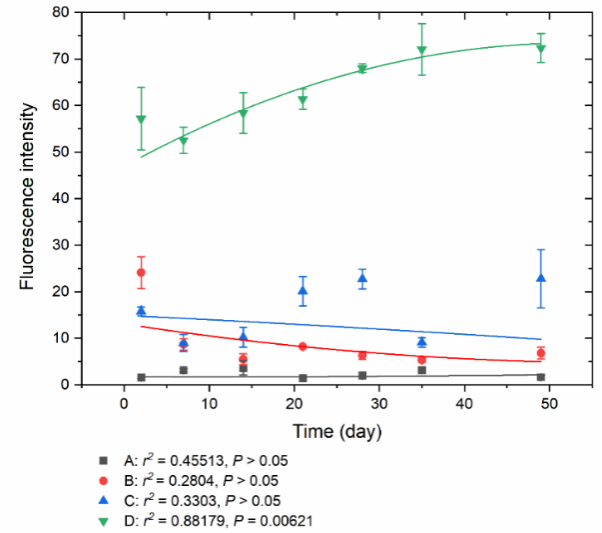

Fig. 2. Changes in fluorescence intensity of *D. magna* during the experiment.

size of *D. magna* was CK > C > A > B > D. The body length of *D. magna* exhibited a significant increase over the course of the study period. (P < 0.0001).

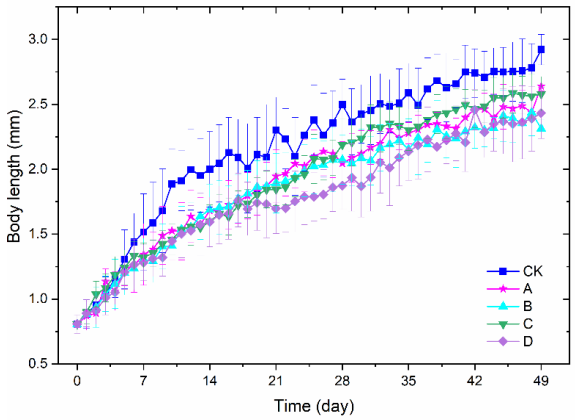

Fig. 3. Changes in body length of *D. magna* during the experiment.

3.2 Survival impacts of MPs on *D. magna*

In this study, the survival rates of the control and experimental group and were analyzed by one-way analysis of variance (ANOVA), and it was found that there was no significant difference (P = 0.319084), which was consistent with the previous studies [9, 10]. However, several studies have demonstrated that the toxicity of MPs towards *D. magna* populations exhibits a decrease in response to increased food intake; however, the extent of this reduction is highly contingent upon the size of MPs[11].

3.3 Growth and development impacts of MPs on *D. magna*

The results of ANOVA found that the body length of *D. magna* in different groups (CK, A, B, C, and D) was different significantly (P = 0.003). Fig. 3 indicated that the

3.4 Reproductive impacts of MPs on *D. magna*

There were significant differences in the time to first brood, the number of neonates produced at first brood, and total number of neonates (Fig. 4): the time to first brood was CK < C < A < B < D (P < 0.0001); the number of neonates was CK > C > A > B > D (P = 0.00088); total number of neonates was CK > C > A > B > D (P = 0.0013). However, total number of broods did not differ significantly among the five groups (P = 0.258). The reproductive impact of 0.1 μm MPs was more pronounced on *D. magna* at a low concentration (0.1 mg/L) compared to 5 μm MPs. At the high concentration (1 mg/L), the consumption of 5 μm MPs in *D. magna* increased continuously, while that of 0.1 μm MPs remained unchanged, suggesting that the high concentration of 5 μm MPs had a greater impact on the reproduction of *D. magna*. This effect was similar to that of MPs on the growth and development of *D. magna*.

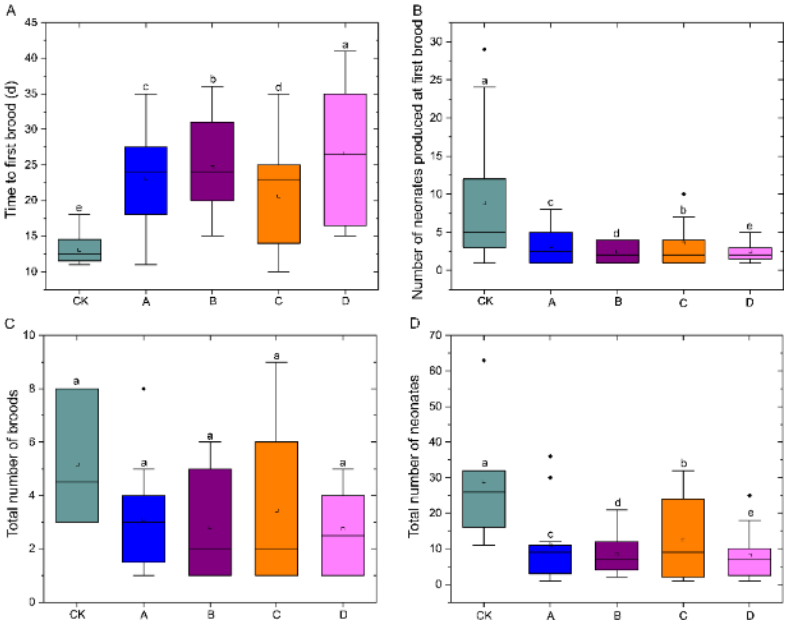

Fig. 4. Changes in reproduction of *D. magna* (A. time to first brood, B. number of neonates produced at first brood, C. Number of broods, D. total number of neonates) during the experiment.

Studies have found that MPs with smaller particle size accumulated more and stayed longer in the organism, resulting in lower feeding rate and damage to the antioxidant defense system [8]. Even at the lowest concentrations, the introduction of plastic microspheres had a detrimental impact on both the growth and reproductive capabilities of the parental D. magna, consequently affecting the development of their offspring. The influences may arise directly from the microspheres, such as causing mechanical damage to bodily structures or inducing malnutrition due to excessive consumption. Alternatively, they could be indirect consequences of reduced microalgae availability, which serves as the primary food source for *D. magna* and leads to food shortages. Moreover, the sensitivity of larvae to MPs exposure was higher than that of adults [12].

4 Conclusion

In this paper, the effects of two MPs sizes at two concentrations on the life history of *D. magna* were studied. The intake of MPs with large particle sizes by *D. magna* increased gradually over the time, which had the most severe impact on *D. magna*. However, MPs with small particle sizes were more harmful at low concentrations. The results will provide important scientific support for the protection of aquatic life and ecological environment, as well as the research on the eco-toxicity and health risks of MPs.

Acknowledgments

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