EFFECTS OF MICROPLASTIC CONCENTRATIONS ON THE SURVIVABILITY AND REPRODUCTION OF MOINA MACROCOPA

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Abstract. In recent years, a large number of microplastics (MPs) appeared in the aquatic environment, causing serious pollution. In order to study the influence of MP concentrations on freshwater zooplankton, polystyrene microspheres (PS) of 0.5 µm were used in this study as experimental reagents, and Moina macrocopa, which was dominant zooplankton in the Pearl River Basin of China, was selected as the experimental object. The experiment was divided into four treatment groups plus a control group, and it was conducted for 15 days. The treatment groups had MP concentrations of 10, 10², 10³ and 10⁴ pieces/mL. The survival time, reproduction rate, the total offspring, and the time of first pregnancy of Moina macrocopa were counted every day, and the intrinsic growth rate, generation time, and net reproduction rate were calculated. The results showed that the survival time of Moina macrocopa decreased with the increase of the MP concentration, and the MP concentration had more influence on the reproduction rate. Except for the 10 pieces/mL treatment group, no reproduction occurred in other treatment groups, and the reproduction rate gradually decreased with the increase of age. The life table showed that only the control group and the 10 pieces/mL treatment group had an intrinsic growth rate, and the intrinsic growth rate of the treatment group was far lower than that of the control group. Our experimental results provide basic data and a theoretical basis for further understanding the toxic effects of MPs on freshwater zooplankton.

Keywords: zooplankton, polystyrene microsphere, intrinsic growth rate, generation time, net reproduction rate

Introduction

In the past several decades, with the mass production and widespread use of plastic products across the world, the global coastal and estuarine environment has directly and indirectly received a large amount of plastic waste from terrestrial and marine sources, which poses potential risks to aquatic wildlife primarily through entanglement and ingestion (Jasna et al., 2018; Su et al., 2019). Plastic pollutants in water gradually decompose into fragments and become microplastics (MPs) (<5 mm) due to mechanical crushing, water erosion, UV degradation, biodegradation, and photodegradation (Xue et al., 2022). The primary particles inside personal care and cosmetics products and the secondary particles produced by the degradation of larger plastic articles are expected to cause environmental pollution by these nanoplastics (Sivan, 2011). MPs are widely

distributed in the marine environment and have been detected in oceans (Enders et al., 2015), coastal waters (Wang et al., 2018), and polar regions (Morgana et al., 2018).

In recent years, MPs have also been found in freshwater ecosystems, such as rivers and lakes. The research on MP pollution in these waters has increasingly supported that MP pollution is a serious problem in the freshwater environment (Eriksen et al., 2013; Erkes-Medrano et al., 2015; Lechner et al., 2014; Moore et al., 2011; Wagner et al., 2014; Zhang et al., 2015).

Zooplanktons are the primary consumers of the aquatic food chain; they ingest phytoplankton, bacteria, and debris, and are also preyed on by species in higher trophic levels. Therefore, zooplanktons play an important role in aquatic ecosystems and have important ecological functions (Hui et al., 2021).

Cladocerans are a type of major zooplankton. They can control the scale and quantity of phytoplankton by ingestion, and they serve as food for species in the higher trophic levels, such as fish. They are considered the link between producers and species in the higher trophic levels (Lampert et al., 2006) and also occupy a central position in the lake food web (Carpenter et al., 2001; Thomas et al., 2008). Cladocerans have become a model organism widely used in ecotoxicology because of their rapid reproduction, easy cultivation, and sensitivity to most toxic substances.

The size and color of MPs are similar to the food of zooplankton, and they are easily ingested by zooplankton (Li et al., 2018). Previous studies have demonstrated the possibility of MP ingestion by zooplankton in the natural environment, such as the South China Sea (Sun et al., 2017), Indian Blair Bay (Goswami et al., 2020), Portuguese coast (Frias et al., 2014), Mediterranean (Collignonet al., 2012), and Kenya coast (Kosore et al., 2018). In the laboratory, the ingestion of MPs by zooplankton has also been verified, and the location of MP enrichment in zooplankton was detected with fluorescence micro-imaging systems (Cheng et al., 2020; Ziajahromi et al., 2017). At present, most studies mainly focus on the toxicity of marine organisms, but few focus on freshwater zooplankton. Among them, most of the previous experimental studies have focused on *Daphnia magna*. In this study, the effects of concentrations of polystyrene microspheres (PS) of 0.5 μ m on the survivability and reproduction of *M* polystyrene microspheres to provide a baseline for scientific assessment of the ecological risks of MPs.

Materials and methods

Instruments and reagents

PS (0.5 μ m) were purchased from Tianjin Beisile Chromatographic Technology Development Center, China, with a concentration of 3.59×10^{11} pieces/mL.

Tested biological culture

Moina macrocopas were acquired from the ponds in the Pearl River Fisheries Research Institute of the Chinese Academy of Fishery Sciences, China, and were inoculated and cultured after identification and separation. *Moina macrocopas* were cultured in 500 mL beakers (GG-17, Sichuan Shubo Co., Ltd., Sichuan, China) at $28 \pm 0.5^{\circ}$ C in a light incubator (MCG-250P, Shanghai Yiheng Technology Co., Ltd., Shanghai, China). The light cycle was 12 h:12 h (L:D), and the light intensity was 2000–3000 lux. *Moina macrocopas* were fed daily with *Chlorella pyrenoidosa* (FACHB-5, Freshwater Algae Culture Collection at the Institute of Hydrobiology, FACHB, China) as the only food source, which were cultured in BG-11 medium (HB8793, Qingdao Hope Bio-Technology Co., Ltd., Qingdao, China).

Concentration preparation of PS

In order to avoid the influence of impurities in the water on the calculation of PS, the experimental water was filtered with a cellulose nitrate membrane (0.45 μ m) (114H6-47-ACN, Sartorius Stedim Biotech GmbH, Germany). The water temperature was maintained at about 25°C, and the pH was sustained between 6.4 and 6.7. The microsphere solution was diluted and finally prepared into exposure concentrations of 10, 10², 10³, and 10⁴ pieces/mL (diameter: 0.5 μ m). The experimental solution was ultrasonically dispersed for 1 min with an ultrasonic cleaner (SB-3200D, Ningbo Scientz Biotechnology Co., Ltd., Ningbo, China) before calculating PS.

Experimental methods

On the night before the experiment, *Moina macrocopa* in their breeding period were selected and placed into a 500 mL beaker (GG-17, Sichuan Shubo Co., Ltd., Sichuan, China) for observation in every 12 h. We selected the *Moina macrocopa* with a birth time of less than 12 h and set five experimental groups (a control group and four treatment groups) according to the concentration of MPs (0, 10, 10^2 , 10^3 , 10^4 pieces/mL) referring to OECD 21 day chronic toxicology experiment. Each group had 10 parallel runs; the volume of exposed solution in each run was 25 mL. We added *M. macrocopa* in each run and cultured it in the constant temperature incubator (MCG-250P, Shanghai Yiheng Technology Co., Ltd., Shanghai, China) at $27 \pm 0.5^{\circ}$ C, with a light intensity of about 1800 lux, and a photoperiod of 12 h:12 h (L:D). We changed the MP solution every three days to ensure that the MP concentration remained stable.

We observed the survivability and reproduction of the neonate body separated from the initial mother using a screening equipment every 12 h and counted the survival time, generation time (T), total offspring, and the offspring of the first brood and calculated the intrinsic growth rate (r_m). The experiments were carried out until the initial mother died. The number of days that *Moina macrocopa* survived were determined as its lifetime, reproductive time referred to the time for newly hatched *Moina macrocopa* growing into maturity, whereas maturity was defined as the time when it first laid eggs.

We used the following formula to calculate the net reproduction rate (R_0) , generation time (T), and the intrinsic growth rate (r_m) :

$$R_0 = \sum l_x m_x$$

$$T = \sum x l_x m_x / l_x m_X$$

rm adopted the iterative approximation method in Excel according to the formula

$$\sum_{x=0}^{n} e^{-rx} l_x m_x = 1,$$

where r was r_m (Hu, 2010).

In the formula, the survival rate (l_x) referred to the percentage of the number of survivors in the total population in the x age group; m_x represented the number of newborn *M. macrocopa* at each time; x was the number of exposure days; the net reproductive rate (R₀) referred to the net growth rate of the population after an era; generation time (T) referred to the time from the birth of parents to the birth of children; and intrinsic growth rate (r_m) was the maximum growth rate of the population under specific experimental conditions.

Statistical analysis

Excel was used for statistical analysis, GraphPad Prism 8 was used for one-way ANOVA, and the Dunnett method was used to compare the treatment group with the control group to test the significance of the difference. When P < 0.05, the difference was significant; When P < 0.01, the difference was extremely significant.

Results and discussion

Survival of Moina macrocopa in different concentrations of MPs

Table 1 shows the life table of *Moina macrocopa* over 15 days under the effect of different concentrations of MPs. The numbers presented in *Table 1* were averages of the 10 parallel runs. The survival time of *Moina macrocopa* decreased with the increased concentration of MPs. Mortality began on the fourth day in the 10, 10^2 , and 10^4 pieces/mL concentration groups, and more than half died on the seventh day when the concentration of MPs was 10 pieces/mL and 10^2 pieces/mL. When the concentration of MPs was 10 pieces/mL and 10^2 pieces/mL. When the concentration of MPs was 10^3 pieces/mL, death occurred on the second day, and more than half died on the fifth day. At the MP concentration of 10^4 pieces/mL, more than half died on the fifth day. In the same age group, the lowest survival rate was the experimental group of 10^3 pieces/mL.

The increase of the concentration of MPs had a great effect on the reproduction rate. *Moina macrocopa* had zero reproduction in the experimental groups of MPs with the concentrations of 10^2 , 10^3 , and 10^4 pieces/mL. *Moina macrocopa* produced only a few offspring when the concentration of MPs was 10 pieces/mL. The reproduction rate gradually decreased with the increase of age in the 10 pieces/mL MP concentration group.

Ogonowski et al. (2016) found that the mortality of *D. magna* rose with the increase of MP concentration when exposed in 1–5 μ m polyethylene (PE) MPs, which was consistent with our results. The reasons may be as follows: (1) It was related to the feeding choice of cladocera. Cladocera is a type of small filter feeding zooplankton that lives by filtering food particles or nutrients suspended in the water during the filter feeding process, so the food selectivity of cladocera is not high (Gophen and Geller, 1984). Therefore, cladocera easily ingested other substances and produced toxicological reactions. (2) It was related to the size of MPs ingested. The algae fed in this study was *Chlorella pyrenoidosa*, whose diameter was generally 3–5 μ m. The size of MPs selected in this study was 0.5 μ m, which is much smaller than the particle size of food, so it was easily ingested and thus more likely to affect the survivability and reproduction of *Moina macrocopa*. Jeong et al. (2017) found that the smaller the particle size of MPs, the longer the MPs remained in the body of the *Paracyclopina nana*. This result showed that the smaller the particle size, the greater the impact of MPs on zooplankton. (3) It was related to the exposure time in the MP solution. The longer the exposure time, the greater the impact on survival. Cole et al. (2015) found that the mortality of copepods fed with MPs increased over time, indicating that the toxicity of MPs to zooplankton was time-dependent, which was consistent with the results of our study.

	Survival rate (l _x)					Reproduction rate (m _x)				
Age	0 (pieces/mL)	10 (pieces/mL)	10 ² (pieces/mL)	10 ³ (pieces/mL)	10 ⁴ (pieces/mL)	0 (pieces/mL)	10 (pieces/mL)	10² (pieces/mL)	10 ³ (pieces/mL)	10 ⁴ (pieces/mL)
1	1	1	1	1	1	0	0	0	0	0
2	1	1	1	0.8	1	0	0	0	0	0
3	1	1	1	0.5	1	0	0	0	0	0
4	0.8	0.8	0.9	0.3	0.7	0	0.8	0	0	0
5	0.8	0.7	0.9	0.3	0.4	1.1	0.6	0	0	0
6	0.8	0.7	0.8	0.3	0	0.5	0.1	0	0	0
7	0.6	0.3	0.4	0.1	0	0.7	0	0	0	0
8	0.5	0.3	0.2	0	0	0.6	0	0	0	0
9	0.4	0.1	0.2	0	0	1.2	0	0	0	0
10	0.4	0.1	0	0	0	0	0	0	0	0
11	0.4	0.1	0	0	0	0	0	0	0	0
12	0.2	0.1	0	0	0	0	0	0	0	0
13	0.2	0.1	0	0	0	0	0	0	0	0
14	0.2	0	0	0	0	0	0	0	0	0
15	0.2	0	0	0	0	0	0	0	0	0

Table 1. Life table of Moina macrocopa in different concentrations of microplastics

Effects of MP concentrations on reproductive parameters of Moina macrocopa

The concentrations of MPs had a significant effect on the survival time, the offspring of the first brood of a mother, the total offspring of a mother, and the time of first pregnancy of *Moina macrocopa* (P < 0.05). In the concentrations of 10^2 , 10^3 , and 10^4 pieces/mL, there were significant differences in the offspring of the first brood of a mother, the total offspring of a mother, and the time of first pregnancy of *Moina macrocopa* (P < 0.001). The survival time, the offspring of the first brood of a mother, the total offspring of a mother, and the time of first pregnancy of *Moina macrocopa* (P < 0.001). The survival time, the offspring of the first brood of a mother, the total offspring of a mother, and the time of first pregnancy of *Moina macrocopa* all gradually reduced with the increase of the concentration of MPs. Notably, the neonate body would no longer reproduce when the concentration of MPs reached 10^2 pieces/mL (*Table 2*).

The results of this study showed that the reproduction of *Moina macrocopa* was greatly affected by high concentrations of MPs. The increase of MPs in the water would inevitably affect the proportion of food ingested by *Moina macrocopa*, which would lead to malnutrition and affect the survivability and reproduction of *Moina macrocopa*. Gong et al. (2020) found that the MPs with sizes ranging from 1–10 μ m, 10–22 μ m, and 46–53 μ m could be ingested by *Daphnia magna* and accumulated in the intestine, causing inhibition of *Daphnia magna*'s activity and therefore possibly affecting its lipid metabolism. A high concentration of PE-MPs in water could adhere to the body surface of *Daphnia magna*, limiting its activities and affecting its ingestion. Jeong et al. (2017) showed that the fecundity of copepods exposed to 50 nm polystyrene beads of 20 mg/L decreased, and the activity of antioxidant enzymes changed. This study demonstrated that the higher the concentration of MPs, the greater the harm to zooplankton. MP concentration was an important factor affecting the survival and reproduction of zooplankton.

Concentration of microplastics (pieces/mL)	Survival time (d)	The offspring of the first brood of a mother (individual)	The total offspring of a mother (individual)	The time of first pregnancy (d)
0	$8.5\pm4.2^{\ast}$	$1.5 \pm 1.0*$	$2.1\pm1.6^*$	$4.7 \pm 2.5*$
10	$6.3\pm2.8*$	$1 \pm 1.0^*$	$1.1 \pm 1.1*$	$2.7\pm2.2*$
10 ²	$6.4\pm1.7*$	0**	0**	0**
10 ³	$4.3 \pm 2.1*$	0**	0**	0**
10^{4}	$4.1\pm0.8*$	0**	0**	0**

Table 2. Reproductive parameters of Moina macrocopa in different concentrations of microplastics

*Significant differences; **extremely significant differences

Life history characteristic parameters of Moina macrocopa in different concentrations of MPs

We calculated the net reproduction rate (R_0), the generation time (T), and the intrinsic growth rate (r_m) of *Moina macrocopa* in different concentrations of MPs according to *Table 1*. As shown in *Table 3*, the net reproductive rate and the generation time showed a downward trend with the increase of the concentration of MPs. The net reproductive rate of *Moina macrocopa* in the control group was more than twice that of the treatment group of 10 pieces/mL. The intrinsic growth rate, which was very low, only existed in the treatment group of 10 pieces/mL and was far lower than the control group. The results showed that the concentration of MPs had a significant effect on the life cycle parameters of *Moina macrocopa*.

Zhang et al. (2020) revealed the molecular mechanism of slow growth, weak reproductive capacity, and imbalance of sex ratio of offspring of *Daphnia pulex* after being exposed to PS for a long time. Trehalose transporter 1 (TRET1), trehalose-6-phosphate synthase (TPS), and chitinase (CHI), which were related to metabolism and cathepsin (CTSL) and involved in growth regulation, were all downregulated, while DMRTA and DMRT1 genes involved in gender differentiation were significantly upregulated. This may also be the underlying reason why the life cycle parameters of *Moina macrocopa* were affected by the concentration of MPs in our study.

Concentration of microplastics (pieces/mL)	Ro	Т	r _m
0	2.49	6.63	0.14
10	1.10	4.54	0.02
10 ²	0	0	0
10 ³	0	0	0
10^{4}	0	0	0

Table 3. Life history characteristic parameters of Moina macrocopa in different concentrations of microplastics

Conclusion

Moina macrocopa was exposed to the 0.5 μ m MPs in different concentrations, and the survivability and reproduction experiment was conducted for 15 days. The results showed that the concentration of MPs had a significant effect on the survivability and reproduction of *Moina macrocopa*. The higher the concentration of MPs, the shorter the survival time of *Moina macrocopa*, and the reproduction rate of *Moina macrocopa*

decreased with the increase of the concentration of MPs. When the concentration of MPs reached 10^2 pieces/mL, *Moina macrocopa* would no longer reproduce. It is strongly possible that the MPs entered the body of *Moina macrocopa*, causing a reduction in food ingestion, thereby giving rise to the malnutrition of *Moina macrocopa*, which directly affected the intrinsic growth rate, generation time, and net reproductive rate.

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