Short-term toxicity of polystryrene microplastics on mysid shrimps *Neomysis japonica*

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Abstract. Plastic debris especially microplastics (MPs) have become a global concern for the aggravating pollution in the oceans. In this study, the physico-chemical properties of fluorescently labeled polystyrene (PS) beads and the effects of PS-MPs on the survival of mysid shrimps (*Neomysis japonica*) were investigated. PS-MPs were identified to have spherical shape, uniform size and stable green fluorescence. The results showed that PS beads had little effects on the mortality of shrimps under a short-term (72 h) exposure with concentrations of 50 μg L\(^{-1}\) and 500 μg L\(^{-1}\). However, PS-MPs had severe short-term toxicity on the survival of mysid shrimps, resulting in 30% mortality especially in a 72 h exposure with the higher concentration of 1000 μg L\(^{-1}\). These findings provide new insights into the toxic effects of MPs on marine invertebrates.

1. Introduction

Global plastic production increased rapidly annually due to the widespread use in both industrial products and consumer goods, which was estimated to reach up to 322 million tons in 2015 [1]. However, lack of appropriate disposal measures of plastic waste led to excessive accumulation of plastic debris in the ocean ecosystem [2], which further aggravated environmental threats such as smothering the seabed, preventing gas-exchange and threatening functioning of marine ecosystems [2,3]. Moreover, large plastic items can be gradually fractured into microplastics (MPs), which defined as plastic particles less than 5 mm in diameter, because of photodegradation, mechanical breakdown and microbiological decomposition [4]. In recent years, concern is continuously rising on the ubiquitous and persistent contamination of MPs in marine ecosystems, especially their threats to marine biota.

Polystyrene (PS), one of the five main types of plastic items, is a primary component of plastic debris observed in the environment [2,4]. Increasing evidences have supported that PS derived MPs (PS-MPs) could be ingested by diverse organisms (e.g. fish, crab and copepod) [5,6,7,8]. Entanglement and blocking of PS-MPs in the tissues may disrupt physiological processes [6], hamper energy flows [7] and even affect the survival and growth of marine biota [8]. Mysid shrimps (*Neomysis japonica*), as an ecotoxicological model for environmental sensitivity [9], have been used in evaluating toxicity of heavy metal [10], PAHs [11] and other environmental pollutants. However, few studies have been focused on the effects of different concentrations of MPs on mysid shrimps. The aim of this study was to investigate the physico-chemical properties of PS-MPs and evaluate the effects of different concentrations of MPs on the survival of mysid shrimps.
2. Materials and methods

2.1. Preparation and characterization of PS-MPs
Fluorescently labeled PS-MPs (5μm, 450 nm excitation, 498 nm emission) were purchased from Phosphorex, Inc. (Lot #: 2106B, Hopkinton, USA). PS suspensions (10 mg/mL) were prepared in filtered natural seawater (NSW) (0.45 μm filter membrane, Jinteng, China) and kept at 4 °C in dark. The suspensions were vortexed for 10 seconds prior to ecotoxicological studies. Primary size and shape of PS-MPs were identified by transmission electron microscopy (TEM, JEM-1011, JEOL, Japan) and scanning electron microscope (SEM, Hatachi S4800, Hatachi, Japan). The fluorescent intensity of PS-MPs was ascertained by fluorescence microscope (DM2500, Leica, Germany). In addition, the zeta-potential of the suspensions was determined using dynamic light scattering (Nano-ZS90, Malvern, UK).

2.2. Mysid shrimps maintenance and PS-MPs exposure
Mysid Shrimps (Neomysis japonica) at the age of 27 days were obtained from an aquafarm in western part of Liaoning province. They were maintained in 18 L glass cylinder containing 12 L of filtered NSW (pH 8.14 ± 0.03, salinity 32.5 ± 0.5 ‰, temperature 22.6 ± 0.6 °C) with continuously aeration for 1 day to allow full gut depuration. The short-term toxicity test was performed in glass beakers containing 2 L of filtered NSW. The mysid shrimps were exposed to different concentrations of PS suspensions (0, 50, 500, 1000 μg L⁻¹) for 72 h, and were fed with brine shrimp nauplius (40 baits/shrimp) twice a day (08:00-09:00 and 17:00-18:00). Dead mysid shrimps, faeces and excess food were removed at regular intervals and the mortality was recorded every day during the experiment.

2.3. Statistical analysis
The significance of the various parameters was tested by one-way analysis of variance (ANOVA) using Duncan’s multiple range test (P = 0.05) for different exposure concentrations (i.e. 0, 50, 500, 1000 μg L⁻¹). All of the analyses were performed by means of Statistical Product and Service Solutions Software (SPSS, version 20.0).

3. Results and discussions

3.1. Properties of PS-MPs
Both the SEM and TEM images confirmed that the pristine diameter of PS-MPs was 5 μm and they had regularly spherical shape (figure 1 a-c). PS microspheres were labeled with stable green fluorescence, which dispersed evenly in the aqueous phase (figure 1 b, d). Additionally, the zeta-potential of PS microspheres was -17.25 ± 2.84 mV.

3.2. Effects of PS-MPs on the survival of mysid shrimps
Regardless of the differences in the exposure time, the toxicity of PS-MPs on the survival of mysid shrimps increased with increasing exposure concentrations, indicating that PS-MPs exhibited significant concentration-dependent toxicity of mortality for the tested mysid shrimps. PS-MPs exposure at the highest concentration (1000 μg L⁻¹) caused 30% mortality of the shrimps after waterborne exposure for 72 h (P < 0.01), but no similar effect was observed at lower concentrations (≤ 500 μg L⁻¹). Additionally, there was also no significant difference between PS-MPs exposure concentrations of 50 μg L⁻¹ and 500 μg L⁻¹ and control treatment during the whole incubation (figure 2). These results could be attributed to the large amounts of MPs accumulation in stomach, intestine or other tissues of the mysid shrimps [12] at the higher concentrations of PS-MPs exposure, which could interfere feeding, digestion, excretion and other normal physiological processes [2,7], consequently suppressing the growth and further posing threats to the survival of the mysid shrimps.
4. Conclusion
In this study, the spherical PS-MPs with particle size of 5 μm, were stable and dispersed uniformly in aqueous phase. The effects of PS-MPs on the mortality of mysid shrimps were concentration-dependent. The higher concentration of PS-MPs (1000 μg L⁻¹) caused a 30% mortality, but lower concentrations (≤ 500 μg L⁻¹) had no obvious adverse effect on the survival of mysid shrimps. These results could contribute to verify threats of MPs to marine biota.

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