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Accumulation, tissue distribution, and biochemical effects of polystyrene microplastics in the freshwater fish red tilapia (*Oreochromis niloticus*)☆

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ABSTRACT

While the presence of microplastics (MPs) in marine environments has been detected worldwide, the importance of MPs pollution in freshwater environments has also been emphasized in recent years. However, the body of knowledge regarding the biological effects of MPs on freshwater organisms is still much more limited than on marine organisms. The aim of the present study was to evaluate the accumulation and tissue distribution of MPs in the freshwater fish red tilapia (*Oreochromis niloticus*), as well as the biochemical effects of MPs on *O. niloticus*. During 14 days of exposure to 0.1 μm polystyrene-MPs at concentrations of 1, 10, and 100 μg L^{-1}, the MPs concentrations in various tissues of *O. niloticus* generally increased over time following the order gut > gills > liver ≈ brain. Moreover, the acetylcholinesterase (AChE) activity in the fish brain was inhibited by MPs exposure, with a maximum inhibition rate of 37.7%, suggesting the potential neurotoxicity of MPs to freshwater fish. The activities of cytochrome P450 (CYP) enzymes [7-ethoxyresorufin *O*-deethylase (EROD) and 7-benzyloxy-4-trifluoromethyl-coumarin *O*-dibenzyloxylase (BFCOD)] in the fish liver exhibited clear temporal variabilities, with significant decreases followed by elevations compared to the control. The alterations of the EROD and BFCOD activities indicate the potential involvement of CYP enzymes for the metabolism of MPs. The activity of antioxidative enzyme superoxide dismutase (SOD) in the liver was significantly induced throughout the exposure period, while the malondialdehyde (MDA) content did not vary with MPs exposure, suggesting that the antioxidative enzymatic system in *O. niloticus* could prevent oxidative damage. These results highlight the ingestion and accumulation of MPs in different tissues of freshwater fish, which lead to perturbations in fish biological systems and should be considered in environmental risk assessment.

1. Introduction

Due to massive production and imperfect waste management, many plastics are discarded into the aquatic environment (McDevitt et al., 2017). Plastic waste in the aquatic environment degrades into numerous tiny plastic fragments/fibres/spheroids/granules/pellets/flakes/beads between 0.1 and 5000 μm in size; these particles are known as microplastics (MPs) (EFSA, 2016). As emerging environmental contaminants, MPs have received increasing attention from both the scientific and public communities (McDevitt et al., 2017; UNEP, 2014).

Over the past decade, the presence of MPs in marine environments has been reported worldwide (Browne et al., 2011; do Sul and Costa, 2014; Waller et al., 2017), and more recently, the importance of MPs pollution in freshwater environments has been emphasized (Duis and Coors, 2016; Eerkes-Medrano et al., 2015; Horton et al., 2017b; Wagner et al., 2014). From the available literature, MPs appear to be widespread in the waters and

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sediments of freshwater environments in Europe (Fischer et al., 2016; Horton et al., 2017a; Klein et al., 2015), North America (Baldwin et al., 2016; Corcoran et al., 2015; Driedger et al., 2015), and China (Su et al., 2016; Wang et al., 2017; Zhang et al., 2016, 2017). MPs share a similar size range with plankton species. Thus, MPs in water can be easily picked and ingested by invertebrates and fish. Several field studies have reported the occurrence of MPs in invertebrates (Su et al., 2016) and the gut contents of fish in freshwater habitats (Dantas et al., 2012; Jabeen et al., 2017; Zhang et al., 2017). This issue raises general concerns about the ecological and human health impacts of MPs across food-chains (Duis and Coors, 2016; Miranda and de Carvalho-Souza, 2016).

Thus, there is an urgent need to investigate the bioaccumulation potential of MPs in freshwater organisms. Some laboratory studies have documented MPs uptake in freshwater invertebrates at organism level, including the water flea (*Daphnia magna*) (Frydkjær et al., 2017; Jemec et al., 2016; Ogonowski et al., 2016; Rehse et al., 2016; Rist et al., 2017; Rosenkranz et al., 2009), amphipods (*Hyalella Azteca* and *Gammarus fossarum*) (Au et al., 2015; Blarer and Burkhardt-Holm, 2016), and oligochaetes (*Lumbricus variegates*) (Imhof et al., 2013). However, information on the bioaccumulation of MPs in freshwater fish, especially at tissue level, is still limited. Lu et al. (2016) confirmed the uptake and tissue accumulation (gills, liver and gut) of polystyrene-MPs (PS-MPs; 5 μm diameter) in zebrafish (*Danio rerio*) during a 7 d waterborne exposure to 20 mg L^{-1} of PS-MPs; the time curves showed a rapid increase of PS-MPs accumulation, reaching a steady state within 48 h. By contrast, Grigorakis et al. (2017) found that due to limited retention time, microbeads and microfibers greater than 63 μm were unlikely to accumulate within the gut contents of goldfish (*Carassius auratus*) *via* dietary exposure with 50 MPs particles per food pellet. These results suggest that accumulation of MPs may be a variation of different factors, such as species, time, particle size, and exposure schemes. Collectively, there are still many gaps in our knowledge of uptake, accumulation, and tissue distribution of MPs in freshwater fish species.

The body of knowledge regarding the biological effects of MPs on freshwater organisms, especially fish, is much more limited than on marine organisms (Duis and Coors, 2016; Eerkes-Medrano et al., 2015; Wagner et al., 2014). It is known that ingestion of MPs by marine organisms can cause blockage of the gastrointestinal tract or inflammatory responses and consequently trigger a range of adverse effects, such as lower energy reserves, reduced reproduction/growth, oxidative damage, metabolism disruption, and cellular lesions (reviewed in Cole et al., 2011; Eerkes-Medrano et al., 2015; Horton et al., 2017b). Within the limited information on freshwater fish, Rochman et al. (2013) reported that exposure to polyethylene-MPs (PE-MPs) induced hepatic stress in Japanese medaka (*Oryzias latipes*), including glycogen depletion, fatty vacuolization and cell necrosis. Moreover, bioaccumulation of PS-MPs can disturb lipid and energy metabolism as well as induce oxidative stress in the liver of zebrafish (*D. rerio*) (Lu et al., 2016). These studies indicate that bioaccumulation of MPs can induce toxic effects in different freshwater fish species, which should be carefully considered.

In the present study, red tilapia (*Oreochromis niloticus*), a common freshwater fish in China, was employed as the model organism. The bioaccumulation and distribution of PS-MPs (0.1 μm) in various tissues (gut, liver, gills and brain) were investigated. Additionally, a suite of biomarkers at the molecular level in fish tissues was applied to evaluate the biological effects of PS-MPs on *O. niloticus*. Among the applied biomarkers, a nervous system enzyme [acetylcholinesterase (AChE)] in the brain was utilized to assess the potential neurotoxicity of PS-MPs; cytochrome P450 (CYP) enzymes [7-ethoxyresorufin *O*-deethylase (EROD) and 7-benzyloxy-4-trifluoromethyl-coumarin *O*-dibenzyloxylase (BFCOD)] in the liver were utilized to assess metabolic disturbances in fish; and an antioxidant enzyme [superoxide dismutase (SOD)] and by-product of lipid peroxidation (LPO) [malondialdehyde (MDA)] in the liver were utilized to assess potential oxidative damage induced by PS-MPs.

2. Materials and methods

2.1. PS-MPs and chemicals preparation

Stocks of green fluorescent PS-MPs beads with a size of 0.1 μm (excitation: 488 nm, emission: 518 nm) were purchased from Da'e Scientific Co., Ltd. (Tianjin, China). They were supplied in deionized water as dispersions (10 mg mL^{-1}, 18198×10^9 particles mL^{-1}). According to the manufacturer, the fluorescent labelled microbeads were prepared using the swelling method, and the fluorescence components were contained inside the polymer matrix. The stocks were stored at 4 °C in the dark and sonicated before each use. Transmission electron microscope (TEM) images for the stocks of PS-MPs used in this study are presented in Fig. 1. The PS-MPs showed a homogeneous size distribution and spherical shape. Kits for analyzing the protein concentration, MDA content, and activities of AChE and SOD were purchased from Comin Biotechnology Co., Ltd. (Suzhou, China). Reduced nicotinamide adenine dinucleotide phosphate (NADPH), resorufin, ethoxyresorufin, and 7-benzyloxy-4-trifluoromethyl coumarin were purchased from Sigma Chemical (St. Louis, MO, USA).

2.2. Ethics statement

This study was approved by the Animal Care and Use committee of Jiangnan University. The methods of all experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals of China.

2.3. Animals and exposure

Red tilapia (*O. niloticus*) were supplied by the Qiting Pilot Research Station (Wuxi, China), which is affiliated to the Freshwater Fisheries Research Center of the Chinese Academy of Fishery

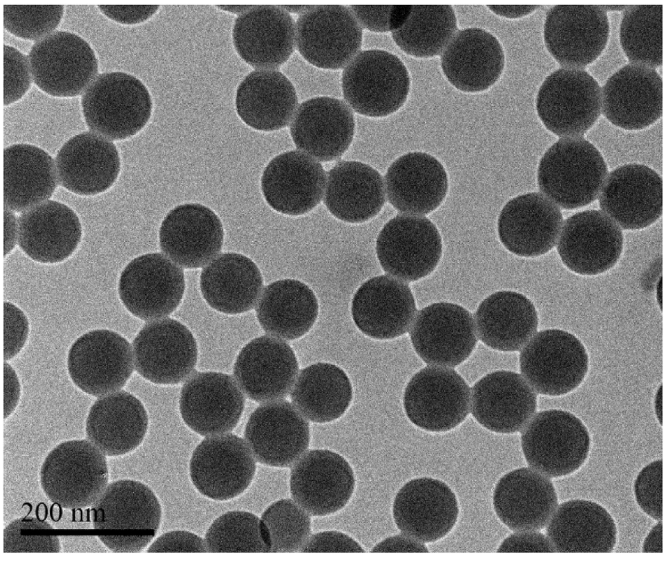

Fig. 1. TEM images of PS-MPs (0.1 μm) used in this study.