Ecotoxicology and Environmental Safety 241 (2022) 113843

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Environmentally relevant concentrations of microplastics modulated the immune response and swimming activity, and impaired the development of marine medaka *Oryzias melastigma* larvae

Jin-Can Chen [a,d], Chao Fang [a], Rong-Hui Zheng [a], Ming-Liang Chen [a], Duck-Hyun Kim [b], Young-Hwan Lee [b], Christyn Bailey [c], Ke-Jian Wang [d], Jae-Seong Lee [b], Jun Bo [a,*]

[a] Laboratory of Marine Biology and Ecology, Third Institute of Oceanography, Ministry of Natural Resources, Xiamen 361005, China
[b] Department of Biological Sciences, College of Science, Sungkyunkwan University, Suwon 16419, South Korea
[c] Fish Immunology and Pathology Laboratory, Animal Health Research Center (CISA-INIA), Madrid 28130, Spain
[d] State Key Laboratory of Marine Environmental Science, College of Ocean & Earth Sciences, Xiamen University, Xiamen 361102, China

ARTICLE INFO

Edited by Professor Bing Yan

Keywords:
Microplastic accumulation
Body length
Swimming behaviour
Mucins
Immunomodulation

ABSTRACT

Microplastics (MPs), due to their impacts on the ecosystem and their integration into the food web either through trophic transfer or ingestion directly from the ambient environment, are an emerging class of environmental contaminants posing a great threat to marine organisms. Most reports on the toxic effects of MPs exclusively focus on bioaccumulation, oxidative stress, pathological damage, and metabolic disturbance in fish. However, the collected information on fish immunity in response to MPs is poorly defined. In particular, little is known regarding mucosal immunity and the role of mucins. In this study, marine medaka (*Oryzias melastigma*) larvae were exposed to 6.0 μm beads of polystyrene microplastics (PS-MPs) at three environmentally relevant concentrations (10^2 particles/L, 10^4 particles/L, and 10^6 particles/L) for 14 days. The experiment was carried out to explore the developmental and behavioural indices, the transcriptional profiles of mucins, pro-inflammatory, immune, metabolism and antioxidant responses related genes, as well as the accumulation of PS-MPs in larvae. The results revealed that PS-MPs were observed in the gastrointestinal tract, with a concentration- and exposure time-dependent manner. No significant difference in the larval mortality was found between the treatment groups and the control, whereas the body length of larvae demonstrated a significant reduction at 10^6 particles/L on 14 days post-hatching. The swimming behaviour of the larvae became hyperactive under exposure to 10^4 and 10^6 particles/L PS-MPs. In addition, PS-MP exposure significantly up-regulated the mucin gene transcriptional levels of *muc7-like* and *muc13-like*, however down-regulated the mucin gene expression levels of *heg1, muc2*, *muc5AC-like* and *muc13*. The immune- and inflammation and metabolism-relevant genes (*jak*, *stat-3*, *il-6*, *il-1β*, *tnf-a*, *ccl-11*, *nf-κb*, and *sod*) were significantly induced by PS-MPs at 10^4 and 10^6 particles/L compared to the control. Taken together, this study suggests that PS-MPs induced inflammation response and might obstruct the immune functions and retarded the growth of the marine medaka larvae even at environmentally relevant concentrations.

1. Introduction

Plastic refers to a group of polymers made from petroleum sources, including polystyrene (PS), polypropylene (PP), polyvinylchloride (PVC) and polyethylene (PE) (Vert et al., 2012). The plastic debris could be categorised as mesoplastics, microplastics and nanoplastics based on their sizes (Andrady, 2011). Microplastic (MP) are referred to as plastic smaller than 5 mm in size (Anon, 2009; Barnes et al., 2009) and MP debris is documented ubiquitously in marine ecosystems worldwide, including coastal and sea surface waters (Gao et al., 2022; Lusher et al., 2014), deep-sea sediment (Woodall et al., 2014), arctic regions (Mu et al., 2019; von Friesen et al., 2020), and marine organisms (Fang et al., 2021). Great variations in MP concentrations have been observed worldwide in marine surface waters, with concentrations ranging from 10^{-4} to 381 particles/L (Lorenz et al., 2019; Shruti et al., 2021; Song et al., 2018, 2015; Zhao et al., 2015).

* Correspondence to: Third Institute of Oceanography, Ministry of Natural Resources, Xiamen 361005, Fujian Province, China.
E-mail address: bojun@tio.org.cn (J. Bo).

https://doi.org/10.1016/j.ecoenv.2022.113843
Received 6 April 2022; Received in revised form 17 June 2022; Accepted 1 July 2022
Available online 6 July 2022

MPs in marine environments are of particular concern since they are posing an increasing threat to marine organisms. An extensive range of marine species can ingest some MPs, because the sizes, shapes, and colours of MPs are similar to those of food (Alimba and Faggio, 2019). Besides, MPs can also be transferred along the food chain to higher trophic level organisms (Hasegawa and Nakaoka, 2021; Sucharitakul et al., 2021; Wang et al., 2021; Zhang et al., 2022). Numerous studies have already focused on the toxicity of MPs on marine organisms, including crustaceans, fish, molluscs and microbiota (Ajith et al., 2020; Farrell and Nelson, 2013; Lee et al., 2013; Martins and Guilhermino, 2018). For instance, the most obvious growth inhibition of the marine diatom (*Skeletonema costatum*) has been observed after exposure to 50 mg/L MPs (PVC, 1 μm) at 96 h (Zhang et al., 2017). However, no mortality of African catfish (*Clarias gariepinus*) juveniles was observed in all treatments except in the highest concentration (2 g/L) of low-density polyethylene (LDPE) MPs (50–500 μm) exposure for 4 days (Tongo and Erhunmwunse, 2022). In addition, the ingested MPs could accumulate in the fish gastrointestinal tract (GIT) and resulting blockages (Tongo and Erhunmwunse, 2022; Wright et al., 2013). MPs have shown to cause adverse effects including oxidative stress (Jovanović, 2017; Kang et al., 2021), reproductive disturbances (Sussarellu et al., 2016), and modulate a series of immunological functions, such as the transcriptional levels of immune genes in fish (Espinosa et al., 2017; Gu et al., 2020). Furthermore, Choi et al. (2018) reported that irregular MPs decreased the swimming behaviour of sheepshead minnow (*Cyprinodon variegatus*).

The most frequently detected synthetic polymers in the aquatic environment are low- and high-density polyethene (LDPE and HDPE), PS, PP, and polyethene terephthalate (PET) (Bratovcic, 2017). These synthetic polymers increase the occurrence of MPs in the aquatic environment as they are discarded after use (Turra et al., 2014). Among the above-mentioned multiple types of polymers, PS is the most frequently used plastic polymer, making it the most commonly used microplastic in toxicological studies and often detected in the aquatic environment (Browne et al., 2010). PS-MPs negatively impact the body length, survival rate and metabolism of marine organisms under stressful exposure (Wang et al., 2019b; Yin et al., 2019). In addition, the increased expression of genes encoding the antioxidants superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in the Chinese mitten crab (*Eriocheir sinensis*) decreased after PS-MP exposure (Yu et al., 2018). Furthermore, some studies of MP exposure demonstrated the inhibitory effects on the locomotor ability of aquatic organisms (Qiang and Cheng, 2019; Sun et al., 2021; Yin et al., 2019). On the contrary, the swimming behaviour of zebrafish became hyperactive after MP exposure (Chen et al., 2020b).

The immune system defends against exogenous microorganisms and xenobiotics, and the negative effects of MPs on the immune system of aquatic animals have been previously reported (Kang et al., 2021; Limonta et al., 2019). MP exposure can modulate a series of immunological functions, such as lysozyme activity, neutrophil levels, immunoglobulin levels, respiratory burst, phagocytosis, and the transcriptional levels of immune genes in fish (Choi et al., 2018; Espinosa et al., 2017; Gu et al., 2020; Kim et al., 2021). However, the collected information of PS-MPs on fish immunity is poorly defined (Abihssira-García et al., 2020; Kim et al., 2021). Secreted and membrane-associated mucins are classes of multifunctional glycosylated proteins involved in cell proliferation, cell differentiation and immune response (Pérez-Sánchez et al., 2013), which play important roles in mucosal immunity (Good et al., 2017). The expression patterns and distribution of mucin in human organs are relatively well known (Dhanisha et al., 2018; Gollub et al., 1992; Larivée et al., 1994; Smirnova et al., 2001; Sperber et al., 1991; Wang et al., 2007). However, knowledge of mucins in fish has just started to advance in the last few years (Marcos-López et al., 2018). PS-MP exposure induced mucin secretion in the gut of zebrafish (Jin et al., 2018), meanwhile, viral infections resulted in a mucin disturbance expression in mucosal tissues in the common carp (*Cyprinus carpio*) (Adamek et al., 2017). In addition, it is reported that MP exposure tends to cause intestinal flora disorders, reduce intestinal mucus secretion, and finally led to the destruction of the digestive system (Qiao et al., 2019; Yin et al., 2021). Our previous research showed that eight out of twenty-one differentially expressed genes were mucin genes of marine medaka larvae exposed to 6.0 μm PS-MPs at a concentration of 10^6 particles/L (Chen et al., 2020a). Herein, we hypothesise that mucin genes may play an important role in the immune protection of marine medaka larvae against PS-MP stress.

The goals of the present study were to examine the toxicity of PS-MP exposure (14 days) on the immune response, swimming activity and development of marine medaka (*Oryzias melastigma*), a marine fish model (Bo et al., 2011; Kong et al., 2008). Firstly, the distribution and accumulation patterns of fluorescently labelled PS-MPs inside *O. melastigma* larvae were examined. Then, the developmental effects of PS-MPs on marine medaka larvae were investigated in terms of body length, body width, and survival rate. Furthermore, the effects of PS-MPs on the swimming behaviour of the larvae were determined. Finally, the transcriptional profiles of a panel of target genes involved in immune- and inflammation-related genes, mucin genes, metabolic genes, and oxidative stress-relevant genes were also analyzed.

2. Materials and methods

2.1. Polystyrene microplastics

PS-MPs were selected in the present study because they are abundant in the marine ecosystems (Han et al., 2020; Sadri and Thompson, 2014). Fluorescent-labelled and fluorescent-free PS-MPs with a diameter of 6.0 μm were obtained from Polysciences (Warrington, PA, USA). The size and morphology of the PS-MPs were confirmed by scanning electron microscopy (SEM). The PS-MPs were spherical and had a consistent size of 6.0 μm (Fig. 1a and b), and the MPs were homogeneously dispersed in the artificial seawater (ASW) under an inverted fluorescence microscope (Fig. 1c and d), and no dense aggregates were observed on the water surface or walls of the plate.

In the present study, we used two types of PS-MPs, green fluorescent-labelled and fluorescent-free. Green fluorescent-labelled PS-MPs were used to determine the distribution of PS-MPs in the medaka larvae, while fluorescent-free PS-MPs were used in all other toxic experiments. Regarding the fluorescent dyes of PS-MPs, the supplier of the green fluorescent-labelled PS-MPs used in this study indicates that there is no leaching effect of the fluorescence and they show high stability in organic solvents, and these products feature water-insoluble dyes, which minimizes the incidence of dye leaching. In addition, a previous study reported that the fluorescent dyes of PS-MP leakage in the aqueous phase can be assumed to be negligible (Li et al., 2020).

2.2. Marine medaka maintenance

Marine medaka were kindly provided by Dr. Doris W.T. Au (City University of Hong Kong). Maintenance of the fish was in accordance with the standard methods as previously described (Chen et al., 2020a). Adult marine medaka was maintained in 30‰ ASW at 28.5 °C under a photoperiod of 14 h light and 10 h darkness, and fish were fed with 2.0 % body weight newly hatched brine shrimp (*Artemia salina*) and fed three times daily at 9:00, 12:00 and 17:00. The ASW was filtered with 0.22 μm pore diameter GF/A cellulose nitrate membrane filters (Millipore Sigma Corp., Burlington, MA, USA). The preparation of fish embryos and larvae was carried out according to a previous study (Zhang et al., 2021). In brief, adult marine medaka were transferred into a breeding aquarium at a ratio of 1:1 male: to female. Breeding crosses were set at 17:30, and embryos were collected the next morning between 8:00 and 9:00. The collected fertilised eggs were randomly assigned to glass petri dishes with a diameter of 12 cm, and incubated in 100 mL ASW. The hatched larvae were reared in PS-MP solution for 14 days in petri dishes (100 larvae in each petri dish and 500 larvae in each

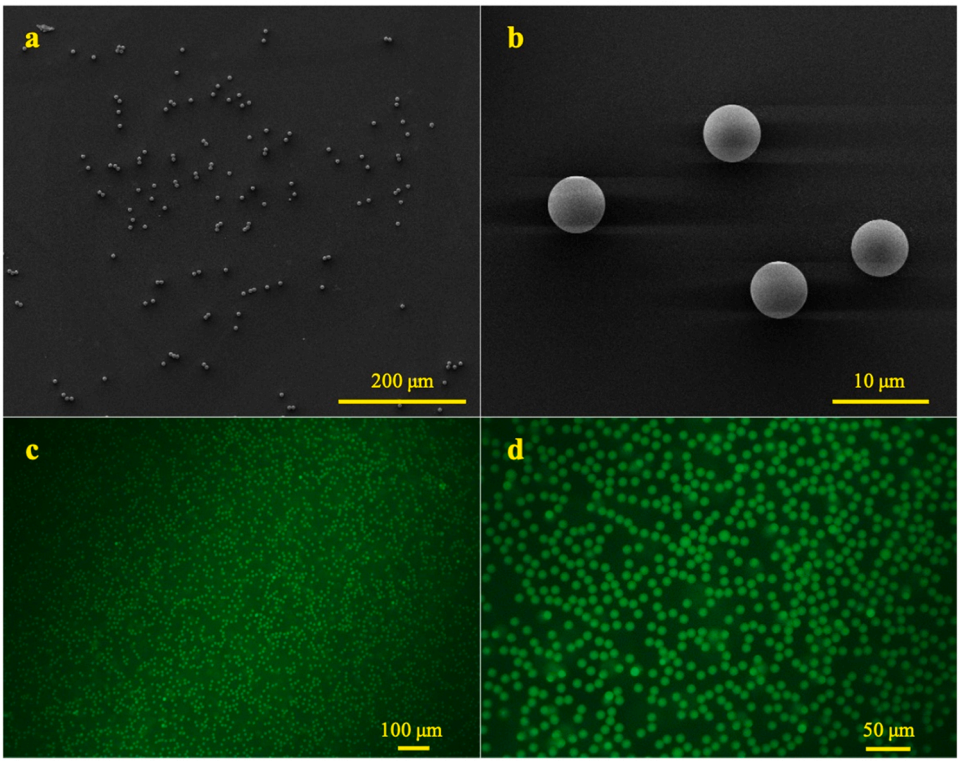

Fig. 1. The morphology and distribution of polystyrene microplastics in artificial seawater (ASW) confirmed by scanning electron microscopy (a) 200 μm, (b) 10 μm, and inverted fluorescence microscope (c) 100 μm, (d) 50 μm.

group; for mortality test: 40 larvae in each petri dish and 200 larvae in each group). During PS-MP exposure period, the larvae were fed commercially formulated feed purchased from Zeigler (East Berlin, PA, USA) twice daily at 2.0 % body weight. All animal experiments were carried out under a guideline approved by Ethics committee of Xiamen University (XMULAC20190066).

2.3. Experimental design

After the embryos were hatched (most embryos were hatched at 11 dpf, marked as 0 day post-hatching, dph), the healthy larvae were randomly separated and exposed to the control group, and PS-MP treatment groups (100 larvae per replicate, five replicates), for a total duration of 14 days. PS microspheres with the size of 6.0 μm were used and four concentrations were used as treatments: 0 (control group), 1×10^2 particles/L (equal to 1.1 μg/L), 1×10^4 particles/L (equal to 1.1×10^3 μg/L) and 1×10^6 particles/L (equal to 1.1×10^5 μg/L). The PS-MP size of 6.0 μm was chosen due to such a small size of MPs being frequently found in the marine environment and that they be easily ingested by marine biota (Kane and Clare, 2019). The concentration of PS-MPs used in this study was set in a range closed to the environmentally relevant MP concentrations (10^{-4} to 381 particles/L), as well as the commonly used concentrations as previous studies reported (0.88×10^2 to 6.82×10^{10} particles/L) (Davarpanah and Guilhermino, 2015; Leslie et al., 2017; Niu et al., 2021; Prata et al., 2022; Song et al., 2015; Zhang et al., 2017).

During the light period, glass pipettes were used to agitate the incubating solution every 4 h to keep the PS-MPs homogeneously dispersed in the water column. The incubating solutions were refreshed every 24 h and dead larvae were removed. At 3-, 7- and 14-dph, the larvae were sampled for the measurement of morphological parameters (body length and body width, n = 9, three larvae from each replicate, three replicates), PS-MP accumulation (n = 3, a pooled sample of three larvae from each replicate, three replicates), larvae swimming behaviour assay (n = 25, five larvae from each replicate, five replicates) and relative gene expression (n = 5, a pooled sampled of 15 larvae from each replicate, five replicates).

2.4. Measurements of survival rate, body length, and body width of marine medaka larvae

The marine medaka larvae were inspected three times daily at 9:30, 12:30 and 17:30, and the survival rates (%) were recorded at the end of the 14-day PS-MP exposure (40 larvae in each replicate, five replicates). The survival rates (%) were measured by dividing the number of surviving larvae by the total number of larvae. Growth was measured by individual body length and body width from photographs taken at 3, 7, and 14 d post-exposure, and nine larvae from each group were sampled at each time point. The body width and body length of marine medaka larvae were determined by a stereomicroscope (Nikon SMZ1270; Nikon Corp., Tokyo, Japan).

2.5. Accumulation of polystyrene microplastics in marine medaka larvae

To determine the accumulation of ingested fluorescent PS-MPs in marine medaka, three larvae from each replicate were sampled and imaged at 3, 7, and 14 dph (n = 9, three larvae sampled from each replicate, three replicates), followed by digested and transfer to a filter to count the number of MP beads as per a previous study (Fang et al., 2018). In brief, marine medaka larvae were sampled and rinsed immediately with Milli-Q water three times to remove the PS-MPs adhered to the larval skin. The larvae were then euthanized by rapid cooling in ice water. After alkaline digestion with 10 % KOH (w/v), followed by floatation and filtration, the microplastic was then transferred to the filter. The number of MPs was counted under a fluorescence microscope (Olympus IX81; Olympus Corp., Tokyo, Japan).

2.6. Larvae behavioural assay

The behaviour assay of marine medaka larvae was performed according to a previous study with slight modification (Zhang et al., 2021). In brief, five larvae were randomly selected from each petri dish at 3, 7, and 14 dph and gently transferred to 96-well plates with one larva per well (n = 25, five larvae from each replicate, five replicates). The behaviour assay was determined between 14:30 and 16:30 under 26 ± 0.5 °C. After a 10 min acclimation in the dark, the swimming behaviour of each larvae was monitored using the DanioVision system (Noldus, Netherlands) in response to light-to-dark transitions (10 min light: 10 min darkness cycle, repeat once). The data (movement duration and distance moved) were exported from EthoVision XT v11.5, followed by calculating in Excel (Microsoft Corp., USA), and carried out the significance test in SPSS v26.0.0.0 (SPSS Software Inc., USA).

2.7. Real-time quantitative polymerase chain reaction (RT-qPCR)

The following genes expression levels were determined by RT-qPCR: (i) immune and inflammation: *c3* (complement component 3, a central complement component), *hep1* and *hep2* (antimicrobial peptide genes, hepcidin1 and 2), *il-6* (interleukin 6), *il-8* (interleukin 8), *il-1β* (interleukin 1β), *tnf-α* (tumour necrosis factor *α*), *jak* (Janus kinase), *stat-3* (signal transducer and activator of transcription 3), *nf-κb* (Nuclear transcription factor-κb), and *ccl-11* (c-c motif chemokine 11); (ii) mucin genes: *heg1* (mucin-like membrane protein, sialylated protein heg homolog 1), *intestinal muc-like*, *intestinal muc-like2*, *intestinal muc-like4*, *muc1-like*, *muc2*, *muc4-like*, *muc6-like*, *muc7-like*, *muc5AC-like*, *muc13*, *muc13-like*, and *muc19*; (iii) metabolism-related genes *cyp1a1* (cytochrome P450 1a1) and *ahr* (aryl hydrocarbon receptor); (iv) antioxidant genes: *sod* (superoxide dismutase), *cat* (catalase) and *gpx* (glutathione peroxidase). The genes were identified from the genome of marine medaka (Kim et al., 2018). In addition, the chromosomal location and classification of mucin genes in marine medaka are shown in Fig. S1.

The pooled samples of 15 larvae obtained at 3, 7, and 14 dph from each replicate were collected from glass petri dishes (n = 5, five replicates), flash frozen in liquid nitrogen and stored at − 80 °C until further analysis. Total RNA in the larvae was isolated using *TRIzol* reagent (Takara Biochemicals, Beijing, China). The RT-qPCR primers are shown in Table S1. The amplification efficiency of all the tested genes ranged from 90 % to 120 %. 18 S ribosomal RNA (*18 S rRNA*) was used as the housekeeping gene and RT-qPCR was performed as previous description (Bo et al., 2012).

2.8. Statistical analysis

Results are reported as mean ± standard error (S.E.). The statistical analysis was performed with SPSS v26.0.0.0 (SPSS Software Inc., USA). Normal distribution of the data was first checked with Shapiro-Wilk's test, followed by Levene's test to check the homogeneity of variances of the data. The biometric data were analysed with a one-way analysis of variance (one-way ANOVA) analysis followed by a Tukey's (honestly significant difference) HSD test when the data were normally distributed. If the data is non-normal distributed, then non-parametric Kruskal-Wallis tests were used. Pearson's correlation between accumulated PS-MPs number and exposure time/concentration was assessed using GraphPad Prism v8.4.0 (GraphPad Software, San Diego, CA, USA). Significant differences were accepted at * $p < 0.05$, * * $p < 0.01$. Graphs were generated using GraphPad Prism v8.4.0 and organized with Microsoft PowerPoint (Microsoft Corp., USA).

3. Results

3.1. Survival rate, body length, and body width of marine medaka larvae

The survival rates of marine medaka larvae were generally high than 99 % (99.17–100 %) in the control and treatment groups (Table S2) after PS-MP exposure for 14 days, with no significant difference between each group. No significant difference in the body width of larvae was observed between the PS-MP treatment groups and control at 3, 7 and 14 days. Compared to the control, a significant reduction ($p < 0.05$) in body lengths of larvae was observed in the PS-MP treatment group for 14 days (Table S3). The width of the larvae ranged from 0.55 mm to 0.94 mm and the body length ranged from 1.53 mm to 2.83 mm (Table S3).

3.2. Accumulation of polystyrene microplastics in marine medaka larvae

The fluorescently labelled PS-MPs were observed in the GIT of marine medaka larvae at 7 and 14 dph (Fig. 2a). Regarding the exposure concentration, fluorescent PS-MPs were only detected in the larvae exposed to 10^4 and 10^6 particles/L PS-MPs (Fig. 2a). A blank control filter (only ASW, no marine medaka larvae) was set up during the larvae digestion process, and no fluorescent PS-MPs were found. The number of PS-MPs in marine medaka larvae in different groups indicated a positive correlation (Pearson correlation) with concentration and exposure duration. The number of PS-MPs in marine medaka larvae reached the highest level (327.3 items/replicate) on day 14 under the exposure concentration of 10^6 particles/L (Fig. 2b), which is consistent with the visible fluorescence signals shown in Fig. 2a.

3.3. Locomotor activity

For the distance moved (per 10 min), no significant changes in locomotor activity of marine medaka larvae were observed ($p > 0.05$) during the light period in all treatment groups at 3-, 7- and 14- day post PS-MP exposure relative to the control group (Fig. 3a and b). However, the distance moved by the larvae from 10^4 particles/L and 10^6 particles/L PS-MPs groups was significantly increased during the dark period (Fig. 3a and b, * $p < 0.05$ and ** $p < 0.01$) at all the three-time points except 14 d (Fig. 3c). In terms of average distance moved per minute, the larvae from 10^2 particles/L, 10^4 particles/L and 10^6 particles/L groups did not significantly differ ($p > 0.05$) in the locomotor activity at 3, 7 and 14 d compared to the control (Fig. 3d, e and f). Furthermore, the average distance moved every minute of the marine medaka larvae from all the groups was similar to each other at 14 days post-PS-MP exposure (Fig. 3f) compared to that of day 3 and day 7 (Fig. 3d and e).

3.4. Effects of polystyrene microplastics on the transcriptional expression levels of target genes

3.4.1. Expression profiles of immune- and inflammation-related marker genes

Compared to the control, the expression level of *il-1β* mRNA showed a significant increment ($p < 0.05$) of the larvae from the 10^2 particles/L PS-MP group at 3 d, and a significant increase ($p < 0.05$) of *il-6*, *tnf-α, jak* and *stat-3* transcripts at 7 d (Fig. 4). Interestingly, the transcript level of *nf-κb* was significantly increased ($p < 0.05$) in the 10^2 particles/L PS-MPs group at 7 d, but significantly decreased ($p < 0.05$) at 14 d compared to the control. For the 10^4 particles/L PS-MPs group, the transcriptional level of *nf-κb* was significantly lower ($p < 0.05$) at 14 d among the nine immune- and inflammation-related genes. For the 10^6 particles/L PS-MPs group, the expression of *stat-3* was significantly greater ($p < 0.05$) at 7 d than that of the control. Moreover, significant decreases ($p < 0.05$) were observed in the *il-6*, *il-1β* and *nf-κb* transcripts from the 10^6 particles/L PS-MPs group at 14 d, and a significant decrease ($p < 0.01$) in *nf-κb* transcripts was observed.

3.4.2. Expression profiles of mucin transcripts

Among the 13 mucin genes/transcripts, six genes (*heg1*, *muc2*, *muc5AC-like*, *muc7-like*, *muc13* and *muc13-like*) were significantly regulated ($p < 0.05$) compared to the control (Fig. 5). (i) For the 10^2 particles/L PS-MPs group, no significant ($p > 0.05$) changes in the 13

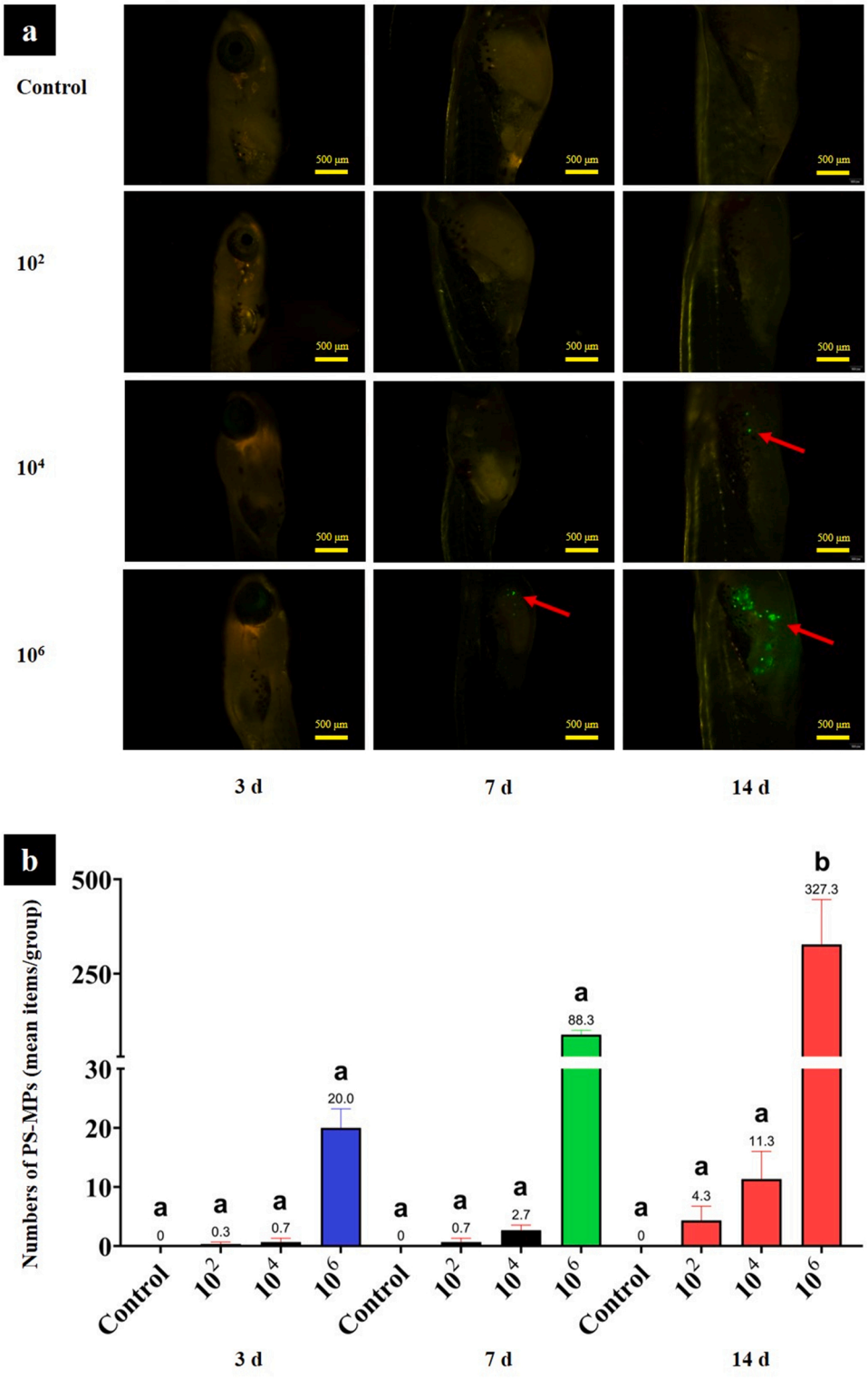

Fig. 2. Accumulation of polystyrene microplastics (PS-MPs) in marine medaka larvae. (a) Images of fluorescent PS-MPs in the gastrointestinal tract (GIT) of marine medaka larvae (n = 9 per group). Representative photos are shown for each group. Red closed arrows indicate PS-MPs in the GIT of the marine medaka larvae. (b) The number of PS-MPs taken up by marine medaka larvae exposed to different concentrations of PS-MPs at different times (n = 3 per group). When there is no common sign between two entries, there is a significant difference ($p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mucin genes were observed at 3, 7 and 14 d, except *muc7-like* demonstrated a significant increase ($p < 0.05$) at day 7; (ii) In terms of 10^4 particles/L PS-MPs group, a significant increase ($p < 0.05$) in *muc13-like* transcripts was reported in the larvae at 3 days post-exposure, but the expression levels of *heg1* and *muc5AC-like* were significantly decreased ($p < 0.01$ for *heg1*, $p < 0.05$ for *muc5AC-like*) at 14 d; (iii) Regarding the 10^6 particles/L PS-MPs group, the *muc2* and *muc13* mRNA levels were significantly downregulated ($p < 0.05$) in the larvae at 3 days post-exposure, while a significant increase ($p < 0.05$) in *muc13-like* transcripts was observed at 7 days post-exposure.

3.4.3. Expression profiles of oxidative stress related genes

Compared to the control, 10^2 particles/L PS-MP exposure group showed a significant increase ($p < 0.05$) of *sod* mRNA expression at 7 d, and then it decreased to a basic level at 14 d (Fig. 6). Compared with the control, no significant change ($p > 0.05$) of *cat* and *gpx* mRNA

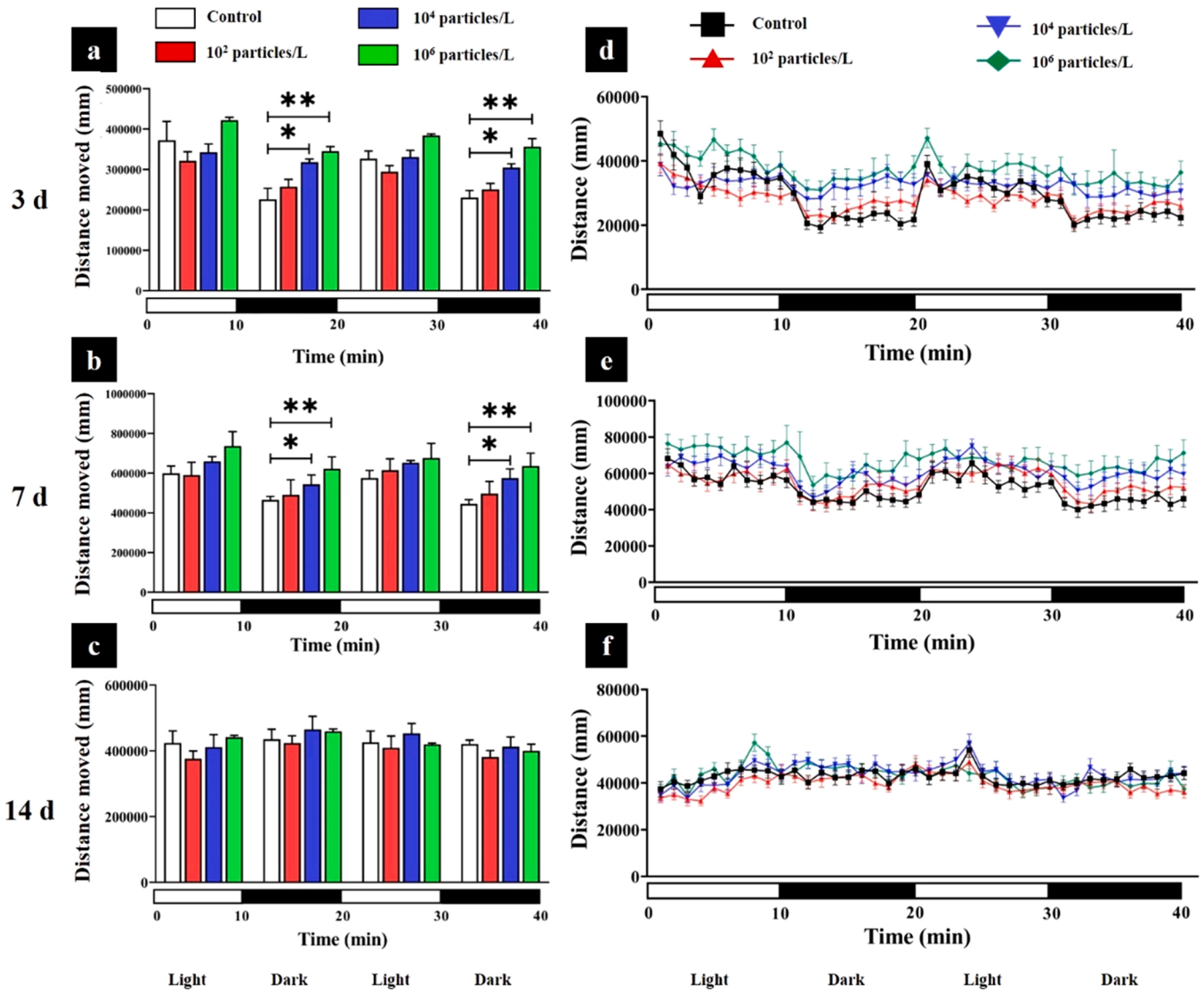

Fig. 3. Locomotor activity of larval marine medaka exposed to different concentrations of polystyrene microplastics (PS-MPs) for (a) 3, (b) 7 and (c) 14 d. Average distance moved per minute of larval marine medaka exposed to different concentrations of PS-MPs for (d) 3, (e) 7 and (f) 14 d. Significant differences were accepted at * $p < 0.05$, ** $p < 0.01$, n = 25 per group (5 larvae from each replicate, five replicates).

expression was observed in the larvae exposed to PS-MPs at any concentration or any exposure time point (Fig. 6).

3.4.4. *Expression profiles of metabolism-related genes*

Compared with the control, no significant changes ($p > 0.05$) of *ahr* and *cyp1a1* mRNA expression were observed in the marine medaka larvae exposed to PS-MPs at 3, 7, and 14 d ($p > 0.05$, Fig. 7).

4. Discussion

4.1. *Polystyrene microplastic uptake delayed the growth of marine medaka larvae*

Body length of fish is an important marker for fishery resource management, which determines the economic value of fish (Tseng et al., 2020). In addition, from a sustainable development perspective, body length can also be used as an important index regardless of mature fish. The body length of larval zebrafish was significantly decreased under polystyrene nanoplastic (PS-NP) exposure with the concentration of 1 mg/L for 5 days (Chen et al., 2017). Similarly, PVC-MPs inhibited the body length growth of common carp (*Cyprinus carpio* var.) larvae under 60-day dietary exposure (Xia et al., 2020). In this study, the body length inhibitory effect of PS-MPs on marine medaka larvae is positively correlated with the MP concentration after 14-day exposure. One explanation for the decreased growth in larvae is that the accumulated MPs may stimulate larval hyperactivity and thus activate the pro-inflammatory immune response, resulting in an increase in energy consumption (Chen et al., 2020b). Another possible explanation is that the MPs blockage in larval GIT may decrease the food intake, as the MPs may occupy space in the intestine, causing a decrease in food intake and digestive capacity of the fish foregut (Bellehumeur et al., 2016; Chen et al., 2020b; Watts et al., 2015).

4.2. *Accumulation of polystyrene microplastics in the body of fish larvae*

MPs seem to build up in fish GIT (mainly intestinal) and translocate among tissues, resulting in adverse effects such as physical blockage, intestinal damage, inhibition of biological functions and even inducing a reduction in larval mortality (Bhuyan, 2022; Browne et al., 2010; Jeong et al., 2018; Kang et al., 2021; Lusher et al., 2013; Wright et al., 2013). In this study, the fluorescently labelled PS-MPs were detected in the GIT of marine medaka larvae, with the number of accumulated PS-MPs

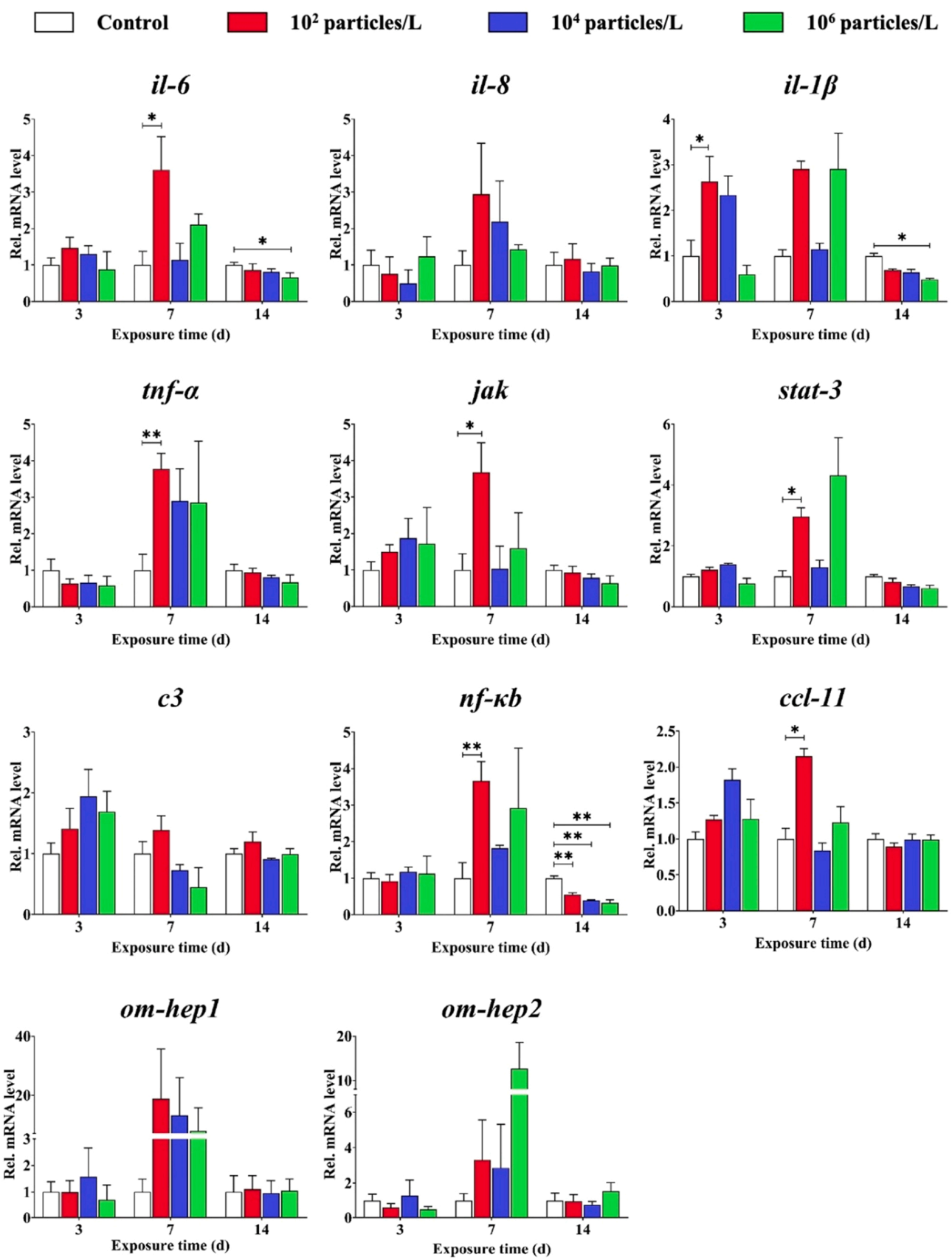

Fig. 4. Relative expression levels of immune- and inflammation-related genes in marine medaka larvae exposed to different concentrations of polystyrene microplastics at different times (n = 5 per group, five replicates). Significant differences were accepted at * = $p < 0.05$, ** = $p < 0.01$.

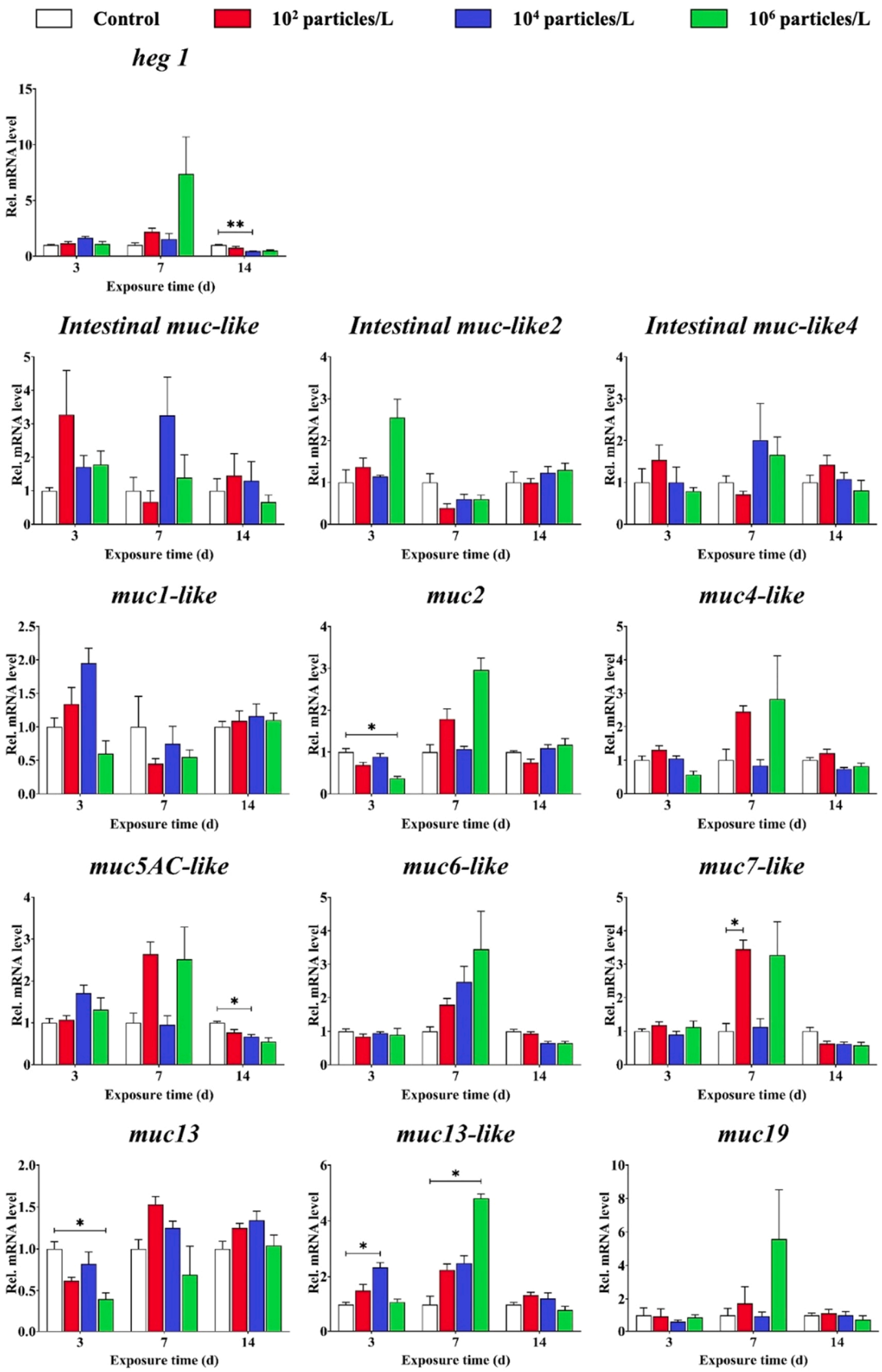

Fig. 5. Relative expression levels of mucin genes in marine medaka larvae exposed to different concentrations of polystyrene microplastics at different times (n = 5 per group, five replicates). Significant differences were accepted at * = $p < 0.05$, ** = $p < 0.01$.

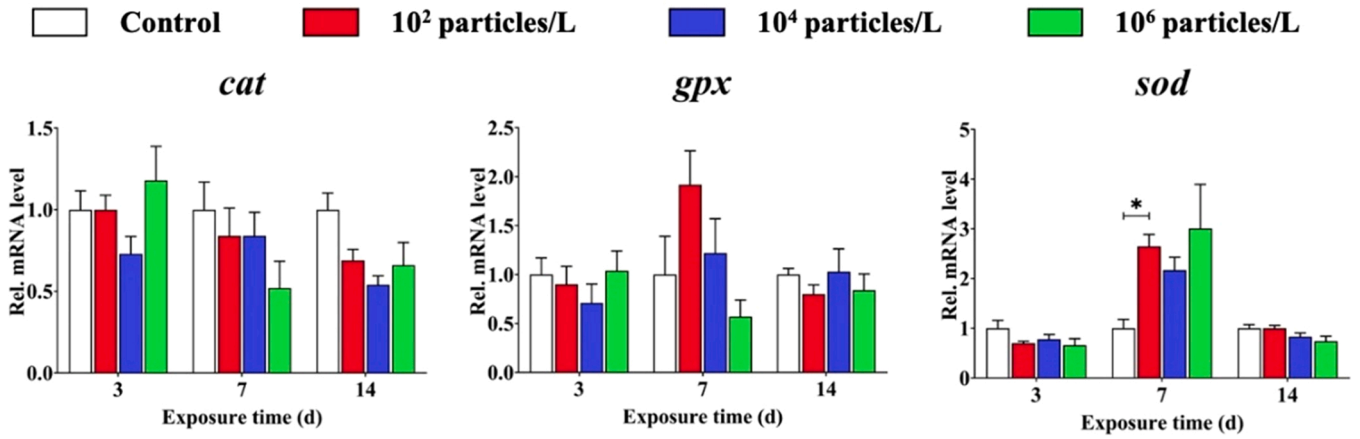

Fig. 6. Relative expression levels of oxidative stress related genes in marine medaka larvae exposed to different concentrations of polystyrene microplastics at different times (n = 5 per group, five replicates). Significant differences were accepted at * = $p < 0.05$.

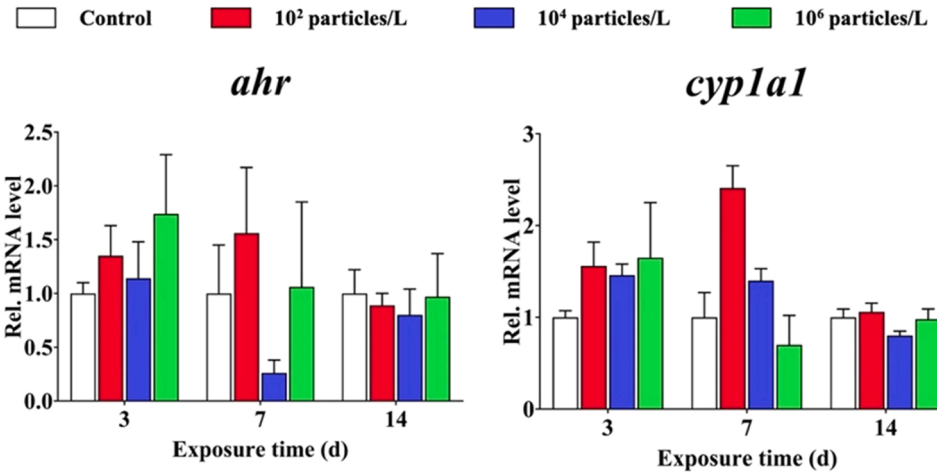

Fig. 7. Relative expression levels of metabolism-related genes in marine medaka larvae exposed to different concentrations of polystyrene microplastics at different times (n = 5 per group, five replicates).

increased in a time- and dose-dependent manner, suggesting blockage of MPs in the GIT. However, the survival rates of *O. melastigma* larvae did not significantly differ between the PS-MP treatment groups and the control after 14-day exposure, which in line with the results obtained by Zhu et al. (2020). Although MPs can accumulate in the GIT of marine medaka larvae, they will not cause the death of larvae under the present experimental condition.

Mounting studies suggested that MP exposure may disturb the gut microbial community in fish (Kang et al., 2021; Yin et al., 2021) and cause intestinal flora disorders, and result in inflammation in the digestive system (Yin et al., 2021). In this study, the larvae are too tiny to separate the intestine for transcriptional analysis of the inflammation-relevant genes, and it is hard to know whether the inflammatory responses were caused directly by MPs or by intestinal flora disorder, despite that we observed the accumulation of PS-MP particles in the GIT (Fig. 2) and the inflammation-relevant genes were significantly induced in the medaka larvae (Fig. 4).

4.3. Polystyrene microplastics led to hyperactive swimming behaviour in marine medaka larvae

Locomotor behaviour has an indispensable role in the daily activities of aquatic organisms, featuring in such activities as finding food and avoiding predators (da Silva Souza et al., 2020; Sun et al., 2021). MPs and further pollutants may affect the swimming behaviour of fish larvae such as swimming speed and swimming distance (Chen et al., 2020b; Yin et al., 2019; Zhang et al., 2021). In addition, the swimming distance and velocity of zebrafish larvae were significantly decreased and showed a correlation after exposure to 1 μm PS-MPs at concentrations of 100 and 1000 μg/L (Qiang and Cheng, 2019). Furthermore, no significant change was reported in the locomotor activity of zebrafish larvae exposed to PS-MPs (45 μm, 1000 μg/L), whereas PS-NPs (45 μm, 1000 μg/L) resulted in a significant decrease in the swimming behaviour of zebrafish larvae (Chen et al., 2017). In the present study, the marine medaka larvae became hyperactive after exposure to 6 μm PS-MPs at the concentrations of 10^4 and 10^6 particles/L, which is consistent with a previous study reporting that the locomotor activity exhibited a significantly increased in zebrafish exposed to MPs with a size of 5 μm (Chen et al., 2020b). In addition, the hyperactive states of larval marine medaka prolonged as the exposure concentration increased. The hyperactivity of fish locomotor activity may attribute to oxidative stress, energy metabolism and particulate matter stimulation (Chen et al., 2020b; Zhu et al., 2016). Neither positive nor negative correlations between the locomotor activity alteration and oxidative stress were observed in this study, therefore the abnormal hyperactivity in marine medaka larvae was likely to be linked with the ingested MP particle stimulation or energy metabolism.

4.4. Polystyrene microplastic exposure activated pro-inflammatory and immune responses

The mucus layer in the GIT is considered to be the first line of defense to the external environment (Pelaseyed et al., 2014), which contains diverse microorganisms that play vital roles in immune function and intestinal barrier function (Hsiao et al., 2013; Yano et al., 2015). The mucin proteins, encoded by approximately 20 mucin genes, shape the skeleton of the intestinal mucus and protect the intestinal tract (Johansson and Hansson, 2016). A previous study reported that mucins are disturbed in reaction to a broad range of injuries and/or challenges (Pérez-Sánchez et al., 2013). For instance, MP exposure caused dysbiosis of gut microbiota associated with immune dysfunction in marine medaka and resulting in an increased mucin volume (Kang et al., 2021). This is the first report on the distinct expression profiles of mucin genes in fish under MP exposure. MP exposure can result in physical damage to the fish intestine which is often associated with gut inflammation (Bhagat et al., 2020). *Muc13*, a transmembrane mucin, plays a crucial protective role in reaction to injury and inflammation in mice (Sheng et al., 2011). In this study, the mRNA expression level of *muc13-like* in marine medaka larvae was significantly increased at 3 and 7 d under PS-MP exposure, and it is speculated that *muc13-like* may have a similar protective function in both fish and mammals. M*uc2* and *muc5AC*, secreted mucins, are thought to be involved in the formation of the mucus layer over the epithelium in mammals (Cornick et al., 2015). The expression levels of *muc2* and *muc5AC-like* mRNA were significantly decreased in the larvae in the present study, which indicates the inhibitory effects of PS-MPs on the secreted mucins. Altogether, the existence of multiple mucin genes of fish and the distinct expression profiles suggest a complex and highly redundant protective mechanism of fish under MP stressors.

Intestinal epithelial cells and goblet cells are also related to the immune response process, and they can function as luminal sensors of the immune system (Pelaseyed et al., 2014). The expression levels of inflammatory- and immune-related genes, such as *il-6*, *jak*, *stat-3*, *tnf-α*, *nf-κb*, and *il-1β*, even at the lowest concentration (10^2 particles/L), were significantly upregulated, indicating an immunomodulating and pro-inflammatory response occurred in marine medaka larvae in response to environmentally relevant concentrations of PS-MPs. Immunotoxicological mechanisms of xenobiotics are involved in different signalling networks, such as NF-κB and JAK-STAT pathways through which the immune system is thought to be activated during pollutant exposure (Cui et al., 2019). IL-6 can activate the JAK-STAT signal pathway (Heinrich et al., 1998). On the contrary, NF-κB and STAT3 can collaboratively induce IL-6 expression in cancer cells (Yoon et al., 2012). In addition, lipopolysaccharide mediated anti-virus activity can be reversed by the suppression of NF-κB and JAK-STAT signalling (Yu et al., 2020). Therefore, this means that IL-6, NF-κB and JAK-STAT signalling pathways interact. Our results clearly showed that the immune response of MPs could be mediated by the JAK-STAT and NF-κB signalling pathways, and the activation of the JAK-STAT and NF-κB signalling pathways could hypothetically increase the susceptibility of fish to bacterial infections.

4.5. Microplastic exposure modulated metabolism and antioxidant responses

The activities of antioxidant and transcriptional expression of antioxidant enzyme-coding genes in fish were induced by environmental pollutants and stresses (Rhee et al., 2013). The relative transcription of antioxidant enzyme-related genes can accurately reflect the antioxidant activity of fish when no biochemical factors exhibit interference (Malandrakis et al., 2014; Wang et al., 2019a). Furthermore, oxidative stress may induce inflammation and impair physiological functions (Liguori et al., 2018). Therefore, monitoring the expression and alteration of related genes encoding antioxidant enzymes can assist us in a great elucidation regarding the potential mechanism of aquatic organisms in response to environmental stresses. The comparative expression levels of *sod* in marine medaka larvae were significantly up-regulated in response to 10^2 particles/L PS-MPs for 7 d and then decreased to normal levels at 14 d, indicating that the SOD enzyme of marine medaka larvae was generated in a timely manner in response to external stresses to scavenge excess free radicals, thus preventing damage to the organism (Wang et al., 2018; Xikeranmu et al., 2019; Zheng et al., 2016).

5. Conclusions

This study demonstrated that PS-MP exposure at levels at which they are found in the environment resulted in modulation of the immune relevant genes, mediated by pro-inflammatory and mucin transcript modulation as well as NF-κB and JAK-STAT signalling pathway activation, hyperactive swimming behaviour, and body length shortening that ultimately obstructed the development of marine medaka larvae. The present study is the first report on the expression profiles of mucin genes in fish in response to MP exposure, and the existence of multiple mucin genes of fish and the distinct expression profiles propose a complex and highly redundant protective mechanism of fish under MP stress. Further consideration should be given to the regulation mechanisms of mucins in aquatic organisms exposed to MPs.

CRediT authorship contribution statement

Jin-Can Chen: Writing – original draft, Formal analysis, Investigation, Visualization. **Chao Fang**: Methodology, Writing – review & editing. **Rong-Hui Zheng**: Investigation. **Ming-Liang Chen**: Methodology. **Duck-Hyun Kim**: Investigation. **Young-Hwan Lee**: Investigation. **Christyn Bailey**: Writing – review & editing. **Ke-Jian Wang**: Supervision. **Jae-Seong Lee**: Writing – review & editing. **Jun Bo**: Conceptualization, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was sponsored by the National Key Research and Development Program of China (No. 2019YFD0901101), the Youth Innovation Fund Project of Xiamen (3502Z20206099) and the Natural Science Foundation of Fujian Province, China (No. 2021J01506). Jincan Chen wishes to express sincere thanks to Mayday and Jason Zhang, whose songs instilled endless spiritual support to him over the past 20 years.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2022.113843.

References

Abihssira-García, I.S., Park, Y., Kiron, V., Olsvik, P.A., 2020. Fluorescent microplastic uptake by immune cells of Atlantic salmon (*Salmo salar* L.). Front. Environ. Sci. 8 https://doi.org/10.3389/fenvs.2020.560206.

Adamek, M., Hazerli, D., Matras, M., Teitge, F., Reichert, M., Steinhagen, D., 2017. Viral infections in common carp lead to a disturbance of mucin expression in mucosal tissues. Fish. Shellfish Immunol. 71, 353–358. https://doi.org/10.1016/j.fsi.2017.10.029.

Ajith, N., Arumugam, S., Parthasarathy, S., Manupoori, S., Janakiraman, S., 2020. Global distribution of microplastics and its impact on marine environment—a review. Environ. Sci. Pollut. Res. 27, 25970–25986. https://doi.org/10.1007/s11356-020-09015-5.

Alimba, C.G., Faggio, C., 2019. Microplastics in the marine environment: Current trends in environmental pollution and mechanisms of toxicological profile. Environ. Toxicol. Pharmacol. 68, 61–74. https://doi.org/10.1016/j.etap.2019.03.001.
Andrady, A.L., 2011. Microplastics in the marine environment. Mar. Pollut. Bull. 62 (8), 1596–1605. https://doi.org/10.1016/j.marpolbul.2011.05.030.
AnonArthur, C., Baker, J., Bamford, H. (eds)., 2009. Proceedings of the International Research Workshop on the Occurrence, Effects and Fate of Microplastic Marine Debris. Sept 9–11, 2008. NOAA Technical Memorandum NOS-OR&R-30. Tacoma, Washington, USA.
Barnes, D.K.A., Galgani, F., Thompson, R.C., Barlaz, M., 2009. Accumulation and fragmentation of plastic debris in global environments. Philos. Trans. 364, 1985–1998. https://doi.org/10.1098/rstb.2008.0205.
Bellehumeur, K., Lapointe, D., Cooke, S.J., Moon, T.W., 2016. Exposure to sublethal levels of PCB-126 impacts fuel metabolism and swimming performance in rainbow trout. Comp. Biochem. Phys. B: Biochem. Mol. Biol. Dr. Thomas W. Moon 199, 97–104. https://doi.org/10.1016/j.cbpb.2016.01.005.
Bhagat, J., Zang, L., Nishimura, N., Shimada, Y., 2020. Zebrafish: an emerging model to study microplastic and nanoplastic toxicity. Sci. Total Environ. 728, 138707 https://doi.org/10.1016/j.scitotenv.2020.138707.
Bo, J., Cai, L., Xu, J.H., Wang, K.J., Au, D.W.T., 2011. The marine medaka Oryzias melastigma – A potential marine fish model for innate immune study. Mar. Pollut. Bull., 6th International Conference on Marine Pollution and Ecotoxicology 63, 267–276. https://doi.org/10.1016/j.marpolbul.2011.05.014.
Bhuyan, Md.S., 2022. Effects of microplastics on fish and in human health. Front. Environ. Sci. 10. https://doi.org/10.3389/fenvs.2022.827289.
Bo, J., Giesy, J.P., Ye, R., Wang, K.J., Lee, J.S., Au, D.W.T., 2012. Identification of differentially expressed genes and quantitative expression of complement genes in the liver of marine medaka *Oryzias melastigma* challenged with *Vibrio parahaemolyticus*. Comp. Biochem. Physiol. Part D Genom. Proteom. 7, 191–200. https://doi.org/10.1016/j.cbd.2012.02.005.
Bratovcic, A., 2017. Degradation of micro- and nano-plastics by photocatalytic methods. J. Nanosci. Nanotechnol. Appl. 3, 1–9. https://doi.org/10.18875/2577-7920.3.304.
Browne, M.A., Galloway, T.S., Thompson, R.C., 2010. Spatial patterns of plastic debris along estuarine shorelines. Environ. Sci. Technol. 44, 3404–3409. https://doi.org/10.1021/es903784e.
Chen, J.C., Chen, M.Y., Fang, C., Zheng, R.H., Jiang, Y.L., Zhang, Y.S., Wang, K.J., Bailey, C., Segner, H., Bo, J., 2020a. Microplastics negatively impact embryogenesis and modulate the immune response of the marine medaka *Oryzias melastigma*. Mar. Pollut. Bull. 158, 111349 https://doi.org/10.1016/j.marpolbul.2020.111349.
Chen, Q., Gundlach, M., Yang, S., Jiang, J., Velki, M., Yin, D., Hollert, H., 2017. Quantitative investigation of the mechanisms of microplastics and nanoplastics toward zebrafish larvae locomotor activity. Sci. Total Environ. 584–585, 1022–1031. https://doi.org/10.1016/j.scitotenv.2017.01.156.
Chen, Q., Lackmann, C., Wang, W., Seiler, T.-B., Hollert, H., Shi, H., 2020b. Microplastics lead to hyperactive swimming behaviour in adult zebrafish. Aquat. Toxicol. 224, 105521 https://doi.org/10.1016/j.aquatox.2020.105521.
Choi, J.S., Jung, Y.J., Hong, N.H., Hong, S.H., Park, J.W., 2018. Toxicological effects of irregularly shaped and spherical microplastics in a marine teleost, the sheepshead minnow (*Cyprinodon variegatus*). Mar. Pollut. Bull. 129, 231–240. https://doi.org/10.1016/j.marpolbul.2018.02.039.
Cornick, S., Tawiah, A., Chadee, K., 2015. Roles and regulation of the mucus barrier in the gut. Tissue Barriers 3, e982426. https://doi.org/10.4161/21688370.2014.982426.
Cui, Q., Chen, F.Y., Zhang, M., Peng, H., Wang, K.J., 2019. Transcriptomic analysis revealing hepcidin expression in *Oryzias melastigma* regulated through the JAK-STAT signaling pathway upon exposure to BaP. Aquat. Toxicol. 206, 134–141. https://doi.org/10.1016/j.aquatox.2018.11.015.
da Silva Souza, J.G., Libeck, L.T., do Carmo Rodrigues Virote, B., Egger, R.C., de Sá, G.C. R., Machado, G.J., Murgas, L.D.S., 2020. A method to analyze the relationship between locomotor activity and feeding behaviour in larvae of *Betta splendens*. Aquacult. Int. 28, 1141–1152. https://doi.org/10.1007/s10499-020-00516-1.
Davarpanah, E., Guilhermino, L., 2015. Single and combined effects of microplastics and copper on the population growth of the marine microalgae *Tetraselmis chuii*. Estuar. Coast. Shelf Sci. Coast. Syst. Chang. Tuning Assess. Manag. Tools 167, 269–275. https://doi.org/10.1016/j.ecss.2015.07.023.
Dhanisha, S.S., Guruvayoorappan, C., Drishya, S., Abeesh, P., 2018. Mucins: structural diversity, biosynthesis, its role in pathogenesis and as possible therapeutic targets. Crit. Rev. Oncol. Hematol. 122, 98–122. https://doi.org/10.1016/j.critrevonc.2017.12.006.
Espinosa, C., Cuesta, A., Esteban, M.Á., 2017. Effects of dietary polyvinylchloride microparticles on general health, immune status and expression of several genes related to stress in gilthead seabream (*Sparus aurata* L.). Fish. Shellfish Immunol. 68, 251–259. https://doi.org/10.1016/j.fsi.2017.07.006.
Fang, C., Zheng, R., Hong, F., Jiang, Y., Chen, J., Lin, H., Lin, L., Lei, R., Bailey, C., Bo, J., 2021. Microplastics in three typical benthic species from the Arctic: occurrence, characteristics, sources, and environmental implications. Environ. Res. 192, 110326 https://doi.org/10.1016/j.envres.2020.110326.
Fang, C., Zheng, R., Zhang, Y., Hong, F., Mu, J., Chen, M., Song, P., Lin, L., Lin, H., Le, F., Bo, J., 2018. Microplastic contamination in benthic organisms from the Arctic and sub-Arctic regions. Chemosphere 209, 298–306. https://doi.org/10.1016/j.chemosphere.2018.06.101.
Farrell, P., Nelson, K., 2013. Trophic level transfer of microplastic: *Mytilus edulis* (L.) to *Carcinus maenas* (L.). Environ. Pollut. 177, 1–3. https://doi.org/10.1016/j.envpol.2013.01.046.
Gao, L., Wang, Z., Peng, X., Su, Y., Fu, P., Ge, C., Zhao, J., Yang, L., Yu, H., Peng, L., 2022. Occurrence and spatial distribution of microplastics, and their correlation with petroleum in coastal waters of Hainan Island, China. Environ. Pollut. 294, 118636 https://doi.org/10.1016/j.envpol.2021.118636.
Gollub, E., Goswami, S., Sperber, K., Marom, Z., 1992. Isolation and characterization of a macrophage-derived high molecular weight protein involved in the regulation of mucus-like glycoconjugate secretion. J. Allergy Clin. Immunol. 89, 696–702. https://doi.org/10.1016/0091-6749(92)90376-d.
Good, M., Kolls, J.K., Empey, K.M., 2017. 130 - Neonatal Pulmonary Host Defense. In: Polin, R.A., Abman, S.H., Rowitch, D.H., Benitz, W.E., Fox, W.W. (Eds.), Fetal and Neonatal Physiology. Elsevier, Amsterdam, The Netherlands, pp. 1262–1293.e12. https://doi.org/10.1016/B978-0-323-35214-7.00130-X.
Gu, W., Liu, S., Chen, L., Liu, Y., Gu, C., Ren, H., Wu, B., 2020. Single-cell RNA sequencing reveals size-dependent effects of polystyrene microplastics on immune and secretory cell populations from zebrafish intestines. Environ. Sci. Technol. 54, 3417–3427. https://doi.org/10.1021/acs.est.9b06386.
Han, M., Niu, X., Tang, M., Zhang, B.-T., Wang, G., Yue, W., Kong, X., Zhu, J., 2020. Distribution of microplastics in surface water of the lower Yellow River near estuary. Sci. Total Environ. 707, 135601 https://doi.org/10.1016/j.scitotenv.2019.135601.
Hasegawa, T., Nakaoka, M., 2021. Trophic transfer of microplastics from mysids to fish greatly exceeds direct ingestion from the water column. Environ. Pollut. 273, 116468 https://doi.org/10.1016/j.envpol.2021.116468.
Heinrich, P.C., Behrmann, I., Müller-Newen, G., Schaper, F., Graeve, L., 1998. Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway1. Biochem. J. 334, 297–314. https://doi.org/10.1042/bj3340297.
Hsiao, E.Y., McBride, S.W., Hsien, S., Sharon, G., Hyde, E.R., McCue, T., Codelli, J.A., Chow, J., Reisman, S.E., Petrosino, J.F., Patterson, P.H., Mazmanian, S.K., 2013. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. Cell 155, 1451–1463. https://doi.org/10.1016/j.cell.2013.11.024.
Jeong, C., Kang, H., Lee, Y., Kim, M., Lee, J., Seo, J., Wang, M., Lee, J., 2018. Nanoplastic ingestion enhances toxicity of persistent organic pollutants (POPs) in the monogonont rotifer *Brachionus koreanus* via multixenobiotic resistance (MXR) disruption. Environ. Sci. Technol. 52, 11411–11418. https://doi.org/10.1021/acs.est.8b03211.
Jin, Y., Xia, J., Pan, Z., Yang, J., Wang, W., Fu, Z., 2018. Polystyrene microplastics induce microbiota dysbiosis and inflammation in the gut of adult zebrafish. Environ. Pollut. 235, 322–329. https://doi.org/10.1016/j.envpol.2017.12.088.
Johansson, M.E.V., Hansson, G.C., 2016. The Mucins. In: Ratcliffe, M.J.H. (Ed.), Encyclopedia of Immunobiology. Academic Press, Oxford, pp. 381–388. https://doi.org/10.1016/B978-0-12-374279-7.02019-1.
Jovanović, B., 2017. Ingestion of microplastics by fish and its potential consequences from a physical perspective. Integr. Environ. Assess. Manag. 13, 510–515. https://doi.org/10.1002/ieam.1913.
Kane, I.A., Clare, M.A., 2019. Dispersion, accumulation, and the ultimate fate of microplastics in deep-marine environments: a review and future directions. Front. Earth Sci. 7. https://doi.org/10.3389/feart.2019.00080.
Kang, H.M., Byeon, E., Jeong, H., Kim, M.S., Chen, Q., Lee, J.S., 2021. Different effects of nano- and microplastics on oxidative status and gut microbiota in the marine medaka *Oryzias melastigma*. J. Hazard. Mater. 405, 124207 https://doi.org/10.1016/j.jhazmat.2020.124207.
Kim, H.S., Lee, B.Y., Han, J., Jeong, C.B., Hwang, D.S., Lee, M.-C., Kang, H.M., Kim, D.-H., Lee, D., Kim, J., Choi, I.Y., Lee, J.S., 2018. The genome of the marine medaka *Oryzias melastigma*. Mol. Ecol. Resour. 18, 656–665. https://doi.org/10.1111/1755-0998.12769.
Kim, J., Maruthupandy, M., An, K.S., Lee, K.H., Jeon, S., Kim, J.-S., Cho, W.-S., 2021. Acute and subacute repeated oral toxicity study of fragmented microplastics in Sprague-Dawley rats. Ecotoxicol. Environ. Saf. 228, 112964 https://doi.org/10.1016/j.ecoenv.2021.112964.
Kong, R.Y.C., Giesy, J.P., Wu, R.S.S., Chen, E.X.H., Chiang, M.W.L., Lim, P.L., Yuen, B.B. H., Yip, B.W.P., Mok, H.O.L., Au, D.W.T., 2008. Development of a marine fish model for studying in vivo molecular responses in ecotoxicology. Aquat. Toxicol. 86, 131–141. https://doi.org/10.1016/j.aquatox.2007.10.011.
Larivée, P., Levine, S.J., Martinez, A., Wu, T., Logun, C., Shelhamer, J.H., 1994. Platelet-activating factor induces airway mucin release via activation of protein kinase C: evidence for translocation of protein kinase C to membranes. Am. J. Respir. Cell Mol. Biol. 11, 199–205. https://doi.org/10.1165/ajrcmb.11.2.8049080.
Lee, K.W., Shim, W.J., Kwon, O.Y., Kang, J.H., 2013. Size-dependent effects of micro polystyrene particles in the marine copepod *Tigriopus japonicus*. Environ. Sci. Technol. 47, 11278–11283. https://doi.org/10.1021/es401932b.
Leslie, H.A., Brandsma, S.H., van Velzen, M.J.M., Vethaak, A.D., 2017. Microplastics en route: Field measurements in the Dutch river delta and Amsterdam canals, wastewater treatment plants, North Sea sediments and biota. Environ. Int. 101, 133–142. https://doi.org/10.1016/j.envint.2017.01.018.
Li, L., Luo, Y., Peijnenburg, W.J.G.M., Li, R., Yang, J., Zhou, Q., 2020. Confocal measurement of microplastics uptake by plants. MethodsX 7, 100750. https://doi.org/10.1016/j.mex.2019.11.023.
Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., Gargiulo, G., Testa, G., Cacciatore, F., Bonaduce, D., Abete, P., 2018. Oxidative stress, aging, and diseases. Clin. Interv. Aging 13, 757–772. https://doi.org/10.2147/cia.s158513.
Limonta, G., Mancia, A., Benkhalqui, A., Bertolucci, C., Abelli, L., Fossi, M.C., Panti, C., 2019. Microplastics induce transcriptional changes, immune response and behavioral alterations in adult zebrafish. Sci. Rep. 9, 15775. https://doi.org/10.1038/s41598-019-52292-5.
Lorenz, C., Roscher, L., Meyer, M.S., Hildebrandt, L., Prume, J., Löder, M.G.J., Primpke, S., Gerdts, G., 2019. Spatial distribution of microplastics in sediments and surface waters of the southern North Sea. Environ. Pollut. 252, 1719–1729. https://doi.org/10.1016/j.envpol.2019.06.093.

Lusher, A.L., Burke, A., O'Connor, I., Officer, R., 2014. Microplastic pollution in the Northeast Atlantic Ocean: validated and opportunistic sampling. Mar. Pollut. Bull. 88 (1–2), 325–333. https://doi.org/10.1016/j.marpolbul.2014.08.023.

Lusher, A.L., McHugh, M., Thompson, R.C., 2013. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. Mar. Pollut. Bull. 67 (1–2), 94–99. https://doi.org/10.1016/j.marpolbul.2012.11.028.

Malandrakis, E.E., Exadactylos, A., Dadali, O., Golomazou, E., Klaoudatos, S., Panagiotaki, P., 2014. Molecular cloning of four glutathione peroxidase (GPx) homologs and expression analysis during stress exposure of the marine teleost *Sparus aurata*. Comp. Biochem. Physiol. Part B Biochem. Mol. Biol. 168, 53–61. https://doi.org/10.1016/j.cbpb.2013.11.005.

Marcos-López, M., Calduch-Giner, J.A., Mirimin, L., MacCarthy, E., Rodger, H.D., O'Connor, I., Sitjà-Bobadilla, A., Pérez-Sánchez, J., Piazzon, M.C., 2018. Gene expression analysis of Atlantic salmon gills reveals mucin 5 and interleukin 4/13 as key molecules during amoebic gill disease. Sci. Rep. 8, 13689. https://doi.org/10.1038/s41598-018-32019-8.

Martins, A., Guilhermino, L., 2018. Transgenerational effects and recovery of microplastics exposure in model populations of the freshwater cladoceran *Daphnia magna* Straus. Sci. Total Environ. 631–632, 421–428. https://doi.org/10.1016/j.scitotenv.2018.03.054.

Mu, J., Zhang, S., Qu, L., Jin, F., Fang, C., Ma, X., Zhang, W., Wang, J., 2019. Microplastics abundance and characteristics in surface waters from the Northwest Pacific, the Bering Sea, and the Chukchi Sea. Mar. Pollut. Bull. 143, 58–65. https://doi.org/10.1016/j.marpolbul.2019.04.023.

Niu, Z., Vandegehuchte, M.B., Catarino, A.I., Everaert, G., 2021. Environmentally relevant concentrations and sizes of microplastic do not impede marine diatom growth. J. Hazard. Mater. 409, 124460 https://doi.org/10.1016/j.jhazmat.2020.124460.

Pelaseyed, T., Bergström, J.H., Gustafsson, J.K., Ermund, A., Birchenough, G.M.H., Schütte, A., van der Post, S., Svensson, F., Rodríguez-Piñeiro, A.M., Nyström, E.E.L., Wising, C., Johansson, M.E.V., Hansson, G.C., 2014. The mucus and mucins of the goblet cells and enterocytes provide the first defense line of the gastrointestinal tract and interact with the immune system. Immunol. Rev. 260, 8–20. https://doi.org/10.1111/imr.12182.

Pérez-Sánchez, J., Estensoro, I., Redondo, M.J., Calduch-Giner, J.A., Kaushik, S., Sitjà-Bobadilla, A., 2013. Mucins as diagnostic and prognostic biomarkers in a fish-parasite model: Transcriptional and functional analysis. PLoS One 8, e65457. https://doi.org/10.1371/journal.pone.0065457.

Prata, J.C., Venâncio, C., Girão, A.V., da Costa, J.P., Lopes, I., Duarte, A.C., Rocha-Santos, T., 2022. Effects of virgin and weathered polystyrene and polypropylene microplastics on *Raphidocelis subcapitata* and embryos of *Danio rerio* under environmental concentrations. Sci. Total Environ. 816, 151642 https://doi.org/10.1016/j.scitotenv.2021.151642.

Qiang, L., Cheng, J., 2019. Exposure to microplastics decreases swimming competence in larval zebrafish (*Danio rerio*). Ecotoxicol. Environ. Saf. 176, 226–233. https://doi.org/10.1016/j.ecoenv.2019.03.088.

Qiao, R., Deng, Y., Zhang, S., Wolosker, M.B., Zhu, Q., Ren, H., Zhang, Y., 2019. Accumulation of different shapes of microplastics initiates intestinal injury and gut microbiota dysbiosis in the gut of zebrafish. Chemosphere 236, 124334. https://doi.org/10.1016/j.chemosphere.2019.07.065.

Rhee, J.S., Kim, B.M., Choi, B.S., Choi, I.Y., Wu, R.S.S., Nelson, D.R., Lee, J.S., 2013. Whole Spectrum of cytochrome p450 genes and molecular responses to water-accommodated fractions exposure in the marine medaka. Environ. Sci. Technol. 47, 4804–4812. https://doi.org/10.1021/es400186r.

Sadri, S.S., Thompson, R.C., 2014. On the quantity and composition of floating plastic debris entering and leaving the Tamar Estuary, Southwest England. Mar. Pollut. Bull. 81, 55–60. https://doi.org/10.1016/j.marpolbul.2014.02.020.

Sheng, Y.H., Lourie, R., Lindén, S.K., Jeffery, P.L., Roche, D., Tran, T.V., Png, C.W., Waterhouse, N., Sutton, P., Florin, T.H.J., McGuckin, M.A., 2011. The MUC13 cell-surface mucin protects against intestinal inflammation by inhibiting epithelial cell apoptosis. Gut 60, 1661–1670. https://doi.org/10.1136/gut.2011.239194.

Shruti, V.C., Pérez-Guevara, F., Kutralam-Muniasamy, G., 2021. The current state of microplastic pollution in the world's largest gulf and its future directions. Environ. Pollut. 291, 118142 https://doi.org/10.1016/j.envpol.2021.118142.

Smirnova, M.G., Kiselev, S.L., Birchall, J.P., Pearson, J.P., 2001. Up-regulation of mucin secretion in HT29-MTX cells by the pro-inflammatory cytokines tumor necrosis factor-alpha and interleukin-6. Eur. Cytokine Netw. 12, 119–125.

Song, Y.K., Hong, S.H., Eo, S., Jang, M., Han, G.M., Isobe, A., Shim, W.J., 2018. Horizontal and vertical distribution of microplastics in Korean coastal waters. Environ. Sci. Technol. 52, 12188–12197. https://doi.org/10.1021/acs.est.8b04032.

Song, Y.K., Hong, S.H., Jang, M., Han, G.M., Shim, W.J., 2015. Occurrence and distribution of microplastics in the sea surface microlayer in Jinhae Bay, South Korea. Arch. Environ. Contam. Toxicol. 69, 279–287. https://doi.org/10.1007/s00244-015-0209-9.

Sperber, K., Goswami, S.K., Gollub, E., Mayer, L., Marom, Z., 1991. Mucus secretagogue production by a human macrophage hybridoma. J. Allergy Clin. Immunol. 87, 490–498. https://doi.org/10.1016/0091-6749(91)90007-b.

Sucharitakul, P., Pitt, K.A., Welsh, D.T., 2021. Trophic transfer of microbeads to jellyfish and the importance of aging microbeads for microplastic experiments. Mar. Pollut. Bull. 172, 112867 https://doi.org/10.1016/j.marpolbul.2021.112867.

Sun, T., Zhan, J., Li, F., Ji, C., Wu, H., 2021. Environmentally relevant concentrations of microplastics influence the locomotor activity of aquatic biota. J. Hazard. Mater. 414, 125581 https://doi.org/10.1016/j.jhazmat.2021.125581.

Sussarellu, R., Suquet, M., Thomas, Y., Lambert, C., Fabioux, C., Pernet, M.E.J., Le Goïc, N., Quillien, V., Mingant, C., Epelboin, Y., Corporeau, C., Guyomarch, J., Robbens, J., Paul-Pont, I., Soudant, P., Huvet, A., 2016. Oyster reproduction is affected by exposure to polystyrene microplastics. Proc. Natl. Acad. Sci. U. S. A. 113, 2430–2435. https://doi.org/10.1073/pnas.1519019113.

Tongo, I., Erhunmwunse, N.O., 2022. Effects of ingestion of polyethylene microplastics on survival rate, opercular respiration rate and swimming performance of African catfish (*Clarias gariepinus*). J. Hazard. Mater. 423, 127237 https://doi.org/10.1016/j.jhazmat.2021.127237.

Tseng, C.H., Hsieh, C.L., Kuo, Y.F., 2020. Automatic measurement of the body length of harvested fish using convolutional neural networks. Biosyst. Eng. 189, 36–47. https://doi.org/10.1016/j.biosystemseng.2019.11.002.

Turra, A., Manzano, A.B., Dias, R.J.S., Mahiques, M.M., Barbosa, L., Balthazar-Silva, D., Moreira, F.T., 2014. Three-dimensional distribution of plastic pellets in sandy beaches: shifting paradigms. Sci. Rep. 4, 4435. https://doi.org/10.1038/srep04435.

Vert, M., Doi, Y., Hellwich, K., Hess, M., Hodge, P., Kubisa, P., Rinaudo, M., Schué, F., 2012. Terminology for biorelated polymers and applications (IUPAC Recommendations 2012). Pure Appl. Chem. 84 (2), 377–410. https://doi.org/10.1351/PAC-REC-10-12-04.

von Friesen, L.W., Granberg, M.E., Pavlova, O., Magnusson, K., Hassellöv, M., Gabrielsen, G.W., 2020. Summer sea ice melt and wastewater are important local sources of microlitter to Svalbard waters. Environ. Int. 139, 105511 https://doi.org/10.1016/j.envint.2020.105511.

Wang, C., Liang, Y., Fang, Y., Chang, X., 2019a. Effects of cyclical short-term food deprivation and refeeding on compensatory growth and gene expression of SOD, GPX and HSP70 in *Schizothorax wangchiachii*. Fish. Shellfish Immunol. 94, 628–633. https://doi.org/10.1016/j.fsi.2019.09.047.

Wang, I.J., Wu, C.Y., Hu, F.R., 2007. Effect of proinflammatory cytokines on the human MUC5AC promoter activity in vitro and in vivo. Clin. Ophthalmol. 1, 71–77.

Wang, J., Li, Y., Lu, L., Zheng, M., Zhang, X., Tian, H., Wang, W., Ru, S., 2019b. Polystyrene microplastics cause tissue damages, sex-specific reproductive disruption and transgenerational effects in marine medaka (*Oryzias melastigma*). Environ. Pollut. 254, 113024 https://doi.org/10.1016/j.envpol.2019.113024.

Wang, T., Hu, M., Xu, G., Shi, H., Leung, J.Y.S., Wang, Y., 2021. Microplastic accumulation via trophic transfer: Can a predatory crab counter the adverse effects of microplastics by body defence? Sci. Total Environ. 754, 142099 https://doi.org/10.1016/j.scitotenv.2020.142099.

Wang, Y., Yi, L., Zhang, J., Sun, L., Wen, W., Zhang, C., Wang, S., 2018. Functional analysis of superoxide dismutase of *Salmonella typhimurium* in serum resistance and biofilm formation. J. Appl. Microbiol. 125, 1526–1533. https://doi.org/10.1111/jam.14044.

Watts, A.J.R., Urbina, M.A., Corr, S., Lewis, C., Galloway, T.S., 2015. Ingestion of plastic microfibers by the crab *Carcinus maenas* and its effect on food consumption and energy balance. Environ. Sci. Technol. 49, 14597–14604. https://doi.org/10.1021/acs.est.5b04026.

Woodall, L.C., Sanchez-Vidal, A., Canals, M., Paterson, G.L.J., Coppock, R., Sleight, V., Calafat, A., Rogers, A.D., Narayanaswamy, B.E., Thompson, R.C., 2014. The deep sea is a major sink for microplastic debris. R. Soc. Open Sci. 1, 140317 https://doi.org/10.1098/rsos.140317.

Wright, S.L., Rowe, D., Thompson, R.C., Galloway, T.S., 2013. Microplastic ingestion decreases energy reserves in marine worms. Curr. Biol. 23, R1031–R1033. https://doi.org/10.1016/j.cub.2013.10.068.

Xia, X., Sun, M., Zhou, M., Chang, Z., Li, L., 2020. Polyvinyl chloride microplastics induce growth inhibition and oxidative stress in *Cyprinus carpio* var. larvae. Sci. Total Environ. 716, 136479 https://doi.org/10.1016/j.scitotenv.2019.136479.

Xikeranmu, Z., Abdunasir, M., Ma, J., Tusong, K., Liu, X., 2019. Characterization of two copper/zinc superoxide dismutases (Cu/Zn-SODs) from the desert beetle *Microdera punctipennis* and their activities in protecting *E. coli* cells against cold. Cryobiology 87, 15–27. https://doi.org/10.1016/j.cryobiol.2019.03.006.

Yano, J.M., Yu, K., Donaldson, G.P., Shastri, G.G., Ann, P., Ma, L., Nagler, C.R., Ismagilov, R.F., Mazmanian, S.K., Hsiao, E.Y., 2015. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. Cell 161, 264–276. https://doi.org/10.1016/j.cell.2015.02.047.

Yin, K., Wang, Y., Zhao, H., Wang, D., Guo, M., Mu, M., Liu, Y., Nie, X., Li, B., Li, J., Xing, M., 2021. A comparative review of microplastics and nanoplastics: Toxicity hazards on digestive, reproductive and nervous system. Sci. Total Environ. 774, 145758 https://doi.org/10.1016/j.scitotenv.2021.145758.

Yin, L., Liu, H., Cui, H., Chen, B., Li, L., Wu, F., 2019. Impacts of polystyrene microplastics on the behavior and metabolism in a marine demersal teleost, black rockfish (*Sebastes schlegelii*). J. Hazard. Mater. 380, 120861 https://doi.org/10.1016/j.jhazmat.2019.120861.

Yoon, S., Woo, S.U., Kang, J.H., Kim, K., Shin, H.-J., Gwak, H.S., Park, S., Chwae, Y.J., 2012. NF-κB and STAT3 cooperatively induce IL6 in starved cancer cells. Oncogene 31, 3467–3481. https://doi.org/10.1038/onc.2011.517.

Yu, P., Li, Y., Wang, Y., Peppelenbosch, M.P., Pan, Q., 2020. Lipopolysaccharide restricts murine norovirus infection in macrophages mainly through NF-kB and JAK-STAT signaling pathway. Virology 546, 109–121. https://doi.org/10.1016/j.virol.2020.04.010.

Yu, P., Liu, Z., Wu, D., Chen, M., Lv, W., Zhao, Y., 2018. Accumulation of polystyrene microplastics in juvenile *Eriocheir sinensis* and oxidative stress effects in the liver. Aquat. Toxicol. 200, 28–36. https://doi.org/10.1016/j.aquatox.2018.04.015.

Zhang, C., Chen, X., Wang, J., Tan, L., 2017. Toxic effects of microplastic on marine microalgae *Skeletonema costatum*: Interactions between microplastic and algae. Environ. Pollut. 220, 1282–1288. https://doi.org/10.1016/j.envpol.2016.11.005.

Zhang, S., Wang, N., Gong, S., Gao, S., 2022. The patterns of trophic transfer of microplastic ingestion by fish in the artificial reef area and adjacent waters of Haizhou Bay. Mar. Pollut. Bull. 177, 113565 https://doi.org/10.1016/j.marpolbul.2022.113565.

Zhang, Y.T., Chen, Mengyun, He, S., Fang, C., Chen, Mingliang, Li, D., Wu, D., Chernick, M., Hinton, D.E., Bo, J., Xie, L., Mu, J., 2021. Microplastics decrease the toxicity of triphenyl phosphate (TPhP) in the marine medaka (*Oryzias melastigma*) larvae. Sci. Total Environ. 763, 143040 https://doi.org/10.1016/j.scitotenv.2020.143040.

Zhao, S., Zhu, L., Li, D., 2015. Microplastic in three urban estuaries, China. Environ. Pollut. 206, 597–604. https://doi.org/10.1016/j.envpol.2015.08.027.

Zheng, J.L., Zhu, Q.L., Shen, B., Zeng, L., Zhu, A.Y., Wu, C.W., 2016. Effects of starvation on lipid accumulation and antioxidant response in the right and left lobes of liver in large yellow croaker *Pseudosciaena crocea*. Ecol. Indic. 66, 269–274. https://doi.org/10.1016/j.ecolind.2016.01.037.

Zhu, B., Wang, Q., Shi, X., Guo, Y., Xu, T., Zhou, B., 2016. Effect of combined exposure to lead and decabromodiphenyl ether on neurodevelopment of zebrafish larvae. Chemosphere 144, 1646–1654. https://doi.org/10.1016/j.chemosphere.2015.10.056.

Zhu, M., Chernick, M., Rittschof, D., Hinton, D.E., 2020. Chronic dietary exposure to polystyrene microplastics in maturing Japanese medaka (*Oryzias latipes*). Aquat. Toxicol. 220, 105396 https://doi.org/10.1016/j.aquatox.2019.105396.