Species-Specific Effects of Microplastics On Juvenile Fishes

Chaonan Zhang
South China Agricultural University
Qiujie Wang
South China Agricultural University
Zhiheng Zuo
South China Agricultural University
Zhengkun Pan
South China Agricultural University
Shaodan Wang
South China Agricultural University
Guohuan Xu
Guangdong Institute of Microbiology
Jixing Zou (zoujixing@scau.edu.cn)
South China Agricultural University

Research Article

Keywords: Microplastics, Species-specific, Juvenile fish, Gene expression, Intestinal morphology.

Posted Date: December 8th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1057867/v1

Abstract

Microplastics contamination have been extensively reported in aquatic ecosystem and organisms. It is wildly acknowledged that the ingestion, accumulation and elimination of microplastics in fishes are species-specific, which mainly depending on the feeding behavior. This study aims to investigate the effects of microplastics on the morphology and inflammatory response in intestines of fishes with different feeding types. Largemouth bass (carnivorous fish), grass carp (herbivorous fish) and Jian carp (omnivorous fish) were used as organism model. The contributing concentration and size of microplastics are explored as well as the response time and legacy effect in fishes. Two different sizes of polystyrene microplastics (8 μm and 80 nm) were set at three concentrations. And samples were analyzed at different exposure times and depuration times. Histological analysis indicated that multiple abnormalities in intestines are presented in three species fishes after acute exposure microplastics. The mRNA abundance of immune-related genes in the intestine tissues of fishes were significantly induced or restrained. There were differential expressions of genes coping with differential sizes and concentrations of microplastics exposure in different fishes. The reason for the difference effects of microplastics on fishes was still unclear but could be due to the difference in the structure and function of the digestive system. These results provide a theoretical basis to further analysis of the mechanism of fish intestinal pathology caused by microplastics.

1. Introduction

Over the last century, plastics have been remarkable materials in peoples' daily life due to its versatile, durable, and incredibly adaptable. Plastics production reached 368 million tonnes in 2019 worldwide with approximately 9% increasing rate every year and China contributed to 31% of world's plastics production (Plastics Europe, 2020). In the meanwhile, the global total of plastic waste reached 380 Tg in 2018 with an exponential growth every year (Rai et al., 2021). Once entering the environment, plastic may degrade or fragment into microplastics through UV radiation, mechanical transformation or biological degradation by microorganisms (Cole et al., 2011; Alimi et al., 2018). Microplastics are small plastic pieces or fibers that are smaller than 5 mm in size (NOAA, 2015). They come in many forms, not only secondary sources, but also primary sources, such as microbeads found in personal care products (McDevitt et al., 2017). Microplastics contamination have been extensively reported in marine, freshwater and terrestrial ecosystems (Peng et al., 2020; Wang et al., 2020; Xu et al., 2020), thus identified as one of the top 10 emerging global environmental problems by the United Nations Environment Program.

Adverse effects of microplastics on fishes have been found in many literatures (Jacob et al., 2020; Abhijit et al.,2021; Anna et al.,2021). Due to the attractive color, buoyancy, and food-like properties, fish are particularly prone to ingesting microplastics (Garrido Gamarro et al., 2020). The ingestion of microplastics by fish can cause a variety of consequences: 1) microplastics can lead to physical damage and histopathological alterations (Peda et al., 2016; Jabeen et al., 2018; Ahrendt et al., 2020); 2) microplastics can cause impairments in oxidative, and disorders of inflammatory balance and intestinal microflora (Gu et al., 2020; Huang et al., 2020; Iheanacho and Odo, 2020); 3) microplastics can also lead

to fish behavior changes (Brun et al., 2019; Guimarães et al., 2021; Rios-Fuster et al., 2021; Wei Shi et al., 2021); 4) microplastics can act as carriers to intensify further adverse effects of other pollutants on fish (Karami et al., 2016; Banaee et al., 2019; Zhang et al., 2019).

It is wildly acknowledged in the literatures that the ingestion, accumulation and elimination of microplastics in fishes are species-specific (Mizraji et al., 2017; Xu and Li, 2021), which was also verified with our previous studies (Wang et al., 2020; Zhang et al., 2021). Our field investigation found microplastic amounts in filter-feeding and omnivorous fish were higher than that of carnivorous species. Our laboratory experiment proved that microplastics ingestion in fish larvae was influenced by feeding type of fish, and omnivores fish were less able to eliminate microplastics than filter-feeding fish. An analysis of more than two thousand gut content surveys indicated that the largest plastic may ingest is about one-twentieth the length of the animal body length (Jams et al., 2020).

In this study, we will investigate species-specific effect of microplastics on three commercial fish species with different feeding types. Largemouth bass, *Micropterus salmoides* is a typical freshwater carnivorous fish species and widely farmed in China due to its strong adaptability, fast growth, delicious taste, and high economic value (Wang et al., 2020). Grass carp (*Ctenopharyngodon idella*), a herbivorous fish species, is one of the most important freshwater cultivars in China, which annual production exceeded 5.53 million tons in 2019 (China Fishery Statistical Yearbook, 2020). Jian carp (*Cyprinus carpio* var. Jian) is an omnivorous freshwater fish species with an annual production of 24.2 million tons worldwide (Yong Lin et al., 2019). This study aims to investigate the effects of microplastics on the morphology and inflammatory response in intestines of fishes with different feeding types. To achieve this goal, histopathological sections were examined, and immune-related genes profiles were used to study the changes in the intestinal tissue of three fishes after microplastics exposure. These results will provide a theoretical basis to further analysis of the mechanism of fish intestinal pathology caused by microplastics.

2. Material And Method

2.1 Materials

Polystyrene microplastics with diameters of 80 nm and 8 μm were purchased from Dae Technology Company (Tianjin, China).

Largemouth bass, grass carp and Jian carp were bought from a livestock farm in Shunde City (Guangdong, China). Largemouth bass was (5.23±0.62) cm in length and (2.97±0.64) g in weight. Grass carp was (5.81±0.50) cm in length and (3.82±0.91) g in weight. Jian carp was (3.46±0.16) cm in length and (0.93±0.19) g in weight. Fish were acclimatized at 25.2 ± 1.5°C in culture water (pH 7.1 ± 0.4; dissolved oxygen 6.4 ± 0.5 mg/L) with a 12 h light/dark cycle. Before the experiment, fish were acclimated in 100 L glass tanks for 5 d and were fed with 5.0% body weight fodder (Haid Group, Guangdong, China) twice daily.

2.2 Experimental design

In the exposure experiment, tanks (20 cm × 15 cm × 15 cm) were filled with 2 L of culture water with four groups of fish. Two different sizes of fluorescent microplastics (8 μm and 80 nm) were set at four concentrations for grass carp and Jian carp: 0, 0.02 mg/L, 0.2 mg/L and 2 mg/L. Based on previous findings in our lab (Zhang et al., 2021), carnivorous fish seem to be more tolerant to microplastics than other fishes. So, we set the higher microplastic exposure concentration for largemouth bass: 0.05 mg/L, 0.5 mg/L and 5 mg/L. The concentrations of exposure for MPs were selected based on the other studies (Karami et al., 2016; Ding et al., 2018; Zhang et al., 2021) and our pre-experiments. As for the experimental size of microplastics, we want to compare the nanoscale microplastics to the micron scale. 80 nm microplastics seem to be the smallest microplastics available, while microplastics on the micron scale are commonly used in experiments. A total of twenty-four tanks were set for each fish species, including control group and replicate group. Three fish species we chosen were not exposed at the same time. Each species of fish was tested separately. Three replicate tanks were used for 24 h and 48 h sampling times. After 48 h exposure, the surviving fish were moved to an aquarium with clean water containing no microplastics for 48 h. No feeding was done during exposure and depuration. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the National Institute of Health Guide for the Care and Use of Laboratory Animals of China. All surgery was performed under anesthesia, and all efforts were made to minimize suffering.

2.3 Histopathological Analysis

Fish from the control and experimental groups were anesthetized on ice and intestines were dissected. Intestinal tissue fixed in general-purpose tissue fixator (Servicebio, Wuhan, China), embedded in paraffin wax, sectioned at 4 μm thickness, and stained with hematoxylin-eosin (H&E). Tissue slices were examined and photographed by a microscopy (Nikon, Tokyo, Japan) with the Mshot Image Analysis System.

2.4 RNA extraction and cDNA synthesis

The experimental methods of RNA extraction and cDNA synthesis are presented in Supplement Text 1. The cDNA was stored at −80°C until further analysis.

2.5 Immune and enzyme-related gene expression

The SYBR green real-time PCR assay was performed on the CFX Connect TM Real-Time System (BIO-RAD, Hercules, CA, USA) using the SYBR® Green Premix Pro Taq HS qPCR kit (Accurate Biotechnology Co., Ltd, Hunan, China) following the manufacturer's approach. Specific primer sequences are listed in Table 1. Details of the PCR program are presented in Supplement Text 2. Expression levels of target genes were normalized to the internal reference, and the data were calculated as the fold change in comparison to the control group.

Table 1
List of gene primers used for qPCR.

Fish	Genes	Sequence, forward/reverse (5'-3')
Largemouth bass	*β-actin*	F: ATCGCCGCACTGGTTGTTGAC
		R: CCTGTTGGCTTTGGGGTTC
	IL-8	F: GAGCCATTTTTCCTGGTGACT
		R: TCCTCATTGGTGCTGAAAGATC
	Caspase 3	F: GCTTCATTCGTCTGTGTTC
		R: CGAAAAAGTGATGTGAGGTA
Grass carp	*β-actin*	F: GGCTGTGCTGTCCCTGTA
		R: TTATTGTGGTTACGCTGGA
	IL-1β	F: AGAGTTTGGTGAAGAAGAGG
		R: TTATTGTGGTTACGCTGGA
	IL-8	F: ATGAGTCTTAGAGGTCTGGGT
		R: ACAGTGAGGGCTAGGAGGG
	TGF-β1	F: TTGGGACTTGTGCTCTAT
		R: AGTTCTGCTGGGATGTTT
	TNF-α	F: CGCTGCTGTCTGCTTCAC
		R: CCTGGTCCTGGTTCACTC
Jian carp	*18S*	F: CTGAGAAACGGCTACCATTC
		R: GCCTCGAAAGAGACCTGTATTG
	IL-1β	F: GAGTGAACTGCACCAAACAAC
		R: GTCGGCACTGTCAGAGTAAAT
	IL-10	F: CTCCGTTCTGCATACAGAGAAA
		R: TCATGACGTGACAGCCATAAG
	TGF-β	F: ACGTTTCCAGATGGTTCAGAG
		R: GCCACTTTCTTTGTTTGGGAATA
	TLR-2	F: GTGCTCCTGTGAGTTTGTATCT
		R: TGGAGTGTCGCACACATAATAG

2.6 Statistical analysis

All data were quantified as the mean ± standard error (S.E) and performed by one-way ANOVA using SPSS 17.0 and Excel 2016. Statistical significance between the control and the experimental groups was conducted by the Duncan's multiple range test. A value of $P < 0.05$ was set with statistical significance.

3. Results

3.1 Intestinal morphology

After HE staining, intestinal histomorphology of three fishes were examined using a light microscope. Histopathological sections showed that the intestinal folds of largemouth bass juvenile were in disorder and shortened, infiltrated cells, especially when fish were exposure in higher concentration microplastics of micron scale (Fig. 1). In the intestine of grass carp juvenile, there was no difference between the control and the treatments for vacuolization, goblet cell hyperplasia or villus shortening (Supplementary figure 1). After combing all the scored histopathology features together, there was no significant difference in the intestinal muscular thickness and intestinal villi length between the groups ($p > 0.05$) (Supplementary figure 2). Juvenile Jian carp showed multiple abnormal intestines after microplastics exposure (Supplementary figure 3). The intestinal folds in the experimental group were not full or regular. However, no significant difference was found in the muscle thickness or villi length in Jian carp either ($p > 0.05$). Histopathological data of Jian carp are listed in Supplementary table 1.

3.2 Transcriptional responses of target genes

After 8 μm microplastics exposure 48 h, the expression levels of the immune-related gene (*IL-8*) were significantly upregulated in the intestines of largemouth bass juvenile ($p<0.05$) (Fig. 2C). 80 nm microplastics caused upregulation of *IL-8* in 48 h depuration after exposure 48 h (Fig. 2A). Whereas the situation of high concentration exposure was different with mid and low concentration exposure (Fig. 2A/C). Expression of *Caspase 3* gene in the intestines of fish exposed 80 nm microplastics 48 h and cleaned 8 μm microplastics 48 h were significantly lower than that in the intestines of fish in the control group ($p < 0.01$) (Fig. 2B/D).

The effects of microplastics on the expression of levels of immune-related genes in intestine tissues of grass carp are shown in Fig. 3. The relative expression levels of *IL-1β*, *IL-8*, *TGF-β1* and *TNF-α* were all observably up-regulated ($P<0.01$) when exposure 80 nm microplastics at low concentration (20 μg/L) in the start of 24 h. *TGF-β1* and *TNF-α* expression level when exposure 80 nm microplastics 24 h at middle and high concentration (200 μg/L and 2000 μg/L) were significantly upregulated, rather than *IL-1β* and *IL-8* expression level. However, there was different gene expression pattern when exposure 8 μm microplastics.

The mRNA expression levels of *IL-1β*, *IL-10*, *TGF-β* and *TLR-2* in intestines of Jian carp juvenile exposed to microplastics of 80 nm and 8 μm are shown in Fig. 4. The upregulation of pro-inflammatory cytokines, such as *IL-1β* and *TLR-2*, or/and downregulation of anti-inflammatory cytokines including *TGF-β1* and *IL-*

10 could cause inflammation in fish. Noteworthily, Jian carp cured better in 8 μm microplastics treatment than in 80 nm microplastics treatment.

4. Discussion

4.1 Effects of microplastics on intestinal morphology of fish

The intestinal morphological effects of microplastics with a dose-dependent way have been explored in various fishes. Over secretion of goblet cells was found in juvenile guppy (*Poecilia reticulata*) after exposing microplastics with 32-40 μm diameter, and the higher concentration of microplastics, the more goblet cells secreted (Huang et al., 2020). However, the loss of villus and crypt cells was significantly increased due to microplastic physical abrasion in the intestine of juvenile intertidal fish (*Girella laevifrons*), and leukocyte infiltration and hyperemia exposure in the high concentration group were more serious than those in the low concentration group (Ahrendt et al., 2020). In the European sea bass (*Dicentrarchus labrax* L.), intestinal tissues were altered after fish were fed with polyvinyl chloride (PVC) pellets for 90 days (Peda et al., 2016). Another morphometric analyses of sea bass fed polyethylene (PE) microplastics in the diets for 21 days showed a significant reduction in the amounts of goblet cells as well as a decrease in villus height (Espinosa et al., 2019). Histological analysis indicated that multiple abnormalities in intestines are presented in three species fishes after acute exposure microplastics in this study.

As we all known, intestine is vital for the digestion and absorption of nutrients, and intestinal morphology characters, such as muscular layer thickness, villi length, and the number of goblet cells indicate intestine health in fish. To some extent, abnormal in the intestinal sections is an immune response to external stimulus. On one hand, pathological changes of intestinal tract might be the result of microplastics intrusion. On the other hand, it is crucial to determine whether this intrusion outpaces the organism's ability to repair itself. From histopathological analysis of intestines of largemouth bass juvenile exposed to 8 nm and 8 μm microspheres after exposure 48 h and clean 48 h (Fig. 1), we found microplastics of lager size and higher concentration cause more serious damage, and the damage seems to be irreversible. Obviously, this change makes fish more sensitive to infection by pathogens. Compared with the intestinal slices of grass carp and Jian carp, Jian carp with smaller intestinal diameter and less perfect villus structure was more seriously damaged by microplastic invasion.

4.2 Effects of microplastics on immune-related genes expression of fish

Many animal studies have indicated that exposure to microplastics impairs oxidative and inflammatory bowel balance (Choi et al., 2018; Ding et al., 2018). Especially, microplastics cause intestinal inflammation, manifested by a significant increase in *IL-1α* levels in the intestine (Hirt and Body-Malapel, 2020). The immune function of organs is highly correlated with the inflammatory response, which is

generally considered to be a typical defense response that protects the host from pathogens (Zhong et al., 2020). Cytokines mediate the inflammatory response in fish, which are mainly divided into pro-inflammatory factors (e.g., *TNF-α*, *IL-1β* and *IL-10*) and anti-inflammatory factors (e.g., *IL-10* and *TGF-β*). For example, interleukin is a typical class of cytokines which is mainly involved in regulating all kinds of lymphocytes in the immune system. Tumor necrosis factor α (*TNF-α*), as pleiotropic proinflammatory and potent regulatory cytokines, can regulate cell proliferation, apoptosis or differentiation in the immune system (Cao et al., 2020). Toll-like receptors (*TLRs*), as a crucial innate receptor, can identify pathogen-associated molecular patterns (PAMPs) of invading microorganisms and induce downstream *NF-κB* activation and the production of *TNF-α*, *IL-10* and other cytokines (Meng et al., 2021).

Previous research in adult male zebrafish (*Danio rerio*) showed that exposure to 1000 μg/L of 0.5 μm microplastics for 14 days significantly up-regulated the transcription levels of *IL-1α*, *IL-1β*, and *Ifn* in the intestine (Jin et al., 2018). In the present study, microplastics exposure significantly induced or restrained the mRNA expression of immune-related genes in the intestine tissues of fishes. There were differential expressions of genes coping with differential sizes and concentrations of microplastics in different fishes. Similarly, in other species, such as rats (Wei et al., 2021) and bivalve (Li et al., 2020), the mRNA abundance of immune-related genes was increased with microplastics exposure.

4.3 Response time and legacy effect of microplastics with different concentration and size

In terms of damage to intestinal morphology, acute exposure did not cause significant damage at the size and concentration of microplastics exposed in this paper. From the perspective of gene expression level, when exposed to nanoscale microplastics at low concentration, fish can promote self-repair through the upregulation of some inflammatory factors. For micron-scale microplastics, we hypothesized that part of microplastics could be removed by fish excretion after ingestion. Therefore, there was no significant difference in gene expression between the experimental fish and the control group during the recovery period. The effects of microplastics on juvenile fishes are species-specific, the specific mechanism needs to be further studied.

Although time had no significant effect on intestinal morphology, we hypothesized that it was related to exposure conditions. Thankfully, even when exposed to extremely high concentration (mg/L) of microplastics, there is no immediate visible damage to the intestinal morphology of fish. Response time and recovery time of gene expression was species-specific. Grass carp has the longest intestinal tract, followed by Jian carp, and largemouth bass has the shortest intestinal tract, which is related to their feeding habits. We hypothesize that the lag time of microplastics in fish intestine is related to the length of the intestine. A methodology to assess how effective Mediterranean fish species, that are known to have ingested marine plastic, were considered gut length as well, which showed fish with smaller gut length is more representative (Bray et al., 2019).

5. Conclusion

In this study, we investigate species-specific effect of microplastics on three fishes with different feeding types. We also try to explore the contributing concentration and size of microplastics, as well as the response time and legacy effect in fishes. Two different sizes of fluorescent microplastics (8 μm and 80 nm) were set at four concentrations. And samples were taken at different exposure times and depuration times. Histopathological sections were examined, immune-related genes profiles were used to study the changes in the intestinal tissue of three fishes after microplastics exposure. The results of this study will be beneficial for extrapolating microplastics contamination risks to commercial fishes. The reason for the difference effects of microplastics on fishes is still unclear but could be due to the difference in the structure and function of the digestive system. This study will provide a valuable steppingstone for future research, where we hope to address the microplastics research gap between various fish species.

Statements & Declarations

Ethics approval/declarations

All experiments were approved by the Animal Care and Use Committee of South China Agricultural University (identification code: 20200127; date of approval: 20 May 2020).

Funding

This study was funded by the Key Realm R&D Program of Guangdong Province (No. 2020B0202080005), and China Agriculture Research System of MOF and MARA (CARS-45-50).

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

“All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Chaonan Zhang, Guohuan Xu and Jixing Zou. The first draft of the manuscript was written by Chaonan Zhang and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.”

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

References

1. Abhijit Mallik, K.A. Martin Xavier, Bejawada Chanikya Naidu, Binaya Bhusan Nayak, 2021. Ecotoxicological and physiological risks of microplastics on fish and their possible mitigation

measures. Sci. Total Environ., 779, 146433.

2. Anna Kukkola, Stefan Krause, Iseult Lynch, Gregory H. Sambrook Smith, Holly Nel, 2021. Nano and microplastic interactions with freshwater biota – Current knowledge, challenges and future solutions. Environ. Int., 152, 106504.
3. Ahrendt, C., Perez-Venegas, D.J., Urbina, M., Gonzalez, C., Echeveste, P., Aldana, M., Pulgar, J., Galbán-Malagón, C., 2020. Microplastic ingestion cause intestinal lesions in the intertidal fish Girella laevifrons. Mar. Pollut. Bull., 151, 110795.
4. Alimi, O.S., Farner Budarz, J., Hernandez, L.M., Tufenkji, N., 2018. Microplastics and Nanoplastics in Aquatic Environments: Aggregation, Deposition, and Enhanced Contaminant Transport. Environ. Sci. Technol., 52, 1704-1724.
5. Banaee, M., Soltanian, S., Sureda, A., Gholamhosseini, A., Haghi, B.N., Akhlaghi, M., Derikvandy, A., 2019. Evaluation of single and combined effects of cadmium and micro-plastic particles on biochemical and immunological parameters of common carp (*Cyprinus carpio*). Chemosphere, 236, 124335.
6. Bray, L., Digka, N., Tsangaris, C., Camedda, A., Gambaiani, D., de Lucia, G.A., Kaberi, H., 2019. Determining suitable fish to monitor plastic ingestion trends in the Mediterranean Sea. Environ. Pollut. 247,1071-1077.
7. Brun, N.R., van Hage, P., Hunting, E.R., Haramis, A.G., Vink, S.C., Vijver, M.G., Schaaf, M., Tudorache, C., 2019. Polystyrene nanoplastics disrupt glucose metabolism and cortisol levels with a possible link to behavioural changes in larval zebrafish. Commun. Biol, 2, 382.
8. Cao, S., Xiong, D., Luo, W., Tang, J., Qu, F., Zhou, Y., He, Z., Xie, S., Liu, Z., 2020. Effects of dietary soy isoflavones on growth, antioxidant status, immune response and resistance of juvenile grass carp (*Ctenopharyngodon idella*) to Aeromonas hydrophila challenge. Aquac. Res., 51, 2472-2482.
9. Choi, J.S., Jung, Y., Hong, N., Hong, S.H., Park, J., 2018. Toxicological effects of irregularly shaped and spherical microplastics in a marine teleost, the sheepshead minnow (*Cyprinodon variegatus*). Mar. Pollut. Bull., 129, 231–240.
10. Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: A review. Mar. Pollut. Bull., 62, 2588-2597.
11. Ding, J., Zhang, S., Razanajatovo, R.M., Zou, H., Zhu, W., 2018. Accumulation, tissue distribution, and biochemical effects of polystyrene microplastics in the freshwater fish red tilapia (*Oreochromis niloticus*). Environ. Pollut., 238, 1–9.
12. Espinosa, C., Esteban, M.Á., Cuesta, A., 2019. Dietary administration of PVC and PE microplastics produces histological damage, oxidative stress and immunoregulation in European sea bass (*Dicentrarchus labrax* L.). Fish Shellfish Immun., 95, 574-583.
13. Garrido Gamarro, E., Ryder, J., Elvevoll, E.O., Olsen, R.L., 2020. Microplastics in Fish and Shellfish - A Threat to Seafood Safety? J. Aquat. Food Prod. T., 29, 417-425.
14. Gu, H., Wang, S., Wang, X., Yu, X., Hu, M., Huang, W., Wang, Y., 2020. Nanoplastics impair the intestinal health of the juvenile large yellow croaker *Larimichthys crocea*. J. Hazard. Mater., 397,

122773.

15. Guimarães, A.T.B., Estrela, F.N., Rodrigues, A.S.D.L., Chagas, T.Q., Pereira, P.S., Silva, F.G., Malafaia, G., 2021. Nanopolystyrene particles at environmentally relevant concentrations causes behavioral and biochemical changes in juvenile grass carp (*Ctenopharyngodon idella*). J. Hazard. Mater., 403, 123864.
16. Hirt, N., Body-Malapel, M., 2020. Immunotoxicity and intestinal effects of nano- and microplastics: a review of the literature. Part. Fibre Toxicol., 17, 57.
17. Huang, J.N., Wen, B., Zhu, J.G., Zhang, Y.S., Gao, J.Z., Chen, Z.Z., 2020. Exposure to microplastics impairs digestive performance, stimulates immune response and induces microbiota dysbiosis in the gut of juvenile guppy (*Poecilia reticulata*). Sci. Total Environ., 733, 138929.
18. Iheanacho, S.C., Odo, G.E., 2020. Dietary exposure to polyvinyl chloride microparticles induced oxidative stress and hepatic damage in *Clarias gariepinus* (Burchell, 1822). Environ. Sci. Pollut Res. Int., 27, 21159-21173.
19. Jabeen, K., Li, B., Chen, Q., Su, L., Wu, C., Hollert, H., Shi, H., 2018. Effects of virgin microplastics on goldfish (*Carassius auratus*). Chemosphere., 213, 323-332.
20. Jacob, H., Besson, M., Swarzenski, P.W., Lecchini, D., Metian, M., 2020. Effects of Virgin Micro- and Nanoplastics on Fish: Trends, Meta-Analysis, and Perspectives. Environ. Sci. Technol., 54, 4733-4745.
21. Jams, I.B., Windsor, F.M., Poudevigne-Durance, T., Ormerod, S.J., Durance, I., 2020. Estimating the size distribution of plastics ingested by animals. Nat. Commun., 11, 1594.
22. 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.
23. Karami, A., Romano, N., Galloway, T., Hamzah, H., 2016. Virgin microplastics cause toxicity and modulate the impacts of phenanthrene on biomarker responses in African catfish (*Clarias gariepinus*). Environ. Res., 151, 58-70.
24. McDevitt, J.P., Criddle, C.S., Morse, M., Hale, R.C., Bott, C.B., Rochman, C.M., 2017. Addressing the Issue of Microplastics in the Wake of the Microbead-Free Waters Act—A New Standard Can Facilitate Improved Policy. Environ. Sci. Technol., 51, 6611-6617.
25. Meng, X., Wu, S., Hu, W., Zhu, Z., Yang, G., Zhang, Y., Qin, C., Yang, L., Nie, G., 2021. Clostridium butyricum improves immune responses and remodels the intestinal microbiota of common carp (*Cyprinus carpio* L.). Aquaculture, 530, 735753.
26. Mizraji, R., Ahrendt, C., Perez-Venegas, D., Vargas, J., Pulgar, J., Aldana, M., Patricio Ojeda, F., Duarte, C., Galbán-Malagón, C., 2017. Is the feeding type related with the content of microplastics in intertidal fish gut? Mar. Pollut. Bull., 116, 498-500.
27. N.O.A.A. National Oceanic and Atmospheric Administration, 2015. Microplastics. https://marinedebris.noaa.gov/sites/default/files/MicroplasticsOnePager_0.pdf.
28. Plastics Europe, 2019. Plastics - the facts 2020: An analysis of European plastics production, demand and waste data. https://www.plasticseurope.org/en/resources/publications/4312-plastics-facts2020.

29. Peda, C., Caccamo, L., Fossi, M.C., Gai, F., Andaloro, F., Genovese, L., Perdichizzi, A., Romeo, T., Maricchiolo, G., 2016. Intestinal alterations in European sea bass *Dicentrarchus labrax* (Linnaeus, 1758) exposed to microplastics: Preliminary results. Environ. Pollut., 212, 251-256.
30. Peng, L., Fu, D., Qi, H., Lan, C.Q., Yu, H., Ge, C., 2020. Micro- and nano-plastics in marine environment: Source, distribution and threats — A review. Sci. Total Environ., 698, 134254.
31. Rai, P.K., Lee, J., Brown, R.J.C., Kim, K., 2021. Environmental fate, ecotoxicity biomarkers, and potential health effects of micro- and nano-scale plastic contamination. J. Hazard. Mater., 403, 123910.
32. Rios-Fuster, B., Arechavala-Lopez, P., García-Marcos, K., Alomar, C., Compa, M., Álvarez, E., Julià, M.M., Solomando Martí, A., Sureda, A., Deudero, S., 2021. Experimental evidence of physiological and behavioral effects of microplastic ingestion in Sparus aurata. Aquat. Toxicol., 231, 105737.
33. Wang, S., Zhang, C., Pan, Z., Sun, D., Zhou, A., Xie, S., Wang, J., Zou, J., 2020. Microplastics in wild freshwater fish of different feeding habits from Beijiang and Pearl River Delta regions, south China. Chemosphere, 258, 127345.
34. Wang, Y., Ni, J., Nie, Z., Gao, J., Sun, Y., Shao, N., Li, Q., Hu, J., Xu, P., Xu, G., 2020. Effects of stocking density on growth, serum parameters, antioxidant status, liver and intestine histology and gene expression of largemouth bass (*Micropterus salmoides*) farmed in the in-pond raceway system. Aquac. Res., 51, 5228-5240.
35. Wang, W., Ge, J., Yu, X., Li, H., 2020. Environmental fate and impacts of microplastics in soil ecosystems: Progress and perspective. Sci. Total Environ., 708, 134841.
36. Wei, J., Wang, X., Liu, Q., Zhou, N., Zhu, S., Li, Z., Li, X., Yao, J., Zhang, L., 2021. The impact of polystyrene microplastics on cardiomyocytes pyroptosis through NLRP3/Caspase-1 signaling pathway and oxidative stress in Wistar rats. Environ. Toxicol., 36, 935-944.
37. Wei Shi, Shuge Sun, Yu Han, Yu Tang, Weishang Zhou, Xueying Du, Guangxu Liu, 2021. Microplastics impair olfactory-mediated behaviors of goldfish Carassius auratus. J. Hazard. Mater., 409, 125016.
38. Xu, J., Li, D., 2021. Feeding behavior responses of a juvenile hybrid grouper, *Epinephelus fuscoguttatus*♀ × *E. lanceolatus*♂, to microplastics. Environ. Pollut., 268, 115648.
39. Xu, S., Ma, J., Ji, R., Pan, K., Miao, A., 2020. Microplastics in aquatic environments: Occurrence, accumulation, and biological effects. Sci. Total Environ., 703, 134699.
40. Yong Lin, Zeng, D., He, P., Wei, P., Hui, W., Wu, T., Zhuo, X., Lin, Y., 2019. mRNA and microRNA transcriptomics analyses in intermuscular bones of two carp species, rice flower carp (*Cyprinus carpio* var. Quanzhounensis) and Jian carp (*Cyprinus carpio* var. Jian). Comp Biochem Physiol Part D Genomics Proteomics, 30, 71-80.
41. Zhang, C., Wang, J., Zhou, A., Ye, Q., Feng, Y., Wang, Z., Wang, S., Xu, G., Zou, J., 2021. Species-specific effect of microplastics on fish embryos and observation of toxicity kinetics in larvae. J. Hazard. Mater., 403, 123948.

42. Zhang, S., Ding, J., Razanajatovo, R.M., Jiang, H., Zou, H., Zhu, W., 2019. Interactive effects of polystyrene microplastics and roxithromycin on bioaccumulation and biochemical status in the freshwater fish red tilapia (*Oreochromis niloticus*). Sci. Total Environ., 648, 1431-1439.
43. Zhong, J., Wu, P., Feng, L., Jiang, W., Liu, Y., Kuang, S., Tang, L., Zhou, X., 2020. Dietary phytic acid weakened the antimicrobial activity and aggravated the inflammatory status of head kidney, spleen and skin in on-growing grass carp (*Ctenopharyngodon idella*). Fish Shellfish Immun., 103, 256-265.

Figures

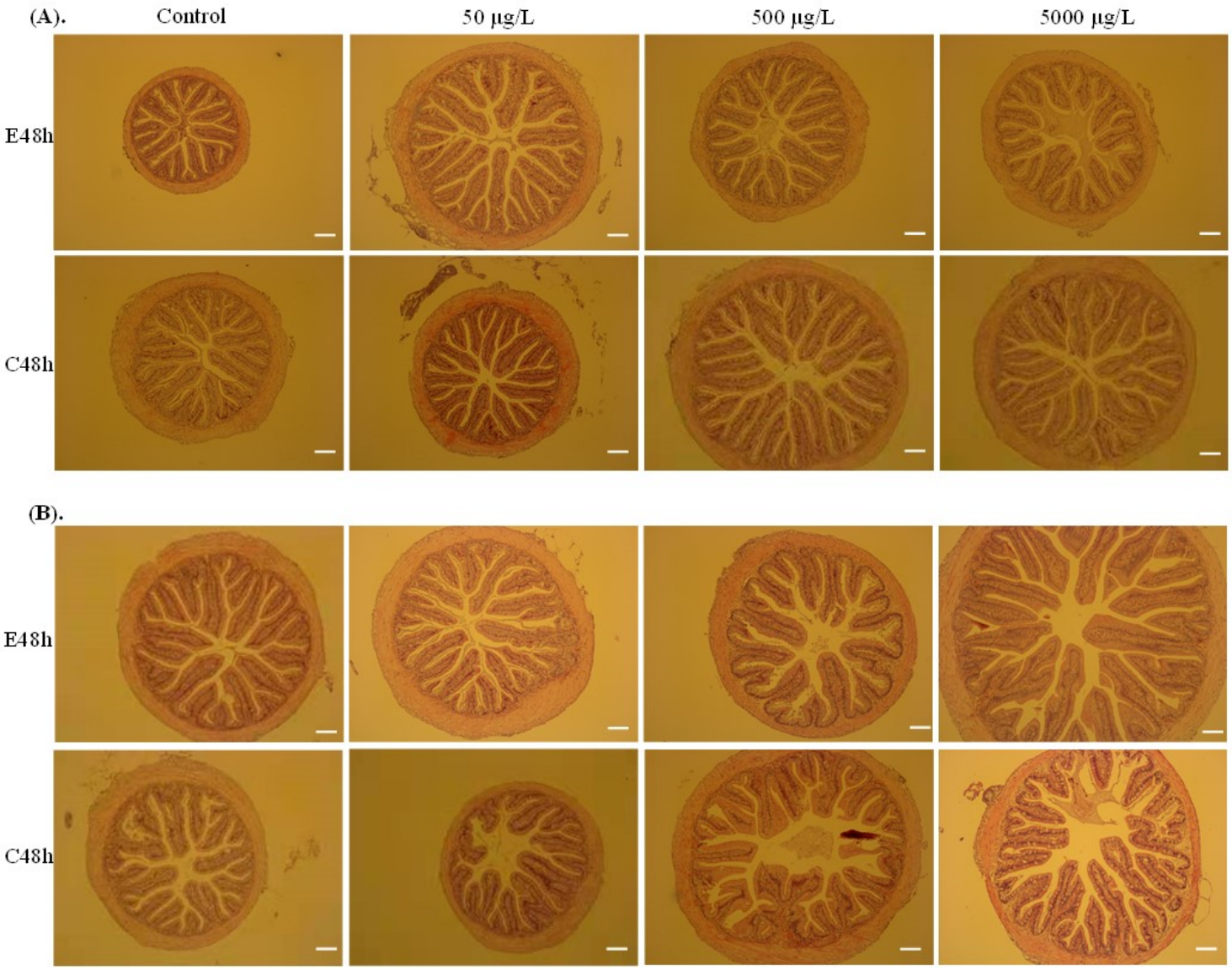

Figure 1

Histopathological analysis of intestines of largemouth bass juvenile exposed to polystyrene microspheres of 80 nm (Fig.1A) and 8 μm (Fig.1B) after exposure 48 h and clean 48 h. Exposure

concentration and time were shown in the picture. Scale bar = 20

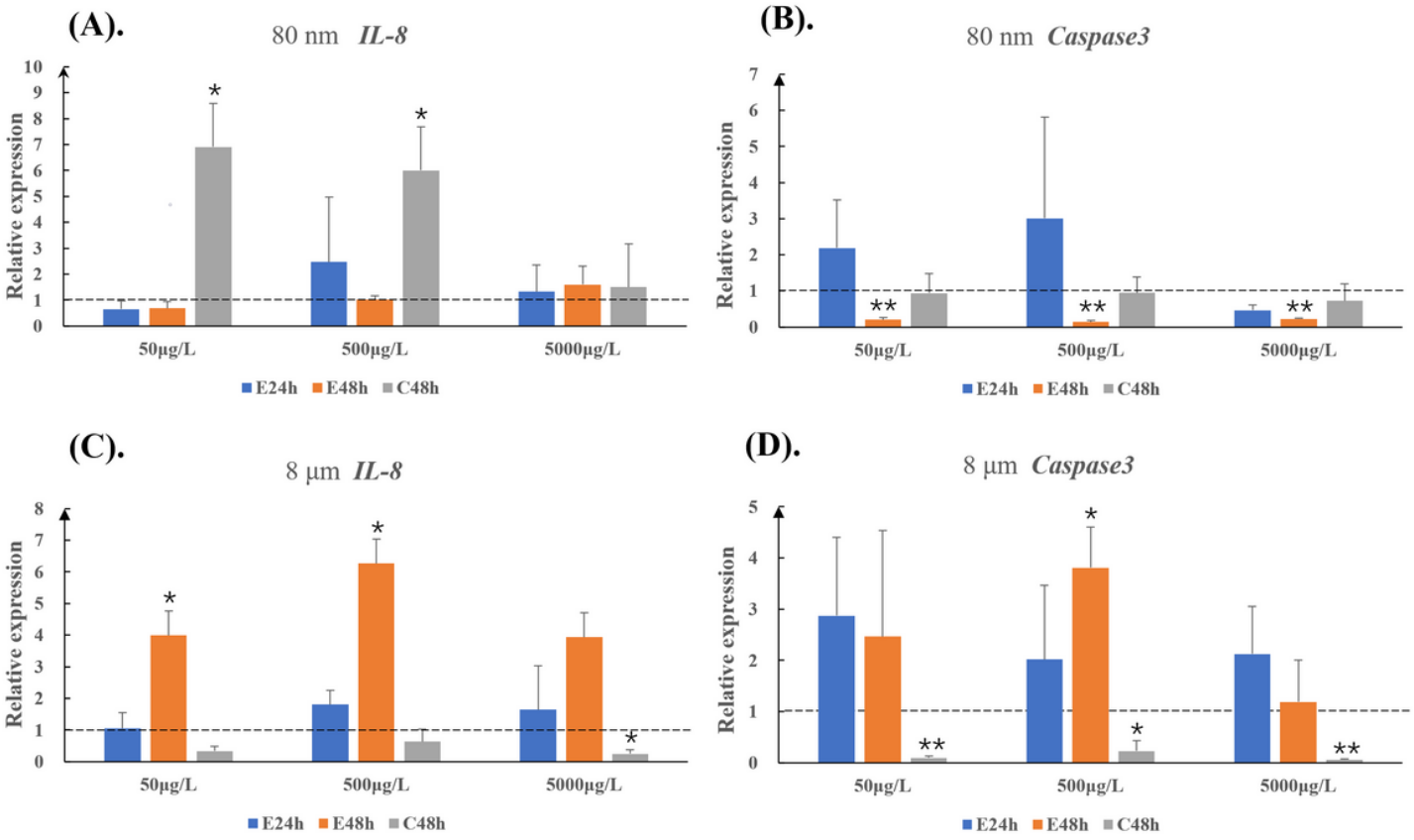

Figure 2

The relative gene expression levels (fold change) of IL-8 (A/C) and Caspase 3 (B/d) in intestines of largemouth bass juvenile exposed to microplastics. Data are expressed as mean ± standard deviation. Significant differences from control are shown (*p < 0.05; **p < 0.01).

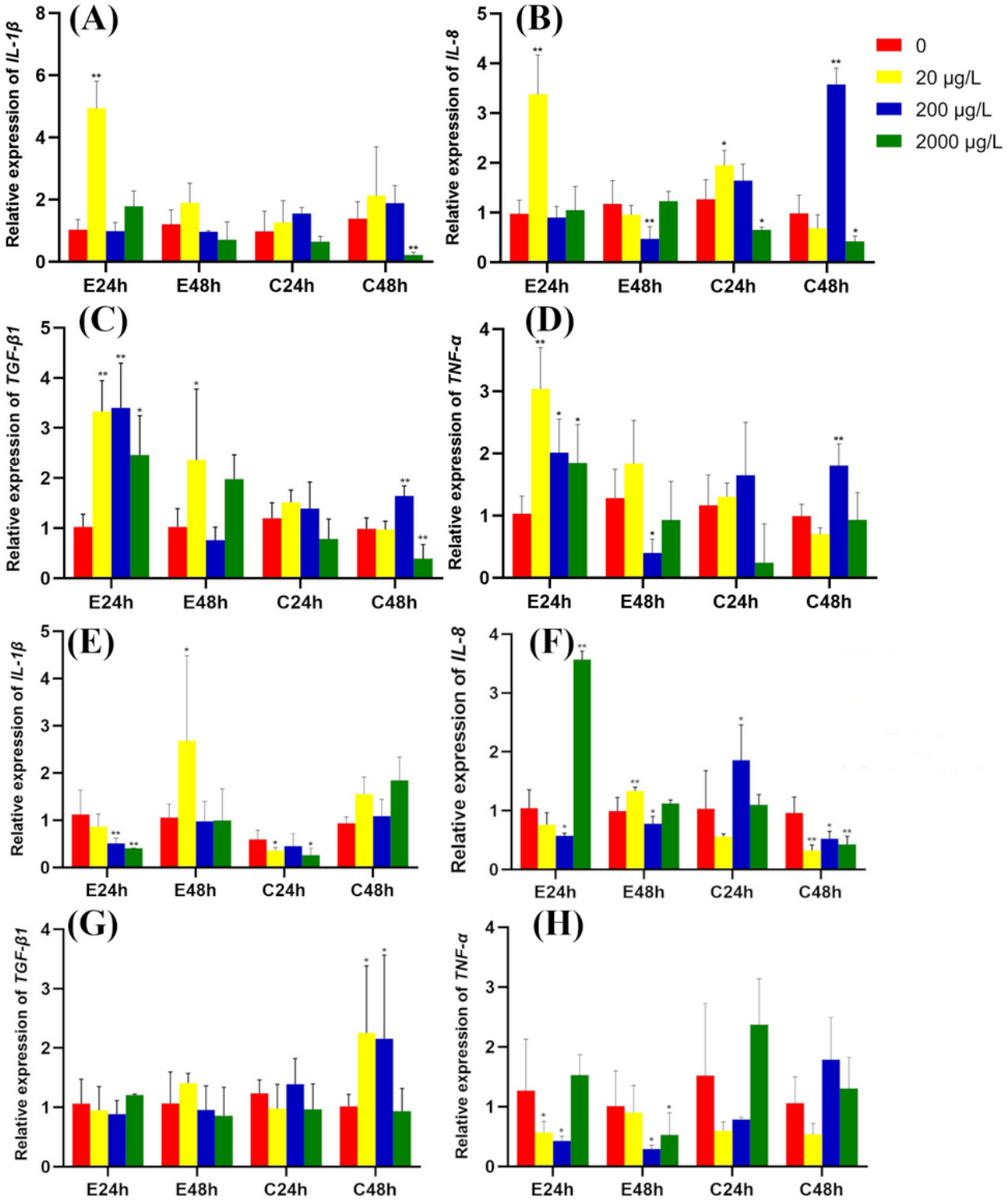

Figure 3

The relative gene expression levels (fold change) in intestines of grass carp juvenile exposed to microplastics of 80 nm (A-D) and 8 μm (E-H). Data are expressed as mean ± standard deviation. Significant differences from control are shown (*p < 0.05; **p < 0.01).

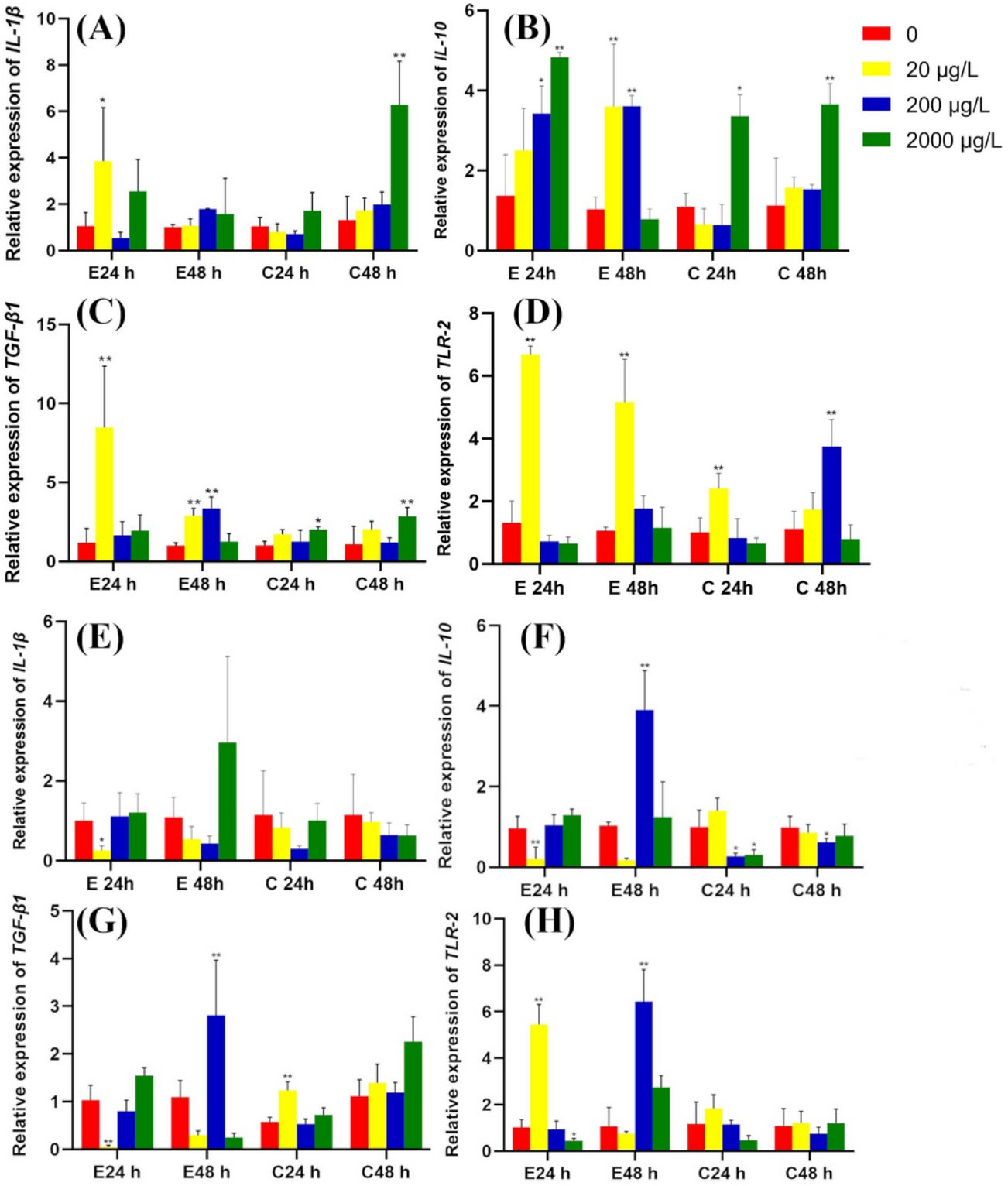

Figure 4

The relative gene expression levels (fold change) in intestines of Jian carp juvenile exposed to microplastics of 80 nm (A-D) and 8 μm (E-H). Data are expressed as mean ± standard deviation. Significant differences from control are shown (*p < 0.05; **p < 0.01).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- suplementarymaterials.docx