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Accumulation and ecotoxicological effects induced by combined exposure of different sized polyethylene microplastics and oxytetracycline in zebrafish☆

Ziyue Yu [a,b], Changzhou Yan [a,*], Donghua Qiu [a,b], Xin Zhang [a,b], Ce Wen [a,b], Sijun Dong [c]

[a] Key Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen, 361021, China
[b] University of Chinese Academy of Sciences, Beijing, 100049, China
[c] College of Life Sciences, Institute of Life Sciences and Green Development, Hebei University, Baoding 071002, China

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ABSTRACT

Microplastics have been widely reported as carriers of antibiotics, yet studies investigating the combined ecotoxicology of microplastics and antibiotics on organisms is limited. In this study, different sized polystyrene plastics and oxytetracycline (OTC) were used to carry out a 30-day single and binary-combined exposure experiment of zebrafish, and the microplastics and OTC accumulation, liver histological alteration, biomarkers and transcriptomic response of zebrafish were evaluated. Our results indicated that 300 nm and 50 nm plastic particles increased the OTC accumulation in liver by 33.8% and 44.5%, respectively. Microplastics and OTC induced severe liver histological damage, and the damage is size-dependent, increasing with the decrease of microplastics sizes. The liver biomarkers indicated a different response pattern in single microplastics exposure and combined with OTC, single or co-exposure of 50 nm nano-plastics and OTC induced intense responses of integrated biomarker response values. The 50 nm nano-plastics, OTC and their combined exposure induced 1330, 2693 and 3965 significantly differentially expressed genes, respectively, in which the steroid biosynthesis pathway was significantly affected by all the three treatments. This study elucidated the size-dependent effects of microplastics and provided detailed data from histopathology to transcriptome profile, enhancing our understanding of the ecotoxicity of microplastics and OTC.

1. Introduction

The global plastic production has reached 367 million tonnes in 2020, and only 6% of waste plastics was collected for recycling or energy recovery (Association of Plastic Manufacturers, 2021). The excessive applications of plastic inevitably lead to the ubiquity of microplastics in ecosystems because of the inefficient recycling rate, as confirmed by numerous research (Imhof et al., 2016; Piyawardhana et al., 2022; Zhao et al., 2014).

The ingestion of microplastics in organisms has been proved by different trophic levels in numerous studies (Cole and Galloway, 2015; Neto et al., 2020; Rummel et al., 2016; Wright et al., 2013). Moreover, the duration of microplastic-retention in organisms is size-dependent. Studies have revealed that the majority of large-sized microplastics (diameter >25 μm) can be depurated from the bodies of molluscs after three days (Van Cauwenberghe and Janssen, 2014); nano-sized particles were harder to depurate than micro-sized ones (Jeong et al., 2016).

The uptake of microplastics by organisms may induce necrosis, infiltrated histological alterations in the zebrafish liver (Lu et al., 2016), lysed muscle fibres (Hsieh et al., 2021), and even brain abnormalities (Jeong et al., 2022). The liver detoxicates xenobiotics and excretes toxic substances, making the organ a sensitive indicator (Bernet et al., 1999; Goulding, 1987; Yancheva et al., 2016). However, the detoxification process might lead to a high accumulation of microplastics (Ding et al., 2018; Lu et al., 2016; Sheng et al., 2021). The effects of microplastics are also inevitably related to particle size; however, studies comparing the influence of micro- and nano-particles have conflicting results (Deng et al., 2017; Ding et al., 2020; Jin et al., 2018).

As the major class of tetracycline antibiotics, oxytetracycline (OTC) is one of the most commonly used broad-spectrum antibiotics for the

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* Corresponding author.
E-mail address: czyan@iue.ac.cn (C. Yan).

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veterinary and aquaculture applications (European Medicines Agency, 2021). The excessive use of OTC leads to pollution in both aquatic and terrestrial ecosystems worldwide (Monteiro et al., 2016), and the contamination level of OTC in the untreated wastewater of farms is the second highest of veterinary antibiotics in China, at approximately 14–82 μg/L (Chen et al., 2015; Zhi et al., 2018). The ecotoxicological effects of OTC have been documented with valid evidence, including changes in antioxidant enzyme activity, related gene expression (Almeida et al., 2019; Pês et al., 2018) and tissue damage (Rodrigues et al., 2019).

Ingested microplastics in aquatic organisms may behave as vectors of chemicals, leading to synergetic or antagonistic effects when in co-existence with other pollutants (Oliveira et al., 2013; Varg et al., 2021). Synergetic effects include increased accumulation of polychlorinated biphenyls and bisphenol A co-exposure with microplastics (Chen et al., 2017; Jiang et al., 2018), as well as severe acute toxicity (Na et al., 2021). Yang et al. (2020a) found that the co-exposure with polystyrene (PS) microplastics for seven days decreased the accumulation of 6:2 chlorinated polyfluorinated ether sulfonate in zebrafish, Zhang et al. (2019) found that co-exposure with PS nano-plastics alleviated the neurotoxicity caused by roxithromycin in red tilapia.

Considering the large amount of OTC and microplastics in freshwater and seawater environments (Chen et al., 2015; Monteiro et al., 2016; Triebskorn et al., 2019), in addition to the frequently used fishery equipment and aquaculture systems utilizing plastics (FAO, 2016), OTC inevitably co-exists with microplastics in different ecosystems. Furthermore, PS particles are reportedly capable of absorbing tetracycline (Xu et al., 2018). However, the ecotoxicology of microplastics and OTC combinations, as well as the different mechanisms underlying the size effects of microplastics and combined exposure to OTC, remains unclear.

In this study, zebrafish (*Danio rerio*) were used as test organisms to investigate the possible effects of microplastics and OTC under single and combined exposure. We speculated that nano-plastics could have a greater effect on zebrafish. Moreover, co-exposure with OTC would aggravate the accumulation of pollutants, thereby inducing synergistic effects; however, co-exposure would disturb the size-dependent effects of microplastics. The following aspects were analysed to verify the above speculations: (i) the accumulations of two kinds of pollutants in liver, (ii) liver histological alterations induced by different sizes of microplastics and OTC, (iii) response of the biomarkers in liver, and (iv) transcriptomic response of zebrafish liver. This study provides valuable insights into the potential impacts of microplastics and OTC on aquatic organisms.

2. Materials and methods

2.1. Materials and test organism

Five size ranges (200 μm, 40 μm, 10 μm, 300 nm and 50 nm) of PS particles were purchased from Narui Materials Ltd. (Shanghai, China). Specific information of microplastics (i.e. size distribution, polymeric composition and zeta potentials) is provided in the supplementary materials (Table S1, Figs. S1–S2). PS was selected for our study as it is highly produced owing to its frequent use in fisheries and aquaculture equipment (Lusher et al., 2017), thereby leading to its great contribution toward plastic pollution in the environment (Jambeck et al., 2015). Microplastic stock solutions (200 mg/L) for each size range were prepared and sonicated for 30 min before use. OTC (purity >95%, CAS number 2058-46-0, reference code: G1802040, Aladdin) was suspended in water to prepare the stock solution (100 mg/L, refreshed every week). Adult zebrafish (*Danio rerio,* wild-type, 6 month old, mixed sex, male to female ratio to 1:1, with the average weight and lengths of 0.26 ± 0.07g and 3.50 ± 0.2 cm, respectively) were purchased from Yudu Aquatic Fishery (Yindou Road, Xiamen), acclimated for 6 weeks at 24 ± 1.5 °C (14:10h light-dark) and fed on commercial diet.

2.2. Experimental design

Twelve experimental groups were set as follows: control (CR), single oxytetracycline (OTC), 200-μm micro-plastics (LM), 40-μm micro-plastics (MM), 10-μm micro-plastics (SM), 300-nm nano-plastics (LN), 50-nm nano-plastics (SN), OTC combined with each size of micro-plastics (LMO, MMO, and SMO) and OTC combined each size of nano-plastics (LNO and SNO). The exposure concentration of all pollutants in every treatment was 100 μg/L, except control. Microplastics concentrations were selected based on the reported environmentally relevant concentrations in freshwater and seawater (Dubaish and Liebezeit, 2013; Lenz et al., 2016; Leslie et al., 2017; Yan et al., 2019). Based on previous studies, chosen OTC concentration exhibited significant effects on organisms (Keerthisinghe et al., 2020; Li et al., 2020a). Furthermore, this concentration can aid in determining the mechanism through which OTC affects organisms; higher OTC concentration was also detected in special scenario (Li et al., 2008). Triplicates were set for all exposure treatments. For each replicate, 10 fish were distributed to the exposure solution (2L) made with previously aerated water in a 5L glass baker, with continuously aerated to ensure comparable O_2 saturation levels. To prevent microplastics from aggregating and sinking, the temperature was set as 24 ± 1.5 °C at a 14:10 h light-dark cycle. Chemicals were supplemented during the daily refreshment of the exposure solution. Working concentrations of OTC in the exposure solutions were measured and no significant difference was observed among the OTC-treated groups (Table S2).

2.3. Accumulation of OTC and microplastics in the livers of zebrafish

For the chemical accumulation experiments, each group was sampled at the end of 30 days-exposure. Triplicates were set for each treatment; the livers of five fish in each replicate were pooled together for microplastic detection, three fish in each replicate were pooled together for OTC detection.

The methods used for OTC detection was followed as previous study (Chemello et al., 2016). Fish livers were homogenized after weighing and suspended in 1% phosphoric acid ultrapure water. Samples were then thoroughly mixed in ultrasonic bath (5 min) and centrifuged (13, 000 rpm, 10 min, 25 °C). After membrane filtration (0.22 μm), 20 μL of supernatant was collected for detection. OTC concentration was detected by previously described method (Zhang et al., 2022).

Microplastics accumulation was detected following the method modified by Ding et al. (2020) and Chagas et al. (2021). Briefly, 100 g/L KOH solution was used to digest the zebrafish liver at 60 °C, heated up to 80 °C after 24 h (Li et al., 2018). Serial dilutions of micro- and nano-plastic suspensions were used to generate a standard curve, and the suspensions were then digested as applied to tissue samples. Subsequently, Nile red fluorescent dye was used to dye aliquots (Sigma-Aldrich, CAS number 7385-67-3, Maes et al., 2017). Samples were then analysed in microplate reader after 5 min of incubation (TECAN, Spark) at 630 nm. Detailed information on the standard curve of the microplastic concentration was provided in Table S3.

2.4. Histological analysis and assessment of biomarkers of zebrafish livers

Four fish in each treatment, in total, 48 fish were euthanised and dissected for histological analysis after 30 days of exposure. The livers were immediately collected and weighed. The hepatosomatic index was calculated as liver weight (mg)/body weight (mg) × 100%. Segments of the livers were fixed, stained and examined as described in our previous study (Yu et al., 2022). The ballooning degeneration rate in livers was determined using ImageJ 1.53q. The histopathological indices was calculated based on previous studies(Antunes et al., 2017; Araújo et al., 2020; Bernet et al., 1999).

Three fish from each replicate were used for the enzyme activity analysis. The activities of superoxide dismutase (SOD), glutathione

(GSH) and catalase (CAT) in the zebrafish livers was measured to evaluate the effects of microplastics and OTC on antioxidant defence. In addition, the activities of aspartate transaminase (AST) and alanine transaminase (ALT) in the livers were measured to evaluate hepatic injury caused by microplastics and OTC. Briefly, liver samples were homogenized in phosphate buffer solution (0.1 mol/L, pH = 7.4) and centrifuged for 15 min (10,000 rpm) at 4 °C. The supernatant was used for total protein concentration and enzymatic activity determination by commercial kits (Nanjing Jiancheng Bioeng. Inst., China, specific reference codes and method descriptions were presented in the supplementary material) following the manufacturer's instructions.

2.5. Transcriptome analysis in livers of zebrafish

At the end of 30-days exposure, 5 livers of zebrafish from each replicate in CR, OTC, SN and SNO treatments were dissected for RNA sequencing. Total RNA was isolated using the M5 universal plus RNA mini kit (Mei5bio, China, MF167-01) according to the manufacturer's specifications. After purity examination, the Illumina HiSeq™ 4000 platform (Illumina, San Diego, CA, USA) was used to sequencing 12 transcriptome libraries (CR, OTC, SN and SNO). RNA data was then analysed as previously described methods (Anders and Huber, 2010; Trapnell et al., 2014), differences with $q \leq 0.05$, false discovery rate (FDR) < 0.05 and $\log_2$(fold-change)> 1 were identified as differentially expressed genes (DEGs). Enrichment analyses of Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway were then performed based on DEGs.

2.6. Statistical analyses

A normal distribution test was performed before data analysis. One-way ANOVA or two-way ANOVA as the parametric methods, and Kruskal-Wallis test as the nonparametric methods were performed using SPSS 20.0 (IBM, USA) to evaluate the difference among treatments. Differences were considered significant when $P < 0.05$. The summaries of statistical analyses were provided in the supplementary material (Tables S4 and S5). Graphs were constructed using Origin 9.0 or R Studio (version 1.3.1093). Moreover, the 'Integrated Biomarker Response version 2' (IBR v2) was calculated based on liver biomarkers (SOD, CAT, GST, AST, and ALT) according to published research (Li et al., 2020b; Sanchez et al., 2013), specific method of calculation was provided in the Supplemental Material. All data were shown as means ± SE (standard error).

3. Results

3.1. Accumulation of OTC and microplastics in zebrafish livers

The accumulation of OTC in zebrafish livers after 30 days-exposure of OTC and microplastics was shown in Fig. 1. No OTC was detected in the non-OTC exposed groups (control, microplastics single exposure).

The concentration of OTC in the liver increased with the decrease of plastic size in the combined treatments, and the greatest OTC concentration was detected in the SNO treatment (4668.8 ± 855.8 ng/g). The addition of micro-plastics exhibited no significant effect on the accumulation of OTC compared with OTC treatments ($P > 0.05$). However, the addition of nano-plastics significantly increased the OTC accumulation in zebrafish liver ($P < 0.05$), reaching 33.8% and 44.5% in LNO and SNO treatments, respectively.

The accumulation of microplastics in livers after exposure for 30-days were presented in Table S6. No microplastics were detected in the livers of control, OTC, LM and LMO treatments. Based on the two-way ANOVA results, significant differences of detected microplastic concentrations were observed between single and OTC-combined groups ($F = 6.995$, df = 1, $P < 0.05$) and among different plastic sizes ($F = 50.943$, df = 4, $P < 0.05$), suggesting that the accumulated micro- or nano-plastics varied with plastic sizes and were affected by the OTC-combination. The greatest weight of PS particles in liver was detected in the MMO treatment (6.91 ± 0.41 μg/g). In the liver, all OTC-combined treatments exhibited higher concentrations of microplastics across all size ranges, except for the largest microplastic treatments (no microplastics detected, Table S6), although the increase was not significant ($P > 0.05$).

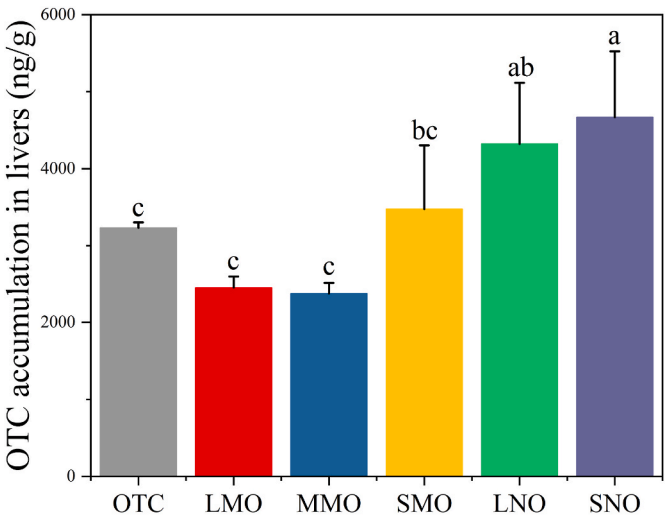

Fig. 1. The oxytetracycline accumulation in zebrafish livers under the single exposure of OTC exposure and combined exposure with different size of microplastics (LMO: OTC combined with 200-μm micro-plastics; MMO: OTC combined with 40-μm micro-plastics; SMO: OTC combined with 10-μm micro-plastics; LNO: OTC combined with 300-nm nano-plastics; SNO: OTC combined with 50-nm nano-plastics). The presented values are the means ± SE (n = 3).

3.2. Histological and damage biomarker alteration in livers

No significant alteration of hepatosomatic index was observed between the different treatments (Fig. S3). Pathological changes of the liver sections and histopathological indices from different treatments were shown in Fig. 2 and Fig. S4. The structure of the livers in the control was normal (Fig. 2A). Microplastics, OTC and their combined exposure induced pathological changes at different degrees (Fig. 2B–L). Most slices exposed to microplastics or OTC presented the hepatocyte ballooning (vacuolization), pyknotic nuclei or peripherally located nuclei, and inflammatory cell infiltration was also observed in some treatments. We further evaluated the degree of hepatocyte ballooning rate in the different treatments (Fig. 3A). Compared with control, microplastics or OTC exposure significantly increased the ballooning rate in livers ($P < 0.05$), and the ballooning rate increased with the decrease of microplastic sizes, regardless of the combination with OTC. Moreover, the ballooning rates in the combined treatments were decreased than those in single OTC treatments, and the decrease was significant in the micro-sized plastic combined treatments ($P < 0.05$). Two-way ANOVA indicated that both the microplastic size and OTC addition affected the liver ballooning rate (Table S4 microplastic size: df = 4, $F = 13.856$, $P = 0.000$; OTC addition: df = 1, $F = 5.469$, $P = 0.027$). Significant differences in zebrafish liver histopathological indices were observed between treatments, the two-way ANOVA also indicate that the microplastic size, the addition of OTC and their interaction exhibited significant effects on histopathological indices. The changes in ALT and AST activities also exhibited similar patterns: ALT and AST activity was also intensified in a size-dependent manner in the single microplastic exposure treatments and decreased in the combined treatments than in the single OTC treatments (Fig. 3 B, C, Table S4).

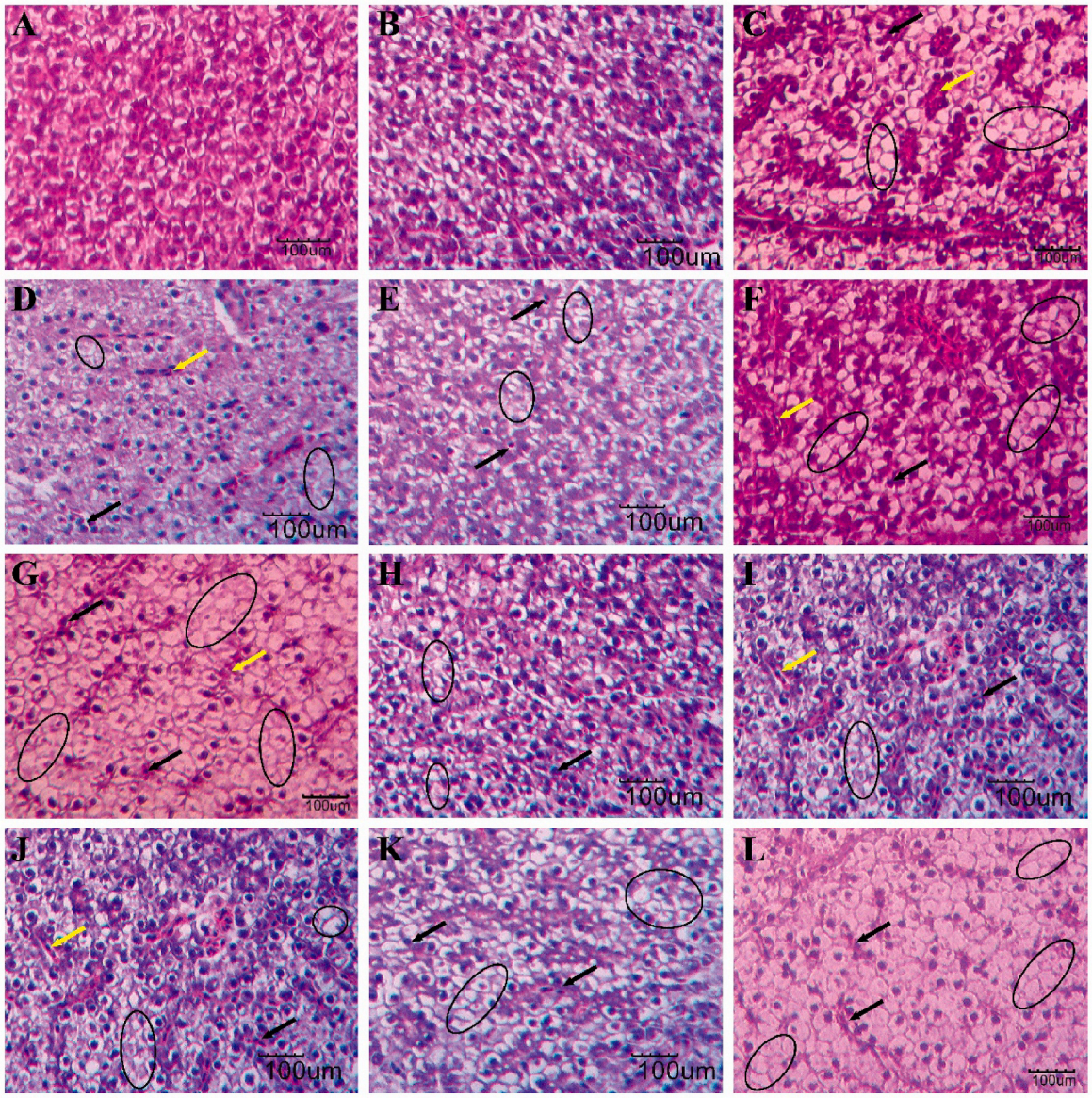

Fig. 2. Representative image of zebrafish liver section after H&E staining. **A.** Control group; **B-D.** 200-μm, 40-μm and 10-μm micro-plastics exposed liver section, respectively; **E-F.** 300-nm and 50-nm nano-plastics exposed liver section; **G.** Liver exposed to single OTC exposure; **H-J.** The combination of OTC and 200-μm, 40-μm and 10-μm micro-plastics exposed liver section, respectively; **K-L.** The combination of OTC and 300-nm and 50-nm nano-plastics exposed liver section. Black arrow: peripherally located nucleus or pyknotic nucleus; Yellow arrow: inflammatory cell infiltration; Black cycle: ballooning degeneration and vacuole formation. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.3. Change of antioxidant enzyme activity and IBR index

The changes in antioxidant enzyme activities after 30-days exposure were shown in Fig. 3D–F. The CAT activity decreased as the microplastic size decreased, and single and combined exposure to small sized nano-plastics (SN) and OTC significantly decreased the CAT activity (Fig. 3D, $P < 0.05$). In the microplastic single exposure groups, SOD activity increased as plastic size decreased (Fig. 3E). OTC exposure significantly increased SOD activity, but decreased in a size-dependent manner with the combination of microplastics. The GSH content was significantly increased in the single microplastic treatments (Fig. 3F, $P < 0.05$), and slightly elevated in the combined exposure treatments. The elevation of GSH decreased with the decrease of microplastic size. Moreover, the combined exposure decreased the GSH content compared with the single microplastic exposure treatments. Two-way ANOVA analysis indicated that the microplastic size significantly affected the CAT activity (Table S4, df = 4, $F = 18.246$, $P = 0.000$), and OTC significantly affected SOD activity and GSH content (Table S4, SOD: df = 1, $F = 16.755$, $P = 0.001$; GSH: df = 1, $F = 54.442$, $P = 0.000$). Moreover, the interaction of microplastics and OTC exhibited a significant effect on SOD activity in zebrafish liver (Table S4, df = 4, $F = 5.564$, $P = 0.005$).

The IBR star plots and values of liver damage calculated using ALT, AST, CAT, SOD and GSH were shown in Fig. 4. In the microplastic single exposure treatments, the most sensitive biomarkers were GSH content, ALT and AST activities (Fig. 4A). In OTC and combination with microplastic treatments, the most sensitive biomarkers were SOD and AST activities (Fig. 4B). The greatest IBR value was detected in the SN treatment, and the IBR values in three micro-sized plastic treatments increased after co-exposure with OTC; however, it decreased in two nano-sized plastic treatments when combined with OTC (Fig. 4C).

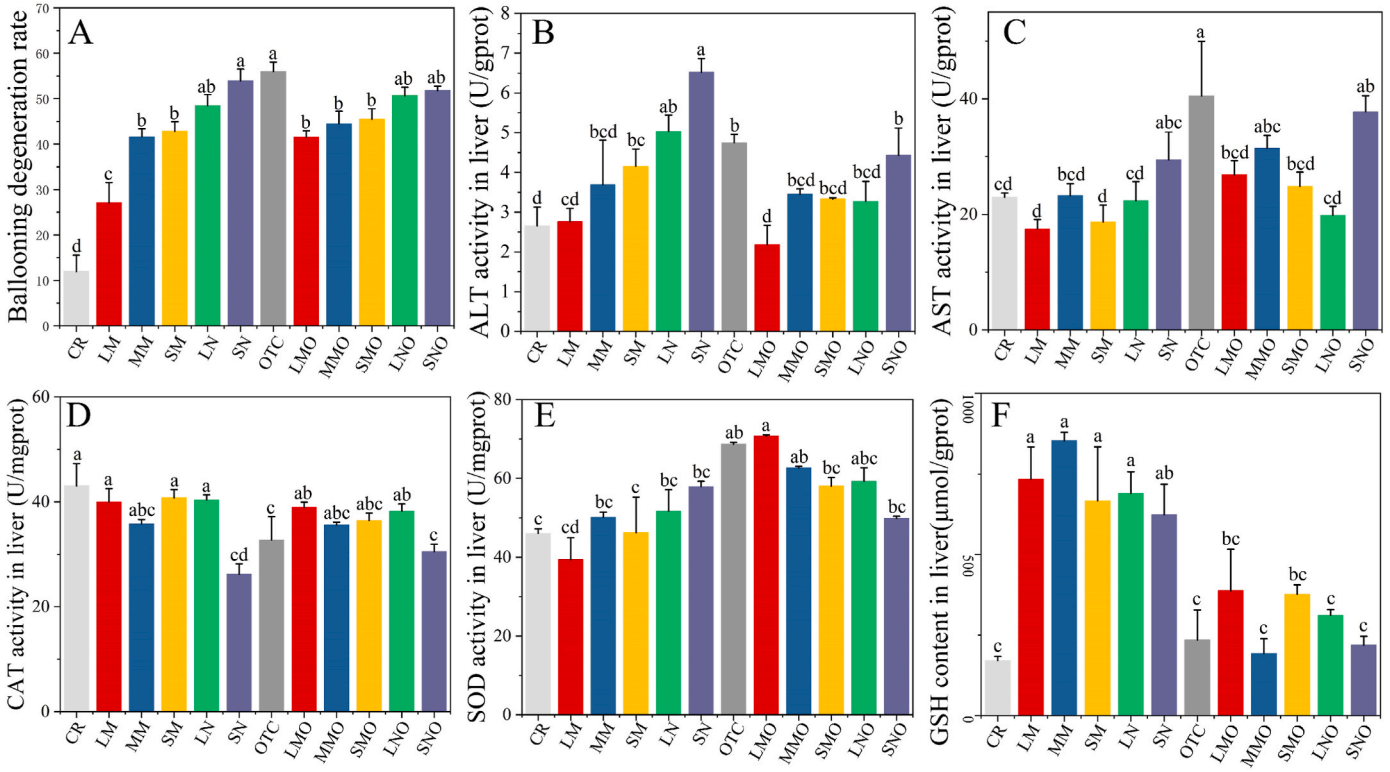

Fig. 3. Hepatocyte ballooning rate (A), alanine transaminase (B)aspartate transaminase (C) activity, the activities of catalase (D), superoxide dismutase (E) and the content of glutathione (F) of zebrafish liver after microplastics and OTC exposure. CR: control; OTC: single oxytetracycline exposure; LM, LMO: 200-μm micro-plastics single and combined with OTC, respectively; MM, MMO: 40-μm micro-plastics single and combined with OTC, respectively; SM, SMO: 10-μm micro-plastics single and combined with OTC, respectively; LN, LNO: 300-nm nano-plastics single and combined with OTC, respectively; SN, SNO: 50-nm nano-plastics single and combined with OTC, respectively. The presented values are the means ± SE (n = 3).

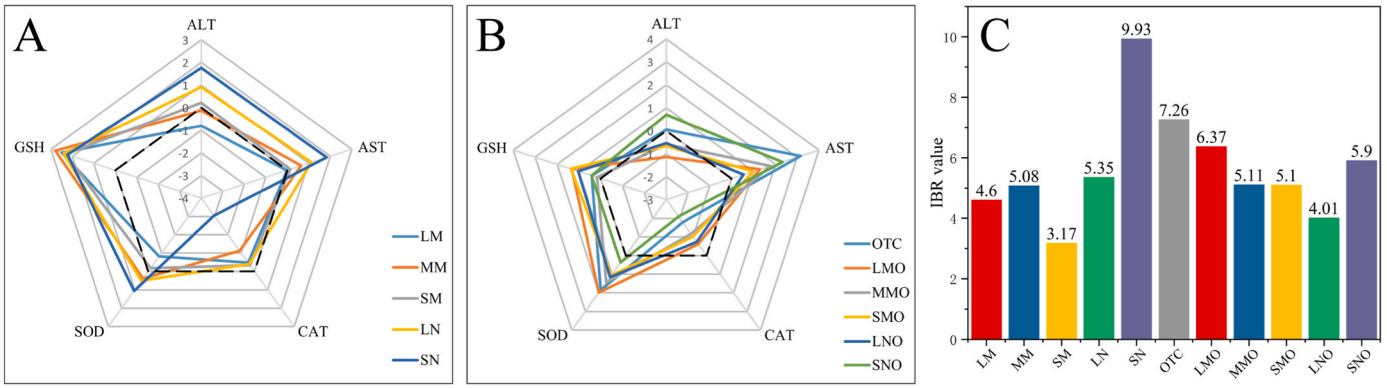

Fig. 4. The IBR star plot under the single exposure of microplastics (A); The IBR star plot under the single exposure of OTC and combined with microplastics (B); and the IBR values in each treatment (C). OTC: single oxytetracycline exposure; LM, LMO: 200-μm micro-plastics single and combined with OTC, respectively; MM, MMO: 40-μm micro-plastics single and combined with OTC, respectively; SM, SMO: 10-μm micro-plastics single and combined with OTC, respectively; LN, LNO: 300-nm nano-plastics single and combined with OTC, respectively; SN, SNO: 50-nm nano-plastics single and combined with OTC, respectively.

3.4. Transcriptomic profiles responding to nano-plastic and OTC exposure

Based on the histological and biomarker response of zebrafish livers, zebrafish exposed to OTC, small size nano-plastics (SN) and their combined exposure (SNO) treatments were selected to further investigate the transcriptomic response of livers. The analysis of the sequencing data quality is presented in the supplementary material (Fig. S5, Tables S7–S8).

The number, degree and distribution of DEGs between different treatments were analysed (Fig. S6). Compared with control, greatest number of DEGs were detected under the combined exposure to OTC and nano-plastics (3965 DEGs: 1601 up-regulated genes and 2364 down-regulated genes). Compared with control, there were 579 overlapping DEGs under OTC, nano-plastics and their combined exposure. .

GO analysis was conducted to further investigate the roles of OTC, nano-plastics and combined exposure in molecular function (MF) and biological process (BP) of genes. Remarkably enriched GO terms in the OTC, nano-plastics and combined exposure treatments were summarised in Table S9 (q value < 0.05). Briefly, in the OTC exposure treatment, the significantly enriched GO pathway was an oxidation-reduction process (GO: 0055,114). After a single exposure to nano-plastics, the DEGs were associated with heterocyclic compound and ion binding

processes (GO:0020,037 heme binding, GO:0046,906 tetrapyrrole binding, GO:0005506 iron ion binding), as well as oxidation-reduction (GO:0016,705 oxidoreductase activity, GO:0055,114 oxidation-reduction process). In the combined exposure treatment, the significantly enriched GO pathways covered those in single exposure treatments, and were also associated with other GO terms such as carbohydrate binding (GO:0030,246) and carbohydrate metabolic process (GO:0005975).

To better illustrate the biological functions of DEGs and mechanisms of contaminant effects, DEGs were mapped to KEGG metabolic and regulatory pathways. All significantly enriched terms that mapped to KEGG pathways were presented in Fig. 5 ($P < 0.05$), and DEGs were primarily enriched in metabolism, biosynthesis and signaling pathways. After OTC exposure, nine KEGG terms, such as steroid biosynthesis, stress defence signaling pathways like PPAR and FoxO, and some amino acid metabolisms pathways, were significantly enriched. In the nano-plastic single exposure group, KEGG terms related to steroid biosynthesis, primary bile acid biosynthesis, amino acid metabolisms and saccharide metabolism, and defence pathways such as ABC transporters, were significantly enriched. Pathways related to fatty acid metabolism, glycolysis/gluconeogenesis, insulin signaling pathway and Tight junction were uniquely enriched in combined exposure group. Notably, steroid biosynthesis was enriched in all three treatments, and therefore we further compared the up- and down-regulated genes in steroid biosynthesis in three treatments (Fig. S7). Similar but slightly differentiated pattern was observed in three treatments, indicating the similar response of zebrafish under different contaminant exposure in steroid biosynthesis pathway.

4. Discussion

4.1. The accumulation and effects of microplastics and OTC exposure

To date, the adsorption of micro- and nano-plastics on antibiotics has been established in many studies (Xiong et al., 2020; Yang et al., 2022; Yu et al., 2020), the absorption of microplastics on OTC was up to 894 μg/g (Zhang et al., 2018). However, the mechanisms underlying the combined effects of microplastics and antibiotics remain unclear. Our results suggested that the accumulation of OTC is significantly affected by the presence of nano-plastics, the OTC amount in three micro-sized plastic combined groups kept comparable ($P > 0.05$); however, it was significantly increased in two nano-plastics combined groups. We speculated that owing to the high adsorption ability of nanoparticles, those OTC-adsorbed nano-plastics translocated into livers via hamolymph and hamocytes (Collard et al., 2017), without depuration through excretion and deficient detoxification processes (Moos et al., 2012; Urquhart et al., 2007), thereby increasing liver OTC accumulation in nano-plastic groups.

The amount of microplastic of all size ranges in zebrafish liver (except for the large micro-sized plastics) increased after combined with OTC, and the increase was significant in LNO treatment, indicating the effects of OTC combination, which was also manifested in the two-way ANOVA test. Notably, the effects of microplastics may not be solely based on weight, although higher accumulated amounts of micro-sized plastic particles are obtained in this study, the number of particles of nano-plastics is actually several orders of magnitude greater than that of micro-sized particles, considering the smaller diameter and volume of nano-plastics. The larger amount of nano-plastics in liver was also positively correlated with the increased OTC accumulation in two nano-plastic combined groups.

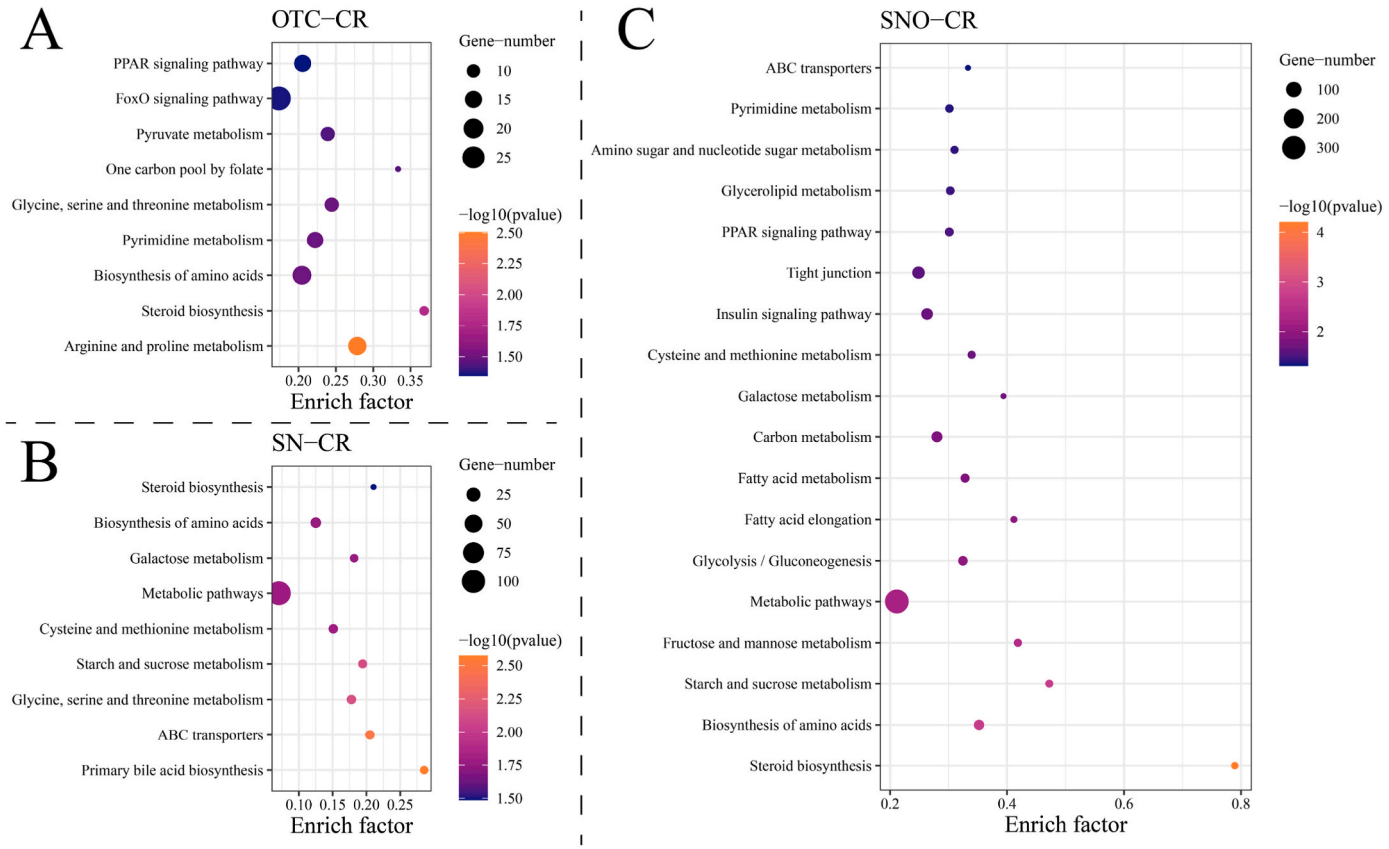

Fig. 5. Dot plots exhibiting the enrichment of KEGG pathways under the OTC exposure (A), 50 nm nano-plastics exposure (B) and combined exposure (C). The x axis shows the enrich factor of each KEGG pathway; the color of the dot indicates the p-value (-$\log_{10}$); the size of the dot denotes the gene number involved in each KEGG pathway. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

4.2. Histological and biomarker response of zebrafish

The high accumulation of OTC or microplastics in livers confirmed their function of defending xenobiotics; however, excessive accumulation might lead to hepatic histopathological changes. Our results demonstrate that hepatic alteration in zebrafish is induced by single and combined exposure of microplastics and OTC at varying degrees. Severe hepatocyte lipid-type vacuolization with nuclear displacement were observed in OTC single exposure treatment, which was positively related to the greatest accumulation of OTC in this group. Regressive changes in liver sections induced by OTC have been detected in previous studies (Rodrigues et al., 2019, 2017), and cytoplasmic vacuolization in livers can be regarded as glycogen and lipid storage (Wolf et al., 2015). Another study suggested that vacuolization in hepatic cells might be related to the excessive accumulation of fat (steatosis) in the cytoplasm (Soler, 1996; Zhang et al., 2021). Pyknosis induced by tetracyclines-based antibiotics was allegedly regulated by activating calpains, thereby triggering apoptosis, leading to chromatin condensation and nuclear disintegration (Guerra et al., 2016). The ballooning rate of hepatic cells in all single or combined exposure treatments was significantly higher than that in control, indicating severe liver damage induced by microplastics and OTC. As anticipated, the ballooning rate of hepatic cells increased in a size-dependent manner, the lesser the size of the microplastics, more severe the liver damage. A previous study has revealed that exposure to 2 μm microplastic induced more severe liver injury in fish than exposure to 10 μm microplastic (Lu et al., 2016), which also supports our speculation on the size-specific influence of microplastics. In the large micro-sized plastic groups, the ballooning rate significantly increased after the addition of OTC, suggesting its relation with OTC accumulation in this group. However, we found the increased OTC accumulation was observed in SNO, while the ballooning rate decreased compared with single OTC exposure. We speculated that the accumulated OTC in single OTC treatment was bioavailable, whereas that in the SNO treatment might be attached to nano-plastic particles with low bioavailability (Gao et al., 2020; Sleight et al., 2017; Yang et al., 2020a), resulting in a slightly lower ballooning rate. Furthermore, the ballooning rate may not fully indicate the extent of liver damage, and the effects of SNO treatment may be reflected in other aspects. Moreover, the histopathological indices increased in three micro-plastics combined with OTC treatments than in single micro-plastics exposure, suggesting a more severe histological damage being induced by combined exposure.

As frequently used indicators of liver damage (Li et al., 2020a,b; Talesa et al., 1992; Yu et al., 2018), the ALT and AST levels in single microplastics groups increased in a size-dependent pattern, which is consistent with the ballooning rate in livers, indicating severe histological damage induced by smaller microplastics. However, such size-dependent pattern was not observed in the OTC-combined treatments, indicating that the addition of OTC disturbed the size-dependent effect of microplastics. Two-way ANOVA analysis further suggested that both the microplastic size and the addition of OTC significantly affected the ALT and AST activities; however, the interaction of microplastics and OTC exhibited no significant influence. The presence of microplastics and OTC in the zebrafish liver were also associated with alterations in the antioxidant enzyme activity. These alterations represent a defensive mechanism against oxidative stress and damage (Dar and Barzilai, 2009; Doyotte et al., 1997). The inhibition of CAT activity in the SN, OTC and their combined exposure might indicate the destruction or deficiency of antioxidant capacity in zebrafish after small nano-plastics and OTC exposure. Other study also suggested this defensive “threshold” of CAT that the CAT activity might be inhibited under severe oxidative damage (Song et al., 2022; Yang et al., 2020b). In the single microplastics exposure groups, SOD activity increased with the decrease of plastic particle sizes; however, a decreasing tendency was observed after the addition of OTC, indicating a differentiated response pattern of SOD under the combination of OTC and microplastics, and the disturbance of OTC on microplastics size-dependent pattern. Two-way ANOVA also indicated a significant effect of microplastics and OTC interaction. GSH can effectively prevent the accumulation of reactive oxygen species and damage (Doyotte et al., 1997; Song et al., 2022). The significantly increased GSH content in microplastics single-exposure treatments and, the unaffected GSH content in OTC and their combination treatments also corresponded the distinct response pattern mentioned in SOD activity. IBR star plots and values were developed to evaluate the responses of individual biomarkers under different treatments (Andrade et al., 2019; Baudou et al., 2019). The star plots indicate the predominant role of GSH in response to microplastic single-exposure, while in OTC combined treatments, SOD and AST activities dominated as sensitive biomarkers. The greatest IBR value (9.93) in SNP treatments indicated the most intense response of zebrafish liver under exposure to 50 nm plastic particles. Furthermore, the IBR value increased after the addition of OTC in three micro-sized plastic groups, while decreased in two nano-sized plastic groups. Notably, the decrease of IBR values in the combined exposure than in single microplastic exposure treatments did not necessarily indicate an alleviation induced by OTC, as the IBR value used in this study is calculated with limited biomarkers.

4.3. Microplastics and OTC perturb the transcriptome profile of zebrafish

Based on the high pollutant accumulations, severe liver damage and enzyme response, we further analysed the transcriptome profiles of zebrafish liver under OTC, SN and SNO exposure. A large amount of significantly up-/down-regulated genes revealed a remarkable response in zebrafish. Moreover, the same GO terms involved in iron- and heme-binding processes were significantly enriched in SN and SNO treatments, which might be a response to the accumulation of nano-plastics. Owing to the large specific area, microorganisms can be easily attached, secrete extracellular polymeric substances and proliferate on microplastics, thereby forming the biofilm (Palmer et al., 2007; Schluter et al., 2015; Zettler et al., 2013). Some iron-binding proteins play unique roles in preventing bacterial biofilms formation by chelating iron and stimulating bacterial twitching (Cizmeci et al., 2014; Good et al., 2017).

The KEGG pathway enriched in OTC, SN and SNO also suggested the effects of OTC and microplastics on endocrine regulation, which could alter various steroid dehydrogenases/reductases, thereby affecting the steroid biosynthetic pathway (Jiang et al., 2020; Norman and Henry, 2015; Sanderson, 2006). Similar results were also recorded in zebrafish under mixture of antibiotics exposure (Qiu et al., 2020). We also observed the sharing enriched KEGG pathways in single and combined exposure, such as the PPAR signaling pathway in OTC and SNO, ABC transporters, the starch and sucrose metabolism pathways in SN and SNO. The significantly increased ballooning rate in OTC and SNO treatments might be influenced by the enriched PPAR signaling pathway, which regulate the hepatic lipogenesis (Nguyen et al., 2008). Starch and sucrose are essential in energy metabolism regulation in livers (Roncal-Jimenez et al., 2011), the disturbances of glucose metabolism induced by microplastics has been reported in previous research (Sheng et al., 2021; Zhao et al., 2020). Similar to our results, microplastics and their combination with triclosan induced an enriched pathway of starch and sucrose metabolism (Sheng et al., 2021).

Furthermore, microplastics affected oyster reproduction through the insulin signaling pathway (Gardon et al., 2018; Sussarellu et al., 2016), which plays an essential role in mobilising reserves during gametogenesis, thereby influencing germinal cell maturation (Gricourt et al., 2006). Our results correspond with their results, that the insulin signaling pathway was also enriched in SNO treatment. The results of this study illustrated the influence of microplastics and OTC on zebrafish reproduction, lipid, glucose and energy metabolism. However, further data like metabolite and protein profiles are required to achieve a more comprehensive understanding on the molecular mechanism of microplastics and OTC in zebrafish.

5. Conclusion

This study emphasized the effects of different sized microplastics and OTC on the liver histopathology, biomarker activity and the transcriptomic response, as well as the accumulation of OTC and microplastics in livers of zebrafish. We found that nano-sized plastics significantly increased the accumulation of OTC in zebrafish liver. Single and combined exposure of OTC and microplastics induced liver histological alteration, and the nano-sized plastics caused more severe damage than micro-plastics. The biomarkers indicated that the addition of OTC perturbed the influence pattern of different sized microplastics. Transcriptomic analysis revealed combined exposure of nano-plastics and OTC remained the shared pathways in single exposure groups (steroid biosynthesis), but also induced exclusive response (reproduction, lipid, glucose and energy metabolism), which provides new insights into interaction between OTC and microplastics. However, more extensive and specific data are required to elucidate the potential ecotoxicological impacts under the combination of different sized microplastics and antibiotics.

Credit author statement

Ziyue Yu: Methodology, Investigation, Writing - original draft. **Changzhou Yan**: Conceptualization, Supervision, Funding acquisition, Writing - review & editing. **Donghua Qiu**: Formal analysis, Writing - review & editing. **Xin Zhang**: Resources, Writing - review & editing. **Ce Wen**: Resources, Writing - review & editing. **Sijun Dong**: Writing - review & editing, Funding acquisition.

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.

Data availability

The authors do not have permission to share data.

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Appendix A. Supplementary data

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References

Almeida, A.R., Tacao, M., Machado, A.L., Golovko, O., Zlabek, V., Domingues, I., Henriques, I., Tacão, M., Machado, A.L., Golovko, O., Zlabek, V., Domingues, I., Henriques, I., 2019. Long-term effects of oxytetracycline exposure in zebrafish: a multi-level perspective. Chemosphere 222, 333–344. https://doi.org/10.1016/j.chemosphere.2019.01.147.

Anders, S., Huber, W., 2010. Differential expression analysis for sequence count data. Genome Biol. 11, 1–12. https://doi.org/10.1186/GB-2010-11-10-R106.

Andrade, L.L. de, do Espirito Santo Pereira, A., Fernandes Fraceto, L., Bueno dos Reis Martinez, C., 2019. Can atrazine loaded nanocapsules reduce the toxic effects of this herbicide on the fish *Prochilodus lineatus*? A multibiomarker approach. Sci. Total Environ. 663, 548–559. https://doi.org/10.1016/J.SCITOTENV.2019.01.380.

Antunes, A.M., Rocha, T.L., Pires, F.S., de Freitas, M.A., Leite, V., Arana, S., Moreira, P. C., Saboia-Morais, S.M.T., 2017. Gender-specific histopathological response in guppies *Poecilia reticulata* exposed to glyphosate or its metabolite aminomethylphosphonic acid. J. Appl. Toxicol. 37, 1098–1107. https://doi.org/10.1002/jat.3461.

Araújo, A.P. da C., Gomes, A.R., Malafaia, G., 2020. Hepatotoxicity of pristine polyethylene microplastics in neotropical *physalaemus cuvieri* tadpoles (Fitzinger, 1826). J. Hazard Mater. 386, 121992 https://doi.org/10.1016/j.jhazmat.2019.121992.

Association of Plastic Manufacturers (Organization), 2021. Plastics – the Facts 2021. PlasticEurope 16.

Baudou, F.G., Ossana, N.A., Castañé, P.M., Mastrángelo, M.M., González Núñez, A.A., Palacio, M.J., Ferrari, L., 2019. Use of integrated biomarker indexes for assessing the impact of receiving waters on a native neotropical teleost fish. Sci. Total Environ. 650, 1779–1786. https://doi.org/10.1016/J.SCITOTENV.2018.09.342.

Bernet, D., Schmidt, H., Meier, W., Burkhardt-Holm, P., Wahli, T., 1999. Histopathology in fish: proposal for a protocol to assess aquatic pollution. J. Fish. Dis. 22, 25–34. https://doi.org/10.1046/J.1365-2761.1999.00134.X.

Chagas, T.Q., Araújo, A.P. da C., Malafaia, G., 2021. Biomicroplastics versus conventional microplastics: An insight on the toxicity of these polymers in dragonfly larvae. Sci. Total Environ. 761, 143231. https://doi.org/10.1016/j.scitotenv.2020.143231.

Chemello, G., Piccinetti, C., Randazzo, B., Carnevali, O., Maradonna, F., Magro, M., Bonaiuto, E., Vianello, F., Radaelli, G., Fifi, A.P., Gigliotti, F., Olivotto, I., 2016. Oxytetracycline delivery in adult female zebrafish by iron oxide nanoparticles. Zebrafish 13, 495–503. https://doi.org/10.1089/zeb.2016.1302.

Chen, H., Liu, S., Xu, X.R., Zhou, G.J., Liu, S.S., Yue, W.Z., Sun, K.F., Ying, G.G., 2015. Antibiotics in the coastal environment of the Hailing Bay region, South China Sea: spatial distribution, source analysis and ecological risks. Mar. Pollut. Bull. 95, 365–373. https://doi.org/10.1016/J.MARPOLBUL.2015.04.025.

Chen, Q., Yin, D., Jia, Y., Schiwy, S., Legradi, J., Yang, S., Hollert, H., 2017. Enhanced uptake of BPA in the presence of nanoplastics can lead to neurotoxic effects in adult zebrafish. Sci. Total Environ. 609, 1312–1321. https://doi.org/10.1016/j.scitotenv.2017.07.144.

Cizmeci, M.N., Kara, S., Kanburoglu, M.K., Simavli, S., Duvan, C.I., Tatli, M.M., 2014. Detection of cord blood hepcidin levels as a biomarker for early-onset neonatal sepsis. Med. Hypotheses 82, 310–312. https://doi.org/10.1016/J.MEHY.2013.12.017.

Cole, M., Galloway, T.S., 2015. Ingestion of nanoplastics and microplastics by Pacific oyster larvae. Environ. Sci. Technol. 49, 14625–14632. https://doi.org/10.1021/ACS.EST.5B04099.

Collard, F., Gilbert, B., Compère, P., Eppe, G., Das, K., Jauniaux, T., Parmentier, E., 2017. Microplastics in livers of European anchovies (*Engraulis encrasicolus,* L.). Environ. Pollut. 229, 1000–1005. https://doi.org/10.1016/J.ENVPOL.2017.07.089.

Dar, I., Barzilai, A., 2009. Cellular responses to oxidative stress. The DNA Damage Response: Implications on Cancer Formation and Treatment 65–79. https://doi.org/10.1007/978-90-481-2561-6_4.

Deng, Y., Zhang, Y., Lemos, B., Ren, H., 2017. Tissue accumulation of microplastics in mice and biomarker responses suggest widespread health risks of exposure. Sci. Rep. 7 https://doi.org/10.1038/SREP46687.

Ding, J., Huang, Y., Liu, S., Zhang, S., Zou, H., Wang, Z., Zhu, W., Geng, J., 2020. Toxicological effects of nano- and micro-polystyrene plastics on red tilapia: are larger plastic particles more harmless? J. Hazard Mater. 396 https://doi.org/10.1016/j.jhazmat.2020.122693.

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. https://doi.org/10.1016/J.ENVPOL.2018.03.001.

Doyotte, A., Cossu, C., Jacquin, M.C., Babut, M., Vasseur, P., 1997. Antioxidant enzymes, glutathione and lipid peroxidation as relevant biomarkers of experimental or field exposure in the gills and the digestive gland of the freshwater bivalve *Unio tumidus*. Aquat. Toxicol. 39, 93–110. https://doi.org/10.1016/S0166-445X(97)00024-6.

Dubaish, F., Liebezeit, G., 2013. Suspended microplastics and black carbon particles in the Jade system, southern North Sea. Water. Air. Soil Pollut. 224 https://doi.org/10.1007/s11270-012-1352-9.

European Medicines Agency, 2021. Sales of veterinary antimicrobial agents in 31 European countries in 2018. Eur. Surveill. Vet. Antimicrob. Consum. 2020.

Gao, M., Xu, Y., Liu, Y., Wang, S., Wang, C., Dong, Y., Song, Z., 2020. Effect of polystyrene on dibutyl phthalate (DBP) bioavailability and DBP-induced phytotoxicity in lettuce. Environ. Pollut. 268, 115870 https://doi.org/10.1016/j.envpol.2020.115870.

Gardon, T., Reisser, C., Soyez, C., Quillien, V., Moullac, G. Le, 2018. Microplastics affect energy balance and gametogenesis in the pearl oyster *Pinctada margaritifera*. Environ. Sci. Technol. 52, 5277–5286. https://doi.org/10.1021/ACS.EST.8B00168.

Good, M., Kolls, J.K., Empey, K.M., 2017. Neonatal pulmonary host defense. Fetal Neonatal Physiol 12, 1262–1293. https://doi.org/10.1016/B978-0-323-35214-7.00130-X.

Goulding, R., 1987. Casarett and Doull's toxicology: the basic science of poisons. Postgrad. Med. 63 https://doi.org/10.1136/PGMJ.63.746.1112-B, 1112–1112.

Gricourt, L., Mathieu, M., Kellner, K., 2006. An insulin-like system involved in the control of Pacific oyster *Crassostrea gigas* reproduction: hrIGF-1 effect on germinal cell proliferation and maturation associated with expression of an homologous insulin receptor-related receptor. Aquaculture 251, 85–98. https://doi.org/10.1016/J.AQUACULTURE.2005.05.015.

Guerra, W., Silva-Caldeira, P.P., Terenzi, H., Pereira-Maia, E.C., 2016. Impact of metal coordination on the antibiotic and non-antibiotic activities of tetracycline-based drugs. Coord. Chem. Rev. 327–328, 188–199. https://doi.org/10.1016/J.CCR.2016.04.009.

Hsieh, S.L., Wu, Y.C., Xu, R.Q., Chen, Y.T., Chen, C.W., Singhania, R.R., Dong, C.D., 2021. Effect of polyethylene microplastics on oxidative stress and histopathology

damages in *Litopenaeus vannamei*. Environ. Pollut. 288, 117800 https://doi.org/10.1016/J.ENVPOL.2021.117800.
Imhof, H.K., Laforsch, C., Wiesheu, A.C., Schmid, J., Anger, P.M., Niessner, R., Ivleva, N. P., 2016. Pigments and plastic in limnetic ecosystems: a qualitative and quantitative study on microparticles of different size classes. Water Res. 98, 64–74. https://doi.org/10.1016/J.WATRES.2016.03.015.
Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Science 347, 768–771. https://doi.org/10.1126/SCIENCE.1260352.
Jeong, B., Baek, J.Y., Koo, J., Park, S., Ryu, Y.K., Kim, K.S., Zhang, S., Chung, C.H., Dogan, R., Choi, H.S., Um, D., Kim, T.K., Lee, W.S., Jeong, J., Shin, W.H., Lee, J.R., Kim, N.S., Lee, D.Y., 2022. Maternal exposure to polystyrene nanoplastics causes brain abnormalities in progeny. J. Hazard Mater. 426, 127815 https://doi.org/10.1016/J.JHAZMAT.2021.127815.
Jeong, C.B., Won, E.J., Kang, H.M., Lee, M.C., Hwang, D.S., Hwang, U.K., Zhou, B., Souissi, S., Lee, S.J., Lee, J.S., 2016. Microplastic size-dependent toxicity, oxidative stress induction, and p-JNK and p-p38 activation in the monogonont rotifer (*Brachionus koreanus*). Environ. Sci. Technol. 50, 8849–8857. https://doi.org/10.1021/ACS.EST.6B01441.
Jiang, J., Chen, L., Wu, S., Lv, L., Liu, X., Wang, Q., Zhao, X., 2020. Effects of difenoconazole on hepatotoxicity, lipid metabolism and gut microbiota in zebrafish (*Danio rerio*). Environ. Pollut. 265, 114844 https://doi.org/10.1016/j.envpol.2020.114844.
Jiang, R., Lin, W., Wu, J., Xiong, Y., Zhu, F., Bao, L.J., You, J., Ouyang, G., Zeng, E.Y., 2018. Quantifying nanoplastic-bound chemicals accumulated in *Daphnia magna* with a passive dosing method. Environ. Sci. Nano 5, 776–781. https://doi.org/10.1039/C7EN00932A.
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.
Keerthisinghe, T.P., Wang, F., Wang, M., Yang, Q., Li, J., Yang, J., Xi, L., Dong, W., Fang, M., 2020. Long-term exposure to TET increases body weight of juvenile zebrafish as indicated in host metabolism and gut microbiome. Environ. Int. 139, 105705 https://doi.org/10.1016/j.envint.2020.105705.
Lenz, R., Enders, K., Nielsen, T.G., 2016. Microplastic exposure studies should be environmentally realistic. Proc. Natl. Acad. Sci. U.S.A. https://doi.org/10.1073/pnas.1606615113.
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, D., Yang, M., Hu, J., Ren, L., Zhang, Y., Li, K., 2008. Determination and fate of oxytetracycline and related compounds in oxytetracycline production wastewater and the receiving river. Environ. Toxicol. Chem. 27, 80–86. https://doi.org/10.1897/07-080.1.
Li, F., Li, F., Hou, X., Luo, X., Tu, H., Zou, Y., Sun, C., Shi, M., Zheng, H., 2018. Comparison of six digestion methods on fluorescent intensity and morphology of the fluorescent polystyrene beads. Mar. Pollut. Bull. 131, 515–524. https://doi.org/10.1016/j.marpolbul.2018.04.056.
Li, J., Dong, T., Keerthisinghe, T.P., Chen, H., Li, M., Chu, W., Yang, J., Hu, Z., Snyder, S. A., Dong, W., Fang, M., 2020a. Long-term oxytetracycline exposure potentially alters brain thyroid hormone and serotonin homeostasis in zebrafish. J. Hazard Mater. 399, 123061 https://doi.org/10.1016/j.jhazmat.2020.123061.
Li, Z., Feng, C., Wu, Y., Guo, X., 2020b. Impacts of nanoplastics on bivalve: fluorescence tracing of organ accumulation, oxidative stress and damage. J. Hazard Mater. 392, 122418 https://doi.org/10.1016/J.JHAZMAT.2020.122418.
Lu, Y., Zhang, Y., Deng, Y., Jiang, W., Zhao, Y., Geng, J., Ding, L., Ren, H., 2016. Uptake and accumulation of polystyrene microplastics in zebrafish (*Danio rerio*) and toxic effects in liver. Environ. Sci. Technol. 50, 4054–4060. https://doi.org/10.1021/acs.est.6b00183.
Lusher, A., Hollman, P., Mandoza-Hill, J., 2017. Microplastics in fisheries and aquaculture. FAO Fisheries and Aquaculture Technical Paper, Rome.
Maes, T., Jessop, R., Wellner, N., Haupt, K., Mayes, A.G., 2017. A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile Red. Sci. Reports 2017 71 7, 1–10. https://doi.org/10.1038/srep44501.
Monteiro, S.H., Francisco, J.G., Andrade, G.C.R.M., Botelho, R.G., Figueiredo, L.A., Tornisielo, V.L., 2016. Study of spatial and temporal distribution of antimicrobial in water and sediments from caging fish farms by on-line SPE-LC-MS/MS. J. Environ. Sci. Health B 51, 634–643. https://doi.org/10.1080/03601234.2016.1181917.
Moos, N. von, Burkhardt-Holm, P., Köhler, A., 2012. Uptake and effects of microplastics on cells and tissue of the blue mussel *Mytilus edulis* L. after an experimental exposure. Environ. Sci. Technol. 46, 11327–11335. https://doi.org/10.1021/ES302332W.
Na, J., Song, J., Achar, J.C., Jung, J., 2021. Synergistic effect of microplastic fragments and benzophenone-3 additives on lethal and sublethal *Daphnia magna* toxicity. J. Hazard Mater. 402, 123845 https://doi.org/10.1016/j.jhazmat.2020.123845.
Neto, J.G.B., Rodrigues, F.L., Ortega, I., Rodrigues, L.D.S., Lacerda, A., Coletto, J.L., Kessler, F., Cardoso, L.G., Madureira, L., Proietti, M.C., 2020. Ingestion of plastic debris by commercially important marine fish in southeast-south Brazil. Environ. Pollut. 267, 115508 https://doi.org/10.1016/j.envpol.2020.115508.
Nguyen, P., Leray, V., Diez, M., Serisier, S., Le Bloc'H, J., Siliart, B., Dumon, H., 2008. Liver lipid metabolism. J. Anim. Physiol. Anim. Nutr. 92, 272–283. https://doi.org/10.1111/J.1439-0396.2007.00752.X.
Norman, A.W., Henry, H.L., 2015. Steroid hormones: chemistry, biosynthesis, and metabolism. Hormones 27–53. https://doi.org/10.1016/B978-0-08-091906-5.00002-1.
Oliveira, M., Ribeiro, A., Hylland, K., Guilhermino, L., 2013. Single and combined effects of microplastics and pyrene on juveniles (0+ group) of the common goby *Pomatoschistus microps* (Teleostei, Gobiidae). Ecol. Indicat. 34, 641–647. https://doi.org/10.1016/J.ECOLIND.2013.06.019.
Palmer, J., Flint, S., Brooks, J., 2007. Bacterial cell attachment, the beginning of a biofilm. J. Ind. Microbiol. Biotechnol. 34, 577–588. https://doi.org/10.1007/S10295-007-0234-4.
Pês, T.S., Saccol, E.M.H., Londero, É.P., Bressan, C.A., Ourique, G.M., Rizzetti, T.M., Pretes, O.D., Zanella, R., Baldisserotto, B., Pavanato, M.A., 2018. Protective effect of quercetin against oxidative stress induced by oxytetracycline in muscle of silver catfish. Aquaculture 484, 120–125. https://doi.org/10.1016/J.AQUACULTURE.2017.10.043.
Piyawardhana, N., Weerathunga, V., Chen, H.S., Guo, L., Huang, P.J., Ranatunga, R.R.M. K.P., Hung, C.C., 2022. Occurrence of microplastics in commercial marine dried fish in Asian countries. J. Hazard Mater. 423, 127093 https://doi.org/10.1016/J.JHAZMAT.2021.127093.
Qiu, W., Liu, X., Yang, F., Li, R., Xiong, Y., Fu, C., Li, G., Liu, S., Zheng, C., 2020. Single and joint toxic effects of four antibiotics on some metabolic pathways of zebrafish (*Danio rerio*) larvae. Sci. Total Environ. 716, 137062 https://doi.org/10.1016/j.scitotenv.2020.137062.
Rodrigues, S., Antunes, S.C., Nunes, B., Correia, A.T., 2019. Histopathological effects in gills and liver of *Sparus aurata* following acute and chronic exposures to erythromycin and oxytetracycline. Environ. Sci. Pollut. Res. 26, 15481–15495. https://doi.org/10.1007/s11356-019-04954-0.
Rodrigues, S., Antunes, S.C., Nunes, B., Correia, A.T., 2017. Histological alterations in gills and liver of rainbow trout (*Oncorhynchus mykiss*) after exposure to the antibiotic oxytetracycline. Environ. Toxicol. Pharmacol. 53, 164–176. https://doi.org/10.1016/J.ETAP.2017.05.012.
Roncal-Jimenez, C.A., Lanaspa, M.A., Rivard, C.J., Nakagawa, T., Sanchez-Lozada, L.G., Jalal, D., Andres-Hernando, A., Tanabe, K., Madero, M., Li, N., Cicerchi, C., McFann, K., Sautin, Y.Y., Johnson, R.J., 2011. Sucrose induces fatty liver and pancreatic inflammation in male breeder rats independent of excess energy intake. Metabolism 60, 1259–1270. https://doi.org/10.1016/J.METABOL.2011.01.008.
Rummel, C.D., Löder, M.G.J., Fricke, N.F., Lang, T., Griebeler, E.M., Janke, M., Gerdts, G., 2016. Plastic ingestion by pelagic and demersal fish from the north sea and baltic sea. Mar. Pollut. Bull. 102, 134–141. https://doi.org/10.1016/J.MARPOLBUL.2015.11.043.
Sanchez, W., Burgeot, T., Porcher, J.M., 2013. A novel "Integrated Biomarker Response" calculation based on reference deviation concept. Environ. Sci. Pollut. Res. 20, 2721–2725. https://doi.org/10.1007/S11356-012-1359-1/TABLES/2.
Sanderson, J.T., 2006. The steroid hormone biosynthesis pathway as a target for endocrine-disrupting chemicals. Toxicol. Sci. 94, 3–21. https://doi.org/10.1093/TOXSCI/KFL051.
Schluter, J., Nadell, C.D., Bassler, B.L., Foster, K.R., 2015. Adhesion as a weapon in microbial competition. ISME J. 9, 139–149. https://doi.org/10.1038/ISMEJ.2014.174.
Sheng, C., Zhang, S., Zhang, Y., 2021. The influence of different polymer types of microplastics on adsorption, accumulation, and toxicity of triclosan in zebrafish. J. Hazard Mater. 402 https://doi.org/10.1016/j.jhazmat.2020.123733.
Sleight, V.A., Bakir, A., Thompson, R.C., Henry, T.B., 2017. Assessment of microplastic-sorbed contaminant bioavailability through analysis of biomarker gene expression in larval zebrafish. Mar. Pollut. Bull. 116, 291–297. https://doi.org/10.1016/j.marpolbul.2016.12.055.
Soler, F., 1996. Anatomo-pathological effect of oxytetracycline in tench (*Tinca tinca*). Toxicol. Lett. 88, 104. https://doi.org/10.1016/0378-4274(96)88724-8.
Song, P., Jiang, N., Zhang, K., Li, X., Li, N., Zhang, Y., Wang, Q., Wang, J., 2022. Ecotoxicological evaluation of zebrafish liver (*Danio rerio*) induced by dibutyl phthalate. J. Hazard Mater. 425, 128027 https://doi.org/10.1016/J.JHAZMAT.2021.128027.
Sussarellu, R., Suquet, M., Thomas, Y., Lambert, C., Fabioux, C., Pernet, M.E.J., Goïc, N. Le, 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.
Talesa, V., Contenti, S., Principato, G.B., Pascolini, R., Giovannini, E., Rosi, G., 1992. Cholinesterases from *Maia verrucosa* and *Palinurus vulgaris*: a comparative study. Comp. Biochem. Physiol. Part C, Comp. 101, 499–503. https://doi.org/10.1016/0742-8413(92)90077-K.
Trapnell, C., Roberts, A., Goff, L., Pertea, G., Kim, D., Kelley, D.R., Pimentel, H., Salzberg, S.L., Rinn, J.L., Pachter, L., Mishra, A., O'farrell, F.M., Reynell, C., Hamilton, N.B., Hall, C.N., Attwell, D., Ichikawa, T., Nakazato, K., Keller, P.J., Kajiura-Kobayashi, H., Stelzer, E.H.K., Mochizuki, A., Nonaka, S., 2014. Correction: corrigendum: Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nat. Protoc. 9, 2513. https://doi.org/10.1038/nprot1014-2513a.
Triebskorn, R., Braunbeck, T., Grummt, T., Hanslik, L., Huppertsberg, S., Jekel, M., Knepper, T.P., Krais, S., Müller, Y.K., Pittroff, M., Ruhl, A.S., Schmieg, H., Schür, C., Strobel, C., Wagner, M., Zumbülte, N., Köhler, H.R., 2019. Relevance of nano- and microplastics for freshwater ecosystems: a critical review. TrAC, Trends Anal. Chem. 110, 375–392. https://doi.org/10.1016/J.TRAC.2018.11.023.
Urquhart, B.L., Tirona, R.G., Kim, R.B., 2007. Nuclear receptors and the regulation of drug-metabolizing enzymes and drug transporters: implications for interindividual variability in response to drugs. J. Clin. Pharmacol. 47, 566–578. https://doi.org/10.1177/0091270007299930.

Van Cauwenberghe, L., Janssen, C.R., 2014. Microplastics in bivalves cultured for human consumption. Environ. Pollut. 193, 65–70. https://doi.org/10.1016/j.envpol.2014.06.010.

Varg, J.E., Kunce, W., Outomuro, D., Svanbäck, R., Johansson, F., 2021. Single and combined effects of microplastics, pyrethroid and food resources on the life-history traits and microbiome of *Chironomus riparius*. Environ. Pollut. 289, 117848 https://doi.org/10.1016/J.ENVPOL.2021.117848.

Wolf, J.C., Baumgartner, W.A., Blazer, V.S., Camus, A.C., Engelhardt, J.A., Fournie, J.W., Frasca, S., Groman, D.B., Kent, M.L., Khoo, L.H., Law, J.M., Lombardini, E.D., Ruehl-Fehlert, C., Segner, H.E., Smith, S.A., Spitsbergen, J.M., Weber, K., Wolfe, M.J., 2015. Nonlesions, misdiagnoses, missed diagnoses, and other interpretive challenges in fish histopathology studies:A guide for investigators, authors, reviewers, and readers. Toxicol. Pathol. 43, 297–325. https://doi.org/10.1177/0192623314540229.

Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: a review. Environ. Pollut. 178, 483–492. https://doi.org/10.1016/J.ENVPOL.2013.02.031.

Xiong, Y., Zhao, J., Li, L., Wang, Y., Dai, X., Yu, F., Ma, J., 2020. Interfacial interaction between micro/nanoplastics and typical PPCPs and nanoplastics removal via electrosorption from an aqueous solution. Water Res. 184, 116100 https://doi.org/10.1016/J.WATRES.2020.116100.

Xu, B., Liu, F., Brookes, P.C., Xu, J., 2018. Microplastics play a minor role in tetracycline sorption in the presence of dissolved organic matter. Env. Pollut 240, 87–94. https://doi.org/10.1016/j.envpol.2018.04.113.

Yan, M., Nie, H., Xu, K., He, Y., Hu, Y., Huang, Y., Wang, J., 2019. Microplastic abundance, distribution and composition in the pearl river along guangzhou city and pearl river estuary, China. Chemosphere 217, 879–886. https://doi.org/10.1016/J.CHEMOSPHERE.2018.11.093.

Yancheva, V., Velcheva, I., Stoyanova, S., Georgieva, E., 2016. Histological biomarkers in fish as a tool in ecological risk assessment and monitoring programs: a review. Appl. Ecol. Environ. Res. 14, 47–75. https://doi.org/10.15666/AEER/1401_047075.

Yang, H., Lai, H., Huang, J., Sun, L., Mennigen, J.A., Wang, Q., Liu, Y., Jin, Y., Tu, W., 2020a. Polystyrene microplastics decrease F–53B bioaccumulation but induce inflammatory stress in larval zebrafish. Chemosphere 255, 127040. https://doi.org/10.1016/J.CHEMOSPHERE.2020.127040.

Yang, H., Wang, Y., Wang, Z., Yuan, S., Niu, C., Liu, Y., Gao, Y., Li, Y., Su, D., Song, Y., 2022. Effect of polytetrafluoroethylene nanoplastics on combined inhibition of ciprofloxacin and bivalent copper on nitrogen removal, sludge activity and microbial community in sequencing batch reactor. Bioresour. Technol. 360, 127627 https://doi.org/10.1016/J.BIORTECH.2022.127627.

Yang, H., Xiong, H., Mi, K., Xue, W., Wei, W., Zhang, Y., 2020b. Toxicity comparison of nano-sized and micron-sized microplastics to Goldfish *Carassius auratus* Larvae. J. Hazard Mater. 388, 122058 https://doi.org/10.1016/J.JHAZMAT.2020.122058.

Yu, F., Li, Y., Huang, G., Yang, C., Chen, C., Zhou, T., Zhao, Y., Ma, J., 2020. Adsorption behavior of the antibiotic levofloxacin on microplastics in the presence of different heavy metals in an aqueous solution. Chemosphere 260, 127650. https://doi.org/10.1016/j.chemosphere.2020.127650.

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.

Yu, Z., Zhang, L., Huang, Q., Dong, S., Wang, X., Yan, C., 2022. Combined effects of micro-/nano-plastics and oxytetracycline on the intestinal histopathology and microbiome in zebrafish (*Danio rerio*). Sci. Total Environ. 843, 156917 https://doi.org/10.1016/J.SCITOTENV.2022.156917.

Zettler, E.R., Mincer, T.J., Amaral-Zettler, L.A., 2013. Life in the “plastisphere”: microbial communities on plastic marine debris. Environ. Sci. Technol. 47, 7137–7146. https://doi.org/10.1021/ES401288X/SUPPL_FILE/ES401288X_SI_001.PDF.

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 Env 648, 1431–1439. https://doi.org/10.1016/j.scitotenv.2018.08.266.

Zhang, H., Wang, J., Zhou, B., Zhou, Y., Dai, Z., Zhou, Q., Chriestie, P., Luo, Y., 2018. Enhanced adsorption of oxytetracycline to weathered microplastic polystyrene: kinetics, isotherms and influencing factors. Environ. Pollut. 243, 1550–1557. https://doi.org/10.1016/J.ENVPOL.2018.09.122.

Zhang, L., Yan, C., Wang, D., Zhen, Z., 2022. Spatiotemporal dynamic changes of antibiotic resistance genes in constructed wetlands and associated influencing factors. Environ. Pollut. 303, 119176 https://doi.org/10.1016/J.ENVPOL.2022.119176.

Zhang, X., Wen, K., Ding, D., Liu, J., Lei, Z., Chen, X., Ye, G., Zhang, J., Shen, H., Yan, C., Dong, S., Huang, Q., Lin, Y., 2021. Size-dependent adverse effects of microplastics on intestinal microbiota and metabolic homeostasis in the marine medaka (*Oryzias melastigma*). Environ. Int. 151, 106452 https://doi.org/10.1016/j.envint.2021.106452.

Zhao, S., Zhu, L., Wang, T., Li, D., 2014. Suspended microplastics in the surface water of the Yangtze Estuary System, China: first observations on occurrence, distribution. Mar. Pollut. Bull. 86, 562–568. https://doi.org/10.1016/j.marpolbul.2014.06.032.

Zhao, Y., Bao, Z., Wan, Z., Fu, Z., Jin, Y., 2020. Polystyrene microplastic exposure disturbs hepatic glycolipid metabolism at the physiological, biochemical, and transcriptomic levels in adult zebrafish. Sci. Total Environ. 710, 136279 https://doi.org/10.1016/J.SCITOTENV.2019.136279.

Zhi, S., Zhou, J., Yang, F., Tian, L., Zhang, K., 2018. Systematic analysis of occurrence and variation tendency about 58 typical veterinary antibiotics during animal wastewater disposal processes in Tianjin, China. Ecotoxicol. Environ. Saf. 165, 376–385. https://doi.org/10.1016/J.ECOENV.2018.08.101.