Received: 16 May 2023 | Revised: 27 October 2023 | Accepted: 16 December 2023

DOI: 10.1111/jwas.13046

ORIGINAL RESEARCH

Journal of the World Aquaculture Society Open Access | WORLD AQUACULTURE Society | WILEY

Assessing the impact of dietary polystyrene nanoplastics on growth performance, immunological parameters, and antioxidant defense in zebrafish (*Danio rerio*)

Ehsan Ahmadifar[1] | **Poulin Shohreh**[2] | **Naser Kalhor**[3] | **Mohsen Shahriari Moghadam**[4] | **Sevdan Yilmaz**[5] | **Sedigheh Mohammadzadeh**[6] | **Mostafa Khajeh**[7] | **Safoura Abarghouei**[8]

[1]Department of Fisheries, Faculty of Natural Resources, University of Zabol, Zabol, Iran

[2]Department of Clinical Science, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Iran

[3]Department of Mesenchymal Stem Cell, Academic Center for Education, Culture and Research Qom Branch, Qom, Iran

[4]Department of Environmental Sciences, Faculty of Natural Resources, University of Zabol, Zabol, Iran

[5]Department of Aquaculture, Faculty of Marine Sciences and Technology, Çanakkale Onsekiz Mart University, Çanakkale, Turkey

[6]Graduated from Fisheries Department, Faculty of Animal Sciences and Fisheries, Sari Agricultural Sciences and Natural Resources University, Sari, Iran

[7]Department of Chemistry, University of Zabol, Zabol, Iran

[8]Department of Fisheries, Faculty of Fisheries and Environmental Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

Abstract

This trial was performed in order to investigate the response of zebrafish (*Danio rerio*) to dietary exposure to polystyrene nanoplastics (PS-NPs) under laboratory conditions on fish growth and health. Healthy zebrafish (n=240) were divided into 12 tanks and fed with diets including 0 (T0), 100 (T1), 500 (T2), and 1000 (T3) mg kg^{-1} synthesized polystyrenes nanoplastics (PS-NPs) for 30 days. At the end of trial, fish fed the PS-NPs supplementation showed weight gain percentages of 79.45%, 70.35%, and 61.88% for T1, T2, and T3 groups, respectively, compared with 87.39% in the control fish. The SOD, GPX and MDA activities and cortisol levels increased by 47%, 32% and 35% and 46%, respectively, especially at high-dose administration ($p < 0.05$). The expression of GPX (T2-81% and T3-82%) and SOD (T2-101% and T3-187%) were remarkably upregulated in T2 and T3 groups. Moreover, the relative gene expression of HSP70, interleukin-1 (IL1), Interferon γ (IFN-γ) and tumor necrosis factor-α (TNF-α) increased by 178%, 202%, 154% and 307%, respectively, especially at

Correspondence
Ehsan Ahmadifar, Department of Fisheries, Faculty of Natural Resources, University of Zabol, Zabol, Iran.
Email: ehsan.ahmadifar@uoz.ac.ir

Sevdan Yilmaz, Department of Aquaculture, Faculty of Marine Sciences and Technology, Çanakkale Onsekiz Mart University, Çanakkale 17100, Turkey.
Email: sevdanyilmaz@comu.edu.tr

Funding information
Iran National Science Foundation (Sandoogh-e-Hemayat az Pajooheshgaran va Fannavarn-e-Keshvar), Grant/Award Number: 98026642

high-dose administration ($p < 0.05$). The results of the present study demonstrated that exposure to PS-NPs especially at high concentrations (500 and 1000 mg kg^{-1} of diet) negatively influenced growth, health status-, antioxidant-, and immunity-related gene expression responses of zebrafish.

KEYWORDS
aquatic pollution, nanoplastic, polystyrene, zebrafish

1 | INTRODUCTION

The aquatic ecosystems have become a sink for large amounts of human waste (Kershaw et al., 2011; Muruganandam et al., 2023). Plastics are the most extensive source of marine pollution, which constitute 60%–95% of marine debris (Schnurr et al., 2018). Because of the low degradation rate of plastics in the environment, their accumulation in the marine environment is increasing (Thompson & Napper, 2018). Plastic marine pollution includes both macroplastics (particles larger than 5 mm) and microplastics (MPs, particles smaller than 5 mm) (Thompson et al., 2004). Dumping or poor waste disposal management around the rivers caused plastics to enter the marine environments, and they harm the environment through entanglement and erosion or habitat destruction (Burgos-Aceves et al., 2022; Multisanti et al., 2022; Savuca et al., 2022; Vegter et al., 2014). When macroplastic is exposed to environmental conditions, it is fragmented through mechanical abrasion, photo-oxidation, and biological gradation and produces particles less than 5 mm, which are known as microplastics (MPs) and nanoplastics (NPs) (Alimi et al., 2018; Andrady, 2011; Oliveira et al., 2019). MPs are synthetic solid particles of regular or irregular shape with sizes ranging from 1 μm to 5 mm (Pettipas et al., 2016). There is no specific definition for NPs, but they are particles of the same origin as MPs, with a size of less than 100 nm (Koelmans et al., 2015).

One of the major plastic polymers with a high production is polystyrene microplastic (PS-MP), which is extensively used in fishing activities. PS-MPs are found in large quantities in estuarine and aquatic ecosystems (Browne et al., 2010). The similar density of PS-MPs to water causes its dispersion in the water column and maximum interaction between PS-MPs and organisms (Assas et al., 2020).

MPs have various environmental effects, such as they can have a direct toxic effect on biota in the environment, or the additives used in plastic production can act as carriers of other environmental pollutants and pathogens, and invasive microorganisms (Ferreira et al., 2019). Various MPs were found in freshwater and marine fishes (Bessa et al., 2018; Lusher et al., 2013). Marine and freshwater organisms can ingest and accumulate microplastics (Abbasi et al., 2018). A big challenge is the possibility of transferring these compounds to the edible part of fish and mollusks, which has caused concern about the quality and safety of these food compounds (Asmonaite et al., 2018; Martyniuk et al., 2023). Neurotoxicity is the main toxic effect of PS-MPs reported in various aquatic organisms (Xiong et al., 2022). Moreover, transgenerational toxicity of PS-MPs has been confirmed by recent studies (Siddiqui et al., 2023). Many studies are being carried out to assess the physiological, ecological, and environmental consequences of plastic particles (Banaee et al., 2023; Barría et al., 2020; Impellitteri et al., 2023); however, most of this research focuses on MPs and there are few studies on NPs. But the concern about NPs is increasing, due to their small size, they are absorbed by microorganisms that are at the base of the food chain and accumulate in secondary consumers, thus potentially affecting the food chain of ecosystems (Barría et al., 2020; Cedervall et al., 2012). Although there was a little information about the effects of NPs on aquatic organisms, these compounds cause

changes in hormone levels, enzyme activities, changes in the immune system, and disturbances in the reproductive process (De Sá et al., 2018).

Growth performance, antioxidant parameters, innate immune responses, and glucose and cortisol levels are important indicators of health and stress levels used in exploring the toxic effects of microplastics and nanoplastics in fish (Barría et al., 2020; Kim et al., 2021). The gene transcriptome analysis is important to understand pollutant toxicity and microplastics and nanoplastics-induced gene expressions (Patra et al., 2022). To our best knowledge, no study has investigated together both the growth performance on and the effects on antioxidant parameters and immunity-related responses of zebrafish. Therefore, in this study, we investigated the response of zebrafish (*Danio rerio*) to dietary exposure to polystyrene nanoplastic under laboratory conditions on their growth performance-, antioxidant-, and immunity-related gene expression responses and stress-related variables.

2 | MATERIALS AND METHODS

2.1 | PS-NPs preparation and diets

The synthesis of polystyrene nanoparticles (PS-NPs) was carried out by an emulsion polymerization method. The polymerization process was performed in a 500-mL round bottom three neck round flask under 250 rpm at 70°C for 24 h. Styrene (7.63 g), divinylbenzene (1.12 g), and sodium dodecyl sulfate (1.24 g) were added to 78 mL of distilled water, and the temperature was kept constant. The polymerization method was started by adding potassium persulfate (0.05 g) under nitrogen purging. The polymerization process under nitrogen purging was carried out for 24 h. After that, the mixture was cooled to ambient temperature and illuminated by sonication for 20 min. The rhodamine b (0.1 g) dye and above-prepared polystyrene nanoparticles (0.3 g) were added in a small amount of tetrahydrofuran (THF). Then, the mixture was heated at 70°C, 250 rpm. Finally, the dye mixture was removed and allowed to cool to an ambient temperature. The LEO-1455VP device with a scanning electron microscope (SEM) was recorded SEM images (Figure S1).

Four iso-nitrogenous (38.9% crude protein) and iso-lipidic (15.1% crude fat) diets (BioMar SAS, Nersac, France) were used for experimental diets preparation. This food completely free from NPs contamination, was soaked in tap water to soften. PS-NPs suspensions of 0, 100, 500, and 1000 mg kg^{-1} were mixed with the softened food and then they were pelleted (0.5 mm pellets) by using an extruder machine and finally air-dried under sunlight (Ahmadifar et al., 2019; Jabeen et al., 2018).

2.2 | Rearing condition

A number of 240 healthy zebrafish were obtained from an ornamental fish breeding center (Guilan. Iran) and moved to a wet lab located in the North of Iran (Gorgan, Iran). The fish were adapted to experimental conditions for 2 weeks and during this time, they were fed with a commercial diet. After 2 weeks, zebrafish were divided into 12 tanks (20 fish per tank) and fed with test diets including 0 (control), 100, 500, and 1000 mg kg^{-1} synthesized polystyrenes for 30 days. There were four treatments with three replicates: control (C, 0 mg kg^{-1} synthesized polystyrenes), T1 (100 mg kg^{-1}), T2 (500 mg kg^{-1}), and T3 (1000 mg kg^{-1}). During the experimental period, well-aerated water was used and fish were fed twice a day with experimental diets. The water temperature, pH value, and dissolved oxygen were recorded daily during the experiment and controlled at 25.2 ± 1.1°C, 7.2 ± 0.3, 6.7 ± 0.43 mg L^{-1}. Dead fish were removed and survival rates were reported at the end of the experiment. After 30 days, a clove oil bath (50 μL L^{-1}) was used to anesthetize the fish and they were weighed individually (Ahmadifar et al., 2019). Weight gain (WG), specific growth rate (SGR), Survival rate, and feed conversion ratio (FCR) indices were estimated using the common formula.

2.3 | Sampling

After 30 days, 15 fish per tank were randomly taken and anesthetized with a clove oil bath (50 μL L^{-1}) and excess water was blotted with absorbent paper. After removing head and fins, fish samples were frozen in liquid nitrogen and a hand homogenizer was used to homogenize this samples individually. Homogenates from five fish were applied for real-time PCR. Fish homogenates (10 fish per tank) were suspended in Tris–HCl buffer (25 mM, pH 7.2) for immunological- and stress-related analysis (Sheikhzadeh et al., 2017).

2.4 | Antioxidant, immunity, and stress factors assay

For measurement of lipid peroxidation products, fish homogenates (0.25 mL) was mixed with trichloroacetic acid (20%, 1.25 mL) and the mixture was centrifuged at 2000 g for 10 min. After centrifugation, the upper layer was discarded, and the collected sediment at the bottom of the vial was dissolved with 1.25-mL sulfuric acid (0.05 M) and 1-mL thiobarbituric acid (0.2%) and boiled at 30 min. Finely, n-butanol (2 mL) was added to the mixture and centrifuged at 2000 g for 10 min and finally absorbance was reported at 532 nm (Satoh et al., 1995).

To determine of catalase activity (CAT), fish samples was mixed with reaction buffer for 1 min at 37°C and then, ammonium molybdate (32.4 mM L^{-1}, 1 mL) was added to the mixture for terminating the enzymatic reaction and finely absorbance was reported at 405 nm (Goth, 1991).

For measurement of superoxide dismutase activity (SOD), reaction buffer was added to fish homogenates (0.05 mL) and then, 0.1 mL NADH (2.34 mM) was added to the mixture and finally, the absorbance was reported at 560 nm for a duration of 3.5 min (Nishiimi et al., 1972).

Glutathione peroxidase activity was measured by mixing fish homogenates with reaction buffer and the absorbance was recorded at 340 nm for 1 min (Pagalia & Valentin, 1967).

Then, 75 μL of *Micrococcus lysodeikticus* bacterial solution was prepared in 0.1 M citrate phosphate buffer with 5.8 acidity and spread in a 96-well pellet. Then 25 μL of the homogenate was added and mixed well. Optical absorption was read for 5 min with a time interval of 30 seconds at a wavelength of 450 nm (Ahmadifar et al., 2019). The determination of total immunoglobulin (Ig) level was carried out according to the method of Siwicki et al. (1994). Briefly, total protein was extracted from the homogenate sample using the microprotein method, then immunoglobulin molecules were precipitated with 12% polyethylene glycol solution and the protein level was measured again, the difference in protein content as Ig content was considered. The amount of total serum protein was measured by ELISA method and using the kit (CUSABIO-CSB-E12045Fh-Chin) at a wavelength of 450 nm and based on the method available in the kit.

2.5 | Gene expression

To extract total RNA according to the kit manufacturer's protocol (RNX Plus) (Cinagen, Iran, RN7713C), the tissue of five fish from each replication was washed twice with cold PBS. Then, 1 mL of a cold RNX Plus solution (Cinagen, Iran, RN7713C) was added to tissue samples and transferred to a microtube by adding 200 μL of chloroform, and the microtube was incubated at laboratory temperature for 5 min. Then, the microtube was centrifuged at 12,000 rpm at 4°C for 15 min. The (transparent) RNA-containing supernatant was kept at −20°C to increase RNA precipitation. After the incubation time, the microtube was removed from −20°C and centrifuged at 12,000 rpm and 4°C for 15 min (at this stage, a white deposit was observed at the bottom of the microtube). The isopropanol-containing supernatant was discarded; 1 mL of 75% ethanol was added to the microtube and centrifuged at 7500 rpm at 4°C for 8 min. Fifty microliter of DEPC was added to the RNA precipitate, and the microtube was kept

at 55°C for 5 min to completely dissolve the precipitate. In the end, the quality and quantity of extracted RNA were examined using 1% agarose gel and a NanoDrop spectrophotometer, respectively. The extracted RNA was kept at −80°C for further use.

The cDNA was synthesized using a Yekta Tajhiz kit according to the manufacturer's instructions. To this aim, 1 μL of random hexamer and 250 ng of extracted RNA were added to a 0.2 cc microtube. After centrifugation for 20 s, the sample was incubated at 75°C for 5 min. Mastermix (4 μL of 5× first-strand buffer, 1 μL of dNTPs each 10 mM, 0.5 μL of RNasin 40 U μL^{-1}, and 1 μL of M-MLV) was added to each microtube. After centrifugation for 20 s, the sample was incubated at 37 and 75°C for 60 and 5 min, respectively, and the cDNA was stored at −70°C. Gene expression was measured quantitatively using SYBR Green/ROX qPCR Master Mix (fluorescence dye) by a Real-Time PCR system. Some of the structural genes in the cell include GAPDH and 18S rRNA. Because of no change in the expression of the GAPDH gene in different tissues and cell lines in similar studies and the present study, it was used as a reference gene for the expression analysis. The expression of immune genes (HSP70, IL-1, IFN-Y, TNF-α, and lysozyme), and oxidative stress (CAT, SOD, and GPX; Table 1) was analyzed using primers extracted from a previous study (Ahmadifar et al., 2019). The results were analyzed using the replication curves. The product melting temperature can be estimated using the location of the melting curve. The average ΔCt of the test and control groups are compared for statistical analysis and drawing the graphs.

2.6 | Statistical analysis

All data were analyzed using SPSS version 22 software at the 95% confidence level. First, the normality and homogeneity of the variance were checked using Kolmogorov–Smirnov test and Levene's test respectively. One-way analysis of variance (ANOVA) was carried out, Tukey's test was chosen to specify the differences between treatments. All data were reported as mean ± SD.

TABLE 1 The list of primers and their nucleotide sequences used in this study for amplifying genes.

Gene	Primer	Length (pb)	Tm (°C)	NCBI reference
CAT	F: 5′ ACAAGTTAAATGCGATGACG 3′	20	54.74	XM_021470442.1
	R: 5′ CGTCTTTCCAATATGCTCAA 3′	20	53.71	
GPX	F: 5′ GACACGCCCACAAAAACTT 3′	19	56.97	NM_200222.1
	R: 5′ CTTTGAACAGTCCTGCTCGT 3′	20	58.31	
SOD	F: 5′ GTCGGTTTCTTTCACTCTCTC 3′	21	56.32	NM_131294.1
	R: 5′ ACTGGCTTCTTTTCACCCT 3′	19	56.44	
IL-1	F: 5′ CGGGCAATATGAAGTCACC 3′	19	56.8	NM_212844.2
	R: 5′ GTCCACATCTCCAGCCTGA 3′	19	58.71	
INF-γ	F: 5′ GCTTTGCCTGGGGAGTATGT 3′	20	55.00	NM_212864.1
	R: 5′ TCTTGTCTAGGTTCTCGGGC 3′	20	55.00	
HSP70	F: 5′ TGGAAAAGTGGAGATCATCG 3′	20	55.17	NM_001113589.1
	R: 5′ GTCTCCAATCAGCCTCTCT 3′	19	55.8	
TNF-α	F: 5′ TAGAACAACCCAGCAAACTC 3′	20	55.59	NM_212859.2
	R: 5′ TCTCCTTCTTCAACATCCAA 3′	20	53.87	
Lysozyme	F: 5′ AATATGAGGCTGGCAGTGG 3′	19	59.92	NM_139180.1
	R: 5′ AGTCCTTCCCCGTATCAGC 3′	19	60.67	

3 | RESULTS

3.1 | Growth results

The growth performance of zebrafish fed with dietary PS-NPs is presented in Table 2. No significant effect was seen in the final weight and survival rate between treatments ($p > 0.05$). WG and SGR of zebrafish were markedly decreased when the fish were fed with different NPs levels ($p < 0.05$). The FCR was lower in zebrafish fed with the control diet ($p < 0.05$).

3.2 | Antioxidant enzymes

No significant difference was seen in CAT activity (Figure 1a) among treatments ($p > 0.05$). The GPX (Figure 1b), MDA (Figure 1c), and SOD (Figure 1d) activities were significantly enhanced by increasing PS-NPs levels and the highest levels were measured in T3 ($p < 0.05$).

3.3 | Serum immunity

There were no significant differences in lysozyme activity (Figure 2a), total protein (Figure 2b), and total immunoglobulin (Figure 2c) between treatments ($p > 0.05$).

3.4 | Stress factors

Dietary PS-NPs led to a remarkable increase in cortisol levels (Figure 3a) in all treatments and T3 had the highest level ($p < 0.05$). No marked effects were observed for glucose (Figure 3b) levels between treatments ($p > 0.05$).

3.5 | Gene expression

No marked effects were reported for the expression of CAT (Figure 4a, $p > 0.05$). The expression of GPX (Figure 4b) and SOD (Figure 4c) were markedly upregulated and the highest RNA levels were observed in T3 ($p < 0.05$). The HSP70 (Figure 5a) and IL1 (Figure 5b) gene expression were notably increased in fish fed with PS-NPs and they were upper in T3 ($p < 0.05$). The relative gene expression of IFN-Y (Figure 5c) and TNF-α (Figure 5d) were upper in fish

TABLE 2 Growth performance of zebrafish fed with different nanoplastic levels for 30 days.

	Initial weight (mg)	Final weight (mg)	Weight gain (mg)	FCR	SGR	Survival rate %
T0	53.78 ± 3.39^{a}	100.78 ± 3.62^{a}	47.00 ± 1.17^{b}	1.92 ± 0.05^{a}	2.11 ± 0.11^{b}	100.00 ± 0.00^{a}
T1	53.00 ± 2.33^{a}	95.11 ± 3.66^{a}	42.11 ± 1.42^{ab}	2.14 ± 0.07^{a}	1.95 ± 0.04^{ab}	100.00 ± 0.00^{a}
T2	52.44 ± 2.30^{a}	89.33 ± 5.17^{a}	36.89 ± 2.89^{a}	2.47 ± 0.19^{ab}	1.77 ± 0.05^{ab}	96.67 ± 1.93^{a}
T3	53.33 ± 2.84^{a}	86.33 ± 4.73^{a}	33.00 ± 2.65^{a}	2.76 ± 0.23^{b}	1.61 ± 0.09^{a}	94.44 ± 2.94^{a}

Note: Different letters designate significant differences as determined by Tukey's post hoc tests (mean ± SE). T0: 0 mg kg^{-1} diet; T1: 100 mg kg^{-1} diet; T2: 500 mg kg^{-1} diet; T3: 1000 mg kg^{-1} diet.

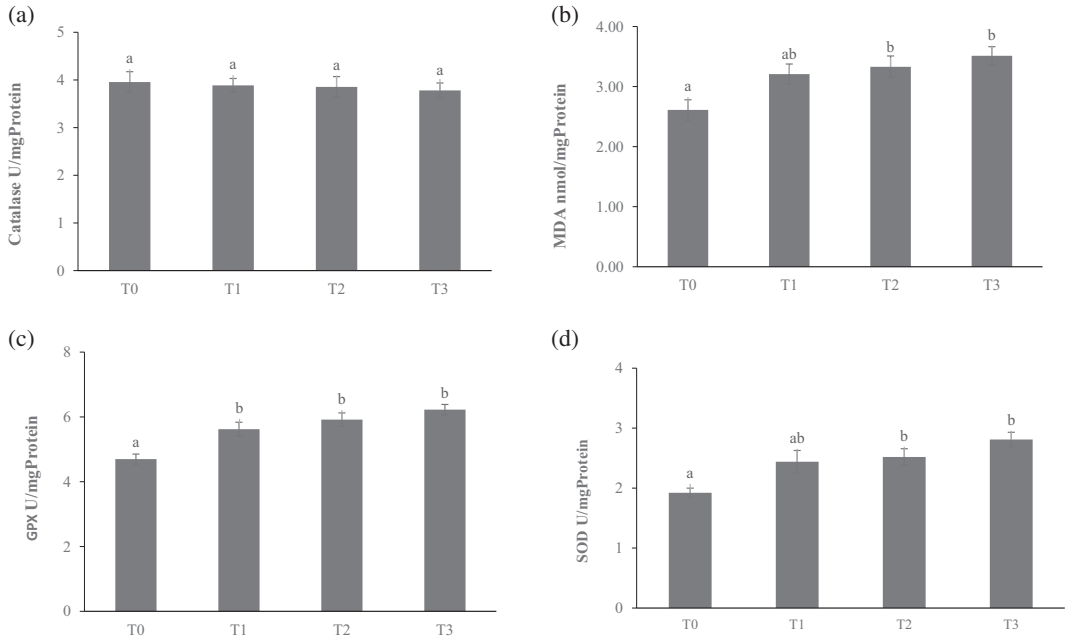

FIGURE 1 Catalase (a, CAT), glutathione peroxidase (b, GPX), Malondialdehyde (c, MDA), and superoxide dismutase (d, SOD) of zebrafish (*Danio rerio*) fed with 0 (T0), 100 (T1), 500 (T2), and 1000 (T3) mg kg^{-1} diet polystyrene nanoplastic for 30 days. Different letters designate significant differences as determined by Tukey's post hoc tests (mean ± SE).

with fed PS-NPs ($p > 0.05$). The relative gene expression of lysozyme (Figure 5e) was not affected by test diets ($p > 0.05$).

4 | DISCUSSION

Nanoplastics are an emerging and still unknown environmental pollutant causing global concern. According to their characteristics, these particles can exist in the form of float, sink, and settle in water (De Villiers, 2018). There are micro and nanoplastics in all parts of aquatic ecosystems, but their effects on freshwater aquatic animals have not been investigated adequately, and most studies evaluated the effect of micro and nanoplastics on ecologically, economically, and conservationally important species (Hollerova et al., 2023; Schirinzi et al., 2020; Setala et al., 2018).

The present experiment did not show a significant effect of feeding with PS-NPs on survival of zebrafish. Consistent with our results, any mortality was not reported in European sea bass (*Dicentrarchus labrax*), zebrafish, and Japanese medaka (*Oryzias latipes*) exposed to MPs (Assas et al., 2020; Lei et al., 2018; Peda et al., 2016). In our test, weight gain and SGR were significantly lower in treatments fed PS-NPs. Likewise, Wang et al. (2022) who reported that 3000 μg L^{-1} PS-NP caused considerable damage to intestinal villi and decreased growth of juvenile orange-spotted groupers (Wang et al., 2022). Moreover, SGR and weight gain in large yellow croaker (*Larimichthys crocea*) decreased significantly with high concentrations of PS-NPs (Lai et al., 2021). Reduced growth may result from NPs absorption by zebrafish during feeding, which leads to gastrointestinal obstruction intestinal villi damage and loss of appetite (Wang et al., 2022; Wright et al., 2013).

The findings of this study showed that feeding with PS-NPs negatively influenced the growth of zebrafish. Growth efficiency and survival rate are related to the physiological state of organisms (Lai et al., 2021). Reduced

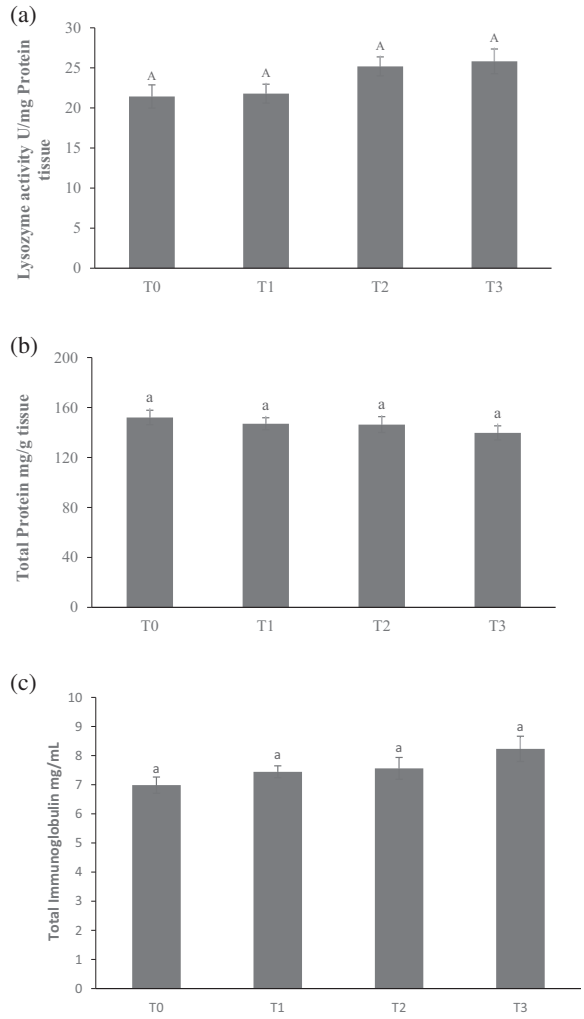

FIGURE 2 Lysozyme activity (a), total protein (b), and total immunoglobulin (c) of zebrafish (*Danio rerio*) fed with 0 (T0), 100 (T1), 500 (T2), and 1000 (T3) mg kg^{-1} diet polystyrene nanoplastic for 30 days. Different letters designate significant differences as determined by Tukey's post hoc tests (mean ± SE).

growth in organisms caused by NPs consumption is probably associated with increased oxidative stress and inflammation (Yu et al., 2018) and decreased nutritional activities and digestive capacity (Sendra et al., 2021).

Reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) are important free radical scavengers for antioxidant defense systems of aquatic organisms (Burgos-Aceves et al., 2018; Livingstone, 2001). In the present study, the CAT activity did not change significantly, but SOD and GPX activities and the expression of their related genes were significantly upper in the treatments fed with PS-NPs. SOD protects the cells against oxidative stress via the effectively neutralizes harmful superoxide ions (Perry et al., 2010). Thanks to its enzymatic activity, SOD converts superoxide ions into hydrogen peroxide, which is then broken down into harmless water and oxygen by other enzymes such as CAT and GPx (Flohe & Ursini, 2008). The relative gene expression of SOD and GPX increased with increasing NPs concentrations, which corresponds to the results obtained for the activities of SOD and GPX enzymes. Similar results were reported in zebrafish, *Eriocheir sinensis*, and *Macrobrachium nipponense* juveniles, indicating the activation of the oxidative defense system (Li et al., 2020; Lu et al., 2016;

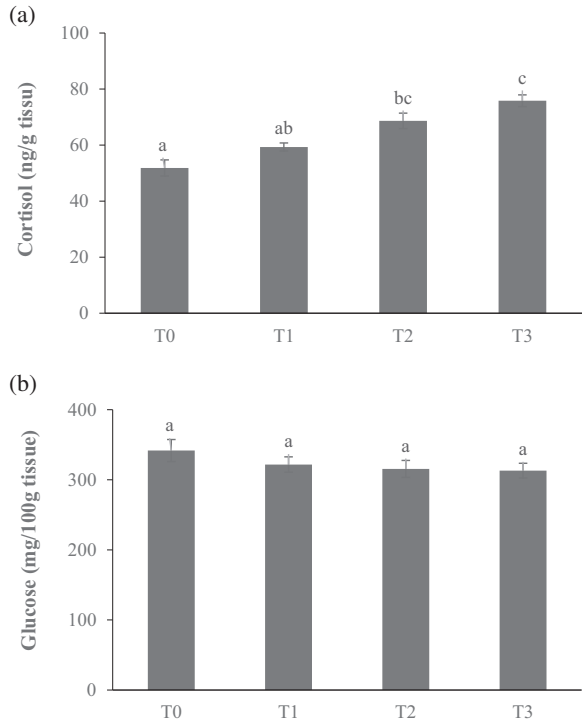

FIGURE 3 Cortisol level (a) and glucose (b) of zebrafish (*Danio rerio*) fed with 0 (T0), 100 (T1), 500 (T2), and 1000 (T3) mg kg^{-1} diet polystyrene nanoplastic for 30 days. Different letters designate significant differences as determined by Tukey's post hoc tests (mean ± SE).

Yu et al., 2018). Increased GPX activity was reported in studies on Nile tilapia (*Oreochromis niloticus*) exposed to a dose of 5 μg L^{-1} PS-MPs (Ahmadifar et al., 2021) and on green discus (*Symphysodon aequifasciatus*) exposed to 50 μg L^{-1} of PS-MPs (Wen et al., 2018).

Elevated GPX activity inhibits ROS formation by peroxide neutralization after exposure to PS-NPs (Kim et al., 2021). The observed changes in enzyme activity and gene expression levels demonstrate that exposure to NPs increases ROS levels in zebrafish, stimulates the expression of antioxidant-related genes, and helps excessive ROS removal. In the present study, MDA activity was significantly upper in the treatments fed with PS-NPs. Similar to the present study, an increase in MDA activity was observed in large yellow croaker exposed to a nanoplastic-containing diet (Lai et al., 2021). The increased MDA content indicates the occurrence of lipid peroxidation in the liver, which can increase the permeability of hepatocytes and exacerbate liver damage (Poli et al., 1987). Altogether, the present consequence proved that dietary exposure to PS-NPs could induce liver damage in zebrafish.

Blood parameters are used as physiological indicators in response to external or internal changes in fish. Several studies in aquatic animals revealed that exposure to nano and microplastics caused disorders in the immune system (Barathinivas et al., 2022; Li et al., 2020; Saha, Dhara, Pal, et al., 2023; Saha, Dhara, Chukwuka, et al., 2023).

Our study showed no significant effect on lysozyme activity, total protein, and the total immunoglobulin level between different treatments, similar to a report in rainbow trout fed with PS-MPs for 4 weeks (Asmonaite et al., 2018). This can be explained by limited innate immune response of fish fed with PS-MPs added feed (Asmonaite et al., 2018). To our knowledge, there is no immunological memory in the innate immune system of fish. The peaks of different innate immune parameters' values reported in previous studies may not be completely similar in magnitude and time (Asmonaite et al., 2018; Wang et al., 2022).

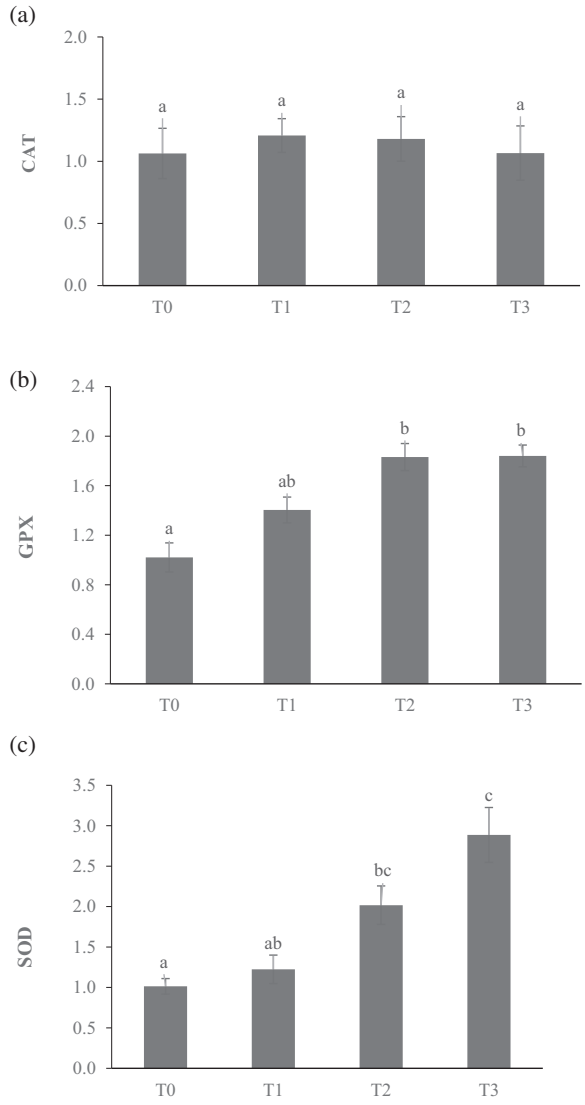

FIGURE 4 Relative expression of catalase (CAT, a), glutathion peroxidase (GPX, b), and superoxide dismutase (SOD, c) of zebrafish (*Danio rerio*) fed with 0 (T0), 100 (T1), 500 (T2), and 1000 (T3) mg kg^{-1} diet polystyrene nanoplastic for 30 days. Different letters designate significant differences as determined by Tukey's post hoc tests (mean ± SE).

In this study, the expression of immunity-related genes, including HSP70, IL1β, IFN-γ, and TNF-α, was higher in the PS-NPs-fed treatments than in the control group. Although oxidative stress through ROS production mainly caused the toxic impact of MPs and NPs on cells, these free radicals subsequently induce various biological responses including inflammation (Hu & Palic, 2020). The present results proved that the expression of pro-inflammatory cytokines, such as IL1β, IFN-γ, and TNF-α, increased by PS-NPs that have negative impact on the immune system. Cytokines are secreted by immune cells to regulate the immune response and inflammation in the body during stressful conditions (Ahmadifar et al., 2021; Hollerova et al., 2023). Similar to our results, the IL1β expression rose in zebrafish (Lu et al., 2018), European sea bass larvae (Mazurais et al., 2015), and rainbow trout (*Oncorhynchus mykiss*) (Hollerova et al., 2023) after exposure to PS-MPs. Our data demonstrated an increase in the TNF-α gene expression

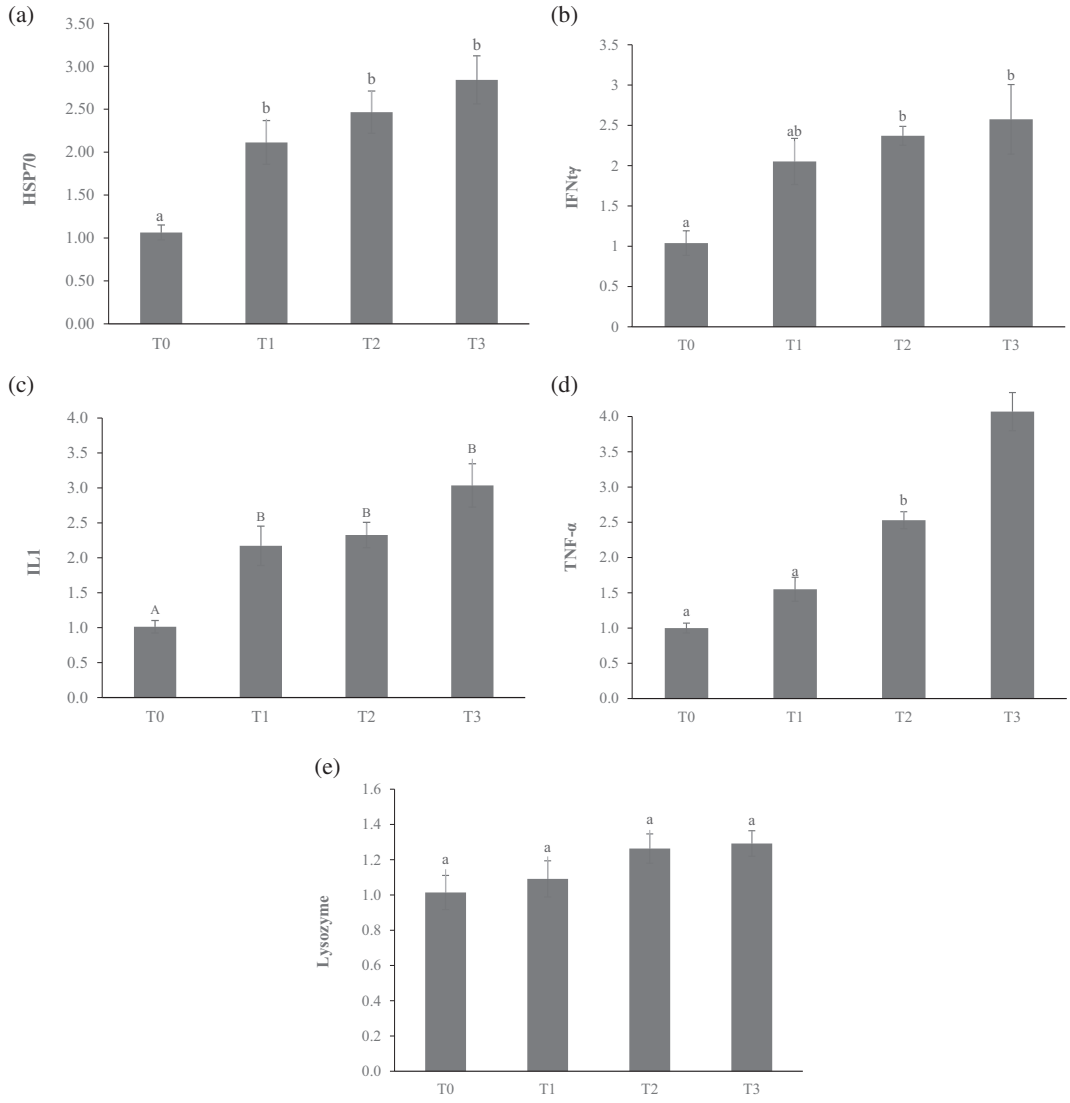

FIGURE 5 Relative expression of HSP70 (a), relative expression of interleukin-1 (b, IL1), interferon γ (c, IFN-γ), tumor necrosis factor-α (d, TNF-α), and lysozyme (e) of zebrafish (*Danio rerio*) fed with 0 (T0), 100 (T1), 500 (T2), and 1000 (T3) mg kg^{-1} diet polystyrene nanoplastic for 30 days. Different letters designate significant differences as determined by Tukey's post hoc tests (mean ± SE).

along with increasing PS-NP levels, which is in line with a report on rainbow trout (Hodkovicova et al., 2021). In this research, the IFN-γ gene expression was remarkably upper in the treatments fed with PS-NPs, which was similarly reported in zebrafish (Jin et al., 2018). The increased expression of inflammatory genes in response to MPs is associated with increased MDA and oxidative stress levels (Choi et al., 2018), which is expected to regulate apoptosis and immune response (Ahmadifar et al., 2021). In general, changes in the expression of inflammatory cytokine genes, including IL1β, IFN-γ, and TNF-α, that are involved in chronic diseases, can be related to liver and gill damage caused by PS-NPs.

An increase in cortisol levels in a stressful situation diverts energy expenditure on growth, reproduction, and activities, such as tissue repair. Growth reduction is usually observed during exposure to toxins and periods of

increased cortisol. In the current study, cortisol levels were significantly higher in the nanoplastic-fed treatments than in the control group, but the growth was lower in these groups than in the control group. Significantly increased cortisol levels were reported in larval and adult zebrafish exposed to PS-NPs (Sarasamma et al., 2020). Cortisol is considered the main corticosteroid secreted by the adrenal system of bony fish in response to acute and chronic stresses, and the high cortisol levels observed in this study might have resulted from the stress induced by PS-NPs.

5 | CONCLUSION

In conclusion, these results demonstrated that exposure to PS-NPs negatively influenced growth and health parameters of zebrafish and could cause liver damage. PS-NPs caused less effect at lower concentrations (100 mg kg^{-1} of diet), but the utmost damage and disturbance in growth and health of fish were observed at high concentrations (500 and 1000 mg kg^{-1} of diet). This consequence proved that different concentrations of PS-NPs increase oxidative stress-related factors and subsequently the expression of inflammatory genes, thereby weakening the immune system. Future studies should focus on the effects of different sizes and types of nanoplastics on the intestinal tract, which is the first involved organ after feeding with nanoplastics.

ACKNOWLEDGMENTS

This work was funded by Iran National Science Foundation (Sandoogh-e-Hemayat az Pajooheshgaran va Fannavarn-e-Keshvar) (Grant No. 98026642) and supported by University of Zabol, Sistan and Baluchestan, Iran.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, Ehsan Ahmadifar, upon reasonable request.

ORCID

Ehsan Ahmadifar https://orcid.org/0000-0001-5245-1231
Sevdan Yilmaz https://orcid.org/0000-0002-4809-5809

REFERENCES

Abbasi, S., Soltani, N., Keshavarzi, B., Moore, F., Turner, A., & Hassanaghaei, M. (2018). Microplastics in different tissues of fish and prawn from the Musa Estuary, Persian Gulf. *Chemosphere*, *205*, 80–87.

Ahmadifar, E., Kalhor, N., Dawood, M. A., Ahmadifar, M., Moghadam, M. S., Abarghouei, S., & Hedayati, A. (2021). Effects of polystyrene microparticles on inflammation, antioxidant enzyme activities, and related gene expression in Nile tilapia (*Oreochromis niloticus*). *Environmental Science and Pollution Research*, *28*(12), 14909–14916.

Ahmadifar, E., Sheikhzadeh, N., Roshanaei, K., Dargahi, N., & Faggio, C. (2019). Can dietary ginger (*Zingiber officinale*) alter biochemical and immunological parameters and gene expression related to growth, immunity and antioxidant system in zebrafish (*Danio rerio*)? *Aquaculture*, *507*, 341–348.

Alimi, O. S., Farner Budarz, J., Hernandez, L. M., & Tufenkji, N. (2018). Microplastics and nanoplastics in aquatic environments: Aggregation, deposition, and enhanced contaminant transport. *Environmental Science & Technology*, *52*(4), 1704–1724.

Andrady, A. L. (2011). Microplastics in the marine environment. *Marine Pollution Bulletin*, *62*(8), 1596–1605.

Asmonaite, G., Sundh, H., Asker, N., & Carney Almroth, B. (2018). Rainbow trout maintain intestinal transport and barrier functions following exposure to polystyrene microplastics. *Environmental Science & Technology*, *52*(24), 14392–14401.

Assas, M., Qiu, X., Chen, K., Ogawa, H., Xu, H., Shimasaki, Y., & Oshima, Y. (2020). Bioaccumulation and reproductive effects of fluorescent microplastics in medaka fish. *Marine Pollution Bulletin*, *158*, 111446.

Banaee, M., Faraji, J., Amini, M., Multisanti, C. R., & Faggio, C. (2023). Rainbow trout (*Oncorhynchus mykiss*) physiological response to microplastics and enrofloxacin: Novel pathways to investigate microplastic synergistic effects on pharmaceuticals. *Aquatic Toxicology*, *261*, 106627.

Barathinivas, A., Ramya, S., Neethirajan, K., Jayakumararaj, R., Pothiraj, C., Balaji, P., & Faggio, C. (2022). Ecotoxicological effects of pesticides on hematological parameters and oxidative enzymes in freshwater catfish, *Mystus keletius*. *Sustainability*, *14*(15), 9529.

Barría, C., Brandts, I., Tort, L., Oliveira, M., & Teles, M. (2020). Effect of nanoplastics on fish health and performance: A review. *Marine Pollution Bulletin*, *151*, 110791.

Bessa, F., Barría, P., Neto, J. M., Frias, J. P., Otero, V., Sobral, P., & Marques, J. C. (2018). Occurrence of microplastics in commercial fish from a natural estuarine environment. *Marine Pollution Bulletin*, *128*, 575–584.

Browne, M. A., Galloway, T. S., & Thompson, R. C. (2010). Spatial patterns of plastic debris along estuarine shorelines. *Environmental Science & Technology*, *44*(9), 3404–3409.

Burgos-Aceves, M. A., Cohen, A., Smith, Y., & Faggio, C. (2018). MicroRNAs and their role on fish oxidative stress during xenobiotic environmental exposures. *Ecotoxicology and Environmental Safety*, *148*, 995–1000.

Burgos-Aceves, M. A., Faggio, C., Betancourt-Lozano, M., González-Mille, D. J., & Ilizaliturri-Hernández, C. A. (2022). Ecotoxicological perspectives of microplastic pollution in amphibians. *Journal of Toxicology and Environmental Health, Part B*, *25*(8), 405–421.

Cedervall, T., Hansson, L. A., Lard, M., Frohm, B., & Linse, S. (2012). Food chain transport of nanoparticles affects behaviour and fat metabolism in fish. *PLoS One*, *7*(2), e32254.

Choi, J. S., Jung, Y. J., Hong, N. H., Hong, S. H., & Park, J. W. (2018). Toxicological effects of irregularly shaped and spherical microplastics in a marine teleost, the sheepshead minnow (*Cyprinodon variegatus*). *Marine Pollution Bulletin*, *129*(1), 231–240.

De Sá, L. C., Oliveira, M., Ribeiro, F., Rocha, T. L., & Futter, M. N. (2018). Studies of the effects of microplastics on aquatic organisms: What do we know and where should we focus our efforts in the future? *Science of the Total Environment*, *645*, 1029–1039.

De Villiers, S. (2018). Quantification of microfibre levels in South Africa's beach sediments, and evaluation of spatial and temporal variability from 2016 to 2017. *Marine Pollution Bulletin*, *135*, 481–489.

Ferreira, G. V., Barletta, M., & Lima, A. R. (2019). Use of estuarine resources by top predator fishes. How do ecological patterns affect rates of contamination by microplastics? *Science of the Total Environment*, *655*, 292–304.

Flohe, L., & Ursini, F. (2008). Peroxidase: A term of many meanings. *Antioxidants & Redox Signaling*, *10*(9), 1485–1490.

Goth, L. (1991). Simple method for determination of serum catalase activity and revision of reference range. *Clinica chimica acta*, *196*, 143–151.

Hodkovicova, N., Hollerova, A., Caloudova, H., Blahova, J., Franc, A., Garajova, M., Lenz, J., Tichy, F., Faldyna, M., Kulich, P., & Mares, J. (2021). Do foodborne polyethylene microparticles affect the health of rainbow trout (*Oncorhynchus mykiss*)? *Science of the Total Environment*, *793*, 148490.

Hollerova, A., Hodkovicova, N., Blahova, J., Faldyna, M., Franc, A., Pavlokova, S., Tichy, F., Postulkova, E., Mares, J., Medkova, D., & Kyllar, M. (2023). Polystyrene microparticles can affect the health status of freshwater fish–Threat of oral microplastics intake. *Science of the Total Environment*, *858*, 159976.

Hu, M., & Palić, D. (2020). Micro-and nano-plastics activation of oxidative and inflammatory adverse outcome pathways. *Redox biology*, *37*, 101620.

Impellitteri, F., Yunko, K., Martyniuk, V., Matskiv, T., Lechachenko, S., Khoma, V., Mudra, A., Piccione, G., Stoliar, O., & Faggio, C. (2023). Physiological and biochemical responses to caffeine and microplastics in *Mytilus galloprovincialis*. *Science of the Total Environment*, *890*, 164075.

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.

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. *Environmental Pollution*, *235*, 322–329.

Kershaw, P., Katsuhiko, S., Lee, S., & Woodring, D. (2011). *Plastic debris in the ocean*. United Nations Environment Programme.

Kim, J. H., Yu, Y. B., & Choi, J. H. (2021). Toxic effects on bioaccumulation, hematological parameters, oxidative stress, immune responses and neurotoxicity in fish exposed to microplastics: A review. *Journal of Hazardous Materials*, *413*, 125423.

Koelmans, A. A., Besseling, E., & Shim, W. J. (2015). Nanoplastics in the aquatic environment. Critical review. In *Marine anthropogenic litter* (pp. 325–340). Springer OPEN.

Lai, W., Xu, D., Li, J., Wang, Z., Ding, Y., Wang, X., Li, X., Xu, N., Mai, K., & Ai, Q. (2021). Dietary polystyrene nanoplastics exposure alters liver lipid metabolism and muscle nutritional quality in carnivorous marine fish large yellow croaker (*Larimichthys crocea*). *Journal of Hazardous Materials*, *419*, 126454.

Lei, L., Wu, S., Lu, S., Liu, M., Song, Y., Fu, Z., Shi, H., Raley-Susman, K. M., & He, D. (2018). Microplastic particles cause intestinal damage and other adverse effects in zebrafish *Danio rerio* and nematode *Caenorhabditis elegans*. *Science of the Total Environment*, *619*, 1–8.

Li, Y., Liu, Z., Li, M., Jiang, Q., Wu, D., Huang, Y., Jiao, Y., Zhang, M., & Zhao, Y. (2020). Effects of nanoplastics on antioxidant and immune enzyme activities and related gene expression in juvenile *Macrobrachium nipponense*. *Journal of Hazardous Materials*, *398*, 122990.

Livingstone, D. R. (2001). Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. *Marine Pollution Bulletin*, *42*(8), 656–666.

Lu, C., Kania, P. W., & Buchmann, K. (2018). Particle effects on fish gills: An immunogenetic approach for rainbow trout and zebrafish. *Aquaculture*, *484*, 98–104.

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. *Environmental Science & Technology*, *50*(7), 4054–4060.

Lusher, A. L., Mchugh, M., & Thompson, R. C. (2013). Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. *Marine Pollution Bulletin*, *67*(1-2), 94–99.

Martyniuk, V., Khoma, V., Matskiv, T., Yunko, K., Gnatyshyna, L., Stoliar, O., & Faggio, C. (2023). Combined effect of microplastic, salinomycin and heating on *Unio tumidus*. *Environmental Toxicology and Pharmacology*, *98*, 104068.

Mazurais, D., Ernande, B., Quazuguel, P., Severe, A., Huelvan, C., Madec, L., Mouchel, O., Soudant, P., Robbens, J., Huvet, A., & Zambonino-Infante, J. (2015). Evaluation of the impact of polyethylene microbeads ingestion in European sea bass (*Dicentrarchus labrax*) larvae. *Marine Environmental Research*, *112*, 78–85.

Multisanti, C. R., Merola, C., Perugini, M., Aliko, V., & Faggio, C. (2022). Sentinel species selection for monitoring microplastic pollution: A review on one health approach. *Ecological Indicators*, *145*, 109587.

Muruganandam, M., Rajamanickam, S., Sivarethinamohan, S., Reddy, M. K., Velusamy, P., Gomathi, R., Ravindiran, G., Gurugubelli, T. R., & Munisamy, S. K. (2023). Impact of climate change and anthropogenic activities on aquatic ecosystem–A review. *Environmental Research*, *238*, 117233.

Nishikimi, M., Appaji, N., & Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Bichemical and Biophysical Research Communications*, *46*, 849–854.

Oliveira, M., Almeida, M., & Miguel, I. (2019). A micro(nano)plastic boomerang tale: A never ending story? *TrAC Trends in Analytical Chemistry*, *112*, 196–200.

Paglia, D. E., & Valentine, W. N. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *The Journal of laboratory and clinical medicine*, *70*, 158–169.

Patra, I., Huy, D. T. N., Alsaikhan, F., Opulencia, M. J. C., Van Tuan, P., Nurmatova, K. C., Majdi, A., Shoukat, S., Yasin, G., Margiana, R., Walker, T. R., & Karbalaei, S. (2022). Toxic effects on enzymatic activity, gene expression and histopathological biomarkers in organisms exposed to microplastics and nanoplastics: A review. *Environmental Sciences Europe*, *34*(1), 80.

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. *Environmental Pollution*, *212*, 251–256.

Perry, J. J. P., Shin, D. S., Getzoff, E. D., & Tainer, J. A. (2010). The structural biochemistry of the superoxide dismutases. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics*, *1804*(2), 245–262.

Pettipas, S., Bernier, M., & Walker, T. R. (2016). A Canadian policy framework to mitigate plastic marine pollution. *Marine Policy*, *68*, 117–122.

Poli, G., Albano, E., & Dianzani, M. U. (1987). The role of lipid peroxidation in liver damage. *Chemistry and Physics of Lipids*, *45*(2-4), 117–142.

Saha, S., Dhara, K., Chukwuka, A. V., Pal, P., Saha, N. C., & Faggio, C. (2023). Sub-lethal acute effects of environmental concentrations of inorganic mercury on hematological and biochemical parameters in walking catfish, *Clarias batrachus*. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *264*, 109511.

Saha, S., Dhara, K., Pal, P., Saha, N. C., Faggio, C., & Chukwuka, A. V. (2023). Longer-term adverse effects of selenate exposures on hematological and serum biochemical variables in air-breathing fish *Channa punctata* (Bloch, 1973) and non-air breathing fish *Ctenopharyngodon idella* (Cuvier, 1844): An integrated biomarker response approach. *Biological Trace Element Research*, *201*(7), 3497–3512.

Sarasamma, S., Audira, G., Siregar, P., Malhotra, N., Lai, Y. H., Liang, S. T., Chen, J. R., Chen, K. H. C., & Hsiao, C. D. (2020). Nanoplastics cause neurobehavioral impairments, reproductive and oxidative damages, and biomarker responses in zebrafish: Throwing up alarms of wide spread health risk of exposure. *International Journal of Molecular Sciences*, *21*(4), 1410.

Satoh, N., Nishimura, M., & Watanabe, Y. (1995). Electrophysiologic alterations in the rabbit nodal cells induced by membrane lipid peroxidation. *European Journal of Pharmacology: Environmental Toxicology and Pharmacology*, *292*(3-4), 233–240.

Savuca, A., Nicoara, M. N., & Faggio, C. (2022). Comprehensive review regarding the profile of the microplastic pollution in the coastal area of the Black Sea. *Sustainability*, *14*(21), 14376.

Schirinzi, G. F., Pedà, C., Battaglia, P., Laface, F., Galli, M., Baini, M., Consoli, P., Scotti, G., Esposito, V., Faggio, C., Farré, M., Barceló, D., Fossi, M. C., Andaloro, F., & Romeo, T. (2020). A new digestion approach for the extraction of microplastics from gastrointestinal tracts (GITs) of the common dolphinfish (*Coryphaena hippurus*) from the western Mediterranean Sea. *Journal of Hazardous Materials*, *397*, 122794.

Schnurr, R. E., Alboiu, V., Chaudhary, M., Corbett, R. A., Quanz, M. E., Sankar, K., Srain, H. S., Thavarajah, V., Xanthos, D., & Walker, T. R. (2018). Reducing marine pollution from single-use plastics (SUPs): A review. *Marine Pollution Bulletin*, *137*, 157–171.

Sendra, M., Sparaventi, E., Novoa, B., & Figureueras, A. (2021). An overview of the internalization and effects of microplastics and nanoplastics as pollutants of emerging concern in bivalves. *Science of the Total Environment*, *753*, 142024.

Setala, O., Lehtiniemi, M., Coppock, R., & Cole, M. (2018). Microplastics in marine food webs. In *Microplastic contamination in aquatic environments* (pp. 339–363). Elsevier.

Sheikhzadeh, N., Kouchaki, M., Mehregan, M., Tayefi-Nasrabadi, H., Divband, B., Khataminan, M., Khani Oushani, A., & Shabanzadeh, S. (2017). Influence of nanochitosan/zeolit composite on growth performance, digestive enzymes and serum biochemical parameters in rainbow trout (Oncorhynchus mykiss). *Aquaculture research*, *48*, 5955–5964.

Siddiqui, S. A., Singh, S., Bahmid, N. A., Shyu, D. J., Domínguez, R., Lorenzo, J. M., Pereira, J. A. M., & Câmara, J. S. (2023). Polystyrene microplastic particles in the food chain: Characteristics and toxicity. A review. *Science of the Total Environment*, *892*, 164531.

Siwicki, A. K., Anderson, D. P., & Rumsey, G. L. (1994). Dietary intake of immunostimulants by rainbow trout affects nonspecific immunity and protection against furunculosis. *Veterinary Immunology and Immunopathology*, *41*(1-2), 125–139.

Thompson, R. C., & Napper, I. E. (2018). Microplastics in the environment. In *Plastics and the environment* (Vol. 47, p. 60). Royal Society of Chemistry.

Thompson, R. C., Olsen, Y., Mitchell, R. P., Davis, A., Rowland, S. J., John, A. W., McGonigle, D., & Russell, A. E. (2004). Lost at sea: Where is all the plastic? *Science*, *304*(5672), 838.

Vegter, A. C., Barletta, M., Beck, C., Borrero, J., Burton, H., Campbell, M. L., Costa, M. F., Eriksen, M., Eriksson, C., Estrades, A., & Gilardi, K. V. (2014). Global research priorities to mitigate plastic pollution impacts on marine wildlife. *Endangered Species Research*, *25*(3), 225–247.

Wang, Q., Huang, F., Liang, K., Niu, W., Duan, X., Jia, X., Wu, X., Xu, P., & Zhou, L. (2022). Polystyrene nanoplastics affect digestive function and growth in juvenile groupers. *Science of the Total Environment*, *808*, 152098.

Wen, B., Jin, S. R., Chen, Z. Z., Gao, J. Z., Liu, Y. N., Liu, J. H., & Feng, X. S. (2018). Single and combined effects of microplastics and cadmium on the cadmium accumulation, antioxidant defence and innate immunity of the discus fish (*Symphysodon aequifasciatus*). *Environmental Pollution*, *243*, 462–471.

Wright, S. L., Rowe, D., Thompson, R. C., & Galloway, T. S. (2013). Microplastic ingestion decreases energy reserves in marine worms. *Current Biology*, *23*(23), R1031–R1033.

Xiong, F., Liu, J., Xu, K., Huang, J., Wang, D., Li, F., Wang, S., Zhang, J., Pu, Y., & Sun, R. (2022). Microplastics induce neurotoxicity in aquatic animals at environmentally realistic concentrations: A meta-analysis. *Environmental Pollution*, *318*, 120939.

Yu, Y., Zhou, D., Li, Z., & Zhu, C. (2018). Advancement and challenges of microplastic pollution in the aquatic environment: A review. *Water, Air, & Soil Pollution*, *229*(5), 1–18.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Ahmadifar, E., Shohreh, P., Kalhor, N., Shahriari Moghadam, M., Yilmaz, S., Mohammadzadeh, S., Khajeh, M., & Abarghouei, S. (2024). Assessing the impact of dietary polystyrene nanoplastics on growth performance, immunological parameters, and antioxidant defense in zebrafish (*Danio rerio*). *Journal of the World Aquaculture Society*, 1–15. https://doi.org/10.1111/jwas.13046