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POLYSTYRENE NANOPLASTICS DISRUPT HEPATIC VITELLOGENIN METABOLISM AND IMPAIR THE REPRODUCTION PROCESS IN FEMALE ZEBRAFISH

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Abstract

For this study, 240 adult healthy female zebrafish were divided into 12 tanks (20 fish per tank) and exposed to 0 (control, T0), 0.1 (T1), 1 (T2), and 10 (T3) mg/L synthesized polystyrene nanoplastics (PS-NPs, nanoplastics synthesized in the chemistry laboratory of the Faculty of Basic Sciences at Zabol University) for 4 weeks. At the end of the experiment, exposure to PS-NPs had no significant effect on the survival of female zebrafish (P>0.05). A significant decrease of hepatic vitellogenin (Vtg) was seen in female zebrafish exposed to PS-NPs (P<0.05). The level of 17-β-estradiol (E2) and testosterone (T) were notably declined in female zebrafish of all PS-NPs-exposed groups (P<0.05). Glucose was notably increased in female zebrafish of all PS-NPs-exposed treatments (P<0.05). There was a significant increase in cortisol levels in all PS-NPs-exposed treatments (P<0.05). No significant difference was seen in lactate levels between different treatments (P>0.05). A significant enhancement in aspartate aminotransferase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) was observed in female zebrafish of all PS-NPs-exposed treatments compared to the control (P<0.05). The present results demonstrate that PS-NPs impair the reproduction process so that none of the female zebrafish broodstock exposed to different doses of PS-NPs reached the spawning stage.

Key words: nanoplastics, sex steroids, liver damage, fish, stress parameters

In recent decades, the production of plastics in the world has increased significantly and 359 million metric tons was produced in 2018 (Plastics Europe, 2019; Yang and Wang, 2023). Plastics were used in different aspects of modern life, including packaging (Wang et al., 2019 a). The increase in population and the expansion of the fishing industry have led to an enhanced amount of plastic waste released into the environment (Geyer et al., 2017). It is predicted that in the next few decades, the release of plastic in the marine ecosystem will increase so that the mass of plastic in the oceans exceeds the mass of fish (Simon and Schulte, 2017). Various studies have shown that the plastics released into the marine ecosystem destroy habitats, cause the migration of some species to new habitats, the loss of some different species of marine animals, and damage the tourism industry, fishing industry, and marine food industry (Gregory, 2009; Simon and Schulte, 2017). Large plastics that are released into the marine ecosystem are affected by various factors such as UV rays, oxidation, hydrolysis, and wave action, which lead to the formation of microplastic particles (MPs) and nanoplastics (NPs) (Hidalgo-Ruz et al., 2013; Alimi et al., 2018; Barría et al., 2020). Microplastics are specified as plastic particles with size ranging from 1 μm to 5 mm and are ubiquitous in marine and freshwater ecosystems (Jabeen et al., 2018; Frias and Nash, 2019). There is no specific definition for NPs but they are compounds similar to MPs whose size is below 100 nm (Koelmans et al., 2015; Barría et al., 2020).

Polyethylene and polystyrene are the main types of microplastic particles and they are among the most abundant polymers, forming more than half of the plastic production in the world (Hollerova et al., 2023) and are one of the plastics with high production and the major components of plastic remaining in the environment (Sadri and Thompson, 2014; Jabeen et al., 2018).

Different studies showed that aquatic organisms including fish can ingest and egest these plastic items (Peda et al., 2016). The results of various studies in fish showed that ingested microplastics cause intestinal blockage, and physical damage and reduce feeding due to saturation with these substances (Anbumani et al., 2018; Lei et al., 2018; Assas et al., 2020). They can also cause inflammatory responses or even destroy the fish's immune system

(Greven et al., 2016; Pirsaheb et al., 2020; Solomando et al., 2020; Cao et al., 2023). In addition, many plastics can move to other organs such as gills and the liver, and thus cause damage to these organs (Lu et al., 2016; Yin et al., 2021).

It has been reported that the exposure to MPs has the potential to reduce the transcription of gonadotropin-releasing hormone (GnRH) in the hypothalamus of African catfish (*Clarias gariepinus*), as well as vitellogenin (Vtg) and choriogenin (Chg) in the liver of Japanese medaka (*Oryzias latipes*) (Rochman et al., 2014; Karami et al., 2016). Within the hypothalamus-pituitary-gonadal (HPG) axis, GnRH serves as a physiological regulator for the secretion of gonadotrophins (GtHs), which in turn control steroidogenesis and gametogenesis in vertebrate species (Gutierrez-Hartmann et al., 2007). The observed decrease in the expression of GnRH, Vtg, and Chg genes implies that MPs may induce disturbances in reproductive processes (Sun et al., 2015).

Although most of the studies are related to investigating the effects of MPs on aquatic animals and a few researches have evaluated the effects of NPs, nevertheless, the concern about the effects of NPs on ecosystems is increasing in the scientific community. In this study, we investigated the response of female zebrafish (*Danio rerio*) at the vitellogenin stage to exposure to polystyrene nanoplastic under laboratory conditions. Currently, zebrafish are used as a model species for acute and chronic toxicity testing and water quality assessment due to their small size, low food cost, short reproductive cycle and genome sequenced (Bhagat et al., 2020).

Material and methods

Synthesis of polystyrene nanoparticles

Polystyrene nanoparticles were synthesized in the chemistry laboratory of the Faculty of Basic Sciences at Zabol University (Zabol, Iran). They were prepared by emulsion and suspension polymerization. The polymerization process was performed in a 500 ml round bottom three neck round flask under 250 rpm at 70ºC 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 70ºC, 250 rpm. Finally, the dye mixture was removed and allowed to cool to an ambient temperature (Figure 1).

Fish and experimental design

The Ethics and Animal Care Committee of the University of Tehran (357; 8 November 2000) was used to conduct the experiment. This study was done in a wet lab located in an ornamental fish culture center (Sari, Iran). A total of 240 adult Tuebingen strain female zebrafish were selected and stored in two aquariums (100 L). The fish were adapted to experimental conditions for 2 weeks and during this time, they were fed twice a day with a commercial diet (BioMar SAS, Nersac, France). After two weeks, zebrafish were divided into 12 aquariums (20 fish per aquarium, 40-L) and state exposed to 0 (control), 0.1, 1, and 10 mg/L synthesized polystyrenes for 4 weeks. The adult zebrafish were exposed to four concentrations of PS-NPs for 4 weeks (Abarghouei et al., 2021). Doses were selected based on a previous study (Lei et al., 2018). There were four treatments with three replicates. The test solutions were completely replaced with fresh NPs suspensions every 2 days. During the experimental period, well-aerated water was used and fish hand fed to satiation three times daily with a commercial diet (BioMar SAS, Nersac, France). The water temperature, pH value, and dissolved oxygen were recorded daily during the experiment and controlled at 23.2±1.1°C, 7.2±0.3, 6.7±0.43 mg/L respectively and 12 h light/dark photoperiod. Dead fish were removed and survival rates were reported at the end of the experiment.

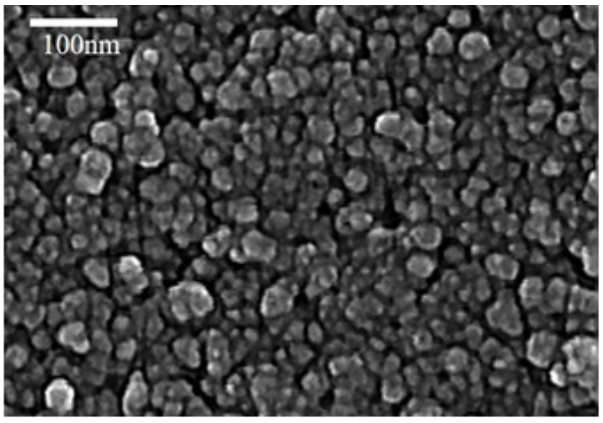


Figure 1. Characterization of polystyrene particles. SEM image of polystyrene particles (scale bar=100nm)

Sample collection

After 4 weeks exposure period, 10 fish per tank were randomly chosen and anesthetized in ice bath and excess water was blotted with absorbent paper. Heads and fins of fish were removed and then the whole body samples were prepared. Samples were moved to sterile tubes and stored at –80°C for future analysis.

Table 1. Stress factors in female zebrafish following exposure to PS-NPs for 28 days (mean ± SE)

Factors/treatments	Control	0.1 mg/L	1 mg/L	10 mg/L
Glucose (mg/100 g tissue)	322±0.5.36 c	558±11.46 ab	541±1.85 b	575±4.42 a
Cortisol (ng/mg protein)	0.85±0.01 b	0.89±0.01 ab	0.92±0.01 ab	0.93±0.02 a
Lactate (mg/100 g tissue)	115.3±2.5	114.6±2.5	116±1	113±1

Bars with a different letter (a, b, and c) denote significant differences between the exposure groups (P<0.05).

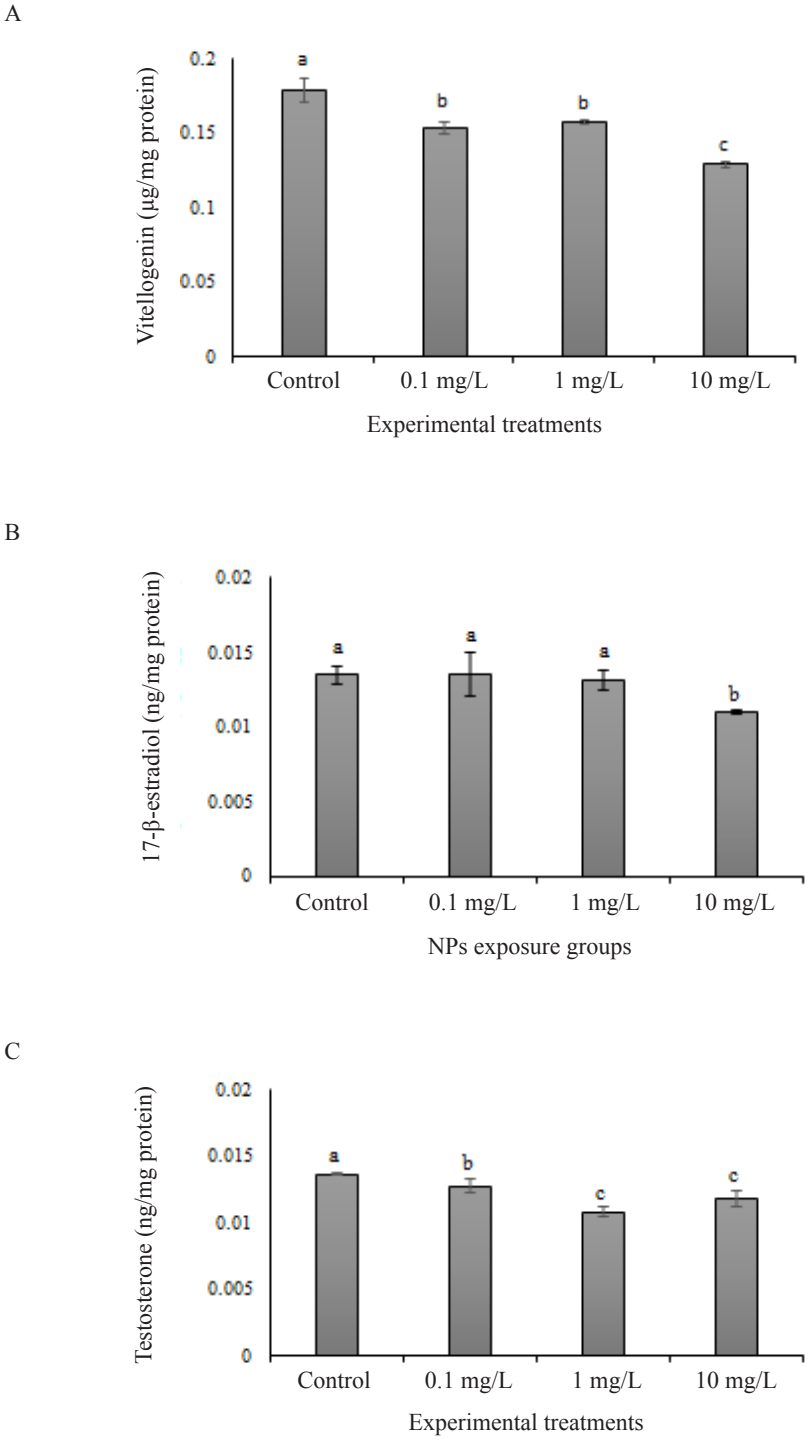


Figure 2. Levels of hepatic vitellogenin (A), 17-β-estradiol (B), testosterone (C) levels in the whole body of female zebrafish following exposure to PS-NPs for 28 days. Data are shown as the mean ± standard errors (SE) of ten female zebrafish in each group. Bars with a different letter (a, b, and c) denote significant differences between the exposure groups (P<0.05)

Sex steroids, hepatic vitellogenin, and stress factors

Commercial kits were purchased to measure sex steroid concentrations (ng/mL) of testosterone (T), 17-β-estradiol (E2) and cortisol (AccuBind ELISA Microwells, Monobind, Inc. Lake Forest, CA, USA). Hepatic vitellogenin (Vtg, ZellBio, GmbH, Germany), glucose (Pars Azmun Co., Karaj, Iran), aspartate aminotransferase (AST, Pars Azmun Co., Karaj, Iran), alanine transaminase (ALT, Pars Azmun Co., Karaj, Iran), alkaline phosphatase (ALP, Pars Azmun Co., Karaj, Iran), and lactate (Biorex, United Kingdom) assays were done with commercial kits according to the protocol.

Liver enzymes

Commercial kits were purchased to measure aspartate aminotransferase (AST, Pars Azmun Co., Karaj, Iran), alanine transaminase (ALT, Pars Azmun Co., Karaj, Iran), alkaline phosphatase (ALP, Pars Azmun Co., Karaj, Iran).

Statistical analysis

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

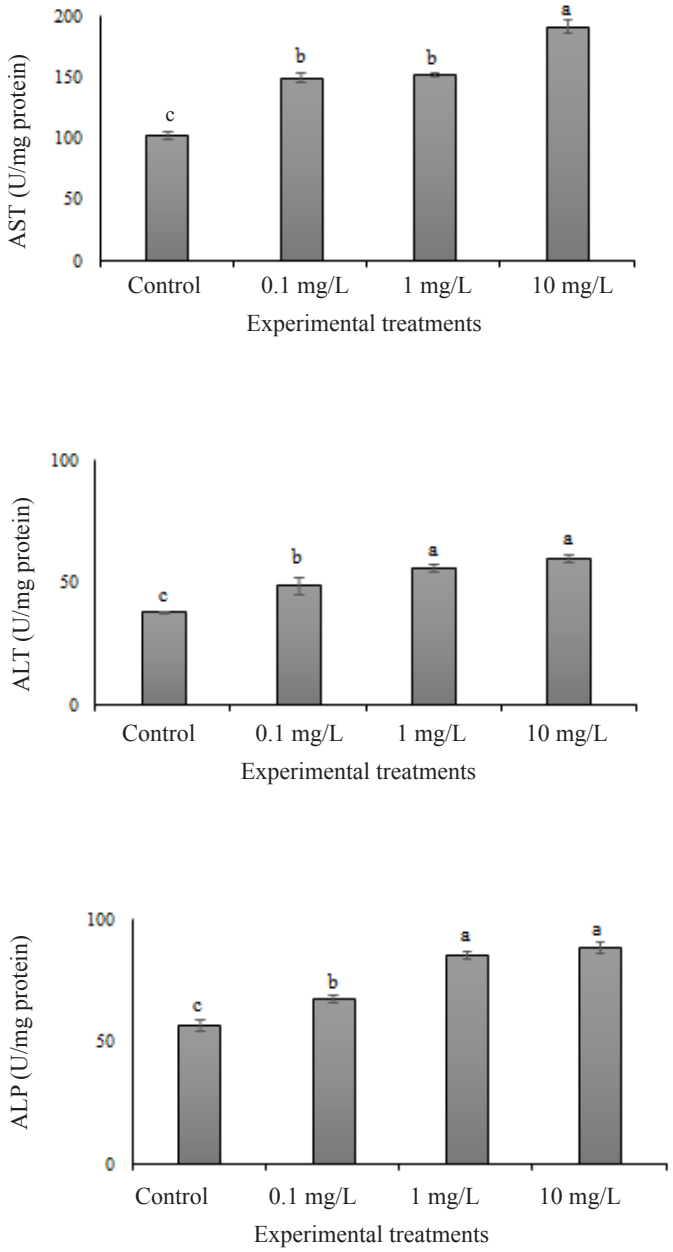


Figure 3. Levels of aspartate aminotransferase (AST, A), alanine transaminase (ALT, B), and alkaline phosphatase (ALP, C) in the whole body of female zebrafish following exposure to PS-NPs for 28 days. Data are shown as the mean ± standard errors (SE) of ten female zebrafish in each group. Bars with a different letter (a, b, and c) denote significant differences between the exposure groups ($P<0.05$)

Results

Survival rate

Exposure to PS-NPs for 28 days did not significantly affect the survival of female zebrafish even at the highest dose ($P>0.05$).

Sex hormones, Vtg, and stress factors

A significant decrease of Vtg was observed in female zebrafish exposed to PS-NPs after 4 weeks and this effect was detectable from 0.1 mg/L. A dose response was also observed as the exposure to the highest concentration of PS-NPs led to the significantly lowest hepatic Vtg content (Figure 2 A, $P<0.05$). After 28 days of exposure to PS-NPs the levels of E2 (Figure 2 B) and T (Figure 2 C) were notably decreased in female zebrafish of all PS-NPS-exposed groups compared to control group ($P<0.05$). It is noticeable that the E2 production was less sensitive than T production to the PS-NPs exposure as the drop of sex-steroid production was observed from 10 and 0.1 ml/L respectively. Glucose was notably increased in female zebrafish of all PS-NPs-exposed treatments compared to the control group, especially at the highest dose (Table 2, $P<0.05$). There was a slight but significant increase in cortisol levels in all PS-NPs-exposed treatments (Table 2, $P<0.05$). However, no difference was seen in lactate levels between different treatments (Table 2, $P>0.05$)

Liver enzymes

The level of AST (Figure 3 A), ALT (Figure 3 B), and ALP (Figure 3 C) were significantly increased in female zebrafish of all PS-NPs-exposed treatments compared to the control and the strongest effect was observed at the dose of 10 mg/L PS-NPs ($P<0.05$).

Discussion

This study aimed to investigate reproductive disorders in fish exposed to polystyrene nanoplastics. Most of studies on fish are limited to the effect of MPs, and the concentration of nanoparticles in aquatic ecosystems has not been measured (Oliveira et al., 2019 a, b; Barria et al., 2020). Recently, Materić et al. (2022) employed an innovative technique, relying on thermal-desorption proton-transfer-reaction mass spectrometry (TD-PTR-MS), in order to identify and quantify various categories of nanoplastics present in the water (Materić et al., 2022).

In this study, survival rate was not significantly affected by different concentrations of PS-NPs. In line with our findings, no mortality was reported in European sea bass, *Dicentrarchus labrax* (Peda et al., 2016), zebrafish, *Danio rerio* (Lei et al., 2018), and Japanese medaka, *Oryzias latipes* (Assas et al., 2020).

Hepatic Vtg in females is a particular protein that acts as a yolk precursor, and its biosynthesis interacts with the gene expression regulation of the hepatic estrogen receptor through endogenous E2 levels (Ding, 2005; Babin et al., 2007). In this study, the Vtg level decreased significantly with increasing the level of PS-NPs and the lowest amount was observed in zebrafish exposed to a dose of 10 mg/L. These results suggest that PS-NPs affect vitellogenesis in female zebrafish and thereby impair ovarian development, potentially acting as a compound impairing reproduction or endocrine regulation depending on the concentration. Similar to our results, different studies have reported that MPs can disrupt sex hormones, reduce the synthesis of hepatic Vtg, and cause delayed ovarian growth by disrupting the hypothalamic-pituitary-gonadal (HPG) axis and the steroidogenesis pathway (Han et al., 2015).

Sex hormones, testosterone (T) and 17-β-estradiol (E2), play an essential role in the growth and function of gonads. In the present investigation, the E2 level decreased significantly with exposure to PS-NPs. Similar results were reported in marine medaka exposed to PS-MPs (Wang et al., 2019 b). Reproduction in fish is controlled by the HPG axis: GnRH secreted by the hypothalamus stimulates the synthesis and release of gonadotropin hormones (FSH and LH) in the pituitary gland, and gonadotropins stimulate steroidogenesis, gametogenesis, and gonadal maturation (Prasad et al., 2015). In the current research, E2 and T levels decreased significantly in female zebrafish exposed to PS-NPs. No study has so far investigated the effects of nanoplastics on the steroidogenesis pathway. However, Wang et al. (2019 b) reported that MPs reduced the expression of reproductive hormones (mGnRH, FSHβ, LHβ, and GtHα) and their related receptors (FSHR and LHR) in female marine medaka, *Oryzias melastigma,* exposed to different doses of MPs. This indicates that MPs negatively influence the HPG axis and, consequently, reduce the levels of sex steroids (T and E2) by inhibiting the synthesis of these steroids (Wang et al., 2019 b). NPs have more neurotoxicity than MPs because the former has a smaller size and easily penetrates barriers, causing high neurotoxicity (Wang et al., 2022). Therefore, the toxicity of MPs in reproductive disorders may also be true for PS-NPs in endocrine and reproductive disorders in fish.

Stress can play an important role in essential life functions in vertebrates. Glucose and cortisol are biomarkers for the health and stress response of fish affected by various environmental contaminants (Lee et al., 2023). In the present study, glucose and cortisol levels were influenced by PS-NPs, and the level of them increased in the whole body (Table 1). The plasma glucose levels of fish increase in response to exposure to toxic substances, thereby stimulating gluconeogenesis, decreasing glucose absorption, and inducing glycogenolysis. These observations suggest the presence of liver dysfunction, as reported in studies by Hamed et al. (2019). The present investigation has revealed that there was a noteworthy elevation in plasma glucose levels after exposure to PS-NPs. This outcome provides confirmation that the exposure of fish to high concentrations of NPs can elicit stress responses. Lee et al. (2023) observed a noteworthy elevation in glucose

levels upon exposure of Korean bullhead (*Pseudobagrus fulvidraco*) to MPs. It is postulated that this rise in glucose levels is attributed to an upsurge in corticosteroids, which may regulate the disrupted glucose levels caused by NP toxicity (Lee et al., 2023).

Due to the increased permeability of the liver membrane caused by tissue damage, the fish liver increases the secretion of enzymes (e.g., ALT and AST) in the blood under environmental stress conditions, and then these enzymes participate in gluconeogenesis resulting from the stress response (Shivashri et al., 2013). Our data revealed significantly elevated ALT and AST levels in zebrafish exposed to PS-NPs, indicating liver damage because of toxicity (Figure 3 A, B). Consistent with our results, increased levels of liver enzymes caused by liver damage were reported in different fish species exposed to MPs in various studies, including tilapia, *Oreochromis niloticus* (Hamed et al., 2019), zebrafish (Umamaheswari et al., 2021), and common carp, *Cyprinus carpio* (Banaei et al., 2022). ALP is another important enzyme that plays a role in fish metabolism, thus changes in the activity of this enzyme can affect fish metabolism (Sharma et al., 2014). In the present study, ALP activity levels increased in zebrafish exposed to PS-NPs compared to the control group (Figure 3 C). Nematdoost Haghi and Banaee (2017) reported that ALP activity rose in common carp exposed to MPs, and similar results were reported in tilapia (Hamed et al., 2019). Elevated ALP levels caused by MPs or NPs may result from the degeneration of liver cells and necrosis caused by these compounds (Nematdoost Haghi and Banaee, 2017). Similar findings by Lee et al. (2023) in Korean bullhead (*Pseudobagrus fulvidraco*) when exposed to MPs (polyethylene) reported that the efflux of ALP was caused by lipid peroxidation and oxidative damage to cell membranes.

In this study, the survival rate was not significantly affected by exposure to different concentrations of PS-NPs, but it led to significant decreases in the levels of Vtg and sex steroids (T and E2). Exposure to PS-NPs, both at low and high doses, caused liver stimulation and increased liver enzymes in female zebrafish, indicating the destruction of liver tissue. Furthermore, stress factors (including cortisol and glucose) were affected by different doses of PS-NPs, and glucose concentrations decreased significantly while a significant increase occurred in cortisol levels. Altogether, the present results demonstrate that PS-NPs impair the reproduction process so that none of the female zebrafish broodstock exposed to different doses of PS-NPs reached the spawning stage.

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References

Abarghouei S., Hedayati A., Raeisi M., Hadavand B.S., Rezaei H., Abed-Elmdoust A. (2021). Size-dependent effects of microplastic on uptake, immune system, related gene expression and histopathology of goldfish (*Carassius auratus*). Chemosphere, 276: 129977.

Alimi O.S., Farner Budarz J., Hernandez L.M., Tufenkji N. (2018). Microplastics and nanoplastics in aquatic environments: aggregation, deposition, and enhanced contaminant transport. Environ. Sci. Technol., 52: 1704–1724.

Anbumani S., Kakkar P. (2018). Ecotoxicological effects of microplastics on biota: a review. Environ. Sci. Pollut. Res., 25: 14373–14396.

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. Mar. Pollut. Bull., 158: 111446.

Babin P.J., Carnevali O., Lubzens E., Schneider W.J. (2007). Molecular aspects of oocyte vitellogenesis in fish. In: The fish oocyte: from basic studies to biotechnological applications, Babin P.J., Cerdá J., Lubzens E. (eds). Springer, pp. 39–76.

Banaei M., Forouzanfar M., Jafarinia M. (2022). Toxic effects of polyethylene microplastics on transcriptional changes, biochemical response, and oxidative stress in common carp (*Cyprinus carpio*). Comp. Biochem. Physiol. C: Toxicol. Pharmacol., 261: 109423.

Barría C., Brandts I., Tort L., Oliveira M., Teles M. (2020). Effect of nanoplastics on fish health and performance: A review. Mar. Pollut. Bull., 151: 110791.

Bhagat J., Zang L., Nishimura N., Shimada Y. (2020). Zebrafish: An emerging model to study microplastic and nanoplastic toxicity. Sci. Total Environ., 728: 138707.

Cao J., Xu R., Wang F., Geng Y., Xu T., Zhu M., Lv H., Xu S., Guo M.Y. (2023). Polyethylene microplastics trigger cell apoptosis and inflammation via inducing oxidative stress and activation of the NLRP3 inflammasome in carp gills. Fish Shellfish Immunol., 1: 108470.

Ding J.L. (2005). Vitellogenesis and vitellogenin uptake into oocyte. In: Hormones and their receptors in fish reproduction, Melamed P., Sherwood N. (eds). World Sci. Pub., Singapore, pp: 254–276.

Frias J.P., Nash R. (2019). Microplastics: Finding a consensus on the definition. Mar. Pollut. Bull., 138: 145–147.

Geyer R., Jambeck J.R., Law K.L. (2017). Production, use, and fate of all plastics ever made. Sci. Adv., 3: e1700782.

Gregory M.R. (2009). Environmental implications of plastic debris in marine settings – entanglement, ingestion, smothering, hangerson, hitch-hiking and alien invasions. Philosophic. Trans. Royal Soc. B: Biol. Sci., 364: 2013–2025.

Greven A.C., Merk T., Karagöz F., Mohr K., Klapper M., Jovanović B., Palić D. (2016). Polycarbonate and polystyrene nanoplastic particles act as stressors to the innate immune system of fathead minnow (*Pimephales promelas*). Environ. Toxicol. Chem., 35: 3093–3100.

Gutierrez-Hartmann A., Duval D.L., Bradford A.P. (2007). ETS transcription factors in endocrine systems. Trends Endocrinol. Metab., 18: 150e158.

Hamed M., Soliman H.A., Osman A.G., Sayed A.E.D.H. (2019). Assessment the effect of exposure to microplastics in Nile tilapia (*Oreochromis niloticus*) early juvenile: I. blood biomarkers. Chemosphere, 228: 345–350.

Han J., Won E.J., Lee M.C., Seo J.S., Lee S.J., Lee J.S. (2015). Developmental retardation, reduced fecundity, and modulated expression of the defensome in the intertidal copepod *Tigriopus japonicus* exposed to BDE-47 and PFOS. Aquat. Toxicol., 165: 136–143.

Hidalgo-Ruz V., Thiel M. (2013). Distribution and abundance of small plastic debris on beaches in the SE Pacific (Chile): a study supported by a citizen science project. Mar. Environ. Res., 87: 12–18.

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. Sci. Total Environ., 858: 159976.

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.

Karami A., Romano N., Galloway T., Hamzah H. (2016). Virgin mi-

croplastics cause toxicity and modulate the impacts of phenanthrene on biomarker responses in African catfish (*Clarias gariepinus*). Environ. Res., 151: 58–70.

Koelmans A.A., Besseling E., Shim W.J. (2015). Nanoplastics in the aquatic environment. Critical review. In: Marine anthropogenic litter, Bergman M., Gutow L., Klages M. (eds). Springer Open, pp. 325–340.

Lee J.H., Kang J.C., Kim J.H. (2023). Toxic effects of microplastic (polyethylene) on fish: Accumulation, hematological parameters and antioxidant responses in Korean bullhead, *Pseudobagrus fulvidraco*. Sci. Total Environ., 877: 162874.

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*. *Sci. Total Environ.*, 619: 1–8.

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.

Materić D., Kjær H.A., Vallelonga P., Tison J.L., Röckmann T., Holzinger R. (2022). Nanoplastics measurements in Northern and Southern polar ice. Environ. Res., 208: 112741.

Nematdoost Haghi B., Banaee M. (2017). Effects of micro-plastic particles on paraquat toxicity to common carp (*Cyprinus carpio*): biochemical changes. IJEST, 14: 521–530.

Oliveira M., Almeida M., Miguel I. (2019 a). A micro (nano) plastic boomerang tale: A never ending story? Trends Anal. Chem., 112: 196–200.

Oliveira M., Ameixa O.M., Soares A.M. (2019 b). Are ecosystem services provided by insects "bugged" by micro (nano) plastics? Trends Anal. Chem., 113: 317–320.

Peda C., Caccamo L., Fossi M.C., Gai F., Andaloro F., Genovese L., Perdichizzi A., Romeo T., Maricchiolo G. (2016). Intestinal alterations in European sea bass *Dicentrarchus labrax* (Linnaeus, 1758) exposed to microplastics: preliminary results Environ. Pollut., 212: 251–256.

Pirsaheb M., Hossini H., Makhdoumi P. (2020). Review of microplastic occurrence and toxicological effects in marine environment: Experimental evidence of inflammation. PSEP, 142: 1–14.

Plastics Europe (2019). An analysis of European plastics production, demand and waste data. Plastics – the Facts 2019. https://www.plasticseurope.org/en/resources/marke t-data

Prasad P., Ogawa S., Parhar I.S. (2015). Role of serotonin in fish reproduction. Front. Neurosci., 9: 195.

Rochman C.M., Kurobe T., Flores I., Teh S.J. (2014). Early warning signs of endocrine disruption in adult fish from the ingestion of polyethylene with and without sorbed chemical pollutants from the marine environment. Sci. Total Environ., 493: 656e661.

Sadri S.S., Thompson R.C. (2014). On the quantity and composition of floating plastic debris entering and leaving the Tamar Estuary, Southwest England. Mar. Pollut. Bull., 81: 55–60.

Sharma U., Pal D., Prasad R. (2014). Alkaline phosphatase: an overview. Indian J. Clin. Biochem., 29: 269–278.

Shivashri C., Rajarajeshwari T., Rajasekar P. (2013). Hepatoprotective action of celery (*Apium graveolens*) leaves in acetaminophen-fed freshwater fish (*Pangasius sutchi*). Fish Physiol. Biochem., 39: 1057–1069.

Simon N., Schulte M.L. (2017). Stopping global plastic pollution: the case for an international convention. Berlin: Heinrich-Böll-Stiftung.

Solomando A., Capó X., Alomar C., Álvarez E., Compa M., Valencia J.M., Pinya S., Deudero S., Sureda A. (2020). Long-term exposure to microplastics induces oxidative stress and a pro-inflammatory response in the gut of *Sparus aurata* (Linnaeus, 1758). Environ. Pollut., 266: 115295.

Sun L., Zuo Z., Chen M., Chen Y., Wang C. (2015). Reproductive and transgenerational toxicities of phenanthrene on female marine medaka (*Oryzias melastigma*). Aquat. Toxicol., 162: 109e116.

Umamaheswari S., Priyadarshinee S., Bhattacharjee M., Kadirvelu K., Ramesh M. (2021). Exposure to polystyrene microplastics induced gene modulated biological responses in zebrafish (*Danio rerio*). Chemosphere, 281: 128592.

Wang M.H., He Y., Sen B. (2019). Research and management of plastic pollution in coastal environments of China. Environ. Pollut., 248: 898–905.

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

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. Sci. Total Environ., 808: 152098.

Yang M., Wang W.X. (2023). Recognition and movement of polystyrene nanoplastics in fish cells Environ. Pollut., 316: 120627.

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

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