Preprints.org

Review

Not peer-reviewed version

Biological Consequences of Microplastic Exposure in Zebrafish (*Danio rerio*): A Systematic Review Across Developmental, Physiological, and Neurobehavioral Endpoints

Assiddik Sapii Yahsin *, Carlito Baltazar Tabelin, Theerayut Phengsaart, Aileen H. Orbecido, William Ka Fai Tse, Yukiko Ogino, Mylah Villacorte-Tabelin *

Posted Date: 12 May 2026

doi: 10.20944/preprints202605.0763.v1

Keywords: microplastics; zebrafish; developmental; physiological; behavioral

Preprints.org is a free multidisciplinary platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC, OpenAlex.

Copyright: This open access article is published under a Creative Commons CC BY 4.0 license, which permit the free download, distribution, and reuse, provided that the author and preprint are cited in any reuse.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

Disclaimer/Publisher's Note: The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

Review

Biological Consequences of Microplastic Exposure in Zebrafish (*Danio rerio*): A Systematic Review Across Developmental, Physiological, and Neurobehavioral Endpoints

Assiddik Sapii Yahsin [1,2,*], Carlito Baltazar Tabelin [3,4], Theerayut Phengsaart [5,6], Aileen H. Orbecido [7], William Ka Fai Tse [8], Yukiko Ogino [8] and Mylah Villacorte-Tabelin [1,2,*]

1 Department of Biological Sciences, College of Science and Mathematics, Mindanao State University-Iligan Institute of Technology, Iligan City 9200, Philippines
2 Molecular and Developmental Biology Laboratory, PRISM, Mindanao State University-Iligan Institute of Technology, Iligan City 9200, Philippines
3 Department of Materials and Resources Engineering and Technology, College of Engineering, Mindanao State University – Iligan Institute of Technology, Iligan City 9200, Philippines
4 Resource Processing and Technology Center, RIEIT, Mindanao State University – Iligan Institute of Technology, Iligan City 9200, Philippines
5 Department of Mining and Petroleum Engineering, Faculty of Engineering, Chulalongkorn University, Bangkok 10330, Thailand
6 Applied Mineral and Petrology Research Unit (AMP RU), Department of Geology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand
7 Department of Chemical Engineering, De LaSalle University, 2401 Taft Avenue, Manila, 0922 Philippines
8 Graduate School of Bioresource and Bioenvironmental Sciences, Faculty of Agriculture, Kyushu University, Fukuoka, 819-0395, Japan
* Correspondence: assiddik.yahsin@g.msuiit.edu.ph (A.S.Y.); mylah.tabelin@g.msuiit.edu.ph (M.V.-T.)

Abstract

Microplastics (MPs) are widespread pollutants in aquatic environments, but their impacts throughout the life cycle remains of organisms are still not well understood. This systematic review integrates recent experimental results on the developmental, physiological, and neurobehavioral effects of MPs exposure on zebrafish (*Danio rerio*), a popular model organism for ecotoxicology research. A PRISMA-guided search using Web of Science (WoS) and Scopus as databases generated 371 articles, which was screened to 60 eligible articles. The collated results showed that MP toxicity strongly related to concentration, size, and extent of weathering or aging at various life stages of zebrafish. For developmental toxicity, a concentration-dependent yielded peer-reviewed publications assessing specific MPs properties, such as polymer identity, size, concentration, shape, and aging status. At various life stages, the toxicity of MPs was most affected by concentration, size, and aging. The developmental toxicity showed a concentration-dependent decrease in the rate of hatching, growth inhibition, and cardiac dysfunction, while, an increase in malformations, especially at concentrations of ≥100 μg/L or ≥10 mg/L has been reported. Non-monotonic and threshold effects have also been observed, the complexity of particle-based versus mass-based concentrations. Weathered and photo-aged MPs were found to exhibit higher embryotoxicity and neurodevelopmental toxicity, including changes in gene expression of neurons, decreased integrity of motor neurons, and impaired retinal development, compared with virgin MPs. Furthermore, physiological endpoints showed that oxidative imbalance was a key mechanistic process, which included changes in the activity of antioxidant enzymes (SOD, CAT, GPx), lipid peroxidation, inflammation, and disruption of tight junctions. Chronic MP exposures caused changes in the gut microbiota, hepatic metabolism, endocrine disruption, reproductive damage, thyroid function disruption, and genotoxicity in zebrafish. Neurobehavioral alterations, such as changes in locomotor

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

activity, anxiety response, neurotransmitter homeostasis, and acetylcholinesterase function, occurred in both larvae and adults, with a potentiation effect in aged MP exposure. Previous, experimental data have also shown that zebrafish are very sensitive to MPs exposure in various biological systems, with toxicity being a function of physicochemical properties and exposure conditions. Finally, this review found major limitations for inter-study comparisons because of inconsistencies and differences in methodology related to MP concentration, simulation of MP aging, and MP dose measurements.

Keywords: microplastics; zebrafish; developmental; physiological; behavioral

1. Introduction

Microplastics (MPs) are emerging pollutants that have become ubiquitous in marine and freshwater environments due to the rapid increase in global plastic production, consumption and disposal, slowly destroying aquatic ecosystems and pressuring aquatic biodiversity [1]. The presence of MPs materials affects not only aquatic life, but also affects the flow rate of water, water depth, and water topography in the bottom [2,3]. In the study of Dokl et al., 2024 [4] (given in Figure 1), the estimated global plastic consumption may increase from 464 Mt in 2020 up to 884 Mt in 2050. China is expected to used plastics for up to ~230-240 Mt, followed by the rest of Asian countries with ~120 Mt, Latin America with ~100-120 Mt, India with ~100Mt, United States of America with ~85-90 Mt, European Union with ~60-7- Mt, Middle East and North Africa with ~30-50 Mt, other African countries with ~30-40 Mt, Japan with ~15 Mt, Canada with <10 Mt, and other countries in the world contributing ~40-60 Mt of plastic consumption by year 2050. And large chunks (48.97 Mt) of these plastics are going to end in landfill areas. Microplastics are plastic particles between 1 μm and 5 mm [3], which are commonly found in most bodies of water with high human population density [5,6]. For instance, in Yangtze River Basin in China, 80% of fishes that were examined showed MP ingestion with polymer types including Polyethylene (PE), Polypropylene (PP), and Polyethylene terephthalate (PET) [7]. In European coastal waters, up to 28.4% of commercially imported fish found to have ingested MP fragments smaller than 1μm [8]. And in the Pasig River in the Philippines, 63,000 particles·km^{-2} of MP concentrations was reported in surface water samples contributing to the ecotoxicological burden in the aquatic ecosystems [9]. These plastic pollutants consist of various classes of polymers, including polyethylene (PE), polypropylene (PP), PET, polystyrene (PS), polyvinyl chloride (PVC), polyethylene terephthalate (PET), poly acrylamide (PAM), and polyurethane (PU) [10].

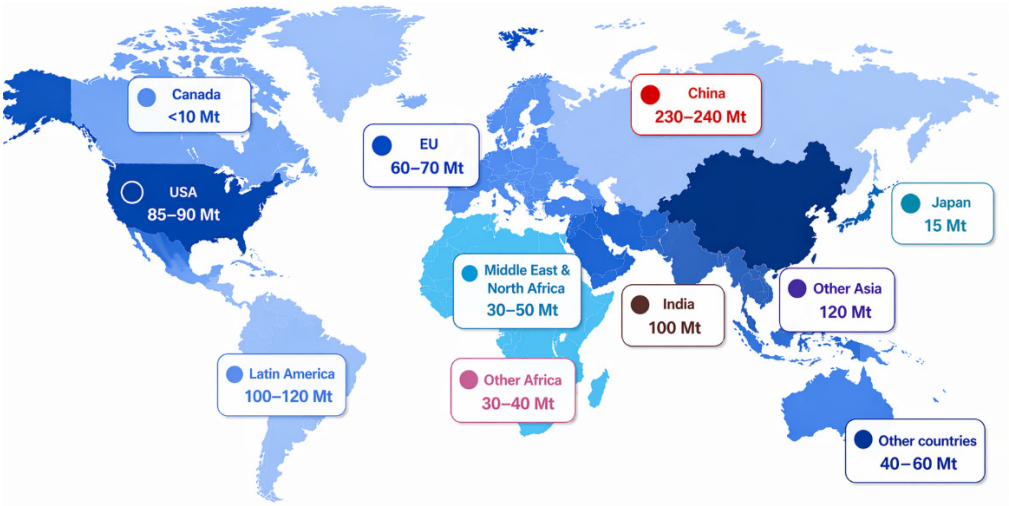

Figure 1. Global distribution of plastic consumption and usage by year 2050 [4].

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

Microplastics are formed in the environment either from the disintegration of large plastics due to prolonged exposure to ultraviolet (UV) radiation, mechanical abrasion, and weathering processes [11] or the inadvertent release of manufactured plastic microbeads found in skin care products and abrasives [12]. Anthropogenic activities, including waste disposal and transportation, textile manufacturing and laundering, construction projects, and medical wastes, are major contributors to the environmental prevalence of microplastic pollution (MPs) [13]. Industrial sectors contribute MP pollution to aquatic ecosystems through different routes. Textiles industry, for example, contribute to MP pollution through the production of microfibers such as polyester, nylon, and acrylic, which are washed away into the aquatic ecosystem through the sewerage system [14]. Transportation is another sector that also contributes to MP pollution, mainly from through the production of tires MP particles during road abrasion, which is then washed away into the aquatic ecosystem through runoff and the sewerage system [15]. Construction industry contributes to MP pollution, mainly through the degradation of synthetic paints, coatings, and insulations, which are then washed away into the aquatic ecosystem through runoffs [16]. Other sources of MP pollution include the abrasion of synthetic materials such as clothing, degradation of plastic packages, degradation of plastic fishing gears, fragmentation of plastic films, and the deposition of synthetic fibers from urban emissions [17].

Due to their small size and hydrophobic properties, MPs have become widespread across diverse environments. They can also be easily transported via air and water, and bioaccumulation in through the food chain, affecting all living organisms and that including humans' population [18]. Moreover, the widespread occurrence of MPs in the aquatic ecosystems indicates that aquatic life, including fishes, are continuously exposed to different levels of MP contaminations [19]. Barboza et al. [12], for example, reported that fish gastrointestinal tract usually held the highest proportion of total MPs at about 40%. This pattern of accumulation has wider implications than just fish health, as seafood is a major dietary source of protein for the majority of human populations, creating a potential exposure pathway for human consumers [20]. Some estimates have put forward numbers as high as 842 MPs particles ingested by adults annually via seafood, with predicted higher intakes in areas where fish show greater contamination levels [21]. Once ingested or inhaled, MP particles may translocate across biological membranes, leading to bioaccumulation in the human body, which may initiate cellular stress responses and inflammatory reactions [22]. Laboratory experiments suggest that MP particles may cause oxidative stress, cytotoxicity, and immune responses in human cells due to the reactivity of the MP particles' surface, as well as the leaching of plastic additives such as bisphenols and phthalates [23].

Among aquatic model organisms, zebrafish (*Danio rerio*) have been widely used in ecotoxicology studies due to their vertebrate physiology combined with experimental practicality [24,25]. The transparent embryos of zebrafish allow for the direct visualization of abnormal development during organogenesis, thus providing precise evaluation of the teratogenic effects of environmental contaminants [25]. The rapid development of zebrafish embryos and their high reproductive capacity make them suitable for conducting toxicity tests on a large scale in the laboratory [26]. Moreover, the genetic similarity of zebrafish to humans is very high, as 70% of the human genome contains at least one ortholog in the zebrafish genome [27]. These characteristics of zebrafish make them an excellent model for studying the effects of environmental contaminants, including microplastic particles [24].

In freshwater ecotoxicology, zebrafish (*Danio rerio*) has been an important vertebrate model organism to study the bioaccumulation effects of MPs and nano plastics, where their bioaccumulation toxicity has been assessed via various methods of experiments tractable life stages and endpoints [25]. The use of zebrafish was first considered in studying anticancer therapy [28]. As their genetic makeup and phenotypic traits were further established, their suitability has expanded and become more broader, finding applications in various research fields such as biomedical and toxicological studies applications. The rapid acceptance of zebrafish in animal studies was further justified by their practical and biological advantages, including low breeding costs, straightforward husbandry, and ethical frameworks that facilitates their use across diverse study methods [29].

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

Previous studies on the short-term ecotoxicity of polystyrene MP using zebrafish showed that tissue distribution, as well as organ-specific effects like hepatic alterations correlated with MP particles size of 5 μm [30]. MP exposure has been linked to intestinal injury phenotypes in zebrafish, as well as oxidative stress related effects in controlled experiments that investigated multiple types of MP such as PE, PS, PP, PVC, PET, PES, PLA, PA, PHA, and PGA [31]. In modern molecular toxicology, the oxidative response is defined as the first line molecular signal for toxic stimuli exposition itself [32]. Although the modulatory activity of oxidative/reducing pathways and Relative oxygen species (ROS)is responsible for oxidative balance, disturbances in such processing of harmful stimuli, caused by unusual dysregulated or disordered in metabolism of ROS, could be a significant source of cellular toxicity [33,34].

Neurobehavioral and neurochemical alterations have been reported in larval zebrafish after exposure to virgin versus aged polystyrene MPs, including changes in locomotor activity and neurotransmission-related parameters [35]. At the molecular level, transcriptomics has demonstrated the possibility for muti-system impacts, which resulted in the exposure associated with MPs causing broad gene expression changes, developmental, and neurobehavioral effects in zebrafish [36]. Aging and weathering can impact MP morphology and potentially their toxicity, thus, it is important to differentiate pristine from environmentally altered MPs when constructing zebrafish hazard evidence [37]. In the study of Mansuri et al. (2024) [38], comparing the effect of weathered and pristine MPs, the results indicated significantly higher mortality in the weathered MPs (80%) compared to the virgin MPs (20%). Zebrafish larvae ingested MPs and exhibited disruptions of key molecular pathways, including oxidative stress response, apoptosis, and DNA damage repair. Evidence in adult zebrafish also indicated that MPs exposure can co-occur with immune related and behavioral effects supported by transcriptional changes, and thus, it is important that developmental, physiological, and neurobehavioral outcomes are considered together [29].Luan et al. (2023) [30], indicated that, Polyglycolic acid (PGA) and Polylactic acid (PLA), MPs exposure at 100 mg/L PGA, showed a significant decrease in the survival and hatching rates at 10 and 24 Hours post-fertilization (hpf), increased wakefulness, reduced sleep in zebrafish for up to 80% in PLA 100 mg/L MPs exposure, and at 5 Days post-fertilization (dpf) Zeitgeber Time (ZT) ZT16, and 6 dpf ZT4 at 100 mg/L MPs exposure, both PGA and PLA affects and decreases the circadian behavior of zebrafish by affecting the brain-derived neurotrophic factor (BDNF).

Recent review papers have addressed the potential of model organisms, especially zebrafish (*Danio rerio*), to evaluate the ecotoxicological effects of MPs and other emerging microcontaminants. Some of the recent review papers highlighted that zebrafish has emerged as one of the most popular model organisms used to investigate aquatic toxicology because of their rapid growth rate, high reproductive potential, transparent embryos, and their genetic similarity to humans to understand the processes of bioaccumulation of pollutants, developmental toxicity, and molecular mechanisms of response to environmental stressors [31]. These characteristics make zebrafish an ideal model organism to understand the processes of microplastic uptake and distribution within the body and to investigate the effects of MPs and nanoplastics on aquatic organisms to understand the mechanisms of their toxicity [32].

However, despite the increasing trend of review articles published on the toxicity of MP exposure to zebrafish, there are still a few limitations existing in the literature. For example, the majority of the published review articles only offered a general summary of the experimental results, without an integrative approach to combine the results obtained from different biological responses. Moreover, there are a few review articles published on the toxicity of MP exposure, such as reproductive toxicity, and oxidative stress, but there is still a lack of comprehensive review articles published on the toxicity of MP exposure, including the development, physiology, and neurobehavioral responses of zebrafish. Other review articles discussed MP pollution in aquatic organisms, but did not provide a detailed synthesis of the experimental results obtained from zebrafish.

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

Thus, it is necessary to offer a systematic review to integrate the experimental results obtained from different biological responses, which would provide a clear idea regarding the biological consequences of microplastic exposure, along with the justification for the use of zebrafish as a model species for studying the ecotoxicology of MP exposure. This systematic review collates existing evidence on the biological impacts of MP exposure in zebrafish, focusing on developmental, physiological, and neuro-behavioral endpoints. A structured and systematic search of prominent scientific databases was performed using the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) 2020 [39], to identify experimental studies assessing the biological impacts of different MP types, sizes, concentrations, and exposure times on zebrafish (*Danio rerio*). The systematic review combined the results from different life stages, including embryos, larvae, juveniles, and adults, to provide a comprehensive evaluation of biological impacts.

The current study aims to answer the following research questions:

i. How does exposure of zebrafish to MPs affect their developmental, physiological, and neurobehavioral endpoints in relation to the underlying toxicity mechanisms, dose-response relationships, and severity of biological impacts?

ii. What are the key methodological constraints, exposure variables (such as polymer type, particle size, concentration, and aging status), and inconsistencies in experimental studies that limit the interpretation of biological impacts and risk assessment in zebrafish models?

2. Materials and Methods

2.1. Search Criteria

The Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) 2020 guidelines were employed for article identification, screening, eligibility assessment, and inclusion/exclusion criteria. No formal review protocol was registered prior to conducting the study. A systematic search was conducted using two major scientific databases: Web of Science (WoS) and Scopus. The search was conducted in August 2025 and the strategy implemented used the terms "microplastic" AND "zebrafish", and restricted to studies published between 2015 and 2024 to capture recent advances in MP aquatic ecotoxicology. The specific search string used was "TITLE-ABS-KEY (zebrafish AND microplastic) AND PUBYEAR > 2014 AND PUBYEAR < 2025".

2.2. Selection Process (Inclusion and Exclusion Criteria)

The initial search of the databases produced a substantial number of results. Following the removal of duplicates, the articles were filtered according to document type and language. Only peer-reviewed research articles published in English were included. Articles classified as reviews, conference papers, proceedings, editorials, letters, corrections, book chapters, and meeting abstracts were excluded. Further filtering was done at the title and abstract level to identify relevance to experimental studies investigating MP exposure in zebrafish (Danio rerio). Studies not conducted on zebrafish, not investigating MPs exposure, or not evaluating biological endpoints were excluded.

Full-text screening further narrowed the dataset to include only original experimental studies investigating measurable developmental (e.g., hatching rate, malformations, survival, growth), physiological (e.g., oxidative stress, histopathology, metabolic changes, bioaccumulation), or neurobehavioral (e.g., locomotor activity, anxiety-like responses, predator evasion, cognitive responses) endpoints. Studies that investigated environmental occurrence without biological endpoint evaluation were also excluded. One of the challenges in the search strategy was the general use of the term "microplastics," which often included studies on pollution assessment of soil, water and sediments, environmental distribution, polymer chemistry, or wastewater treatment without considering biological effects in zebrafish. Manual screening was necessary to ensure relevance to the endpoints of development, physiology, and neurobehavioral effects.

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

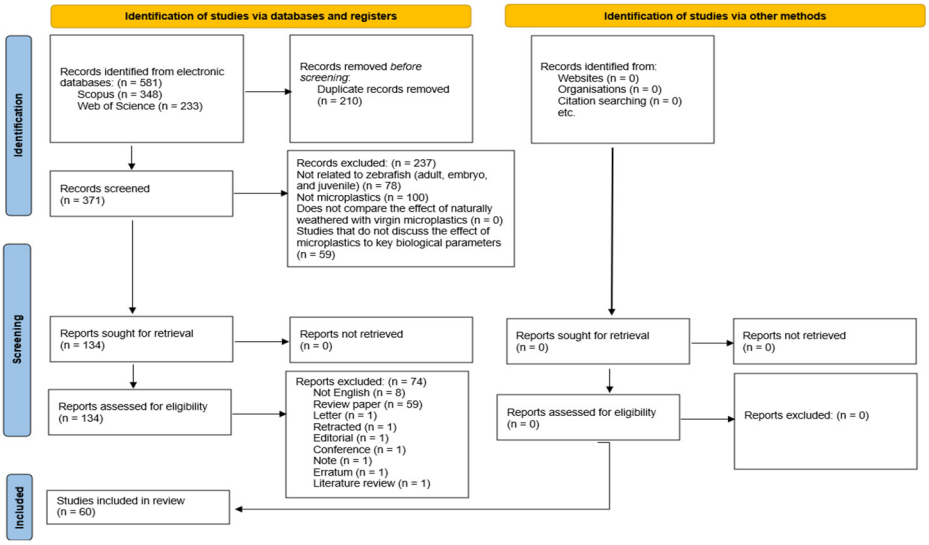

Figure 2. The PRISMA flow diagram for the systematic review procedure.

2.3. Data Synthesis

Using VOSviewer an analysis of keyword co-occurrence and bibliographic coupling was carried out to visualize the intellectual structure of the literature related to MP toxicity in zebrafish models [40]. This analysis identified frequently occurring keywords and research clusters that define the current scope of MP-related biological research in zebrafish models. As shown in Figure 3, dominant keywords which showed the strongest connections, included "microplastics", "zebrafish", and "toxicity. These terms showed strong connections with frequently used keywords, such as "oxidative stress", "development", "neurobehavior", "accumulation", and "gene expression", which are the primary endpoints of MPs related research in zebrafish models. Moreover, the bibliographic coupling and co-authorship network analysis revealed authors who contributed significantly to the field of MP toxicity research in zebrafish (Figure 4). The authors who showed the strongest links are the ones whose publications showed a large number of references to other publications within the dataset, showing the strong intellectual connections within the research community. These clusters of authors demonstrate the collaborative nature of the MP toxicity research, where some authors have contributed significantly to the understanding of the impact of MP on the development, physiology, and neurobehavior of zebrafish.

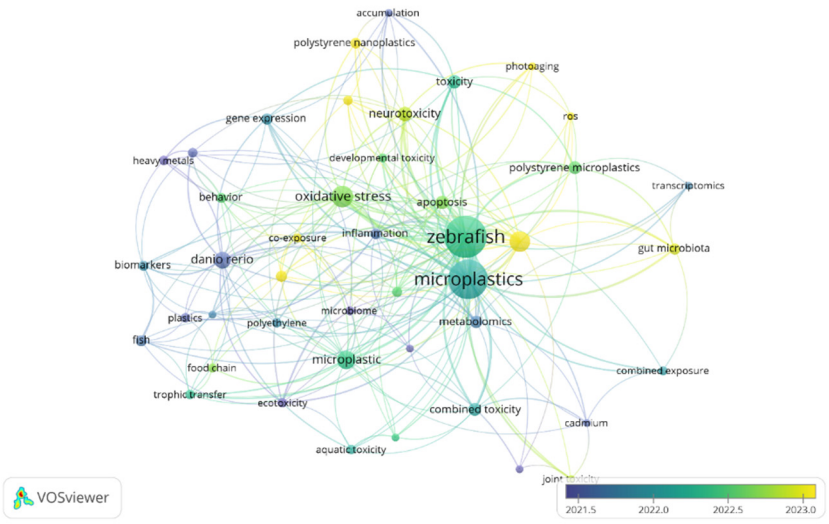

Figure 3. Keyword co-occurrence analysis for effects of MPs exposure to zebrafish.

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

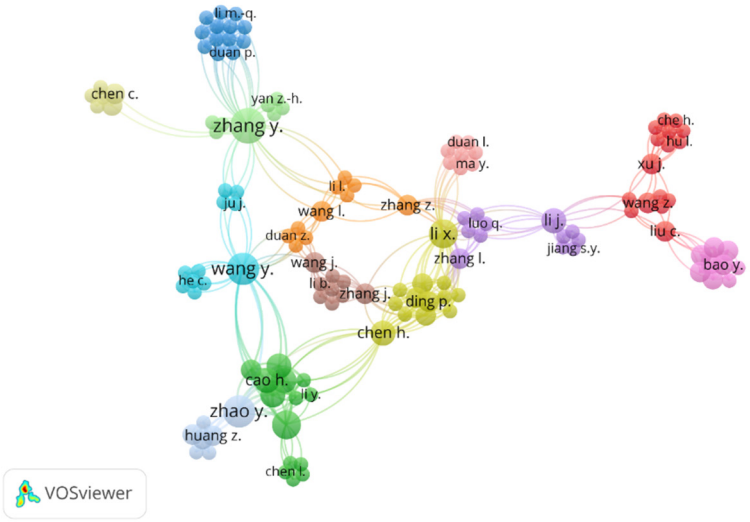

Figure 4. Bibliographic coupling of co-authorship using VOSviewer [46].

3. Developmental Anomalies and Consequences of Microplastic Exposure in Zebrafish

Embryonic and larval exposure to MPs demonstrated clear concentration-dependent developmental toxicity. Higher concentrations of polystyrene (PS) with particle size of 1-5 μm, at 10 μg/L, the results showed no significant effects on survival or hatching, and minor to non-significant increase in pericardial edema (5%). At 100 μg/L, hatching rate reduced to 10% and a slight increase in tail curvature (8-10%). PS MP exposure at 1000 μg/L, it significantly delayed hatching to -25%, heart rate to -13% vs control, and sudden increased in embryos deformities for up to 22% [36]. However, certain particle-based exposures revealed non-linear dose responses, with moderate particle loads producing measurable cardiac and growth suppression while higher mass-based concentrations did not proportionally increase embryotoxicity [37]. For instance, particle aggregation and bioavailability have been found to decrease with increased concentration in zebrafish studies where MP were found to cluster in a way that reduced their interaction with embryos [Prata et al. 2022] [37]. In addition, saturation of uptake pathways and limits to internal particle accumulation have also been described in zebrafish experiments where increasing exposure concentrations did not proportionally increase internal particle burden or toxicity, indicating biological thresholds in uptake or transport processes [Medriano & Bae. 2022] [42].

Low-dose exposures to PS, PE, and PES frequently showed no mortality or significant change in body length and survival as reported by Medriano & Bae, 2024 [42], Luo et al. 2021 [45], and Yang et al. 2024 [56]. Developmental toxicity increases with concentration but may exhibit non-monotonic or threshold-like patterns depending on particle metrics. For example, exposure to fragmented polyethylene and polyester microplastics at 0.2 mg/L and 1 mg/L produced no significant differences in survival, cardiac endpoints, or developmental parameters, indicating a plateau response where increasing concentration did not correspond to increased toxicity [44]. Similarly, experiments using particle-based exposure metrics for artificially weathered polypropylene and polystyrene showed that moderate particle loads (2,000–20,000 $MP \cdot L^{-1}$) resulted in reduced heart rate and body length in embryos, whereas a much higher exposure level (200,000 $MP \cdot L^{-1}$) did not further intensify embryotoxic effects, suggesting a non-linear relationship between particle abundance and developmental outcomes [37]. Another example is observed in polystyrene microbead exposure experiments where embryos exposed to 10 μg/L showed no significant developmental effects, while intermediate concentrations such as 100 μg/L produced measurable reductions in hatching and mild deformities, and extremely high concentrations (1000 μg/L) caused developmental delay and increased malformations, demonstrating a threshold-like transition from negligible to pronounced

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

toxicity as concentration increased [36]. These findings indicate that developmental responses to microplastics are not always proportional to concentration and may depend on particle number, size distribution, and physicochemical interactions within the exposure system.

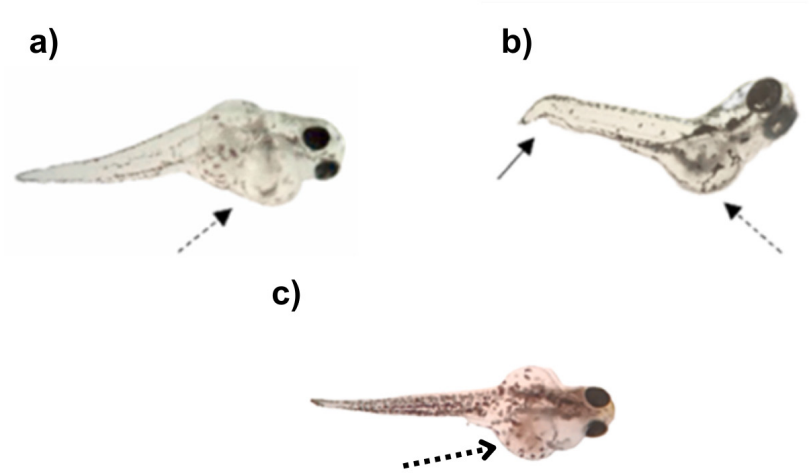

Figure 5. Noticeable yolk sac deformities (a), bent tail (arrow) (b), cardiac edema (dotted arrow) (c), and spinal curvature/scoliosis (red arrow) of zebrafish larvae (b) [36].

Furthermore, particle aging significantly amplified developmental and neurodevelopmental toxicity. Photo-aged and weathered PS and PE MPs consistently resulted to (~80–82%) compared with virgin PS particles, which produced only ~10–20% mortality and were not significantly different from controls. In the same study, weathered MP treatments also produced markedly higher malformation rates, including spinal deformities (~13–14%) and bent tails (~21%), whereas virgin PS exposures resulted in low or statistically insignificant malformation frequencies [38]. Similarly, photo-aged PS particles (1 μm) produced stronger growth inhibition and survival reduction in zebrafish larvae compared with virgin PS at equivalent concentrations (0.1–100 μg/L), indicating enhanced toxicity after environmental aging [58]. Another study examining UV-aged polyamide (PA) microplastics demonstrated clear concentration-dependent growth impairment: exposure to 10 μg/L caused slight growth inhibition, 100 μg/L significantly reduced larval body length and induced intestinal structural damage, and 1,000 μg/L resulted in severe growth inhibition accompanied by pronounced intestinal damage and impaired lipid absorption. In this experiment, photo-aged PA particles produced significantly stronger toxic effects than pristine particles across all exposure levels [60]. Artificially aged PS reduced motor neuron fluorescence and dysregulated neurodevelopment-related genes including gabra1, manf, nestin, and gfap, indicating interference with neuronal differentiation and central nervous system formation [48]. And Retinal exposure to fluorescent microspheres altered proliferation markers and downregulated neurogenesis genes such as sox2 and neuroD, suggesting compensatory but disrupted neurodevelopmental signaling [55]. The enhanced toxicity observed in aged or weathered MPs is primarily attributed to physicochemical transformations that occur during environmental aging processes such as ultraviolet (UV) irradiation, oxidative degradation, and mechanical abrasion. These processes introduce oxygen-containing functional groups (e.g., carbonyl and hydroxyl groups) on the polymer surface, increasing surface polarity and reactivity, which enhances interactions between particles and biological membranes (Koelmans et al. 2015 [97]; Lambert & Wagner, 2016 [98]). Another mechanism contributing to enhanced toxicity is the increased ability of weathered MPs to adsorb environmental contaminants, including heavy metals, hydrophobic organic pollutants, and plastic additives. These adsorbed compounds can be transferred to organisms during exposure, allowing aged microplastics to act as vectors of secondary toxicants and amplifying biological effects (Rochman et al. 2013 [99]; Koelmans et al. 2015 [97]). In zebrafish

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

models, these combined effects can stimulate excessive reactive oxygen species (ROS) production and disrupt antioxidant defense systems, leading to oxidative stress, inflammation, and altered expression of genes involved in neurodevelopmental processes (Savuca et al. 2023 [29]). Additionally, polymer type and particle size further influenced developmental outcomes. For instance, exposure to PVC MPs with a mean particle size of approximately 250 μm at concentrations of 100, 200, 300, and 400 ppm showed increasing developmental abnormalities in zebrafish embryos. While survival and hatching were not significantly affected across these exposure levels, significant edema formation was observed at the highest concentration (400 ppm) by 120 hpf, and tail malformation rates reached up to ~19.1% in the 300–400 ppm exposure groups, indicating concentration-dependent teratogenic effects [53]. In contrast, fragmented PE and PES MPs with average sizes of ~180 ± 210 μm (PE) and ~350 ± 220 μm (PES) produced minimal developmental toxicity at lower concentrations. Acute exposure experiments using 0.2 mg/L and 1 mg/L of these particles showed no significant differences in survival, cardiac parameters, or major developmental abnormalities, suggesting that relatively large fragmented particles at low concentrations exert limited embryotoxic effects under short-term exposure conditions [42,43].

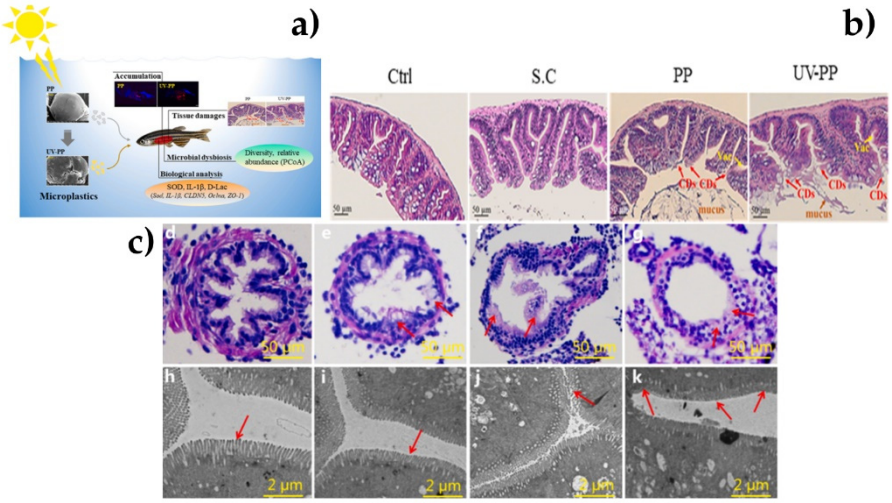

Figure 6. (a) Schematic overview of MP (PP and UV-PP) toxicity in zebrafish, illustrating accumulation, tissue damage, microbial dysbiosis, and associated biological responses. (b) intestinal histology (Control, S.C, PP, UV-PP) showing normal villi in controls and villus damage, increased mucus, and inflammatory cell infiltration in exposed groups [41]. (c) higher magnification images indicating epithelial disruption and structural abnormalities (red arrows) with scale bar 50 μm. (d) TEM images revealing ultrastructural damage, including microvilli loss and cellular disruption in PP and UV-PP groups (red arrows) with scale bar 2 μm [59].

Microbead PS at 10 mg/L resulted in measurable malformation and mortality across different size classes, demonstrating that even microscale particles can induce structural abnormalities in zebrafish embryos. In one study, exposure to 5 ± 3 μm PS microbeads produced 31.25% malformation and 11.11% mortality, with 72 hpf hatching at 72.22% and 96 hpf hatching at 87.50%. A similar experiment using larger 50 ± 3 μm PS microbeads at the same concentration resulted in 27.78% malformation and 8.33% mortality, with 68.05% hatching at 72 hpf and 88.89% at 96 hpf, indicating that smaller particles produced slightly stronger developmental effects under equivalent exposure conditions [55]. Additional studies further demonstrate the influence of particle size on developmental toxicity. For example, exposure to 1 and 5 μm PS particles at concentrations of 10, 100, and 1000 μg/L showed increasing embryotoxicity with both concentration and particle size characteristics. At 1000 μg/L, zebrafish embryos exhibited approximately 25% reduction in hatching rate, a 13% decrease in heart rate, and malformations in about 22% of embryos, including pericardial edema and tail deformities [36].

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

Lastly, growth endpoints at various stages of life reveal that chronic exposure refers to longer experimental durations ranging from 7 days to several weeks (e.g., 14–60 days) and frequently involves higher cumulative exposure levels. Chronic studies commonly used concentrations between 1 mg/L and 100 mg/L, with stronger physiological and developmental effects observed at the upper end of this range. For example, long-term exposure to ≥10 mg/L PS MPs resulted in reduced body length, metabolic disruption, and increased oxidative stress biomarkers, while very high chronic exposures such as 50–100 mg/L produced pronounced physiological damage, including hepatic injury and oxidative imbalance in zebrafish tissues [56,59]. Nevertheless, some low-dose exposures did not present statistically significant differences in growth parameters, reiterating the significance of concentration thresholds. In several zebrafish experiments, these thresholds appear to occur at relatively low exposure ranges. For example, exposure to spherical PS of ~5 µm at 20 µg/L for 21 days showed no significant changes in body length, body weight, or survival, indicating that concentrations at or below this level may fall below the threshold required to induce measurable growth impairment under chronic exposure conditions [45]. These thresholds are not universal and vary depending on the polymer type, particle size, and particle morphology. Polymer composition influences toxicity because different plastics contain distinct additives and surface chemistries that affect particle reactivity and interaction with biological tissues. For instance, PVC MPs produced developmental abnormalities at high concentrations (300–400 ppm), while PE fragments showed minimal effects at lower mg/L levels, indicating polymer-dependent toxicity differences [53].

Table 1. Developmental/Neurodevelopmental effects in pristine/virgin/unspecifies and UV/Photo-aged MPs exposure.

Life Stage	Plastic Characterization (Type/Shape/Size/Concentrations)	Endpoints	Exposure Time	Effects	Ref.
Adult	PP/Spherical/Virgin: 33.20 ± 14.42 µm/ UV: 20.83 ± 10.46 µm/ 50 mg/L for PP and UV-PP	Survival rate, hatching rate, time to hatch	14 days	Excretion began immediately after MPs removal, GI residual after 1 day: PP = 7.9%, UV-PP = 12.3%, Elimination half-life: PP = 0.78 days, UV-PP = 0.38 days, 99.9% excreted after 5 days.	[41]
	PE & PES/Fragmented beads/average size of 180±210 µm/1 mg/L both PE & PES	Growth and development: Body length and survival rate	96 hours (4 days) acute exposure	No significant developmental toxicity.	[42]
	PS/5 µm/2 mg/L	Hatching rate (72hpf), body length (7 dpf), heart rate, and malformation rate (pericardial edema, yolk sac edema, spinal deformity)	14 days	No recorded mortality for PS MPs exposure.	[43]
	PE & PES/Fragments/PE mean size: 180 ± 210 µm PES mean length: 350 ± 220 µm/ 0.2 mg/L, 1 mg/L both PE & PES	Cardiac and Developmental: Heart rate, Pericardial edema, Cardiac looping, Blood flow abnormalities, Survival and hatching	30 days	No mortality observed, No major difference between 0.2 and 1 mg/L (threshold effect).	[44]
	PS/Spherical beads/5 µm diameter/20 µg/L	Growth indices: Body weight, Body length, Condition factor ($K = W/L^3 \times 100$)	21 days	No mortality occurred; final body length and body weight did not change.	[45]
	PGA/~1 µm in diameter/1 mg/L & 100 mg/L	Neurochemistry: brain 5-HT system	28 days	↓ brain 5-HT both 1 mg/L & 100 mg/L. Serotonin-pathway gene expression: ↑ tph1b while ↓ tph1a.	[46]

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

				Brain inflammatory gene expression: ↑ (il-1β, tnf-α, il-10) after PGA exposure.	
Embryos	PS/Spherical beads/1, 5 µm /10, 100, 1000 µg/L	Survival rate, hatching rate, time to hatch	96 h (4 days post-fertilization)	PS (10 µg/L) No significant effects on survival or hatching. Minor but non-significant increase in pericardial edema (5%). PS (100 µg/L) ↓ Hatching rate (~10% reduction); slight increase in tail curvature (8–10%). PS (1000 µg/L) Significant developmental delay; ↓ hatching (−25%), ↓ heart rate (−13% vs control), ↑ deformities (22% embryos malformed).	[36]
	Virgin & Photo-aged PS/1 µm diameter/0, 0.1, 1, 10, 100 µg/L for Virgin and Photo-aged PS MPs	Embryo development and mortality (daily observation); Neurotoxicity-focused endpoints: locomotor behavior, neurotransmitter changes, neuronal development and gene expression related to neurotransmission/adipocytokine signaling.	24 h	Motor neuron development (Tg(hb9-GFP), 120 hpf): A-PS exposure reduced motor neurons in brain and spinal cord relative to control; GFP fluorescence intensity decreased from 238 ± 2.58 AU (control) to 234 ± 2.88 (0.1 µg/L), 232 ± 4.97 (1 µg/L), 230 ± 3.95 (10 µg/L), and 229 ± 2.53 AU (100 µg/L) ($P < 0.05$). Neurodevelopment- and nervous system–related gene expression (120 hpf): A-PS significantly altered expression of nervous system function/development genes (nestin, gfap, manf, shha, alpha–tubulin, mbp). Relative to control, A-PS significantly inhibited gabra1 and manf, and significantly elevated nestin, gfap, alpha–tubulin, and mbp.	[47]
	Virgin & Artificially weathered PP & PS/PS: ~15–36 µm PP: ~50–148 µm; After weathering: (≤230 µm)/Environmentally relevant (particle based): 2,000, 20,000, 200,000 MP·L⁻¹; High concentrations (mass based):12.5, 25, 50, 100 mg·L⁻¹	Mortality, hatching rate	96 hours (outcomes recorded at 24/48/72/96 h; heart rate at 72 h; length at 96 h)	2,000 MP·L⁻¹: ↓ heart rate, ↓ body length. 20,000 MP·L⁻¹: Sublethal growth effects. 200,000 MP·L⁻¹: No linear increase in toxicity. 12.5–100 mg·L⁻¹: No significant embryotoxicity.	[37]
	PS/Spherical/0.1 µm diameter/0, 0.1, 1, 10, 50, 100 mg/L	Development: cumulative mortality, hatching, malformation (48–96 hpf); Morphometrics: yolk sac area, pericardial edema, body length, eye size, heart rate.	96 h	PS attenuated AgNP toxicity rather than acting as a strong developmental toxicant at the selected dose.	[48]

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

Embryos → Larvae	Fluorescent plastic microspheres/Spherical/ 1-5 µm/ 2 mg/L (~1.09 × 10^8 particles/L)	Developmental and Biodistribution: Mortality, Growth, Distribution of MPs in tissues	2 hpf to 14 dpf	Increased PCNA-positive cells in retina → increased cell proliferation, Downregulation of neurogenesis genes (sox2, neuroD, olig2), Altered DNMT expression (epigenetic modulation), Presence of plastic particles in retina.	[49]
	Not disclosed/MPs: ~1 µm diameter/MPs suspended at 0.006%, 0.0045%, 0.003%, 0.0015% solids	Mortality rate, tail and vascular morphology (larval stage), angiogenesis, growth metric (caudal body length), caudal vein morphometrics, and heart morphology	Exposed for 3 days (1 dpf → 4 dpf)	Developmental toxicity included: Pathological changes of caudal vein plexus (angiogenesis abnormalities), Caudal tissue impairment and reduced growth/body length, Peripheral microcirculation dysfunction (caudal region) Mortality (1 dpf → +1day exposure): Mortality from 29.2% (MP1) to 95.8% (MP4), with intermediate values 33.3% (MP2) and 58.3% (MP3) Heart morphology: described as largely transformed (malformed) relative to control zebrafish	[50]
	PS/Fragmented/2 µm/10 mg/L	Developmental toxicity: blood disorder, heartbeat, hatch/death rates, malformations, morphometrics; Neurodevelopmental marker: atoh1a expression in cerebellar area (fluorescent reporter)	24–72 hpf	Developmental toxicity: Single PS 157 µm showed no observed effect. Neurodevelopmental toxicity: µ-PS did not change cerebellar fluorescence.	[51]
	PGA, PLA, PBS, PHA, PBAT/No data/1 mg/L and 100 mg/L for each polymer	Survival: assessed repeatedly from 3–96 hpf; hatching assessed at 48/72/96 hpf.	3, 6, 10, 24, and 96 hpf	Early morphology (3-95 hpf): No significant morphological changes at 6, 10, and 24 hpf across groups Survival (96 hpf): Survival rate significantly decreased in mg/L PHA and 1 mg/L PBAT, and in 100 mg/L PGA, PLA, PBS, and PHA groups Hatching: At 48 hpf, hatching rates were significantly increased in high-concentration MPs groups; at 72 and 96 hpf, hatching showed a decreased trend. Larval morphometrics (96 hpf): No significant malformations at 96 hpf, but body length and head area were markedly reduced in all exposure groups except 1 mg/L	[52]

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

			PGA; eye area decreased except in 1 mg/L PGA and 1 mg/L PBAT. Retinal histology (5 dpf): IPL thickness was significantly reduced in 1 mg/L PGA and 100 mg/L PBAT; ONL thickness significantly decreased in 1 mg/L PBS and 1 mg/L PBAT; RGL thickness significantly decreased in all treatment groups. Eye/retina gene expression (5 dpf): 100 mg/L PGA, PLA, and PBAT significantly decreased pax6a, pax6b, rx1, gnat2, grk1b, and opn1mw1; 100 mg/L PBS increased pax6b, gnat2, grk1b, and opn1mw1 but reduced rx1; 100 mg/L PHA increased pax6a, gnat2, and grk1b but reduced rx1.	
PVC/Mean size ~250 µm/ 100, 200, 300, 400 ppm	Phenotype endpoints: survival, hatching, edema, tail malformation.	30 days	Survival: No mortality reported through 120 hours post-fertilization (hpf) across all exposure scenarios (MP-only, phenanthrene-only, co-exposure). Hatching: Hatching rate was reported as not affected, including under the study's "extremely high" exposure conditions. Morphology / teratogenic endpoints (monitored at 48, 72, 96, 120 hpf): Edema rate and tail malformation rate were recorded as developmental indicators. • Edema: Significant edema increase occurred in the MP-only highest concentration group (A4; 400 ppm) at 120 hpf; phenanthrene-only groups showed no significant edema effect vs control. • Co-exposure edema: Increased edema was observed at lower MP concentration (200 ppm) in co-exposure groups AB3	[53]

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

			and AB4, interpreted in the paper as a synergistic toxicological effect • because phenanthrene alone did not produce edema. • Tail malformation: MP-only exposure produced a significantly higher tail malformation rate at high MP concentrations (300 and 400 ppm) at 120 hpf (reported up to 19.1%). • Phenanthrene-only tail malformation: Significant differences vs control was observed at 72, 96, and 120 hpf, but the paper states the degree was smaller than in MP-only high-dose effects. • Co-exposure tail malformation: A slight increase was noted at 48 and 72 hpf, which was reported to disappear with longer exposure.	
PET/Irregular fragments/Average 30–100 µm/1 mg/L & 10 mg/L	Growth (length/weight)	0 to 96- or 120-hours post-fertilization (hpf)	Significant growth alterations	[54]
Virgin & Weathered PS & PE/Spherical/Virgin 10 µm and 30 µm; Weathered PS (lab prepared, ~1–7 µm range) and Weathered PE (lab prepared, ~1–10 µm range)/Virgin MPs targeted ~10^5–10^6 particles/L, Weathered MPs targeted ~10^4 particles/L	Survival/motality, teratogenic outcomes (spinal/tail defects, edema),	10 days	Virgin PS: 10–20% (PS 30 µm ~10%; PS 10 µm ~20%; not significant vs control), Weathered MPs: ~80–82% (weathered PS ~80%; weathered PE ~82%; highly significant). Malformations: Weathered groups: spinal malformations ~13–14%, bent tails ~21%, significantly higher than control, Virgin PS groups: low/non-significant malformations.	[38]

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

	PS/Microsphere/5 μm/1 mg/L	Mortality/survival and body length	2 h post-fertilization (hpf) and continued to 7 days post-fertilization (dpf).	Embryo hatching: No significant effect on hatching. Heart rate: No significant effect on heart rate. Mortality: Increaser embryo and larval cumulative mortality during the 7-day exposure Growth (body length): No significant effect on growth.	[55]
Larvae	PS & PVC/Spherical/PS (Spherical ~7.0 μm mean size) PVC (Spherical ~3.8 μm mean size)/ Both MPS 20 mg L^{-1}	Survival and Development: Daily mortality, Morphological abnormalities	10 days	PS MPs alone: Higher mortality than PVC (10–20%), Strong locomotion suppression. PVC MPs alone: Moderate mortality, moderate suppression.	[56]
	PS/Virgin & Photoaged/Spherical beads/1 μm/0, 1, 10, and 100 μg/L both Virgin & Photoaged	Growth and development: Body length and survival rate	96 hours post-fertilization (hpf)	More strongly inhibited larval growth, and ↓ survival rate in photoaged PS.	[57]
	PS/Microbeads/5 ± 3 μm & 50 ± 3 μm/10 mg/L at 5 μm (MPs-5) or 50 μm (MPs-50)	Growth indices: Body weight, Body length, Condition factor ($K = W/L^3 \times 100$)	Up to 96 hpf	Developmental endpoints: PS-MPs 10 mg/L (5 μm): malformation 31.25%; mortality 11.11%; hatching 72 hpf 72.22%, 96 hpf 87.50%; PS-MPs 10 mg/L (50 μm): malformation 27.78%; mortality 8.33%; hatching 72 hpf 68.05%, 96 hpf 88.89%.	[58]
	Virgin & UV-aged PA/Irregular fragments/~5 μm (mean particle diameter)/0, 10, 100, and 1,000 μg/L	Growth and development: Body length and survival rate	2 days post-fertilization (dpf) → 10 dpf	10 (μg/L): Slight growth inhibition (photoaged > pristine) 100 (μg/L): Reduced body length; intestinal structural changes 1,000 (μg/L): Significant growth inhibition; severe intestinal damage; impaired lipid adsorption; photo-aging significantly enhanced PA toxicity	[59]
	Virgin & Photo-aged PS/Virgin: 10 μm Photoaged: 6.5 μm/0.1–100 μg/L for both V-PS and P-PS.	Neurotransmission: neurotransmitters (5-HT, GABA, DA, ACh); enzymes (AChE, ChAT, ChE)	1 hfp to 120 hpf	V-PS significantly increased neurotransmitter levels and cholinergic enzyme activity; response trends with concentration and IBR weighting toward neurotransmitter disruption; only V-PS has the effect on the neurotransmitter level of the zebrafish	[60]
	PS/~25 μm in diameter/25, 250 μg/L	Neurobehavior: light–dark locomotor response (movement distance, max acceleration, average velocity); Neurodevelopment genes: gap43, α1-tubulin	Early dpf stages	No significant change in neurobehavioral or neurodevelopment-related gene expression (P>0.05).	[61]

Abbreviations: PS—polystyrene; PP—polypropylene; PE—polyethylene; PES—polyester; PVC—polyvinyl chloride; PGA—polyglycolic acid; PBAT—polybutylene adipate terephthalate; PLA—polylactic acid; PHA—

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

polyhydroxyalkanoate; PBS—polybutylene succinate; PET—polyethylene terephthalate; PA—polyamide; MPs—microparticles; hpf—hours post-fertilization; dpf—days post-fertilization; DNMT—DNA methyltransferase; PCNA—proliferating cell nuclear antigen; 5-HT—5-hydroxytryptamine; GABA—gamma-aminobutyric acid; DA—dopamine; Ach—acetylcholine; AChE—acetylcholinesterase; ChAT—choline acetyltransferase; ChE—cholinesterase; AgNP—silver nanoparticles; GFP—green fluorescent protein; UV—ultra-violet; PPM—parts per million; ↑—increased; ↓—decreased; →—progression.

4. Physiological and Oxidative Response of Zebrafish Exposed to Microplastics

Across life stages, oxidative stress emerged as the most consistent physiological response of zebrafish to MPs exposure. For instance, PE MPs with an average diameter of 40 ± 10 μm at 100 μg/L for 21 days resulted in increased superoxide dismutase (SOD) and catalase (CAT) activities, accompanied by mild intestinal villi damage, suggesting activation of antioxidant defense mechanisms in response to oxidative stress [62]. In another study using 1–5 μm spherical microplastics at 2 mg/L, zebrafish larvae exhibited significant increases in ROS production, lipid peroxidation (LPO), and alterations in multiple antioxidant biomarkers including SOD, CAT, GPx, GST, GR, and GSH/GSSG, together with inhibition of acetylcholinesterase activity and reduced physiological capacity [64]. And chronic exposure to weathered polyethylene microplastics at 1 μg/L for 40 days caused significant modulation of immune and oxidative stress responses, including elevated plasma cortisol levels, increased antimicrobial and lysozyme activity, and changes in hematological parameters, indicating that oxidative stress responses were associated with systemic physiological stress during prolonged exposure [81].

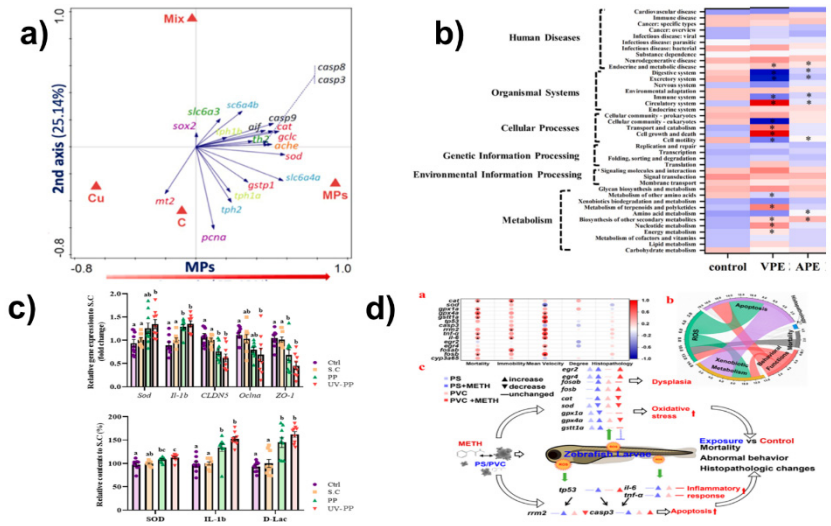

Figure 7. Oxidative stress responses in zebrafish following MPs exposure. (a) Multivariate analysis showing changes in oxidative stress–related and apoptosis-related gene expression following microplastic exposure [64]. (b) Heatmap illustrating altered metabolic and cellular pathways associated with oxidative stress responses after exposure to virgin and aged polyethylene microplastics [62]. (c) and biochemical markers (SOD, IL-1β, D-Lac) across treatments, indicating altered oxidative stress, inflammation, and intestinal barrier function [59]. (d) integrated schematic summarizing toxicological outcomes in zebrafish larvae, including oxidative stress, inflammation, apoptosis, dysplasia, behavioral changes, and histopathological effects following exposure to MPs and co-contaminants [57].

Lower concentrations of MPs often induced compensatory antioxidant responses, whereas higher concentrations produced sustained oxidative imbalance and activation of cellular damage pathways. For example, exposure of zebrafish to PGA MPs at 1 mg/L and 100 mg/L for 28 days resulted in significant physiological alterations associated with oxidative stress. At 1 mg/L, early antioxidant responses and intestinal barrier disturbances were observed, including dysregulation of

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

gut-related signaling pathways and microbial imbalance. At 100 mg/L, stronger systemic responses occurred, including enhanced inflammatory signaling and neurochemical alterations linked to oxidative stress pathways [46]. Similarly, experiments using PS microbeads (5 ± 3 μm and 50 ± 3 μm) at 10 mg/L in zebrafish embryos demonstrated strong oxidative stress responses. Exposure increased reactive oxygen species (ROS) production, elevated lipid peroxidation markers such as malondialdehyde (MDA), and activated antioxidant enzymes including SOD and CAT. In addition, oxidative damage was associated with DNA oxidative damage markers (8-OHdG) and altered expression of apoptosis-related genes, including upregulation of p53, Bax, and caspase-3/8/9, and downregulation of the anti-apoptotic gene Bcl-2, indicating activation of apoptosis pathways following oxidative stress exposure [55].

Intestinal toxicity and barrier dysfunction were recurrent findings. Histopathological changes included villus shortening, epithelial shedding, vacuolization, goblet cell reduction, and tight-junction gene downregulation (cldn5, zo-1), accompanied by increased D-lactate levels indicating compromised barrier integrity. Zebrafish exposed to PP and UV-weathered PP microplastics (33.20 ± 14.42 μm for virgin PP and 20.83 ± 10.46 μm for UV-PP) at 50 mg/L for 14 days resulted in clear intestinal mucosal damage, characterized by villus structural disruption, vacuolization, ciliary defects, and a 34% decrease in goblet cells in PP treatments and 51% reduction in UV-PP groups. Molecular analysis further showed significant downregulation of tight junction genes including cldn5 and zo-1, together with increased D-lactate levels, which is a recognized marker of intestinal permeability and barrier dysfunction [66]. Inflammatory responses were also evident following microplastic exposure. In the same experiment, expression of inflammatory cytokines was significantly elevated, including interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), indicating activation of intestinal immune responses associated with tissue injury and oxidative stress [66]. Similarly, exposure to fragmented PE and PES microplastics (180 ± 210 μm and 350 ± 220 μm) at concentrations of 0.2 and 1 mg/L for 30 days resulted in metabolic disturbances in intestinal tissues and disruption of lipid metabolism pathways, suggesting that microplastics interfere with normal intestinal physiological processes [42].

Gut microbiota profiling further confirmed significant dysbiosis after microplastic exposure. Studies reported increases in Proteobacteria abundance and shifts in Fusobacteria and Firmicutes populations, indicating disruption of microbial community structure. In addition, metabolomic analyses revealed disturbances in key metabolic pathways, including purine metabolism, bile acid biosynthesis, arginine metabolism, and lipid metabolism, demonstrating that intestinal microbial imbalance can influence host metabolic regulation [66,90]. Also, chronic exposure of zebrafish to PS MPs (5 μm) at 20 μg/L for 21 days resulted in significant alterations in hepatic metabolic parameters. Specifically, hepatic pyruvate, triglycerides (TG), and total cholesterol (T-CHO) levels were significantly reduced, indicating disruption of lipid and carbohydrate metabolism [45].

Although several glycolipid metabolism genes (e.g., gk, hk1, pepckc, aco, cpt1, ppar-α, acc, and fas) did not show significant transcriptional changes, oxidative stress markers were affected, including increased catalase (CAT) activity and reduced glutathione (GSH) levels, together with suppression of oxidative stress–related genes such as Mn-sod, Nrf2, Keap1, Gpx, and Bcl2, demonstrating impaired antioxidant regulatory pathways in hepatic tissue [45]. Histopathological studies also demonstrated liver tissue damage associated with MP exposure. Exposure to PS MPs of varying particle sizes (10 μm, 40 μm, and 200 μm) at 100 μg/L for 30 days resulted in hepatocyte vacuolization, nuclear abnormalities, and structural alterations of liver tissue, indicating hepatic injury. Oxidative stress biomarkers further showed size-dependent responses, where superoxide dismutase (SOD) activity increased as particle size decreased, while CAT activity significantly decreased in groups exposed to the smallest particles, demonstrating that smaller microplastics induced stronger oxidative stress responses in liver tissue [65].

Metabolomic analyses further revealed systemic metabolic disturbances in zebrafish exposed to MPs. In the study of Dimitriadi et al. 2021 [88], zebrafish were exposed to PS MPs with a particle size of approximately 5 μm at concentrations of 1, 10, and 100 mg/L, and metabolomic profiling of cardiac

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

tissues revealed substantial alterations in central energy metabolism pathways. Several intermediates of the tricarboxylic acid (TCA) cycle were significantly reduced, including succinic acid (~75%) and α-ketoglutaric acid (~56%), while pyruvic acid increased by approximately 38% relative to the control group. These changes indicate disruption of mitochondrial metabolic processes and oxidative phosphorylation, suggesting that MP exposure interferes with cellular energy production and metabolic regulation.

Furthermore, endocrine and reproductive endpoints demonstrate interference with hormonal regulation following MPs exposure. For example, exposure of adult zebrafish to PS MPs with a particle size of ~100 μm at a concentration of 40.1 μg/L for 21 days resulted in significant endocrine disruption [55]. This exposure increased ovarian testosterone levels by approximately 75% and brain testosterone levels by about 39.3% compared with controls. Histological examination of ovarian tissue revealed oocyte maturation arrest characterized by an increased proportion of immature stage I–II follicles and reduced numbers of mature follicles, indicating impaired reproductive function and chronic anovulation-like conditions. In addition, oxidative stress biomarkers such as malondialdehyde (MDA) increased to approximately 0.015 μM/mg ovarian tissue, suggesting oxidative damage associated with endocrine disruption [55].

Moreover, chronic exposure to PE MPs with a particle size of approximately 25 μm at 100 μg/L for 35 days significantly altered endocrine regulation in zebrafish. Hormonal assays showed disruption of reproductive hormones including estradiol (E2), testosterone (T), and 11-keto testosterone (11-KT), accompanied by altered expression of genes within the hypothalamic–pituitary–gonadal–liver (HPGL) axis such as gnrhr2, gnrhr3, cyp11a, cyp19a, erα, and vtg, which are essential regulators of steroidogenesis and reproductive development. Histological analysis also revealed pathological ovarian changes including loss of contact between the oocyte membrane and follicular cell layers and cellular degeneration, confirming reproductive toxicity following PE exposure [78]. Additionally, endocrine disruption involving the thyroid axis has been reported following exposure to PS MPs with a particle size of approximately 2 μm at concentrations of 0.1 mg/L and 1 mg/L over 63 days.

These exposures enhanced maternal transfer of thyroid hormones (T3 and T4) to offspring while simultaneously reducing thyroid hormone levels in the F2 generation, demonstrating that microplastics can interfere with endocrine signaling across generations [75]. While artificially aged PLA MPs with an approximate particle size of ~100 μm at a concentration of 5 mg/L also induced reproductive toxicity during 5 weeks of exposure. In this study, aged PLA particles caused ovarian structural damage and disrupted steroid hormone balance, and these maternal exposures produced offspring with increased mortality, reduced hatching rates, and decreased body length, indicating that weathered bioplastic particles can exert transgenerational developmental effects [69].

Additionally, neurophysiological biomarkers and cardiovascular endpoints further demonstrate systemic involvement following microplastic exposure. Neurotoxicity was frequently assessed through acetylcholinesterase (AChE) activity, which was significantly altered. For instance, zebrafish exposed to undisclosed type of MPs (~1–5 μm) at a concentration of 2 mg/L from 2 hpf to 14 dpf exhibited significant inhibition of AChE activity, accompanied by elevated oxidative stress biomarkers including ROS, lipid peroxidation, and changes in antioxidant enzymes (SOD, CAT, GST, and GR), linking oxidative stress responses with neurophysiological dysfunction [64]. Similarly, exposure of zebrafish embryos and larvae to PS MPs of 1 μm diameter at concentrations of 0.1, 1, and 10 mg/L for 21 days induced oxidative stress responses characterized by increased MDA levels and altered antioxidant enzyme activity, which were associated with reduced swimming performance and behavioral changes indicative of neurotoxicity [85].

Cardiovascular effects of MPs have also been widely reported. Exposure of zebrafish embryos to PS MPs (1–5 μm) at concentrations of 10, 100, and 1000 μg/L resulted in significant reductions in heart rate at higher concentrations, demonstrating cardiotoxic responses during early developmental stages [36]. In addition, experiments assessing PS microplastics at concentrations of 1, 10, and 100 μg/L under co-exposure with microcystin-LR showed thrombus formation in the caudal vein,

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

inhibition of angiogenesis in vascular tissues, and increased oxidative stress and inflammatory gene expression, indicating disruption of cardiovascular development pathways [92]. Further metabolomic and histopathological studies using 5 μm PS MPs at concentrations of 1, 10, and 100 mg/L demonstrated cardiac oxidative damage characterized by a 528.5% increase in lipid peroxidation and approximately 100-fold increase in DNA damage markers, together with activation of apoptosis pathways including increased Bax/Bcl-2 ratio and elevated caspase-3 and caspase-9 expression, indicating severe cardiac injury following high-dose exposure [88].

Lastly, bioaccumulation and depuration dynamics demonstrated rapid gastrointestinal uptake and variable elimination kinetics in zebrafish exposed to MPs. For example, zebrafish exposed to PP MPs with an average particle size of 33.20 ± 14.42 μm (virgin PP) and 20.83 ± 10.46 μm (UV-weathered PP) at a concentration of 50 mg/L for 14 days showed rapid ingestion and accumulation within the gastrointestinal tract. Following removal of MPs from the exposure medium, depuration occurred relatively quickly, with residual particles remaining at 7.9% for virgin PP and 12.3% for UV-weathered PP after 1 day, and approximately 99.9% of particles eliminated within 5 days. The elimination half-life was 0.78 days for virgin PP and 0.38 days for weathered PP, indicating faster depuration but greater short-term retention of weathered particles due to stronger biological interaction [42].

Although studies examining PS MPs (~5 μm) at concentrations of 1, 10, and 100 mg/L reported widespread tissue distribution following ingestion, with particles detected in the gastrointestinal tract, liver, and other internal tissues, suggesting systemic bioavailability of smaller particles after intestinal uptake [88]. Sex-specific responses were also observed during chronic exposure experiments. In one study, zebrafish exposed to PS MPs (~5 μm) at 20 μg/L for 21 days exhibited significant alterations in gut microbiota composition and metabolic responses. Female zebrafish displayed stronger microbiota dysbiosis, including greater shifts in dominant microbial taxa and associated metabolic pathways, which coincided with reproductive impairment and altered lipid metabolism, indicating that sex-dependent physiological differences can influence the biological impact of microplastic accumulation [45].

Table 2. Physiological effects of pristine/virgin/unspecifies and UV/Photo-aged in MPs exposure.

Life Stage	Plastic Characterization (Type/Shape/Size/Concentrations)	Endpoints	Exposure Time	Effects	Ref.
Adult	PE/Spherical/Average diameter of 40 ± 10 μm /100 μg/L	Oxidative stress biomarkers: SOD, CAT, GPx, MDA, T-AOC	21 d	100 μg/L: Slight oxidative stress (↑ SOD, CAT activities); minor intestinal villi damage.	[62]
	PS/Spherical beads/1 μm & 3 μm/0.01, 0.1, 1.0, 10.0 mg/L	Cardiac physiology (heart rate at 72 h); Redox homeostasis/oxidative stress biomarkers	96 hours (4 days post-fertilization)	↑ in heart rate both concentrations with more strongly at 10 mg/L. ↓ ROS content at 10 mg/L; ↑ Lipid hydroperoxides but more significant in 10 mg/L. Antioxidant enzymes: GPX: unaffected; GR: ↑ significantly only in 1 mg/L; SOD: ↑ significantly only in 10 mg/L.	[36]
	Virgin & UV PP/Virgin: 33.20 ± 14.42 μm UV: 20.83 ± 10.46 μm/50 mg/L both V & UV	Histopathology: Intestinal villi length, mucus secretion, goblet cell number, vacuolization, ciliary defects.	14 d	Tissue distribution and accumulation: both V & UV weathered PP particles were detected in the GI tract, liver, and gills. Peak GI burden (day 3): PP = 383.4 ± 50.3 particles; UV-PP = 2053.7 ± 371.4 particles.	[41]

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

	Gene expression: Sod1, IL-1β, CLDN5, Oclna, ZO-1. Enzyme biomarkers: SOD, IL-1β (inflammation), and D-lactate (intestinal permeability). Gut microbiome: Alpha diversity (Chao1, Shannon), Beta diversity (PCoA, UniFrac), OTU abundance, Taxonomic shifts at phylum & genus levels, KEGG pathway analysis using PICRUSt & MinPath.		Excretion kinetics (GI): elimination half-life PP = 0.78 d; UV-PP = 0.38 d; after 5 days of depuration, both MPs reached 99.9% excretion. Intestinal histopathology: exposure to PP and UV-PP produced mucosal damage characterized by structural damage, vacuolization, ciliary defects, mucus secretion, and reduced goblet cells. Goblet cells: decreased by 34% (PP) and 51% (UV-PP). Oxidative stress / inflammation markers (gut): sod mRNA upregulated significantly in UV-PP; il-1β mRNA upregulated significantly in both PP and UV-PP vs S.C; SOD and IL-1β enzyme-level changes were consistent with gene-expression patterns. Tight junction / barrier markers: cldn5 and zo-1 transcription downregulated in PP and UV-PP; D-lactate (D-Lac) increased. Gut microbiota diversity and composition: Shannon index increased significantly in the PP group; PCoA indicated microbiome shifts after PP and UV-PP exposure. Differential taxa (reported comparisons): Rhizobium, Gemmobacter, and Cloacibacterium were significantly higher in PP than UV-PP; Luteolibacter and Rodobacter were significantly higher in UV-PP; Porphyromonadaceae and Aeromonas tended to decrease in MP-exposed groups.	
Virgin MPs (propriety polymer)/Spherical/ 1-5 µm/ 2 mg/L (~1.09 × 10^8 particles/L).	Biochemical Biomarkers: ROS, LPO, SOD, CAT, GPx, GST, GR, GSH/GSSG, LDH, AChE, and MT. Molecular responses: Lipid metabolism genes (fabp, apoa1, etc.), and Intestinal barrier genes (zo-1, claudin).	2 hpf to 14 dpf	Significant inhibition of acetylcholinesterase (AChE) activity compared with control, indicating neurotoxicity. AChE inhibition showed correlation with mortality increase and reduced growth. And other biomarkers such as ROS, LPO, SOD, CAT, GST, GR, GSH/GSSG, LDH, has significantly affect the overall physiological capacity of the zebrafish.	[63]
PE & PES/Fragmented beads/average size of 180±210 µm/1 mg/L both PE & PES	Molecular responses: Lipid metabolism genes (fabp, apoa1, etc.), and Intestinal barrier genes (zo-1, claudin). Oxidative stress: ROS production, Antioxidant enzymes (SOD, CAT), Lipid peroxidation (MDA).	96 hours (4 days) acute exposure	PE exposure: significantly altered lipid metabolism in the intestine and liver, and perturbation of lipid metabolism, fatty acid metabolism, vitamin metabolism, TCA cycle, and amino acid metabolism. PES exposure: upregulated metabolites included phosphocholine and 2-lysophosphatidylcholine; and downregulation metabolites included triglyceride, 13-HDoHE, n-triacontanol, and phosphatidylserine.	[42]
Propriety polymer (composition undisclosed/Spherical/1–5 µm /2 mg/L	Molecular responses: Oxidative stress-related genes, Detoxification-related genes.	30 d	MPs (2 mg/L): ↑ Metallothionein (MT), ↑ tph1a (serotonin synthesis gene), ↓ LDH (metabolic alteration).	[64]

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

	Metabolomics: Whole-body metabolomics, LC-MS/MS (QTOF) untargeted metabolomics.		Gut microbiota: Increased; Fusobacteria, and Protobacteria; Decreased: Firmicute; Clear dysbiosis in both PE and PES groups.	
PS/Microbeads/ 200 µm, 40 µm, 10 µm/100 µg/L	Biochemical biomarkers: ALT, AST (hepatic injury), SOD, CAT, GSH (oxidative stress), Integrated Biomarker Response (IBR).	30 d	MPs exposure resulted in liver pathological changes, wherein liver damage included the presence of ballooning of the hepatocytes (vacuolization), nuclear abnormalities (including the formation of pyknotic or peripherally located nuclei), and the presence. CAT activities decreased with the decrease of MPs size, and the smallest MPs group showed a significant decrease in CAT under single exposure. In the MPs single-exposure groups, SOD activity increased with decreasing plastic size. GSH content significantly rose under the single MPs treatments, but the magnitude of GSH increase showed a decline with a decrease in MPs size.	[65]
PS/5 µm/2 mg/L	Oxidative stress biomarkers: Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), Malondialdehyde (MDA). Histopathology: Liver tissue, Hematoxylin & eosin (H&E) staining, Hepatocyte vacuolization, Cellular degeneration, Histopathological scoring. Gene Expression (qRT-PCR): Metallothionein (mt2), Antioxidant-related genes, Apoptosis-related genes.	14 d	Gills (Histopathology): There were no histopathological changes in the gills of the fish that were exposed to not contaminated. Intestine (Histopathology): Intestinal changes are minor, including cracking of villi, caused by MPs alone. Liver (histopathology): There was no obvious impact of MPs alone on histopathology.	[43]
PE & PES/Fragments/PE mean size: 180 ± 210 µm PES mean length: 350 ± 220 µm/ 0.2 mg/L, 1 mg/L both PE & PES	Whole-body untargeted LC-MS/MS metabolomics to quantify MPs associated shifts in molecular metabolites and enriched metabolic pathways.	30 d	PE specific metabolomic changes: (0.2 mg/L) and (1 mg/L) overlapped with minimal separation, while both separated from controls. Both concentrations had no significant different and does not change metabolomics of zebrafish. Gut microbiome (16S metagenomics): Community composition: dominant phyla included Proteobacteria, Fusobacteria, Firmicutes, Actinobacteria, Bacteroidetes, and Verrucomicrobia; Fusobacteria increased (0.3–11.7%) in microplastics-exposed groups vs control, while Proteobacteria decreased (0.4–9.0%) across exposures vs control. Potential pathogens were reported only in exposed groups (examples:	[44]

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

	Gut microbiome physiology and gut microbiota profiling after MPs exposure.		Mycobacterium in PES2; Aeromonas in PE1/PE2). Diversity: alpha diversity indices (observed OTU, Shannon, Faith FD, Simpson) were reported as not significantly altered across exposure groups. PE: Physical epithelial damage, oxidative stress, inflammation, disrupted membrane and energy metabolism. PES: fibers interfered more strongly with gut microbiota interactions, lipid digestion, and endocrine-related metabolism.	
PVC & PP/Irregular fragments/5–50 μm/PVC MPs (100 μg/L), and PP MPs (100 μg/L)	Intestinal histopathology (H&E); Intestinal inflammation biomarkers/gene expressions: TNF-α levels and IL-1β gene expression. Oxidative stress biomarkers/antioxidant-related expression; Intestinal metabolomics; and Intestinal microbiota	21 d	Intestinal histopathology (H&E): Control intestines showed intact villi shape and regularly arranged epithelial cells. PVC-only and PP-only groups showed slight shedding of intestinal villi. Intestinal inflammation biomarkers/gene expressions: TNF-α levels significantly increased in all treatment groups except the cadmium-only group; this includes PVC-only and PP-only. IL-1β gene expression significantly increased in all treatment groups; this includes PVC-only and PP-only. Oxidative stress biomarkers/antioxidant-related expression: No remarkable shifts in SOD activity or MDA levels were reported after exposure to PVC alone or PP alone. PP (but not PVC) significantly increased GPx expression (detoxification-related). Intestinal metabolomics: PVC-only: 32 significant differential metabolites down-regulated and 24 up-regulated vs control. PP-only: 85 significant differential metabolites down-regulated and 10 up-regulated vs control. KEGG pathway enrichment: both PVC-only and PP-only showed disturbances in purine metabolism and arginine/proline metabolism. PVC-only additionally disrupted primary bile acid biosynthesis and arachidonic acid metabolism. PP-only additionally affected arginine biosynthesis and glutamate metabolism. Intestinal microbiota: At the phylum level, Firmicutes relative abundance increased in PVC-only and PP-only groups. Proteobacteria relative abundance increased in all treatment groups, including PVC-only and PP-only.	[66]
PS/Spherical beads/2 μm diameter/ 0.44 mg/L (~10^8 items/L)	Oxidative biomarkers: SOD, CAT, GSH, and MDA,	7 d	Ocular oxidative-stress biomarkers (eyes; ELISA; normalized to protein): MPs exposure showed no significant main effect on SOD, CAT, GSH, or MDA in the model analysis ($p > 0.1$). GSH specifically: the MPs-only group had significantly higher ocular GSH	[67]

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

			than the control group. MDA: no significant difference was detected in ocular MDA among groups.	
PS/Spherical beads/2–4 μm diameter/440 μg/L	Oxidative stress biomarkers: SOD, CAT activities, MDA content, and protein normalization. Histopathology: villus number, villus height and width, and intestinal wall thickness; and gut microbiota analysis.	21 d	PS beads were observed in the gut at day 1 and day 21 (control showed only autofluorescence). PS distributed along anterior–middle–posterior gut and tended to accumulate in the mid–posterior gut and/or be excreted with fecal pellets. Oxidative stress/anti-oxidant response: CAT activity was significantly increased in the PS group compared with the control group (and AMI group). SOD activity was not reported as significantly increased in the PS group (significance was reported for the PS+AMI group vs control/PS/AMI). MDA was not reported as significantly increased in the PS group (the significant change reported was a decrease in the AMI group vs control/PS). Morphometrics: villus width was significantly increased in the PS-MPs group; intestinal wall thickness was significantly decreased in the PS-MPs group. And the number of villi showed decreasing tendency in PS-MPs group. Gut microbiota: PS-MPs showed increasing tendency in Proteobacteria which includes exiguobacterium, Candidatus paracaedibacter, and staphylococcus.	[68]
PS/Spherical beads/5 μm diameter/20 μg/L	Hepatic biochemical parameters: Energy metabolism: glucose, pyruvate, Lipid metabolism: TG, T-CHO, LDL-C, NEFA, Oxidative stress: SOD, CAT, GSH, MDA Glycolipid metabolism genes: Glycolysis / gluconeogenesis: Gk, Hk1, Pepckc, β-oxidation: Aco, Cpt1, Ppar-α, Lipid synthesis: Acc, Fas, Ppar-γ, Lipid transport: Apo, Fabp6. Oxidative stress genes: Mn-sod, Cu/Zn-sod, Cat, Nrf2, Keap1, Gpx, Bcl2; Inflammatory genes: IL-1β, IL-6, IL-8, TNF-α, IFN, C3, IL-10.	21 d	Hepatic metabolism: PS: ↓ pyruvate, TG, T-CHO. PS specific significantly changes: hepatic pyruvate, TG, and T-CHO with significant decreased; PS has no significant effect on NEFA. Hepatic glycolipid metabolism genes: Based from the results, PS has no significant effect on glycolipid metabolism genes. Oxidative biomarkers: CAT activity has a significant increase in PS exposure vs control.; GSH content reduced after 21 days of exposure; Oxidative stress related mRNA such as Mn-sod, Nrf2, Keap1, Gpx, and Bcl2 mRNA levels were significantly inhibited in PS-MPs.	[45]
Virgin & Photo-aged PLA/~100 μm/5 mg·L^{-1}	Female reproductive endpoints: Gonadosomatic index (GSI), Ovary histology (H&E), Sex homones:	5 w	DPLA > UPLA toxicity, Ovarian structural damage, disrupted steroid hormones, Altered metabolomic pathways, Offspring: ↑ mortality, ↓ hatching, ↓ body length.	[69]

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

	Testosterone (T), Estradiol (E2), Emphasis on E2/T; Ovray metabolomics.			
PE/Spherical/146.2 ± 8.9 μm/ 5 μg/L and 50 μg/L	Oxidative stress & Antioxidant Biomarkers: Catalase (CAT), Glutathione-S-transferase (GST), Lipid peroxidation (MDA); Ion regulation: Na^+/K^+-ATPase activity (gills).	10 d & 20 d	5 μg/L: 10 days: ↓ CAT and GST (liver); 20 days: ↑ GST activity (adaptive response), ↑ lipid peroxidation (brain). 50 μg/L: Stronger oxidative stress than 5 μg/L, Significant ↑ MDA (brain), Marked ↑ Na^+/K^+-ATPase activity in gills.	[70]
PS/Spherical beads/1 μm/30 mg/L	MP distribution (gills vs gut), gut histopathology, oxidative stress enzyme, liver metabolomics, gut microbiota (16S rRNA), sex hormones, and reproductive output.	Chronic (multi-week).	Females accumulated more PS, Gut microbiota dysbiosis stronger in females, Altered hepatic lipid & energy metabolism, Reduced egg production, Clear sex-specific toxicity.	[71]
PS/Fragmented/5 μm/20 & 200 mg/L	Cd accumulation (liver, gut, gill), Oxidative stress enzymes, Histopathology, Inflammatory gene expression.	Chronic (multi-week).	MPs enhanced Cd bioaccumulation, there is an increased trend in oxidative damage and inflammation synergistically increased, MPs acted as toxic vector.	[72]
PS/Fragmented/5 μm/LMPs: 0.1, 1, 10, 50, 100 mg/L; SMPs: 0.1, 1, 10, 50, 100 mg/L	Antioxidant enzymes: SOD, CAT, GPx, Lipid peroxidation (MDA).	96 hours post-exposure	SOD activity: At day 4, PS-MPs exposure significantly increased hepatic SOD. CAT activity: At day 4, CAT activity is significantly higher and remained significantly higher at day 8. GPx: At day 4, GPx activity is significantly higher and remained significantly higher at day 8. Lipid peroxidation (MDA): PS-MPs has no significant effect on hepatic MDA at day 4, but evidently reduced at day 8.	[73]
PS/Not disclosed/0, 50, 500 μg/L	Gene expression: qPCR normalization to β-actin, Ovarian histology: (oocyte stage scoring; H&E), Oxidative stress markers: MDA (lipid peroxidation), SOD, CAT.	60 d	50 and 500 μg/L: decreased ovarian SOD/CAT activity (oxidative stress), increased NO, increased apoptosis; dose-response noted (500 μg/L highest TUNEL-positive), All dosage groups: MDA boosted (membrane damage).	[73]
PS/Spherical/2 μm/0.1 mg/L and 1.0 mg/L	Endpoints measured: Thyroid axis & maternal transfer: co-exposure increased maternal transfer of T3 and T4, and reduced thyroid hormones in the "F2 generation."	63 d	Results: "At both concentrations" (0.1 and 1.0 mg/L), μ-PS exacerbated acetochlor-induced reductions in thyroid hormones and promoted maternal transfer.	[75]
PE/Not disclosed/60 mg/L	Genotoxicity / cytotoxicity (blood cell biomarkers): Increased nuclear abnormalities, changes in erythrocyte and nuclear size/shape (mutagenic + cytotoxic signals), Morphometric RBC nuclear/shape endpoints are shown in later figures (e.g., elongation/circularity).	10 d	Results: "At both concentrations" (0.1 and 1.0 mg/L), μ-PS exacerbated acetochlor-induced reductions in thyroid hormones and promoted maternal transfer. Genotoxicity (erythrocytes; comet assay): The DNA damage index was ~64% higher than control, and tail intensity increased by >60% relative to unexposed fish. Hydrogen peroxide (H2O2): PE-MP exposure was associated with higher	[76]

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

			H2O2 in brain (vs control) and higher H2O2 in liver (PE-MPs alone). In gills, PE-MP exposure showed reduced H2O2 versus control (this reduction was reported for PE-MPs alone).	
PSE/Spherical/100 μm /40.1 μg/L	Endocrine profile: Serum LH, FSH, and β-estradiol (E2) quantified by ELISA; kit ranges provided; absorbance read at 450 nm for E2; Metabolomic/oxidative stress markers: measured via glucometer; Molecular biomarkers (qPCR).	21 d	Ovary testosterone: PS-MP increased ovarian testosterone by 75% vs control; Brain testosterone: PS-MP increased brain testosterone by 39.3% vs control after 21 days. Ovarian histology and oocyte maturation arrest (PCOS-like morphology): PS-MP ovaries: more developing immature (stage I/II) follicles and significantly fewer mature follicles (interpreted as chronic anovulation-like). Oxidative stress: MDA was significantly higher in PS-MP than control (direction/significance stated), with PS-MP MDA reported as 0.015 μM/mg ovarian tissue (LET: 0.104 μM/mg).	[77]
PGA/~1 μm in diameter/1 mg/L & 100 mg/L	Gut barrier / intestinal permeability: regulation downstream of Wnt/β-catenin; supported by qPCR, ELISA, tissue section analysis; Gut microbiome: 16S rRNA sequencing; Liver injury + histopathology and molecular assays (qPCR/ELISA) connecting gut disruption to systemic effects.	28 d	1 mg/L PGA MPs (28 d): evidence of gut barrier disruption, microbiota dysbiosis, and behavioral/neurochemical disturbance (anxiety-like and impaired cognition/visual preference; altered 5-HT system). Wnt/β-catenin pathway genes (intestine): low PGA reduced wnt-4a, high PGA increased wnt-4a and wnt-10b expression, while both doses reduced gsk3β and dkk1 expression, and high PGA increased β-catenin expression. Taxonomic changes (phylum-level trends): reduced Proteobacteria, and increased Fusobacteria and Bacteroidetes after PGA exposure.	[46]
PE//Spherical/25 μm 100 μg/L	Endocrine biomarkers (adults; sex hormones + VTG): Analytes: Estradiol (E2), testosterone (T), 11-keto testosterone (11-KT), vitellogenin (VTG); Genes: 17 HPGL-axis related genes listed in the paper (e.g., gnrh2/3, gnrhr2/3, lhβ, fshβ, fshr, lhr, star, cyp11a, cyp19a/b, erα/β, ar, vtg1, etc.); qPCR approach: SYBR Green real-time PCR; three biological replicates per treatment (as stated for this assay)	35 d	Pathological changes described including the loss of contact between oocyte membrane and follicular cell layer, yolk cell breakdown, and cell lysis; gnrhr2/gnrhr3: significantly reduced in all groups except MP group; Additional gene-level guidance is mentioned in the text (e.g., cyp11a, lhr, erα, vtg downregulation; lhβ and erβ upregulation with MA significant for erβ; and MA-specific trends for cyp19a/cyp19b/star).	[78]
PE/Microsphere/10–300 μm/0.1, 2, and 300 mg/L	Primary endpoints: uptake, accumulation, and elimination of MPs (counts of fluorescent microspheres) in daphnia and zebrafish; Fish tissues assessed for MPs: gill and digestive gland; feces collected for removal	72 h	MP10 (0.1 mg/L): only a few particles at 1 h and only individual particles detectable at 12 h; interpreted by authors as inadvertent ingestion. (2 mg/L): accumulation peaked at 6 h with 163.18 particles/fish; then declined with time. MPs in gut (67.39% at 6 h; 53.85% at 12 h), while Exp2–Exp4 showed most MPs in feces; after 12 h in	[79]

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

		quantification in pathway experiment.		Exp2–Exp4, fecal proportions ranged ~87.86% to 98.22%.	
	PE/Not disclosed/ PE-MPs: VPE or APE at 1 mg/L (non-lethal; LC50 of MPs alone >100 mg/L)	Oxidative stress: Enzymes: SOD, CAT; Histopathology; Immune gene expression: Genes: TLR-2, TLR-4, MyD88, NF-κB, NF-κB1, c-Rel, TNF-α, TNF-β, IL-1β; Immune protein: NF-κB; Gut microbiome.	35 d	Virgin polyethylene microplastics (VPE) and aged polyethylene microplastics (APE) caused significant changes in the intestinal microbial community. Both VPE and APE increased the relative abundance of Proteobacteria and reduced Fusobacteria. Oxidative stress responses: Lipid peroxidation (MDA) was increased in the APE group (~3.99%). Antioxidant enzyme activities indicated compensatory responses: Superoxide dismutase (SOD) activity was increased in VPE-treated fish. Catalase (CAT) activity indicated adaptive responses related to oxidative stress. Immune-related gene expression Microplastic exposure increased intestinal immune signaling pathway markers: TLR-2, c-Rel APE treatment caused more immune activation than VPE. Intestinal barrier and inflammation Indications of intestinal stress and inflammation related to microbiota imbalance and oxidative status.	[80]
	Virgin & Artificially weathered PP & PS/PS: ~15–36 μm PP: ~50–148 μm; After weathering: (≤230 μm)/Environmentally relevant (particle based): 2,000, 20,000, 200,000 MP·L^{-1}; High concentrations (mass based):12.5, 25, 50, 100 mg·L^{-1}	Malformations (edema, scoliosis, hemorrhage), Heart rate, Body length, Swimming bladder, microplastic–chorion interaction	96 hours (outcomes recorded at 24/48/72/96 h; heart rate at 72 h; length at 96 h)	2,000 MP·L^{-1}: ↓ heart rate, ↓ body length. 20,000 MP·L^{-1}: Sublethal growth effects. 200,000 MP·L^{-1}: No linear in-crease in toxicity. 12.5–100 mg·L^{-1}: No significant embryotoxicity.	[37]
	Weathered PE/Fragmented/32 μm/1 μg/L	Innate immune metrics: lysozyme, antimicrobial, antiprotease activity; Hematology: differential counts + RBC indices (MCV/MCH/MCHC discussion indicates anemia typing); Stress physiology: plasma cortisol elevated (stress response).	40 d	Results 1 μg/L: Significant modulation of lysozyme, antimicrobial, antiprotease activity, plus altered blood differential counts; Male fish more susceptible than females after chronic exposure; Hematological interpretation suggests macrocytic-type anemia signatures; (MCV/MCH changes; MCHC decreased in both sexes after 40 d, with weaker change in females); Cortisol increased, consistent with chronic stress from MP accumulation	[81]
Embryos	PE/Spherical/10–150 μm/100 mg/L, 500 mg/L, and 1000 mg/L	Cellular & Physiological: Microplastic accumulation (fluorescence microscopy), Excretion patterns, Reactive oxygen species (ROS) – H_2DCFDA staining,	Up to 120 hpf (5 days); excretion observed up to 11 d	100 mg/L: Accumulation: Detected at ≥96 hpf (eye, gut, liver); ROS: ↑ ROS (moderate); Cell Death: ↑ apoptosis; Gene Expression: Early antioxidant gene upregulation. 500 mg/L: Accumulation: Increased accumulation; ROS: ↑↑ ROS; Cell Death: ↑↑ apoptosis; Gene Expression: Downregulation of antioxidant genes.	[82]

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

	Cell death – Acridine Orange staining. Molecular (qRT-PCR): Antioxidant genes: sod2, cat, hmox1, nfe2l2a, keap1a, DNA damage / apoptosis genes: puma, mdm2, tp53.		1000 mg/L: Accumulation: Highest accumulation; ROS: ↑↑↑ROS; Cell Death: ↑↑↑ apoptosis; Gene Expression: Strong oxidative stress & DNA damage response.	
Virgin & Chlorinated PS/Both Pristine and Chlorinated MPs ~5 μm /0.25 mg/L, 1.0mg/L, and 4.0 mg/L	Intestinal histopathology, gut microbiota, oxidative stress biomarkers in gut (SOD, CAT, MDA), immune signaling (gene transcription) and nf-κb protein.	96–120 hpf	Gut microbiota: Chao1 index: increased significantly in VPE-only and APE-only groups vs control; VPE-only higher than APE-only. Shannon index: increased significantly in VPE-only and APE-only groups vs control; VPE-only showed the largest increase (~2.88× control). After 7 days: SOD and CAT activities in "other treatment groups" (i.e., non-PTH-alone) increased significantly (range given 17.32%–143.84%). After 14 days: CAT activity remained elevated in each treatment group vs control (increase ~55.00%–113.18%); MDA in the APE-only group was significantly increased by 3.99% vs control. After 21 days: SOD activity in the PE-MPs treatment group returned to baseline levels; MDA content decreased in all treatment groups by ~17.03%–57.70% vs control. VPE-only (gene expression): TLR-2, MyD88, c-Rel, TNF-β, and IL-1β increased by 1.13–1.79× vs control. APE-only (gene expression): TLR-2 and c-Rel upregulated (~2.10× and ~1.95×), while NF-κB1 and IL-1β downregulated (~0.61× and ~0.73×) vs control. nf-κb protein content in gut: VPE-only and APE-only did not show a significant change vs control (the significant change described is for penthiopyrad alone and for combined groups).	[83]
PE/Microsphere/8.0 μm/50 μg/L and 500 μg/L	qRT-PCR gene expression: IGF-related: igf1, igf2a, igf2b, igfra, igfrb; GH-related: ghrh, gh1, ghra, ghrb.	72 h	Low MPs (50 μg/L) and low PFOS (0.02 μg/L) can activate gene expression rapidly (short time window). High MPs (500 μg/L) and high PFOS (0.1 μg/L) activate genes rapidly and sustain elevated expression longer.	[74]
PS/Spheres/0.1 μm diameter/0, 0.1, 1, 10, 50, 100 mg/L	Oxidative stress: ROS (DCFH-DA), antioxidant/related markers (later sections); Apoptosis: acridine orange staining; Transcriptomics + metabolomics; DEG/GO/KEGG (cell cycle, retinol, ferroptosis, p53).	96 h	Oxidative stress: ROS production was measured in larvae following embryo-larval exposure, with a PS-MPs-alone group added ("Larvae exposed to 1 mg/L PS"). Apoptosis: acridine orange (AO) staining-based apoptosis analysis included a PS-MPs-alone group ("Larvae exposed to 1 mg/L PS").	[48]

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

Embryos → Larvae	PS/Spherical/1 μm in diameter/0.1, 1, and 10 mg/L	Oxidative biomarkers: SOD, CAT, GPx, MDA, ROS	21 d	0.1 mg/L: Slight oxidative stress; mild increase in SOD and CAT activities; no behavioral alteration. 10 mg/L: High oxidative stress (↑MDA levels 2× control); reduced swimming velocity; histopathological changes in liver	[85]
	PE/Mean diameter 58.9 ± 4.52 μm / 0.0, 12.5, 50 and 100 mg. L-1	Juveniles: Gastrointestinal retention and depuration of PE microplastics. Adults: Histology: Organs analyzed: intestine, gills, liver. Adult: Genotoxicity and Cytotoxicity: Micronucleus test, nuclear abnormalities, Comet assay (alkaline). Adults: Biochemical Biomarkers: Acetylcholinesterase (AChE), Glutathione-S-transferase (GST), Lactate dehydrogenase (LDH)	Embryo: 96 h, Juvenile: 72 h, Adult: 96 h	Juveniles' depuration: after exposure, the gastrointestinal tract eliminated the microplastics gradually; after 15 days in clean water (post-exposure), the intestinal lumen agglomerate disappeared (recovery test). Adult tissue distribution/histology: PE microplastics agglomerated with fecal content in the intestinal lumen and were detectable there, but were not observed in the intestinal wall/villi, nor in gill or liver. Genotoxicity/cytotoxicity: micronucleus test showed no chromosome breaks/malsegregation and no nuclear abnormalities across exposure levels ($p > 0.05$). Comet assay: no increase in DNA break indices across exposure levels ($p > 0.05$), except the positive control (H_2O_2 0.1%, $p < 0.05$). Neurotoxicity-related biomarker (AChE): AChE activity in adult head showed significant differences at 50 and 100 mg/L versus control (*** $p < 0.001$), while tail AChE showed no difference ($p > 0.05$). LDH (tail): LDH activity was not modified versus control ($p > 0.05$). GST: body GST activity significantly decreased at 50 and 100 mg/L (* $p < 0.05$), while gill GST activity increased with a concentration–effect relationship (*** $p < 0.001$).	[86]
	Fluorescent plastic microspheres/Spherical/ 1-5 μm/ 2 mg/L (~1.09 × 10^8 particles/L).	Gene expression: Neurogenesis / proliferation: sox2, pcna, ngn1, neuroD, olig2; Motor neuron development: islet1, islet2a, islet2b; Epigenetic regulation; dnmt1, dnmt3–dnmt8; Related to antioxidant activity (sod1, cat), apoptosis (casp3, casp8, casp9), neurogenesis (pcna, sox2), and neurotransmitter systems (cholinergic, serotonergic, dopaminergic) Cellular and Histopathology endpoints: Immunohistochemistry (PCNA, ISL1&2), Stereological analysis of	2 hpf to 14 dpf.	Virgin MPs (proprietary polymer): Inhibited GPx activity; upregulated sod1, casp8, casp9, casp3, th, and slc6a3 genes; increased AChE activity. Induced behavioral changes in mean speed and distance moved. Apoptosis: MPs alone significantly increased the expression of apoptosis-related genes (casp3, casp8, casp9).	[49]

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

	retina and brain, Histopathology (retina, brain).			
PS/Spherical/20 µm/2 mg/L	Thyroid axis parameters: T3, T4, TSH. Metabolomics: metabolites such as BHA, arachidonic acid and glycerophospholipid pathways.	7 d	Uptake/distribution: After 7 days, the 20 µm fluorescent polystyrene MPs were mainly found accumulated in the GI tract of the larvae (none were found in the controls). Thyroid axis endpoint: MPs did not significantly change T3. No significant difference in the results in metabolomic analysis.	[87]
PS/Spherical/5 µm and 10 µm/1, 10, and 100 mg/L	Molecular: Oxidative stress biomarkers, Antioxidant enzyme activities, Lipid peroxidation, Expression of heart-related genes. Histopathological: Heart tissue structure, Cardiomyocyte alterations, Apoptotic markers.	96 hpf	Oxidative damage and genotoxicity in heart tissue after dietary PS-MPs exposure (21 days): lipid peroxidation was higher in PS-MPs treated fish (reported as +528.5% vs control), and DNA damage was higher (reported as ~100× higher vs control). Autophagy markers: LC3 II/I ratio increased (reported as 2.2-fold higher) and SQSTM1/p62 decreased (reported as 2.8-fold lower) in PS-MPs treated fish vs control. Apoptosis markers: Bax/Bcl-2 ratio increased (reported as 5.1-fold higher) and caspase-3 and caspase-9 increased (reported as 2.5-fold higher) in PS-MPs treated fish vs control. Heart metabolomics (PS-MPs exposed vs control): the metabolic profile of heart tissue was altered, with most metabolites reduced; pyruvic acid (+38%) and acetylcarnitine (+14%) increased, while TCA intermediates (e.g., succinic acid −75%, α-ketoglutaric acid −56%) and multiple amino acids were reduced.	[88]
PS/Microbeads/5 ± 3 µm & 50 ± 3 µm/10 mg/L at 5 µm (MPs-5) or 50 µm (MPs-50)	Oxidative stress and oxidative damage genes: ROS, MDA, DNA damage markers, CAT; Apoptosis related gene expression such as p53, Bax, and Bcl-2; Dioxin-like marker genes (CYP1A1 and CYP1B1).	Up to 96 hpf	Oxidative stress and oxidative damage: increased ROS with associated increases in lipid peroxidation (MDA) and oxidative DNA damage marker (8-OHdG), and activation/induction of antioxidant enzymes like SOD and CAT. Apoptosis related genes: p53 and Bax were upregulated and Bcl-2 was downregulated vs controls and caspase 3, 8, 9 were upregulated in PS-MPs exposed treatments. At 5µm PS-MPs: CYP1A1 and CYP1B1 significantly upregulated vs control (CK). And at 50 µm PS-MPs: no significant difference vs control (CK) for these CYP responses.	[55]
PP/Mixed fragments/11.9–44.6 µm/0 mg/L, 1 mg/L, 10 mg/L, 100 mg/L	Gut MP load; Oxidative stress; ROS; SOD, CAT; Neurotoxicity; Acetylcholinesterase (AChE);	28 d	0 (control): No effect 1 mg/L: Mild ROS elevation, Early antioxidant imbalance.	[89]

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

	Histopathology; Liver; Brain.		10 mg/L: Significant oxidative stress, Liver histological damage, Increased AChE activity. 100 mg/L: Severe oxidative stress, Marked hepatic and neural injury, Apoptosis of blood cell, High MP bioaccumulation in gut.	
PE/0–10 mm/ 0 mg/L, 10 mg/L, 100 mg/L, 1000 mg/L	Gut microbiota: qPCR at phylum level (all 4 groups) + 16S sequencing. Biochemical indicators (physiology): TG, GLU, TCHO, TBA, LDL, HDL, pyruvic acid, NEFA. Gene expression (glycolipid & phospholipid metabolism): RT-qPCR in 0/10/100/1000 mg/L groups. Metabolomics: Nontargeted LC-MS metabolomics only for Control vs 1000 mg/L (6 parallels; 400 larvae/sample).	7 d	0 mg/L (control): Nontargeted LC-MS metabolomics only for Control vs 1000 mg/L (6 parallels; 400 larvae/sample). 10 mg/L: Minimal to lower significant. 100 mg/L: Microbiome (qPCR, phylum level): Firmicutes and Bacteroidetes significantly lower vs control; Actinobacteria, β-Proteobacteria, γ-Proteobacteria significantly reduced vs control (also true at 1000). 1000 mg/L: Microbiome (16S sequencing vs control): alpha diversity shifts; OTUs decrease; clear separation in beta diversity (PCA), Proteobacteria/Chloroflexi/Fusobacteria ↑; Firmicutes/Bacteroidetes/Actinobacteria and others ↓, Genus-level dysbiosis (Aeromonas/Shewanella etc ↑; many beneficial taxa ↓), Biochemical indicators: TG, TCHO, NEFA, TBA, GLU significantly increased; pyruvic acid significantly decreased; LDL/HDL show a decreasing trend, Glycolysis/glucose genes: PK, HK1, GK decreased significantly at 1000 mg/L; PEPCKc decreased at 100 and 1000.	[90]
PS/Spherical/1 μm diameter /100 μg/L and 1000 μg/L	Gene expression (il1b, cat, sod).	4 hpf → 96–120 hpf.	100 μg/L: No effect on the gene expression. 1000 μg/L: There is significant ↑ inflammatory gene (il1b), ↑ oxidative stress marker (cat).	[91]
Not disclosed/~1 μm diameter/MPs suspended at 0.006%, 0.0045%, 0.003%, 0.0015% solids	Microcirculation / RBC velocity using high-speed CCD and micro-PIV analysis. And heart morphology.	Exposed for 3 days (1 dpf → 4 dpf)	The caudal artery (CA), the systole–diastole cycle duration was elongated in MP3-ZF without changing RBC velocity. And despite the duration and magnitude of RBC velocity at the dorsal artery (DA) were not significantly altered.	[52]
PS/Not disclosed/1, 10, 100 μg/L	Endpoints measured: Cardiac development: heart rate (HR) at multiple hpf; cardiac morphology; SV–BA distance; histopathology, Thrombosis: thrombus in caudal vein in 100 μg/L MC-LR + PS-MPs/NPs group, Angiogenesis: inhibited angiogenesis (DLAV/ISV loss) in Tg(kdrl:EGFP) model; amelioration by ASTA, Oxidative stress + inflammation: ROS staining; MDA/SOD/GSH; IL-	7 d	Results: At 96 & 168 hpf: MC-LR + PS-MPs → lower heart rate vs control and MC-LR alone groups. 100 μg/L MC-LR + PS-MPs: thrombus observed; vascular loss; oxidative stress/inflammation increased; ASTA provides partial rescue.	[92]

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

	6/IL-8 mRNA by qRT-PCR at 168 hpf, Gene expression pathways: cardiovascular development genes & calcium signaling pathway genes altered (heatmaps).			
PGA, PLA, PBS, PHA, PBAT/No data/1 mg/L and 100 mg/L for each polymer	Thyroid axis / molecular endpoints: Hormones (ELISA): T3, T4, TSH in 120 hpf larvae (tested using 100 mg/L groups); Gene expression (qRT-PCR): HPT axis genes + eye/retina development genes (samples taken at 72 hpf; again, focusing on 100 mg/L groups for gene work).	3, 6, 10, 24, and 96 hpf	1 mg/L: Survival: significantly decreased for PHA (1 mg/L) and PBAT (1 mg/L) at 96 hpf; Body length / head area: reduced in most low-dose groups except PGA 1 mg/L (which showed no significant reduction). Eye area: decreased in most low-dose groups except PGA 1 mg/L and PBAT 1 mg/L (no significant decrease reported for those two). Retina thickness (5 dpf): IPL decreased at PGA 1 mg/L (reported significant reduction); ONL decreased at PBS 1 mg/L and PBAT 1 mg/L; RGL decreased in all 1 mg/L groups (and also all 100 mg/L groups). Visually-mediated behavior (light–dark): In light, locomotor parameters were reduced in all treatment groups (interpreted as inhibited motor ability during light period. 100 mg/L: Early embryonic development: PBS, PHA, PBAT (100 mg/L) caused developmental delay at 3 hpf (PBS notably with embryonic cell mound defects). Retina & eye-development gene expression: Eye/retina genes (e.g., pax6a, pax6b, rx1, gnat2, grk1b, opn1mw1) were abnormally altered; notably, PGA/PLA/PBAT 100 mg/L significantly decreased multiple eye/retina development genes. RGL thickness decreased in all groups, supporting retinal injury across polymers/doses. Thigmotaxis (3 dpf): Thigmotaxis increased significantly in PGA, PLA, PBS, PBAT at 100 mg/L, described as anxiety-like behavior. Visually-mediated behavior (5 dpf): PBS 100 mg/L is explicitly flagged as disrupting visually-mediated behavior (impaired light-to-dark response pattern). Thyroid hormones (ELISA, 100 mg/L only): TSH: no significant change overall. T3 and T4: significantly decreased in PHA 100 mg/L and PBAT 100 mg/L; not significantly changed for PGA/PLA/PBS 100 mg/L. HPT axis gene disruption (100 mg/L groups): crh down-regulated (notably PGA/PBS/PHA), tshβ up-regulated in	[52]

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

				all groups. dio1 and ttr generally down; dio2 up with PLA 100 mg/L. TH synthesis genes: tg down in PBAT; nis down in PLA and PBAT; tpo up in PBAT. TH receptors: thrab increased in PGA/PBS/PHA/PBAT; thrb decreased in PLA but increased in PBS/PHA/PBAT.	
	PS/Microsphere/5 µm/1 mg/L	Oxidative stress biomarkers: CAT, SOD, GPX activities; MDA level; ROS measurement (DCFH-DA assay described); TG level (lipid-related index) at 7 dpf.	2 h post-fertilization (hpf) and continued to 7 days post-fertilization (dpf).	Oxidative stress biomarker: CAT down by 1.55× (NPs) and 1.22× (MPs), SOD down by 1.76× (NPs) and 1.62× (MPs), GPX down by 1.96× (NPs) and 1.58× (MPs), ROS up by 1.20× (NPs) and 1.41× (MPs).	[43]
	PET & PE/Irregular fragments/Average 30–100 µm/1 mg/L & 10 mg/L	Enzymatic biomarkers (EROD, AChE), reproductive output (fecundity/fertility), and offspring (F1) fitness.	0 to 96- or 120-hours post-fertilization (hpf)	Biomarkers: EROD and AChE activities were altered only in marine medaka, not zebrafish.	[54]
Larvae	PS/ 0.2, 1.0, and 10 µm/20 µg/mL for all particle sizes (0.2, 1.0, 10 µm)	Mortality; Vascular development; CYP1A Activity (EROD Assay). Oxidative Stress (ROS); Cell Death (Acridine Orange Staining).	Larvae: Chorion removed at 24 hpf, Exposure from 24–96 hpf (72 h post-initiation).	CYP1A activity (EROD, heart region): PS-MPs alone did not induce CYP1A activity. ROS in heart region: PSMPs alone were not significantly different from control for ROS. Cell death (acridine orange signal, heart region): PSMPs alone did not affect cell death.	[93]
	PS & PVC/Spherical/PS (Spherical ~7.0 µm mean size) PVC (Spherical ~3.8 µm mean size)/ Both MPS 20 mg L^{-1}	Histopathology: Intestinal MP deposition, Inflammation, Pleural effusion, Cellular damage (H&E staining). Molecular and Biochemical: Oxidative stress genes (sod, cat, gpx1a, gpx4a, gstt1a), Inflammation genes (tnf-α, il-6), Apoptosis / p53 pathway genes (tp53, casp3, rrm2), Development & metabolism genes (egr2, egr4, fosab, fosb, cyp3a65).	10 d	MPs visibly accumulated in intestinal tract, Inflammatory infiltration and pleural effusion observed, PS caused slightly more damage than PVC. Gene expression: MPs alone significantly upregulated oxidative stress genes, PS induced stronger oxidative stress than PVC. PS MPs were more toxic than PVC MPs to zebrafish larvae, Joint toxicity depended strongly on MP polymer type; Oxidative stress was the central mechanism linking mortality, behavior, histopathology, and gene expression.	[57]
	PP/Irregular fragments/8–10 µm/ 0 mg/L (control), 1 mg/L, 10 mg/L, 100 mg/L	Cytoxicity and tissue damage: Liver histopathology, Intestinal histopathology, Cellular vacuolization, Inflammatory infiltration. Oxidative stress biomarkers: Reactive oxygen species (ROS), Superoxide dismutase (SOD), Catalase (CAT), Malondialdehyde (MDA). Apoptosis and Molecular markers: Caspase-3 activity, bax, bcl-2 gene transcription, p53 transcription.	96 h (acute exposure)	Control: Normal liver and intestinal morphology, Baseline ROS, enzyme activity, and gene transcription. 1 mg/L: Slight increase in ROS, No significant histological damage, Minor antioxidant response. 10 mg/L: Significant ROS elevation, Decreased SOD and CAT, Increased MDA, Mild liver vacuolization, Increased bax and caspase-3 expression, Reduced ATP levels. 100 mg/L: Severe oxidative stress, Strong lipid peroxidation, marked liver and intestinal tissue damage; High apoptosis activation, Suppressed AChE activity, Significant metabolic disruption.	[94]

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

	Neurotoxicity and metabolic Endpoints: Acetylcholinesterase (AChE) & ATP content.			
PS/5 μm/1 mg/L	Toxicity endpoints (larval): Oxidative stress gene expression such as GSH/GSSG.	7 d	Oxidative-stress–linked metabolite: Glutathione (GSH) increased in the MPs-only group (fold change 1.17). GSH/GSSG: No significant different in the results.	[95]
PS/~25 μm in diameter/250 μg/L	Apoptosis markers: bax/bcl2, caspase3; AO staining brain.	Early dpf stages	Apoptosis-related genes (whole larvae): PS-MPs upregulated the bax/bcl2 ratio and caspase3 expression at 1 dpf, but not at 3 dpf and 5 dpf. Apoptosis staining (brain region): At 5 dpf, there were no apoptotic bodies in the larval brain regions exposed to PS-MPs.	[61]
Virgin & UV-aged PA/Irregular fragments/~5 μm (mean particle diameter)/0, 10, 100, and 1,000 μg/L	Intestinal health: Intestinal morphology (H&E staining), Goblet cell number, Tight junction–related gene expression. Lipid absorption: Oil Red O staining and Triglyceride content. Molecular responses: Lipid metabolism genes (fabp, apoa1, etc.), and Intestinal barrier genes (zo-1, claudin).	2 d post-fertilization (dpf) → 10 dpf	Mild changes in the intestinal structure were noticed, such as slight desquamation of enterocytes and vacuolation in the intestinal mucosa. Oxidative stress responses were triggered, as evidenced by enhanced production of reactive oxygen species and changes in the activity of antioxidant enzymes. There was impairment in lipid metabolism, as evidenced by: Decreased expression of lipid metabolites (triglycerides, diglycerides, monoglycerides, cholesterol esters, phospholipids). Inhibition of lipoprotein lipase activity: Decreased expression of genes involved in fat digestion and absorption. Decreased triglyceride and cholesterol concentrations, suggesting impaired triglyceride and cholesterol absorption.	[59]
Virgin & Photo-aged PS/Virgin: 10 μm Photoaged: 6.5 μm/0.1–100 μg/L for both V-PS and P-PS.	Oxidative stress biomarkers: SOD, CAT, GST, MDA; \Molecular: expression of neurotransmission genes (ache, drd3, 5ht2c, gat1, etc.) and oxidative stress genes (cat1, sod1, gpx1a, gstrl, etc.).	1 hfp to 120 hpf	Oxidative stress: antioxidant enzymes + MDA significantly altered at 10–100 μg/L P-PS. As well as the SOD, CAT, and GST are significantly altered.	[60]
PS/Virgin & Photoaged/Spherical beads/1 μm/0, 1, 10, and 100 μg/L both Virgin & Photoaged	Molecular responses: Lipid metabolism genes (fabp, apoa1, etc.), and Intestinal barrier genes (zo-1, claudin).	96 h post-fertilization (hpf)	Photoaged PS MPs: caused intestinal epithelial damage, Reduced goblet cell number, disrupted intestinal barrier genes, severely impaired lipid absorption.	[58]

Abbreviations: PS—polystyrene; PP—polypropylene; PE—polyethylene; PES—polyester; PVC—polyvinyl chloride; PGA—polyglycolic acid; PBAT—polybutylene adipate terephthalate; PLA—polylactic acid; PHA—polyhydroxyalkanoate; PBS—polybutylene succinate; PET—polyethylene terephthalate; PA—polyamide; MPs—microplastics; hpf—hours post-fertilization; dpf—days post-fertilization; h—hours; d—days; w—weeks; DNMT—DNA methyltransferase; PCNA—proliferating cell nuclear antigen; 5-HT—5-hydroxytryptamine; GABA—gamma-aminobutyric acid; DA—dopamine; Ach—acetylcholine; AChE—acetylcholinesterase; ChAT—choline acetyltransferase; ChE—cholinesterase; AgNP—silver nanoparticles; GFP—green fluorescent protein; SOD—Superoxide dismutase; CAT—Catalase; GPx—Glutathione peroxidase; MDA—

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

Malondialdehyde; ROS—Reactive oxygen species; GR—Glutathione reductase; GI—Gastrointestinal; PCoA—Principal Coordinate Analysis; LDH—Lactate dehydrogenase; GST—Glutathione S-transferase; CYP1A—Cytochrome P450 1A; EROD—Ethoxyresorufin-O-deethylase; HMOX1—Heme oxygenase 1; nfe212a—Nuclear factor erythroid 2-related factor 2a; keap1a—Kelch-like ECH-associated protein 1a; tp53—tumor protein p53; NEFA—Non-esterified fatty acids; LDL—Low-density lipoprotein; T-CHO—Total cholesterol; TG—Triglycerides; GSH—Reduced glutathione; Mn-sod—Manganese superoxide dismutase; Bcl2—B-cell lymphoma 2; UV—ultra-violet; PPM—parts per million; ↑—increased; ↓—decreased; →—progression.

5. Neurobehavioral and Their Corresponding Effects in Zebrafish Exposed to Microplastics

Neurobehavioral results across studies show that microplastic exposure can significantly alter locomotor activity in larval and juvenile zebrafish, with responses strongly influenced by particle concentration, size, and exposure duration. In zebrafish larvae exposed to PS and PVC MPs at 20 mg/L for 10 days resulted in clear locomotor impairment. In this experiment, PS exposure produced higher mortality (~10–20%) and markedly suppressed swimming activity, whereas PVC exposure produced moderate locomotor inhibition and lower mortality, indicating polymer-specific differences in behavioral toxicity [57]. Also, zebrafish larvae exposed to PS MPs with a particle size of approximately 25 μm at concentrations of 25 and 250 μg/L showed no significant changes in locomotor behavior, including movement distance, average velocity, and maximum acceleration during light–dark cycle assays [61]. In comparison, zebrafish exposed to PS microbeads (5 ± 3 μm) at 10 mg/L resulted in 31.25% larval malformation and 11.11% mortality, with associated reductions in swimming performance due to developmental abnormalities affecting tail morphology and neuromuscular coordination [56].

Also, neurotransmitter regulation and cholinergic function were strongly affected by MP exposure under defined experimental conditions. In the study of Zhang et al. 2022 [59], zebrafish larvae exposed to virgin PS MPs with a particle size of ~1 μm at concentrations of 1, 10, and 100 μg/L from 1 hpf to 120 hpf showed significant alterations in several neurotransmitters, including serotonin (5-HT), dopamine (DA), γ-aminobutyric acid (GABA), and acetylcholine (ACh). These exposures also altered cholinergic enzyme activities, including acetylcholinesterase (AChE), choline acetyltransferase (ChAT), and cholinesterase (ChE), indicating disruption of neurotransmission pathways associated with neural development and behavioral regulation.

Additional experiments demonstrate that the direction of AChE modulation varies depending on polymer type, particle size, and exposure conditions. For instance, zebrafish larvae exposed to spherical MPs (1–5 μm) at 2 mg/L from 2 hpf to 14 dpf showed significant inhibition of AChE activity, accompanied by increased reactive oxygen species (ROS), lipid peroxidation (LPO), and altered antioxidant biomarkers including SOD, CAT, GPx, GST, and GR, linking oxidative stress to neurophysiological impairment [64]. And neurochemical alterations were observed when zebrafish were exposed to PS particles of 50 nm and 45 μm from 4 to 120 hpf, where AChE activity decreased while antioxidant enzymes including CAT and GPx increased, together with elevated glutathione (GSH) levels and reduced body length (≈6.1%), suggesting that oxidative stress-mediated neuronal disruption contributed to impaired neurodevelopment and locomotor activity [89].

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

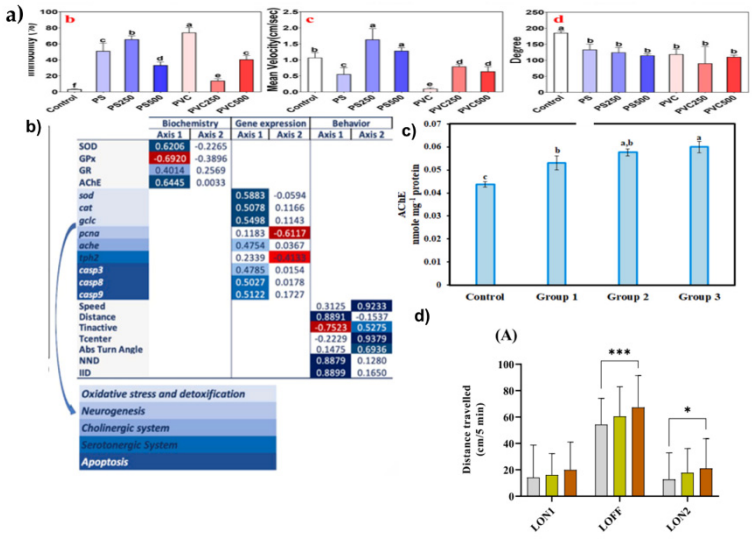

Figure 8. Neurobehavioral, neurochemical, and cardiovascular effects of microplastic exposure in zebrafish. (a) Locomotor behavior parameters (immobility, mean velocity, and movement angle) showing dose- and polymer-dependent behavioral alterations following PS and PVC exposure [57]. (b) Multivariate analysis (loading scores) linking biochemical markers, gene expression, and behavioral parameters to oxidative stress, neurogenesis, cholinergic/serotonergic systems, and apoptosis [59]. (c) Acetylcholinesterase (AChE) activity levels across groups, reflecting neurotoxicity effects [64]. (d) locomotor response (distance traveled) under light–dark conditions (LON/LON2, LOFF), showing significant behavioral alterations in exposed groups [54].

Additionally, gene expression studies revealed that MP exposure can disrupt neurodevelopmental and neurofunctional processes in zebrafish under specific particle properties and exposure conditions. In the study of Li et al. 2024 [47], zebrafish embryos exposed to artificially aged PS MPs with a particle size of ~1 μm at concentrations of 0.1, 1, 10, and 100 μg/L exhibited significant alterations in neuronal development. After exposure until 120 hpf, aged PS particles reduced motor neuron fluorescence intensity from 238 ± 2.58 AU in controls to approximately 229 ± 2.53 AU at 100 μg/L, indicating impaired motor neuron formation. Gene expression analysis showed downregulation of neurodevelopment-related genes such as gabra1 and manf and upregulation of neural progenitor and structural genes including nestin, gfap, alpha-tubulin, and mbp, suggesting dysregulated neuronal differentiation and glial activation during early nervous system formation.

In addition, exposure of zebrafish embryos to fluorescent plastic microspheres (1–5 μm) at 2 mg/L from 2 hpf to 14 dpf resulted in abnormal neurodevelopmental signaling in retinal tissues. These particles accumulated in the retina and caused increased PCNA-positive proliferating cells, indicating compensatory cell proliferation. However, transcription of key neurogenesis genes such as sox2, neuroD, and olig2 was significantly downregulated, and DNA methyltransferase (DNMT) expression was altered, suggesting that microplastics can interfere with both neural differentiation pathways and epigenetic regulatory mechanisms controlling neurodevelopment [49]. Additional evidence of neuronal damage was observed in experiments where zebrafish exposed to PS MPs (~5 μm) at concentrations of 1–100 mg/L exhibited significant activation of apoptosis pathways. Neural tissues showed upregulation of apoptosis-related genes including casp3, casp8, and casp9 together with oxidative stress biomarkers, indicating activation of programmed cell death pathways in response to microplastic-induced cellular damage [86].

Cardiovascular and neurobehavioral integration was also documented under defined exposure conditions. In the study of La Pietra et al. 2024 [36], zebrafish embryos exposed to spherical PS microbeads with particle sizes of 1 and 5 μm at concentrations of 10, 100, and 1000 μg/L for 96 hpf showed measurable cardiotoxic effects. At 1000 μg/L, embryos exhibited a ~13% reduction in heart rate relative to controls, accompanied by developmental delay and increased deformities, including ~22% malformed embryos with pericardial edema and spinal curvature. These cardiovascular

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

impairments were associated with reduced locomotor performance and altered physiological activity during larval development [36]. Cardiac oxidative injury was further demonstrated in chronic exposure experiments using spherical PS microplastics (~5 μm) at concentrations of 1, 10, and 100 mg/L. Metabolomic and biochemical analyses revealed severe oxidative damage in cardiac tissue, including a ~528.5% increase in lipid peroxidation markers and an approximately 100-fold increase in DNA oxidative damage indicators compared with control groups. These responses were accompanied by significant activation of apoptosis-related pathways, including increased Bax/Bcl-2 ratio and elevated expression of caspase-3 and caspase-9, indicating oxidative injury and programmed cell death in cardiac tissues [88].

Co-exposure studies demonstrated that MPs can intensify toxicity of other environmental contaminants. Zebrafish embryos exposed to spherical PS MPs (~1 μm) at concentrations of 1, 10, and 100 μg/L together with microcystin-LR (MC-LR) showed pronounced cardiovascular disruption. Histological examination revealed thrombus formation in caudal blood vessels, inhibition of angiogenesis, and increased vascular abnormalities compared with single exposures. These combined exposures also produced elevated oxidative stress biomarkers and inflammatory responses, including increased expression of interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), indicating synergistic toxicity between microplastics and cyanobacterial toxins [48]. Chronic exposure in zebrafish microplastic studies generally refers to long-term exposure periods extending beyond early embryonic stages, typically ≥21 days to several weeks, during which organisms experience continuous or repeated exposure to microplastics at environmentally relevant or experimental concentrations. Chronic exposure designs are commonly used to evaluate endocrine disruption, metabolic alterations, microbiota shifts, and behavioral outcomes that cannot be detected in short-term acute toxicity assays conducted during early embryogenesis (e.g., 24–96 hpf exposure windows) [45,73,76].

Under these chronic conditions, endocrine–neurobehavioral interactions have been reported. In the study of Li et al. 2022 [73], exposed to PS MPs (~2 μm diameter) at concentrations of 0.1 and 1 mg/L for 63 days exhibited disruption of thyroid hormone regulation. The exposure altered circulating triiodothyronine (T3) and thyroxine (T4) levels and enhanced maternal transfer of thyroid hormones to embryos, which subsequently influenced developmental outcomes and behavioral responses in offspring. These endocrine alterations indicate that long-term microplastic exposure can interfere with the hypothalamic–pituitary–thyroid (HPT) axis, a key regulator of neural development and behavioral regulation [73]. Additional chronic experiments demonstrated interactions between gut physiology and behavioral regulation.

Zebrafish exposed to PS MPs (~5 μm) at 20 μg/L for 21 days developed intestinal barrier disruption, microbiota dysbiosis, and metabolic disturbances. Microbiome analyses showed significant shifts in dominant bacterial taxa, including increased Proteobacteria and altered Fusobacteria and Firmicutes abundance, which coincided with behavioral changes such as increased anxiety-like responses and reduced exploratory activity in locomotor assays. These responses were linked to intestinal inflammation and barrier impairment, indicating involvement of the gut–brain axis in behavioral dysfunction [76]. Bioaccumulation characteristics affect the intensity of neurobehavioral toxicity. Smaller and degraded particles produced stronger biological responses than larger or pristine particles. In the study of Kim et al. 2022 [58], zebrafish larvae exposed to photo-aged PS MPs (~1 μm diameter) at concentrations of 0.1, 1, 10, and 100 μg/L showed stronger developmental and neurodevelopmental impairment than larvae exposed to virgin PS at the same concentrations.

Aged PS exposure reduced motor neuron fluorescence intensity from 238 ± 2.58 AU in controls to 229 ± 2.53 AU at 100 μg/L, together with dysregulation of neurodevelopment-related genes including gabra1 and manf, indicating impaired neuronal differentiation and signaling [58]. Finally, MP exposure alters monoaminergic signaling pathways, including serotonin (5-HT), dopamine (DA), and γ-aminobutyric acid (GABA). These neurotransmitters regulate locomotion, anxiety behavior, and neural development. Changes in neurotransmitter levels occur when oxidative stress disrupts

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

enzymes involved in synthesis and metabolism or when MPs interfere with neuronal gene expression controlling neurotransmitter transport and receptor signaling (Chen et al. 2017 [96]; Ding et al. 2023 [60]). And MPs can interfere with endocrine signaling pathways such as the hypothalamic–pituitary–thyroid (HPT) and hypothalamic–pituitary–gonadal (HPG) axes.

Changes in hormones including thyroxine (T4), triiodothyronine (T3), testosterone, and estradiol affect neuronal differentiation, metabolism, and behavior. MPs may act as endocrine disruptors by adsorbing endocrine-active contaminants or by altering gene expression related to steroidogenesis and thyroid regulation (Luo et al. 2021 [45]; Chen et al. 2017 [96]). Also, MP ingestion often leads to intestinal barrier damage and microbiota dysbiosis. Shifts in microbial communities (e.g., increased Proteobacteria and altered Firmicutes/Fusobacteria) can modify microbial metabolite production and immune signaling. These gut-derived signals influence neural function through the gut–brain axis, contributing to anxiety-like behavior, altered locomotion, and cognitive dysfunction [45].

Table 3. Behavioral effects of pristine/virgin/unspecifies and UV/Photo-aged in MPs exposure.

Life Stage	Plastic Characterization (Type/Shape/Size/Concentrations)	Endpoints	Exposure Time	Effects	Ref.
Adult	PS/Irregular fragments/~400 μm/0.5 mg/L	Behavioral Parameters: Latency to first MP capture, Capture frequency, spitting frequency, Swallowing ratio (%), Feeding duration, Time in feeding zone, Swimming activity, Speed, Total distance moved	10 m per trial	Latency to first capture: Most zebrafish started MP capture within ~10 s of adding MPs. Shy zebrafish showed longer latency than bold zebrafish (not significant). Capture frequency: Bold zebrafish captured MPs significantly more often than shy zebrafish on exposure days ($p < 0.05$). Spitting behavior: Zebrafish often spat MPs out after capture. About 30-47% of total capture events happened in the first minute. Spitting frequency was positively correlated with capture frequency. Swallowing ratio: About 40-60% of captured MPs were swallowed. Bold fish had higher effective exposure because of higher capture frequency. Swimming activity during feeding: Zebrafish showed immediate increase in activity after MP addition. Bold zebrafish showed significantly higher levels of feeding activity (activity %, total distance, average speed) than shy zebrafish on days 2-3 ($p < 0.05$). Bold zebrafish spent significantly more time in feeding areas (upper water layer). Ingestion/egestion behavior: Bold zebrafish ingested more	[96]

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

			MPs than shy zebrafish when exposed to MPs only (significant on day 2). Very few MPs were left in intestines after 3 days, suggesting efficient egestion.	
Propriety polymer (composition undisclosed/Spherical/1–5 µm /2 mg/L	Behavior: Locomotor activity (Open Field Test), anxiety (Light-Dark Test), and social.	30 d	Locomotor activity (Open Field Test): Mean speed: markedly reduced in MPs group (584.1 ± 33.5 cm/min) compared to control (695.9 ± 27.1 cm/min) ($p < 0.01$). Total distance moved: significantly reduced in MPs group (2863.4 ± 1.8 cm) compared to control (3445.8 ± 234.4 cm) ($p = 0.0395$). Absolute turn angle: not significantly different between MPs group and control. Time inactive: significantly increased in MPs group (10.4 ± 0.3%) compared to control (3.0 ± 0.3%) ($p < 0.0001$). Time in center zone: no significant difference between MPs group and control. Anxiety-like behavior (Light-Dark Test): MPs did not significantly alter anxiety-like behavior of the zebrafish. Social/Shoaling behavior: MPs altered social behavior significantly, leading to tighter shoaling patterns.	[64]
PS/Spherical beads/2 µm diameter/ 0.44 mg/L (~10^8 items/L)	Locomotion behavior: Average swimming velocity (ASV), Duration of high mobility (DHM), Frequency of high mobility (FHM), Duration of thigmotaxis (DTH).	7 d	Results: No locomotion alteration. No change in thigmotaxis or high mobility metrics. No change in post-stimulation escape response. While there is a significant increase in shoaling duration. While it enhances the shoaling behavior, it did not induced hyperactivity, hypoactivity, and altered startle performance.	[67]
PE/Not disclosed/60 mg/L	Behavioral endpoints: School cohesion, school depth, distance from predator (anti-predator response deficit inferred).	10 d	Results: Direct exposure to PE MPs at 60 mg/L for 10 days did not significantly alter: Latency to reach the top, time spent in the top zone, anxiety index in open field test, swimming speed, and total distance traveled. Additionally, no significant increase in shoal cohesion when predator was present, impaired defensive	[76]

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

			aggregation response, and reduced prey-predatory distance compared to control.	
PGA/~1 μm in diameter/1 mg/L & 100 mg/L	Behavior: anxiety-like behavior + cognitive/visual preference behaviors (novel tank and color preference experiments).	28 d	After zebrafish exposed to 1 mg/L and 100 mg/L of PGA microplastics for 28 days, some clear changes showed up in their behavior. First, in the Novel Tank Test, the fish started sticking to the bottom more as the concentration went up. Like, their movement got really limited to the bottom zone. Significant decreased in: - Time spent in the top zone - Distance traveled in the top zone - The ratio of time spent top versus bottom - Number of times they entered the top zone But interestingly, their average swimming speed didn't change much. For cognitive effects, researchers used a Color Preference Test with a four-arm maze. Normally, the fish prefer colors in this order: Blue, then no color, then green, red, and yellow last. After exposure: - The high concentration group spent way more time in the blue and no-color areas. Their color preference shifted compared to the control group. - The low concentration group didn't show much change in color preference.	[46]
PS/Spheres/0.1 μm diameter/0, 0.1, 1, 10, 50, 100 mg/L	Locomotion: moving distance, swimming speed.	96 h	Swimming pace through the trial did not change reliably when it compares to the control. The pattern of behavior seen in PS-MPs treated subjects matched levels observed in animals getting no treatment. At 1 mg/L, polystyrene MPs it showed no obvious change in fish behavior after 96 hours. Movement patterns stayed the same regardless of exposure.	[48]

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

Embryos → Larvae	PS/Spherical/1 μm in diameter/0.1, 1, and 10 mg/L	Swimming velocity, mobility ratio, avoidance response.	21 d	Swimming velocity: No significant effect in MPs exposure. Mobility ratio: No significant effect Avoidance response: No significant differences relative to the control for MPs alone.	[85]
	PS/ Spherical/1 μm diameter/100 μg/L and 1000 μg/L	\ Swimming distance, swimming speed, and Dark-light avoidance behavior	4 hpf → 96–120 hpf	Swimming speed dropped by 3.5% when fish were exposed to 1000 μg/L in dark water after 120 hpf. That level reduced movement more than any other. Swimming speed dropped slightly - 1.47 plus or minus 0.31 millimeters per second compared to 1.52 plus or minus 0.29 in healthy animals; that difference turned out statistically significant, p equals 0.027. At 1000 micrograms per liter, overall movement shrunk, cutting the total distance by roughly 3.2 percent. A shift appeared with 15.28 ± 2.81 cm measured against 15.78 ± 2.68 cm baseline values - difference found significant at p = 0.021. Yet exposure of 100 μg/L showed no clear impact during dark swimming trials. Light and dark cycles switched every few days in a test lasting around five days at 120 hpf. Light period: Swimming length and speed showed no clear changes across the board - whether at 100 or 1000 μg/L. Dark period: At 100 μg/L, fish swam shorter distances - by 4.6% fewer beats. Swimming slowed down by 4.9% at 100 μg/L A shift appeared - movement slowed in one condition compared to the other. Speed dropped by about 1.41 to 1.48 millimeters per second. That difference? Highly significant, showing up clearly beyond doubt. The p value hit a near-zero mark after testing. At 1000 μg/L, animals swam 2.8 percent slower. Speed rose slightly in treatment versus control (1.44 ± 0.24 mm/s vs. 1.48 ± 0.23 mm/s; p = 0.0065).	[91]

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

PGA, PLA, PBS, PHA, PBAT/No data/1 mg/L and 100 mg/L for each polymer	Behavioral: Thigmotaxis at 3 dpf (edge preference); Light–dark test at 5 dpf: max speed, total distance, movement count in light vs dark.	3, 6, 10, 24, and 96 hpf	Thigmotaxis behavior in 3-day-old larvae Larvae exposed to strong concentrations - like 100 mg/L - of PGA, PLA, PBS, and PBAT showed a clearer tendency toward edge avoidance. This shift toward thigmotaxis became more pronounced, suggesting heightened anxiety behavior. Even at 100 mg/L, PHA didn't cause much thigmotactic response. At just 1 mg/L, there was barely any change in thigmotaxis worth noting. Larvae at five days old responded to changes in light and dark. During shifts in illumination, their actions were observed. Light at 80 lux levels Every single one of the five natural breakdown microplastics - made from PGA, PLA, PBS, PHA, PBAT - dissolved more easily when tested at both light and heavy doses. Their ability to break down happened clearly across every sample exposure. Dark condition (0 lx): – 100 mg/L PBS and 1 mg/L PBAT caused slower movement, lower overall travel, and fewer shifts in position. – 100 mg/L PHA cut down movement speed and overall motion total. Not every option lowered dark-phase movement - some changes didn't affect it significantly. Light–dark responsiveness: Beyond 100 mg/L PBS plus 1 mg/L PBAT, movement patterns stayed largely unchanged across light-to-dark shifts. These conditions weakened the body's reliance on visual cues when responding to sudden changes. Light-driven reactivity simply fell short under such exposure. Other groups still showed clear differences in movement between light and dark periods.	[52]

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

Larvae	PS & PVC/Spherical/PS (Spherical ~7.0 µm mean size) PVC (Spherical ~3.8 µm mean size)/ Both MPS 20 mg L^{-1}	Immobility duration, Mean swimming velocity, Turn angle (locomotor behavior)	10 d	Locomotor activity: PS & PVC MPs significantly suppressed swimming activity compared to control. Immobility duration: Immobility duration significantly increased in larvae and immobility in PVC group showed U-shaped trajectory, indicating high immobility observed under PVC-MPs exposure. Mean velocity and turn angle: Both PS & PVC-MPs reduced the mean swimming velocity of zebrafish. And turn angle was altered in a concentration-dependent manner patterns.	[57]
	Virgin & Photo-aged PS/Virgin: 10 µm Photoaged: 6.5 µm/0.1–100 µg/L for both V-PS and P-PS.	Behavior: locomotor activity (reduced locomotion).	1 hfp to 120 hpf	Locomotor activity: After 120 hours of development, young zebrafish moved less when exposed to 100 µg/L of virgin PS-MPs than those in clean water. Their ability to swim declined noticeably under that condition. Swimming changes showed up when larvae exposed to photoaged PS-MPs at 1–100 µg/L levels. Speed shifted in response to those doses. P-PS given at 10 and 100 µg/L caused fish to swim slower, with lower average speeds than in the control and V-PS groups ($p < 0.05$). Light–dark response: Most changes happened while the lights were off. The P-PS group showed clearer shifts than others. Correlation with neurochemical biomarkers measures of behavior - like how fast zebrafish swam - tended to drop as brain chemicals such as 5-HT, GABA, and ACh went down. What also followed a similar pattern was the activity of enzymes like AChE and ChE, which moved in step with those neurotransmitters but in opposite directions.	[60]

Abbreviations: PS—polystyrene; PP—polypropylene; PE—polyethylene; PES—polyester; PVC—polyvinyl chloride; PGA—polyglycolic acid; PBAT—polybutylene adipate terephthalate; PLA—polylactic acid; PHA—polyhydroxyalkanoate; PBS—polybutylene succinate; PET—polyethylene terephthalate; PA—polyamide; MPs—microplastics; hpf—hours post-fertilization; dpf—days post-fertilization; h—hours; d—days; w—weeks.

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

6. Summary and Future Perspectives

This systematic review demonstrates that MP exposure causes multi-system biological responses in zebrafish, including developmental toxicity, oxidative and metabolic disorders, barrier disruption, endocrine disruption, neurochemical imbalance, and reproductive toxicity. Developmental toxicity is concentration-dependent, but there are nonlinear or threshold effects, especially when particle number concentrations are compared with mass concentrations. Smaller particles and naturally weathered microplastics are more toxic than larger particles and pristine microplastics, which emphasizes the role of surface reactivity and physicochemical transformation in determining the hazard potential of microplastics.

Oxidative stress emerges as a common mechanistic axis connecting developmental, physiological, and neurobehavioral endpoints. Disruption of antioxidant mechanisms, inflammatory responses, mitochondrial and metabolic changes, and apoptosis signaling provide evidence of systemic stress responses. Changes in the gut microbiome and intestinal barrier dysfunction provide a link between local exposures and systemic metabolic and immune effects. Endocrine-related endpoints, such as steroidogenesis disruption, thyroid hormone modulation, and reproductive effects, suggest transgenerational effects.

Despite the increase in experimental complexity, there are still some critical limitations. The lack of standardization in particle characterization, the variability in concentration reporting, the lack of standardization in aging procedures, and the limitations in study design (multi-generational or long-term studies) are some of the limitations.

The integration of multi-omics approaches to understand systemic toxicity mechanisms. Transcriptomics, metabolomics, proteomics, and epigenomics are increasingly applied to identify molecular pathways involved in oxidative stress, endocrine disruption, metabolic reprogramming, and neurodevelopmental impairment. These approaches allow researchers to identify biomarkers of MP exposure and to clarify how oxidative stress, mitochondrial dysfunction, and inflammatory signaling translate into organism-level effects. Multi-omics integration also allows better understanding of transgenerational responses and epigenetic inheritance, particularly in long-term zebrafish exposure studies.

Another promising area is the use of advanced imaging and tracking technologies to study MP uptake and biodistribution in zebrafish. Fluorescent labeling, confocal microscopy, and high-resolution imaging techniques are increasingly used to track particle accumulation in organs such as the intestine, liver, brain, and reproductive tissues. These tools provide direct visualization of MP internalization and tissue interactions, allowing researchers to connect particle distribution with physiological and behavioral outcomes.

Artificial intelligence and machine learning technologies are emerging as promising tools in MP ecotoxicology research. AI image analysis is seen as having the potential to enhance MP detection, identification, and quantification in environmental samples and biological tissues. Machine learning can also be applied to analyze large datasets from multi-omics approaches to identify predictive toxicity pathways and biomarker networks. In zebrafish behavioral assays, AI video tracking systems are being increasingly applied to quantify locomotor activity, anxiety-like behavior, and social behavior with greater accuracy and without subjective bias.

The collated experimental evidences confirmed that MPs are biologically active stressors in zebrafish, which could trigger coordinated dysfunctions in developmental, physiological, and neurobehavioral levels. The results emphasize the importance of standardized experimental approaches to ensure ecological relevance and risk assessment. In the future direction of using zebrafish as a model for conducting MP toxicity studies, it is recommended that there be an increased focus on advancing the knowledge in this field in terms of mechanistic understanding, ecological relevance, and translational value. Future studies should aim to expand beyond the current single-generation design and include multi-generation studies to assess transgenerational toxicity. Moreover, the use of "omics" technologies such as transcriptomics, proteomics, metabolomics, and

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

epigenomics is essential for advancing our knowledge in the field of microplastic toxicity. Furthermore, standardization of the parameters used in conducting microplastic toxicity studies is essential for increasing the reproducibility and comparison of the data generated in this field. Future studies should also aim to include environmentally relevant microplastics in the studies. This can be achieved by using aged microplastics and those with biofilm formation. Also, future studies should also include co-exposure studies with other environmental contaminants. Moreover, the use of imaging technologies will be useful in precisely assessing the accumulation of microplastics in the tissues. Additionally, further neurobehavioral studies, including circadian rhythm studies and cognitive function tests, are necessary to more fully understand the effects on the nervous system. Interactions between microbe-gut interactions involving MPs should also be explored to understand this relationship. Development of high-throughput screening tools to automate testing procedures for toxicity studies is also important to increase efficiency in testing. The integration of zebrafish-derived data into adverse outcome pathways/ecological risk assessment frameworks is important to fully utilize this model in assessing the overall effects of microplastic pollution.

Finally, it is likely that zebrafish model will feature prominently in high throughput ecotoxicological screening systems. And due to the zebrafish's transparent embryos and larvae MP can easily be tracked using automated systems, enabling a high-throughput approach to test the ecotoxicity of various types of polymers, sizes, and aging states in a simulated environmental scenario. Such a high-throughput system in conjunction with computer models could provide a predictive tool in ecotoxicology for predicting risks posed by these new pollutants.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

Author Contributions: Conceptualization, A.S.Y., C.B.T., and M.V.-T.; method-ology, A.S.Y., C.B.T., and M.V.-T..; software, A.S.Y. and C.B.T.; validation, C.B.T. and M.V.-T.; formal analysis, A.S.Y., C.B.T., and M.V.T.; investigation, T.P., A.O., W.K.F.T., Y.O., C.B.T., and M.V.-T..; resources, T.P., A.O., W.K.F.T., Y.O., C.B.T., and M.V.-T.; data curation, A.S.Y., and C.B.T.; writing—original draft preparation A.S.Y., C.B.T., and M.V.-T.; writing—review and editing, T.P., A.O., W.K.F.T., Y.O., C.B.T., and M.V.-T.; visualization, A.S.Y., C.B.T, and M.V.-T.; supervision, C.B.T., and M.V.-T.; project ad-ministration, T.P., A.O., W.K.F.T., Y.O., C.B.T., and M.V.-T.; funding acquisition, A.O., C.B.T., and M.V.-T. All authors have read and agreed to the published version of the manuscript."

Acknowledgments: The authors would like to thank the Department of Science and Technology-Accelerated Science and Technology Human Resource Development Program and the Department of Research of Mindanao State University-Iligan Institute of Technology (MSU-IIT), Iligan City, Philippines (SO#00228-IIT) for the financial assistance.

Abbreviations

The following abbreviations are used in this manuscript:

5-HT	5-hydroxytryptamine
Ach	Acetylcholine
AChE	Acetylcholinesterase
AgNP	Silver nanoparticles
BDNF	Brain-derived neurotrophic factor
Bcl2	B-cell lymphoma 2
CAT	Catalase
ChAT	Choline acetyltransferase
ChE	Cholinesterase
CYP1A	Cytochrome P450 1A
DA	Acetylcholinesterase
DNMT	DNA methyltransferase
Dpf	Days post-fertilization

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

EROD	Ethoxy resorufin-O-demethylase
GABA	Gamma-aminobutyric acid
GFP	Green fluorescent protein
GI	Gastrointestinal
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Reduced glutathione
GST	Glutathione S-transferase
HMOX1	Heme oxygenase 1
Hpf	Hours post-fertilization
keap1a	Kelch-like ECH-associated protein 1a
LDH	Lactate dehydrogenase
LDL	Low-density lipoprotein
MDA	Malondialdehyde
Mn-sod	Manganese superoxide dismutase
MPs	Microplastics
Mt	Million metrics tons
NE-FA	Non-esterified fatty acids
nfe212a	nuclear factor erythroid 2-related factor 2a
OECD	Organization for Economic Co-operation and Development
PA	Polyamide
PBAT	Polybutylene adipate terephthalate
PBS	Polybutylene succinate
PCNA	Proliferating cell nuclear antigen
PCoA	Principal Coordinate Analysis
PE	Polyethylene
PES	Polyester
PET	Polyethylene terephthalate
PGA	Polyglycolic acid
PHA	Polyhydroxyalkanoate
PLA	Polylactic acid
PP	Polypropylene
PPM	Parts per million
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PS	Polystyrene
PVC	Polyvinyl chloride
ROS	Reactive oxygen species
SOD	Superoxide dismutase
T-CHO	Total cholesterol
TEM	Transmission electron microscopy
TG	Triglycerides
tp53	Tumor protein p53
UV	Ultra-violet
W	Weeks

References

1. Borrelle, S.B., Ringma, J., Law, K.L., Monnahan, C.C., Lebreton, L., McGivern, A., Murphy, E., Jambeck, J., Leonard, G.H., Hilleary, M.A., Eriksen, M., Possingham, H. P., De Frond, H., Gerber, L.R., Polidoro, B., Tahir, A., Bernard, M., Mallos, N., Barnes, M., Rochman, C.M., 2020. Predicted growth in plastic waste exceeds efforts to mitigate plastic pollution. Science 369 (6510), 1515–1518. https://doi.org/10.1126/science.aba3656.
2. Andrady, A.L., 2011. Microplastics in the marine environment. Mar. Pollut. Bull. 62 (8), 1596–1605. https://doi.org/10.1016/j.marpolbul.2011.05.030.
3. Gago, J., Galgani, F., Maes, T., Thompson, R.C., 2016. Microplastics in seawater: recommendations from the marine strategy framework directive implementation process. Front. Mar. Sci. 3 https://doi.org/10.3389/fmars.2016.00219.

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

4. Dokl, M., Copot, A., Krajnc, D., Van Fan, Y., Vujanović, A., Aviso, K. B., Tan, R. R., Kravanja, Z., & Čuček, L. (2024). Global projections of plastic use, end-of-life fate and potential changes in consumption, reduction, recycling and replacement with bioplastics to 2050. Sustainable Production and Consumption, 51, 498–518. https://doi.org/10.1016/j.spc.2024.09.025
5. Kramm, J., Völker, C., & Wagner, M. (2018). Superficial or Substantial: Why Care about Microplastics in the Anthropocene? Environmental Science & Technology, 52(6), 3336–3337. https://doi.org/10.1021/acs.est.8b00790
6. Zhao, Y., Jin, R., Chen, Y., Zhang, J., Tao, S., Liu, S., & Shen, M. (2023). Constructed wetlands as neglected fixed source of microplastics and antibiotic resistance genes in natural water bodies? The Science of the Total Environment, 902, 166474. https://doi.org/10.1016/j.scitotenv.2023.166474
7. Kramm, J., Völker, C., & Wagner, M. (2018). Superficial or Substantial: Why Care about Microplastics in the Anthropocene? Environmental Science & Technology, 52(6), 3336–3337. https://doi.org/10.1021/acs.est.8b00790
8. Amobonye, A., Bhagwat, P., Raveendran, S., Singh, S., & Pillai, S. (2021). Environmental Impacts of Microplastics and Nanoplastics: A Current Overview. Frontiers in Microbiology, 12, 768297. https://doi.org/10.3389/fmicb.2021.768297
9. Hsu, W.T., Domenech, T., McDowall, W., 2021. How circular are plastics in the EU?: MFA of plastics in the EU and pathways to circularity. Clean. Environ. Syst. 2, 100004 https://doi.org/10.1016/j.cesys.2020.100004.
10. 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. https://doi.org/10.1016/j.scitotenv.2018.07.207
11. Atamanalp, M.; Köktürk, M.; Uçar, A.; Duyar, H. A.; Özdemir, S.; Parlak, V.; Esenbuğa, N.; Alak, G. (2021). Microplastics in Tissues (Brain, Gill, Muscle and Gastrointestinal) of Mullus barbatus and Alosa immaculata. Archives of Environmental Contamination and Toxicology, 81(3), 460–469. https://doi.org/10.1007/s00244-021-00885-5
12. Barboza, L. G. A.; Lopes, C.; Oliveira, P.; Bessa, F.; Otero, V.; Henriques, B.; Raimundo, J.; Caetano, M.; Vale, C.; Guilhermino, L. (2019). Microplastics in wild fish from North East Atlantic Ocean and its potential for causing neurotoxic effects, lipid oxidative damage, and human health risks associated with ingestion exposure. The Science of the Total Environment, 717, 134625.https://doi.org/10.1016/j.scitotenv.2019.134625
13. Habib, R. Z.; Thiemann, T. (2021). Microplastic in commercial fish in the Mediterranean Sea, the Red Sea and the Arabian Gulf. Part 1: The Mediterranean Sea. Journal of Water Resource and Protection, 13(08), 563–587. https://doi.org/10.4236/jwarp.2021.138031
14. Bhagat, J., Zang, L., Nishimura, N., & Shimada, Y. (2020). Zebrafish: An emerging model to study microplastic and nanoplastic toxicity. Science of the Total Environment, 728, 138707. https://doi.org/10.1016/j.scitotenv.2020.138707
15. Stock, F.; Narayana, V. K. B.; Scherer, C.; Löder, M. G. J.; Brennholt, N.; Laforsch, C.; Reifferscheid, G. (2020). Pitfalls and limitations in microplastic analyses. In The œhandbook of environmental chemistry (pp. 13–42). https://doi.org/10.1007/698_2020_654
16. Khan, F.R.; Alhewairini, S.S. Zebrafish (Danio rerio) as a Model Organism. In Current Trends in Cancer Management; Streba, L., Gheonea, D.I., Schenker, M., Eds.; IntechOpen: London, UK, 2019.
17. Savuca, A., Nicoara, M. N., Ciobica, A., Gorgan, D. L., Ureche, D., & Balmus, I. M. (2023). Current Aspects on the Plastic Nano- and Microparticles Toxicity in Zebrafish—Focus on the Correlation between Oxidative Stress Responses and Neurodevelopment. Animals, 13(11), 1810. https://doi.org/10.3390/ani13111810
18. 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. https://doi.org/10.1021/acs.est.6b00183
19. 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, 619–620, 1–8. https://doi.org/10.1016/j.scitotenv.2017.11.103

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

20. Smith, M., Love, D. C., Rochman, C. M., & Neff, R. A. (2018). Microplastics in seafood and the implications for human health. Current Environmental Health Reports, 5(3), 375–386. https://doi.org/10.1007/s40572-018-0206-z
21. Bhuyan, M. S. (2022). Effects of microplastics on fish and in human health. Frontiers in Environmental Science, 10, 827289. https://doi.org/10.3389/fenvs.2022.827289
22. Atamanalp, M.; Köktürk, M.; Uçar, A.; Duyar, H. A.; Özdemir, S.; Parlak, V.; Esenbuğa, N.; Alak, G. (2021). Microplastics in Tissues (Brain, Gill, Muscle and Gastrointestinal) of Mullus barbatus and Alosa immaculata. Archives of Environmental Contamination and Toxicology, 81(3), 460–469. https://doi.org/10.1007/s00244-021-00885-5
23. Prata, J. C., da Costa, J. P., Lopes, I., Duarte, A. C., & Rocha-Santos, T. (2020). Environmental exposure to microplastics: An overview on possible human health effects. Science of the Total Environment, 702, 134455.
24. Lammer, E., Carr, G. J., Wendler, K., Rawlings, J. M., Belanger, S. E., & Braunbeck, T. (2009). Is the fish embryo toxicity test (FET) with the zebrafish (Danio rerio) a potential alternative for the fish acute toxicity test? Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 149(2), 196–209. https://doi.org/10.1016/j.cbpc.2008.11.006
25. Hill, A. J., Teraoka, H., Heideman, W., & Peterson, R. E. (2005). Zebrafish as a model vertebrate for investigating chemical toxicity. Toxicological Sciences, 86(1), 6–19. https://doi.org/10.1093/toxsci/kfi110
26. Limonta, G., Mancia, A., Benkhalqui, A., Bertolucci, C., Abelli, L., Fossi, M. C., & Panti, C. (2019). Microplastics induce transcriptional changes, immune response and behavioral alterations in adult zebrafish. Scientific Reports, 9, 15775. https://doi.org/10.1038/s41598-019-52292-5
27. Howe, K., Clark, M. D., Torroja, C. F., Torrance, J., Berthelot, C., Muffato, M., Collins, J. E., Humphray, S., McLaren, K., Matthews, L., McLaren, S., Sealy, I., Caccamo, M., Churcher, C., Scott, C., Barrett, J. C., Koch, R., Rauch, G. J., White, S., … Stemple, D. L. (2013). The zebrafish reference genome sequence and its relationship to the human genome. Nature, 496(7446), 498–503. https://doi.org/10.1038/nature12111
28. Khan, F.R.; Alhewairini, S.S. Zebrafish (Danio rerio) as a Model Organism. In Current Trends in Cancer Management; Streba, L., Gheonea, D.I., Schenker, M., Eds.; IntechOpen: London, UK, 2019.
29. Savuca, A., Nicoara, M. N., Ciobica, A., Gorgan, D. L., Ureche, D., & Balmus, I. M. (2023). Current Aspects on the Plastic Nano- and Microparticles Toxicity in Zebrafish—Focus on the Correlation between Oxidative Stress Responses and Neurodevelopment. Animals, 13(11), 1810. https://doi.org/10.3390/ani13111810
30. Luan, J., Liu, J.-L., Xu, Y.-X., Zhang, Z., & Chen, X. (2023). Effects of microplastic exposure on the early developmental period and circadian rhythm of zebrafish (Danio rerio): A comparative study of polylactic acid and polyglycolic acid. Ecotoxicology and Environmental Safety, 258, 114994. https://doi.org/10.1016/j.ecoenv.2023.114994
31. Bhagat, J., Nishimura, N., & Shimada, Y. (2020). Zebrafish: An emerging model to study microplastic and nanoplastic toxicity. Science of the Total Environment, 728, 138707. https://doi.org/10.1016/j.scitotenv.2020.138707
32. Huang, W., Chen, Y., & Zhang, X. (2024). Exploring developmental toxicity of microplastics and nanoplastics in zebrafish: Mechanisms and ecological implications. Science of the Total Environment.
33. Zhang, Y. (2018). Cell toxicity mechanism and biomarker. Clinical and Translational Medicine, 7(1), 34. https://doi.org/10.1186/s40169-018-0212-720.
34. Auten, R. L., & Davis, J. M. (2009). Oxygen toxicity and reactive oxygen species: The devil is in the details. Pediatric Research, 66(2), 121–127. https://doi.org/10.1203/pdr.0b013e3181a9eafb
35. Pedersen, A. F., Meyer, D. N., Petriv, A. M. V., Soto, A. L., & Nelissen, K. (2020). Nanoplastics impact the zebrafish (Danio rerio) transcriptome: Associated developmental and neurobehavioral consequences. Environmental Pollution, 266(Pt 2), 115090. https://doi.org/10.1016/j.envpol.2020.115090
36. La Pietra, A., Fasciolo, G., Lucariello, D., Motta, C. M., Venditti, P., & Ferrandino, I. (2024). Polystyrene microplastics effects on zebrafish embryological development: Comparison of two different sizes. Environmental Toxicology and Pharmacology, 106, 104371. https://doi.org/10.1016/j.etap.2024.10437128.
37. Prata, J. C., Venâncio, C., Girão, A. V., Da Costa, J. P., Lopes, I., Duarte, A. C., & Rocha-Santos, T. (2021). Effects of virgin and weathered polystyrene and polypropylene microplastics on Raphidocelis subcapitata

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

and embryos of Danio rerio under environmental concentrations. The Science of the Total Environment, 816, 151642. https://doi.org/10.1016/j.scitotenv.2021.15164232.

38. Mansuri, A., Trivedi, C., & Kumar, A. (2024). Impact of virgin and weathered microplastics on zebrafish: Bioaccumulation, developmental toxicity and molecular pathway disruptions. The Science of the Total Environment, 957, 177510. https://doi.org/10.1016/j.scitotenv.2024.177510
39. Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., Shamseer, L., Tetzlaff, J. M., Akl, E. A., Brennan, S. E., Chou, R., Glanville, J., Grimshaw, J. M., Hróbjartsson, A., Lalu, M. M., Li, T., Loder, E. W., Mayo-Wilson, E., McDonald, S., . . . Moher, D. (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ, 372, n71. https://doi.org/10.1136/bmj.n71
40. Van Eck, N. J., & Waltman, L. (2009). Software survey: VOSviewer, a computer program for bibliometric mapping. Scientometrics, 84(2), 523–538. https://doi.org/10.1007/s11192-009-0146-3
41. Choi, J. S., Yoon, H., Heo, Y., Kim, T. H., & Park, J. (2024). Comparison of gut toxicity and microbiome effects in zebrafish exposed to polypropylene microplastics: Interesting effects of UV-weathering on microbiome. Journal of Hazardous Materials, 470, 134209. https://doi.org/10.1016/j.jhazmat.2024.13420920.
42. Medriano, C. A., & Bae, S. (2022). Acute exposure to microplastics induces metabolic disturbances and gut dysbiosis in adult zebrafish (Danio rerio). Ecotoxicology and Environmental Safety, 245, 114125. https://doi.org/10.1016/j.ecoenv.2022.114125
43. Marshoudi, M. A., Reasi, H. a. A., Al-Habsi, A., & Barry, M. J. (2023). Additive effects of microplastics on accumulation and toxicity of cadmium in male zebrafish. Chemosphere, 334, 138969. https://doi.org/10.1016/j.chemosphere.2023.13896923.
44. Medriano, C. A., Kim, S., Kim, L. H., & Bae, S. (2024). Chronic exposure of adult zebrafish to polyethylene and polyester-based microplastics: metabolomic and gut microbiome alterations reflecting dysbiosis and resilience. Journal of Hazardous Materials, 484, 136691. https://doi.org/10.1016/j.jhazmat.2024.136691
45. Luo, T., Weng, Y., Huang, Z., Zhao, Y., & Jin, Y. (2021). Combined hepatotoxicity of imidacloprid and microplastics in adult zebrafish: Endpoints at gene transcription. Comparative Biochemistry and Physiology Part C Toxicology & Pharmacology, 246, 109043. https://doi.org/10.1016/j.cbpc.2021.10904326.
46. Luan, J., Wen, L., Bao, Y., Bai, H., Zhao, C., Zhang, S., Man, X., Yin, T., & Feng, X. (2024). Systemic toxicity of biodegradable polyglycolic acid microplastics on the gut-liver-brain axis in zebrafish. The Science of the Total Environment, 954, 176898. https://doi.org/10.1016/j.scitotenv.2024.176898
47. Li, H., Yang, Z., Liu, Y., Sun, P., Wu, B., & Chen, L. (2024). Combined effects of polyvinyl chloride or polypropylene microplastics with cadmium on the intestine of zebrafish at environmentally relevant concentrations. The Science of the Total Environment, 954, 176289. https://doi.org/10.1016/j.scitotenv.2024.17628930.
48. Xiao, Y., Hu, L., Duan, J., Che, H., Wang, W., Yuan, Y., Xu, J., Chen, D., & Zhao, S. (2024). Polystyrene microplastics enhance microcystin-LR-induced cardiovascular toxicity and oxidative stress in zebrafish embryos. Environmental Pollution, 352, 124022. https://doi.org/10.1016/j.envpol.2024.12402233.
49. Santos, D., Luzio, A., Bellas, J., & Monteiro, S. M. (2022). Microplastics- and copper-induced changes in neurogenesis and DNA methyltransferases in the early life stages of zebrafish. Chemico-Biological Interactions, 363, 110021. https://doi.org/10.1016/j.cbi.2022.11002134.
50. Park, S. H., & Kim, K. (2021). Microplastics induced developmental toxicity with microcirculation dysfunction in zebrafish embryos. Chemosphere, 286(Pt 3), 131868. https://doi.org/10.1016/j.chemosphere.2021.13186835.
51. Wang, J., Wu, J., Cheng, H., Wang, Y., Fang, Y., Wang, L., & Duan, Z. (2022). Polystyrene microplastics inhibit the neurodevelopmental toxicity of mercury in zebrafish (Danio rerio) larvae with size-dependent effects. Environmental Pollution, 314, 120216. https://doi.org/10.1016/j.envpol.2022.12021636.
52. Wen, L., Man, X., Luan, J., Zhang, S., Zhao, C., Bao, Y., Liu, C., & Feng, X. (2024). Early-life exposure to five biodegradable plastics impairs eye development and visually-mediated behavior through disturbing hypothalamus-pituitary-thyroid (HPT) axis in zebrafish larvae. Comparative Biochemistry and Physiology Part C Toxicology & Pharmacology, 284, 109981. https://doi.org/10.1016/j.cbpc.2024.10998137.
53. Li, J., Liu, X., Fu, J., Gong, Z., Jiang, S. Y., & Chen, J. P. (2024). Metabolic profile changes of zebrafish larvae in the single- and co-exposures of microplastics and phenanthrene. The Science of the Total Environment, 953, 175994. https://doi.org/10.1016/j.scitotenv.2024.17599451.

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

54. Cormier, B., Cachot, J., Blanc, M., Cabar, M., Clérandeau, C., Dubocq, F., Bihanic, F. L., Morin, B., Zapata, S., Bégout, M., & Cousin, X. (2022). Environmental microplastics disrupt swimming activity in acute exposure in Danio rerio larvae and reduce growth and reproduction success in chronic exposure in D. rerio and Oryzias melastigma. Environmental Pollution, 308, 119721. https://doi.org/10.1016/j.envpol.2022.11972139.
55. Zhang, J., Bai, Y., Meng, H., Zhu, Y., Yue, H., Li, B., Wang, J., Wang, J., Zhu, L., & Du, Z. (2024). Combined toxic effects of polystyrene microplastics and 3,6-dibromocarbazole on zebrafish (Danio rerio) embryos. The Science of the Total Environment, 913, 169787. https://doi.org/10.1016/j.scitotenv.2023.16978741.
56. Yang, H., Ju, J., Wang, Y., Zhu, Z., Lu, W., & Zhang, Y. (2024). Micro-and nano-plastics induce kidney damage and suppression of innate immune function in zebrafish (Danio rerio) larvae. The Science of the Total Environment, 931, 172952. https://doi.org/10.1016/j.scitotenv.2024.172952
57. Xu, J., Yang, W., Wang, D., Wang, Z., Liu, C., & Li, J. (2023). Methamphetamine shows different joint toxicity for different types of microplastics on zebrafish larvae by mediating oxidative stress. Toxics, 12(1), 9. https://doi.org/10.3390/toxics1201000943.
58. Kim, J., Haque, M. N., Lee, S., Lee, D., & Rhee, J. (2022). Exposure to environmentally relevant concentrations of polystyrene microplastics increases hexavalent chromium toxicity in aquatic animals. Toxics, 10(10), 563. https://doi.org/10.3390/toxics1010056344.
59. Zhang, X., Xia, M., Zhao, J., Cao, Z., Zou, W., & Zhou, Q. (2021). Photoaging enhanced the adverse effects of polyamide microplastics on the growth, intestinal health, and lipid absorption in developing zebrafish. Environment International, 158, 106922. https://doi.org/10.1016/j.envint.2021.10692245.
60. Ding, P., Xiang, C., Li, X., Chen, H., Shi, X., Li, X., Huang, C., Yu, Y., Qi, J., Li, A. J., Zhang, L., & Hu, G. (2023). Photoaged microplastics induce neurotoxicity via oxidative stress and abnormal neurotransmission in zebrafish larvae (Danio rerio). The Science of the Total Environment, 881, 163480. https://doi.org/10.1016/j.scitotenv.2023.16348046.
61. Cao, Q., Wei, D., Ma, X., Liu, R., Samra, Qi, Y., Yuan, C., & Huang, D. (2024). Polystyrene microplastics mitigate lead-induced neurotoxicity by reducing heavy metal uptake in zebrafish larvae. The Science of the Total Environment, 919, 170790. https://doi.org/10.1016/j.scitotenv.2024.17079047.
62. Zhao, Y., Chen, H., Liang, H., Liang, H., Zhao, T., Ren, B., Li, Y., Liang, H., Liang, H., Liu, Y., Cao, H., Cui, N., & Wei, W. (2024). Combined toxic effects of polyethylene microplastics and lambda-cyhalothrin on gut of zebrafish (Danio rerio). Ecotoxicology and Environmental Safety, 276, 116296. https://doi.org/10.1016/j.ecoenv.2024.11629648.
63. Santos, D., Luzio, A., Félix, L., Cabecinha, E., Bellas, J., & Monteiro, S. M. (2022). Microplastics and copper induce apoptosis, alter neurocircuits, and cause behavioral changes in zebrafish (Danio rerio) brain. Ecotoxicology and Environmental Safety, 242, 113926. https://doi.org/10.1016/j.ecoenv.2022.11392649.
64. Santos, D., Luzio, A., Félix, L., Bellas, J., & Monteiro, S. M. (2022). Oxidative stress, apoptosis and serotonergic system changes in zebrafish (Danio rerio) gills after long-term exposure to microplastics and copper. Comparative Biochemistry and Physiology Part C Toxicology & Pharmacology, 258, 109363. https://doi.org/10.1016/j.cbpc.2022.109363
65. Yu, Z., Yan, C., Qiu, D., Zhang, X., Wen, C., & Dong, S. (2022). Accumulation and ecotoxicological effects induced by combined exposure of different sized polyethylene microplastics and oxytetracycline in zebrafish. Environmental Pollution, 319, 120977. https://doi.org/10.1016/j.envpol.2022.12097750.
66. Li, H., Yang, Z., Liu, Y., Sun, P., Wu, B., & Chen, L. (2024b). Combined effects of polyvinyl chloride or polypropylene microplastics with cadmium on the intestine of zebrafish at environmentally relevant concentrations. The Science of the Total Environment, 954, 176289. https://doi.org/10.1016/j.scitotenv.2024.176289
67. Zhang, Y., Chen, C., & Chen, K. (2023). Combined exposure to microplastics and amitriptyline induced abnormal behavioral responses and oxidative stress in the eyes of zebrafish (Danio rerio). Comparative Biochemistry and Physiology Part C Toxicology & Pharmacology, 273, 109717. https://doi.org/10.1016/j.cbpc.2023.10971752.
68. Shi, Y., Chen, C., Han, Z., Chen, K., Wu, X., & Qiu, X. (2023). Combined exposure to microplastics and amitriptyline caused intestinal damage, oxidative stress and gut microbiota dysbiosis in zebrafish (Danio rerio). Aquatic Toxicology, 260, 106589. https://doi.org/10.1016/j.aquatox.2023.10658953.

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

69. Zhang, L., Luo, Y., Zhang, Z., Pan, Y., Li, X., Zhuang, Z., Li, J., Luo, Q., & Chen, X. (2023). Enhanced reproductive toxicity of photodegraded polylactic acid microplastics in zebrafish. The Science of the Total Environment, 912, 168742. https://doi.org/10.1016/j.scitotenv.2023.168742
70. Rangasamy, B., Malafaia, G., & Maheswaran, R. (2021). Evaluation of antioxidant response and Na+-K+-ATPase activity in zebrafish exposed to polyethylene microplastics: Shedding light on a physiological adaptation. Journal of Hazardous Materials, 426, 127789. https://doi.org/10.1016/j.jhazmat.2021.127789
71. Wu, D., Carter, L., Kay, P., Holden, J., Yin, Y., & Guo, H. (2024). Female zebrafish are more affected than males under polystyrene microplastics exposure. Journal of Hazardous Materials, 482, 136616. https://doi.org/10.1016/j.jhazmat.2024.136616
72. Lu, K., Qiao, R., An, H., & Zhang, Y. (2018). Influence of microplastics on the accumulation and chronic toxic effects of cadmium in zebrafish (Danio rerio). Chemosphere, 202, 514–520. https://doi.org/10.1016/j.chemosphere.2018.03.14556.
73. Li, C., Yuan, S., Zhou, Y., Li, X., Duan, L., Huang, L., Zhou, X., Ma, Y., & Pang, S. (2021). Microplastics reduce the bioaccumulation and oxidative stress damage of triazole fungicides in fish. The Science of the Total Environment, 806(Pt 4), 151475. https://doi.org/10.1016/j.scitotenv.2021.15147557.
74. Gupta, P., Mahapatra, A., Suman, A., Ray, S. S., Malafaia, G., & Singh, R. K. (2023). Polystyrene microplastics disrupt female reproductive health and fertility via sirt1 modulation in zebrafish (Danio rerio). Journal of Hazardous Materials, 460, 132359. https://doi.org/10.1016/j.jhazmat.2023.13235958.
75. Jiang, X., Yan, S., Sun, H., Kong, X. Z., Li, S., Shi, H., Zhu, X., & Gu, X. (2024). Preparation of fluorescent polyurethane microspheres and their applications as reusable sensor for 4-nitrophenol detection and as microplastics model for visualizing polyurethane in cells and zebrafish. Journal of Colloid and Interface Science, 673, 550–563. https://doi.org/10.1016/j.jcis.2024.06.07059.
76. Da Costa Araújo, A. P., Da Luz, T. M., Rocha, T. L., Ahmed, M. a. I., De Melo E Silva, D., Rahman, M. M., & Malafaia, G. (2022). Toxicity evaluation of the combination of emerging pollutants with polyethylene microplastics in zebrafish: Perspective study of genotoxicity, mutagenicity, and redox unbalance. Journal of Hazardous Materials, 432, 128691. https://doi.org/10.1016/j.jhazmat.2022.12869160.
77. Adhikari, M., Biswas, C., Mazumdar, P., Sarkar, S., & Pramanick, K. (2024). Evaluating the potential of daily intake of polystyrene microplastics via drinking water in inducing PCOS and its ovarian fibrosis progression using female zebrafish. NanoImpact, 34, 100507. https://doi.org/10.1016/j.impact.2024.10050761.
78. Xue, M., Jia, M., Qin, Y., Francis, F., & Gu, X. (2024). Toxicity of parental co-exposure of microplastic and bisphenol compounds on adult zebrafish: Multi-omics investigations on offspring. The Science of the Total Environment, 955, 176897. https://doi.org/10.1016/j.scitotenv.2024.17689762.
79. Gong, N., Wang, Z., Wang, X., & Shao, K. (2024). Uptake, removal and trophic transfer of fluorescent polyethylene microplastics by freshwater model organisms: the impact of particle size and food availability. Aquatic Toxicology, 277, 107165. https://doi.org/10.1016/j.aquatox.2024.107165
80. Zhao, Y., Ma, C., Wei, W., Wang, Y., Cao, H., Cui, N., Liu, Y., & Liang, H. (2024). Effects of single and combined exposure of virgin or aged polyethylene microplastics and penthiopyrad on zebrafish (Danio rerio). The Science of the Total Environment, 921, 171160. https://doi.org/10.1016/j.scitotenv.2024.171160
81. Harikrishnan, T., Paramasivam, P., Sankar, A., Sakthivel, M., Sanniyasi, E., Raman, T., Thangavelu, M., Singaram, G., & Muthusamy, G. (2024). Weathered polyethylene microplastics induced immunomodulation in zebrafish. Environmental Toxicology and Pharmacology, 109, 104478. https://doi.org/10.1016/j.etap.2024.104478
82. Mohan, M., Gaonkar, A. A., Nanjappa, D. P., K, K., Vittal, R., Chakraborty, A., & Chakraborty, G. (2023). Screening for microplastics in drinking water and its toxicity profiling in zebrafish. Chemosphere, 341, 139882. https://doi.org/10.1016/j.chemosphere.2023.13988265.
83. Wang, X., Ding, N., & Liu, H. (2024). Effect of microplastics on sodium hypochlorite disinfection and changes in its toxicity on zebrafish. Chemosphere, 363, 142594. https://doi.org/10.1016/j.chemosphere.2024.142594
84. Shu, Q., Xie, S., Junaid, M., Zheng, R., Tang, H., Zou, J., & Zhou, A. (2024). MPs and PFOS single and combined exposure significantly alter genetic expressions of growth hormone and insulin growth factor-related biomarkers during zebrafish embryonic development. The Science of the Total Environment, 949, 174925. https://doi.org/10.1016/j.scitotenv.2024.17492567.

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 May 2026 doi:10.20944/preprints202605.0763.v1

85. Yan, Z., Zhao, H., Zhu, P., Wang, Y., Hou, J., Lu, G., & He, C. (2024b). Polystyrene microplastics alter the trophic transfer and biotoxicity of fluoxetine in an aquatic food chain. Journal of Hazardous Materials, 470, 134179. https://doi.org/10.1016/j.jhazmat.2024.134179
86. De Souza Freire, I., Fascineli, M. L., Piau, T. B., Pereira, B. F., & Grisolia, C. K. (2023). Multilevel Toxicity Evaluations of Polyethylene Microplastics in Zebrafish (Danio rerio). International Journal of Environmental Research and Public Health, 20(4), 3617. https://doi.org/10.3390/ijerph2004361768.
87. Zhao, H., Xu, J., Yan, Z., Ren, H., & Zhang, Y. (2020). Microplastics enhance the developmental toxicity of synthetic phenolic antioxidants by disturbing the thyroid function and metabolism in developing zebrafish. Environment International, 140, 105750. https://doi.org/10.1016/j.envint.2020.10575069.
88. Dimitriadi, A., Papaefthimiou, C., Genizegkini, E., Sampsonidis, I., Kalogiannis, S., Feidantsis, K., Bobori, D. C., Kastrinaki, G., Koumoundouros, G., Lambropoulou, D. A., Kyzas, G. Z., & Bikiaris, D. N. (2021). Adverse effects polystyrene microplastics exert on zebrafish heart – Molecular to individual level. Journal of Hazardous Materials, 416, 125969. https://doi.org/10.1016/j.jhazmat.2021.12596971.
89. Priyadharshini, S., Jeyavani, J., Al-Ghanim, K. A., Govindarajan, M., Karthikeyan, S., & Vaseeharan, B. (2024). Eco-toxicity assessment of polypropylene microplastics in juvenile zebrafish (Danio rerio). Journal of Contaminant Hydrology, 266, 104415. https://doi.org/10.1016/j.jconhyd.2024.104415
90. Zhao, Y., Qin, Z., Huang, Z., Bao, Z., Luo, T., & Jin, Y. (2021). Effects of polyethylene microplastics on the microbiome and metabolism in larval zebrafish. Environmental Pollution, 282, 117039. https://doi.org/10.1016/j.envpol.2021.11703972.
91. Qiang, L., & Cheng, J. (2019). Exposure to microplastics decreases swimming competence in larval zebrafish (Danio rerio). Ecotoxicology and Environmental Safety, 176, 226–233. https://doi.org/10.1016/j.ecoenv.2019.03.08873.
92. Xiao, Q., Xiang, H., Tian, Y., Huang, J., Li, M., Wang, P., Lian, K., Yu, P., Xu, M., Zhang, R., Zhang, Y., Huang, J., Zhang, W., & Duan, P. (2024). Polystyrene microplastics alleviate the developmental toxicity of silver nanoparticles in embryo-larval zebrafish (Danio rerio) at the transcriptomic level. The Science of the Total Environment, 954, 176485. https://doi.org/10.1016/j.scitotenv.2024.17648574.
93. Sim, Y., Cho, H., Lee, J., Lee, W. S., Kim, H., & Jeong, J. (2023). Combined effects of microplastics and benz[a]anthracene on cardiotoxicity in zebrafish (Danio rerio) larvae: Size matters. Chemosphere, 330, 138723. https://doi.org/10.1016/j.chemosphere.2023.13872375.
94. Bobori, D. C., Feidantsis, K., Dimitriadi, A., Datsi, N., Ripis, P., Kalogiannis, S., Sampsonidis, I., Kastrinaki, G., Ainali, N. M., Lambropoulou, D. A., Kyzas, G. Z., Koumoundouros, G., Bikiaris, D. N., & Kaloyianni, M. (2022). Dose-Dependent cytotoxicity of polypropylene microplastics (PP-MPs) in two freshwater fishes. International Journal of Molecular Sciences, 23(22), 13878. https://doi.org/10.3390/ijms23221387876.
95. Zhu, J., Zhang, Y., Xu, Y., Wang, L., Wu, Q., Zhang, Z., & Li, L. (2022). Effects of microplastics on the accumulation and neurotoxicity of methylmercury in zebrafish larvae. Marine Environmental Research, 176, 105615. https://doi.org/10.1016/j.marenvres.2022.10561577.
96. Chen, Y., Li, W., Xiang, L., Mi, X., Duan, M., & Wu, C. (2022). Fish personality affects their exposure to microplastics. Ecotoxicology and Environmental Safety, 233, 113301. https://doi.org/10.1016/j.ecoenv.2022.113301
97. Koelmans, A. A., Besseling, E., & Shim, W. J. (2015). Nanoplastics in the aquatic environment: Critical review. In M. Bergmann, L. Gutow, & M. Klages (Eds.), Marine anthropogenic litter (pp. 325–340). Springer. https://doi.org/10.1007/978-3-319-16510-3_12
98. Lambert, S., & Wagner, M. (2016). Formation of microscopic particles during the degradation of different polymers. Chemosphere, 161, 510–517. https://doi.org/10.1016/j.chemosphere.2016.07.042
99. Rochman, C. M., Hoh, E., Hentschel, B. T., & Kaye, S. (2013). Long-term field measurement of sorption of organic contaminants to five types of plastic pellets: Implications for plastic marine debris. Environmental Science & Technology, 47(3), 1646–1654. https://doi.org/10.1021/es303700s

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

© 2026 by the author(s). Distributed under a Creative Commons CC BY license.