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REVIEW ARTICLE

Ecological Toxicity, Oxidative Stress and Impacts of Microplastics on the Gills of Fish

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ABSTRACT

The fish, which comes into contact with Microplastics (MPS) in aquatic systems, accumulates these particles internally, affecting the storage pattern with particle size. MPs entering the body of fish can reach the bloodstream, changing blood properties and hemodynamics. They additionally disrupt the equilibrium between reactive oxygen species (ROS) production and antioxidant defense, resulting in oxidative stress and harm to the aquatic system. MPS also causes immunomodulatory effects through physical and chemical poisoning and induces neurotoxicity, which is often indicated by changes in acetylco-lestrase (ACHE) activity. This review analyzes MP toxicity in fish, focusing on major indicators, bioaccumulation, hematological changes, antioxidant reactions, immune reactions, and neurotoxicity -exposure levels. This approach facilitates the identification of biomarkers to assess MP poisoning. This research uncovers the cellular and molecular processes that control the prolonged toxicity of MPs in fish gills. Nevertheless, further investigations are required to comprehensively grasp the joint impacts of MPs along with their rising pollutants and possible outcomes for aquatic ecosystems.

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INTRODUCTION

Plastics have colorful applications in several sectors, such as holders, construction, buses, electronics, electrical, cloth, husbandry, and industry (Bhuyan, 2022). According to Zhang *et al*. (2023), world plastic production in 2018 amounted to nearly 360 million tons, with an estimated 80,000 tons entering the marine environment. Roughly 10 of the plastic waste generated worldwide, equivalent to 9.5 million tons per year, is present in the marine environment. This substance raises significant environmental pitfalls (Iheanacho and Odo, 2020). Plastics are categorized based on their composition and structure into several primary types, such as polyethylene (PE), polystyrene (PS), polyvinyl chloride (PVC), polyethylene terephthalate (PET), polyamide (PA), polypropylene (PP), and ethylene-vinyl acetate (EVA), each possessing distinct properties and applications (Bhuyan, 2022). The global plastic production reached 360 million tons in 2018, with an estimated 80,000 tons entering submarine systems annually (Zhang *et al*., 2023). Roughly 9.5 million tons of plastic waste, representing 10 of global production, accumulate in marine surroundings every time, which constitute an important dangerous material (Iheanacho and Odo, 2020).

Microplastics (MPs), defined as synthetic polymer patches under 5 mm, form through plastic degradation in submarine ecosystems or enter directly as small solids, posing direct pitfalls to marine life (Frias and Nash, 2019). Recent exploration suggests classifying MPs within the 1-

1000 µm size range (Qiao *et al*., 2020). Ingestion by brackish and marine fish is well- proved (Wright, 2013), leading to both bodily harm and chemical poisoning. Physical impacts include digestive tract blockages, internal injuries, ulcerative lesions, and implicit gastric rupture (Du *et al.,* 2021). Chemical effects involve energy reduction and reduced growth/reduplication, oxidative stress and metabolic dysfunction, cellular damage and endocrine disruption, immunosuppression and neurotoxicity, and genotoxic effects that could result in death (Ding *et al*., 2018; Lu *et al*., 2016). The types of MP found in the gills and digestive tract of *Gambusia affinis* are shown in Fig. 1.

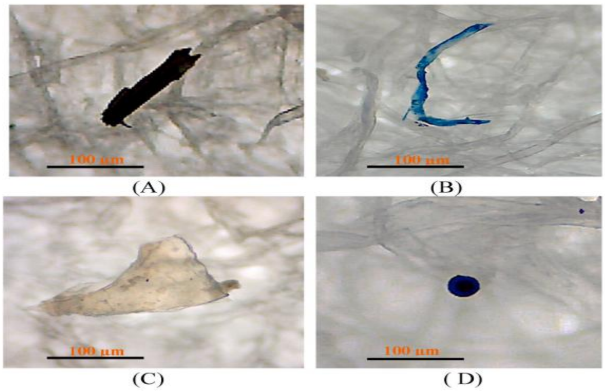

Fig 1. Type of MP found in gills and digestive tract of *Gambusia affinis*: A (fragment), B (fiber), C (film), D (pellet) (Buwono *et al*. 2021)

Plastics incorporate multitudinous chemical complements during manufacturing, including plasticizers (like phthalates and bisphenol A), colorings, UV pollutants, and heavy metal retardants. Also, as Microplastics (MPs) move through submarine systems, they accumulate adsorbed environmental pollutants such as polychlorinated biphenyls (PCBs), organochlorine pesticides, polycyclic aromatic hydrocarbons (PAHs), and fragrances. Due to their small size, submarine organisms frequently mistake MPs for food, leading to accidental ingestion (Barboza *et al.,* 2021). This initiates entry into food webs, where dangerous substances can concentrate precipitously in blood suckers through bio-magnification. Consequently, MPs negatively affect diverse organisms, including zooplankton, mollusks, crustaceans, fish at all life stages, marine turtles, aquatic mammals, and seabirds. Fish, as top predators in many aquatic systems, serve as critical indicators for assessing MP toxicity. Therefore, MP contamination not only disrupts aquatic ecosystems but also threatens human food safety, given fish's importance as a global protein source (Adamovsky *et al.,* 2021)

MPs are microscopic pollutants (1–1000µm). Their widespread presence, persistence, and documented adverse effects on living organisms have elevated them to a major global environmental concern. MPs originate from two primary pathways: direct release of manufactured particles (e.g., microbeads in cosmetics, microfibers from textiles) and the fragmentation of larger plastic debris. Common environmental plastics include polyethylene, polypropylene, and polyvinyl chloride. A key environmental risk is the high bioavailability of MPs to diverse aquatic species, particularly fish (Frias and Nash, 2019)

Exposure to MPs can induce oxidative stress in fish. This occurs when the production of damaging reactive oxygen species (ROS) overwhelms the organism's natural antioxidant defenses, which include enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GR), and glutathione S-transferase (GST). ROS, generated internally or acquired from the environment, cause significant harm to cellular components like lipids, DNA, and proteins. SOD acts first by converting ROS into hydrogen peroxide (H_2O_2), which is then broken down into water and oxygen by CAT and GPX. GR and GST play vital roles in combating oxidative stress and detoxifying pollutants (Ahrendt *et al.,* 2023). Physical damage from MPs themselves may contribute to this stress response. Key indicators of oxidative damage include elevated levels of malondialdehyde (MDA) and H_2O_2. A broader stress response often involves increased cortisol production, a hormone known to modulate immune function and inhibit growth (Xia *et al.,* 2020)

While nanoplastics have been shown to elevate cortisol in zebrafish, research on MP-specific effects on fish stress responses remains limited. Likewise, physiological and cellular processes are unnaturally regulated by gene expression. Probing how MPs alter the expression of genes controlling stress and growth is thus pivotal. Histopathological examinations reveal that MP exposure causes tissue damage in fish, including liver and intestinal inflammation, hepatic lesions, necrosis, hemorrhaging, and significant gill injury. Physical abrasion by MPs likely contributes to gill and gut damage (Limonta *et al.,* 2019)

Gills are vital respiratory organs directly exposed to waterborne contaminants like MPs. These particles can diffuse across gill membranes and enter the bloodstream, reaching internal organs like the liver and brain. In freshwater fish, gills are also essential for ion regulation. Key enzymes like basolateral Na^+/K^+-K-ATPase generate the electrochemical gradient driving sodium uptake, aided by apical H^+-H-ATPase and sodium channels. Various environmental stressors are known to disrupt the activity of these ion transporters (Evans *et al.,* 2005)

Similarly, shrimp gills perform critical functions in respiration, osmoregulation, nitrogen excretion, and immune defense. Their direct contact with water makes them largely susceptible to environmental stressors, potentially causing physiological dysfunction. Given this vulnerability, assessing the impact of combined stressors like nitrite and MPs on gill function in shrimp species (e.g. *Litopenaeus vannamei*) is highly relevant (Henry *et al.,* 2012).

Need for the research: Fish gills function as mucosal lymphoid tissues crucial for immune defense. Microplastics trigger oxidative stress that impairs gill structure and immune capacity, although the precise mechanisms remain unclear. Building on existing evidence, we propose that oxidative stress contributes to polyethylene micro plastic-induced cell apoptosis through modulation of the NF-κB signaling pathway. Furthermore, we seek to establish if NLRP3 inflammasome activation is necessary for initiating the immune-inflammatory response caused by Microplastics. (Buchmann *et al.,* 2022)

Objective of the study: This study aimed to characterize Gill histopathological alterations, Gill oxidative stress responses, and shifts in Gill metabolic function patterns. These findings advance understanding of the individual and concerted poisonous impacts of nitrite and Microplastics on shrimp gills. (Xing *et al.,* 2023)

Micro plastic and Diet Preparation

Polypropylene (PP) micro plastic particles were generated from pristine fibers (diameter: 0.9 mm; Crystal Tec, Korea) using an optimized cryotome protocol. Fibers were sectioned into lengths of approximately 0.9-1.0 mm (Bucci *et al.,* 2022). Given the importance of particle size, shape, and surface characteristics for toxicity, PP particles were characterized using Scanning Electron Microscopy (SEM; Hitachi SU 1510) coupled with Energy Dispersive X-ray Spectroscopy (EDX). Particle size distribution was quantified using Image J software (Ver. 1.53). Two dietary treatments were formulated: a basal diet (55% crude protein, 20% crude oil, 12% crude ash, 11% vitamins, 2% crude fiber) and experimental diets incorporating PP Microplastics at concentrations of 100 mg/g and 250 mg/g (Bucci *et al.,* 2022).

Micro plastic Impact on Contaminant Bioaccumulation in Gills: Exposure to Microplastics in water elevated mercury concentrations within the gills of *D. labrax* juveniles. Both Microplastics and mercury, individually and combined, induced oxidative stress in gill tissue. Analysis of overall antioxidant enzyme activity induction indicated an additive toxicological interaction for the mixture containing the lowest concentrations of both contaminants in the gills. These results underscore the importance of further research into micro plastic interactions influencing the bioconcentration, bioaccumulation, and toxicity of co-occurring environmental pollutants across species (Bank *et al.,* 2019)

Measured gill mercury concentrations (mean ± SD) ranged from 1.519 ± 0.369 μg/g to 4.825 ± 0.881 μg/g. Bioconcentration Factors (BCF) in gills (152 ± 37 to 302 ± 55) confirm direct metal uptake from water and bioconcentration within gill tissue, consistent with prior reports of mercury accumulation in *D. labrax*. Microplastics ingested via the gills, potentially carrying adsorbed mercury, may explain increased gill mercury levels in co-exposure groups (Feng *et al.,* 2022) Subsequent release and partial absorption of mercury from particles within the gills could facilitate increased accumulation in internal organs like the liver. A similar process may occur within the digestive system, further elevating hepatic mercury. Microplastics present in the gills might also interfere with local metal uptake and elimination mechanisms. Additionally, physical obstruction by particles could impede oxygen diffusion, potentially causing hypoxia and reducing aerobic energy production, as hypothesized for *Daphnia magna*. If occurring, this energy deficit could impair mercury elimination in fish exposed to micro plastic-contaminant mixtures (Gautam *et al.,* 2024).

Gill Response to Combined Micro plastic and Copper Exposure: Exposure to bobby (Cu) and Microplastics (MPs) generated reactive oxygen species (ROS) exceeding the gills' antioxidant capacity, dismembering oxidative balance. A significant increase in lipid peroxidation (LPO) situations passed in the Cu25 MPs group compared to controls (p=0.02), indicating oxidative damage in zebrafish gills. Elevated LPO in gills, reflecting membrane lipid revision and cellular damage, aligns with findings in European seabass exposed to fusions of fluorescent MPs and mercury (Lu *et al.,* 2020). Although this study didn't perform gill histology or quantify MP entrapment, the observed oxidative damage implies implicit for Cu MPs fusions to beget histopathological changes and vitiate gill function. Supporting this, co-exposure to polystyrene MPs and cadmium convinced both oxidative stress and histopathological differences in zebrafish gills. also,severe necrosis, lamellar emulsion, and inflammation were proved in rainbow trout gills exposed to polystyrene MPs and chlorpyrifos. These collaborative findings punctuate gill perceptivity to MP pollution, emphasizing the environmental trouble posed by plastics (Li *et al.,* 2023).

Lactate dehydrogenase (LDH) activity, a marker of anaerobic metabolism, cell damage, and hypoxia, decreased significantly in groups exposed to MPs alone (p=0.008) and Cu + MPs (p=0.001). This suggests potential suppression of anaerobic glycolysis and a shift towards aerobic respiration in zebrafish gills (Willett *et al.,* 2024). Given the likelihood of MPs causing physical obstruction or histopathological changes in gills, the observed LDH decrease may relate to an increased respiration rate by exposed fish compensating for impaired gill function. Marine mussels exposed to polystyrene Microplastics (MPs) and BDE-47 exhibited an inhibition of lactate dehydrogenase (LDH) activity concurrent with elevated respiration rates, mirroring effects observed in fish gills. These findings suggest that MPs, whether alone or combined with bobby (Cu), can disrupt the metabolic energy force in fish gills, pressing their implicit to vitiate critical physiological processes in submarine organisms (Willett *et al.,* 2024). Acetylcholine (ACh) functions as a critical signaling molecule in fish gills, contributing to oxygen sensing and respiratory regulation. This role is mediated through both neuronal and non-neuronal cholinergic systems, where ACh interacts with nicotinic (nAChR) and muscarinic (mAChR) receptors on neuro epithelial cells (NECs) and other gill cell type (Zachar *et al*., 2017; Santos *et al*., 2022). Table 1 indicating the side effect of different microplastics.

Table 1. Side effect of different microplastics

Treatment	Dose	Model	Side effects	Reference
Micro plastic	MPs 2mg/L	Zebrafish	Decreased body length: 3.59+0.30(control) vs 3.52+0.30 (MPs) Decreased body weight: 0.53+0.17 (control) vs 0.49+0.14 (MPs) SOD 0.281+0.12 (control) vs 0.291+0.16 (MPs) CAT 0.054+0.02 (control) vs 0.057+0.02 (MPs)	(Santos *et al.*, 2022)
Micro plastic + Copper	MPs 2mg/L + Cu 25µg/L	Zebrafish	Decreased body length: 3.59+0.30 (control) vs 3.49 +0.33 (Cu+ MPs) Decreased body weight: 0.53+0.17 (control) vs 0.47+0.14 (Cu + MPs) SOD 0.281+0.12 (control) vs 0.37+0.18 (Cu+ MPs) CAT 0.054+0.02 (control) vs 0.10+0.08 (Cu+ MPs)	(Santos *et al.*, 2022)
Polyethylene MPs	250, 500µg/L	*Cyprinus carpio*	Plasma 250, 500 µg/L	(Banaee *et al.*, 2019).
Polycarbonate MPs	0.025, 0.05, 0.1 0.2µg/L	*Pimephales Promelas*	Kidney, 0.025, 0.05, 0.1 µg/L	(Greven *et al.*, 2016)
Polyethylene + Polystyrene MPs	100, 1000µg/L	*Danio rerio*	Intestine, Gill, 100, 1000 µg/L	(Limonta *et al*., 2019)
MPs	1, 10, 100mg/L	*Oreochromis Niloticus*	Peripheral blood 1 mg/L	(Espinosa *et al.*, 2019)
Polyvinylchloride MPs	100, 500mg/kg	*Dicentrarchus Labrax*	Skin mucus 500 mg/kg	(Espinosa *et al*., 2019)

Effects of bioaccumulation of MPs in fish gills: Freshwater fish physiologically regulate ion balance through gill chloride cells that actively take up ions. To offset ion loss in dilute environments, they excrete large volumes of dilute urine. Marine fish, facing dehydration in concentrated seawater, constantly ingest water. This fundamental difference in osmoregulation leads to greater micro plastic (MP) accumulation in marine fish compared to freshwater species when exposed to identical MP levels (Lee *et al*., 2019; Assas *et al*., 2020). Several factors, including fish species, concentrations and duration of contact, size of the particles of MPs and the behavior of the fish food, influence the accumulation of micro plastic (MP). These variables shape together how MPs make in aquatic organisms (Ding *et al*., 2018).

Microplastics can be in different shapes and sizes and how they accumulate in fish is dependent on the size of the flyspeck. Administered smaller particulates can diffuse the fish's circulatory system and accumulate in fish tissues in different came and varying quantities in certain tissues (Lu *et al*., 2018). Young or tiny Microplastics are little enough to breach the intestinal barrier and enter the systemic circulation where they can be distributed to different tissues. Also, 5-150 micrometer sized particles may also re-enter systemic circulation after crossing the intestinal membrance and the diaphragm (Jovanović *et al*., 2018).

Microplastics also result in intestinal damage due to the formation of cracks, abnormal cell perforation and disrupted inter cell communication (Lei *et al*., 2018). Calcium imbalance as well as the aforementioned issues also result in disrupted gut function. Fish can absorb Microplastics by eating contaminated food, and by directly absorbing particles from the water through their gills. There are reports of Microplastics, including 0.1mm diameter polystyrene particles, gaining access to the fish's brain and other organs through the blood. Presumably due to lower mobility, small Microplastics are able to remain in fish tissues longer over larger Microplastics which are able to stay in the body circulatory system longer (Jovanović *et al*., 2018).

Gills, critical for respiration, ion regulation, and acid-base balance, are directly exposed to aquatic contaminants, providing a major entry route for pollutants into fish tissues (Roda *et al*., 2020). While the exposure route (waterborne vs dietary) is crucial for many toxicants, MP particle size is a more decisive factor for accumulation than the route. MP accumulation in the brain poses a direct threat to the central nervous system, as patches can potentially access the blood- brain hedge depending on size. Accumulation in any towel can beget toxin and functional impairment. The intestine's part in digestion and immersion leads to direct contact with pollutants during feeding, performing in high MP accumulation (Kim and Kang, 2017). The liver and feathers, involved in detoxification and excretion, are also prone to high MP accumulation from recycling patches in the blood (Kim and Kang, 2015).

Accumulation in gills and intestines confirms respiration and ingestion as primary MP uptake pathways. The gills' large surface area facilitates significant accumulation from waterborne exposure. MPs absorbed across cellular barriers can persist toxically in tissues for extended periods. Nevertheless, MPs ingested and excreted are able to be deposited in benthic ecosystem, influencing the bottom organisms as well (Yin *et al*., 2018). Knowledge of MP accumulation patterns in fish is important for deciding target organs to study toxic effects and to interpret MP concentrations in field samples.

The investigation constantly shows that the accumulation of MP depends mainly on the particle size instead of the exhibition route (Zitouni *et al*., 2021), reported that accumulation is easier for smaller MPs, regardless of the absorption path. On the contrary, (Jovanović *et al*., 2018) suggested that smaller MPs could be more easily eliminated from the liver through circulation, while larger particles could persist. In general, MP particle size seems to exert a stronger influence on accumulation and toxic effects than the type of MP polymer or the absorption path. More studies are needed to clarify the bonding patterns of objective organs related to the size of MP (Kim *et al*., 2021).

Changes in gill morphology in response to nitrite and micro plastic exposure: As the primary respiratory organ, shrimp gills also contribute to osmoregulation, nitrogen excretion, hormone metabolism, and sensitive functions (Evans *et al*., 2013). Research highlights the impact of nitrite on the gills of M. amazonicum, specifically on the epithelial kerchief thickening and detachment (Brazão *et al*., 2021). Research highlights the impact of nitrite on the gills of M. amazonicum, specifically on the epithelial kerchief thickening and detachment (Brazão *et al*., 2021). Microplastics also beget gill kerchief damage in shrimp (Choi *et al*., 2020; Saborowski *et al*., 2022). This exploration revealed significant gill structural differences across all stress groups, demonstrating that both individual and combined nitrite and micro plastic exposure vitiate gill integrity and physiological function. especially, the NM group (combined stress) displayed more severe damage, suggesting synergistic negative goods beyond single stressors.

Oxidative Stress Mechanisms in Gills: Environmental pollutants often exert toxicity through oxidative stress. Antioxidant enzymes like SOD and CAT maintain redox balance by converting superoxide anions (O_2^-) to hydrogen peroxide (H_2O_2), then further decomposing H_2O_2 to water and oxygen (Brazão *et al*., 2021). Prior research shows upregulated *SOD* and *CAT* expression in *L. vannamei* gills after 72-hour nitrite exposure (Guo *et al*., 2013) and transient increases in *M. nipponense* following 96-hour micro plastic exposure (Yu *et al*., 2018). Consistent with this, 14-day exposures in the current study elevated *SOD* and *CAT* expression, confirming oxidative stress induction by both individual and combined stressors.

Thioredoxin (Trx) serves as a key antioxidant protein (Zuo *et al*., 2019), while heat shock proteins (HSPs) aid stress defense (Shi *et al*., 2016). *Trx* upregulation occurred in *Daphnia magna* after micro plastic exposure (Tang *et al*., 2019). Differential antioxidant gene expression patterns among treatment groups here may reflect distinct mechanisms: Microplastics directly induce gill oxidative stress, whereas nitrite likely impairs oxygen transport, secondarily causing stress. Altered *Trx*, *HSP70*, and *HSP90* expression confirms disrupted antioxidant protein homeostasis, adversely impacting shrimp physiological balance.

Metabolic Disruption in Stressed Gills: Untargeted metabolomics identified altered metabolic profiles in *L. vannamei* gills under all stress conditions. The PPAR signaling pathway was notably affected, with concurrent changes in 9-OxoODE (a PPARγ activator). As PPARγ regulates adipocyte differentiation and glucose uptake (Dong *et al*., 2015), these findings imply disrupted carbohydrate and lipid metabolism homeostasis.

Amino acid metabolism pathways were also modified, particularly ornithine, lysine, and niacin alkaloid biosynthesis. Stressor-specific effects emerged: nitrite primarily altered D-arginine/D-ornithine metabolism; Microplastics affected phenylalanine, tyrosine, and tryptophan pathways; combined stress targeted phenylalanine metabolism. This suggests shrimp gills deploy distinct metabolic strategies against different stressors, with PPAR signaling and amino acid metabolism as key response mechanisms.

Metabolite analysis revealed physiological implications: Reduced quinolinic acid (inhibits gluconeogenesis) suggests enhanced glucose synthesis for energy under single stressors (Ribeiro *et al*., 2006). Altered L-tryptophan (involved in immunity/nutrient metabolism; Tejpal *et al*., 2009), L-tyrosine (precursor for lipolytic norepinephrine; Saavedra *et al*., 2008), alanine (promotes glucose production/mucosal repair; Kudsk *et al*., 2006), N-acetylornithine (arginine biosynthesis intermediate; Xing *et al*., 2023), and urocanic acid (supports mucosal repair via glutamate; Filipec *et al*., 1998) indicate disrupted nutrient metabolism and immune function. Decreased O-acetylcarnitine (fatty acid β-oxidation marker; Miyazaki *et al*., 2021) and 8-HETE (arachidonic acid metabolite; Caijo *et al*., 2005) imply impaired fatty acid and inflammatory regulation. Changes in bile acids (hydrocholic acid, deoxycholic acid) suggest disrupted organic acid balance (Caijo *et al*., 2005; Gallimore and Godkin, 2013). Altered 2-pyrrolidone (GABA metabolism marker; Gallimore and Godkin, 2013; Kennedy *et al*., 2019) indicates GABA pathway involvement in stress response (Xing *et al*., 2023).

Micro plastic Accumulation Dynamics: Microplastics were found in gills and digestion systems in all the sampled locations. Gill accumulation may be temporary. Microplastics can stick to mucus or filaments and be released (Su *et al*., 2019). Retention depends on MP type, size, and filtration efficiency (Barboza *et al*., 2021). Gill-entangled MPs can impair respiration, cause physical damage (e.g., filament fractures), and increase infection risk (Jabeen *et al*., 2018; Movahedinia *et al*., 2012). While the gut is the primary uptake route, gills represent a significant pathway for waterborne MP uptake, causing short-term toxicity (Su *et al*., 2019; Sun *et al*., 2019). MPs persist longer in the intestines due to slow digestion (Hastuti *et al*., 2019).

Fragment, fiber, film, and pellet MPs were identified, consistent with other studies (Jabeen *et al*., 2017; Lusher *et al*., 2013; Ory *et al*., 2018). Fragments predominated (gills: 43.7%; digestive tract: 48%), likely originating from abundant plastic bags/bottles in the watershed (Ariyunita *et al*., 2020; Eerkes-Medrano and Thompson, 2018).

The prevalent MP size was 0.05–0.1 mm (gills: 40.7%; digestive tract: 40%). Smaller MPs (<0.05 mm) can translocate more readily, increasing chemical bioavailability (Wright *et al*., 2013). FTIR analysis identified polymers: Polyethylene (PE), Polystyrene (PS), Polyvinylchloride (PVC), Polyamide (PA/Nylon), and Polycarbonate (PC). Fragments contained PE, PS, PVC, PC; fibers comprised PE and PA; films were mainly PE; pellets consisted of PS and PC. PVC poses significant ecological risks due to phthalate additives that disrupt hormones (Ding *et al*., 2020b). PS, PE, PC, and PA prevalence aligns with their buoyancy and widespread use (Klein *et al*., 2015). Polymer types matched known sources: fragments (PE, PS, PVC, PC), fibers (PE, PA), films (PE), pellets (PS, PC) (Chércoles *et al*., 2009; Ding *et al*., 2020a; Scherer *et al*., 2018; Tanaka and Takada, 2016).

Conclusion

The digestive tract shows greater micro plastic (MP) contamination than gills in the studied fish. Both organs contained fragments, fibers, films, and pellets. FTIR analysis identified these MPs as composed of polyethylene (PE), polystyrene (PS), polyvinyl chloride (PVC), polyamide (PA), and polycarbonate (PC). This MP pollution induced oxidative stress responses, evidenced by alterations in SOD, CAT, and MDA levels within the gills and digestive tract of Gambusia from the East Java Brantas River. These findings demonstrate that MP abundance directly and indirectly causes oxidative damage.

Notably, exposure to PVC-MPs did not significantly impair gill histopathology, ion-regulation function, or plasma ion homeostasis in goldfish. This resistance may stem from an efficient detoxification capacity, potentially involving the upregulation of enzymes like GST and CYP1A, which could explain the maintenance of H_2O_2 and MDA at basal levels.

Separate research on zebrafish revealed that one-month exposure to MPs, either alone or combined with copper (Cu), negatively impacts gills. This exposure inhibited antioxidant defenses, induced oxidative stress and damage, and elevated ROS levels. The increased ROS likely triggered apoptosis via the intrinsic pathway, indicated by upregulated *casp3* transcript levels. Furthermore, the combination of MPs and Cu dysregulated the serotonergic pathway in gills, increasing *tph1a* transcript levels. For most biomarkers assessed, the combined MPs+Cu exposure proved more toxic than either pollutant alone, highlighting that plastics interacting with heavy metals pose amplified risks to aquatic organisms. This study reveals molecular and cellular mechanisms governing the long-term toxicity of MPs and Cu in fish gills. However, further research is required to fully understand the combined effects of MPs and their sorbed pollutants, along with potential consequences for aquatic ecosystems.

Conflict of Interest: The authors have no competing interests.

Authors' contributions: MAK, MSR, JC, and DM analyzed the data and wrote and revised the manuscript.

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