

INVESTIGATING THE BEHAVIORAL IMPLICATIONS OF MICROPLASTIC EXPOSURE IN ANIMAL SPECIES

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Abstract

Microplastics (MPs) are pervasive pollutants impacting ecosystems worldwide, causing physical harm and behavioral changes in wildlife. MP toxicity mainly depends on size, porosity, weathering, and surrounding compounds. MPs disrupt feeding and metabolism, resulting in reproductive, neurological, and behavioral disorders. These impacts significantly alter ecological niches and reduce animal survival. Understanding these impacts is crucial for assessing the broader ecological effects of plastic pollution and developing effective mitigation strategies. This review investigated 110 papers on quantifying the behavioral consequences of MPs while considering their physiological basis, emphasizing the significance of these changes and their potential long-term influence on populations and ecosystems. This study thoroughly investigates model species, including plankton, fish, amphibians, crustaceans, pollinators, and mammals. Fish exhibited the most significant behavioral impact, while crustaceans showed the least. Mice, representing mammals, displayed the most severe physiological damage.

Key words: microplastics, animal behavior, reactive oxygen species

Microplastics (MPs) have emerged as pervasive contaminants across global ecosystems. Their high bioaccumulation potential and disruptive effects on ecological processes have raised critical environmental concerns, with far-reaching consequences for biodiversity, ecosystem stability, and overall ecological integrity (Feizi et al., 2024; Razeghi et al., 2021 a). MPs originate from diverse sources, including cosmetics, agriculture, household products, and industrial activities (Wu et al., 2022; Razeghi et al., 2021 b). Microplastics have been found in various sample types, including freshwater ecosystems, marine environments, soil, and even living organisms, such as humans (Hamidian and Dalvand, 2023; Dalvand and Hamidian, 2023). Based on their molecular structure, MPs can enhance the bioavailability and toxicity of coexisting environmental pollutants, such as heavy metals, antibiotics, antibiotic-resistant pathogens, and nearly every chemical in their surroundings during their degradation and emergence (Zhang et al., 2021).

Beyond physiological implications, the impact of microplastics on animal behavior has become a pivotal focus of scientific research, as alterations in behavior often serve as early warning indicators of broader ecological disturbances. This review delves into the underlying mechanisms through which MPs influence animal behavioral alterations, providing a comprehensive analysis of the ecological implications of these disruptions. By comprehending how MPs influence animal behavior, this

review aims to inspire conservation efforts and strategies to mitigate the detrimental effects of plastic pollution on the ecosphere. This is further highlighted when comparing it with our current knowledge on conventional pollutants such as heavy metals (Mirzajani et al., 2016 a, b; Mansouri et al., 2013).

This review identifies keystone and laboratory model species that exert a large impact on ecological conditions through behavioral changes. The emphasis is placed on the importance of studying the behavioral responses of ecologically central species to enhance our understanding and prediction of environmental disturbances' effects on ecosystems. Additionally, the review underscores the complexity of examining species interactions amid multiple disturbances and the necessity for collaborative research efforts. A conceptual framework is required to elucidate the connections between behavioral responses and ecosystem processes, contributing to a more integrated understanding of ecological dynamics (Rahman and Candolin, 2022).

Although this study does not specifically address the neurobiological mechanisms underpinning MP exposure, accumulating evidence suggests that MPs disrupt a broad spectrum of behaviors, including foraging efficiency, predatory interactions, social dynamics, and reproductive processes (Sun et al., 2021 a). These disruptions frequently result in adverse outcomes such as malnutrition, inflammatory responses, and elevated mortality rates. No-

tably, the effects of MPs extend beyond physical impacts, such as ingestion or entanglement, and involve complex physiological and neurobehavioral mechanisms. These alterations have consequences that go beyond individual organisms, potentially affecting population dynamics as well as the resilience and functionality of ecosystems (Chellasamy et al., 2023).

Technical perspectives

Certain ecotoxicology studies propose classifying microplastics as primarily physical agents, emphasizing that their impacts predominantly stem from their physical presence rather than their chemical toxicity. This perspective suggests that MPs disrupt critical biological processes such as feeding, growth, and reproduction by physically obstructing digestive tracts and interfering with nutrient absorption without direct chemical harm. This is in contradiction with the effect of well-documented pollutants such as metals, when the effects are more likely chemical disruptions (Alavian et al., 2017; Padashbarmchi et al., 2015).

Microplastics are frequently ingested inadvertently by organisms, leading to trophic transfer and subsequent biomagnification within food webs. This biomagnification can also cause the increasing release of toxic compounds, such as additives and adsorbed pollutants, into the organism's body.

From a more scientific and professional standpoint, the exposure of microplastic research encompasses various facets, including the polymeric structure and chemical properties of pollutants, environmental parameters, species-specific physiological responses, ecological niches, and the influence of emerging pollutants. Further, microplastics frequently serve as vectors for pollutants such as hydrocarbons, pesticides, heavy metals (Moza-farjalali et al., 2023), and pathogens, exacerbating species' health risks.

Metabolic impact

Microplastics are significant environmental pollutants that interfere with physiological and developmental processes in various organisms. By inducing oxidative stress, MPs disrupt the activity of antioxidant enzymes, leading to tissue damage and metabolic imbalances. Reactive oxygen species significantly disturb the balance of essential antioxidants such as superoxide dismutase (SOD) (Wang et al., 2022 a), catalase (CAT) (Yao et al., 2023), and glutathione (GSH) (Pandi et al., 2022). These disruptions weaken the integrity of cell membranes, lysosomes, and mitochondria, disrupting essential cellular functions. This impairment of cellular integrity leads to reduced growth rates and smaller body size. MPs can also trigger mitogen-activated protein kinase (MAPK) pathways, initiating cell apoptosis and predisposing organisms to various diseases (Wang et al., 2022 b). Additionally, MPs stimulate lipid peroxidation (LPO), oxidative damage to lipids, and DNA damage, collectively exacerbating cellular dysfunction. These effects contribute to

the development of diseases such as cancer, atherosclerosis, and neurodegenerative disorders (Kadac-Czapska et al., 2024).

These mechanisms underscore the complex and harmful effects of microplastics on cellular health and the overall integrity of organisms.

Additionally, MPs affect reproductive systems by impairing sperm viability and inducing developmental abnormalities, ultimately impacting population dynamics. Prolonged exposure to MPs intensifies these effects, resulting in higher mortality rates and reduced species resilience. The cumulative consequences of MPs underscore their profound threat to ecological stability and biodiversity.

Cellular biochemical impact

Microplastics exert multifaceted cellular impacts that compromise cellular homeostasis and functionality. They induce endoplasmic reticulum (ER) stress, triggering the cell unfolded protein response (UPR), which results in oxidative damage and pro-inflammatory signaling. Additionally, MPs cause mitochondrial dysfunction, characterized by disrupted electron transport chains, increased production of reactive oxygen species (ROS), and subsequent activation of apoptotic pathways. MPs also impair lysosomal function, interfering with autophagic processes and leading to the accumulation of cellular debris, further exacerbating cell damage and apoptosis. Furthermore, MPs directly compromise cell membranes through physical interactions and ROS-induced lipid peroxidation, resulting in structural instability and loss of membrane integrity. These cellular disruptions collectively highlight the profound and multifactorial toxicity of MPs at the molecular level, with far-reaching implications for organismal health and ecological stability (Kadac-Czapska et al., 2024).

Neurological impacts

Acetylcholinesterase dysfunction represents a subset of the broader and extensively studied field of genotoxicity induced by external pollutants and chemical exposures. Meta-analyses have identified key indices for assessing genotoxic endpoints, including the percentage of DNA in the tail (TDNA%), and the number of micronuclei (NM). These biomarkers have consistently shown significant increases following microplastic exposure, underscoring MPs' genotoxic potential. Specifically, TDNA% and NM were elevated by 20% and 81%, respectively, compared to control groups. TDNA% measures the proportion of DNA migrating into the comet tail during a comet assay, with higher values reflecting greater DNA damage. Similarly, micronuclei, which are small extranuclear structures formed from chromosome fragments or whole chromosomes excluded during cell division, serve as critical indicators of chromosomal damage and genotoxicity. These findings collectively highlight the multifaceted genotoxic impacts of MPs and their role in disrupting cellular integrity (Sun et al., 2021 b).

Acetylcholinesterase (AChE) is an essential enzyme found in all organisms with a nervous system that relies on acetylcholine as a neurotransmitter, underscoring its critical evolutionary role. Despite its universal presence, AChE's structure and sensitivity to inhibitors, such as pesticides, can vary among species, reflecting their ecological adaptations. Recent research has revealed that microplastics exert neurotoxic effects on AChE activity in a size-dependent manner, with smaller particles inducing greater inhibition. Species-specific responses to MP exposure further highlight the variability in neurotoxicity across taxa. For example, exposure to polystyrene nanoparticles (PSNP) at a concentration of 3 mg/L resulted in a 20% reduction in AChE activity, significantly impairing the enzyme's ability to degrade acetylcholine and potentially disrupting normal neurotransmission. Additionally, the severity of MP-induced genotoxicity has been found to correlate more strongly with particle size, species characteristics, and habitat than with MP composition, morphology, or exposure parameters (Torres-Ruiz et al., 2023). These findings emphasize the complex and multifaceted impacts of MPs on neurological and cellular functions across species.

Environmental studies point of view

Microplastics infiltrate ecosystems from diverse sources and circulate across various environmental compartments. Their structural properties influence retention times within each medium before transitioning to another. However, once inside an organism, MPs exhibit prolonged persistence, posing significant ecological and health risks. Even when organisms expel these particles, they inadvertently facilitate their availability to other species, perpetuating the exposure cycle.

Animal behavior involves intricate responses to internal and external stimuli, deeply rooted in genetic frameworks and regulated by the nervous and hormonal systems. These behaviors, shaped by environmental triggers and evolutionary adaptations, are critical in defining a species' ecological niche and sustaining environmental balance. However, exposure to mutagenic or oxidative agents can disrupt these behaviors by altering gene expression and causing nerve blockages, potentially undermining ecosystem stability.

Thus, microplastic pollution profoundly influences behavior through a complex cascade of physiological and biochemical disruptions. Upon infiltration, MPs are ingested by organisms through respiratory or digestive pathways. Once entrapped, MPs induce oxidative stress, characterized by the overproduction of reactive oxygen species, which cause cellular damage and inflammation. The mass of microplastics, accompanied by their oxidative byproducts, migrates to vital organs, including the brain, where it disrupts neural signaling pathways and impairs neurological functions.

Simultaneously, MPs and their chemical additives act as endocrine disruptors, altering hormonal homeostasis and interfering with processes regulating stress, reproduction, and metabolism. This oxidative imbalance dam-

ages neuronal structures, disrupts synaptic function, and impairs neurotransmitter synthesis, leading to alterations in cognition, movement, and social interactions manifest as impaired foraging, social interaction, reproduction, and predator avoidance.

Such behavioral dysfunctions jeopardize individual species and cascade through populations, destabilizing trophic interactions, disrupting ecological processes, and ultimately threatening biodiversity and ecosystem resilience.

As previously noted, behavioral changes undermine the health of individual animals and entire populations, resulting in far-reaching consequences for critical ecosystem services, including climate regulation, nutrient cycling, erosion control, pest management, disease regulation, and pollination.

Humanity and future generations will be the ultimate sufferers of these ecological convulsions, bearing the consequences of diminished biodiversity, weakened ecosystem services, and compromised environmental stability (Relić and Đukić-Stojčić, 2023).

Figure 1 illustrates the cycle of plastic pollution across five interconnected stages:

Stage 1: Human activities introduce plastic debris into the environment as primary or secondary microplastics via degradation processes based on particle size and exposure duration.

Stage 2: Microplastics disperse across environmental compartments, including air, water, and soil, and are consumed indiscriminately by organisms.

Stage 3: The retention of MPs within organisms causes health impairments due to prolonged exposure and bioaccumulation and biomagnification of the particles and toxic compounds.

Stage 4: Surviving organisms carrying MPs (or those still processing them) progress through the food chain, culminating with humans as the ultimate recipients.

Stage 5: The disruption caused by microplastics extends to vital ecological processes, hindering nutrient cycling, energy flow, and biomass distribution and triggering interspecies behavioral dysfunctions, ultimately threatening the stability of ecospheres.

This comprehensive overview underscores the multifaceted impacts of MPs on organisms and ecosystems, with implications for biodiversity and human health.

Pollution impact assessment approaches

Historically, manual observation was the primary method for assessing behaviors such as feeding and movement. Today, advanced bio-logger technologies track physiological parameters, such as heart rate, providing deeper insights into animal stress and welfare (Glencross et al., 2023). Controlled behavioral experiments and longitudinal studies have expanded our understanding of environmental stressors. These studies highlight key behaviors influenced by pollution, such as altered movement patterns, disrupted predation strategies, and changes in reproductive behaviors (Santos et al., 2021 a).

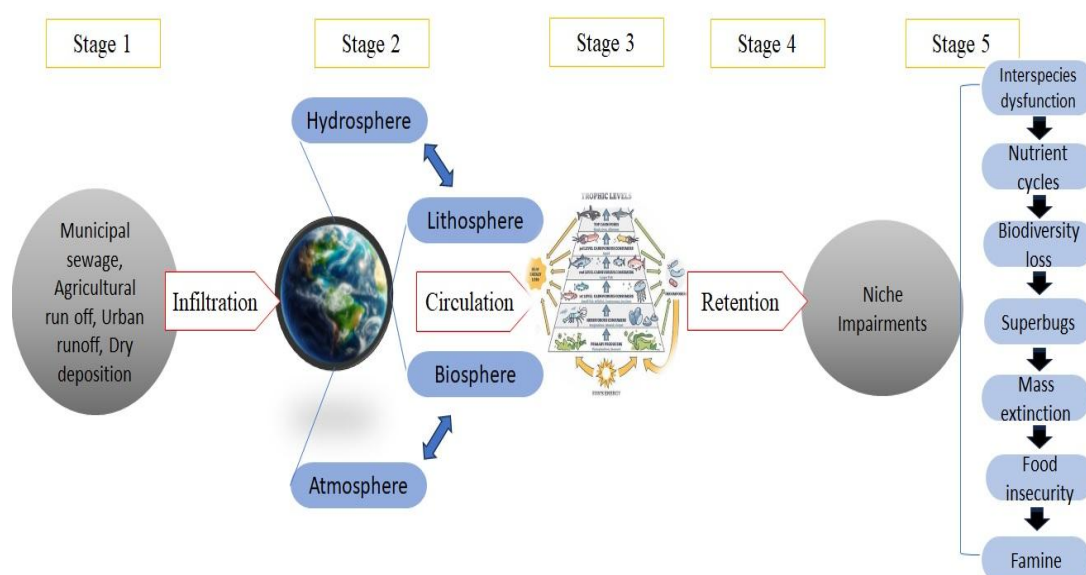


Figure 1. Cycle of plastic pollution in the environment

Researchers have developed a range of methodologies to capture distinct aspects of animal behavior and associated physiological responses. Laboratory-based assays are pivotal for specific behaviors, including feeding, locomotion, reproduction, and predator-prey interactions.

Feeding behavior assessments, for example, measure food intake and prey capture efficiency, revealing potential disruptions in energy balance and nutritional health due to microplastic exposure.

Locomotion and activity level measurements, often conducted using sophisticated video tracking systems, offer insights into changes in motion patterns and energy expenditure, indicating potential impairments in physical health. Reproductive studies explore the effects of microplastics on mating behaviors, nest building, and offspring care, where disruptions could lead to significant population declines. Predatory interaction assessments evaluate how microplastics influence pursuit success and escape maneuvers.

Physiological and biochemical assessments, such as stress response measurements and neurochemical analyses, elucidate the internal mechanisms driving observed behavioral changes, offering critical insights into the systemic effects of microplastics on animal health.

Cognitive neuroscience approaches assess the neurotoxicity of microplastics on learning and memory, uncovering possible impairments in sensory responses and potential damage to neural pathways involved in perception. Field studies complement laboratory investigations by observing long-term behavioral shifts and habitat utilization in natural environments, often employing telemetry and tracking methods. Together, these methodologies form a comprehensive framework for understanding the multifaceted impact of microplastic pollution on ani-

mal behavior and conservation. The subsequent pathway leads to behavior alteration (Sun et al., 2021 a).

Food web

Trophic-level studies have revealed that the predominant microplastics identified were polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), and polystyrene (PS). Microplastic concentrations were higher in organisms at higher trophic levels, indicating potential transfer along the food chain. The detected shapes included fibers, fragments, films, and pellets, with fibers being the most common. These findings indicate that microplastics transfer through trophic levels, accumulating in higher-level organisms and potentially impacting consumer health. By tracing trophic levels (plankton, worms, snails, bees, amphibians, fish, and mice) and simulating the course of microplastic flow, we can confirm the transfer and accumulation of microplastics within higher-level organisms (microplastic biomagnification).

The grazer plankton, ciliate *Euplotes vannus*, reportedly ingested 20 mg/L of polyester microplastics, resulting in significant reductions in ingestion rate (28.18%), growth rate (32.01%), biovolume (30.46%), and carbon biomass (82.27%) (Zhou et al., 2024). In contrast, heterotrophic dinoflagellate *Oxyrrhis marina* showed no significant changes in growth rate, biovolume, or carbon biomass under similar microplastic exposure conditions (Rauen et al., 2023). These differences might stem from variations in feeding mechanisms, energy allocation strategies, or physiological resilience between the two species.

The unproductive consumption of microplastics led to a reduction in dinoflagellate abundance, as the organisms expended significant energy in searching

for, phagocytosing, and excreting plastic particles (Rauen et al., 2024). On the other end of the web, fish species are particularly vulnerable to microplastic contamination through genotoxicity, foraging speed, and reproductive disorders, making them more susceptible than many other organisms. The detection rate of microplastics increased from sandworms (42.86%) to fish (91.95%) (Wang et al., 2021 a). We investigated the behavioral impacts of microplastics on various organisms, including plankton, worms, snails, bees, amphibians, fish, and mice, to provide further clarification on this issue.

Fish

Fish are prone to ingesting microplastics due to their small size and similarity to natural prey, leading to unintentional consumption. Once ingested, MPs can release toxic chemicals into fish tissues, potentially compromising their health. Research has shown that MPs disrupt marine ecosystems at multiple levels, ranging from physiological to molecular impacts. These disruptions include interference with microalgal photosynthesis, growth, gene expression, and nutrient exchange, while in fish, MPs cause inflammation, oxidative stress, and metabolic disturbances, ultimately impairing health and growth (Parolini et al., 2023; Wang et al., 2023). Moreover, studies have demonstrated size-dependent effects, with smaller particles causing more severe genotoxicity, and found a significant reduction in acetylcholinesterase levels in the brains of exposed aquatic species. It is recommended to evaluate changes in animal behavior by assessing various genotoxic endpoints following MP exposure (Sun et al., 2021 b).

Recent research on juvenile jacobever (*Sebastes schlegelii*) has demonstrated that exposure to polystyrene can significantly affect behavior, physiology, and nutritional quality. This exposure has been linked to impaired feeding, reduced swimming speed, and restricted movement, leading to decreased hunting abilities. Additionally, microplastics were found to accumulate in the gills and intestines, causing damage to the gallbladder and liver, ultimately resulting in diminished growth, protein, and lipid content, indicating reduced energy reserves and nutritional quality (Yin et al., 2018).

Furthermore, *Danio rerio* (zebrafish) exposed to microplastics showed decreased intestinal D-lactate levels, indicating gut dysfunction, inflammation, and oxidative stress. Similarly, *Dicentrarchus labrax* exposed to PVC particles exhibited intestinal changes, including increased goblet cells and villus thickness, potentially affecting feeding and nutrient absorption (Covello et al., 2024). Exposure to plastics, metals, drugs, and pesticides has led to neurobehavioral changes in *Danio rerio*, including reduced aggression, impaired predator avoidance, anxiety, and cognitive dysfunction, highlighting both their sensitivity to environmental stressors and the broader ecological risks for aquatic life (Orger and Polavieja, 2017; Sarasamma et al., 2020).

Fish exhibits a range of complex behaviors, including locomotion, thigmotaxis, social interactions, reproduction, and foraging, which have been thoroughly investigated in neurobiology, toxicology, and behavioral genetics. These behaviors are critical for activity, anxiety, and social dynamics, providing insights into group behavior, dominance hierarchies, and reproductive strategies. Plastic particles disrupt courtship and mating by altering swimming behavior, reducing locomotion, and triggering anxiety-like responses, hindering effective mating rituals and territorial defense. Social interactions are also disrupted, with changes in gathering frequency and time spent in spawning areas, which are critical for ensuring reproductive success.

Zebrafish exhibited significantly reduced exploratory behavior, preferring to stay near the tank bottom, along with heightened anxiety, hyperreactivity, and altered predator avoidance. Disruptions in circadian rhythms, abnormal movement orientation, reduced locomotor activity, and decreased aggression and predator avoidance highlight the extensive negative impacts of MP pollution on zebrafish behavior (Mak et al., 2019; Yu et al., 2022; Proca et al., 2024).

Intestinal MP accumulation did not significantly damage villi but reduced foregut muscularis thickness at low concentrations. Despite no oxidative stress or histological changes, zebrafish showed marked hyperactivity, likely due to particulate stimulation and elevated estrogen; this led to energy depletion, reduced glucose and acetaldehyde, and increased amino acids (Chen et al., 2020). These behavioral alterations may result from intricate mechanisms, including direct effects on sensory organs and the central nervous system, as well as potential hormonal imbalances, underscoring the multifaceted impact of PSNP on neurobehavioral health in affected organisms. The biochemical analysis findings indicate that polymers inhibited acetylcholinesterase activity and disrupted endocrine-related gene expression in the thyroid and glucocorticoid axes (Torres-Ruiz et al., 2023).

1. Behavioral alterations experiment in fish

To study the effects of microplastic exposure on fish behavior, researchers employed advanced methods to observe swimming patterns, social interactions, and courtship behaviors, with particular attention to circling, figure-eight movements, territorial displays, and other specific actions. Advanced video recording techniques and three-dimensional behavior analysis are required to capture and analyze subtle changes in zebrafish behavior in real-time. Social behaviors can be evaluated by social interactions, aggression levels, and predator avoidance assessment, providing insights into the social dynamics affected by plastics. Additionally, neurotransmitter levels are measured using enzyme-linked immunosorbent assay (ELISA) to quantify brain neurotransmitters such as dopamine, serotonin, and GABA, highlighting potential neurochemical changes. Finally, histological examination of brain tissue is required to analyze struc-

tural changes, inflammation, and apoptosis, allowing for a comprehensive understanding of the neurotoxic effects of pollutants on zebrafish behavior (Santos et al., 2022).

Recent advancements in research methodologies have significantly enhanced our understanding of the effects of environmental pollutants on zebrafish. Notably, ZF-AutoML, a machine-learning technique, has been developed for detecting anomalies in fluorescent-labeled zebrafish, providing a robust framework for behavioral analysis (Sawaki et al., 2019). Furthermore, optogenetics is employed to manipulate olfactory responses in transgenic zebrafish, allowing for precise exploration of sensory processing and behavioral responses. The reviewers also examined the effects of biodegradable polymers on fish development and neurobehavior, revealing significant developmental stunting in post-fertilization embryos. Microplastics resulted in reduced survival and hatching rates, increased wakefulness, and decreased larvae sleep, indicating disruptions in circadian behavior potentially linked to alterations in brain-derived neurotrophic factor (BDNF) levels (Kardgar et al., 2023; Luan et al., 2023).

2. Aquacultures

Based on published studies and the author's previous experiments, aquaculture ponds have been reported to be impacted by microplastics, with concentrations in water ranging from 6.6 to 263.6 items per liter. The predominant type of MPs in water are fibers, typically less than 0.5 mm in size, with red, blue, and transparent colors. The primary polymer types found in the water are cellulose, polystyrene (PS), polyethylene terephthalate (PET), and polyethylene (PE). In sediments, the concentration ranges from 556.67 to 2500 items/kg, with fibers being the most common form and less than 0.5 mm in size. These fibers are listed as red, yellow, green, white, pink, blue, and transparent, with the polymer types being cellulose, polypropylene (PP), polymethyl methacrylate (PMMA), and polyethylene (Li et al., 2021; Wu et al., 2022). For the aquatic species in China, rice-fish (*Procambarus clarkia*) ingests fewer than single fiber particles smaller than < 1 mm (Lv et al., 2019), while Florida bass (*Micropterus salmoides*) consumed between 12 to 92 items per individual, and Nile tilapia (*Oreochromis niloticus*) ingests 14 to 94 items per individual in natural water (Tien et al., 2020).

Aquaculture animal behavior can be systematically analyzed using traditional and advanced methodologies. Manual observation has long been the standard approach for monitoring feeding behavior and food intake, relying on visual assessments of the animals' responses to feeding. Growth and energetics models further enhance this analysis by evaluating feeding rates, diet formulation, environmental conditions, feeding frequency, and behavioral patterns. In aquatic environments, microplastics can enter organisms through respiratory or digestive pathways, while terrestrial species may inhale airborne particles or drink them through water (Sonwani et al., 2021).

Studies investigating trophic-cycle impacts of sediment plastic particles on amphipods and fish species, such as those conducted by Tusetto et al. (2017), reported no significant changes in fish personality. However, a general increase in shyness emerged, disrupting schooling behavior and social interactions. In their experiments, Tusetto et al. (2017) used analytical-grade plastic particles placed on the beachside for 60 days to absorb pollutants (pyrene) before being collected and utilized in feeding trials. This methodology, however, has been criticized by multiple authors for its lack of alignment with the structural characteristics of polymers typically found in environmental samples. Microplastics serve as carriers for hazardous chemicals like phthalates and PCBs, which can gradually leach into the bodies of animals, thereby increasing the risks to both environmental and human health. Studies detected bisphenol A (BPA) in fish liver and muscle tissues. Fish with microplastics showed significantly higher bisphenol levels, suggesting that microplastics may increase bisphenol accumulation in aquatic organisms (Barboza et al., 2020; Fuster et al., 2022).

Histological changes, hormonal imbalances, and impaired reproductive functions were also observed, emphasizing the multifaceted reproductive toxicity of MPs (Wu et al., 2024). Although the number of proven contaminants that have neglected to assess their effects combined with plastics are tributyltin (Lan et al., 2020), norethindrone (Hou et al., 2020), tebuconazole (Yan et al., 2023), and thiamethoxam (Yang et al., 2023). Microplastics worsen cadmium-induced oxidative damage and inflammation, further compromising behavior and reproduction. Fipronil disrupts neurotransmitter functions, while deltamethrin inhibits enzymes and alters swimming behavior, contributing to neurotoxic effects (Santos et al., 2021 a, b; Proca et al., 2024). Nanoparticles carrying these compounds penetrate the embryonic brain, causing neuronal loss and disrupting GABAergic, cholinergic, and serotonergic systems, leading to behavioral abnormalities (Li et al., 2025).

Plankton communities

In plankton communities, behavioral alterations have been quantified by selective factors such as algal growth, grazer behavior, trophic cascades, and overall ecosystem stability. The studies on zooplankton have demonstrated a reduction in heart rate and hopping frequency, which ultimately diminishes their grazing rates efficiency and heightens their vulnerability to predation by competitors. This alteration strengthens the trophic cascade effects and enhances algal growth due to reproductive capacity depletion and grazing pressure (De Felice et al., 2019; Pan et al., 2022; Khoshnamvand et al., 2024).

The studies demonstrated a series of behavioral assays, including a spontaneous tail movement assay, a light/dark activity assay, thigmotaxis anxiety assays using auditory and visual stimuli, and a startle response habituation assay in reaction to auditory stimuli. In co-

pepods, MPs can cause inflammation, stress responses, reduced egg production, toxicity, and ultimately death. Survival rates were size- and concentration-dependent, with 50 nm particles significantly delaying developmental time. The ultraspiracle gene, associated with molting and metamorphosis, was downregulated. In contrast, the vitellogenin gene expression, a precursor for protein synthesis and reproduction, remained unaffected by larger particles. However, these particles led to an overall decline in reproductive success. Moreover, genes associated with oxidative stress (such as catalase) and inflammation (such as lipopolysaccharide-induced TNF factor) showed increased expression due to MP exposure (Kim et al., 2022; Rodríguez-Torres et al., 2020).

Amphibians

In anuran families, the tadpoles exhibited notable external changes, including reduced body length, abnormal tail enlargement. They decreased ocular and mouth areas, alongside an increasing number of melanophores and pigmentation rates in the skin (camouflage capabilities). The reports also highlighted mutagenic effects characterized by an increase in nuclear erythrocyte abnormalities, alongside cytotoxicity evidenced by alterations in erythrocyte size, nuclei area, and nucleus/cytoplasm ratio (Araújo et al., 2020).

The affected tadpoles exhibited reduced swimming speeds and shorter travel distances, indicating impaired locomotion. Furthermore, they spent increased time in the periphery of test arenas, reflecting elevated anxiety levels (Araújo and Malafaia, 2020; Boyero et al., 2020). Also, the tadpoles of *Pelophylax nigromaculatus* exposed on PLA-MPs showed significant increase in the proportion of freezing behavior positively correlated by particle concentration. In tank tests, PLA-MPs increased tadpole aggregation, with a tendency for tadpoles to cluster in edge regions. The combination of PLA-MPs and Cd²⁺ resulted in an increase in aggregation, which then decreased with higher concentrations of Cd²⁺ (Li and Chen, 2024).

To evaluate the MP exposure effects on the defensive responses of tadpoles, the researchers measured the total distance covered by individual tadpoles before and after exposure to cues from tadpole-fed larvae. Although predation risk significantly reduced the tadpole's total distance travelled, the varying MP concentrations did not affect their defensive behaviors. Contradictorily, some studies showed no influence of MP concentration on tadpole growth or mortality rates (De Felice et al., 2018; Scribano et al., 2023).

Recently, microplastic contamination was reported for the first time in adult many-banded tree frogs (*Bonana multifasciata*) and *Physalaemus ephippifer* (Souza-Ferreira et al., 2025). These Amazonian anuran species exhibited high contamination levels across all exposure pathways, including the integument, respiratory tract, and gastrointestinal tract. Health issues, including tissue damage, chronic inflammation, and granuloma forma-

tion in the respiratory tract have been documented. The significance of this study lies in its emphasis on the integumentary system of amphibians as a critical exposure pathway, demonstrating significant levels of microplastic contamination. Notably, the integumentary system exhibited higher levels of microplastic contamination than the respiratory tract, though lower than the gastrointestinal tract.

Crustaceans

Some species facilitate microplastic dissemination by ingesting, accumulating, and breaking down MPs, re-introducing them into the environment, sustaining their presence in the food chain (Swank et al., 2022). However, chronic exposure causes them physiological stress, leading to behavioral changes like altered burrowing and emergence patterns in Norway lobster (*Nephrops norvegicus*), which affect their ecological roles and vulnerability to fishing (Aguzzi et al., 2023).

Research indicates that heavy metals, microplastics, and organic contaminants significantly disrupt the behaviors of hermit crab (*Dardanus calidus*), such as resource assessment, risk coping, and shell selection. For instance, copper impairs the ability to evict opponents during shell fights, while microplastics affect cognitive functions and shell selection. Organic pollutants, such as certain pharmaceuticals, also alter learning behaviors and increase predation risk (Crump et al., 2020; Cunningham et al., 2021; Briffa et al., 2023; Crump et al., 2023). However, recent findings reveal that shore crabs (*Hemigrapsus anguineus*) showed notable resilience and adaptability after exposure to polluted plastic leachates, with no significant behavioral changes observed. This resilience is underscored by high inter-individual variability in behavior, suggesting adaptive traits that enhance their ability to cope with environmental stressors. Behavioral observations showed no significant changes in anxiety-related behaviors in crabs, including startle response, scototaxis, thigmotaxis, and vigilance, after exposure to leachates. However, high inter-individual variability suggested adaptive traits that may enhance resilience and contribute to their success as an invasive species (Delaeter et al., 2023; McDaid et al., 2023).

Soil media

Microplastics, particularly polyethylene, significantly alter soil properties such as pH, stability, and nutrient cycling, which can disrupt soil-dwelling organisms vital for maintaining soil structure and ecosystem health. These changes can also negatively impact seed germination, crop growth, and plant development, potentially reducing agricultural productivity. Studies reveal that microplastics lead to notable changes in the feeding behavior, locomotion, avoidance responses, and reproductive capacity of soil-dwelling organisms. Although some MPs may enhance plant growth by improving soil structure, most studies revealed generally impaired seed germination, root activity, and photosynthesis due to pore

blockage, root damage, and the release of toxic additives (Zhou et al., 2021).

Positively charged MPs (MP⁺) can infiltrate plant tissues, reducing biomass, altering gene expression, and increasing oxidative stress while inhibiting beneficial bacteria and acting as vectors for pathogens (Li et al., 2022). Most studies overlooked measuring the zeta potential of microplastics, which typically ranges from −30 to −40 mV. Zeta potential is crucial for understanding MP toxicity, as it affects particle stability and interactions with biological membranes. A more negative charge can enhance colloidal stability and influence the bioavailability and toxicity of microplastics, making these measurements vital for assessing their impact on organisms and ecosystems (Wang et al., 2022 a). Wastewater treatment plants, which produce sludge as fertilizer, are a significant source of MPs in soils, alongside dry deposition and plastic waste degradation. These soaked MPs disrupt soil health, potentially leading to long-term ecological consequences by altering soil properties and hindering plant development (Hamidian et al., 2021).

In soil-dwelling species, smaller particles are particularly harmful due to easier ingestion and prolonged retention in the gut. Laboratory-produced plastics have demonstrated ecotoxicity in organisms such as *Caenorhabditis elegans*, impacting energy metabolism and reproductive fitness. Exposure to microplastics in *C. elegans* causes significant physiological and behavioral disruptions, including impaired growth, reduced reproduction, oxidative stress, premature aging, and circadian rhythm disturbances. These effects, often linked to oxidative stress and neurodegeneration, underscore the potential neurotoxic impact of plastics (Gubert et al., 2023). In earthworms, exposure results in histopathological damage, immune responses, oxidative stress, and disrupted energy metabolism, all of which indicate compromised health and functionality. Similarly, springtails exhibit decreased mobility, increased avoidance behavior, suppressed reproduction, and reduced gut bacterial diversity, further disrupting their ecological roles. Nematodes are ideal for assessing microplastic ingestion, accumulation, and toxicity due to their transparency, sensitivity, and well-characterized genetics.

Nematodes show reduced body length, decreased intestinal calcium levels, increased enzyme expression, and inhibited reproduction, suggesting long-term adverse effects on population dynamics. Interestingly, isopods display no significant impacts on feeding or energy reserves, indicating variability in response among different taxa (Qiang et al., 2023). Similarly, analysis of nematode showed a decrease in body length, intestinal calcium levels, and reproductive inhibition (Lei et al., 2018; Kim and An, 2019).

Mobility tests have been conducted to quantify changes in movement rate and distance travelled by organisms such as springtails and nematodes. Avoidance tests assess the reaction of springtails to microplastic-contaminated soil by measuring their avoidance rates. Further, feed-

ing behavior in isopods and earthworms is assessed by evaluating their energy reserves. Reproductive effects in nematodes and earthworms are determined by examining body length, intestinal health, embryo number, and brood size. Reproductive behavior should be assessed through egg-laying rates and offspring viability metrics.

Pollinators

The complex behaviors of honey bee (*Apis mellifera*), one of the most influential insect species, including foraging, navigation, and communication, are closely linked to their physiology and environmental conditions. Research shows that environmental pollution disrupts the efficiency of their foraging systems, which rely on sensory cues such as light direction and landmarks. Few studies have investigated the MP physiobiological effects on *Apis mellifera*. Findings indicate that prolonged exposure to PSMP alters antioxidative, detoxification, and immune system-related genes in young honey bees, potentially disrupting their physiological functions (Wang et al., 2021 b). Ingested polyethylene is transferred to the hemolymph, causing histological changes and increasing susceptibility to viral infections. Exposure to MPs has also been linked to disrupted locomotion and altered light-seeking behavior in honey bees, particularly at higher doses compared to lower doses and controls, further underscoring the health risks posed by microplastic ingestion (Deng et al., 2021; Keteoglou et al., 2023). High PE concentrations significantly increased bee mortality, while lower levels did not. PE exposure also altered feeding behavior, with bees at low concentrations consuming more food. However, in behavioral tests, high concentration of PE specifically impaired bees' consistent response to sucrose, while sucrose sensitivity, habituation, learning, and memory remained unaffected (Balzani et al., 2022; Ahmed, 2023).

Although previous reviewers do not directly address the combined effects of plastics contaminated with metals, they recognize the potential for synergistic interactions among various environmental stressors. The documented impact of pesticide exposure and air pollution on animals' immune functions suggests that plastic and metal contaminants could pose similar risks. For instance, heavy metal contamination significantly threatens foraging sources, health, and colony stability of honey bees. Researchers employ biomarker analyses, measuring metal concentrations and examining related enzymes and proteins in bee tissues. These combined threats can severely compromise bee health, weaken immune systems, and increase disease susceptibility, ultimately undermining colony resilience.

Mammals

Plastics significantly impact the metabolic, reproductive, and physiological health of mice, serving as a representative model for understanding mammalian health effects. Maternal plastic exposure during gestation led to metabolic disorders in offspring, highlighting the po-

tential for long-term health consequences and leading to disrupted metabolic homeostasis not only in the dams but also in their offspring (F1 and F2), indicating that the effects of microplastic exposure can transcend generations (Luo et al., 2019 a, b). The plastics-induced pyroptosis and apoptosis in ovarian granulosa cells through the NLRP3/Caspase-1 caused reproductive toxicity (Hou et al., 2021).

Notably, transplacental transfer has been documented and potentially exposed developing embryos to potential harm. Ingested MPs, especially those within biofilms enriched with microorganisms, can release absorbed pollutants, exacerbating toxicological effects. The parental transfer of microplastics to offspring, whether through regurgitation or contaminated nesting materials, further increases exposure risks. It has been demonstrated that PSMP leads to excessive production of reactive oxygen species in mice, which disrupts skeletal muscle regeneration by converting myoblasts into adipocytes (Shengchen et al., 2021). To assess mice's behavioral alterations, they should be exposed to microplastics and evaluated using cognitive tests such as the Morris water maze and novel object recognition. A significant accumulation of MPs in brain tissues, leading to blood-brain barrier disruption, reduced dendritic spine density, and hippocampal inflammation has been reported. Exposed mice exhibited cognitive and memory deficits, reflected in reduced movement and lower novelty scores, indicating impaired short-term recognition memory. Additional effects included deeper nucleolar staining, fewer hippocampal neurons, elevated pro-apoptotic mRNA levels, decreased synaptogenesis-related proteins, and increased inflammation-related genes and proteins. These results suggest that PS-MP exposure may cause learning and memory dysfunctions and induce neurotoxic effects, raising serious public health concerns (Jin et al., 2022). These studies underscore the wide-ranging and multigenerational impacts of polystyrene exposure on mammalian health, necessitating further investigation into their ecological and human health implications.

Microplastic studies overlooked the essential role of circadian rhythms in regulating biological processes such as cellular activities, reproduction, and behavior in organisms. Disruptions to these rhythms caused by environmental pollution can lead to significant issues for both animal and human behaviors. Integrating circadian disturbances as a parameter in MP pollution studies may elucidate the compounded effects of environmental stressors on behavior and overall health, enhancing our understanding of anthropogenic impacts on biological systems. Circadian rhythms regulate gametogenesis, ovulation, and critical behaviors such as sleeping, feeding, and locomotion. Environmental factors such as hypoxia, light pollution, and temperature fluctuations significantly impact these rhythms in humans and fish. Understanding these dynamics is crucial for addressing the circadian disruption's broader ecological and behavioral effects (Zheng et al., 2021; Zeman et al., 2023).

Studies classified the impact of environmental chemicals on disrupting rhythms in aquatic organisms, mainly fish, into six groups: steroid hormones (e.g., glucocorticoids), metals (e.g., lithium), pesticides (e.g., thifluzamide, flutolanil, and 2,4-dichlorophenol), PCBs, neuroactive drugs, and other compounds such as cyanobacterial toxins and bisphenol A. Unfortunately, microplastics can act as vectors for all of these compounds especially in nanoparticles joined with metals (Mozafarjalali et al., 2023).

1. Mice maternal exposure

Luo et al. (2019 a, b) investigated the intergenerational effects of maternal exposure to polystyrene microplastics during gestation, focusing on metabolic disruptions in offspring. Key findings revealed that pregnant mice exposed to 0.5 mm and 5 mm MPs at 100 and 1000 mg/L exhibited significant metabolic alterations in their offspring. These changes included disrupted serum triglycerides, total cholesterol, HDL-C, and LDL-C levels, with more pronounced effects observed in the 5 mm MPs-treated group. Significant shifts in serum metabolites, including amino acids and acyl-carnitines, were also observed alongside evidence of fatty acid metabolism disorders, as confirmed by altered hepatic gene expression.

A recent study investigated the effects of polystyrene microplastic (PS-MP) exposure on female mice and their offspring with a test dose of 1.357 $\mu\text{g/g}$ body weight by artificial feedings. Female mice (F0) exposed to PS-MPs during lactation, and their reproductive health were assessed from puberty to adulthood (Dou et al., 2024). The findings revealed delayed puberty onset, disruptions in the estrous cycle, reduced fertility marked by fewer pups per litter, elevated testosterone levels, and ovarian abnormalities, including impaired follicular development and inflammation. In the offspring (F1), male mice exhibited significant reproductive toxicity, characterized by reduced sperm count and viability, while no notable reproductive abnormalities were detected in female offspring (F1 and F2). Notably, the transgenerational effects of reproductive toxicity were pronounced in male offspring (F1) but did not persist into the second generation (F2), highlighting the potential for limited generational impact. These findings underscore the intergenerational health risks associated with MP exposure, particularly highlighting the increased susceptibility linked to larger particles.

Discussion on the noticed implications and miscalculations and neglections

The implications of experimental design factors such as circadian rhythms, environmental stimuli, and genetic variability are critical for accurately interpreting behavioral and physiological data. Theoretically, assembling favorable circumstances is achievable; however, replicating optimal conditions within a laboratory environment presents significant challenges, potentially leading to confounding results (Venâncio et al., 2022).

The incarceration stress induced in experimental set-ups can also affect behavioral outcomes, highlighting the importance of minimizing stressors in study designs. Genetic variability, including specific compromises, can lead to increased resilience and adaptability, often without noticeable physical changes. Species such as landfill scavengers challenge many established assumptions about the hazards of plastic debris on animal health and survival.

Circadian rhythms require delicate metabolic and environmental pathways leading to overall animal health. Environmental stimuli include temperature fluctuations, humidity, natural odor, pheromone reactions, light angle, temporary food preference alterations, interspecies interaction, wind directions, moisture, and other parameters. Therefore, laboratory lighting conditions and the color of human operators' clothing may inadvertently influence animal behavior, introducing biases that can skew data. Temperature variations and seasonal changes further complicate results by altering metabolic rates and behavior, emphasizing the need to control these variables for experimental consistency.

Moreover, discrepancies between F1 and F2 generations, arising from genetic and epigenetic changes, must be carefully considered when assessing long-term and transgenerational plastic effects. The second generation reacts differently to the establishment due to altered environmental stimuli. Furthermore, species diversity and group size within experiments can impact social interactions and data interpretation, necessitating careful consideration of these factors to enhance the generalizability of findings. Moreover, the chronic natural exposure of microplastics in wildlife and subsequent evolutionary adaptations by animals can bias experimental results, as these animals may already have adapted to the effects of MPs, thus skewing the observed responses. Addressing these methodological considerations is essential for refining experimental protocols and achieving reliable and valid conclusions in scientific research.

Recent studies have introduced an innovative, cost-effective detection method that involves the artificial biofilm feeding embedded with fluorescent microplastic particles. The microplastics can then be tracked by observing their transitions under UV light, which makes them visible in feces. This method is notable for its speed and accessibility, allowing researchers without specialized training or expensive equipment to utilize it. However, using this approach, no behavioral alterations were observed in snails (*Radix balthica*) (Ehlers et al., 2020).

The authors recommend utilizing multiple applications, emphasizing cost-effectiveness, open-source accessibility, user-friendly modular design, and straightforward infrastructure. Notable examples include *AnimApp* and *GoFish*, which are effective for analyzing small animal movements (such as distance and speed) and studying fish and insect larvae through video recordings (Rao et al., 2019; Ajuwon et al., 2024). These tools offer reproducible and precise analysis of behavioral alterations.

Conclusion

Microplastics significantly impact living organisms by causing physical damage, altering feeding behaviors, and impairing growth. MPs can block metabolic pathways, sink due to biofouling, and increase the risk of novel diseases. Microplastics compromise animals' ecological niches through a cascade of events, beginning with physiological stress and progressing to metabolic, neurological, and behavioral alterations. The integrity of the ecosystem relies on the interactions among ecological niches; and any disruption in species fulfilling their roles can lead to the collapse of the entire ecosystem. Prolonged exposure to microplastics reduces reproductive success, increases mortality in species such as copepods, and causes intestinal infections in small fish and seabirds. In plants, MPs obstruct sunlight, hinder photosynthesis, and disrupt nutrient transport, leading to oxidative and genotoxic damage. These effects highlight the broad ecological risks of microplastics in environments.

This review highlights the need for interdisciplinary approaches to understand and mitigate the impacts of environmental pollution on animal behavior. Integrating perspectives from ecology, toxicology, and behavioral science is essential for developing effective strategies to protect wildlife and preserve ecosystem stability amid escalating environmental challenges. Ongoing research and proactive policy measures are crucial for safeguarding animal behavior and maintaining the vital services provided by healthy ecosystems. Future research should focus on developing safe techniques for emerging contaminants, such as magnetic and organic nanoparticles, ensuring that these technologies do not pose additional risks to ecosystems or human health. As previously noted, certain crabs, fish, and mammals exhibit notable resistance to oxidative agents. It is strongly encouraged to explore the physiological mechanisms that underpin this resistance, especially in landfill scavengers, to gain a more comprehensive understanding of the factors influencing their adaptability.

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