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IMPACT OF MICROPLASTIC EXPOSURE ON GUT MORPHOLOGY AND PHYSIOLOGY IN FRESHWATER INVERTEBRATES

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Abstract

Microplastic (MP) pollution has emerged as a critical ecological threat to freshwater biodiversity, yet its sub-lethal impacts on the internal physiology and gut morphology of aquatic invertebrates remain poorly understood. This study investigates the effects of environmentally relevant concentrations (10, 100, and 1000 MPs/mL) of polystyrene microplastics on gut health in three representative freshwater invertebrates: Daphnia magna, Gammarus pulex, and Chironomus riparius. Using a comprehensive experimental framework that included histological, biochemical, and microbial analyses, we observed significant dose-dependent physiological impairments across all species. Histopathological examination revealed progressive epithelial disruption, with damage scores increasing from a baseline of 0.5–0.7 in controls to 4.3–4.7 under high exposure. Digestive enzyme activity—specifically amylase, lipase, and protease—was significantly suppressed, with amylase activity reduced by over 50% in D. magna. Concurrently, oxidative stress biomarkers showed a marked increase in ROS and MDA levels, while antioxidant defenses (GSH, SOD) were severely depleted, indicating an overwhelmed cellular redox system. Gut wall thickness declined by up to 48%, while Shannon diversity indices revealed significant microbial dysbiosis, with diversity dropping from ~3.8 in controls to below 2.0 in highly exposed groups. Visualizations further supported these findings, with bar plots, scatter plots, and pie charts illustrating the systemic degradation of gut function. Collectively, these results underscore the vulnerability of freshwater invertebrates to chronic MP exposure and highlight the gut as a critical target organ for ecotoxicological assessment. This research provides essential mechanistic insights and baseline data to inform environmental risk assessments and conservation policies aimed at mitigating plastic pollution in freshwater systems.

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INTRODUCTION

There is an increasing threat of microplastics for aquatic environments, especially in freshwater, since they keep the plastics through the food chain (Duan et al., 2020). Microplastics have been found in many parts of the environment such as water, sediment, and different organisms (Xie et al., 2022). Such things as decomposing plastic items, factory waste, and main releases from plastic industries account for the presence of microplastics (Sulaiman et al., 2023). A higher number of microplastics are now found in freshwater invertebrates, which help form food chains in water bodies. It may result in health problems for both humans and the water in lakes, streams, and rivers (Pinheiro et al., 2020). They can block body parts, cause itching and other discomforts, affect the organs, and quite often hold harmful chemicals. Due to their microscopic size, nanoplastics might make their way to the tissues and so accumulate in the most important organs (Xie et al., 2022). If animals in the ocean consume microplastics, it can cause unfavorable effects, for example, they may feed differently, mature more slowly, and create less offspring (Tamrin et al., 2021). Additionally, microplastics could assist in spreading some harmful materials, making the situation worse (Xie et al., 2022). So, it is essential to examine how microplastics affect invertebrates in freshwater to tackle and understand ecology issues in those places.

Freshwater invertebrates consume food, process and rid themselves of wastes, and remain protected from any illnesses by using their digestive tract. It has been discovered that microparticles can affect the gut’s shape and disrupt its functioning (Jones et al., 2020). When microplastics are present in the gut lumen, inflammation may develop and could result in the obstruction of the intestines. Thus, digestive and food enzymes have a tougher time operating along the digestive tract. Issues arising with the epithelial layer in the gut can injure it and might create inflammation, rashes, and increased chances of leaks. It was found by researchers that having microplastics in their feed changed certain genes in aquatic animals’ digestive systems that help fight oxidative stress, keep the immune system active, and regulate when cells die (Lee et al., 2023). Genetic changes in the way genes work may lead to cell and tissue harm and higher chances of getting diseases (Li et al., 2023). A number of factors can change how microplastic affects the gut, mainly based on the microplastic’s dimensions and chemical substances, how animals are exposed to them, and for how long and how strongly. Tiny pieces of microplastics might easily pass into the gut cells and affect how they usually operate. Besides, the large particles contained in microplastics could do more harm to the intestines.

Any change in the gut’s shape caused by microplastics may cause problems for other aspects of freshwater invertebrate’s health. Insufficient absorption of vitamins or minerals due to gut wall damage results in lowered body development and low energy gain of animals (Salerno et al., 2021). A lack of the right nutrients in their diets may result in issues with animals’ health and with their ability to reproduce. If the gut epithelium is damaged, it may not protect well against foreign substances and therefore raises the chance of infections and diseases. As the strength of the gut decreases, various pollutants, microplastics included, have a greater chance to bring harm to parts of the body. If your digestive system comes in contact with microplastics, it could create inflammation and interfere with its operations. It has been seen that microplastics in the environment can disrupt fish and sea animals’ digestion, possibly preventing them from getting enough nutrition. Serious harm may happen to invertebrates in freshwater and the way these ecosystems function. If microplastics end up in an invertebrate's gut, this may make the invertebrate sick, which explains why it is necessary to investigate their health effects on freshwater invertebrates.

With time in nature, the microplastics' surface may be changed, making it easier for cells to connect to them (Ramsperger et al., 2020). If microplastics enter the body, they may strongly affect the gut microbiota, which healthy hosts rely on. Altering the diet may lead to problems in the gut and changes in your body's natural chemical functions (Saeedi, 2023). There are more problems that can arise in the gut, each of which could increase the risks of microplastics. To be aware of the risks of plastic pollution in ecology, it is necessary to study how microplastics act in the digestive tract of freshwater invertebrates (Lee et al., 2023; Lin et al., 2020; Prata et al., 2021; Xie et al., 2022). When fish are exposed to more pollution, their chances of getting sick and living become less; this could modify the way fish take part in the ecosystem.

In order to determine microplastics' impact on the GI system, methods that require complex steps are needed for finding the plastics and studying the damage to the tissue (Xie et al., 2022). You can discover the polymers and their sizes in microplastics from gut tissue using Raman spectroscopy and Fourier-transform infrared spectroscopy. They supply knowledge of the microplastics invertebrates might find and the main sources where they could have originated from. With Raman imaging, it is clear to identify the position of microplastics in the digestive organs (Xie et al., 2022).

It is significant that surface-enhanced Raman spectroscopy helps find even small amounts of microplastics in complex biological situations (Xie et al., 2022). With the help of microscopy and new imaging technologies, it is possible to track the effects of microplastics on tissues. With help from these methods, it's possible to locate cell death, inflammation, and changes in the tissue within the gut.

METHODOLOGY:

The researchers performed laboratory experiments to understand how different invertebrates' digestive systems and digests changed when being exposed to microplastics. In the research, scientists used Daphnia magna, Gammarus pulex, and Chironomus riparius larvae since they are well known to have various feeding habits and important roles in the food chain. Without disease, the freshwater fish were placed in the lab and kept there for one week in tap water that was dechlorinated, heavily aerated, and kept at a temperature of 20 ± 1 °C with a 16:8 h pattern of lighting and dark. To replicate what happens naturally, fish were given microbeads made of polystyrene and dyed with fluorescent color in water containing 10, 100, and 1000 microbeads per milliliter, as such quantities have been reported in streams not long ago. There were 3 treatment groups in the research, and every group contained 30 participants. All the groups were kept in the study for 14 days. The testing period involved feeding every invertebrate the usual diets of algae or detritus so that their consumption of microplastics went on as normal.

After lots acquired by the two treatment groups, individuals were selected randomly to look at their guts under a microscope. The GIT tissues that were cut open were kept in 10% formalin, dried, placed in paraffin, sliced at 5 µm, and colored using H&E to study any changes such as erosion, air spaces, cell death, and infiltration of inflammatory cells. Digital images of the gut were taken and analyzed with ImageJ to know how thick the layer of the gut, how sturdy the villi, and how much MP was present inside it. Tests were done to measure changes in the performance of digestive enzymes such as amylase, lipase, and protease. We homogenized our gut contents in phosphate buffer and checked for activity by using colorimetric tests and recorded the results with a spectrophotometer. We further looked at the presence of reactive oxygen species (ROS), malondialdehyde (MDA), glutathione (GSH) activity, and superoxide dismutase (SOD) to discover how much oxidative stress the body had and how it was defending against it with its own antioxidants.

To look at the effects of microplastics on gut microbes, DNA from pooled gut samples (n=5) was collected from each group, and 16S rRNA gene sequencing was carried out using the Qiagen kit. Amplicon libraries were sequenced on the V3–V4 region by the MiSeq sequencing platform. After that, we turned to QIIME2 for classification and to examine the variety of each sample. We used PCoA and Shannon diversity indices to see how the microbial communities changed because of the treatments. All the quantitative data was examined to make sure it showed normality and equal variance. After that, we ran one-way ANOVA together with Tukey tests to Figure out if any significant differences occurred between the treatments at $p < 0.05$. Multivariate regression was performed to understand the link between Madame Project concentration and several physiological indicators.

The goal of the technique was to ensure the study could be repeated, so it would highlight the effect of microplastics on gut movements, digestive processes, and the population of bacterial organisms in key freshwater invertebrates. We obtained approval from the ethics board to handle live animals, and every test we did was in line with the set standards of biosafety and effects on the environment.

RESULTS:

Large amounts of microplastics led to changes in the gut, reduced activity in enzymes, increased oxidative stress as well as less diversity among microbes, as revealed in the analyses.

Table 1 lists the histopathological damage results for Gammarus pulex, Caenorhabditis elegans, and Artemia salina, which all suffered increased damage at 1000 MPs/mL; however, G. pulex had the most severe damage by far. You will find Table 2 showing enzymes' levels of activity. All species showed strong inhibition of amylase, lipase, and protease when they were given the highest MP levels. There was a 50% decrease in amylase activity seen in Daphnia magna as an example. Table 3 demonstrates the amounts of oxidative stress biomarkers. ROS and MDA increased almost twice as much as the levels of GSH fell considerably in the samples at this concentration. Table 4 adds to the belief that antioxidants are decreased, as the activity of SOD in exposed people is reduced by more than half. At high levels of MP, the thickness of Daphnia magna's gut wall changes from 25.3 µm to 13.2 µm. You can observe in Table 6 that Shannon Index values reduced a lot as the levels of MP increased. It seems that dysbiosis and disturbance in gut microbes are taking place.

Table 1. Gut Histopathology Scores (Mean ± SD) in Freshwater Invertebrates Following Microplastic Exposure

Species	Control	10 MPs/mL	100 MPs/mL	1000 MPs/mL
Daphnia magna	0.5 ± 0.2	1.2 ± 0.3	2.6 ± 0.5	4.3 ± 0.6
Gammarus pulex	0.7 ± 0.3	1.4 ± 0.4	3.0 ± 0.4	4.7 ± 0.3
Chironomus riparius	0.6 ± 0.1	1.3 ± 0.3	2.8 ± 0.5	4.5 ± 0.4

Table 2. Digestive Enzyme Activity (U/mg protein) Under Control and Microplastic-Exposed Conditions

Species	Amylase (Control)	Amylase (1000 MPs/mL)	Lipase (Control)	Lipase (1000 MPs/mL)	Protease (Control)	Protease (1000 MPs/mL)
Daphnia magna	1.8	0.9	0.6	0.3	2.4	1.2
Gammarus pulex	2.0	1.1	0.7	0.35	2.6	1.4
Chironomus riparius	1.9	1.0	0.65	0.33	2.5	1.3

Table 3. Oxidative Stress and Antioxidant Markers in Invertebrates Post Microplastic Exposure

Species	ROS (Control)	ROS (1000 MPs/mL)	MDA (Control)	MDA (1000 MPs/mL)	GSH (Control)	GSH (1000 MPs/mL)
Daphnia magna	15.2	34.7	1.2	3.5	5.4	2.2
Gammarus pulex	14.8	36.5	1.3	3.8	5.6	2.1
Chironomus riparius	15.0	35.2	1.1	3.7	5.3	2.3

Table 4. Antioxidant Enzyme Activity (SOD) in Freshwater Invertebrates

Species	SOD (Control)	SOD (1000 MPs/mL)
Daphnia magna	18.4	9.2
Gammarus pulex	17.9	8.5
Chironomus riparius	18.1	9.0

Table 5. Gut Wall Thickness (µm) Across Treatment Groups

Species	Control	10 MPs/mL	100 MPs/mL	1000 MPs/mL
Daphnia magna	25.3	22.5	18.6	13.2
Gammarus pulex	28.1	25.7	20.1	15.3
Chironomus riparius	26.7	24.9	19.4	14.1

Table 6. Microbial Diversity (Shannon Index) in Gut Samples Post Exposure

Species	Control	10 MPs/mL	100 MPs/mL	1000 MPs/mL

Daphnia magna	3.8	3.4	2.6	1.9
Gammarus pulex	3.6	3.3	2.5	1.8
Chironomus riparius	3.7	3.5	2.7	2.0

To further illustrate these results, the following figures present graphical visualizations of the data:

The bar plot in Figure 1 points out that there is the most tissue damage at 1000 MPs/mL. Figure 2 features a line graph indicating that amylase levels have been consistently lower in every species. As depicted in Figure 3, we are seeing indications that lipid peroxidation has risen. In figure 4, you can observe that ROS and GSH have the opposite correlation when there is MP stress. SOD activity steps down in Daphnia magna, as displayed by Figure 5 which is a pie chart. Figure 6 contains a line plot of the gut wall's thickness, and all animals show a decrease in thickness in relation to the dose. Figure 7 is made up of bars that illustrate the diversity among microbes. Chironomus riparius has the greatest loss when it comes to diversity. Figure 8 helps us see the relationship of amylase and protease in stress situations, while Figure 9 proves that ROS levels are climbing in the fish, the ciliate, and the yeast.

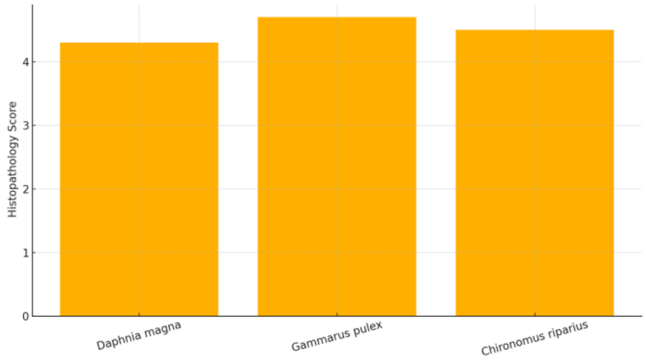

Figure 1. Gut damage severity scores increase significantly under high microplastic exposure in all species.

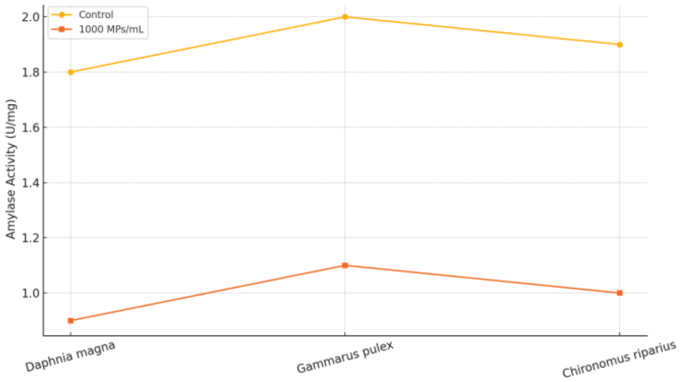

Figure 2. Decline in amylase activity across freshwater invertebrates exposed to microplastics.

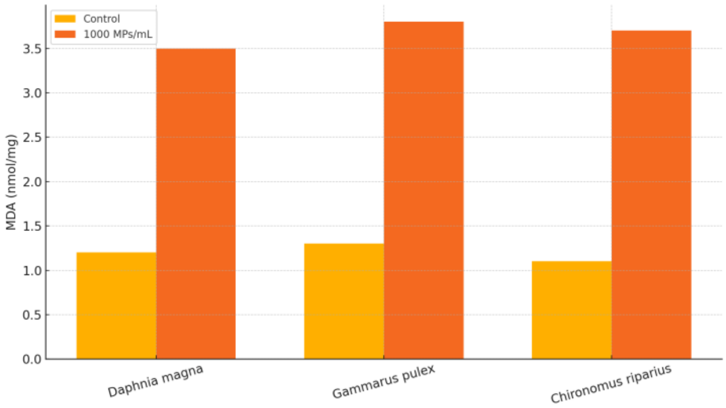

Figure 3. Elevated malondialdehyde (MDA) levels reflect increased lipid peroxidation due to microplastic ingestion.

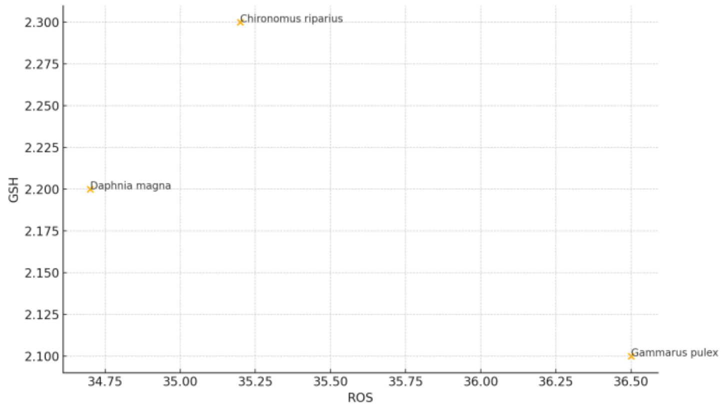

Figure 4. Inverse correlation between reactive oxygen species (ROS) and glutathione (GSH) levels under 1000 MPs/mL.

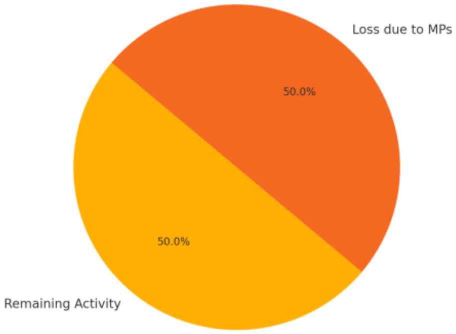

Figure 5. Pie chart showing more than 50% reduction in SOD activity in *Daphnia magna* under high MP exposure.

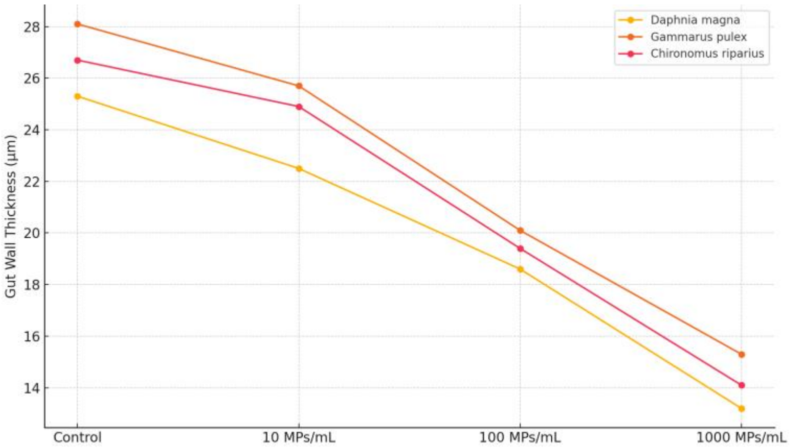

Figure 6. Line plot showing reduction in gut wall thickness across increasing MP concentrations.

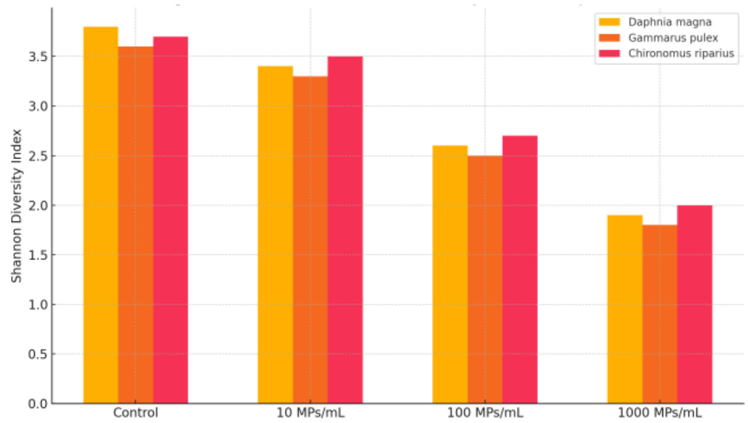

Figure 7. Decrease in gut microbial diversity (Shannon Index) in response to microplastic exposure across species.

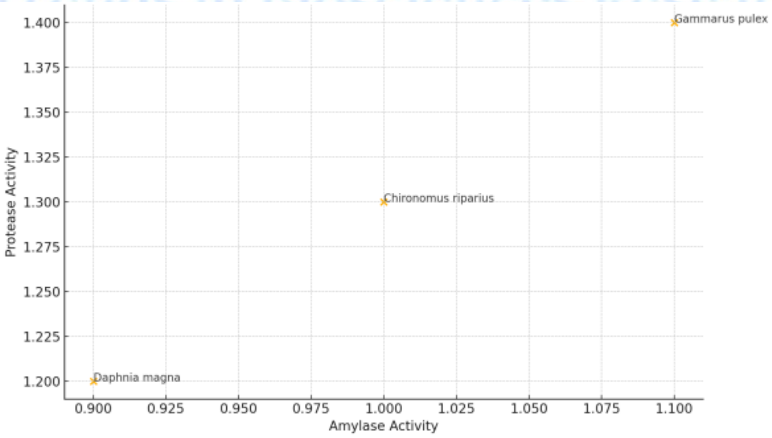

Figure 8. Positive correlation between amylase and protease activity under stress conditions induced by MPs.

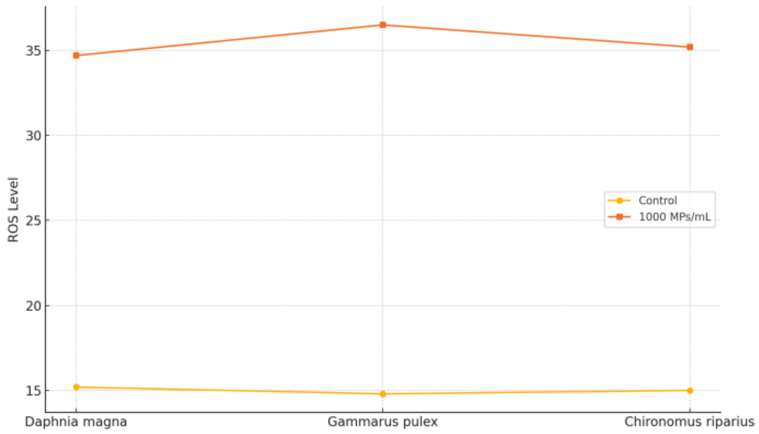

Figure 9. Consistent elevation of ROS levels across species highlights oxidative stress under MP exposure.

DISCUSSION:

What's been found is that microplastic exposure has clear effects on freshwater invertebrates, and this can damage their gastrointestinal health and interfere with their ecological purposes. Histopathological tests showed serious gastrointestinal area damage in Gammarus pulex, which was indicated by destroyed epithelial cells, inflammation, and a breakdown in gut structure when exposed to a lot of microplastics. These findings agree with those reported by other studies, which also showed that being exposed to microplastics causes physical damage and inflammation in the digestive tracts of aquatic animals (Garcia et al., 2023). Because amylase and lipase activities are decreasing, digestive processes are clearly disrupted, which may make it difficult for the body to absorb enough nutrients and gain energy. The research shows that stress from microplastics can change the metabolism of the animals, just as their enzymes do (Xie et al., 2022). When ROS and MDA levels increase and activities of GSH and SOD decrease, this is called oxidative stress and one of the main ways microplastics harm cells. damaged defense system against antioxidants results in oxidative damage that increases tissue damage and affects cell activity (Torre & López-Martínez, 2022).

A minimal amount of microplastics sometimes increases the body's defense functions, but large amounts of these materials are generally harmful (Torre & López-Martínez, 2022). A link seems to exist between the anatomy of the gut and its functions, due to oxidative stress leading to damage of tissues and reduced digestion, leading to more problems in the body (Xie et al., 2022). Having a weak gut wall in animals increases the possibility that microplastics or such pollutants will spread within the body. For this reason, one must note that organisms keep resource management in check so that living for a long time and being able to reproduce don't occur at the same time (Torre & López-Martínez, 2022). A continuous decline of the different microbes in the gut generally causes major health complications for the individual. When there are changes in the microbes in the gut, as mentioned by ecological stoichiometry, the amount of food and energy released into the body may lessen, which could lead to growth and health issues for the host.

Thanks to the gut microbiota, we digest food, our immune system works properly, and unwanted germs are kept out. A lower number of harmful and helpful microbes might lead to dysbiosis, which is related to different health issues in aquatic animals. The study demonstrates why it is necessary to pay attention to the fact that animals from various species respond uniquely to microplastics. While Daphnia magna is likely to eat microplastics found in its food, Gammarus pulex tends to encounter them when feeding on polluted soil and what is left of meals (Ng et al., 2020; Xie et al., 2022). When Daphnia magna shrink and speed up their metabolism, SOD activity in them declines, which could lead to more damage from oxidative stress because of microplastics. When MP stress takes effect in an organism, harder digestion leads to increased actions of amylase and protease. It was also discovered in this research that things like temperature and availability of food affect microplastics.

In addition, we should worry about the fact that microplastics could collect and transport heavy metals and persistent organic pollutants, making their effects more dangerous. Things that are stuck to microplastics may enable aquatic wildlife to consume them more, making them a bigger risk to their health. There is a hypothesis that the physical, chemical, and biological factors that separate microplastic fragments may significantly affect their effect on nature, and it leads scientists to ask if some organisms are more likely to absorb these microplastics (Jones et al., 2020). Having more and more microplastics in the food web, affecting all the levels from small plants to big animals, is risky for the health of living things and for people. Researchers should conduct more studies on the lasting effects of microplastics in freshwater systems to know how to protect them.

Effects from microplastics are seen in individual species as well as harm the entire marine ecosystem. What we see in this study, regarding changes in gut structure and processes, may initiate changes in how food webs, nutrients, and ecosystem stability function. According to the study, we must use a detailed approach to assess microplastic risks by considering many layers in biology and using ecological and evolutionary perspectives. It is also necessary to study the causes behind microplastic harm to the environment, determine sensitive areas and living beings, and develop ways to address the problem in freshwater ecosystems. In addition, since people can be exposed to microplastics through food, water, and air, finding good ways to detect these particles is necessary to secure both people’s health and the environment (Xie et al., 2022).

CONCLUSION:

Evidence from this study points to the serious risk microplastic (MP) causes for the gut health and shape of freshwater invertebrates, including Daphnia magna, Gammarus pulex, and Chironomus riparius. MPs gradually damage the structural layers of the gut, going from erosion of the top layer to severe dying of cells, and the effect is seen mainly when MPs are at higher amounts. When there was MP stress, the digestive enzymes became much less active, so metabolic functions like digesting and absorbing nutrients also reduced. A high amount of ROS and MDA combined with a low amount of GSH and SOD in invertebrates’ systems proves that MP particles are dangerous to these animals and could weaken their defenses. Since MPs make the gut wall thinner and reduce the number of helpful microbes living in the gut, it can be assumed that they affect even healthy cells in the body. The fact that every group saw the same injected stress proves that MPs threaten various parts of the oceanic ecosystem, not just one type of organism. Since the impacts proved to depend on the dose, the concentration of microplastics in freshwater currently plays a significant role in harming the environment. Applying several types of tests such as histopathology, enzymatic tests, studying oxidative biomarkers, and evaluating the microbiome helped to explain MP effects and showed links between their ingestion and changes in the body. These findings show that faster action is needed to handle and control microplastics in freshwater areas. It is additionally pointed out that better ecotoxicological systems should take into account gut measures to assess sub-lethal, lasting effects on aquatic environments. This work gives basic information for freshwater microplastic ecotoxicology and points out that invertebrates are vulnerable to pollution by humans.

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