ISSN: 0973-4929, Vol. 20, No. (1) 2025, Pg. 410-417

Current World Environment

www.cwejournal.org

Effects of Polystyrene Microplastics on Behavioural Response and Histopathology of *Carassius auratus*

MINAKSHI KUMARI* and ARTI KUMARI

University Department of Zoology, LN Mithila University, Darbhanga, Bihar, India.

Abstract

The contamination of aquatic ecosystems by plastic waste is a major environmental challenge worldwide. Microplastics are small plastic particles having size ranging from 1 μm to 5 mm. Their ingestion has been detected in many aquatic organisms. In this study, the aim is to investigate behavioural responses and histopathological changes in the gills and gut of goldfish (*Carassius auratus*) following exposure to 10 mg/L of Polystyrene microplastics (PS-MPs, size: -5 μm) for 28 days. Histopathological alterations were observed in the gills and intestine of the treated group. Significant alterations detected in the gills of the treated group include lamellar aneurysm as well as lamellar deviation, along with protrusion of the secondary lamellar epithelium and fusion of secondary lamellae. In the intestine, structural damage observed included broken villi, muscularis mucosa, and submucosa, along with enlarged goblet cells and detached epithelial layers. The behavioural changes observed in the treated group illustrated signs of distress, lethargic movement, and lack of appetite. Additionally, the fish showed impaired buoyancy control, making it difficult for them to maintain an upright orientation as well as positional control within the water column. The research revealed the physiological dysfunction resulting in fish by toxic effects of PS-MPs.

Article History
Received: 07 February 2025
Accepted: 02 April 2025

Keywords
Behavioural Response; *Carassius Auratus*; Histopathology; Polystyrene Microplastics.

Introduction

The contamination of aquatic ecosystems due to plastic waste is a major environmental concern worldwide. Plastic pollution refers to the accumulation of plastic debris and particles in the environment posing significant risks to all living organisms, including humans. Plastic that acts as a pollutant is categorized by the size into nano-, micro-, meso-, or macro debris.[1]

The increasing global production of plastic since the mid-20th century has led to a heavy buildup of plastic waste in both freshwater[2] and marine[3] ecosystems. The ingestion of plastic has been widely detected in

CONTACT Minakshi Kumari minakshi.lifesciences@gmail.com University Department of Zoology, LN Mithila University, Darbhanga, Bihar, India.

Doi: https://dx.doi.org/10.12944/CWE.20.1.31

many aquatic organisms, raising significant concerns about its ecological consequences.

The discovery of the plastic in 1869 by Hyatt was revolutionary. Since then, it has taken new and better forms and become an integral part of day-to-day life. However, its non-biodegradability has made plastic waste a menace for the environment. Plastic wastes, due to their buoyant properties, flow with the drainage system and finally end up in aquatic ecosystems.[4] In recent years, several new pieces of evidence have accumulated indicating the harm caused to the aquatic ecosystem by microplastics and nanoplastics.[5,6]

Microplastics (MPs) are formed by degradation of larger plastic products and other related synthetic materials, which are of length lesser than 5mm.[7] Polyethene, polystyrene, polyvinylchloride and polyethene terephthalate are among the most abundant microplastics found in the world.[8] Of these, polystyrene (PS) finds its application in a wide range of plastic products.[9]

Polystyrene (PS) is a synthetic polymer synthesized from styrene monomer. Its applications include a wide range of products, including biomedical/ bioanalytical materials, toys, food packaging materials, refrigerator linings etc, Because of these diverse applications, it is one of the most widely used plastic materials. Polystyrene plastic exists in various forms such as solid plastic, rigid foam, and thin PS films. However, despite its widespread use, PS contributes significantly to plastic pollution due to its persistence and potential toxicity.

The ingestion of microplastics by living organisms is a well-known issue, but plastic leaching is also a significant concern. It is well known that the chemicals leaching out of plastic materials react with the food material to produce toxins. These toxins, as per common notion among general people, are said to cause multiple types of cancer. In the case of polystyrene, the leaching of unbound, free styrene monomers, which is a liquid petrochemical, poses elevated risks of its incorporation in the food chain and bioaccumulation among animals at higher trophic levels. The majority of the studies reported tissue-specific accumulation of polystyrene microplastics (PS-MPs), whereas, some also showed oxidative damages caused by its metabolic form-styrene oxide, resulting in micronuclei formation, chromosomal aberration and sister chromatid exchanges.[10]

Hence, in the present study an effort has been made to illustrate histopathological changes in tissues of different organs of *Carassius auratus* exposed to Polystyrene microplastics.

Materials and Methods

The freshwater gold fish, *Carassius auratus* procured from Ali aquarium, Darbhanga. The healthy fish have average length 7.4±0.5 cm (measured using a digital vernier caliper) and weight were 20±0.5g (measured using a digital weighing balance) were brought to laboratory and washed with KMnO4 (0.1%) to avoid external infection. The fish were acclimatized for 15 days under laboratory conditions before the experiment. The fish were fed commercial floating feed (Tetra Goldfish Gold Growth) once daily at 3% of their body weight. Physicochemical water parameters (Temperature- 14 to 22°C, dissolved oxygen- 6.62 to 6.76 mg l-1, alkalinity- 62 to 68 mg l-1, CO2-Nil, pH-6.5 to 8.5) were maintained according to APHA guidelines.[11]

The stock of 5 µm PS-MPs was purchased from Sigma Aldrich Pvt. Ltd. and stored at 4ºC in a laboratory refrigerator (Haier HYC-260) to ensure stability and prevent degradation. Before each use, the stock was sonicated. The fish were subjected to PS-MPs (5 µm) at a concentration of 10 mg/L for a duration of 28 days. Simultaneously, control fish were maintained without PS-MPs parallel with treatment group. The fish were sacrificed on the 29th day, following the completion of the 28 days exposure period. 3-4 mm thick tissue samples from vital organs of fish such as the gills and intestine were taken from both the treated and control group. These samples were fixed in 10% Neutral Buffered Formalin for some time (18-24 hours). The fixed tissue samples were processed and then embedded in paraffin blocks following standard histological procedures.[15] Using a rotary microtome, 5–7 µm thick serial sections were obtained. For routine histological staining, stains like, Ehrlich's Haematoxyline (H.) and alcoholic Eosin (E.) were prepared and applied according to Luna.[12] Photomicrographs were taken to document the most characteristic histopathological lesions in the stained tissue sections.

Experimental Design

Prior literature was reviewed to formulate the experimental design, ensuring relevance and validity. The study involved Gold fish (*Carassius auratus*), which were divided into two groups: a control group and a treated group exposed to 10 mg/L Polystyrene microplastics (PS-MPs) of 5 µm size. The concentration of 10 mg/L of PS-MS was chosen based on previous studies that have showed its biological impact on aquatic organisms like zebrafish.[13] This allows for a meaningful assessment of microplastic toxicity in fish. Each group was maintained in triplicates, requiring a total of 6 tanks.

The experiment was conducted in 120 L aquaria, each containing 100 L of water. In each tank, 7 fish (n=7) were housed to maintain consistent stocking density. The PS-MPs were prepared from stock solution and administered to the treated groups, while the control groups were maintained without exposure. Optimization of exposure duration and doses was conducted to reflect realistic environmental conditions.

Results

The Gold fish, *Carassius auratus* exposed to PS-MPs (5 µm) at a concentration of 10 mg/L, exhibited clinical signs, histopathological changes in gills and intestine, as well as alterations in behavioural responses.

Behavioural Response

Behavioural changes were observed in treated fish (*Carassius auratus*), exposed to PS-MPs (5 µm) at a concentration of 10 mg/L. This study utilized a single concentration (10 mg/L) of PS-MPs (5 µm), chosen based on prior research that reported significant biological effects at this level. The focus was to evaluate behavioural responses under a defined exposure condition. Future studies will incorporate multiple concentrations to better understand dose-dependent toxicity and establish a broader toxicological profile.

The treated fish exhibited signs of distress, lethargy, and reduced appetite, which progressively worsened over time. Among the treated fish, 60% exhibited erratic swimming behavior, 40% showed signs of lethargy, and 30% displayed reduced feeding activity. The severity of these behaviors was assessed using a three-point scale (mild, moderate, severe), with 35% of affected fish classified as exhibiting severe behavioral impairments. With prolonged exposure, the fish showed impaired buoyancy control, making it difficult for them to maintain an upright orientation and positional control within the water column. The impaired buoyancy and lethargic movements observed in the treated fish suggest neurological disturbances, potentially linked to microplastic ingestion. Previous studies[14,15] , have also reported similar behavioral disruptions in fish exposed to PS-MPs, indicating that these effects may be associated with neurotoxicity or gut-brain interactions caused by microplastic exposure.

Additionally, microplastic accumulation in fish tissues, particularly in the gut and gills, may affect their survival, growth, and reproductive success, ultimately disrupting aquatic food webs. Since microplastics have been detected in commercially important fish species, there is a potential risk of human exposure through trophic transfer, particularly via seafood consumption. This aligns with previous studies that have raised concerns about the bioaccumulation of microplastics and their associated toxicants in higher trophic levels.

Clinical Inspection

All fish (*Carassius auratus*) from the entire test group were clinically examined. Traces of microplastics were detected in the gill chamber and mouth. Oedematous patches were detected at sites where microplastic particles had deposited.

Histopathology

The histopathological alterations were detected in gills and intestine of C. auratus exposed to PS-MPs. Gill: The gills of goldfish exhibit a typical teleostean plan. They comprise of primary as well as secondary gill lamellae, supported by a cartilaginous skeletal framework, multilayered epithelium, and a well-developed vascular system. The primary gill lamellae appear as flat, leaf-like structures arranged in double rows, with alternately arranged secondary lamellae extending laterally. These secondary lamellae are lined with squamous epithelium and supported by pillar cells (Figure 1).

In the gills of the treated group, significant histopathological alterations were observed, including lamellar aneurysm, protrusion of the secondary lamellar epithelium, lamellar deviation, and secondary lamellar fusion (Figure 2). Statistical analysis revealed that gill lamellar fusion was significantly higher in the treated group compared to the control (t = 4.57, p = 0.02), confirming the severity of microplastic-induced damage. These structural changes indicate impaired respiratory function, which could lead to reduced oxygen uptake and increased physiological stress in fish. Similar gill pathologies have been reported in other fish species exposed to polystyrene microplastics,[16,17] reinforcing the concern that PS-MPs contribute to respiratory dysfunction in aquatic organisms.

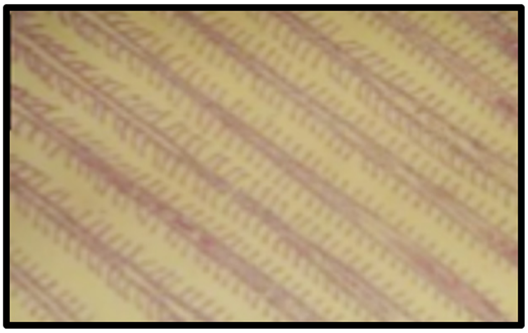

Fig. 1: Micrograph of gill tissue of normal Gold Carp (*Carassius auratus*) (H&E 100x).

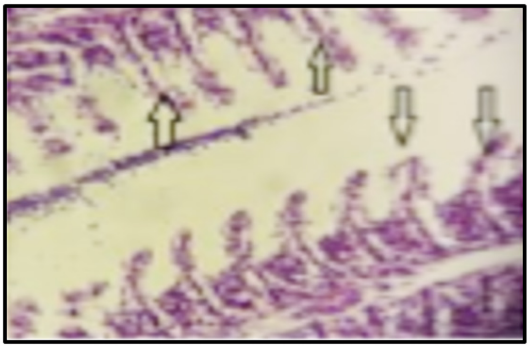

Fig. 2: Micrograph of gill tissue of treated *Carassius auratus*, exposed to PS-MPs (5 µm) at concentration of 10 mg/L for 28 days, showed lamellar aneurysm, secondary lamellar fusion, (H&E 150x).

Gastrointestinal tract

The intestinal wall of control Gold carp, *C. auratus* have four distinct layers, which are: *mucosa, submucosa, muscularis* and *serosa*. The mucosal layer is thrown into finger-like villi, which are composed of many goblet cells (mucous cells) with nuclei positioned in the center and simple, long columnar cells. The lamina propria is made up of mucosal folds into which the thin sub-mucosa is projected. This layer is made up of blood cells, collagen fibers, and loose connective tissue. There are two layers of muscle in the muscularis: the thick, circular inner layer and the thin, longitudinal outer layer. Blood capillaries and the peritoneal layer make up the serosa. (Figure 3)

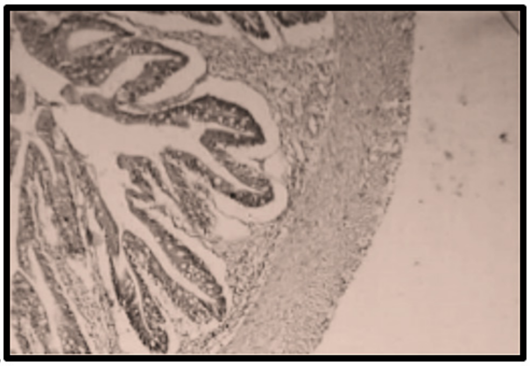

Fig. 3: Photomicrograph of a transverse section (T. S.) of the intestine of a normal Gold Carp (*Carassius auratus*) showing the typical structure of circular muscles, longitudinal muscles, serosa, and villi (H&E, 50x).

Fig. 4: Photomicrograph of the transverse section (T. S.) of the intestine of treated fish, Gold Carp (*Carassius auratus*), exposed to PS- MPs at a concentration of 10 mg/L for 28 days (H&E, 50x).

In the intestine of the treated gold carp, significant structural damages were observed, including broken or detached villi, muscularis mucosa, and submucosa, along with enlarged goblet cells and detached epithelial layers (Figure 4). Statistical analysis showed that intestinal villi degradation was observed in 70% of treated fish compared to 10% in controls ($\chi^2 = 6.89$, $p = 0.01$), highlighting the pronounced impact of polystyrene microplastic exposure on intestinal integrity. These changes suggest a disruption in nutrient absorption and increased susceptibility to intestinal inflammation, potentially affecting overall fish health. Similar findings have been observed in zebrafish (Danio rerio) and Nile tilapia (Oreochromis niloticus) exposed to microplastics,[18,19] highlighting the widespread impact of PS-MPs on the digestive systems of aquatic organisms.

Discussion

The current study evaluated changes in the behavioural responses of experimental fish (*Carassius auratus*) exposed to polystyrene microplastics. Traces of microplastic particles were quite evident in the buccal cavity and gill chamber of fish. The treatment groups exhibited a higher tendency to consume microplastics. These findings align with previous research suggesting that more aggressive fish tend to ingest larger quantities of both pelleted diets and microplastics.[14] Differences in ingestion rates among individuals have been linked to behavioural traits, where bolder zebrafish exposed to PS-MPs showed a greater propensity for consuming microplastics compared to shy zebrafish.[15]

Salerno *et al*.[20] reported that microplastic ingestion capacity in fish varies based on life stages, while Li *et al*.[21] suggested that species with swallowing feeding mechanisms are at greater risk of ingestion compared to filter or suction feeders. The present study also suggests that juvenile fish are more susceptible to microplastic intake compared to adults.

Zhang & Yin *et al*.[22,23] have also shown that exposure to microplastics affects feeding behaviour in fish, leading to reduced food intake, altered hunting strategies, and diminished overall activity. Similar patterns were observed in the present work, as fish in the experimental groups exhibited a decline in foraging activity and swimming behaviour after a 48-hour exposure period. These results are consistent with earlier studies that have documented behavioural alterations following microplastic ingestion.[24,25]

A significant behavioural alteration observed in the experimental group was impaired swimming, potentially due to swim bladder dysfunction, as documented in several previous studies. Disruptions in swim bladder activity may be driven by greater susceptibility to inflammation[26] or the potential accumulation of microplastics in the pneumatic duct.[27] Yong *et al*.[15] also reported difficulty in swim bladder formation in zebrafish larvae exposed to nanoplastics during development.

To evaluate the reversibility of buoyancy impairment, a follow-up observation was conducted for 10 days post-exposure. The treated fish were maintained in microplastic-free water under the same controlled conditions. It was observed that approximately 75% of the fish showed partial recovery of normal buoyancy, while 25% continued to display minor orientation issues. This suggests that while some effects may be reversible, prolonged exposure could lead to persistent physiological impairments.

Histopathological analysis revealed structural alterations in response to microplastic exposure, particularly in the gill chamber and buccal cavity. Oedematous changes and the presence of prominent red patches were observed, indicating potential tissue damage. Prolonged exposure may increase these effects, leading to more severe physiological impairments. Similar histopathological alterations have been reported in previous studies, where microplastic exposure resulted in tissue inflammation and lesions in fish.[16,17]

Microplastic exposure has been detected in aquatic organisms across different trophic levels, suggesting that its impact extends beyond a single species. Laboratory studies confirm that many marine, estuarine, and freshwater organisms, including fish, can absorb or consume microplastics, making them prone to various health risks.[28]

Additionally, microplastic accumulation in fish tissues, particularly in the gut and gills, may affect their survival, growth, and reproductive success, ultimately disrupting aquatic food webs. Since microplastics have been detected in commercially important fish species, there is a potential risk of human exposure through trophic transfer, particularly via seafood consumption. This aligns with previous studies that have raised concerns about the bioaccumulation of microplastics and their associated toxicants in higher trophic levels.

These findings highlight the urgent need for further research on the long-term effects of microplastic pollution on aquatic ecosystems and its potential consequences for human health.

Conclusion

This research demonstrated that exposure to polystyrene microplastics (PS-MPs) in Gold fish (*Carassius auratus*) induced histopathological alterations in the gills and gut, as well as behavioural modifications in the fish. Treated fish exhibited signs of distress, reduced activity, and loss of appetite. Furthermore, impairment in buoyancy was observed, which make it difficult for the fish to sustain vertical orientation and positional control within the water column. These findings highlight the biological disturbances caused by polystyrene microplastic toxicity, underscoring potential risks to aquatic organisms.

Acknowledgement

The authors are thankful to the Department of Zoology, C.M.Sc. college, Darbhanga, L.N. Mithila University, Darbhanga, for the provision of laboratory facilities used in this study.

Funding Sources

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest

The authors do not have any conflict of interest

Data Availability Statement

The manuscript incorporates all datasets produced or examined throughout this research study.

Ethics Statement

This study did not use human subjects. However, animal models were used and the authors declare that all experiments were performed in accordance with the guidelines and regulations of the Institutional Animal Ethics Committee (IAEC). Fish handling and maintenance followed humane treatment protocols to minimize stress and discomfort.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Permission to Reproduce Material from other sources

Not Applicable.

Author Contributions

- **Minakshi Kumari:** Conceptualization, Methodology, Data Collection, Formal Analysis, Writing – Original Draft Preparation.
- **Dr. Arti Kumari:** Supervision, Review & Editing

References

1. Hammer J, Kraak MHS, Parsons JR. Plastics in the Marine Environment: The Dark Side of a Modern Gift. In: ; 2012:1-44. doi:10.1007/978-1-4614-3414-6_1
2. Bordós G, Urbányi B, Micsinai A, *et al*. Identification of microplastics in fish ponds and natural freshwater environments of the Carpathian basin, Europe. *Chemosphere.* 2019;216:110-116. doi:10.1016/j.chemosphere.2018.10.110
3. Barnes DKA, Galgani F, Thompson RC, Barlaz M. Accumulation and fragmentation of plastic debris in global environments. Philosophical Transactions of the Royal Society B: *Biological Sciences.* 2009;364(1526):1985-1998. doi:10.1098/rstb.2008.0205
4. Thompson R, Moore C, Andrady A, Gregory M, Takada H, Weisberg S. New Directions in Plastic Debris. *Science* (1979). 2005;310(5751):1117-1117. doi:10.1126/science.310.5751.1117b
5. Avio CG, Gorbi S, Regoli F. Experimental development of a new protocol for extraction and characterization of microplastics in fish tissues: First observations in commercial species from Adriatic Sea. *Mar Environ Res*.

2015;111:18-26. doi:10.1016/j.marenvres.2015.06.014

6. Chae Y, Kim D, Kim SW, An YJ. Trophic transfer and individual impact of nano-sized polystyrene in a four-species freshwater food chain. *Sci Rep.* 2018;8(1):284. doi:10.1038/s41598-017-18849-y
7. Lambert S, Wagner M. Characterisation of nanoplastics during the degradation of polystyrene. *Chemosphere.* 2016;145:265-268. doi:10.1016/j.chemosphere.2015.11.078
8. Alimba CG, Faggio C. Microplastics in the marine environment: Current trends in environmental pollution and mechanisms of toxicological profile. *Environ Toxicol Pharmacol*. 2019;68. doi:10.1016/j.etap.2019.03.001
9. Farrelly TA, Shaw IC. Polystyrene as Hazardous Household Waste. In: Household Hazardous Waste Management. *InTech;* 2017. doi:10.5772/65865
10. International Agency for Research on Cancer (IARC). Genetic and Related Effects: An Updating of Selected IARC Monographs from Volumes 1 to 42.; 1987.
11. American Public Health Association (APHA). Standard Methods for the Examination of Water and Wastewater. 21st ed. *American Public Health Association*; 2005.
12. Luna LG. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. Vol 3. McGraw-Hill Book Company; 1968.
13. Zhang J, Bai Y, Meng H, *et al*. Combined toxic effects of polystyrene microplastics and 3,6-dibromocarbazole on zebrafish (Danio rerio) embryos. *Science of The Total Environment.* 2024;913:169787. doi:10.1016/j.scitotenv.2023.169787
14. Chen Q, Lackmann C, Wang W, Seiler TB, Hollert H, Shi H. Microplastics Lead to Hyperactive Swimming Behaviour in Adult Zebrafish. *Aquatic Toxicology.* 2020;224:105521. doi:10.1016/j.aquatox.2020.105521
15. Yong C, Valiyaveettil S, Tang B. Toxicity of Microplastics and Nanoplastics in Mammalian Systems. *Int J Environ Res Public Health.* 2020;17(5):1509. doi:10.3390/ijerph17051509
16. Kumari R. Estimation of Microplastic Content in Freshwater Culture Ponds and Its Histopathological Manifestations in Fish. PhD Thesis. LNMU; 2021.
17. Pedà C, Caccamo L, Fossi MC, *et al*. Intestinal alterations in European sea bass Dicentrarchus labrax (Linnaeus, 1758) exposed to microplastics: Preliminary results. *Environmental Pollution.* 2016;212:251-256. doi:10.1016/j.envpol.2016.01.083
18. Qiao R, Sheng C, Lu Y, Zhang Y, Ren H, Lemos B. Microplastics induce intestinal inflammation, oxidative stress, and disorders of metabolome and microbiome in zebrafish. *Science of The Total Environment.* 2019;662:246-253. doi:10.1016/j.scitotenv.2019.01.245
19. Lei L, Wu S, Lu S, *et al*. Microplastic particles cause intestinal damage and other adverse effects in zebrafish Danio rerio and nematode Caenorhabditis elegans. *Science of The Total Environment*. 2018;619-620:1-8. doi:10.1016/j.scitotenv.2017.11.103
20. Salerno M, Berlino M, Mangano MC, Sarà G. Microplastics and the functional traits of fishes: A global meta-analysis. *Glob Chang Biol.* 2021;27(12):2645-2655. doi:10.1111/gcb.15570
21. Li B, Liang W, Liu QX, *et al*. Fish Ingest Microplastics Unintentionally. *Environ Sci Technol.* 2021;55(15):10471-10479. doi:10.1021/acs.est.1c01753
22. Zhang T, Song K, Meng L, *et al*. Distribution and Characteristics of Microplastics in Barnacles and Wild Bivalves on the Coast of the Yellow Sea, China. *Front Mar Sci.* 2022;8. doi:10.3389/fmars.2021.789615
23. Yin L, Liu H, Cui H, Chen B, Li L, Wu F. Impacts of polystyrene microplastics on the behavior and metabolism in a marine demersal teleost, black rockfish (Sebastes schlegelii). *J Hazard Mater.* 2019;380:120861. doi:10.1016/j.jhazmat.2019.120861
24. Anbumani S, Kakkar P. Ecotoxicological effects of microplastics on biota: a review. *Environmental Science and Pollution Research.* 2018;25(15):14373-14396. doi:10.1007/s11356-018-1999-x
25. Mattsson K, Johnson E V., Malmendal A, Linse S, Hansson LA, Cedervall T. Brain damage and behavioural disorders in fish induced by plastic nanoparticles delivered through

the food chain. *Sci Rep.* 2017;7(1):11452. doi:10.1038/s41598-017-10813-0

26. Du J, Xu S, Zhou Q, *et al*. A review of microplastics in the aquatic environmental: distribution, transport, ecotoxicology, and toxicological mechanisms. *Environmental Science and Pollution Research*. 2020;27(11):11494-11505. doi:10.1007/s11356-020-08104-9
27. Sado RY, de Souza FC, Behr ER, Mocha PRE, Baldisserotto B. Anatomy of Teleosts and elasmobranchs. In: Biology and Physiology of Freshwater Neotropical Fish. *Elsevier;* 2020:21-47. doi:10.1016/B978-0-12-815872-2.00002-6
28. Dawson AL, Kawaguchi S, King CK, *et al*. Turning microplastics into nanoplastics through digestive fragmentation by Antarctic krill. *Nat Commun.* 2018;9(1):1001. doi:10.1038/s41467-018-03465-9