Microplastic contamination in the Alabama River and effects on *Daphnia magna*

by

Safeerul Islam Hashmi

A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
August 9, 2025

[Microplastics, Alabama River, Spatial and temporal variation, *Daphnia magna*, Toxic effects, Chronic exposure]

Approved by

Dr. Tham Hoang (Supervisor), Associate Professor School of Fisheries, Aquaculture, and Aquatic Sciences, Auburn University
Dr. Jim Stoeckel, Associate Professor School of Fisheries, Aquaculture, and Aquatic Sciences, Auburn University
Dr. Joseph R. Tomasso Jr., Professor School of Fisheries, Aquaculture, and Aquatic Sciences, Auburn University
Dr. Dengjun Wang, Assistant Professor School of Agricultural and Biological Engineering, University of Florida

Abstract

This thesis research focused on assessing microplastic (MP) contamination in the Alabama River and evaluating the impacts of different MP types on *Daphnia magna*. Chapter 1 was an overview of MP pollution including sources, ecological impacts, and rationale for conducting this thesis research. Chapter 2 investigated MP contamination in the Alabama River at which sources, temporal and spatial distributions, and MP load into Mobile Bay were studied. Chapter 3 focused on the chronic effects of three environmentally representative MPs: nylon and Kevlar microfibers and MPs made from a single use plastic cup on *D. magna.* Effects on survival, growth, reproduction, and MP uptake were determined. Chapter 4 was a preliminary risk assessment for MPs in the Alabama River through comparing the results of chapter 2 and chapter 3 using the probabilistic risk assessment approach to determine the bioavailability of MPs and potential risk to aquatic organisms in the Alabama River.

Acknowledgment

I would like to express my deepest gratitude to Allah Almighty who gave me courage and strength to accomplish this degree. I am grateful to my parents for their unending support, care, patience, and belief in me. Their sacrifices and encouragement have been the foundation of my success. I am highly thankful to my advisor, Dr. Tham Hoang, for his continual support and guidance throughout this 2-year MS journey at Auburn University, USA. His expertise and teaching skills were instrumental in doing the research and in enabling me grow as a sound researcher. I would also express my gratitude and thanks to my exam committee for their invaluable input, time, and contributions during this degree program. I would also like to thank the faculty and staff of the School of Fisheries, Aquaculture, and Aquatic Sciences, Auburn University for their continual support and creating a supportive and inclusive environment.

My sincere appreciation extends to my lab mates and friends (Dr. Andrew Barrick, Md. Sayem Ahmed, Alison Boardwine, Sean Parham, Ellis Nabi, and John Ikyoive) who supported me with field samplings and other associated lab work. This thesis is a result of the collective support and inspiration I received from my mentors and teachers who contributed alot in shaping my future and making me who I am today.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

MP	Microplastic
PCPs	Personal Care Products
MRF	Material Recovery Facilities
ABS	Acrylonitrile Butadiene Styrene
PE	Polyethylene
PET	Polyethylene Terephthalate
PBT	Polybutylene Terephthalate
PMMA	Polymethyl Methacrylate
PPS	Polyphenylene Sulfide
PP	Polypropylene
PVC	Polyvinyl Chloride
PMP	Polymethyl Pentene
PS	Polystyrene
PC	Polycarbonate
EVA	Ethylene Vinyl Acetate
POM	Polyoxymethylene
PU	Polyurethane
PETG	Polyethylene Terephthalate Glycol
FEP	Fluorinated Ethylene Propylene
PCTFE	Polychlorotrifluoroethylene

CHAPTER 1

1. LITERATURE REVIEW

1.1. Plastic production

Plastics are organic polymers manufactured for different applications. Their strength, flexibility, high durability, light weight characteristics, and low production costs make them one of the most often used materials in our society. Poly-tetra-fluoroethylene (PTFE), thermoplastic polyurethanes (TPU), poly-sulphones (PSO), polyester sulphone (PES), polyacrylates, polyamide imide (PAI) etc. are examples of common plastics (Hahladakis et al., 2018). Increasing consumer demand has resulted in increased plastic production, especially in recent years. It was estimated that about 2 million tons of plastics were produced globally in 1950. The amount rapidly increased in recent years to an approximate of 391 million tons in 2021 indicating an increase of 180 times in 71 years (Fig. 1.1) (Boschi et al., 2023).

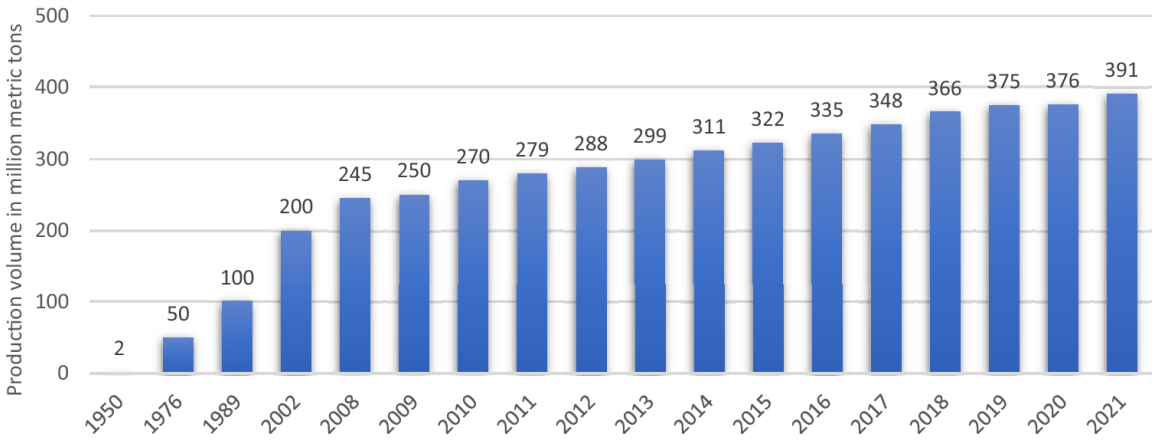

Figure 1.1. Global plastic production from 1950 to 2021.

The United States is one of the major global plastic producers. Plastic resins production has increased from 102,806 to 125,505 million pounds in the past 12 years, from 2010 to 2022 (Fig. 1.2).

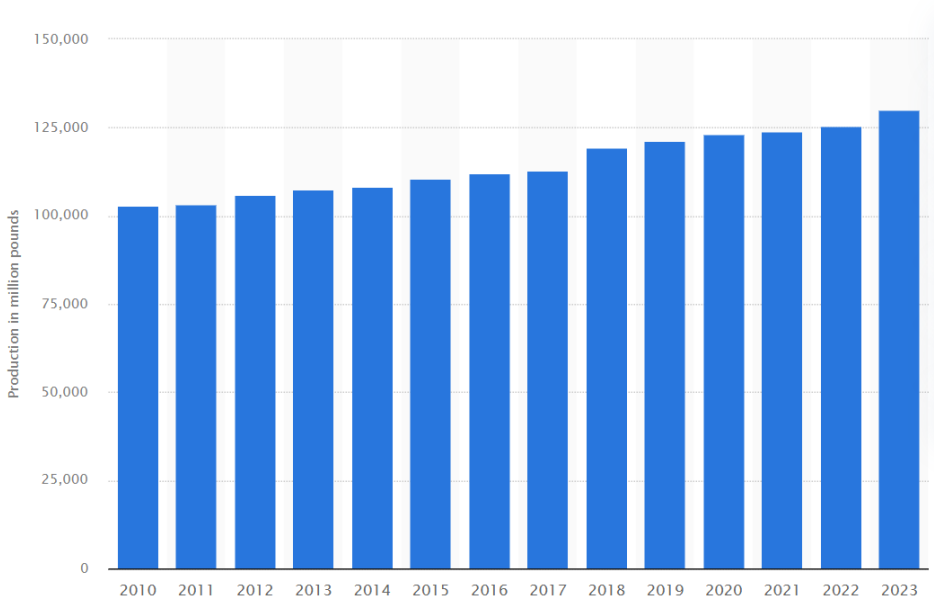

Figure 1.2. Production of plastics in the US from 2010 to 2022 (Jaganmohan, 2024).

The mass scale production of plastics has increased plastic waste to the environment. The management of plastic waste varies, depending on the country and geographic region. The Europe Union has implemented policies to use less plastics and manage plastic waste such as banning or charging the use of plastic bags in grocery stores and separating plastic waste for recycling and therefore discard less plastics into the environment (Kasznik & Łapniewska, 2023). Other parts of the world, such as Asia and South America, have been believed to release more plastic waste into the environment. On average, more than 75% of the world plastic produced ends up in landfill sites. About 15.8% are incinerated whereas only a small proportion (8.7%) is recycled (Thomas et al., 2024). According to the Statistics for 2018, plastics make around 12.2% of the municipal solid waste produced in the US, making it the 3rd largest waste category after paper/paperboard and food wastes. It is evidenced that the world is facing a global plastic pollution crisis. Development of alternative materials that are more environmentally friendly and reduce, reuse, and recycle more plastics are needed to solve the plastic pollution problem.

1.2. How plastics turn into microplastics

Large plastic materials can become microplastics (MPs) through a chain of biodegradable, non-biodegradable, or a combination of both processes. Physical degradation, photodegradation, thermal degradation, thermo-oxidative degradation, and hydrolysis are examples of non-biodegradable processes (Turner et al., 2020). Physical degradation occurs due to weathering and physical collisions that lead to fragmentation of large plastic materials into smaller size plastic particles. Thermal degradation usually occurs at a commercial scale. Typical examples include the breakdown of plastic packaging, containers, synthetic textiles, and recycling of plastic waste using heat. Hydrolysis and photodegradation occurs naturally where chemical bonds in plastic materials are broken by water molecules and UV light. Biodegradable processes are carried out by microorganisms (Lambert & Wagner, 2016). Under biodegradation, chemical bonds in plastic materials are broken by actions of extracellular enzymes produced by microorganisms (J. Yuan et al., 2020). As a result, micro- and nanoplastics particles are created. Consequently, the polymers are converted into monomers. Non-biodegradable processes also degrade the polymer structures, alter mechanical properties, increase their specific surface area, and ultimately result in increasing the rates of physico-chemical reactions and interactions with microorganisms (Lucas et al., 2008). In the aquatic environment, plastic waste can also undergo gradual degradation due to its exposure to water waves, temperature, different biological processes undergoing in the water and other physical processes (Wu et al., 2019).

1.3. Definition of microplastics and classification

Microplastics (MPs) are plastic particles that have a three-dimensional size of 5 mm or less (Viaroli et al., 2022). Recently, this definition has been discussed based on the size that can be ingested by living organisms and their ability to cause negative impacts to biota. Hoang et al, (2024) suggested

that MPs should be limited to plastic particles with sizes of 1 µm to 1000 µm. Particles in this size range can be ingested by many small and sensitive organisms and can have effects on them. Based on their origin, MPs are classified as primary or secondary (Fig. 1.3). Primary microplastics are intentionally created with the size described above. Relevant examples of primary microplastics are polymers synthesized for applications as exfoliants in processes, such as chemical formulations, grinding products, maintenance of various plastic products and manufacturing of synthetic clothing. Microbead is another common type of primary MPs. Their common use is in personal care products (PCPs) and face wash. They were often made of polyethylene (PE), polypropylene (PP) and polystyrene (PS) (Fendall & Sewell, 2009). Secondary microplastics are formed due to the fragmentation of large size plastic materials through physical, chemical, and biological processes in the natural environment and plastic recycling facilities (Browne et al., 2010)

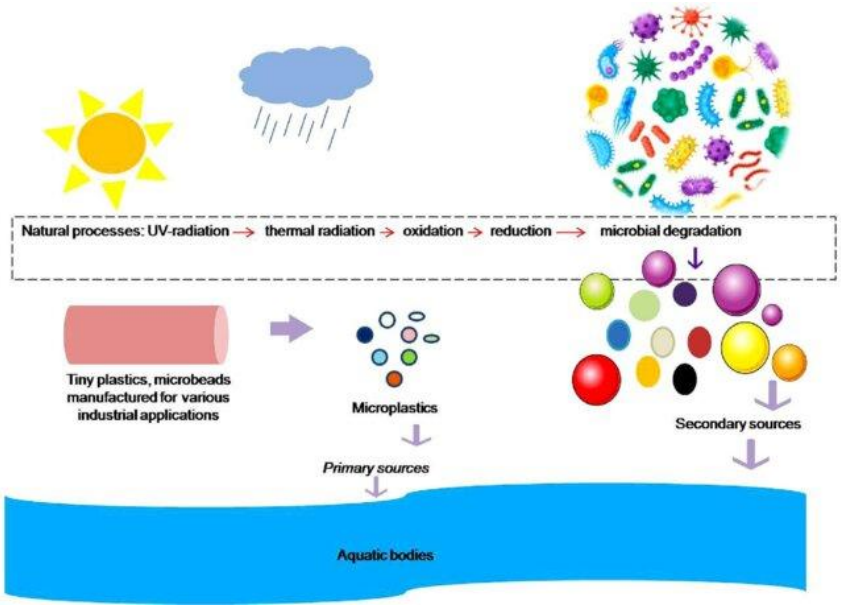

Figure 1.3. Classification of MPs and production mechanisms (Sarma et al., 2022).

1.4. Pathways of microplastics to water systems

Microplastics can enter aquatic ecosystems through point sources, such as wastewater treatment plants, effluents of plastic production plants and solid waste processing facilities, and leachate of landfills (Fig. 1.4). Non-point sources include agriculture and urban runoff, household discharges, and atmospheric deposition.

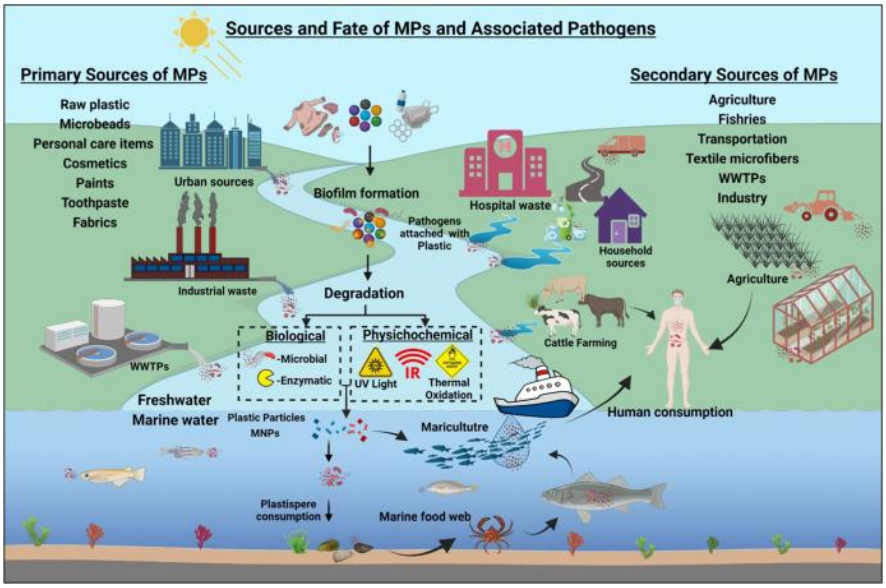

Figure 1.4. Sources of microplastic into waterbodies (Junaid et al., 2022).

Among the point sources, effluent of wastewater treatment plants and plastic recycling industries have been reported to contain high MPs concentrations and contributed to MP pollution in river systems. According to Hoellein et al. (2019), MPs from the effluents of the Chicago wastewater treatment plant are an important source of MPs in the Chicago River. A study from the United Kingdon reported that substantial MPs were present in wastewater released from plastic recycling facilities due to inefficient wastewater treatment process (Brown et al., 2023). Another study in Vietnam also found high MP concentrations in waters near small plastic recycling facilities that had no treatment technology to prevent MPs release into the environment (Le Tran et al., 2023).

Although recycling plastics is important, implementation of treatment processes to contain MPs is necessary.

In addition, the frictional effects between road and tires can release micro-size rubber fragments into the environment. Soil in waste transfer stations has been reported to contain substantial MPs and micro rubber fragments. A recent study in China found a release of 41,297-82,593 MPs from a single waste transfer station in an hour. Based on this finding, researchers estimated that over 110 million MPs can be released from a transfer station in Shanghai to surrounding environments (Hu et al., 2022). Material recovery facility (MRF) sites are also important sources of MP release into the environment. Studies have highlighted the potential of MPs release from different unit processes occurring in these facilities like tipping, screening, waste transfer onto conveyor belts, waste compression, frictional effects, and mechanical abrasion (Zhang et al., 2021).

Plastics from landfill sites can be blown to waterways and enter aquatic systems via leachate discharges. Studies have identified the redistribution of MPs during waste treatments, accumulating MPs in the sludge or treated effluent (He et al., 2019). Strong correlations between landfills and release of MPs to the air, soil, and water have been reported in many studies (Fajaruddin Natsir et al., 2021; Mahesh et al., 2023; Nurhasanah et al., 2021; Sarathana & Winijkul, 2023; Wan et al., 2022). Evidence of groundwater contamination has also been reported by studies due to leachate released from landfill sites. Concentrations of 2-80 MPs/L were found in the groundwater (GW) samples collected from surrounding locations of a landfill site in Chennai, India (Ket al., 2021).

1.5. Microplastics in river systems

Microplastics in water can pose risks to living organisms and ecosystems via different ways. Aquatic organisms have been found to mistakenly ingest MPs and physical damages to their

digestive system was reported (Huang et al., 2020; Jeong et al., 2024). Microplastics can absorb and desorb environmental pollutants and transport them through aquatic ecosystems and into organisms when microplastic associated with environmental contaminants are ingested. Special concerns are with organic toxic chemicals, such as phthalates, polybrominated diphenyl ethers, and other persistent organic pollutants (POPs) (Crawford & Quinn, 2016). Since MPs can last for a long time (up to centuries) in the natural environment, their potential impacts on the ecosystem are of great concern (Born & Brüll, 2022; Chamas et al., 2020). In past years, research on MP pollution was focused on the marine environment, whereas less attention was given to freshwater ecosystems (Eerkes-Medrano & Thompson, 2018). In recent years, research on MPs in terrestrial and aquatic environments has been intensified (Table 1.1). In the US, studies have reported strong correlations between MPs presence in rivers and various anthropogenic factors, such as human population densities and urban land covers in the US (Talbot & Chang, 2022). Similar findings were also reported in Europe (Dris et al., 2015; Lechner et al., 2014; Liu et al., 2019), Asia (Chen et al., 2021; Irfan et al., 2020; Kameda et al., 2021), Australia (Leterme et al., 2023; Su et al., 2020), and North America (Grbić et al., 2020; Talbot et al., 2022; Yonkos et al., 2014).

Table 1.1: MPs found in rivers across the world.

River	Catchment Area (km^2)	Country	Sample Fraction	MPs count	Abundant MPs Size	Types	References
Kaveri River	81,155	India	Sediment	187 ± 103 - 699 ± 66 items/kg	---	PA, PE, PP, PET	(Maheswaran et al., 2022)
Lower Santa Cruz River	22,000	US	Water column/ sediments	19.5 ± 2.2 MP/L 246.9 ± 30.5 per kg	---	---	(Eppehimer et al., n.d.)
Brisbane River	13,560	Australia	Simulation/ Modeling	---	---	PE, PA, PET, PP, PS	(B. He et al., 2021)
Liangfeng River	528	China	Sediment	(14.40-79.45) × 10^3 items/kg (max. value)	50-500 µm	PE, PS, PP, PA, PVC	(Xia et al., 2021)
Dafeng River	---	China	Sediment	9.4-50.3 items/kg (dry) 0.0-21.3 items/kg (rainy)	---	Polyester, PU, PBT, POM, PS, PE, PP	Liu et al. (2021)
Amazon River	7000,000	Brazil	Sediment	417-8178 particles/kg 0-5725 particles/kg	0.063-5 mm 0.063-1 mm	---	(Gerolin et al., 2020)
Wei River	135,000	China	Water/ Sediment	3.67-10.7 items/L 360-1320 items/kg	5-0.5 mm	---	(Ding et al., 2019)
Rhine River	220,000	Germany	Sediment	43.5-459 mg/kg or 786-1368 particles/kg	630-5000 µm	PE, PP, PS, PA	(Klein et al., 2015)
Long Beach Harbor	---	USA	Water	113.627 particles/L	3-500 µm	---	(Wiggin & Holland, 2019)
Los Angeles River	---	USA	Water	641.292 particles/L	3-500 µm	---	(Wiggin & Holland, 2019)
San Gabriel River	---	USA	Water	63.359 particles/L	3-500 µm	---	(Wiggin & Holland, 2019)
Yangtze River	1.8 million	China	Water sediments soil	1.27 MPs/L 286.20 MPs/kg 338.09 MPs/kg	0.6-1 mm	PP, PE, PA, PS, PVC, PET, PC, and PVF	(Yuan et al., 2022)
Amazon River	7,000,000	Brazil	Water	8-39 MPs/m^3	100-1000 µm	Polyester, PP, PS, and PE	(Rico et al., 2023)

Rivers are an integral part of freshwater ecosystems and play a vital role in life sustenance on Earth. Freshwater systems are extensively utilized for different functions and purposes such as (a) life support by fulfilling the drinking water and agricultural demands (Bexeitova et al., 2024), (b) ecosystem services by recycling nutrients, waste assimilation and climate regulation (Darji et al., 2022), (c) biodiversity habitat by enabling fish, aquatic species, invertebrates, plants and animals to survive (Raju et al., 2023; Sulaiman et al., 2023), (d) recreational activities and tourism spots such as boating, fishing, swimming etc. (Allen et al., 2022). The presence of MPs in freshwater bodies worldwide in recent years raised concerns about the potential impacts of MPs on public health and aquatic life. Microplastics have been found in different parts of the body of living organisms including humans. Hence there is a dire need to conduct studies to evaluate MP pollution in river systems and understand their potential risk and impacts on aquatic life.

1.6. Impacts of microplastics

Microplastic can affect living organisms if ingested, relocated, and/or entangled in the digestive system. Microplastic ingestion by terrestrial and aquatic organisms has been reported. Research found MPs of different types in various classes of aquatic organisms (Fig. 1.5, Table 1.2).

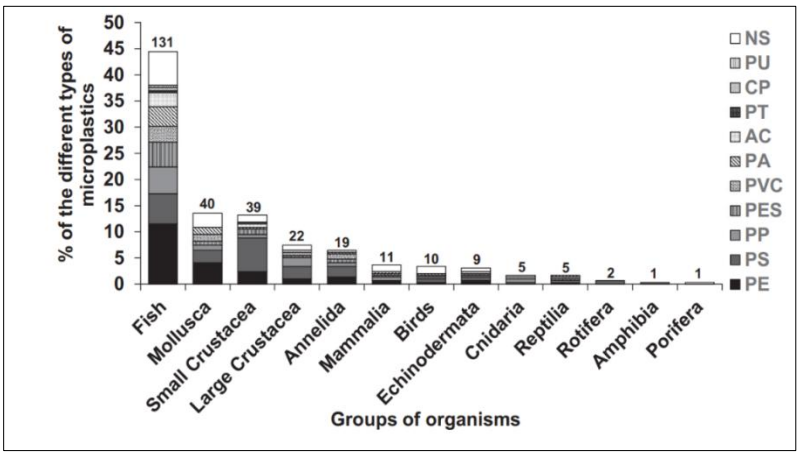

Figure 1.5. Percentage of different MP types present in aquatic organisms (de Sá et al., 2018). Each bar represents the number of studies found MPs in the organisms whereas plastic types

discussed include Not specified (NS). Polyurethane (PU), Cellophane (CP), Polyether (PT), Acrylic (AC), Polyamide (PA), Polyvinylchloride (PVC), Polyester (PES), Polypropylene (PP), Polystyrene (PS), Polyethylene (PE).

The amount and type of ingested MPs varied and was dependent on species and habitat they live in. An amount of 7.5±4.9 MP (PE, PET, PP) particles per organism were found in Nile Tilapia in Egypt (Khan et al., 2020), 8.12±4.26 MP (PA, PE, PS) items per organism in the fish guts in Iran (Makhdoumi, Amin, et al., 2021; Makhdoumi, Naghshbandi, et al., 2021), 3 microbeads/fibers in every 50 Goldfish retained in Canada (Grigorakis et al., 2017), and 0.17±0.07 – 0.92±0.19 microfibers and fragments/individual in crayfish in China (Zhang et al., 2021) were reported. Large MPs can be accumulated and entangled in the digestive system. Small MPs have been reported to relocate into different parts of the body, such as blood stream, livers, kidneys, brain, etc. (Liang et al., 2024; Rahman et al., 2024; Sun et al., 2024; Auta et al., 2017; Hollerová et al., 2021).

Table 1.2: MPs exposure impacts on aquatic organisms.

Organisms	**Influence and Endpoint**	**Reference**
Shrimp (Paratya australiensis)	36% shrimp had MPs, with an average of 24 ± 31 items/g.	(Nan et al., 2020)
Farrer's scallop (Chlamys farreri)	Reported the MPs potential for bioaccumulation. Enhanced impacts of BDE-209 on hemolytic phagocytosis, ultrastructural variations in gills and digestive gland.	(Xia et al., 2020)
Beluga whales (Delphinapterus leucas)	Highlighted the presence of MPs in the gastrointestinal tract. 18 to 147 MP beads were found in the digestive tract of each individual.	(Moore et al., 2020)
Zebrafish (Danio rerio)	Zebrafish ingested the MPs, passed along the intestinal lumen, and passed out of body.	(Batel et al., 2020)
Girella fish (Girella laevifrons)	Inflammation, circulatory disorders; regressive changes in the intestinal tissue, assessing crypt/villi cell loss.	(Ahrendt et al., 2020)
Goldfish (Carassius auratus)	Different properties of MPs increase the risk of exposure to fish.	(Moore et al., 2020)
Sea cucumber (Apostichopus japonicus)	MPs affected the status of growth and physiology in sea cucumber.	(Mohsen et al., 2019)
Nile tilapia (Oreochromis niloticus)	Anemia and perturbation were triggered, causing mortality in juvenile tilapia.	(Xiong et al., 2019)
Planktonic crustacean (Daphnia magna)	The mortality rate was increased. MPs altered the toxicity of pollutants, such as herbicides.	(Zocchi & Sommaruga, 2019)

Once entered and accumulated in the animal body, MPs are not metabolized and can have negative effects on living organisms. The consequences included blockage of their digestive tract leading to effects on energy intake for biological performance, vectoring environmental pollutants and increasing the bioaccumulation of pollutants in organisms' tissue, and transfer of pollutants and MPs through the trophic chain (Jeong et al., 2024). Going through these processes, MPs can affect ecosystem functioning and services (Desforges et al., 2015; Tuuri & Leterme, 2023). Microplastics can eventually be transferred to humans via consumption of MP contaminated food.

Research has found different types of effects on living organisms when they are exposed to MPs. The effects range from molecular responses including gene expression (Marcellus et al., 2024; Poma et al., 2023), enzymatic activity impacts (Khan et al., 2024; Liang et al., 2021; Patra et al., 2022; Yu et al., 2020), physiological processes (Bhuyan, 2022; Jiang et al., 2022; Mkuye et al., 2022), and lethality (Bhuyan, 2022b; Masud & Cable, 2023; Seeley et al., 2023). However, most studies were conducted with primary MPs, that are less abundant in the natural environment than secondary MPs. To better understand the environmental impacts of MPs, it is important to conduct research using environmentally realistic MPs. Research with long time exposure is also more relevant than short time exposure. With MPs being persistent in the natural environment, results of long-time exposure studies are more relevant for ecological risk assessment to support decision and policy making for management of MP pollution.

1.7. Problem statement

As described in the sections above, MPs in the environment are increasing as a result of the ongoing acceleration of plastic production and use around the world. Research assessing MP pollution in big rivers in the world has been conducted. In the US, a few studies on MPs in river systems have been reported, such as the Hudson River (Polanco et al., 2020) or Chesapeake Bay

(Bikker et al., 2020), San Francisco Bay and Long Beach, California (Miller et al., 2021; Wiggin & Holland, 2019), Neuse River in North Carolina (Kurki-Fox et al., 2023), Eel, Salamonie, Tippecanoe, Lower East Fork White, Muscatatuck, and Patoka Rivers, Indiana (Conard et al., 2023), coastal sites of North Carolina, Texas, and US Virgin Islands (Yu et al., 2018), and Snake River of Idaho and Oregon (Kapp & Yeatman, 2018). The Alabama River is the largest river in Alabama in the US. The Alabama River is the major drainage of urban and agricultural runoff and city effluents, and therefore, is expected to receive MP release from various sources along its waterway, such as wastewater treatment plants, agricultural activities, recreational and fishing activities. However, information on MP contamination in the Alabama River system is limited. Mobile Bay- the drainage basin of the Alabama River is the home to a diversity of aquatic species and has been identified as one of the most important nearshore habitats in the Gulf of America. Microplastic contamination can affect aquatic life in the Alabama River, Mobile Bay, and the Gulf of America if the contamination is high. Considering these existing gaps in the literature and the lack of information, this research is designed to evaluate and characterize MP contamination in the Alabama River and potential impacts of environmentally realistic MPs on aquatic organisms using *Daphnia magna*, a model species for ecotoxicology research.

1.7.1. Research Objectives

The main objectives of this study are as follows

Objective 1: To determine MPs contamination in the Alabama River.

- To understand the spatial distribution of MPs in the Alabama River
- To understand the temporal distribution of MPs in the Alabama River
- To estimate the MPs load into Mobile Bay

Objective 2: To determine the effects of chronic exposure of environmentally realistic MPs on *Daphnia magna*

- To evaluate MPs ingestion by *D. magna* over time
- To assess the effects of MPs on growth, reproduction, and survival of *D. magna*

1.8. References

1. Ahrendt, C., Perez-Venegas, D. J., Urbina, M., Gonzalez, C., Echeveste, P., Aldana, M., Pulgar, J., & Galbán-Malagón, C. (2020). Microplastic ingestion cause intestinal lesions in the intertidal fish Girella laevifrons. *Marine Pollution Bulletin*, *151*. https://doi.org/10.1016-/j.marpolbul.2019.110795
2. Allen, S., Allen, D., Karbalaei, S., Maselli, V., & Walker, T. R. (2022). Micro(nano)plastics sources, fate, and effects: What we know after ten years of research. *Journal of Hazardous Materials Advances*, *6*, 100057. https://doi.org/10.1016/J.HAZADV.2022.100057
3. Auta, H. S., Emenike, C. U., & Fauziah, S. H. (2017). Distribution and importance of microplastics in the marine environment: A review of the sources, fate, effects, and potential solutions. *Environment International*, *102*, 165–176. https://doi.org/10.1016/J.ENVINT.-2017.02.013
4. Batel, A., Baumann, L., Carteny, C. C., Cormier, B., Keiter, S. H., & Braunbeck, T. (2020). Histological, enzymatic and chemical analyses of the potential effects of differently sized microplastic particles upon long-term ingestion in zebrafish (Danio rerio). *Marine Pollution Bulletin*, *153*. https://doi.org/10.1016/j.marpolbul.2020.111022
5. Bexeitova, K., Baimenov, A., Varol, E. A., Kudaibergenov, K., Zhantikeyev, U., Sailaukhanuly, Y., Toshtay, K., Tauanov, Z., Azat, S., & Berndtsson, R. (2024). Microplastics in freshwater systems: A review of classification, sources, and environmental impacts. *Chemical Engineering Journal Advances*, *20*, 100649. https://doi.org/10.1016/J.CEJA.2024.100649
6. Bhuyan, M. S. (2022a). Effects of Microplastics on Fish and in Human Health. *Frontiers in Environmental Science*, *10*, 827289. https://doi.org/10.3389/FENVS.2022.827289/XML-/NLM

7. Bhuyan, M. S. (2022b). Effects of Microplastics on Fish and in Human Health. *Frontiers in Environmental Science*, *10*, 827289. https://doi.org/10.3389/FENVS.2022.827289/XML/NLM

8. Bikker, J., Lawson, J., Wilson, S., & Rochman, C. M. (2020). Microplastics and other anthropogenic particles in the surface waters of the Chesapeake Bay. *Marine Pollution Bulletin*, *156*. https://doi.org/10.1016/j.marpolbul.2020.111257

9. Born, M. P., & Brüll, C. (2022). From model to nature — A review on the transferability of marine (micro-) plastic fragmentation studies. *Science of The Total Environment*, *811*, 151389. https://doi.org/10.1016/J.SCITOTENV.2021.151389

10. Boschi, A., Scieuzo, C., Salvia, R., Arias, C. F., Perez, R. P., Bertocchini, F., & Falabella, P. (2023). Beyond Microbial Biodegradation: Plastic Degradation by Galleria mellonella. *Journal of Polymers and the Environment 2023 32:5*, *32*(5), 2158–2177. https://doi.org/10.1007/S10924-023-03084-6

11. Brown, E., MacDonald, A., Allen, S., & Allen, D. (2023). The potential for a plastic recycling facility to release microplastic pollution and possible filtration remediation effectiveness. *Journal of Hazardous Materials Advances*, *10*. https://doi.org/10.1016/j.hazadv.2023.100309

12. Browne, M. A., Galloway, T. S., & Thompson, R. C. (2010). Spatial patterns of plastic debris along estuarine shorelines. *Environmental Science and Technology*, *44*(9), 3404–3409. https://doi.org/10.1021/es903784e

13. Chamas, A., Moon, H., Zheng, J., Qiu, Y., Tabassum, T., Jang, J. H., Abu-Omar, M., Scott, S. L., & Suh, S. (2020). Degradation Rates of Plastics in the Environment. *ACS Sustainable Chemistry and Engineering*, *8*(9), 3494–3511. https://doi.org/10.1021/ACSSUSCHEMENG.9B06635/ASSET/IMAGES/LARGE/SC9B06635_0009.JPEG

14. Chen, H. L., Gibbins, C. N., Selvam, S. B., & Ting, K. N. (2021). Spatio-temporal variation of microplastic along a rural to urban transition in a tropical river. *Environmental Pollution*, *289*, 117895. https://doi.org/10.1016/J.ENVPOL.2021.117895
15. Conard, W. M., O'Reilly, K. E., Hartlage, C., & Lamberti, G. A. (2023). Widespread microplastic pollution in Indiana, USA, rivers. *River Research and Applications*, *39*(10), 2092–2101. https://doi.org/10.1002/RRA.4204
16. Crawford, C. B., & Quinn, B. (2016). Microplastic Pollutants. *Microplastic Pollutants*, 101–130. https://research-portal.uws.ac.uk/en/publications/microplastic-pollutants
17. Darji, J., Lodha, P., & Tyagi, S. (2022). Assimilative capacity and water quality modeling of rivers: a review. *AQUA - Water Infrastructure, Ecosystems and Society*, *71*(10), 1127–1147. https://doi.org/10.2166/AQUA.2022.063
18. de Sá, L. C., Oliveira, M., Ribeiro, F., Rocha, T. L., & Futter, M. N. (2018). Studies of the effects of microplastics on aquatic organisms: What do we know and where should we focus our efforts in the future? In *Science of the Total Environment* (Vol. 645, pp. 1029–1039). Elsevier B.V. https://doi.org/10.1016/j.scitotenv.2018.07.207
19. Desforges, J. P. W., Galbraith, M., & Ross, P. S. (2015). Ingestion of Microplastics by Zooplankton in the Northeast Pacific Ocean. *Archives of Environmental Contamination and Toxicology*, *69*(3), 320–330. https://doi.org/10.1007/S00244-015-0172-5/TABLES/3
20. Ding, L., Mao, R. fan, Guo, X., Yang, X., Zhang, Q., & Yang, C. (2019). Microplastics in surface waters and sediments of the Wei River, in the northwest of China. *Science of the Total Environment*, *667*, 427–434. https://doi.org/10.1016/j.scitotenv.2019.02.332

21. Dris, R., Gasperi, J., Rocher, V., Saad, M., Renault, N., & Tassin, B. (2015). Microplastic contamination in an urban area: a case study in Greater Paris. *Environmental Chemistry*, *12*(5), 592–599. https://doi.org/10.1071/EN14167

22. Eerkes-Medrano, D., & Thompson, R. (2018). Occurrence, Fate, and Effect of Microplastics in Freshwater Systems. *Microplastic Contamination in Aquatic Environments: An Emerging Matter of Environmental Urgency*, 95–132. https://doi.org/10.1016/B978-0-12-813747-5.00004-7

23. Eppehimer, D. E., Hamdhani, H., Hollien, K. D., Nemec, Z. C., Lee, L. N., Quanrud, D. M., & Bogan, M. T. (n.d.). *Impacts of baseflow and flooding on microplastic pollution in an effluent-dependent arid land river in the USA*. https://doi.org/10.1007/s11356-021-13724-w/Published

24. Fajaruddin Natsir, M., Selomo, M., Ibrahim, E., Arsin, A. A., & Alni, N. C. (2021). Analysis on microplastics in dug wells around Tamangapa Landfills, Makassar City, Indonesia. *Gaceta Sanitaria*, *35*, S87–S89. https://doi.org/10.1016/j.gaceta.2020.12.024

25. Fendall, L. S., & Sewell, M. A. (2009). Contributing to marine pollution by washing your face: Microplastics in facial cleansers. *Marine Pollution Bulletin*, *58*(8), 1225–1228. https://doi.org/10.1016/j.marpolbul.2009.04.025

26. Gerolin, C. R., Pupim, F. N., Sawakuchi, A. O., Grohmann, C. H., Labuto, G., & Semensatto, D. (2020). Microplastics in sediments from Amazon rivers, Brazil. *Science of the Total Environment*, *749*. https://doi.org/10.1016/j.scitotenv.2020.141604

27. Grbić, J., Helm, P., Athey, S., & Rochman, C. M. (2020). Microplastics entering northwestern Lake Ontario are diverse and linked to urban sources. *Water Research*, *174*, 115623. https://doi.org/10.1016/J.WATRES.2020.115623

28. Grigorakis, S., Mason, S. A., & Drouillard, K. G. (2017). Determination of the gut retention of plastic microbeads and microfibers in goldfish (Carassius auratus). *Chemosphere*, *169*, 233–238. https://doi.org/10.1016/J.CHEMOSPHERE.2016.11.055

29. Hahladakis, J. N., Velis, C. A., Weber, R., Iacovidou, E., & Purnell, P. (2018). An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling. In *Journal of Hazardous Materials* (Vol. 344, pp. 179–199). Elsevier B.V. https://doi.org/10.1016/j.jhazmat.2017.10.014

30. He, B., Smith, M., Egodawatta, P., Ayoko, G. A., Rintoul, L., & Goonetilleke, A. (2021). Dispersal and transport of microplastics in river sediments. *Environmental Pollution*, *279*. https://doi.org/10.1016/j.envpol.2021.116884

31. He, P., Chen, L., Shao, L., Zhang, H., & Lü, F. (2019). Municipal solid waste (MSW)landfill: A source of microplastics? -Evidence of microplastics in landfill leachate. *Water Research*, *159*, 38–45. https://doi.org/10.1016/j.watres.2019.04.060

32. Hoellein, T. J., & Rochman, C. M. (2021). The "plastic cycle": a watershed67 20 scale model of plastic pools and fluxes. *Frontiers in Ecology and the Environment*, *19*(3), 176–183. https://doi.org/10.1002/FEE.2294;WGROUP:STRING:PUBLICATION

33. Hollerová, A., Hodkovicová, N., Blahová, J., Faldyna, M., Maršálek, P., & Svobodová, Z. (2021). Microplastics as a potential risk for aquatic environment organisms – a review. *Acta Veterinaria Brno*, *90*(1), 99–107. https://doi.org/10.2754/AVB202190010099

34. Hu, T., He, P., Yang, Z., Wang, W., Zhang, H., Shao, L., & Lü, F. (2022). Emission of airborne microplastics from municipal solid waste transfer stations in downtown. *Science of the Total Environment*, *828*. https://doi.org/10.1016/j.scitotenv.2022.154400

35. Huang, J. N., Wen, B., Zhu, J. G., Zhang, Y. S., Gao, J. Z., & Chen, Z. Z. (2020). Exposure to microplastics impairs digestive performance, stimulates immune response and induces microbiota dysbiosis in the gut of juvenile guppy (Poecilia reticulata). *Science of The Total Environment*, *733*, 138929. https://doi.org/10.1016/J.SCITOTENV.2020.138929

36. Irfan, M., Qadir, A., Mumtaz, M., & Ahmad, S. R. (2020). An unintended challenge of microplastic pollution in the urban surface water system of Lahore, Pakistan. *Environmental Science and Pollution Research*, *27*(14), 16718–16730. https://doi.org/10.1007/S11356-020-08114-7/TABLES/2

37. Jaganmohan, M. (2024, January 10). *Total plastics production in the United States from 2010 to 2022*. https://www.statista.com/statistics/203398/total-us-resin-production-from-2008/

38. Jeong, E., Lee, J. Y., & Redwan, M. (2024). Animal exposure to microplastics and health effects: A review. *Emerging Contaminants*, *10*(4), 100369. https://doi.org/10.1016/J.EMCON-.2024.100369

39. Jiang, W., Fang, J., Du, M., Gao, Y., Fang, J., & Jiang, Z. (2022). Microplastics influence physiological processes, growth and reproduction in the Manila clam, Ruditapes philippinarum. *Environmental Pollution*, *293*, 118502. https://doi.org/10.1016/J.ENVPOL.-2021.118502

40. Junaid, M., Siddiqui, J. A., Sadaf, M., Liu, S., & Wang, J. (2022). Enrichment and dissemination of bacterial pathogens by microplastics in the aquatic environment. *Science of The Total Environment*, *830*, 154720. https://doi.org/10.1016/J.SCITOTENV.2022.154720

41. K, M. B., Natesan, U., R, V., R, P. K., R, R., & S, S. (2021). Spatial distribution of microplastic concentration around landfill sites and its potential risk on groundwater. *Chemosphere*, *277*. https://doi.org/10.1016/j.chemosphere.2021.130263

42. Kameda, Y., Yamada, N., & Fujita, E. (2021). Source- and polymer-specific size distributions of fine microplastics in surface water in an urban river. *Environmental Pollution*, *284*, 117516. https://doi.org/10.1016/J.ENVPOL.2021.117516

43. Kapp, K. J., & Yeatman, E. (2018). Microplastic hotspots in the Snake and Lower Columbia rivers: A journey from the Greater Yellowstone Ecosystem to the Pacific Ocean. *Environmental Pollution*, *241*, 1082–1090. https://doi.org/10.1016/J.ENVPOL.2018.06.033

44. Khan, F. R., Shashoua, Y., Crawford, A., Drury, A., Sheppard, K., Stewart, K., & Sculthorp, T. (2020). 'The Plastic Nile': First Evidence of Microplastic Contamination in Fish from the Nile River (Cairo, Egypt). *Toxics 2020, Vol. 8, Page 22*, *8*(2), 22. https://doi.org/10.3390/TOXICS-8020022

45. Khan, T. F., Sikder, A. H. F., Khan, T. F., & Sikder, A. H. F. (2024). Microplastic Can Decrease Enzyme Activities and Microbes in Soil. *Open Journal of Soil Science*, *14*(1), 1–12. https://doi.org/10.4236/OJSS.2024.141001

46. Klein, S., Worch, E., & Knepper, T. P. (2015). Occurrence and spatial distribution of microplastics in river shore sediments of the rhine-main area in Germany. *Environmental Science and Technology*, *49*(10), 6070–6076. https://doi.org/10.1021/acs.est.5b00492

47. Kurki-Fox, J. J., Doll, B. A., Monteleone, B., West, K., Putnam, G., Kelleher, L., Krause, S., & Schneidewind, U. (2023). Microplastic distribution and characteristics across a large river basin: Insights from the Neuse River in North Carolina, USA. *Science of The Total Environment*, *878*, 162940. https://doi.org/10.1016/J.SCITOTENV.2023.162940

48. Lambert, S., & Wagner, M. (2016). Characterisation of nanoplastics during the degradation of polystyrene. *Chemosphere*, *145*, 265–268. https://doi.org/10.1016/j.chemosphere.2015.11.078

49. Le Tran, M., Thi Van Nguyen, H., Phuong Thai, V., & Ngoc Nguyen, T. (2023). Potential contamination of microplastic from plastic recycling enterprises in Ho Chi Minh City, Vietnam. *Human and Ecological Risk Assessment: An International Journal*, *29*(7–8), 1031–1043. https://doi.org/10.1080/10807039.2023.2228414

50. Lechner, A., Keckeis, H., Lumesberger-Loisl, F., Zens, B., Krusch, R., Tritthart, M., Glas, M., & Schludermann, E. (2014). The Danube so colourful: A potpourri of plastic litter outnumbers fish larvae in Europe's second largest river. *Environmental Pollution*, *188*, 177–181. https://doi.org/10.1016/J.ENVPOL.2014.02.006

51. Leterme, S. C., Tuuri, E. M., Drummond, W. J., Jones, R., & Gascooke, J. R. (2023). Microplastics in urban freshwater streams in Adelaide, Australia: A source of plastic pollution in the Gulf St Vincent. *Science of The Total Environment*, *856*, 158672. https://doi.org/10.1016/J.SCITOTENV.2022.158672

52. Liang, J., Ji, F., Abdullah, A. L. B., Qin, W., Zhu, T., Tay, Y. J., Li, Y., & Han, M. (2024). Micro/nano-plastics impacts in cardiovascular systems across species. *Science of The Total Environment*, *942*, 173770. https://doi.org/10.1016/J.SCITOTENV.2024.173770

53. Liang, Y., Lehmann, A., Yang, G., Leifheit, E. F., & Rillig, M. C. (2021). Effects of Microplastic Fibers on Soil Aggregation and Enzyme Activities Are Organic Matter Dependent. *Frontiers in Environmental Science*, *9*, 650155. https://doi.org/10.3389/FENVS-.2021.650155/BIBTEX

54. Liu, F., Olesen, K. B., Borregaard, A. R., & Vollertsen, J. (2019). Microplastics in urban and highway stormwater retention ponds. *Science of The Total Environment*, *671*, 992–1000. https://doi.org/10.1016/J.SCITOTENV.2019.03.416

55. Lucas, N., Bienaime, C., Belloy, C., Queneudec, M., Silvestre, F., & Nava-Saucedo, J. E. (2008). Polymer biodegradation: Mechanisms and estimation techniques - A review. In *Chemosphere* (Vol. 73, Issue 4, pp. 429–442). https://doi.org/10.1016/j.chemosphere.-2008.06.064

56. Mahesh, S., Gowda, N. K., & Mahesh, S. (2023). Identification of microplastics from urban informal solid waste landfill soil; MP associations with COD and chloride. *Water Science and Technology : A Journal of the International Association on Water Pollution Research*, *87*(1), 115–129. https://doi.org/10.2166/wst.2022.412

57. Maheswaran, B., Karmegam, N., Al-Ansari, M., Subbaiya, R., Al-Humaid, L., Sebastin Raj, J., & Govarthanan, M. (2022). Assessment, characterization, and quantification of microplastics from river sediments. *Chemosphere*, *298*. https://doi.org/10.1016/j.chemo-sphere.2022.134268

58. Makhdoumi, P., Amin, A. A., Karimi, H., Pirsaheb, M., Kim, H., & Hossini, H. (2021). Occurrence of microplastic particles in the most popular Iranian bottled mineral water brands and an assessment of human exposure. *Journal of Water Process Engineering*, *39*, 101708. https://doi.org/10.1016/J.JWPE.2020.101708

59. Makhdoumi, P., Naghshbandi, M., Ghaderzadeh, K., Mirzabeigi, M., Yazdanbakhsh, A., & Hossini, H. (2021). Micro-plastic occurrence in bottled vinegar: Qualification, quantification and human risk exposure. *Process Safety and Environmental Protection*, *152*, 404–413. https://doi.org/10.1016/J.PSEP.2021.06.022

60. Marcellus, K. A., Bugiel, S., Nunnikhoven, A., Curran, I., & Gill, S. S. (2024). Polystyrene Nano- and Microplastic Particles Induce an Inflammatory Gene Expression Profile in Rat

Neural Stem Cell-Derived Astrocytes In Vitro. *Nanomaterials*, *14*(5). https://doi.org/10.3390/NANO14050429,

61. Masud, N., & Cable, J. (2023). Microplastic exposure and consumption increases susceptibility to gyrodactylosis and host mortality for a freshwater fish. *Diseases of Aquatic Organisms*, *153*, 81–85. https://doi.org/10.3354/DAO03721
62. Miller, E., Sedlak, M., Lin, D., Box, C., Holleman, C., Rochman, C. M., & Sutton, R. (2021). Recommended best practices for collecting, analyzing, and reporting microplastics in environmental media: Lessons learned from comprehensive monitoring of San Francisco Bay. *Journal of Hazardous Materials*, *409*, 124770. https://doi.org/10.1016/J.JHAZMAT.2020-.124770
63. Mkuye, R., Gong, S., Zhao, L., Masanja, F., Ndandala, C., Bubelwa, E., Yang, C., & Deng, Y. (2022). Effects of microplastics on physiological performance of marine bivalves, potential impacts, and enlightening the future based on a comparative study. *Science of The Total Environment*, *838*, 155933. https://doi.org/10.1016/J.SCITOTENV.2022.155933
64. Mohsen, M., Wang, Q., Zhang, L., Sun, L., Lin, C., & Yang, H. (2019). Heavy metals in sediment, microplastic and sea cucumber Apostichopus japonicus from farms in China. *Marine Pollution Bulletin*, *143*, 42–49. https://doi.org/10.1016/j.marpolbul.2019.04.025
65. Moore, R. C., Loseto, L., Noel, M., Etemadifar, A., Brewster, J. D., MacPhee, S., Bendell, L., & Ross, P. S. (2020). Microplastics in beluga whales (Delphinapterus leucas) from the Eastern Beaufort Sea. *Marine Pollution Bulletin*, *150*. https://doi.org/10.1016/j.marpolbul.2019-.110723
66. Nan, B., Su, L., Kellar, C., Craig, N. J., Keough, M. J., & Pettigrove, V. (2020). Identification of microplastics in surface water and Australian freshwater shrimp Paratya australiensis in

Victoria, Australia. *Environmental Pollution*, *259*. https://doi.org/10.1016/j.envpol.2019.-113865

67. Nurhasanah, Cordova, M. R., & Riani, E. (2021). Micro- and mesoplastics release from the Indonesian municipal solid waste landfill leachate to the aquatic environment: Case study in Galuga Landfill Area, Indonesia. *Marine Pollution Bulletin*, *163*. https://doi.org/10.1016/j.marpolbul.2021.111986
68. Patra, I., Huy, D. T. N., Alsaikhan, F., Opulencia, M. J. C., Van Tuan, P., Nurmatova, K. C., Majdi, A., Shoukat, S., Yasin, G., Margiana, R., Walker, T. R., & Karbalaei, S. (2022). Toxic effects on enzymatic activity, gene expression and histopathological biomarkers in organisms exposed to microplastics and nanoplastics: a review. *Environmental Sciences Europe 2022 34:1*, *34*(1), 1–17. https://doi.org/10.1186/S12302-022-00652-W
69. Polanco, H., Hayes, S., Roble, C., Krupitsky, M., & Branco, B. (2020). The presence and significance of microplastics in surface water in the Lower Hudson River Estuary 2016–2019: A research note. *Marine Pollution Bulletin*, *161*. https://doi.org/10.1016/j.marpolbul.-2020.111702
70. Poma, A. M. G., Morciano, P., & Aloisi, M. (2023). Beyond genetics: can micro and nanoplastics induce epigenetic and gene-expression modifications? *Frontiers in Epigenetics and Epigenomics*, *1*, 1241583. https://doi.org/10.3389/FREAE.2023.1241583
71. Rahman, R. R., Baqee, A., Alam, M., Khan, M. W., Muhib, M. I., & Kabir, A. (2024). Organ-specific bioaccumulation of microplastics in market fish of Dhaka and size-dependent impacts of PVC microplastics on growth of Anabus testudineus. *Environmental Pollution*, *361*, 124807. https://doi.org/10.1016/J.ENVPOL.2024.124807

72. Raju, M., Gandhimathi, R., & Nidheesh, P. V. (2023). The cause, fate and effect of microplastics in freshwater ecosystem: Ways to overcome the challenge. *Journal of Water Process Engineering*, *55*, 104199. https://doi.org/10.1016/J.JWPE.2023.104199

73. Rico, A., Redondo-Hasselerharm, P. E., Vighi, M., Waichman, A. V., Nunes, G. S. de S., de Oliveira, R., Singdahl-Larsen, C., Hurley, R., Nizzetto, L., & Schell, T. (2023). Large-scale monitoring and risk assessment of microplastics in the Amazon River. *Water Research*, *232*, 119707. https://doi.org/10.1016/J.WATRES.2023.119707

74. Sarathana, D., & Winijkul, E. (2023). Concentrations of Airborne Microplastics during the Dry Season at Five Locations in Bangkok Metropolitan Region, Thailand. *Atmosphere*, *14*(1). https://doi.org/10.3390/atmos14010028

75. Sarma, H., Hazarika, R. P., Kumar, V., Roy, A., Pandit, S., & Prasad, R. (2022). Microplastics in marine and aquatic habitats: sources, impact, and sustainable remediation approaches. *Environmental Sustainability*, *5*(1), 39–49. https://doi.org/10.1007/s42398-022-00219-8

76. Seeley, M. E., Hale, R. C., Zwollo, P., Vogelbein, W., Verry, G., & Wargo, A. R. (2023). Microplastics exacerbate virus-mediated mortality in fish. *Science of The Total Environment*, *866*, 161191. https://doi.org/10.1016/J.SCITOTENV.2022.161191

77. Su, L., Sharp, S. M., Pettigrove, V. J., Craig, N. J., Nan, B., Du, F., & Shi, H. (2020). Superimposed microplastic pollution in a coastal metropolis. *Water Research*, *168*, 115140. https://doi.org/10.1016/J.WATRES.2019.115140

78. Sulaiman, B., Woodward, J. C., & Shiels, H. A. (2023). Riverine microplastics and their interaction with freshwater fish. *Water Biology and Security*, *2*(4), 100192. https://doi.org/10.1016/J.WATBS.2023.100192

79. Sun, Y., Deng, Q., Zhang, Q., Zhou, X., Chen, R., Li, S., Wu, Q., & Chen, H. (2024). Hazards of microplastics exposure to liver function in fishes: A systematic review and meta-analysis. *Marine Environmental Research*, *196*, 106423. https://doi.org/10.1016/J.MARENVRES-.2024.106423

80. Talbot, R., & Chang, H. (2022). Microplastics in freshwater: A global review of factors affecting spatial and temporal variations. *Environmental Pollution*, *292*, 118393. https://doi.org/10.1016/J.ENVPOL.2021.118393

81. Talbot, R., Granek, E., Chang, H., Wood, R., & Brander, S. (2022). Spatial and temporal variations of microplastic concentrations in Portland's freshwater ecosystems. *Science of The Total Environment*, *833*, 155143. https://doi.org/10.1016/J.SCITOTENV.2022.155143

82. Thomas, D. S., Kneifel, J. D., & Butry, D. T. (2024). *NIST Advanced Manufacturing Series 100-64*. https://doi.org/10.6028/NIST.AMS.100-64

83. Turner, A., Holmes, L., Thompson, R. C., & Fisher, A. S. (2020). Metals and marine microplastics: Adsorption from the environment versus addition during manufacture, exemplified with lead. *Water Research*, *173*. https://doi.org/10.1016/j.watres.2020.115577

84. Tuuri, E. M., & Leterme, S. C. (2023). How plastic debris and associated chemicals impact the marine food web: A review. *Environmental Pollution*, *321*, 121156. https://doi.org/10.1016/-J.ENVPOL.2023.121156

85. Viaroli, S., Lancia, M., & Re, V. (2022). Microplastics contamination of groundwater: Current evidence and future perspectives. A review. *Science of The Total Environment*, *824*, 153851. https://doi.org/10.1016/J.SCITOTENV.2022.153851

86. Wan, Y., Chen, X., Liu, Q., Hu, H., Wu, C., & Xue, Q. (2022). Informal landfill contributes to the pollution of microplastics in the surrounding environment. *Environmental Pollution*, *293*. https://doi.org/10.1016/j.envpol.2021.118586

87. Wiggin, K. J., & Holland, E. B. (2019). Validation and application of cost and time effective methods for the detection of 3–500 μm sized microplastics in the urban marine and estuarine environments surrounding Long Beach, California. *Marine Pollution Bulletin*, *143*, 152–162. https://doi.org/10.1016/J.MARPOLBUL.2019.03.060

88. Wu, P., Cai, Z., Jin, H., & Tang, Y. (2019). Adsorption mechanisms of five bisphenol analogues on PVC microplastics. *Science of the Total Environment*, *650*, 671–678. https://doi.org/10.10-16/j.scitotenv.2018.09.049

89. Xia, B., Zhang, J., Zhao, X., Feng, J., Teng, Y., Chen, B., Sun, X., Zhu, L., Sun, X., & Qu, K. (2020). Polystyrene microplastics increase uptake, elimination and cytotoxicity of decabromodiphenyl ether (BDE-209) in the marine scallop Chlamys farreri. *Environmental Pollution*, *258*. https://doi.org/10.1016/j.envpol.2019.113657

90. Xia, F., Yao, Q., Zhang, J., & Wang, D. (2021). Effects of seasonal variation and resuspension on microplastics in river sediments. *Environmental Pollution*, *286*. https://doi.org/10.1016/j.-envpol.2021.117403

91. Xiong, X., Tu, Y., Chen, X., Jiang, X., Shi, H., Wu, C., & Elser, J. J. (2019). Ingestion and egestion of polyethylene microplastics by goldfish (Carassius auratus): influence of color and morphological features. *Heliyon*, *5*(12). https://doi.org/10.1016/j.heliyon.2019.e03063

92. Yonkos, L. T., Friedel, E. A., Perez-Reyes, A. C., Ghosal, S., & Arthur, C. D. (2014). Microplastics in four estuarine rivers in the chesapeake bay, U.S.A. *Environmental Science*

and Technology, *48*(24), 14195–14202. https://doi.org/10.1021/ES5036317/SUPPL_FILE-/ES5036317_SI_001.PDF

93. Yu, H., Fan, P., Hou, J., Dang, Q., Cui, D., Xi, B., & Tan, W. (2020). Inhibitory effect of microplastics on soil extracellular enzymatic activities by changing soil properties and direct adsorption: An investigation at the aggregate-fraction level. *Environmental Pollution*, *267*, 115544. https://doi.org/10.1016/J.ENVPOL.2020.115544
94. Yu, X., Ladewig, S., Bao, S., Toline, C. A., Whitmire, S., & Chow, A. T. (2018). Occurrence and distribution of microplastics at selected coastal sites along the southeastern United States. *Science of The Total Environment*, *613–614*, 298–305. https://doi.org/10.1016/J.SCITOT-ENV.2017.09.100
95. Yuan, J., Ma, J., Sun, Y., Zhou, T., Zhao, Y., & Yu, F. (2020). Microbial degradation and other environmental aspects of microplastics/plastics. *Science of the Total Environment*, *715*. https://doi.org/10.1016/j.scitotenv.2020.136968
96. Yuan, W., Christie-Oleza, J. A., Xu, E. G., Li, J., Zhang, H., Wang, W., Lin, L., Zhang, W., & Yang, Y. (2022). Environmental fate of microplastics in the world's third-largest river: Basin-wide investigation and microplastic community analysis. *Water Research*, *210*, 118002. https://doi.org/10.1016/J.WATRES.2021.118002
97. Zhang, D., Fraser, M. A., Huang, W., Ge, C., Wang, Y., Zhang, C., & Guo, P. (2021). Microplastic pollution in water, sediment, and specific tissues of crayfish (Procambarus clarkii) within two different breeding modes in Jianli, Hubei province, China. *Environmental Pollution*, *272*, 115939. https://doi.org/10.1016/J.ENVPOL.2020.115939
98. Zhang, K., Hamidian, A. H., Tubić, A., Zhang, Y., Fang, J. K. H., Wu, C., & Lam, P. K. S. (2021). Understanding plastic degradation and microplastic formation in the environment: A

review. In *Environmental Pollution* (Vol. 274). Elsevier Ltd. https://doi.org/10.1016/j.env-pol.2021.116554

99. Zocchi, M., & Sommaruga, R. (2019). Microplastics modify the toxicity of glyphosate on Daphnia magna. *Science of the Total Environment*, *697*. https://doi.org/10.1016/j.scitotenv.-2019.134194

CHAPTER 2

2. DISCOVERY OF MICROPLASTICS IN THE ALABAMA RIVER: DISTRIBUTIONS, TRANSPORT AND LOADS, SOURCES, AND RELATIVE COMPARISON WITH WORLD RIVERS

2.1. Abstract

The present study investigated microplastic (MP) contamination in the Alabama River. Concentration and size distributions, transport and loads, and potential sources of MPs were studied. Results showed that MP concentrations in the Alabama River and world polluted rivers are comparable. The concentration fluctuated by season and location with no consistent trends. The study revealed that MPs in the Alabama River likely did not travel long distances and sedimentation on the riverbed may be a major sink for MPs. Polyethylene terephthalate (PET, 21.7 %) was the most abundant polymer found in the samples. Other less abundant polymer types were polymethyl pentene (PMP, 16.1 %), polypropylene (PP, 13.8 %), polyvinyl chloride (PVC, 12.4 %), polybutylene terephthalate (PBT, 12 %), acrylonitrile butadiene styrene (ABS, 6.7 %), and polyethylene (PE, 5.3 %). Approximately 90 % MPs were less than 2.5 mm long with 50 % being less than 1 mm, which are in consumable size range for many sensitive aquatic organisms. It was estimated that hundreds of billions to trillions of MPs enter Mobile Bay per day. This poses potential exposures and risks to aquatic life in the Bay and the Gulf of Mexico (hereafter called Gulf of America). Microplastic concentrations were quantitatively correlated with the watershed drainage into the river and human pollution suggesting contributions of human activities to MP contamination. The present study provides a first look at MP contamination in the Alabama River.

Keywords – Microplastics abundance and concentrations Alabama River Load and transport Size distribution Spatial and temporal variation

2.2. Introduction

Plastic production in the modern world is increasing continuously and so is plastic waste. Estimates predicted a generation of around 12,000 tons of plastic waste by 2050 (Geyer et al., 2017). The chemical stability of plastics enables them to persist in the natural environment for decades (Xia et al., 2024) and poses an environmental concern for the scientific community and environmental managers around the globe. Plastic debris are believed to be more toxic and harmful as their size is reduced (Chae et al., 2019). Plastics with a size equal to or less than 5 mm are defined as microplastics (MPs) (Viaroli et al., 2022). Based on their origin and applications, MPs are categorized into primary and secondary MPs. Primary MPs are plastic particles intentionally produced with a size of 5 mm or less for specific industrial applications, such as plastic microbeads used in industrial abrasions, cosmetics products, plastic pellets, whereas secondary MPs are produced by fragmentation of large plastic items (Rochman et al., 2016). Although most plastic waste is produced on land, plastics are washed off and/or blown into waterways, such as lakes and rivers and end up in the ocean. Industrial, domestic, and agricultural discharges are all important sources of MPs in the environment.

Once plastics reach the waterbodies, aquatic species are exposed to them. Microplastics have been reported to be ingested by living organisms and caused lethal and sublethal effects on them (Barrick et al., 2025; Hoang & Mitten, 2022; Lee et al., 2023; López-Rojo et al., 2020; Nelms et al., 2019; Yuan et al., 2022). Therefore, understanding MP contamination status and managing sources to reduce MP impacts on the natural ecosystem is necessary. Research on MP contamination in river systems has been carried out around the world including in England (Horton et al., 2018), Germany (Klein et al., 2015), Switzerland (Faure et al., 2015), Canada (Ballent et al., 2016), Austria (Lechner et al., 2014), India (Sarkar et al., 2021; Sarkar et al., 2021), South Korea

(Eo et al., 2019; Wang et al., 2017), Japan (Kabir et al., 2021; Nihei et al., 2020), and Vietnam (Lahens et al., 2018). The level of MP contamination in world river systems varies, depending on sources and management measures. Overall, MP concentrations have been reported to be higher in rivers in Asia where pollution control is less intense than in western countries. In the US, few studies on MP pollution in river systems have been conducted with the most comprehensive research limited to the San Francisco Bay and Long Beach, California (Miller et al., 2021; Wiggin & Holland, 2019), Neuse River in North Carolina (Kurki-Fox et al., 2023), Chesapeake Bay (Yonkos et al., 2014), Eel, Salamonie, Tippecanoe, Lower East Fork White, Muscatatuck, and Patoka Rivers, Indiana (Conard et al., 2023), coastal sites of North Carolina, Texas, and US Virgin Islands (Yu et al., 2018), and Snake River of Idaho and Oregon (Kapp & Yeatman, 2018).

The Alabama River is the largest river in Alabama, USA. The river is formed by the confluence of Coosa and Tallapoosa Rivers-components of the Alabama River Basin and flows approximately 640 km through Montgomery-the capital city of Alabama and Selma to the Gulf of America. Downstream, it joins the Tombigbee River at the bottom of the Alabama River Basin in Mobile-the second largest city of Alabama and forms Mobile River, ultimately forming an estuary at Mobile Bay of the Gulf of America. The Alabama River is the major drainage of urban and agricultural runoff and city effluents, and therefore, is expected to receive MP releases from various sources along its waterway, such as wastewater treatment plants, agricultural activities, recreational and fishing activities. Anthropogenic activities on Alabama's water system have resulted in increased contamination. Both point and non-point sources have contributed to the water quality degradation in the Alabama River. However, information on MP contamination in the Alabama River system is poorly understood. Mobile Bay is the drainage basin of the Alabama River, is the home to a diversity of aquatic species, and has been identified as one of the most

important nearshore habitats in the Gulf of America. Microplastic contamination can affect aquatic life in the Alabama River, Mobile Bay, and the Gulf of America if the contamination is high. Considering these gaps, the present study was conducted to understand different aspects of MP contamination status of the Alabama River. The spatial and temporal variation in MP concentration, characterization of MP types, size distributions which are related to bioavailability for ingestion, transport, loads into Mobile Bay, and potential factors contributing to MP release into the river system were evaluated. To the author's best knowledge, this is the first extensive study on MP contamination in a river system that investigated the sources, distributions, transport, loads, and potential bioavailability of particles using an innovative sampling method that allowed determination of MP distribution in a water column. Results of the present study provided a baseline for future researchers to conduct risk assessments to support management of MP pollution in US river systems.

2.3. Materials and methods

2.3.1. Study site

In the present study, five sites (Wetumpka, Montgomery, Selma, Dixie Landing, and Mobile) along the Alabama River were selected to investigate MP pollution in the river (Fig. A1). Wetumpka (32◦ 32' 20.6" N; 86◦ 12' 22.5" W) is the upper stream site and expected to be less contaminated than Montgomery (32◦ 25' 46.6" N; 86◦ 23' 33.5" W) and Mobile (30◦ 40' 01.6" N; 87◦ 55' 31.4" W) sites. Selma site (32◦ 09" 43.6" N; 87◦ 07' 04.5" W) is downstream of Montgomery and is expected to have lower MP concentrations than the Montgomery site. Dixie Landing (31◦ 17' 43.2" N; 87◦ 45' 52.4" W) is a remote site because it is in a forest area that receives little human activity. It is above the merging point of the Alabama River and Tombigbee River. The Mobile site (30◦ 40' 01.6" N; 87◦ 55' 31.4" W) is at the river mouth at which the river drains into Mobile Bay. This site

selection covers approximately 94 % of the river stream (~ 600 km) and allows determination of the spatial difference and potential contributions of human activities in urban areas to MP concentrations in the Alabama River water.

2.3.2. Water sampling

To determine the temporal and spatial differences in MP concentration in the Alabama River, water samples were collected from each selected site in the summer, fall, and winter of 2023 and spring of 2024. In recent studies, water sampling for MP analysis was conducted using plankton nets that were designed for microplastics (called microplastic net) or straight water collection (Pan et al., 2020; Rico et al., 2023). However, a major drawback of net sampling is that it only collects the surface samples and is also unable to collect the particles that have sizes of less than the opening size of the mesh (usually 333 μm) while smaller plastic particles tend to be the most abundant in natural waters (Song et al., 2014). To avoid this problem, water samples in the present study were collected using a self-designed sampler that was made of a commercial 3.06 m PVC pipe and copper ball valves that allowed collecting samples at three different sections (layers) of the water column to determine the vertical distribution of MPs in the river water (Fig. A2).

The first section was 0.61 m from the surface, which represents the water column of most field studies on MP pollution in surface waters (Akdogan et al., 2023; Matjašič et al., 2023). The middle and bottom layers were 1.20 m each below the surface layer. A silicon rubber cork at the bottom of pipe was used to open and close the sampler each time. The bottom of the cork (larger diameter end) was attached to a string that was secured to the pipe on the outside to keep the sampler open. The top of the cork (smaller diameter end) was hooked to a stainless-steel wire that was connected to the top of the sampler from inside that allowed closing the sampler through pulling the wire from the top of the sampler. The cork was kept half-way open when the sampler was lowered down

in the water to allow water to go through the sampler. After reaching the required depth, the cork was pulled to close the sampler. Water samples were collected in jars by opening the copper ball valves assembled to the sampler. This sampling method was used for all sites except for the Dixie Landing site, at which only surface water was collected because of the lack of boat access. Kemmerer sampler was also used for collecting an additional water sample during Winter and Spring seasons. At each site, triplicate samples were collected at three locations along the river at approximately 300 m apart. At each location, a 3 L composite water samples were collected from the middle and both edges of the river. A total of 164 water samples were collected for MP analysis in the one-year period of the study [(3 layers x 3 replicates x 4 seasons x 4 sites) + (1 layer x 3 replicates x 4 seasons x 1 site (Dixie Landing))]. The PVC sampler was rinsed thoroughly with 10 % HNO_3 acid and DI water to wash off MPs that might be associated with the pipes during manufacturing before using for sampling. At sampling sites, the sampler was rinsed with river water before collecting samples to prevent cross contamination between sites. Water was collected into pre-acid washed glass containers and covered with aluminum foil before capping to avoid potential contamination of microplastics from the container's caps. Samples were placed in coolers and transported to the Ecotoxicology and Risk Assessment Laboratory of Auburn University for processing and analysis of MPs. Water quality parameters were measured at the sampling sites. Water pH was measured using a Toledo Seven 2Go pH meter. Temperature, dissolved oxygen (DO), and conductivity were measured using a YSI Pro 2030 Meter. The river flow on the sampling day was obtained from the online waterflow monitoring database of the USGS. Other water quality parameters, such as hardness (titration, (Eaton et al., 2005a)) and alkalinity (titration method, (Eaton et al., 2005b)), and metal concentrations were analyzed using an Inductively Coupled

Plasma Mass Spectrometry (ICP-MS), Model NexION 2000 in the Ecotoxicology and Risk Assessment Laboratory of Auburn University. Results of water quality are reported in Table A1.

2.3.3. Quality assurance

To minimize environmental contamination, glassware was used to conduct the experiment. All the glassware was rinsed with acetone and MilliQ water prior to the experiment. Glassware was covered with aluminum foil to avoid deposition of airborne MPs. Since ambient MPs have been reported to be present in the laboratory, blank filters were also used for filtration to determine the background MPs in the laboratory, especially for microfibers. The laboratory bench was swept before processing the samples. A cotton laboratory coat was worn during processing and analysis of samples. In addition, a nylon MP sample and a cup MP sample (microplastics made from a single use plastic cup were prepared separately by mixing the MPs in ethanol (Barrick et al., 2024). Six replicate samples of each MP type were collected and filtered to measure MP concentrations in the ethanol solutions. Another six replicate samples were collected into digestion tubes, digested, and measured MPs as for river water samples to determine the recovery of the analysis method.

2.3.4. Analysis of microplastics

Analysis of MPs was conducted in the Ecotoxicology and Risk Assessment Laboratory of Auburn University based on the National Oceanic and Atmospheric Administration (NOAA) guidelines (Masura et al., 2015). Water sample was first mixed thoroughly, and 1 L was passed through a stack of stainless-steel sieves of 5 mm and 38 μm pore sizes. The 38 μm sieve was selected because water can easily pass through it without the use of vacuum pump to avoid potential breaking of microplastics due to pressure caused by vacuum pump. Through this step, the samples were separated into parts. The water passing through 38 μm sieve (hereafter called below 38 μm

fraction) was filtered directly onto a cellulose nitrate grid membrane filter with the pore size of 0.45 µm (VWR International) for identification and characterization of MPs. Materials retained on 38 µm sieve (hereafter called above 38 µm fraction) were rinsed with MilliQ water and collected into a pre-acid washed glass beaker and digested with 30 % H_2O_2 for 24 h at 60°C to break organic materials. After digestion, the solution was diluted three times with MilliQ water and filtered on 0.45 µm membrane filters for MP identification and characterization.

2.3.5. Identification and characterization of microplastics

The filters were first examined under a LAXCO Stereomicroscope to identify and count MPs. Physical characteristics of MPs, such as size, and color were determined and recorded. The dominant MPs in the natural environment have been reported to be microfibers (Mauro et al., 2022; Liu et al., 2022; Weis & Falco, 2022). To be convenient for interpretation and comparison, MPs in water samples of the present study were categorized into two classes: fibers and non-fibers (fragments, beads, and films) (Hoang & Mitten, 2022). Fiber length and width and length of non-fiber MPs were measured using a LAXCO Stereomicroscope. The approximate area of non-fiber MPs (length x width) was also calculated. Microplastic concentrations in the entire water column (three layers combined) were calculated using Eq. 1.

$$C = \frac{Ns+Nm+Nb}{Vs+Vm+Vb} \qquad (1)$$

where C is the MP concentration (MP particles/L, hereafter called MPs/ L) in the water column Ns, Nm, and Nb, are the number of MPs found in surface, middle, and bottom water layers, respectively. V_s, V_m, and V_b are the water volumes (L) of the three layers, respectively. After determining the shape and size, the polymer type was verified through a Shimadzu AIRsight Infrared/Raman Microscope using Fourier Transform Infrared Spectroscopy (FTIR) method. More details of the measurement method are described in Table A7. Particle spectrum was compared

with the reference plastic spectrum to determine the polymer type. According to the manufacturer, a matching score of greater than 550 is considered plastic material.

2.3.6. Analysis of size distribution of microplastics and relative comparison of microplastic concentrations in world rivers

Results of the size measurement of MPs (fiber length and length of non-fiber MPs) were used to analyze the size distribution of MPs. Cumulative frequency distribution method that is usually used for assessment of toxicant exposure and effect distribution for ecological risk assessment was used for the size distribution analyses in the present study (Hoang & Rand, 2015; Li et al., 2025). The lengths of fiber and non-fiber MPs were used to develop cumulative log-logistic distributions for individual MPs for each site of all seasons, all sites in the same season, and all sites and seasons together using least squares regression. The lengths were ranked from the shortest to the longest, and a centile was calculated for each MP using Eq. 2:

$$Centile = Rank * \frac{100}{(n+1)} \quad (2)$$

where n is the total number of MPs. The 10^{th}, 50^{th}, and 90^{th} cumulative percentiles of MP length were calculated. The 10^{th} and 90^{th} percentiles are usually used to represent effect and exposure benchmarks in ecological risk assessment, respectively, and represent the size range of 80 % MPs in the present study. The centile calculation method above was also used to determine the cumulative centile ranks that were used for comparing MP concentrations in the water of world rivers. MP concentrations of world rivers were obtained from the literature. Concentrations reported in units other than MPs/L were converted to MPs/L for the centile rank analysis.

2.3.7. Assessment of microplastic transport in the Alabama River and load into Mobile Bay

MP transport rates in the Alabama River at each sampling site were calculated using Eq. 3.

$$MP\ transport\ rate\ =\ (MP\ concentration)\ \times\ (water\ flowrate) \quad (3)$$

where, MP transport rate is in number MPs/day, MP concentration is in number MPs/L, and water flowrate is in L/day. Water flowrates were calculated based on the hourly water discharge rates at each site on the sampling day that were obtained from the USGS online database. The water discharge data of a monitoring site that is nearest to a sampling site of the present study was used to calculate the average daily flowrates, the seasonal lowest and highest daily average flowrates, and seasonal average of daily flowrates for the sampling site (Table A2).

These water flowrates and average MP concentrations for each sampling day were used to calculate daily MP passing through each sampling site (hereafter called MP transport) using Eq. 3 above. Total MP transport (in number MPs) through each sampling site in a season (Eq. 4) and in the entire study year (Eq. 5) were also calculated. The daily MP transport rates and the total MP transports in a season and the study year at the Mobile site represent the daily MP load rates and total MP loads into Mobile Bay and the Gulf of America in a season and year, respectively.

*Total MP transport in a season = (Seasonal daily average MP transport rate) * (Number of days in a season)* (4)

$$Total\ MP\ transport\ in\ the\ study\ year\ =\ \sum_{i=1}^{4}(Total\ MP\ transport\ in\ a\ season) \quad (5)$$

2.3.8. Assessment of the relationship of microplastics in the Alabama River water with potential sources.

The Pearson correlation method was used to determine the correlation between MP concentrations of the Alabama River water, water flow, watershed area, and human population of the city of the sites studied. The average water flow data were obtained through the USGS online database. Human population data were obtained from the US Census Bureau or the city/county database. Watershed areas for each studied site were extracted through the USGS StreamStat online database. Data used for correlation analysis are shown in Table A3.

2.3.9. Statistical analysis

Comparisons of significant differences in MP concentrations between sites in a season or between seasons at a site were conducted to determine the spatial and temporal variation of MP contamination in the Alabama River. Data were transformed using the logarithmic transformation method to satisfy the assumptions of normal distribution and homogenous variances before comparison. Statistical Analysis System (SAS) – Academics on Demand was used for the statistical analyses. Normality of the data was determined using the Shapiro-Wilks test. ANOVA pairwise comparisons with Tukey's test were used to determine the significant difference between the mean MP concentrations. Statistical significance was set at a p-value < 0.05.

2.4. Results

2.4.1. Microplastic characteristics

Microplastics of different sizes, shapes, colors, and types were found in all water samples (Fig. 2.1).

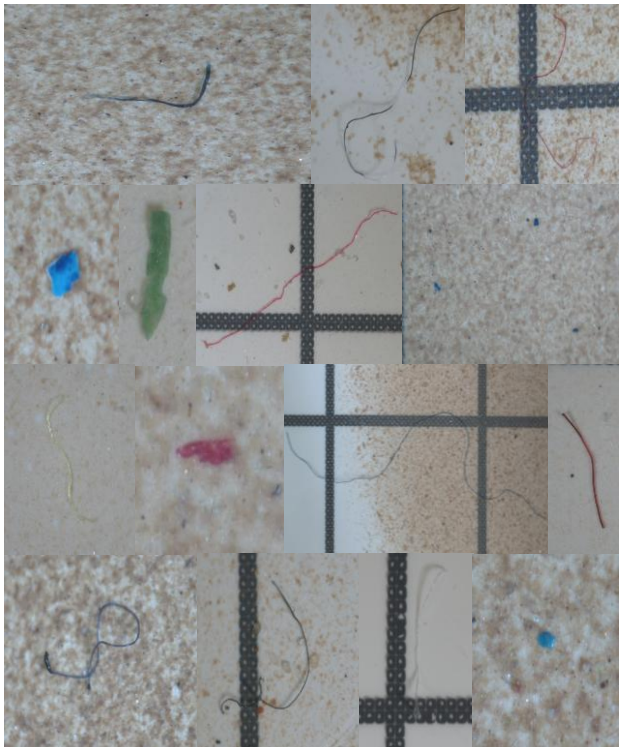

Fig. 2.1: Microplastics in the Alabama River water.

A total of 3156 MPs were identified, sized, and colored in the present study. The microfiber lengths (ranged from 0.1 mm to 5.0 mm), lengths of non-fiber MPs (ranged from 0.02 mm to 5 mm), and the two-dimensional area of non-fiber MPs (ranged from 0.001 mm^2 to 0.8 mm^2) are reported in Table 2.4. The results of polymer type analysis showed that no consistent trends or frequency by site or season for the chemical characteristics of MPs were observed (Table A4, Figure A3).

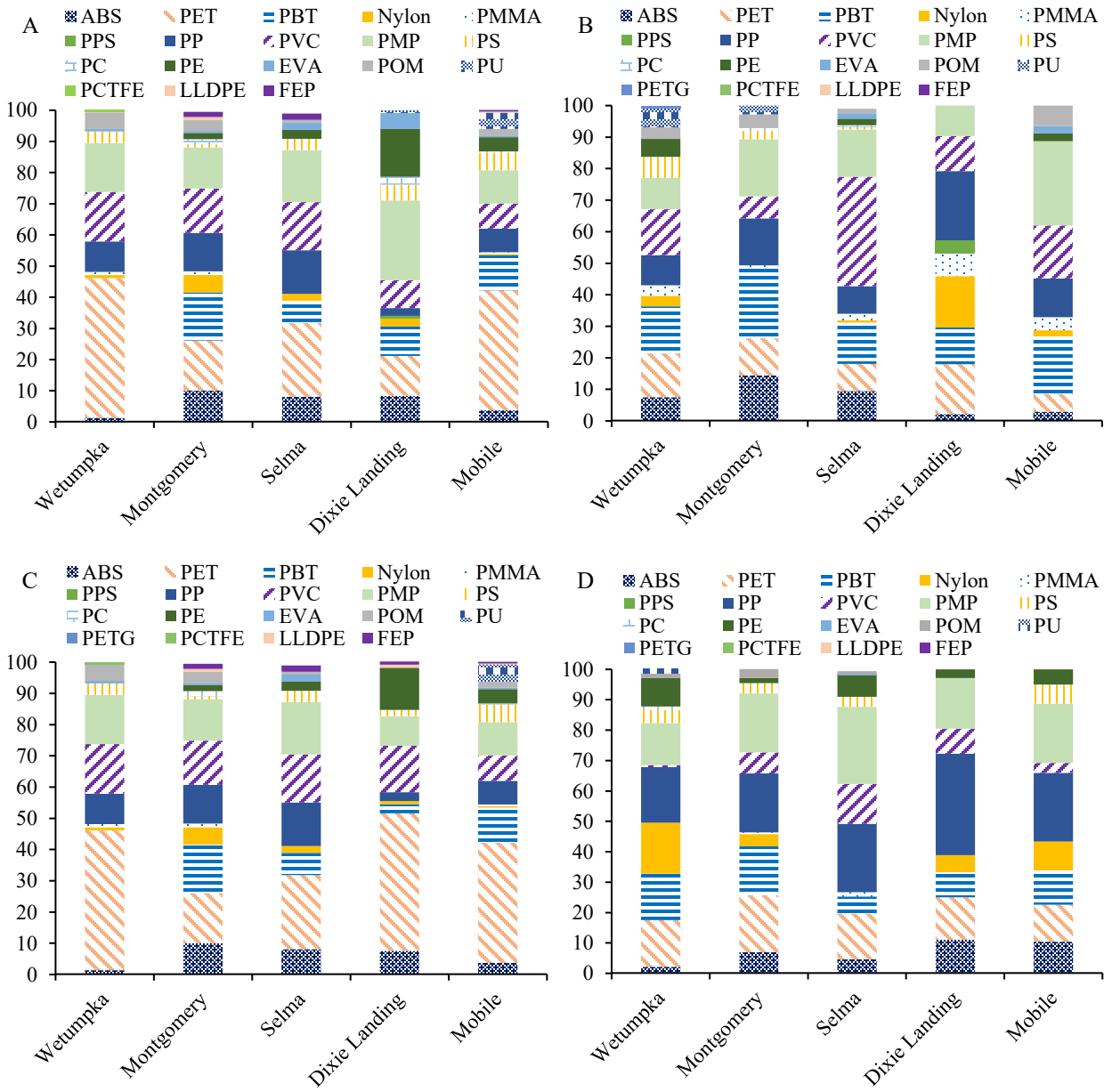

Figure 2.2: Percentage of polymer types in the Alabama River water (A: summer, B: fall, C: winter, D: spring).

However, the most dominant polymer type across all sites was polyethylene terephthalate (PET) with a seasonal average of 21.7 % and a range of 5.7–44.7 % followed by polymethyl pentene (PMP) (16.1 %, 9.5–26.7 %), polypropylene (PP) (13.8 %, 2.4–33.3 %), polyvinyl chloride (PVC) (12.4 %, 0.7–34.7 %), polybutylene terephthalate (PBT) (12.0 %, 2.9–22.2 %), acrylonitrile butadiene styrene (ABS) (6.7 %, 1.4–14.4 %), and polyethylene (PE) (5.3 %, 1.7–15.3 %) (Table A5, Fig. 2.2). Other plastic polymer types were less than 5.0 % on average.

2.4.2. Microplastic concentrations

Microplastic concentrations in samples of different water layers for the below 38 µm and above 38 µm fractions are presented in Figure A4. Statistical analysis showed that except for the Mobile site in the summer and the Wetumpka site in the fall and spring, no statistically significant difference in MP concentration between the two fractions was found for other sites and seasons. The results also showed that MP concentrations of different water layers at the same site and in the same season were not statistically significantly different (Figure A5). In addition to that, the MP concentrations of different water layers were not significantly different from the Kemmerer samples (hereafter called K samples) (Figure A6).

To be convenient for comparison and interpretation, MP concentrations of combined fractions and water layers (without K samples) were used and presented. Results of the QC samples showed that small numbers of fibers were detected on the QC filters with the average of 3.75 + 4.50 MPs/filter for the summer batch of samples and 1.00 + 1.41 MPs/filter for the fall batch of samples (Table A4). Microplastics were not detected in the QC filters for the winter and spring sample batches. Results of the MP spiked samples are reported in Table A6.

The average MP concentrations before and after digestion were 20 and 23 MPs/filter for cup MPs and 75 and 66 MPs/filter for nylon microfibers, respectively. These results indicated a recovery of

112 % for cup MPs and 87 % for nylon microfibers. These recoveries are within 15 % of targeted concentrations, which are usually accepted for chemical analysis and should be accepted for MP analysis.

The size range of MPs before and after digestion overlapped. For cup MPs, the two-dimensional surface area ranged from 0.0001 to 0.08 mm^2 for before digestion and 0.0001–0.06 mm^2 for after digestion. The length range of nylon microfibers were 0.05–1.56 mm and 0.02–4.37 mm for before and after digestion, respectively. These results suggest that the digestion process did not affect MP size.

Microplastic concentrations (all MPs, fibers, non-fiber MPs) in the Alabama River water are shown in Fig. 2.3. The MP concentration varied by site and season. Overall, MP concentrations were the highest in the summer and lowest in the winter. The ranges of the average MP concentrations (all MPs) for the summer, fall, winter, and spring were 31–135 MPs/L, 25–66 MPs/L, 13–48 MPs/L, and 12–75 MPs/L, respectively (Table A4). Those respective ranges for fibers were 15–67 MPs/L, 18–49 MPs/L, 7–36 MPs/L, and 11–52 MPs/L and for non-fiber MPs were 13–83 MPs/L, 9–23 MPs/L, 6–14 MPs/L, and 1–23 MPs/L (Table A4). There were statistically significant differences in MP concentrations between sites in the same season.

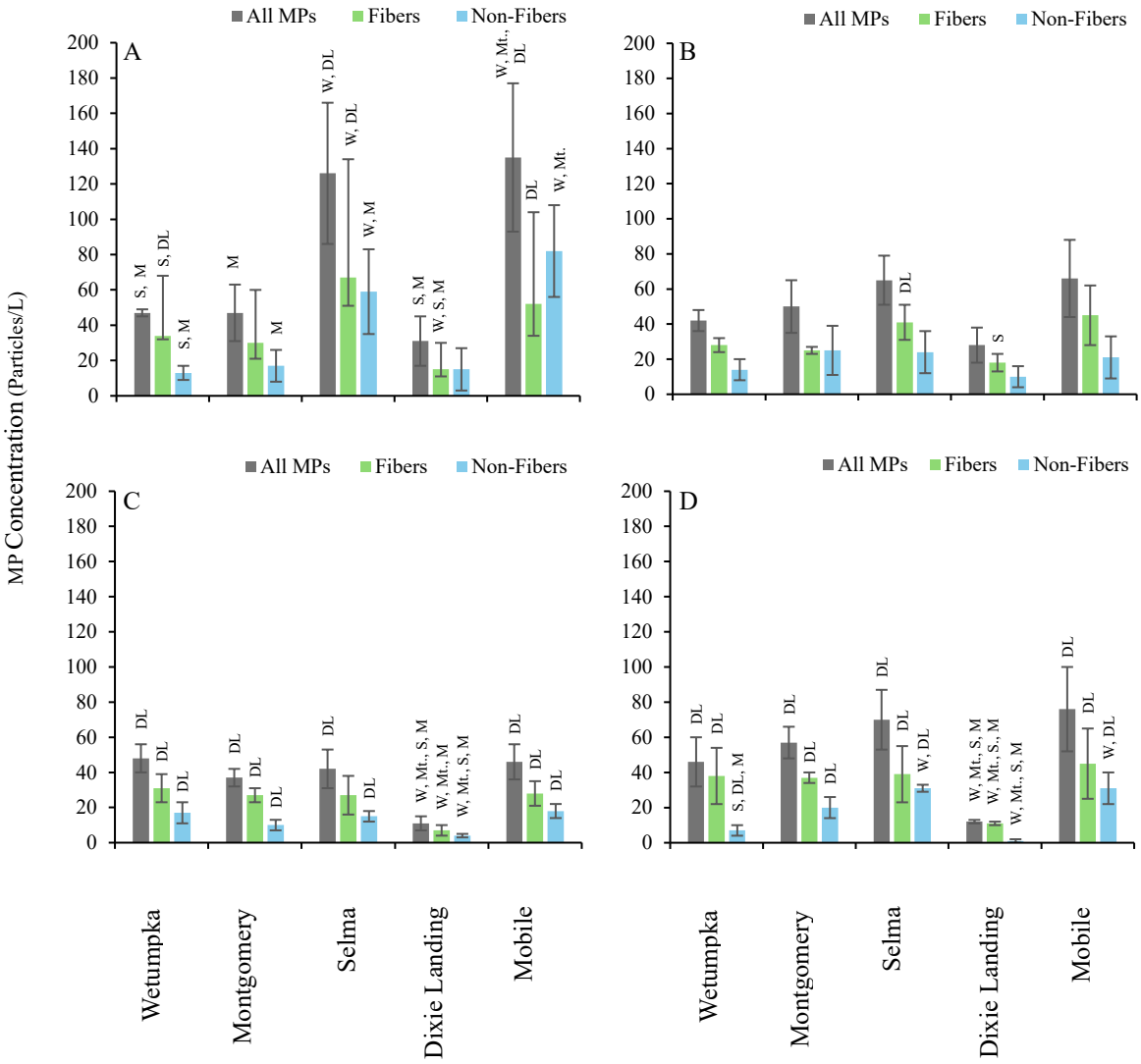

Figure 2.3: Spatial differences in microplastic concentrations of the Alabama River water (A: summer, B: fall, C: winter, D: spring. Error bars represent standard deviations. Letter W, Mt., S, DL, and M are assigned to Wetumpka, Montgomery, Selma, Dixie Landing, and Mobile sites, respectively.

For example, in the summer, the concentration of all MPs at the Mobile site was significantly higher than those at the Wetumpka, Montgomery, and Dixie Landing sites but not significantly higher than the Selma site (Fig. 2.3A). Non-fiber MP concentration was statistically significantly higher at the Mobile site than at the Wetumpka and Montgomery sites (Fig. 2.3A). The fiber concentration was statistically significantly higher at the Selma site than the Wetumpka and Dixie Landing sites (Fig. 2.3A). In the fall, only fiber concentrations at the Selma and Dixie Landing

sites were statistically significant (Fig. 2.3B). For the winter and spring, MP concentrations (all MPs, fibers, non-fiber MPs) at the Dixie Landing site were statistically significantly lower than those at other sites with the exception for no statistically significant difference of the fiber concentrations at the Dixie Landing and Selma sites in the winter (Figs. 2.3C, 2.3D).

The temporal variation of MP concentrations appeared to be less significant than spatial variation (Fig. 2.4).

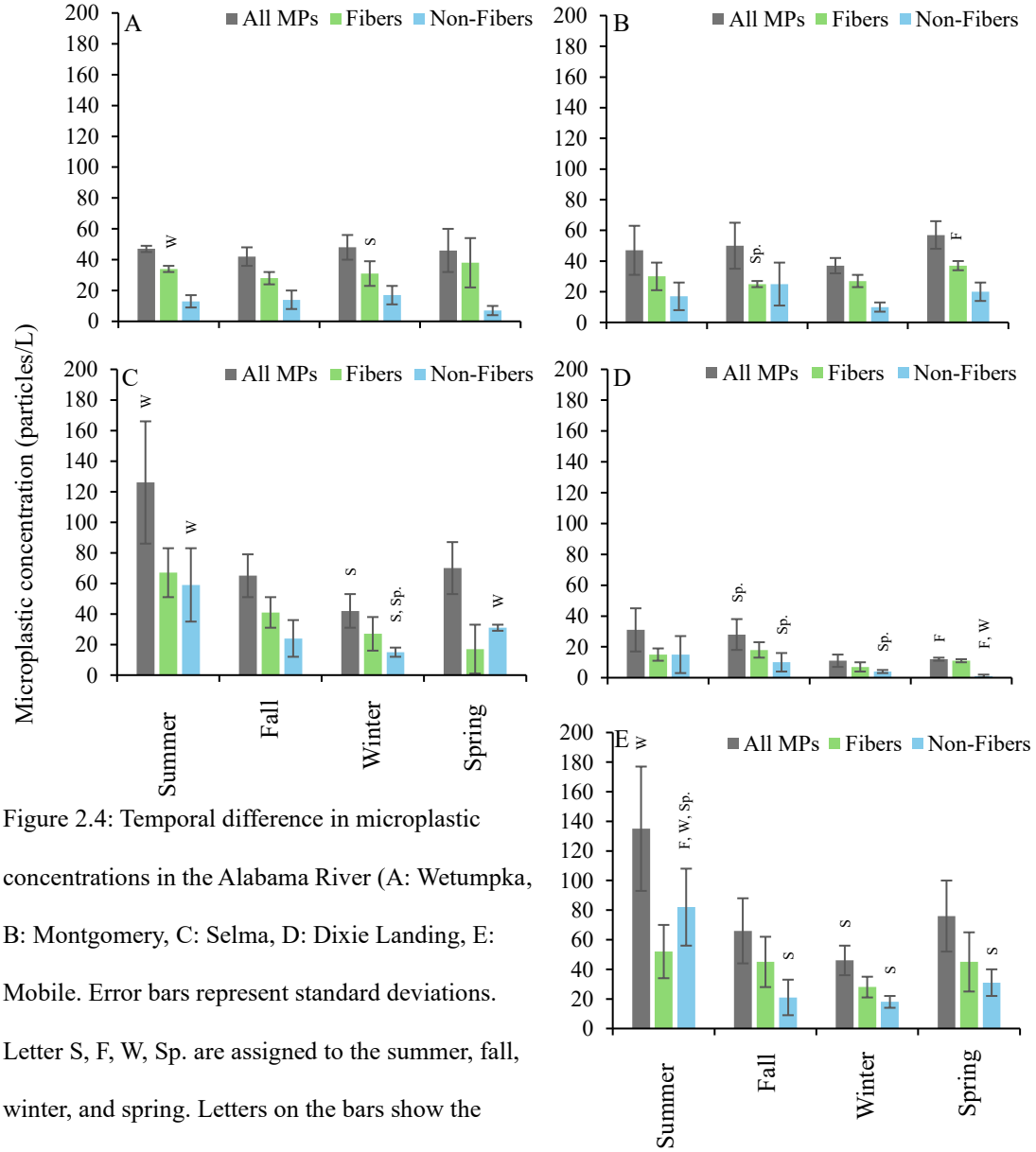

Figure 2.4: Temporal difference in microplastic concentrations in the Alabama River (A: Wetumpka, B: Montgomery, C: Selma, D: Dixie Landing, E: Mobile. Error bars represent standard deviations. Letter S, F, W, Sp. are assigned to the summer, fall, winter, and spring. Letters on the bars show the differences.

At the Wetumpka site, only fiber concentrations in the summer and winter were statistically significantly different (Fig. 2.4A). For the Montgomery site, the MP concentrations between the seasons were not statistically significantly different except for the fiber concentrations in the fall and spring (Fig. 2.4B). At the Selma site, the concentrations of all MPs and non-fiber MPs in the summer and winter were statistically significantly different. Other cases were not statistically significantly different (Fig. 2.4C). Statistically significant differences were found for the concentrations of all MPs and non-fiber MPs between the fall and spring and non-fiber MPs between the winter and spring (Fig. 2.4D). For the Mobile site, the concentration of all MPs was statistically significantly higher in the summer than in the winter. Non-fiber MP concentration was also statistically significantly higher in the summer than in other seasons (Fig. 2.4E).

2.4.3. Relative percentage of microplastics

Relative percentage of microplastics Overall, fiber MPs were found to have higher percentages than non-fiber MPs except for the Dixie Landing site in the summer, winter, and spring and the Mobile site in the summer, at which a reversed relative distribution was found (Fig. 2.5). In the summer, fiber MPs ranged from 39 % (Mobile site) to 72 % (Wetumpka site) compared to non-fiber MPs range of 28 % (Wetumpka site) - 61 % (Mobile site) (Fig. 2.5A). The ranges in the fall were 62 % (Montgomery site) – 74 % (Mobile site) and 26 % (Mobile site) – 38 % (Montgomery site) for fiber and non-fiber MPs, respectively (Fig. 2.5B). The percentages of fiber and non-fiber MPs varied largely in the winter and spring with 39 % (Dixie Landing site) to 81 % (Montgomery site) for fibers and 19 % (Mongomery site) to 61 % (Dixie Landing site) in the winter (Fig. 2.5C) and 35 % (Dixie Landing site) to 85 % (Wetumpka site) for fiber and 15 % (Wetumpka site) to 65 % (Dixie Landing site) for non-fiber MPs in the spring (Fig. 2.5D).

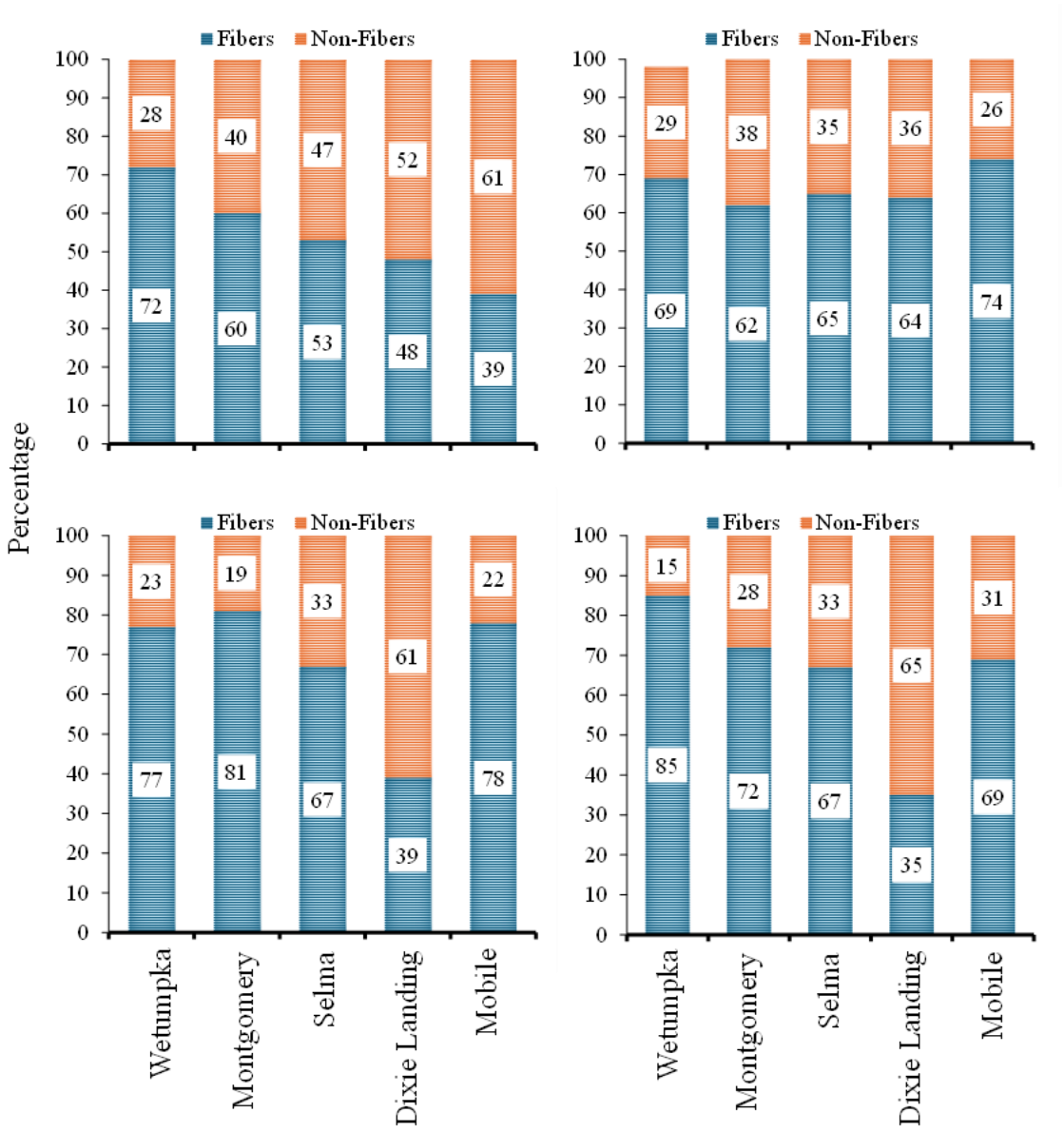

Fig. 2.5. Percentage of microplastics in the Alabama River water (A: summer, B: fall, C: winter, D: spring. Data shown in the columns are percentages of MP types).

2.4.4. Microplastic size distribution

Results of the size distribution analyses for 3156 MPs showed a slight difference in temporal and spatial distributions. Among the four seasons, the MP size distribution pattern for each site in the winter was the most similar with the distribution curves being close to each other. The distribution curves for other seasons, especially for the spring, were more separated between sites (Fig. 2.6).

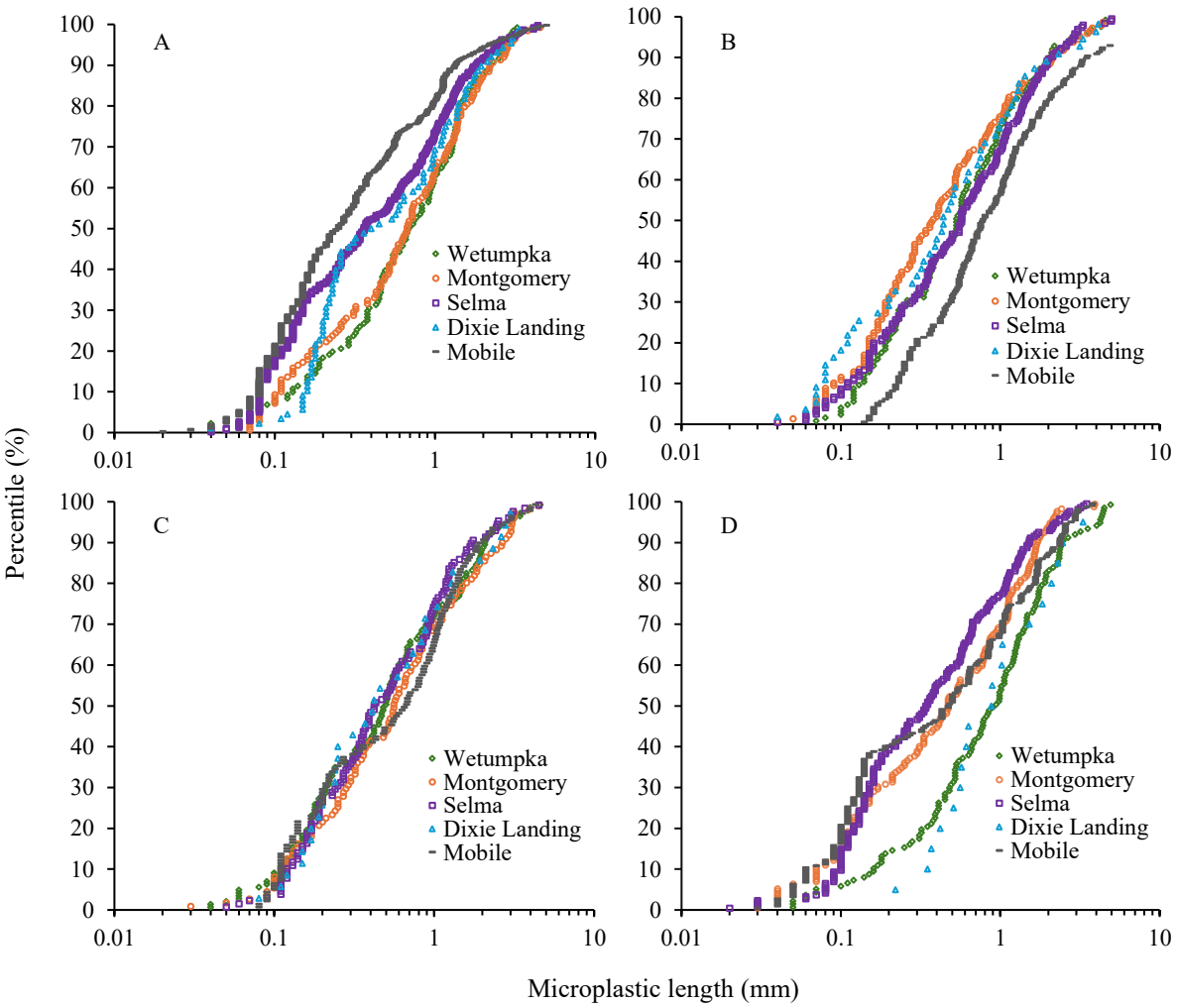

Fig. 2.6: Cumulative frequency distribution of microplastic size by site (A: summer, B: fall, C: winter, D: spring).

Among the five sites, the MP size distribution for each season was more similar to each other at the Montgomery and Selma sites (Figure A7). The distributions were more distant between seasons at other sites (Figure A7). Results of the 10th, 50th, and 90th percentile calculations for the length of MPs are shown in Table 2.1.

Table 2.1: Percentile of MP length (mm) for different seasons and sites in the Alabama River.

Season	Site	Percentile		
		50^{th}	10^{th}	90^{th}
Summer (2023)	Wetumpka	0.70	0.13	2.22
	Montgomery	0.67	0.11	2.30
	Selma	0.36	0.08	1.81
	Dixie Landing	0.40	0.16	2.14

	Mobile	0.25	0.08	1.34
Fall (2023)	Wetumpka	0.53	0.14	2.08
	Montgomery	0.22	0.09	2.11
	Selma	0.57	0.11	2.13
	Dixie Landing	0.44	0.08	1.94
	Mobile	0.66	0.16	2.49
Winter (2023)	Wetumpka	0.50	0.11	2.08
	Montgomery	0.56	0.12	2.67
	Selma	0.42	0.12	1.75
	Dixie Landing	0.42	0.15	2.63
	Mobile	0.67	0.11	2.04
Spring (2023)	Wetumpka	0.94	0.16	2.48
	Montgomery	0.47	0.07	1.71
	Selma	0.34	0.10	1.52
	Dixie Landing	0.88	0.35	2.46
	Mobile	0.47	0.06	2.36
Summer	All sites	0.38	0.08	1.81
Fall	All sites	0.54	0.12	2.12
Winter	All sites	0.52	0.11	2.09
Spring	All sites	0.51	0.09	2.05
All seasons	Wetumpka	0.60	0.13	2.22
All seasons	Montgomery	0.52	0.10	2.06
All seasons	Selma	0.40	0.09	1.80
All seasons	Dixie Landing	0.49	0.12	2.25
All seasons	Mobile	0.42	0.09	1.94
All seasons	All sites	0.48	0.10	1.99

The 50th percentile data varied largely by site and season, ranging from the lowest value at the Montgomery site in the summer (0.22 mm) to the highest value at the Wetumpka site in the spring (0.94 mm, Table 2.1). The variation in MP length at the 10th percentile was less than that at the 50th percentile and ranged from 0.06 mm (Mobile site and spring) to 0.35 mm (Dixie Landing site and spring). The 90th percentile data varied little compared to the 10th and 50th percentile data with the range of 1.34 mm (Mobile site and summer) to 2.67 mm (Montgomery site and winter) (Table 2.1). Overall, the 10th to 90th percentile size range was the smallest for the Mobile site in the summer and the largest at the Montgomery site in the winter.

When analyzing the data of all sites, summer had the lowest 50th percentile value (0.38 mm) with the smallest 10th to 90th percentile size range, whereas those values were highest (0.54 mm) for the fall (Table 2.1, Figure A8-A). Analysis of data for all seasons showed that the Selma site had the lowest 50th percentile value (0.40 mm) and the lowest 10th to 90th percentile size range (Table 2.1, Figure A8-B). The 50th percentile value was highest at the Wetumpka site (0.60 mm) and the highest 10th to 90th percentile size range was at the Dixie Landing site (Table 2.1, Figure A8-B). When the data of all seasons and sites are pooled together, the 50th, 10th, and 90th percentile values were 0.48 mm, 0.10 mm, and 1.99 mm, respectively (Table 2.1, Figure A8-C).

2.4.5. Microplastic transport on the Alabama River

Based on MP concentrations found in the present study and the water flowrates of the Alabama River; billions of MPs were estimated to pass through the river each day (Table 2.2). Overall, MP transport at the Wetumpka site was the lowest and highest at the Mobile site. The estimated daily transport ranged from 310 to 9324, 251–1126, 263–1629, and 606–2271 billion MPs/day in summer, fall, winter, and spring, respectively. Those respective ranges of the seasonal average of daily transport were 366–7376, 273–1240, 1068–4021, and 1055–7631 billion MPs/day. The total number of MPs transport through the sampling sites in the summer, fall, winter, and spring ranged from 33,633 to 677,800, 24,846–112,856, 97,213–365,880, and 90,331–702,053 billion, respectively. In the study year, the total number of MPs transported through Wetumpka, Montgomery, Dixie Landing, and Mobile sites were estimated at 246,074, 681,434, 387,040, and 1858,589 billion. The MP transport estimates for the Mobile site represent the daily load, total loads per season and per year into Mobile Bay and the Gulf of America.

Table 2.2: Estimated MP transport in the Alabama River at the sampling sites and on the sampling days.

Seasons	Sites	Microplastic transport in the Alabama River (billion MPs/day)				Total MP transport in a season (billion MPs)	Total MP transport in a year (billion MPs)
		Sampling day	Seasonal lowest daily	Seasonal highest daily	Average seasonal daily		
Summer (2023)	Wetumpka	310	211	645	366	33,633	N/A
	Montgomery	1308	426	3217	1200	110,364	N/A
	Selma	NA	NA	NA	NA	NA	NA
	Dixie Landing	1282	562	4769	1501	138,088	N/A
	Mobile	9324	1543	17,209	7367	677,800	N/A
Fall (2023)	Wetumpka	251	194	575	273	24,846	N/A
	Montgomery	1126	439	2321	857	77,984	N/A
	Selma	NA	NA	NA	NA	NA	NA
	Dixie Landing	530	426	1519	601	54,685	N/A
	Mobile	853	471	3507	1240	112,856	N/A
Winter (2023)	Wetumpka	263	227	6692	1069	97,264	N/A
	Montgomery	1604	337	10,297	2612	237,682	N/A
	Selma	NA	NA	NA	NA	NA	NA
	Dixie Landing	425	174	2880	1068	97,213	N/A
	Mobile	1629	397	8120	4021	365,880	N/A
Spring (2024)	Wetumpka	606	280	7430	982	90,331	N/A
	Montgomery	2271	961	7207	2776	255,404	N/A
	Selma	NA	NA	NA	NA	NA	NA
	Dixie Landing	612	274	3150	1055	97,055	N/A
	Mobile	8584	2867	12,823	7631	702,053	N/A
Whole year	Wetumpka	N/A	N/A	N/A	N/A	N/A	246,074
	Montgomery	N/A	N/A	N/A	N/A	N/A	681,434
	Selma	N/A	N/A	N/A	N/A	N/A	NA
	Dixie Landing	N/A	N/A	N/A	N/A	N/A	387,040
	Mobile	N/A	N/A	N/A	N/A	N/A	1,858,589

NA: data are not available, N/A: not applicable.

2.4.6. Correlation analysis

Microplastic concentration and total MP transport at the sampling sites along the Alabama River was significantly ($p < 0.01$) correlated with the water flow, watershed area, and human population of the city of the sampling sites (Table 2.3). The total MP transport was highly correlated with water flow (coefficient of 0.83) and watershed area (coefficient of 0.66). Concentration of MPs in the water was strongly correlated with the watershed area (coefficient of 0.76). The correlations

between human population and MP concentration (coefficient of 0.54), and human population and total MP transport (coefficient of 0.49) were weaker than the correlations between watershed area and total MP transport (coefficient of 0.66), watershed area and MP concentration (coefficient of 0.76), and watershed area and human population (coefficient of 0.72).

Table 2.3. Results of correlation analysis.

	Total MP transport	Microplastic concentration	Population	Water flow	Watershed area
Total MP transport	1				
Microplastic concentration	0.81	1			
Population	0.49	0.54	1		
Water flow	0.83	0.38	0.41	1	
Watershed area	0.66	0.76	0.72	0.43	1

Correlations were significant at $p < 0.01$.

2.4.7. Comparison of microplastics in the Alabama River with world rivers

Microplastic concentrations in the water of 25 rivers in the world were obtained from the literature to compare with the MP concentrations in the Alabama River. The MP concentration of world rivers ranged from 0.00002 to 649 MPs/L (Table A7). Data of all rivers including the Alabama River were analyzed to determine the cumulative centile rank for each river. Results of the analysis showed that the cumulative percentile for the Alabama River was from the 61st to 91st (Fig. 2.7). These results indicate that the Alabama River had MP concentrations higher than MP concentrations of 61 % world rivers but lower than MP concentrations of 9 % world rivers.

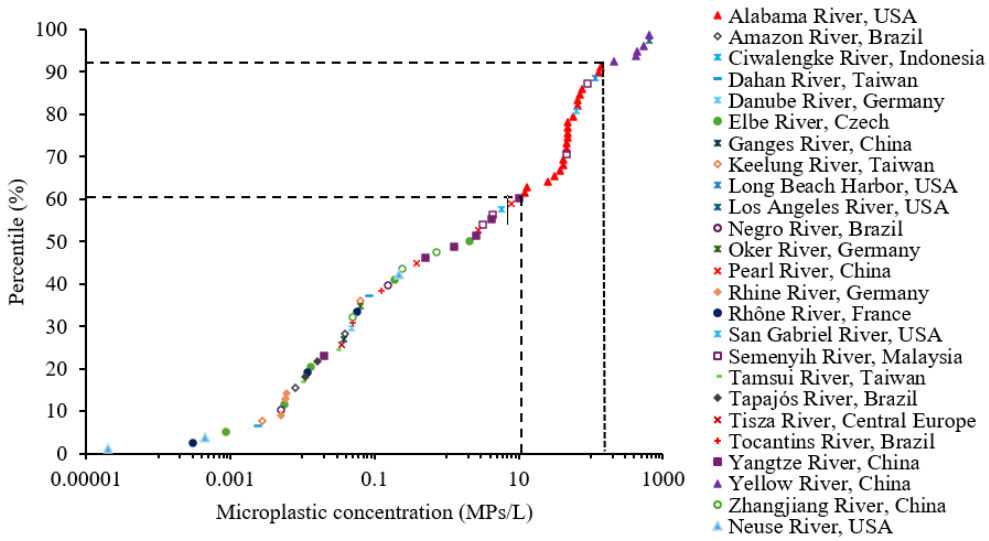

Figure 2.7. Relative distribution of microplastic concentrations of world rivers.

2.5. Discussion

2.5.1. Spatial and temporal distribution of MPs and potential sources and transport

The present study found MPs in the Alabama River water at all sites and seasons. The concentration ranged from 12 to 135 MPs/L (Table A4). Although these concentrations are within the concentration range of world rivers, they are higher than the concentrations of 61 % rivers in the world. These results suggest that the Alabama River is relatively contaminated with MPs. Compared with the Los Angeles River of the USA and Yellow River of China, the Alabama River is less contaminated with the percentile being lower than the percentile of those rivers (> 91 %, Fig. 2.7). However, caution should be taken when comparing MP concentrations of world rivers. Plankton net sampling method was commonly used for collecting samples for MP analyses (Constant et al., 2020; Kurki-Fox et al., 2023; Mani et al., 2015b; Mani & Burkhardt-Holm, 2020; Mohsen et al., 2023; Pan et al., 2020; Rico et al., 2023; Scherer et al., 2020; Schrank et al., 2022b; Wong et al., 2020; Zhao et al., 2014). While plankton net sampling method allows collection of samples in large water surface areas, which would be more representative, the mesh sizes are usually large, such as 300 µm of commercial microplastic sampling net. Microplastics with smaller

sizes than this mesh size can pass through the net and are not collected for analysis. A recent study by Kurki-Fox et al. (2023) using the net sampling method with a mesh size of 335 μm found substantial loss of samples with sizes smaller than 335 μm. In addition, the water volume passing through the net, which is used to calculate MP concentration, might not be accurately measured. The water volume passing through the net was calculated based on the speed of the sampling net. The calculated water volume was a few folds higher than the actual volume passing through the net because the net was clogged with suspended particles and algae that prevented water passing through (author's unpublished data). Moreover, fibers with lengths longer than the mesh can pass through the mesh size used because fiber width is usually narrow. The results of analysis of the fiber lengths in the below 38 μm fraction samples showed that most fibers were greater than 0.5 mm (Figure A9). Fibers with lengths up to 5 mm were detected in this sample fraction. These fibers would have been excluded if the net sampling method were used. Losing samples and overestimating water volume will result in underestimation of MP concentration in water.

Considering concentrations of all MPs along the river, the concentration increased as water flows from upstream (Wetumpka site) to downstream (Selma site) during the summer, fall, and spring (Fig. 2.3). The increase in MP concentration at the downstream sites can be due to MP transport from upstream to downstream and MP inputs from local sources at the downstream sites. However, MP inputs from local sources at the Selma site (~ 160 km south of the Montgomery site) are not expected to be substantial because Selma is a smaller city with less development and has a human population of approximately ten times lower than the human population of Montgomery (Table A3). Therefore, MP transport from upstream would be an important source of MPs at the Selma site. However, if MPs continue to travel further downstream, MP concentrations at the Dixie Landing site (~ 250 km south of Selma site) could have been higher than MP concentrations at the

Selma site. The present study found that MP concentrations were two to four times lower at the Dixie Landing site than at the Selma site (Fig. 2.3, Table A3). These results suggest that MPs in the Alabama River water would have sedimented on their way traveling from the Selma to Dixie Landing sites. The higher MP concentrations at the Mobile site than the Dixie Landing site suggest that most MPs at the Mobile site would be from local sources. The changes in MP concentration at these studied sites reveal an important finding that likely not all MPs can travel all the way from upstream to downstream and to the ocean. The river bottom would be an important destination for MPs. Therefore, estimation of MP loads into oceans from long rivers should focus more on local sources that are not far away from the river mouth. Understanding the potential transport of MPs from upstream to downstream is important for management to minimize MP loads into the ocean from rivers. The consistent lower MP concentrations found at the Dixie Landing than other sites in all seasons suggest that MPs at this site are less influenced by local sources and can be considered as the base MP concentration in the Alabama River water that are associated with permanent sources, such as from wastewater treatment plants in upstream and atmospheric deposition. Microplastic release through the effluents of wastewater treatment plants have been reported to be substantial (Acarer, 2023; Gkatzioura et al., 2021; Kay et al., 2018; Mintenig et al., 2017; Ruan et al., 2019; Sadia et al., 2022) . Microplastics are also found in remote environments, such as in the arctic environment, and are attributed to atmospheric deposition (Bergmann et al., 2022; T. Hoang & Mitten, 2022; Illuminati et al., 2024; Zhao et al., 2014; Ziani et al., 2023).

Among the seasons, summer had the highest MP concentration, and the concentration was lowest in winter regardless of site (Figs. 2.3, 2.4, Table A4). These results reflect a temporal variation of MPs in the Alabama River water, which is believed to be related to non-point sources associating with human's outdoor activities that usually happen more in the summer than winter, such as

fishing, swimming, and kayaking work. The significant positive correlations between MP concentration in the Alabama River water and human population and watershed area (Table 2.3) support the temporal variation of MP concentrations found in the present study. Findings of the present study are also in agreement with a recent study in the USA by Kurki-Fox et al. (2023) demonstrating correlations between MP concentrations in Neuse River water (North Carolina) and human population and watershed areas. Studies in other parts of the world also found direct correlations between MP abundance and human population and density (Jang et al., 2020; Mani et al., 2015b; Nel et al., 2018; Tang et al., 2018).

Results of the size distribution analyses showed that although the distribution pattern did not appear to be markedly different, the distribution values at the 10th, 50th, and 90th percentiles were largely different by season at a specific site and by site in the same season (Table 2.1). For example, the 50th percentiles of MP length at the Wetumpka site in the summer, fall, winter, and spring were 0.70 mm, 0.53 mm, 0.50 mm, and 0.94 mm, respectively. Those respective values at the Mobile site were 0.25 mm, 0.66 mm, 0.67 mm, and 0.47 mm. These sizes reflect a difference by a factor of more than two. The 50th percentile of MP length in the summer ranged from 0.25 mm (Mobile site) to 0.70 mm (Wetumpka site). Those size ranges for the fall, winter, and spring were 0.22–0.66 mm, 0.42–0.67 mm, and 0.34–0.94 mm, respectively. Overall, MPs in the summer (for all sites) were found to have the smallest size with 80 % of MPs being between 0.08 and 1.81 mm in length. The fall appeared to have larger MPs with 80 % MPs had sizes within the 0.12–2.12 mm range. By location, Wetumpka site (for all seasons) had the largest MPs. The size range of 80 % MPs was 0.13–2.22 mm. The respective size range was the lowest at the Selma site (0.40–1.80 mm). River wide, 80 % MPs found in the Alabama River water had sizes between 0.10 and 1.99 mm with 50 % MPs being less than 0.48 mm. The difference in MPs size by site would reflect

different MP inputs from local point sources, while the difference by season would be attributed to non-point sources associating with human's outdoor activities. Understanding particle size of MPs is vitally important for interpretation of their environmental impacts. Microplastics are not expected to produce effects on living organisms if they are not ingested by organisms. Microplastic ingestion is dependent on the relative size of MPs to organisms, specifically the size of the organism's mouth. The smaller MPs at the Selma site regardless of season and in the summer across the sites along the Alabama River would be more bioavailable for ingestion by aquatic life in the river than larger MPs in other seasons at other sites. According to Barrick et al. (2025), water flea, Daphnia magna can ingest microfibers at lengths up to 350 µm. The ingestion of two nylon microfibers can result in up to 50 % lethality to D. magna (Barrick et al., 2025). Another study by Barrick et al. (2024) also found that D. magna ingested environmentally realistic MPs at sizes up to 118 µm. Plastic microbeads at sizes of 63–75 µm have been reported to be ingested by D. magna (Canniff & Hoang, 2018) and benthic organism (Hyalella azeteca) (Au et al., 2015). Larval fathead minnows were found to ingest plastic microbeads at sizes up to 150 µm (Hoang & Felix-Kim, 2020) and 489 µm (Malinich et al., 2018). Another study confirmed the ingestion of MPs ranging between 0.4 and 2 mm by the mussel species Anodonta anatina and less than 41µm in Mytillus galloprovincialis (Başaran Kankılıç et al., 2023). In addition, MPs have been reported to be transferred through the food chain in aquatic ecosystems copepods, crustaceans, decapod crustaceans, mussels, amphipods, fishes, marine mammals, and seabirds (Elizalde-Velázquez et al., 2020; Gündoğdu et al., 2020; Koraltan et al., 2022; Ugwu et al., 2021). Detrimental effects of MPs on aquatic organisms have been observed for multiple levels of biological organization such as physical disruption of digestive tracts and physical abrasions, alterations to energetic reserves, impacts on immune and endocrine systems, and changes sexual development and fecundity (Bai

et al., 2021; Sharma & Chatterjee, 2017). Given that MPs in the Alabama River water are highly abundant, there is a good chance that organisms within the river system ingest MPs, which may influence their biological performance. Research to investigate MP ingestion and potential impacts on living organisms in the Alabama River should be conducted to help assess ecological risks and impacts of MPs in this important river system. Further research to determine local sources of MP loads into the river is also needed.

2.5.2. Common MP shapes and polymers

Microplastic types found in the present study are similar to the MP types found in other parts of the world. Fibers were the dominant plastic type in all seasons and at all sites, with a few exceptions, for example at the Dixie Landing site in the summer, winter, and spring and the Mobile site in the summer. Their wide range of applications, especially in textile products, made them dominant over the non-fiber MPs. This finding is in agreement with the results of other MP studies conducted on river systems in different regions of the world (Shruti et al., 2021; Tan et al., 2022; Yin et al., 2022).

A total of 19 different polymer types were found in the Alabama River water samples. Among those, PET, PP, PE, PVC, PMP, PBT, ABS, and nylon were most often found, ranging between 5.7– 44.7 %, 2.4–33.3 %, 1.7–15.3 %, 0.7–34.7 %, 9.5–26.7 %, 2.9–22.2 %, 1.4–14.4 %, and 0.5–16.8 %, respectively. Polymers, such as PET, PP, and PE were reported to be the dominant polymers in rivers due to their extensive applications in different industrial products (Apetogbor et al., 2023; Qian et al., 2023). The present study also found three of these polymers to be dominant in the Alabama River. Polyethylene terephthalate is commonly used in the manufacturing of daily-use items, such as clothes, carpets, water bottles, and synthetic fibers. Its application in synthetic fiber production is reported to be as high as 60 % by volume (Radzi et al., 2021). Polyethylene

terephthalate fibers can be released from washing different fibrous materials, such as carpets and clothing (Dalla Fontana et al., 2020; Talukdar et al., 2024). Similarly, nylon is used for producing items that have good flexibility and strength (common examples include textile products, airbags, fishing lines, and seatbelts) (Dalla Fontana et al., 2020). Like these two polymers, PE is also widely used polymer for manufacturing bags, pipes, agricultural mulches, fishing gear etc. Xia et al. (2021) reported that PP is extensively used in food packaging, the manufacturing of fishing gears, and the agricultural sector due to its lightweight and stable properties.

2.5.3. Microplastic transport and load into Mobile Bay and Gulf of America

The present study did a first look into MP loads into Mobile Bay from the Alabama River. Microplastic passing at Mobile site is considered as MP loads into Mobile Bay. Various load rates were estimated including daily load, seasonal lowest daily load, seasonal highest daily load, seasonal average daily load, and total loads in a season and year. The study estimated hundreds of billions to trillions of MPs loading into Mobile Bay per day. The loading rates are related to MP concentrations and water discharge rate at the river mouth, which is dependent on rainfall. Overall, the loading rates were higher in the summer (9324 billion MPs/day) and spring (8584 billion MPs/day) than in the fall (853 billion MPs/day) and the winter (1629 billion MPs/day) (Table 2.2). In a year, the total number of MPs entering Mobile Bay from the Alabama River was estimated at 1858,589 billion. The water volume of Mobile Bay is approximately 3.2 × 1012 L. These calculations can result in an approximate addition of MPs into Mobile Bay per year at 581 MPs/L. This estimation was for MPs from the Alabama River only. Microplastic discards from local sources along the shorelines and other tributaries of the Bay are expected to be substantial as the present study demonstrates that MPs entering aquatic environments from local sources are more important than from transport far away from upstream. Therefore, MPs loading into Mobile Bay

are expected to be higher than the estimation of the present study. Data for MP concentration in Mobile Bay water is not available, but MPs in sandy sediment along the coastlines of Mobile Bay were reported to be abundant (Wessel et al., 2016). Once entering the bay, MPs can sink to the bay bottom and flow out to the Gulf of America. These pathways of MPs can result in decreasing their water concentration. Regardless, based on the results of the present study, MP concentration in Mobile Bay water is not expected to be low. It is also necessary to mention that the MP load estimates in the present study were based on the MP concentrations measured at a branche of Mobile River while the river drains water into Mobile Bay via branches. The estimations assumed that MP concentrations in all branches were the same. More research to determine MP concentration of all branches would provide more accurate estimation of MP loads into Mobile Bay. Mobile Bay is one of the four largest estuarine systems in the Gulf of America and the home of many aquatic species and has been known to be one of the most biodiverse estuaries in the US. Understanding MP contamination status and their potential impact on aquatic life is vitally important. More research to determine MPs in Mobile Bay and potential impacts is therefore necessary.

2.6. Conclusions and suggestions

The present study is one of the first attempts to investigate the MP contamination in the Alabama River. The study was designed to discover the temporal and spatial variation of MP concentration, size, sources, and transport and loads into Mobile Bay that allowed interpretation of potential exposure, ingestion, and impacts on living organisms. Microplastics with different types, shapes, sizes, colors were found. Microplastic concentration and size in the Alabama River temporally and spatially varied and comparable with MP concentrations in world polluted rivers. Microplastic concentrations were found to directly correlate with local point and non-point sources that are

associated with human activities and watershed areas. Spatial comparison of MP concentrations among the studied sites along the river revealed an important finding that MPs in the Alabama River water likely did not travel for long distances along the river and mostly sedimented on the way flowing downstream from upstream. Regardless, MP loads into Mobile Bay from the Alabama River is a concern. Analyses of size distribution indicated that over 90 % of MPs in the Alabama River had particle sizes within a size range that can be ingested by many small sensitive aquatic species. The high abundance with suitable size range of MPs for uptake poses potential risks of MP exposure, ingestion, and impacts on aquatic life. However, more monitoring and ecotoxicology research to study MPs contamination in the river system including the estuarine system of Mobile Bay and potential impacts of MPs on aquatic organisms in these import aquatic systems are needed. Understanding the MP contamination sources and effects will help us develop suitable management plans to minimize their impact on the environment to support ecosystem functioning and sustainable development.

2.7. References

1. Acarer, S. (2023). Microplastics in wastewater treatment plants: Sources, properties, removal efficiency, removal mechanisms, and interactions with pollutants. Water Science and Technology, 87(3), 685–710. https://doi.org/10.2166/WST.2023.022
2. Akdogan, Z., Guven, B., & Kideys, A. E. (2023). Microplastic distribution in the surface water and sediment of the Ergene River. Environmental Research, 234, 116500. https://doi.org/10.1016/J.ENVRES.2023.116500
3. Alam, F. C., Sembiring, E., Muntalif, B. S., & Suendo, V. (2019). Microplastic distribution in surface water and sediment river around slum and industrial area (case study: Ciwalengke

River, Majalaya district, Indonesia). Chemosphere, 224, 637–645. https://doi.org/10.1016/J.CHEMOSPHERE.2019.02.188

4. Apetogbor, K., Pereao, O., Sparks, C., & Opeolu, B. (2023). Spatio-temporal distribution of microplastics in water and sediment samples of the Plankenburg river, Western Cape, South Africa. Environmental Pollution, 323. https://doi.org/10.1016/j.envpol.2023.121303
5. Au, S. Y., Bruce, T. F., Bridges, W. C., & Klaine, S. J. (2015). Responses of Hyalella azteca to acute and chronic microplastic exposures. Environmental Toxicology and Chemistry, 34(11), 2564–2572. https://doi.org/10.1002/ETC.3093
6. Bai, Z., Wang, N., & Wang, M. (2021). Effects of microplastics on marine copepods. Ecotoxicology and Environmental Safety, 217, 112243. https://doi.org/10.1016/J.ECOENV.2021.112243
7. Ballent, A., Corcoran, P. L., Madden, O., Helm, P. A., & Longstaffe, F. J. (2016). Sources and sinks of microplastics in Canadian Lake Ontario nearshore, tributary and beach sediments. Marine Pollution Bulletin, 110(1), 383–395. https://doi.org/10.1016/j.marpolbul.2016.06.037
8. Barrick, A., Boardwine, A. J., & Hoang, T. C. (2024). Accumulation, depuration, and potential effects of environmentally representative microplastics towards Daphnia magna. Science of The Total Environment, 950, 175384. https://doi.org/10.1016/J.SCITOTENV.2024.175384
9. Barrick, A., Boardwine, A. J., Nguyen, N. H. A., Sevcu, A., Novotna, J., & Hoang, T. C. (2025). Acute toxicity of natural and synthetic clothing fibers towards Daphnia magna: Influence of fiber type and morphology. Science of The Total Environment, 967, 178751. https://doi.org/10.1016/J.SCITOTENV.2025.178751
10. Başaran Kankılıç, G., Koraltan, İ., Erkmen, B., Çağan, A. S., Çırak, T., Özen, M., Seyfe, M., Altındağ, A., & Tavşanoğlu, Ü. N. (2023). Size-selective microplastic uptake by freshwater

organisms: Fish, mussel, and zooplankton. Environmental Pollution, 336, 122445. https://doi.org/10.1016/J.ENVPOL.2023.122445

11. Bergmann, M., Collard, F., Fabres, J., Gabrielsen, G. W., Provencher, J. F., Rochman, C. M., van Sebille, E., & Tekman, M. B. (2022). Plastic pollution in the Arctic. Nature Reviews Earth & Environment 2022 3:5, 3(5), 323–337. https://doi.org/10.1038/s43017-022-00279-8
12. Büngener, L., Schäffer, S. M., Schwarz, A., & Schwalb, A. (2024). Microplastics in a small river: Occurrence and influencing factors along the river Oker, Northern Germany. Journal of Contaminant Hydrology, 264, 104366. https://doi.org/10.1016/J.JCONHYD.2024.104366
13. Canniff, P. M., & Hoang, T. C. (2018). Microplastic ingestion by Daphnia magna and its enhancement on algal growth. Science of The Total Environment, 633, 500–507. https://doi.org/10.1016/J.SCITOTENV.2018.03.176
14. Chae, Y., Kim, D., & An, Y. J. (2019). Effects of micro-sized polyethylene spheres on the marine microalga Dunaliella salina: Focusing on the algal cell to plastic particle size ratio. Aquatic Toxicology, 216, 105296. https://doi.org/10.1016/J.AQUATOX.2019.105296
15. Chen, H. L., Selvam, S. B., Ting, K. N., & Gibbins, C. N. (2023). Microplastic concentrations in river water and bed sediments in a tropical river: implications for water quality monitoring. Environmental Monitoring and Assessment, 195(2), 1–10. https://doi.org/10.1007/S10661-022-10856-5/FIGURES/4
16. Conard, W. M., O'Reilly, K. E., Hartlage, C., & Lamberti, G. A. (2023). Widespread microplastic pollution in Indiana, USA, rivers. River Research and Applications, 39(10), 2092–2101. https://doi.org/10.1002/RRA.4204
17. Constant, M., Ludwig, W., Kerhervé, P., Sola, J., Charrière, B., Sanchez-Vidal, A., Canals, M., & Heussner, S. (2020). Microplastic fluxes in a large and a small Mediterranean river

catchments: The Têt and the Rhône, Northwestern Mediterranean Sea. Science of The Total Environment, 716, 136984. https://doi.org/10.1016/J.SCITOTENV.2020.136984

18. Dalla Fontana, G., Mossotti, R., & Montarsolo, A. (2020). Assessment of microplastics release from polyester fabrics: The impact of different washing conditions. Environmental Pollution, 264, 113960. https://doi.org/10.1016/J.ENVPOL.2020.113960
19. Di Mauro, R., Castillo, S., Pérez, A., Iachetti, C. M., Silva, L., Tomba, J. P., & Chiesa, I. L. (2022). Anthropogenic microfibers are highly abundant at the Burdwood Bank seamount, a protected sub-Antarctic environment in the Southwestern Atlantic Ocean. Environmental Pollution, 306, 119364. https://doi.org/10.1016/J.ENVPOL.2022.119364
20. Eaton, A., Clesceri, L., Rice, E., & Greenberg, A. (2005a). Standard Methods for the Examination of Water and Wastewater (M. Franson, Ed.; 21st Edition). American Public Health Association.
21. Eaton, A., Clesceri, L., Rice, E., & Greenberg, A. (2005b). Standard Methods for the Examination of Water and Wastewater (M. Franson, Ed.; 21st Edition). American Public Health Association.
22. Elizalde-Velázquez, A., Carcano, A. M., Crago, J., Green, M. J., Shah, S. A., & Cañas-Carrell, J. E. (2020). Translocation, trophic transfer, accumulation and depuration of polystyrene microplastics in Daphnia magna and Pimephales promelas. Environmental Pollution, 259, 113937. https://doi.org/10.1016/J.ENVPOL.2020.113937
23. Eo, S., Hong, S. H., Song, Y. K., Han, G. M., & Shim, W. J. (2019). Spatiotemporal distribution and annual load of microplastics in the Nakdong River, South Korea. Water Research, 160, 228–237. https://doi.org/10.1016/j.watres.2019.05.053

24. Faure, F., Demars, C., Wieser, O., Kunz, M., & de Alencastro, L. F. (2015). Plastic pollution in Swiss surface waters: nature and concentrations, interaction with pollutants. Environmental Chemistry, 12(5), 582. https://doi.org/10.1071/EN14218

25. Geyer, R., Jambeck, J. R., & Law, K. L. (2017). Production, use, and fate of all plastics ever made. Science Advances, 3(7). https://doi.org/10.1126/SCIADV.1700782/SUPPL_FILE/1700782_SM.PDF

26. Gkatzioura, A., Zafirakou, A., & Zorpas, A. A. (2021). Wastewater treatment plants' contribution in microplastics' contamination of surface water. Effluent concentration and detection techniques review. Desalination and Water Treatment, 227, 26–33. https://doi.org/10.5004/DWT.2021.27287

27. Gündoğdu, S., Çevik, C., & Temiz Ataş, N. (2020). Occurrence of microplastics in the gastrointestinal tracts of some edible fish speciesalong the Turkish coast. Turkish Journal of Zoology, 44(4), 312–323. https://doi.org/10.3906/zoo-2003-49

28. Han, M., Niu, X., Tang, M., Zhang, B. T., Wang, G., Yue, W., Kong, X., & Zhu, J. (2020). Distribution of microplastics in surface water of the lower Yellow River near estuary. Science of The Total Environment, 707, 135601. https://doi.org/10.1016/J.SCITOTENV.2019.135601

29. Hildebrandt, L., Zimmermann, T., Primpke, S., Fischer, D., Gerdts, G., & Pröfrock, D. (2021). Comparison and uncertainty evaluation of two centrifugal separators for microplastic sampling. Journal of Hazardous Materials, 414, 125482. https://doi.org/10.1016/J.JHAZMAT.2021.125482

30. Hoang, T. C., & Felix-Kim, M. (2020). Microplastic consumption and excretion by fathead minnows (Pimephales promelas): Influence of particles size and body shape of fish. Science of the Total Environment, 704, 135433. https://doi.org/10.1016/j.scitotenv.2019.135433

31. Hoang, T. C., & Rand, G. M. (2015). Mosquito control insecticides: A probabilistic ecological risk assessment on drift exposures of naled, dichlorvos (naled metabolite) and permethrin to adult butterflies. Science of The Total Environment, 502, 252–265. https://doi.org/10.1016/J.SCITOTENV.2014.09.027
32. Hoang, T., & Mitten, S. (2022). Microplastic accumulation in the gastrointestinal tracts of nestling and adult migratory birds. Science of The Total Environment, 838, 155827. https://doi.org/10.1016/J.SCITOTENV.2022.155827
33. Horton, A. A., Jürgens, M. D., Lahive, E., van Bodegom, P. M., & Vijver, M. G. (2018). The influence of exposure and physiology on microplastic ingestion by the freshwater fish Rutilus rutilus (roach) in the River Thames, UK. Environmental Pollution, 236, 188–194. https://doi.org/10.1016/j.envpol.2018.01.044
34. Illuminati, S., Notarstefano, V., Tinari, C., Fanelli, M., Girolametti, F., Ajdini, B., Scarchilli, C., Ciardini, V., Iaccarino, A., Giorgini, E., Annibaldi, A., & Truzzi, C. (2024). Microplastics in bulk atmospheric deposition along the coastal region of Victoria Land, Antarctica. Science of The Total Environment, 949, 175221. https://doi.org/10.1016/J.SCITOTENV.2024.175221
35. Jang, M., Shim, W. J., Cho, Y., Han, G. M., Song, Y. K., & Hong, S. H. (2020). A close relationship between microplastic contamination and coastal area use pattern. Water Research, 171, 115400. https://doi.org/10.1016/J.WATRES.2019.115400
36. Kabir, A. H. M. E., Sekine, M., Imai, T., Yamamoto, K., Kanno, A., & Higuchi, T. (2021). Assessing small-scale freshwater microplastics pollution, land-use, source-to-sink conduits, and pollution risks: Perspectives from Japanese rivers polluted with microplastics. Science of The Total Environment, 768, 144655. https://doi.org/10.1016/j.scitotenv.2020.144655

37. Kapp, K. J., & Yeatman, E. (2018). Microplastic hotspots in the Snake and Lower Columbia rivers: A journey from the Greater Yellowstone Ecosystem to the Pacific Ocean. Environmental Pollution, 241, 1082–1090. https://doi.org/10.1016/J.ENVPOL.2018.06.033

38. Kasznik, D., & Łapniewska, Z. (2023). The end of plastic? The EU's directive on single-use plastics and its implementation in Poland. *Environmental Science & Policy*, *145*, 151–163. https://doi.org/10.1016/J.ENVSCI.2023.04.005

39. Kay, P., Hiscoe, R., Moberley, I., Bajic, L., & McKenna, N. (2018). Wastewater treatment plants as a source of microplastics in river catchments. Environmental Science and Pollution Research, 25(20), 20264–20267. https://doi.org/10.1007/S11356-018-2070-7/FIGURES/2

40. Klein, S., Worch, E., & Knepper, T. P. (2015). Occurrence and spatial distribution of microplastics in river shore sediments of the rhine-main area in Germany. Environmental Science and Technology, 49(10), 6070–6076. https://doi.org/10.1021/acs.est.5b00492

41. Koraltan, İ., Mavruk, S., & Güven, O. (2022). Effect of biological and environmental factors on microplastic ingestion of commercial fish species. Chemosphere, 303, 135101. https://doi.org/10.1016/J.CHEMOSPHERE.2022.135101

42. Kurki-Fox, J. J., Doll, B. A., Monteleone, B., West, K., Putnam, G., Kelleher, L., Krause, S., & Schneidewind, U. (2023). Microplastic distribution and characteristics across a large river basin: Insights from the Neuse River in North Carolina, USA. Science of The Total Environment, 878, 162940. https://doi.org/10.1016/J.SCITOTENV.2023.162940

43. Lahens, L., Strady, E., Kieu-Le, T.-C., Dris, R., Boukerma, K., Rinnert, E., Gasperi, J., & Tassin, B. (2018). Macroplastic and microplastic contamination assessment of a tropical river (Saigon River, Vietnam) transversed by a developing megacity. Environmental Pollution, 236, 661–671. https://doi.org/10.1016/j.envpol.2018.02.005

44. Lechner, A., Keckeis, H., Lumesberger-Loisl, F., Zens, B., Krusch, R., Tritthart, M., Glas, M., & Schludermann, E. (2014). The Danube so colourful: A potpourri of plastic litter outnumbers fish larvae in Europe's second largest river. Environmental Pollution, 188, 177–181. https://doi.org/10.1016/J.ENVPOL.2014.02.006

45. Lee, Y., Cho, J., Sohn, J., & Kim, C. (2023). Health Effects of Microplastic Exposures: Current Issues and Perspectives in South Korea. Yonsei Medical Journal, 64(5), 301. https://doi.org/10.3349/YMJ.2023.0048

46. Li, A., Boardwine, A. J., & Hoang, T. C. (2025). Metals in sediment of the lower Great Lakes and region-wide discoveries. Journal of Hazardous Materials, 487, 137099. https://doi.org/10.1016/J.JHAZMAT.2025.137099

47. Lin, L., Zuo, L. Z., Peng, J. P., Cai, L. Q., Fok, L., Yan, Y., Li, H. X., & Xu, X. R. (2018). Occurrence and distribution of microplastics in an urban river: A case study in the Pearl River along Guangzhou City, China. Science of The Total Environment, 644, 375–381. https://doi.org/10.1016/J.SCITOTENV.2018.06.327

48. Liu, J., Liu, Q., An, L., Wang, M., Yang, Q., Zhu, B., Ding, J., Ye, C., & Xu, Y. (2022). Microfiber Pollution in the Earth System. Reviews of Environmental Contamination and Toxicology, 260(1), 13. https://doi.org/10.1007/S44169-022-00015-9

49. López-Rojo, N., Pérez, J., Alonso, A., Correa-Araneda, F., & Boyero, L. (2020). Microplastics have lethal and sublethal effects on stream invertebrates and affect stream ecosystem functioning. Environmental Pollution, 259, 113898. https://doi.org/10.1016/J.ENVPOL.2019.113898

50. Malinich, T. D., Chou, N., Sepúlveda, M. S., & Höök, T. O. (2018). No evidence of microplastic impacts on consumption or growth of larval Pimephales promelas. Environmental Toxicology and Chemistry, 37(11), 2912–2918. https://doi.org/10.1002/ETC.4257
51. Mani, T., & Burkhardt-Holm, P. (2020). Seasonal microplastics variation in nival and pluvial stretches of the Rhine River – From the Swiss catchment towards the North Sea. Science of The Total Environment, 707, 135579. https://doi.org/10.1016/J.SCITOTENV.2019.135579
52. Mani, T., Hauk, A., Walter, U., & Burkhardt-Holm, P. (2015a). Microplastics profile along the Rhine River. Scientific Reports 2015 5:1, 5(1), 1–7. https://doi.org/10.1038/srep17988
53. Mani, T., Hauk, A., Walter, U., & Burkhardt-Holm, P. (2015b). Microplastics profile along the Rhine River. Scientific Reports 2015 5:1, 5(1), 1–7. https://doi.org/10.1038/srep17988
54. Masura J., Baker J., Foster G., Arthur C., & Herring C. (2015). Laboratory Methods for the Analysis of Microplastics in the Marine Environment .
55. Matjašič, T., Mori, N., Hostnik, I., Bajt, O., & Kovač Viršek, M. (2023). Microplastic pollution in small rivers along rural–urban gradients: Variations across catchments and between water column and sediments. Science of The Total Environment, 858, 160043. https://doi.org/10.1016/J.SCITOTENV.2022.160043
56. Miller, E., Sedlak, M., Lin, D., Box, C., Holleman, C., Rochman, C. M., & Sutton, R. (2021). Recommended best practices for collecting, analyzing, and reporting microplastics in environmental media: Lessons learned from comprehensive monitoring of San Francisco Bay. Journal of Hazardous Materials, 409, 124770. https://doi.org/10.1016/J.JHAZMAT.2020.124770
57. Mintenig, S. M., Int-Veen, I., Löder, M. G. J., Primpke, S., & Gerdts, G. (2017). Identification of microplastic in effluents of waste water treatment plants using focal plane array-based

micro-Fourier-transform infrared imaging. Water Research, 108, 365–372. https://doi.org/10.1016/J.WATRES.2016.11.015

58. Mohsen, A., Balla, A., & Kiss, T. (2023). High spatiotemporal resolution analysis on suspended sediment and microplastic transport of a lowland river. Science of The Total Environment, 902, 166188. https://doi.org/10.1016/J.SCITOTENV.2023.166188
59. Napper, I. E., Baroth, A., Barrett, A. C., Bhola, S., Chowdhury, G. W., Davies, B. F. R., Duncan, E. M., Kumar, S., Nelms, S. E., Hasan Niloy, M. N., Nishat, B., Maddalene, T., Thompson, R. C., & Koldewey, H. (2021). The abundance and characteristics of microplastics in surface water in the transboundary Ganges River. Environmental Pollution, 274, 116348. https://doi.org/10.1016/J.ENVPOL.2020.116348
60. Nel, H. A., Dalu, T., & Wasserman, R. J. (2018). Sinks and sources: Assessing microplastic abundance in river sediment and deposit feeders in an Austral temperate urban river system. Science of The Total Environment, 612, 950–956. https://doi.org/10.1016/J.SCITOTENV.2017.08.298
61. Nelms, S. E., Barnett, J., Brownlow, A., Davison, N. J., Deaville, R., Galloway, T. S., Lindeque, P. K., Santillo, D., & Godley, B. J. (2019). Microplastics in marine mammals stranded around the British coast: ubiquitous but transitory? Scientific Reports 2019 9:1, 9(1), 1–8. https://doi.org/10.1038/s41598-018-37428-3
62. Nihei, Y., Yoshida, T., Kataoka, T., & Ogata, R. (2020). High-Resolution Mapping of Japanese Microplastic and Macroplastic Emissions from the Land into the Sea. Water, 12(4), 951. https://doi.org/10.3390/w12040951
63. Pan, Z., Sun, Y., Liu, Q., Lin, C., Sun, X., He, Q., Zhou, K., & Lin, H. (2020). Riverine microplastic pollution matters: A case study in the Zhangjiang River of Southeastern China.

Marine Pollution Bulletin, 159, 111516. https://doi.org/10.1016/J.MARPOLBUL.2020.111516

64. Qian, Y., Shang, Y., Zheng, Y., Jia, Y., & Wang, F. (2023). Temporal and spatial variation of microplastics in Baotou section of Yellow River, China. Journal of Environmental Management, 338, 117803. https://doi.org/10.1016/J.JENVMAN.2023.117803
65. Radzi, A. M., Sapuan, S. M., Huzaifah, M. R. M., Noor Azammi, A. M., Ilyas, R. A., & Nadlene, R. (2021). A Review of the Mechanical Properties of Roselle Fiber-Reinforced Polymer Hybrid Composites. Roselle: Production, Processing, Products and Biocomposites, 259–269. https://doi.org/10.1016/B978-0-323-85213-5.00017-2
66. Rico, A., Redondo-Hasselerharm, P. E., Vighi, M., Waichman, A. V., Nunes, G. S. de S., de Oliveira, R., Singdahl-Larsen, C., Hurley, R., Nizzetto, L., & Schell, T. (2023). Large-scale monitoring and risk assessment of microplastics in the Amazon River. Water Research, 232, 119707. https://doi.org/10.1016/J.WATRES.2023.119707
67. Rochman, C. M., Cook, A. M., & Koelmans, A. A. (2016). Plastic debris and policy: Using current scientific understanding to invoke positive change. Environmental Toxicology and Chemistry, 35(7), 1617–1626. https://doi.org/10.1002/ETC.3408
68. Ruan, Y., Zhang, K., Wu, C., Wu, R., & Lam, P. K. S. (2019). A preliminary screening of HBCD enantiomers transported by microplastics in wastewater treatment plants. Science of The Total Environment, 674, 171–178. https://doi.org/10.1016/J.SCITOTENV.2019.04.007
69. Sadia, M., Mahmood, A., Ibrahim, M., Irshad, M. K., Quddusi, A. H. A., Bokhari, A., Mubashir, M., Chuah, L. F., & Show, P. L. (2022). Microplastics pollution from wastewater treatment plants: A critical review on challenges, detection, sustainable removal techniques

and circular economy. Environmental Technology & Innovation, 28, 102946. https://doi.org/10.1016/J.ETI.2022.102946

70. Sarkar, D. J., Das Sarkar, S., Das, B. K., Praharaj, J. K., Mahajan, D. K., Purokait, B., Mohanty, T. R., Mohanty, D., Gogoi, P., Kumar V, S., Behera, B. K., Manna, R. K., & Samanta, S. (2021). Microplastics removal efficiency of drinking water treatment plant with pulse clarifier. Journal of Hazardous Materials, 413, 125347. https://doi.org/10.1016/j.jhazmat.2021.125347
71. Sarkar, D. J., Das Sarkar, S., Das, B. K., Sahoo, B. K., Das, A., Nag, S. K., Manna, R. K., Behera, B. K., & Samanta, S. (2021). Occurrence, fate and removal of microplastics as heavy metal vector in natural wastewater treatment wetland system. Water Research, 192, 116853. https://doi.org/10.1016/j.watres.2021.116853
72. Scherer, C., Weber, A., Stock, F., Vurusic, S., Egerci, H., Kochleus, C., Arendt, N., Foeldi, C., Dierkes, G., Wagner, M., Brennholt, N., & Reifferscheid, G. (2020). Comparative assessment of microplastics in water and sediment of a large European river. Science of The Total Environment, 738, 139866. https://doi.org/10.1016/J.SCITOTENV.2020.139866
73. Schrank, I., Löder, M. G. J., Imhof, H. K., Moses, S. R., Heß, M., Schwaiger, J., & Laforsch, C. (2022a). Riverine microplastic contamination in southwest Germany: A large-scale survey. Frontiers in Earth Science, 10, 794250. https://doi.org/10.3389/FEART.2022.794250/BIBTEX
74. Schrank, I., Löder, M. G. J., Imhof, H. K., Moses, S. R., Heß, M., Schwaiger, J., & Laforsch, C. (2022b). Riverine microplastic contamination in southwest Germany: A large-scale survey. Frontiers in Earth Science, 10, 794250. https://doi.org/10.3389/FEART.2022.794250/BIBTEX
75. Sharma, S., & Chatterjee, S. (2017). Microplastic pollution, a threat to marine ecosystem and human health: a short review. Environmental Science and Pollution Research 2017 24:27, 24(27), 21530–21547. https://doi.org/10.1007/S11356-017-9910-8

76. Shruti, V. C., Pérez-Guevara, F., Elizalde-Martínez, I., & Kutralam-Muniasamy, G. (2021). Current trends and analytical methods for evaluation of microplastics in stormwater. Trends in Environmental Analytical Chemistry, 30, e00123. https://doi.org/10.1016/J.TEAC.2021.E00123

77. Song, Y. K., Hong, S. H., Jang, M., Kang, J. H., Kwon, O. Y., Han, G. M., & Shim, W. J. (2014). Large accumulation of micro-sized synthetic polymer particles in the sea surface microlayer. Environmental Science and Technology, 48(16), 9014–9021. https://doi.org/10.1021/ES501757S/SUPPL_FILE/ES501757S_SI_001.PDF

78. Talukdar, A., Kundu, P., Bhattacharya, S., & Dutta, N. (2024). Microplastic contamination in wastewater: Sources, distribution, detection and remediation through physical and chemical-biological methods. Science of The Total Environment, 916, 170254. https://doi.org/10.1016/J.SCITOTENV.2024.170254

79. Tan, Y., Dai, J., Wu, X., Wu, S., & Zhang, J. (2022). Characteristics, occurrence and fate of non-point source microplastic pollution in aquatic environments. Journal of Cleaner Production, 341, 130766. https://doi.org/10.1016/J.JCLEPRO.2022.130766

80. Tang, G., Liu, M., Zhou, Q., He, H., Chen, K., Zhang, H., Hu, J., Huang, Q., Luo, Y., Ke, H., Chen, B., Xu, X., & Cai, M. (2018). Microplastics and polycyclic aromatic hydrocarbons (PAHs) in Xiamen coastal areas: Implications for anthropogenic impacts. Science of The Total Environment, 634, 811–820. https://doi.org/10.1016/J.SCITOTENV.2018.03.336

81. Ugwu, K., Herrera, A., & Gómez, M. (2021). Microplastics in marine biota: A review. Marine Pollution Bulletin, 169, 112540. https://doi.org/10.1016/J.MARPOLBUL.2021.112540

82. Viaroli, S., Lancia, M., & Re, V. (2022). Microplastics contamination of groundwater: Current evidence and future perspectives. A review. Science of The Total Environment, 824, 153851. https://doi.org/10.1016/J.SCITOTENV.2022.153851

83. Wang, W., Ndungu, A. W., Li, Z., & Wang, J. (2017). Microplastics pollution in inland freshwaters of China: A case study in urban surface waters of Wuhan, China. Science of The Total Environment, 575, 1369–1374. https://doi.org/10.1016/j.scitotenv.2016.09.213

84. Weis, J. S., & Falco, F. De. (2022). Microfibers: Environmental Problems and Textile Solutions. Microplastics 2022, Vol. 1, Pages 626-639, 1(4), 626–639. https://doi.org/10.3390/MICROPLASTICS1040043

85. Wessel, C. C., Lockridge, G. R., Battiste, D., & Cebrian, J. (2016). Abundance and characteristics of microplastics in beach sediments: Insights into microplastic accumulation in northern Gulf of America estuaries. Marine Pollution Bulletin, 109(1), 178–183. https://doi.org/10.1016/J.MARPOLBUL.2016.06.002

86. Wiggin, K. J., & Holland, E. B. (2019). Validation and application of cost and time effective methods for the detection of 3–500 μm sized microplastics in the urban marine and estuarine environments surrounding Long Beach, California. Marine Pollution Bulletin, 143, 152–162. https://doi.org/10.1016/J.MARPOLBUL.2019.03.060

87. Wong, G., Löwemark, L., & Kunz, A. (2020). Microplastic pollution of the Tamsui River and its tributaries in northern Taiwan: Spatial heterogeneity and correlation with precipitation. Environmental Pollution, 260, 113935. https://doi.org/10.1016/J.ENVPOL.2020.113935

88. Xia, F., Yao, Q., Zhang, J., & Wang, D. (2021). Effects of seasonal variation and resuspension on microplastics in river sediments. Environmental Pollution, 286. https://doi.org/10.1016/j.envpol.2021.117403

89. Xia, W., Rao, Q., Liu, J., Chen, J., & Xie, P. (2024). Occurrence and characteristics of microplastics across the watershed of the world's third-largest river. Journal of Hazardous Materials, 480, 135998. https://doi.org/10.1016/J.JHAZMAT.2024.135998

90. Yin, L., Wen, X., Huang, D., Zhou, Z., Xiao, R., Du, L., Su, H., Wang, K., Tian, Q., Tang, Z., & Gao, L. (2022). Abundance, characteristics, and distribution of microplastics in the Xiangjiang river, China. Gondwana Research, 107, 123–133. https://doi.org/10.1016/J.GR.2022.01.019

91. Yonkos, L. T., Friedel, E. A., Perez-Reyes, A. C., Ghosal, S., & Arthur, C. D. (2014). Microplastics in four estuarine rivers in the chesapeake bay, U.S.A. Environmental Science and Technology, 48(24), 14195–14202. https://doi.org/10.1021/ES5036317/SUPPL_FILE/ES5036317_SI_001.PDF

92. Yu, X., Ladewig, S., Bao, S., Toline, C. A., Whitmire, S., & Chow, A. T. (2018). Occurrence and distribution of microplastics at selected coastal sites along the southeastern United States. Science of The Total Environment, 613–614, 298–305. https://doi.org/10.1016/J.SCITOTENV.2017.09.100

93. Yuan, W., Christie-Oleza, J. A., Xu, E. G., Li, J., Zhang, H., Wang, W., Lin, L., Zhang, W., & Yang, Y. (2022). Environmental fate of microplastics in the world's third-largest river: Basin-wide investigation and microplastic community analysis. Water Research, 210, 118002. https://doi.org/10.1016/J.WATRES.2021.118002

94. Yuan, Z., Nag, R., & Cummins, E. (2022). Human health concerns regarding microplastics in the aquatic environment - From marine to food systems. Science of The Total Environment, 823, 153730. https://doi.org/10.1016/J.SCITOTENV.2022.153730

95. Zhao, S., Zhu, L., Wang, T., & Li, D. (2014). Suspended microplastics in the surface water of the Yangtze Estuary System, China: First observations on occurrence, distribution. Marine Pollution Bulletin, 86(1–2), 562–568. https://doi.org/10.1016/J.MARPOLBUL.2014.06.032

96. Ziani, K., Ioniță-Mîndrican, C. B., Mititelu, M., Neacșu, S. M., Negrei, C., Moroșan, E., Drăgănescu, D., & Preda, O. T. (2023). Microplastics: A Real Global Threat for Environment and Food Safety: A State of the Art Review. Nutrients, 15(3), 617. https://doi.org/10.3390/NU15030617

Appendix A

Table A1. Water quality and chemistry of the Alabama River from summer 2023 to spring 2024.

Seasons	Sites	Water quality parameters							
		Hardness	Alkalinity	Salinity	Conductivity	Ca	Mg	K	Na
Summer (2023)	Wetumpka	57	68	0.1	157.18	15,491	5095	919	5458
	Montgomery	52	47	0.1	128.49	11,127	3774	913	4602
	Selma	52	52	0.1	165.04	13,046	3142	1027	8394
	Dixie Landing	44	47	0.1	183.60	12,729	2943	1057	9335
	Mobile	36	44	0.1	308.21	11,893	1565	1057	8973
Fall (2023)	Wetumpka	77	81	0.1	171.70	27,902	15,954	2770	100,895
	Montgomery	37	51	0.1	100.57	8823	3358	937	5070
	Selma	56	103	0.1	171.83	12,994	4391	1408	12,865
	Dixie Landing	55	101	0.1	184.07	13,997	3977	2110	17,579
	Mobile	NA	104	4.71	7911.11	149,501	170,785	4481	NA
Winter (2023)	Wetumpka	75	85	0.1	134.03	14,791	5283	1466	NA
	Montgomery	65	92	0.1	103.84	12,781	4483	1551	NA
	Selma	64	107	0.1	114.62	13,894	4314	1947	NA
	Dixie Landing	55	71	0.1	119.64	13,215	3623	2073	NA
	Mobile	NA	103	8.3	11,246.33	104,837	221,084	4228	NA
Spring (2024)	Wetumpka	44	59	0.1	91.51	NA	NA	NA	NA
	Montgomery	51	51	0.1	88.89	NA	NA	NA	NA
	Selma	63	67	0.1	117.73	NA	NA	NA	NA
	Dixie Landing	49	43	0.1	123.24	NA	NA	NA	NA
	Mobile	NA	77	0.1	115.03	NA	NA	NA	NA

NA: data are not available

Table A2: Estimated average water discharges of the Alabama River (billion L/day).

Seasons	Sites	Sampling day	Seasonal lowest	Seasonal highest	Seasonal average
Summer (2023)	Wetumpka	7.21	4.90	15.01	8.50
	Montgomery	32.71	10.66	80.43	29.99
	Selma	NA	NA	NA	NA
	Dixie Landing	41.34	18.12	153.84	48.42
	Mobile	69.58	11.51	128.43	54.98
Fall (2023)	Wetumpka	5.97	4.63	13.69	6.50
	Montgomery	22.53	8.78	46.42	17.14
	Selma	NA	NA	NA	NA
	Dixie Landing	21.20	17.04	60.74	24.04
	Mobile	12.93	7.13	53.14	18.79
Winter (2023)	Wetumpka	5.48	4.74	139.41	22.27
	Montgomery	43.34	9.11	278.30	70.59
	Selma	NA	NA	NA	NA
	Dixie Landing	38.62	15.82	261.83	97.12
	Mobile	35.41	8.63	176.52	87.41
Spring (2024)	Wetumpka	13.17	6.08	161.51	21.34
	Montgomery	39.84	16.86	126.43	48.70
	Selma	NA	NA	NA	NA
	Dixie Landing	50.99	22.80	262.53	87.91
	Mobile	114.45	38.23	170.97	101.75

Data were from the USGS online database at https://waterdata.usgs.gov/nwis NA: data are not available

Table A3. Microplastic count, concentration, population, water flow, watershed area of the Alabama River watershed.

Seasons	Locations	MP Count	MPs /L	Population	Flow (billion L/day)	Watershed Area (km^2)	MPs/ capita
Summer (2023)	Wetumpka	310.22	43	7218	7214,440,108	26,159	0.04
	Montgomery	1308.45	40	193,702	32,711,292,083	38,988	0.01
	Dixie Landing	1281.59	31	199	41,341,555,208	361	6.44
	Mobile	9323.57	134	181,253	69,578,915,625	113,040	0.05
Fall (2023)	Wetumpka	250.86	42	7218	5,972,904,546	26,159	0.03
	Montgomery	1126.46	50	193,702	22,529,187,440	38,988	0.01
	Dixie Landing	529.92	25	199	21,196,627,708	361	2.66
	Mobile	853.10	66	181,253	12,925,813,590	113,040	0.005
Winter (2023)	Wetumpka	262.92	48	7218	5,477,400,610	26,159	0.04
	Montgomery	1603.67	37	193,702	43,342,487,500	38,988	0.01
	Dixie Landing	424.81	11	199	38,619,267,708	361	2.13
	Mobile	1628.71	46	181,253	35,406,815,521	113,040	0.01
Spring (2024)	Wetumpka	606.00	46	7218	13,174,002,637	26,159	0.08
	Montgomery	2270.73	57	193,702	39,837,414,896	38,988	0.01
	Dixie Landing	611.87	12	199	50,989,362,500	361	3.07
	Mobile	8584.00	75	181,253	114,453,327,083	113,040	0.05

Table A4. Size and concentrations of MPs in the Alabama River water from summer 2023 to spring 2024.

Seasons	Location	Layer	Fibers (Length, mm)		Non-fibers (Size, mm)			Non-fibers (Area, mm^2)	All MPs by layer	Fibers by layer	Non-Fibers by layer	All MPs by site	Fibers by site	Non-Fibers by site
			Range	Avg. ± Sdev	Range	Length	Width	Avg. ± Sdev	MPs/L ± Sdev	MPst/L ± Sdev	MPs/L ± Sdev	Avg MPs/L ± Sdev	Avg. MPs/L ± Sdev	Avg. MPs/L ± Sdev
Summer (2023)	Background*		0.32 – 2.78	1.29 ± 0.69	ND	ND	ND	N/A	N/A	N/A	N/A	3.75 ± 4.50	N/A	N/A
	Wetumpka	Top	0.45 – 3.00	1.30 ± 0.70	0.040 – 0.040	0.39 – 0.43	0.09 – 0.10	0.040 ± 0.000	27±3.46	25±4.20	2±1.20	47 ± 2.10	34 ± 2.00	13 ± 4.00
		Middle	0.12 – 3.26	1.08 ± 0.70	0.005 – 0.120	0.07 – 0.7	0.04 – 0.38	0.013 ± 0.008	66±4.35	47±3.20	19±1.20			
		Bottom	0.29 – 3.09	1.46 ± 0.90	0.001 – 0.150	0.04 – 0.98	0.04 – 0.64	0.050 ± 0.050	47±2.10	30±1.70	17±3.80			
	Montgomery	Top	0.20 – 3.10	1.48 ± 0.82	0.005 – 0.050	0.07 – 0.59	0.08 – 0.40	0.157 ± 0.500	50±11.70	34±6.50	16±5.90	47 ± 16.00	30 ± 9.30	17 ± 9.20
		Middle	0.27 – 3.17	0.87 ± 0.70	0.004 – 0.060	0.07 – 0.79	0.06 – 0.48	0.029 ± 0.020	43±5.70	30±4.00	13±4.00			
		Bottom	0.38 – 4.50	1.60 ± 1.10	0.005 – 0.100	0.07 – 1.00	0.07 – 0.64	0.039 ± 0.040	47±7.40	26±4.50	21±3.60			
	Selma	Top	0.16 – 4.38	1.17 ± 0.90	0.003 – 0.100	0.04 – 0.53	0.05 – 0.49	0.027 ± 0.030	152±4.50	83±4.70	69±1.70	126 ± 40.20	67 ± 16.40	59 ± 24.30
		Middle	0.1 0 – 4.30	1.20 ± 1.00	0.002 – 0.200	0.04 – 1.16	0.05 – 0.34	0.021 ± 0.030	145±25.40	75±11.50	70±14.60			
		Bottom	0.30 – 3.20	1.30 ± 0.60	0.003 – 0.100	0.05 – 0.99	0.05 – 0.56	0.290 ± 0.030	81±17.10	43±9.60	38±8.10			
	Dixie Landing	Top	0.21 – 3.35	1.34 ± 0.81	0.001 – 0.140	0.04 – 0.26	0.10 – 0.29	0.035 ± 0.020	31 ± 14	15 ± 4.00	15 ± 12.00	31 ± 14.00	15 ± 4.00	16 ± 12.00
		Middle	NM	NM	NM	NM	NM	NM	NM	NM	NM			
		Bottom	NM	NM	NM	NM	NM	NM	NM	NM	NM			
	Mobile	Top	0.20 – 4.10	1.20 ± 0.98	0.002 – 0.500	0.04 – 1.33	0.06 – 0.02	0.040 ± 0.070	121±20.60	57±4.60	64±16.40	135 ± 42.00	52 ± 17.50	83 ± 25.70
		Middle	0.17 – 4.96	1.15 ± 1.10	0.002 – 0.800	0.02 – 1.10	0.04 – 0.40	0.060 ± 0.110	139±13.30	45±4.40	94±9.00			
		Bottom	0.28 – 4.60	1.16 ± 0.92	0.001 – 0.290	0.03 – 0.85	0.03 – 0.21	0.040 ± 0.050	144±14.70	55±9.70	89±6.50			
Fall (2023)	Background*		0.71 – 1.08	0.91 ± 0.15	ND	ND	ND	N/A	N/A	N/A	N/A	1.00 ± 1.41	N/A	N/A
	Wetumpka	Top	0.34 – 4.35	1.32 ± 1.09	0.005 – 0.205	0.10 – 0.57	0.06 – 0.25	0.054 ± 0.054	33±4.60	21±1.70	12±3.00	42 ± 6.00	29 ± 3.00	13 ± 5.00
		Middle	0.18 – 3.07	0.87 ± 0.60	0.004 – 0.180	0.07 – 0.45	0.1 0– 0.38	0.048 ± 0.040	57±4.40	39±3.00	18±2.00			
		Bottom	0.34 – 4.58	1.51 ± 1.20	0.014 – 0.074	0.10 – 0.39	0.14 – 0.30	0.044 ± 0.023	35±1.50	28±3.80	7±2.50			
	Montgomery	Top	0.16 – 4.38	1.53 ± 1.17	0.003 – 0.156	0.04 – 0.41	0.05 – 0.61	0.029 ± 0.030	63±4.40	35±2.10	28±6.40	47 ± 15.70	30 ± 9.20	17 ± 9.30

		Middle	0.29 – 5.00	1.20 ± 1.18	0.009 – 0.348	0.05 – 0.53	0.06 – 0.87	0.072 ± 0.075	50±5.10	28±4.90	22±6.70			
		Bottom	0.15 – 3.77	1.08 ± 0.92	0.005 – 0.025	0.07 – 0.68	0.07 – 0.68	0.059 ± 0.064	36±9.20	29±6.80	7±2.50			
	Selma	Top	0.10 – 4.97	1.25 ± 1.19	0.007 – 0.055	0.04 – 0.79	0.05 – 0.51	0.030 ± 0.031	61±7.50	26±1.50	35±6.00			
		Middle	0.28 – 5.00	1.30 ± 0.87	0.011 – 0.144	0.06 – 0.96	0.07 – 1.27	0.052 ± 0.035	74±8.50	53±9.10	21±3.50	65 ± 14.00	42 ± 9.00	23 ± 12.00
		Bottom	0.24 – 2.58	1.09 ± 0.70	0.038 – 0.218	0.06 – 1.04	0.06 – 2.74	0.079 ± 0.056	59±3.50	47±0.60	12±3.00			
	Dixie Landing	Top	0.20 – 4.10	1.31 ± 0.93	0.003 – 0.149	0.04 – 0.72	0.04 – 0.45	0.017 ± 0.031	25 ± 80.00	16 ± 5.00	9 ± 4.00			
		Middle	NM	NM	NM	NM	NM	NM	NM	NM	NM	25 ± 8.00	16 ± 5.00	9 ± 4.00
		Bottom	NM	NM	NM	NM	NM	NM	NM	NM	NM			
	Mobile	Top	0.19 – 4.87	1.48 ± 1.05	0.005 – 0.205	0.11 – 0.25	0.19 – 0.37	0.054 ± 0.054	80±14.00	75±16.10	5±2.10			
		Middle	0.42 – 3.27	1.20 ± 0.84	0.005 – 0.233	0.08 – 0.78	0.06 – 0.72	0.048 ± 0.053	43±5.00	26±3.80	17±6.40	66 ± 22.00	49 ± 17.00	17 ± 14.00
		Bottom	0.27 – 4.73	1.26 ± 1.30	0.007 – 0.403	0.07 – 0.96	0.06 – 0.61	0.071 ± 0.071	75±7.80	47±2.10	28±8.70			
	Background		ND	ND	ND	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	Wetumpka	Top	0.12 – 4.62	1.19 ± 1.04	0.006 – 0.075	0.06 – 0.33	0.10– 0.50	0.039 ± 0.020	66±6.30	47±4.20	19±3.2			
		Middle	0.08 – 3.86	1.32 ± 0.94	0.001 – 0.104	0.04 – 0.45	0.05 – 0.70	0.046 ± 0.029	50±1.20	30±1.70	20±1.5	48 ± 8.00	34 ± 6.00	14 ± 4.00
		Bottom	0.21 – 3.16	0.93 ± 0.90	0.005 – 0.098	0.09 – 0.21	0.05 – 0.75	0.044 ± 0.033	27±1.70	23±2.10	4±0.6			
	Montgomery	Top	0.28 – 3.06	1.08 ± 0.85	0.005 – 0.09	0.03 – 0.33	0.07 – 0.30	0.025 ± 0.028	29±1.50	20±0.60	9±1.00			
		Middle	0.21 – 4.51	1.24 ± 1.00	0.007 – 0.166	0.09 – 0.32	0.06 – 0.78	0.051 ± 0.052	58±1.20	48±1.70	10±0.60	37 ± 5.00	30 ± 5.00	7 ± 1
		Bottom	0.27 – 3.18	1.29 ± 1.07	0.012 – 0.037	0.05 – 0.25	0.11 – 0.28	0.024 ± 0.010	25±4.90	23±4.60	2±0.60			
Winter (2023)	Selma	Top	0.19 – 3.68	1.03 ± 0.83	0.026 – 0.168	0.16 – 0.43	0.16 – 0.39	0.081 ± 0.076	34±5.00	31±5.00	3±0.00			
		Middle	0.19 – 4.5	1.07 ± 1.02	0.004 – 0.352	0.05 – 0.28	0.06 – 0.20	0.078 ± 0.077	47±3.80	21±2.70	26±1.50	42 ± 10.00	28 ± 12.00	14 ± 3
		Bottom	0.24 – 3.12	1.15 ± 0.76	0.012 – 0.281	0.09 – 0.85	0.09 – 0.94	0.073 ± 0.068	45±5.30	31±8.70	14±3.50			
	Dixie Landing	Top	0.25 – 3.02	1.26 ± 0.91	0.010 – 0.230	0.08 – 0.59	0.03 – 0.71	0.068 ± 0.058	13 ± 4.00	7 ± 3.00	6 ± 3.00			
		Middle	NM	NM	NM	NM	NM	NM	NM	NM	NM	13 ± 4.00	7 ± 3.00	6 ± 3.00
		Bottom	NM	NM	NM	NM	NM	NM	NM	NM	NM			
	Mobile	Top	0.31 – 4.01	1.36 ± 0.96	0.010 – 0.274	0.09 – 0.36	0.10 – 0.10	0.048 ± 0.066	46±2.10	26±1.20	20±1.50			
		Middle	0.36 – 4.30	1.36 ± 0.90	0.005 - 0.155	0.06 – 0.33	0.1 0– 0.50	0.031 ± 0.036	47±8.30	44±9.10	3±1.00	46 ± 10.00	36 ± 11.00	10 ± 3.00
		Bottom	0.48 – 3.96	1.27 ± 0.74	0.010 - 0.100	0.04 – 0.45	0.05 – 0.70	0.045 ± 0.034	45±4.40	37±2.10	8±2.50			

	Background		ND	ND	ND	ND	ND	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Spring (2024)	Wetumpka	Top	0.21 – 4.50	1.30 ± 1.19	0.007 – 0.084	0.05 – 0.31	0.10 – 0.47	0.038 ± 0.029	42±6.60	31±8.10	11±2.50	46 ± 14.00	39 ± 15.00	7 ± 2.00
		Middle	0.40 – 3.54	1.48 ± 0.78	0.004 – 0.080	0.05 – 0.32	0.07 – 0.25	0.030 ± 0.027	33±1.00	27±0.00	6±1.00			
		Bottom	0.18 – 4.91	1.11 ± 0.85	0.009 – 0.040	0.06 – 0.41	0.05 – 0.45	0.024 ± 0.017	62±8.50	59±9.50	3±1.00			
	Montgomery	Top	0.16 – 2.21	0.95 ± 0.50	0.004 – 0.070	0.04 – 0.47	0.04 – 0.57	0.020 ± 0.016	66±7.00	39±4.00	27±3.60	57 ± 9.00	41 ± 3.00	16 ± 7.00
		Middle	0.11 – 3.91	1.17 ± 0.76	0.001 – 0.090	0.03 – 0.37	0.03 – 0.67	0.013 ± 0.008	65±5.90	51±6.60	14±3.50			
		Bottom	0.34 – 2.26	0.99 ± 0.69	0.005 – 0.040	0.03 – 0.33	0.04 – 0.23	0.016 ± 0.012	40±2.90	32±3.20	8±0.60			
	Selma	Top	0.25 – 3.47	1.10 ± 0.70	0.002 – 0.090	0.03 – 0.32	0.03 – 0.45	0.023 ± 0.014	78±2.70	43±6.70	35±5.50	70 ± 17.00	47 ± 17.00	23 ± 4.00
		Middle	0.14 – 2.73	1.10 ± 0.43	0.002 – 0.140	0.02 – 0.66	0.04 – 0.82	0.023 ± 0.030	72±5.20	48±5.30	24±1.00			
		Bottom	0.12 – 3.33	0.87 ± 0.58	0.008 – 0.056	0.06 – 0.46	0.04 – 0.36	0.025 ± 0.016	61±9.00	50±7.50	11±1.50			
	Dixie Landing	Top	0.22 – 3.30	1.17 ± 0.74	0.030 – 0.030	0.09	0.32	0.030	12 ± 1.00	11 ± 1.00	1 ± 1.00	12 ± 1.00	11 ± 1.00	1 ± 1.00
		Middle	NM	NM	NM	NM	NM	NM	NM	NM	NM			
		Bottom	NM	NM	NM	NM	NM	NM	NM	NM	NM			
	Mobile	Top	0.20 – 3.75	1.50 ± 1.00	0.002 – 0.100	0.03 – 0.32	0.04 – 0.02	0.027 ± 0.030	94±9.20	54±11.80	40±4.10	75 ± 18.00	52 ± 23.00	23 ± 7.00
		Middle	0.26 – 3.80	1.43 ± 1.00	0.002 – 0.040	0.06 – 0.22	0.03 – 0.21	0.010 ± 0.008	67±2.10	46±6.50	21±4.60			
		Bottom	0.10 – 2.95	1.00 ± 0.70	0.006 – 0.060	0.1 0– 0.34	0.05 – 0.39	0.023 ± 0.019	64±9.00	55±8.60	9±2.70			

ND: not detected, N/A: not applicable, NM: not measured, *Background microplastic concentrations are in MPs/filter

Table A5. Percentage of polymer types for microplastics in the Alabama River water from summer 2023 to spring 2024.

Poly.	Summer (2023)					Fall (2023)					Winter (2023)					Spring (2024)					Avg ± Std.	Range
	Wet.	Mont.	Sel.	Dix.	Mob.	Wet.	Mont.	Sel.	Dix.	Mob.	Wet.	Mont.	Sel.	Dix.	Mob.	Wet.	Mont.	Sel.	Dix.	Mob.		
ABS	1.4	10	8.1	8.4	3.7	1.4	10	8.1	7.6	3.7	7.5	14.4	9.5	2.2	2.9	2.2	6.9	4.7	11.1	10.4	6.7 ± 3.7	1.4 – 14.4
PET	44.7	16.1	23.7	12.8	38.5	44.7	16.1	23.7	44	38.5	13.9	11.8	8.6	15.8	5.7	15.3	18.9	15	13.9	12.2	21.7 ± 12.8	5.7 – 44.7
PBT	–	15.5	7.1	9.4	11.2	–	15.5	7.1	2.9	11.2	14.9	22.2	13.1	11.6	18.2	15.3	16	5.6	8.3	11.3	12.0 ± 4.8	2.9 – 22.2
Nylon	1	5.5	2.2	2.6	0.5	1	5.5	2.2	1	0.5	3.4	–	0.7	16.3	1.9	16.8	4	–	5.6	9.5	4.5 ± 5	0.5 – 16.8
PMMA	1	1.2	–	–	0.5	1	1.2	–	–	0.5	3.3	0.9	2.1	7.2	4.2	–	0.6	1.4	–	–	1.9 ± 1.9	0.5 – 7.2
PPS	–	–	–	0.9	–	–	–	–	–	–	–	–	–	4.2	–	–	–	–	–	–	2.6 ± 2.3	0.9 – 4.2
PP	9.8	12.4	14	2.4	7.6	9.8	12.4	14	2.9	7.6	9.6	14.9	8.7	21.9	12.3	18.2	19.4	22.5	33.3	22.6	13.8 ± 7.5	2.4 – 33.3
PVC	15.9	14.2	15.4	9	8.1	15.9	14.2	15.4	14.8	8.1	14.5	7	34.7	11.1	16.8	0.7	6.9	13.1	8.3	3.2	12.4 ± 7	0.7 – 34.7
PMP	15.7	13.2	16.7	25.6	10.7	15.7	13.2	16.7	9.5	10.7	10	18.1	15	9.7	26.7	13.9	19.4	25.4	16.7	19.5	16.1 ± 5.2	9.5 – 26.7
PS	3.7	1	3.6	5	5.6	3.7	1	3.6	2	5.6	6.6	2.7	0.7	–	–	4.4	3.4	3.3	–	6.3	3.7 ± 1.8	0.7 – 6.6
PC	–	1.6	–	2.6	0.4	–	1.6	–	–	0.4	–	0.9	0.7	–	–	–	–	–	–	–	1.2 ± 0.8	0.4 – 2.6
PE	–	2	2.9	15.3	4.6	–	2	2.9	13.5	4.6	5.7	–	2	–	2.5	9.5	1.7	7	2.8	5	5.3 ± 4.2	1.7 – 15.3
EVA	0.8	0.7	2.3	5.1	0.5	0.8	0.7	2.3	–	0.5	–	–	1.8	–	2.3	–	–	0.5	–	–	1.5 ± 1.4	0.5 – 5.1
Lanoline	–	0.5	1.1	–	–	–	0.5	1.1	–	–	–	–	1.1	–	–	–	–	0.5	–	–	0.8 ± 0.3	0.5 – 1.1
POM	5.3	3.5	0.9	–	2.1	5.3	3.5	0.9	–	2.1	3.7	4.3	1.4	–	6.5	1.5	2.9	0.9	–	–	3.0 ± 1.8	0.9 – 6.5
PU	–	–	–	–	5.2	–	–	–	–	5.2	–	–	–	–	–	1.5	–	–	–	–	3.6 ± 2.2	0.9 – 6.2
PETG	–	–	–	–	–	–	–	–	–	–	0.6	–	–	–	–	–	–	–	–	–	0.6 ± 0	0.6 – 0.6
PCTFE	0.8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.8 ± 0	0.8 – 0.8
LLDPE	–	0.9		–	0.4	–	0.9		1	0.4	–	–	–	–	–	0.7	–	–	–	–	0.7 ± 0.3	0.4 – 1.0
FEP	–	1.7	2	–	0.5	–	1.7	2	1	0.5	–	–	–	–	–	–	–	–	–	–	1.3 ± 0.7	0.5 – 2.0

ABS: acrylonitrile butadiene styrene, PE: polyethylene, PET: polyethylene terephthalate, PBT: polybutylene terephthalate, PMMA: polymethyl methacrylate, PPS: polyphenylene sulfide, PP: polypropylene, PVC: polyvinyl chloride, PMP: polymethyl pentene, PS: polystyrene, PC: polycarbonate**,** EVA: ethylene vinyl acetate ,

POM: polyoxymethylene, PU: polyurethane, PETG: polyethylene terephthalate glycol, FEP: fluorinated ethylene propylene, PCTFE: polychlorotrifluoroethylene, and LLDPE: linear low density polyethylene.

Table A6: Results of recovery of QC samples.

Material	Replicates	Before digestion (#MPs/filter)	Size range (mm or mm^2)	After digestion (#MPs/filter)	Size range (mm or mm^2)	Recovery (%)
Cups	1	13	0.0001-0.007	21	0.0001-0.009	
	2	11	0.0001-0.01	8	0.0003-0.006	
	3	26	0.0002-0.012	12	0.0001-0.008	
	4	13	0.0004-0.005	12	0.0001-0.005	
	5	23	0.0002-0.007	25	0.0001-0.01	
	6	35	0.0001-0.08	57	0.0001-0.06	
Average		20		23		112
Standard deviation		9		18		
Nylon	1	81	0.06-0.98	66	0.05-1.05	
	2	46	0.06-0.93	52	0.02-1.5	
	3	53	0.08-1.56	30	0.03-1.4	
	4	78	0.05-1.53	110	0.07-4.37	
	5	55	0.08-1.43	103	0.09-1.92	
	6	138	0.05-1.33	32	0.09-1.73	
Average		75		66		87
Standard deviation		34		35		

Table A7: Microplastic concentrations in world rivers.

Rivers	Country	Sampling depth	Sampling method	Extraction method	Identification method	MPs/L	References
Alabama River	USA	0-3m from surface of water.	PVC pipe sampler	Sieved through 5mm and 38µm sieves. Fraction less than 38µm was filtered on the filter paper. Greater than 38µm fraction was oxidized at 60°C for 24hrs using H_2O_2 (30%). After digestion the remaining solution was diluted 3x and filtered on the filter paper.	Stereomicroscope/ FTIR microscope	12	
Alabama River	USA					13	
Alabama River	USA					25	
Alabama River	USA					31	
Alabama River	USA					37	
Alabama River	USA					42	
Alabama River	USA					42	
Alabama River	USA					46	
Alabama River	USA					46	
Long Beach Harbor	USA	Surface water samples.	20L grab samples collected with a portable peristaltic water pump.	Sieved through 500, 124, 63, and 20µm sieves; filtered onto a 3µm polycarbonate filter; 16hr oxidation through H_2O_2 at 50°C.	Light microscope (40×-100× total magnification; Olympus CX41) with Dyna Lite 150 (an external light source). Fluorescent microscope.	113.627	(Wiggin & Holland, 2019)
Los Angeles River	USA					641.292	
San Gabriel River	USA					63.359	
Yangtze River	China	0-50cm from surface of water.	Steel sampler.	Sieved through 48µm stainless steel sieve; 24hrs oxidation using KOH:NaClO (1:1) solution (30%); digested samples were filtered on a 0.45µm Whatman filter paper.	Stereomicroscope (SMZ25, Nikon, Japan)/ micro-Raman spectrometer (DXR2, ThermoFisher Scientific, USA)	0.020	(W. Yuan et al., 2022)
Yangtze River	China					2.580	
Yangtze River	China					1.270	
Rhine River	Germany	Surface water samples.	Manta net (rectangular opening of 60cm x 18cm, 300µm mesh)	Sieved through 5, 1, and 300µm sieves; stored at 5°C; organic matter was removed by treating first with 0.25% sodium dodecyl sulfate (stirred at 450 rotations/min; 10 min; incubated at 70°C overnight). Samples cooled to 40°C and incubated with 0.5% each of Biozym F (lipase) and SE (3 days; 37°C). 24hr oxidation through H_2O_2 at 37°C; density separation (23% NaCl/water; density 1.16 g cm^{-3}).	FTIR spectroscopy; software Resolutions pro (Molecular Spectroscopy Solutions FTIR, Version 4.0, Bio-Rad, Cambridge, MA, USA) was used.	0.005	(Mani et al., 2015a)

Rhine River	Germany		Manta Trawl (60x18cm) with a 0.3mm mesh, removable cod end (5Gyres, Los Angeles, CA, USA) and mechanical flowmeter at the aperture centre (model 438 110; HYDRO-BIOS, Kiel, Germany).	Wet sieved through 0.3, 1 and 5mm sieves (0.3–1 mm, 1–5 mm and >5 mm. The 1–5 mm and >5 mm fractions suspended in ~40% EtOH and stored in glass jars. The 0.3–1 mm fraction underwent castor oil separation	stereomicroscope with LED super-lighting (Olympus SZ61, Tokyo, Japan)/ attenuated total reflection Fourier transform infrared spectroscope (ATR-FTIR).	0.006	(Mani & Burkhardt-Holm, 2020)
Rhine River	Germany	15cm surface water.	Manta trawl (opening of 30 × 15cm and a net with a mesh size of 300µm)	Sieved through 500µm sieve; enzymatic-oxidative purification; wet peroxide oxidation (Fenton protocol), and density separation.	stereomicroscope (Leica M50, Leica Microsystems GmbH, Germany) with Olympus DP26, 5 Megapixel camera and CellSens Dimension (Olympus Corporation, Japan) software/ (ATR)-FTIR spectroscopy.	0.0059	(Schrank et al., 2022a)
Danube River	Germany	15cm surface water.	Manta trawl (opening of 30 × 15cm and a net with a mesh size of 300µm)	Sieved through 500µm sieve; enzymatic-oxidative purification; wet peroxide oxidation (Fenton protocol), and density separation.	stereomicroscope (Leica M50, Leica Microsystems GmbH, Germany) with Olympus DP26, 5 Megapixel camera and CellSens Dimension (Olympus Corporation, Japan) software/ (ATR)-FTIR spectroscopy.	0.0487	(Schrank et al., 2022b)
Amazon River	Brazil	Surface water samples.	Plankton net (55µm)	Material on filter was rinsed with reverse osmosis (RO) water and left to settle overnight. Overlying water was filtered onto a Whatman (pore size 0.7 µm) filter paper. The sedimented material was passed through a 53µm sieve. Material retained in the sieve was oxidized using H_2O_2 30% (v/v) at 40°C at 100rpm. Density separation was done using NaCl.	Nikon SMZ 745T stereomicroscope at 20–50x magnification/FTIR.	0.008	(Rico et al., 2023)
Amazon River	Brazil					0.039	
Tapajós River	Brazil					0.011	
Tapajós River	Brazil					0.016	
Negro River	Brazil					0.005	
Negro River	Brazil					0.152	
Tocantins River	Brazil					0.050	
Tocantins River	Brazil					0.126	
Yellow River	China	0–30 cm from surface	Stainless steel bucket	50 mL water centrifuged at 3000r/min for 5min. Density floatation using NaCl solution was used to extract microplastic from the residue.	Optical electron microscope (CX31, OLYMPUS, Philippines)/scanning electron microscope (Quanta 250, Fédération Equestre Internationale,	430.000	(Han et al., 2020)
Yellow River	China					211.670	

				Supernatant was sieved through 50, 100, 200 and 500µm sieves. 12hrs oxidation was done using H_2O_2 (30%) and filtered on 0.45µm glass cellulose fiber membrane filter.	USA). ATR-FTIR (IS50, Thermo Fisher Scientific, USA).		
Yellow River	China	0 – 50cm from the river surface. Surface sediment and soil samples (0 – 5 cm)	Stainless steel drums Stainless steel shovel	Water: Filtered on 10µm stainless-steel filters; 24hrs oxidation with 30% H_2O_2; density flotation using potassium formate solution and the flotation device. Soil and sediment: Air-dried; sieved through 5mm sieve, 24hrs oxidation with 30% H_2O_2; frozen dried in Freeze dryers (Alpha 1–2 LD plus, Christ company); density flotation using potassium formate solution and the flotation device.	Agilent Laser Infrared 8700LDIR (Agilent Technologies, Inc.) coupled with Clarity software.	436.670	(Qian et al., 2023)
Yellow River	China					649.170	
Yellow River	China					542.000	
Tisza River	Central Europe	20–30 cm from surface	Manta trawl net.	The water was sieved through 90 and 2000µm sieves and evaporated at 105°C, sediment was weighed and expressed as g/m^3. Samples with high sediment content were subjected to density separation ($ZnCl_2$). 48hrs oxidation by H_2O_2 (30%) was done. The samples were washed into glass Petri dishes and dried.	Light microscope (Ash Inspex II); 60x magnification/ATR-FTIR.	0.035	(Mohsen et al., 2023)
Oker River	Germany	Surface of water	Submersible pump and stainless-steel filters (300 µm).	Filtrate transferred to an Erlenmeyer feeding bottle; density separation using NaCl followed by 24hrs settling; oxidation using H_2O_2 (30%) and H_2SO_4 (97%). Samples were sieved through 1mm and 200µm copper- or stainless-steel sieve.	Stereomicroscopes (Leica MZ6 or MZ7.5) with up to 40× and 50× magnification. Macroscope with up to 80× magnification (MZM 1, ASKANIA Mikroskop Technik Rathenow), FTIR spectroscope; software: OPUS Version 7.2.	0.063	(Büngener et al., 2024)
Yangtze River	China	1m depth from	12 V DC Teflon pump	Samples stored in 50 mL glass	Dissecting microscope (80x).	10.200	(Zhao et al., 2014)
Yangtze River	China					0.500	

Yangtze River	China	surface of water.		Bottles; oxidized by H_2O_2 (30%); density floatation using $ZnCl_2$.		4.137	
Zhangjiang River	China	Surface of water.	10L steel bucket Manta net (mesh size 330µm) and stored in 1L glass jars.	Fractional filtration (mesh size 5 and 0.3mm); oxidation using H_2O_2 (30%) and $FeSO_4$ (0.05 M); density separation using saturated NaCl solution.	microscopic evaluation/Raman spectroscope.	0.050	(Pan et al., 2020)
Zhangjiang River	China					0.246	
Zhangjiang River	China					0.725	
Pearl River	China	-	-	Samples were filtered through a 20µm membrane filter; residues transferred to a 1000mL conical flask; H_2O_2 (30%) was added for oxidation in an incubator shaker at 65°C and 80rpm (24hrs); density separation using NaCl (24hrs); filtered through a 5µm membrane filter.	Stereo light microscope (OLYMPUS SZX10, Tokyo, Japan); images taken with a digital camera (OLYMPUS DP80, Tokyo, Japan); size analysis using Image J software (1.46r, National Institutes of Health, USA). µ-FTIR (Nicolet iN 10, Thermo Fisher, USA) coupled with MCT detector.	0.379	(Lin et al., 2018)
Pearl River	China					2.724	
Pearl River	China					7.924	
Semenyih River	Malaysia	Surface of water and riverbed.	Plastic cylinder (0.5m dia.; 1m tall).	Samples sieved through 53µm stainless steel sieve; 30min oxidation using H_2O_2 (30%) and iron (II) sulphate (0.05 M) at 60°C. Filtered on 0.7µm Whatman filter; oven dried (24h) at 60°C.	Stereo microscope (Nikon SMZ1500)/hot needle test.	3.120	(Chen et al., 2023)
Semenyih River	Malaysia					4.390	
Semenyih River	Malaysia					45.860	
Semenyih River	Malaysia					90.000	
Ciwalengke River	Indonesia	Water: 45 cm from the surface of water.	Sediments: Ekman grab sampler with shovels. Water: grab sample in 1 L glass container.	Sediments: Wet sediments dried at 100°C (48hrs); density separation using NaCl (30%) with 2min stirring. Floating material in the supernatant was filtered on Whatman filter (1.2µm) paper. Water samples: 500mL water filtered on a Whatman filter (1.2µm) paper.	Light binocular microscope (OPTIKA series B-383FL)/*Bruker Senterra Raman Spectrometer*.	5.850	(Alam et al., 2019)
Elbe River	Czech	Surface of water.	Apstein plankton net (opening: 0.022m^2, Ø 17cm, length: 110cm, mesh size: 150µm)	Water samples: oxidation using 5–15 mL of a 1:1 mixture of potassium hydroxide (10 M) solution and H_2O_2 (30%); samples neutralized with formic acid after 3-4 days. Density separation using potassium	Stereo microscope (Keyence VHX2000) with a digital camera (JVC, KY-F75U, imaging software: Discus, version 4.80.8238)/ ATR-FTIR (Perkin Elmer, Spectrum Two).	0.00557	(Scherer et al., 2020)
Elbe River	Czech					0.00088	
Elbe River	Czech					0.013	

				formate; supernatant filtered on anopore inorganic membrane filters(0.2µm).			
Tamsui River	Taiwan	Surface of the water.	Manta net with a mesh size of 0.3mm.	Sample rinsed with DI water and wet sieved by 5 and 0.3mm sieves. Samples with no organic matter dried at 50°C (12hrs). Samples with organic matter were oxidized with H_2O_2 (35%) overnight at 30°C.	ATR-FTIR spectroscope	0.010	(Wong et al., 2020)
Tamsui River	Taiwan					0.030	
Dahan River	Taiwan					0.002	
Dahan River	Taiwan					0.083	
Keelung River	Taiwan					0.003	
Keelung River	Taiwan					0.064	
Elbe River	Czech	-	Hydro cyclone and centrifuge systems.	Sieved through a 500 and 0.45µm membrane filters. Fractions < 500µm were subjected to oxidative-chemical matrix (i.e. natural organic and inorganic materials) digestion along with density separation.	Stereo microscope (SZ61; Olympus, Hamburg, Germany)/ ATR-FTIR spectroscope.	0.193	(Hildebrandt et al., 2021)
Elbe River	Czech					2.072	
Ganges River	China	0.5m below the surface of water.	Bilge pump connected to PP tubing across a nylon mesh (330µm)	---	Light microscope (S9E-Leica) / FTIR spectroscope.	0.038	(Napper et al., 2021)
Rhône River	France	Surface of the water.	Manta trawl net (10 cm opening radius; 300µm mesh)	Sieved through 10, 5, 1mm and 200µm metal sieves; oxidation was done by H_2O_2 (40%) at 50°C; filtered on a Whatman® filter papers (2µm).	Microscope IR-Plan Spectra Tech® coupled to an ABB® FTLA2000 FTIR spectrometer.	0.0003	(Constant et al., 2020)
Rhône River	France					0.058	
Rhône River	France					0.0116	
Neuse River	USA	40cm from surface of water.	Trawl net (335µm)	Sieved through 200µm sieve; 1hr digestion was done using ($FeSO_4$) and H_2O_2 (32%) at 60°C; filtered the digestate on 100µm nylon filter.	µFTIR (Thermo Fisher Scientific Nicolet iN10 MX) and an Attenuated Total Reflectance attachment (Thermo Fisher Scientific Nicolet iZ10).	0.00002	(Kurki-Fox et al., 2023)
Neuse River	USA					0.00044	
Neuse River	USA					0.221	

Figures

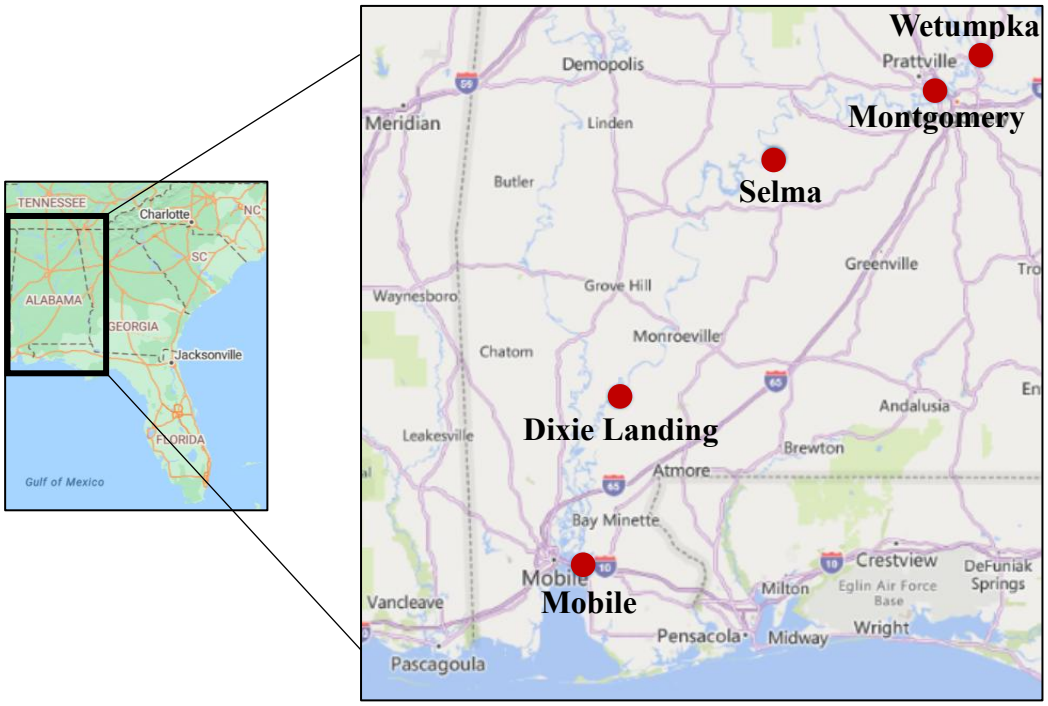

Figure A1: Map of sampling sites along the Alabama River.

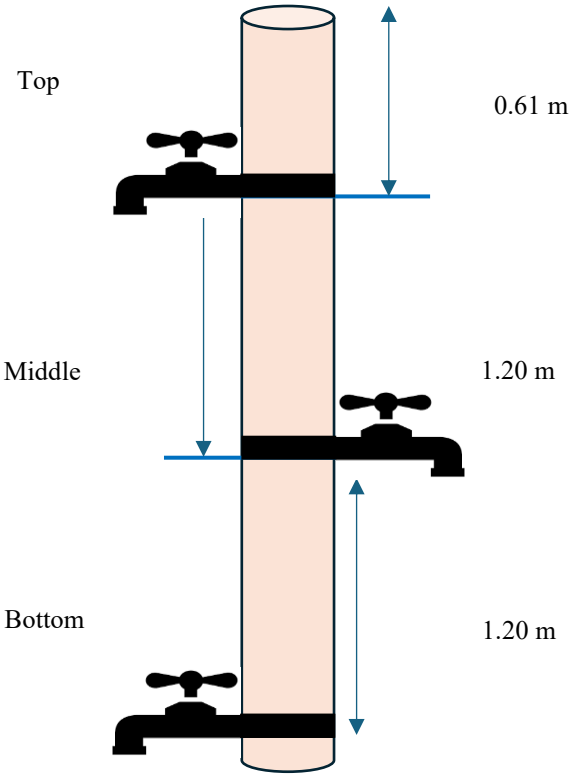

Figure A2: Sampler used for collecting water samples for microplastic analyses.

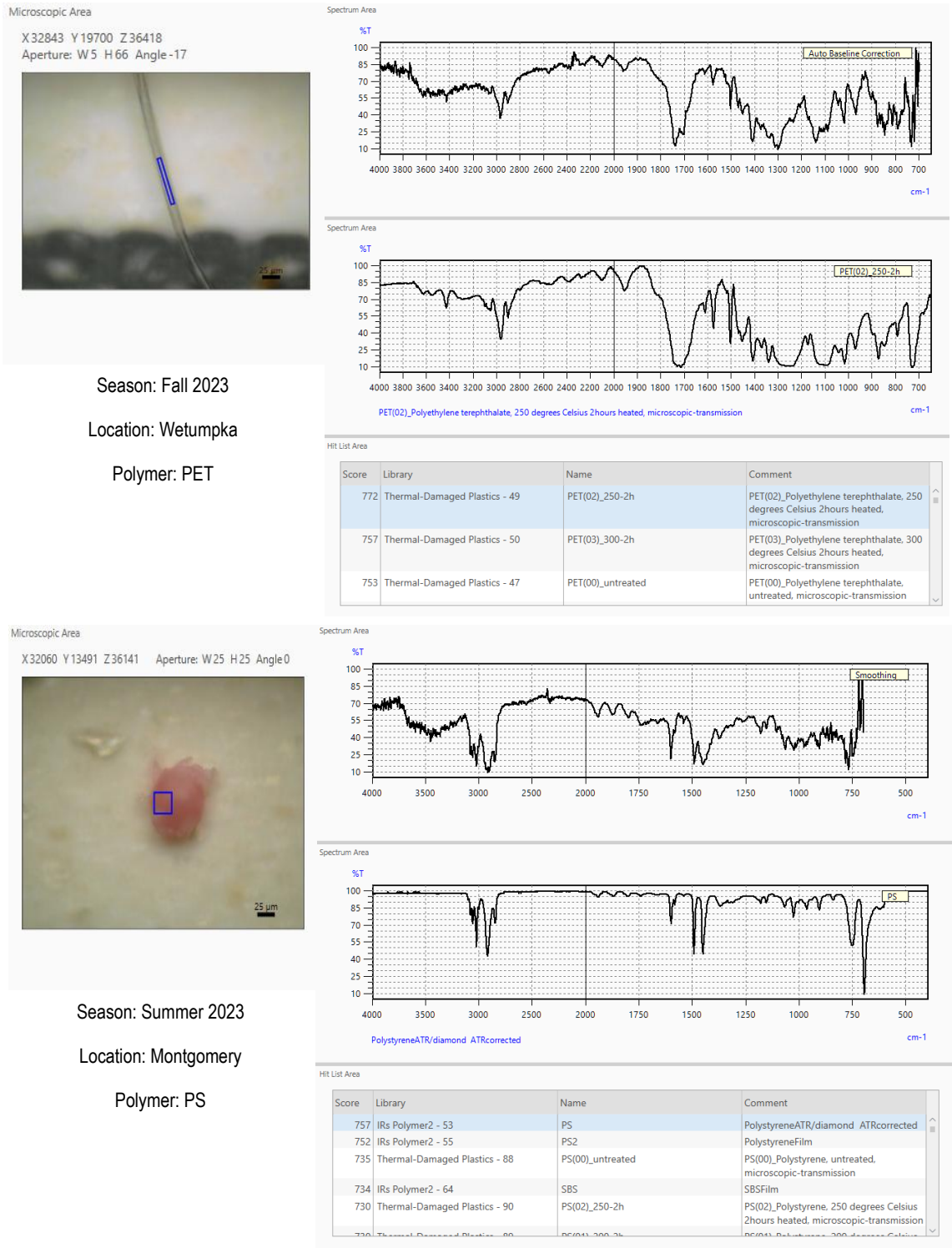

Figure A3: Spectrum of dominant polymer found in water samples.

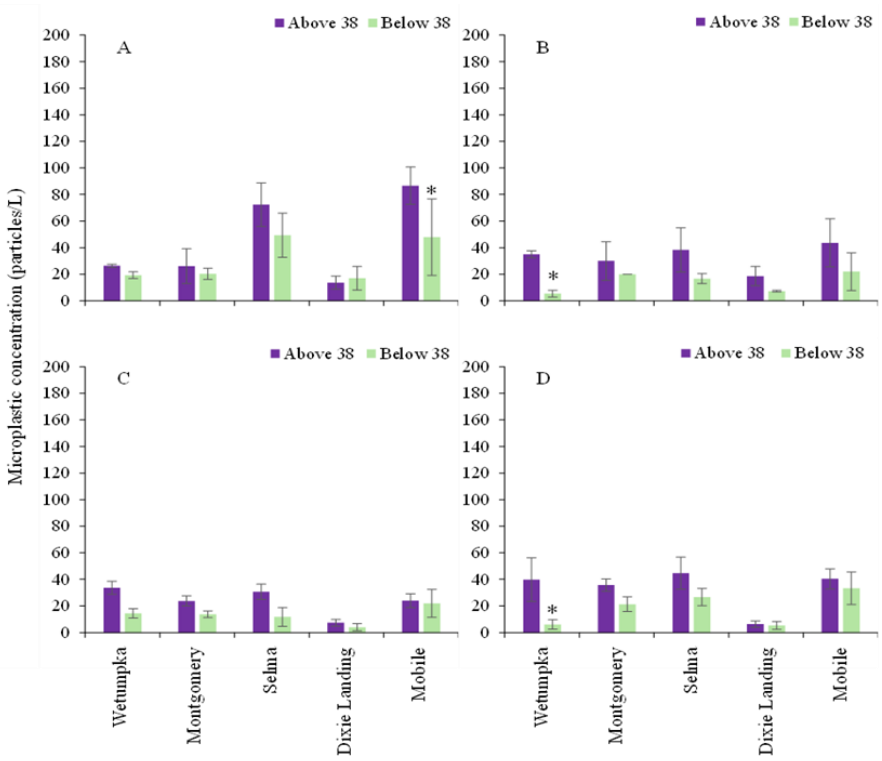

Figure A4: Microplastic concentrations in above and below 38 µm fractions (A: summer, B: fall, C: winter, D: spring. Error bars represent standard deviations. Asterisks are indications of statistically significant differences between two fractions).

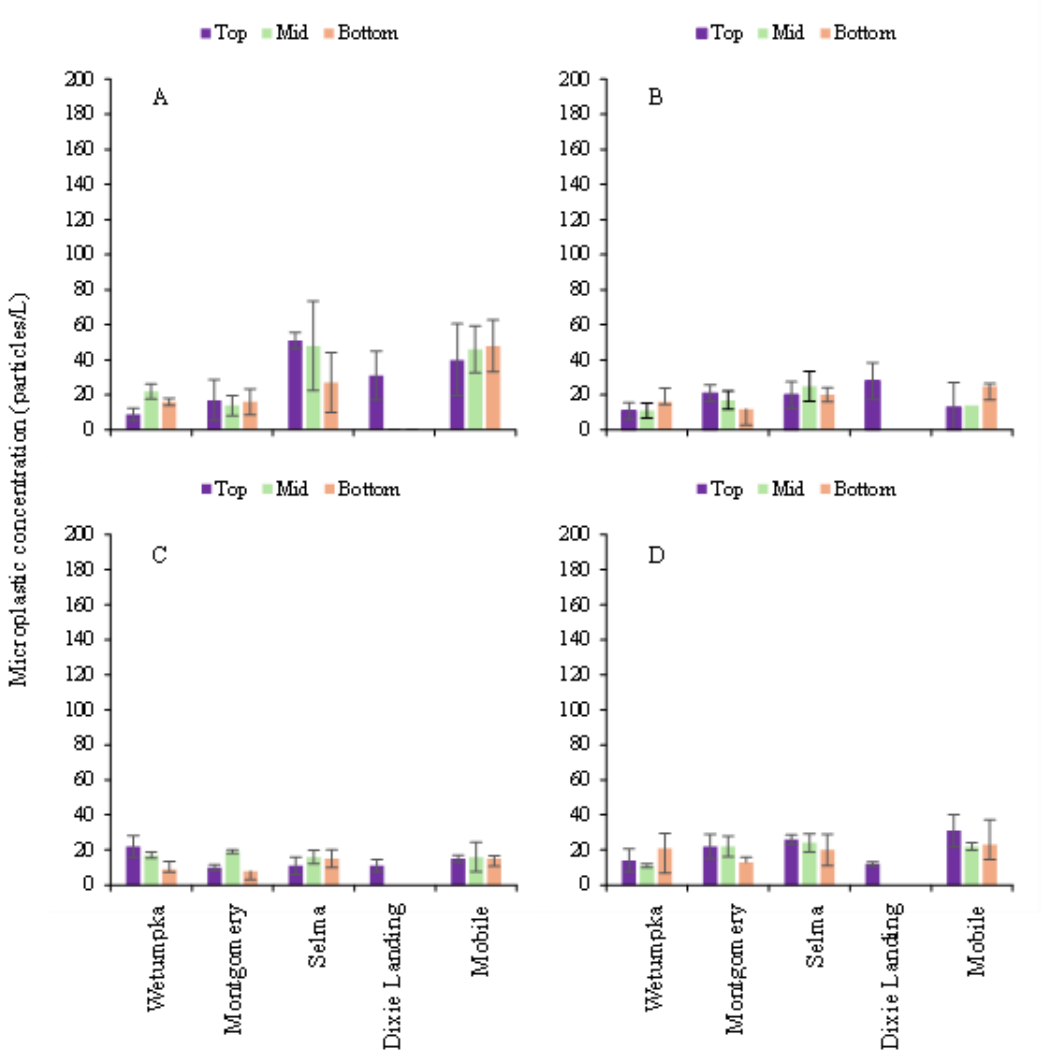

Figure A5: Microplastics in water layers of the Alabama River (A: summer, B: fall, C: winter, D: spring. Error bars represent standard deviations. There was no statistically significant difference in concentrations of water layers).

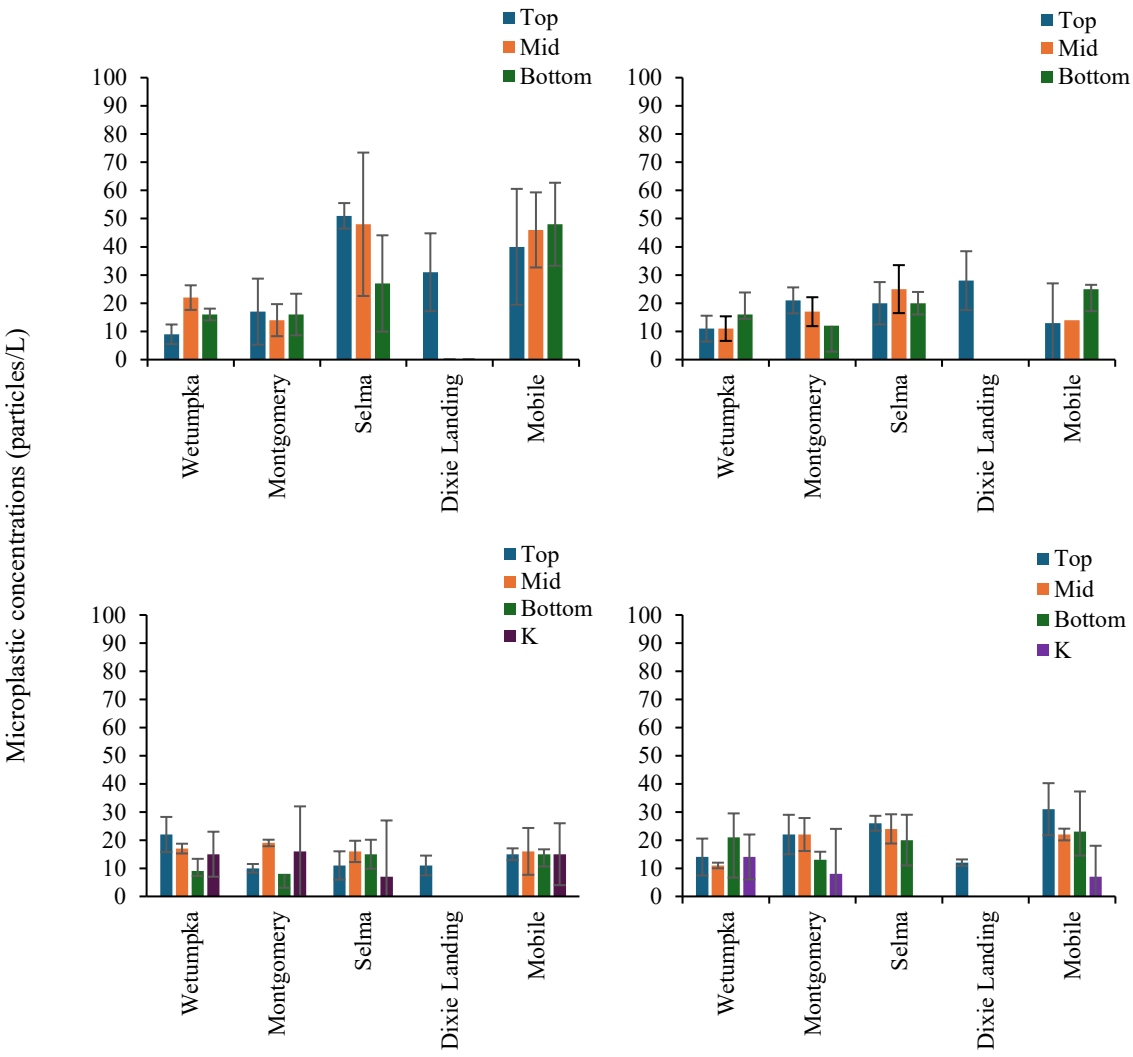

Figure A6: Microplastics in water layers of the Alabama River with K samples (A: summer, B: fall, C: winter, D: spring. Error bars represent standard deviations. There was no statistically significant difference in concentrations of water layers).

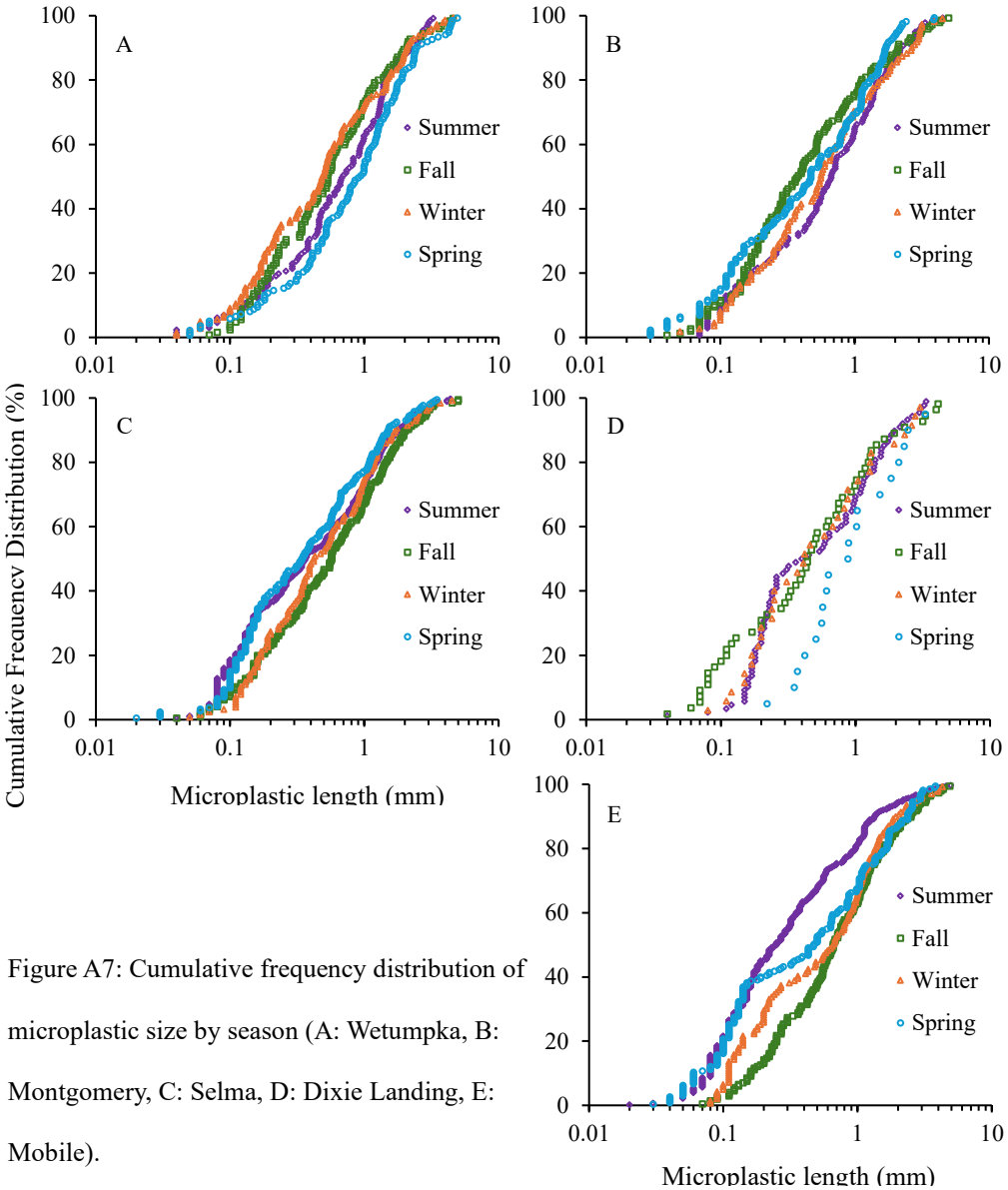

Figure A7: Cumulative frequency distribution of microplastic size by season (A: Wetumpka, B: Montgomery, C: Selma, D: Dixie Landing, E: Mobile).

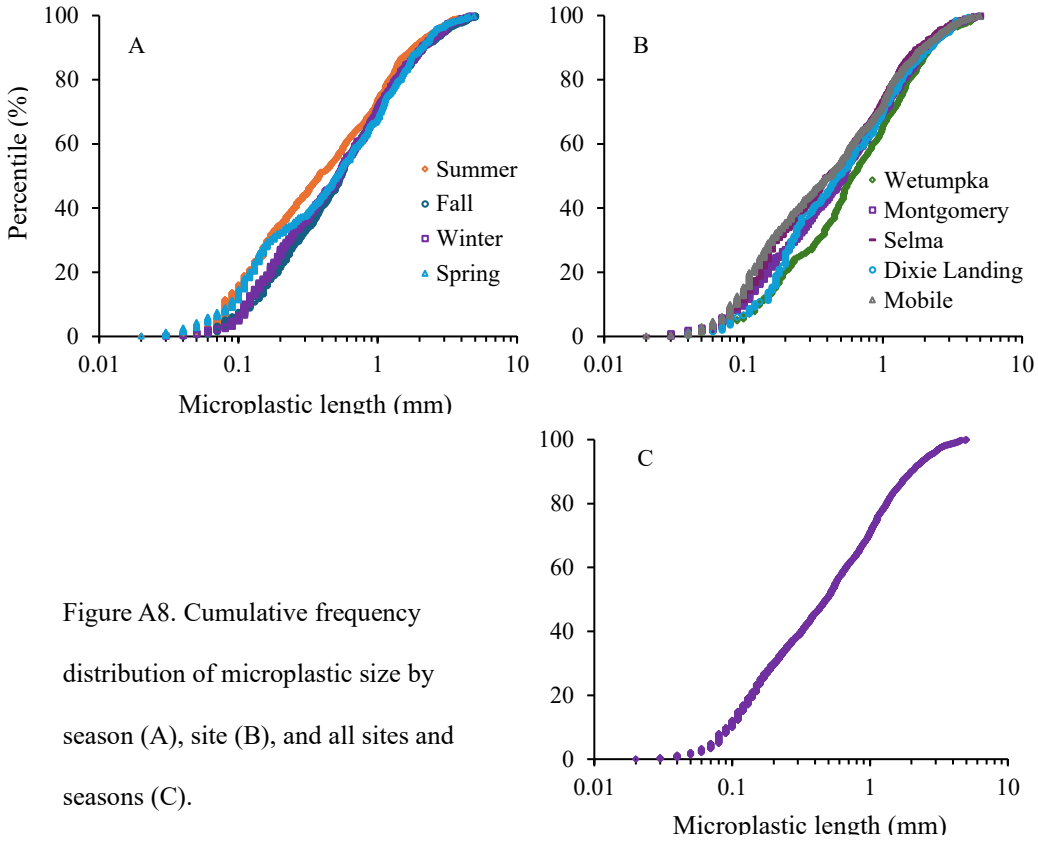

Figure A8. Cumulative frequency distribution of microplastic size by season (A), site (B), and all sites and seasons (C).

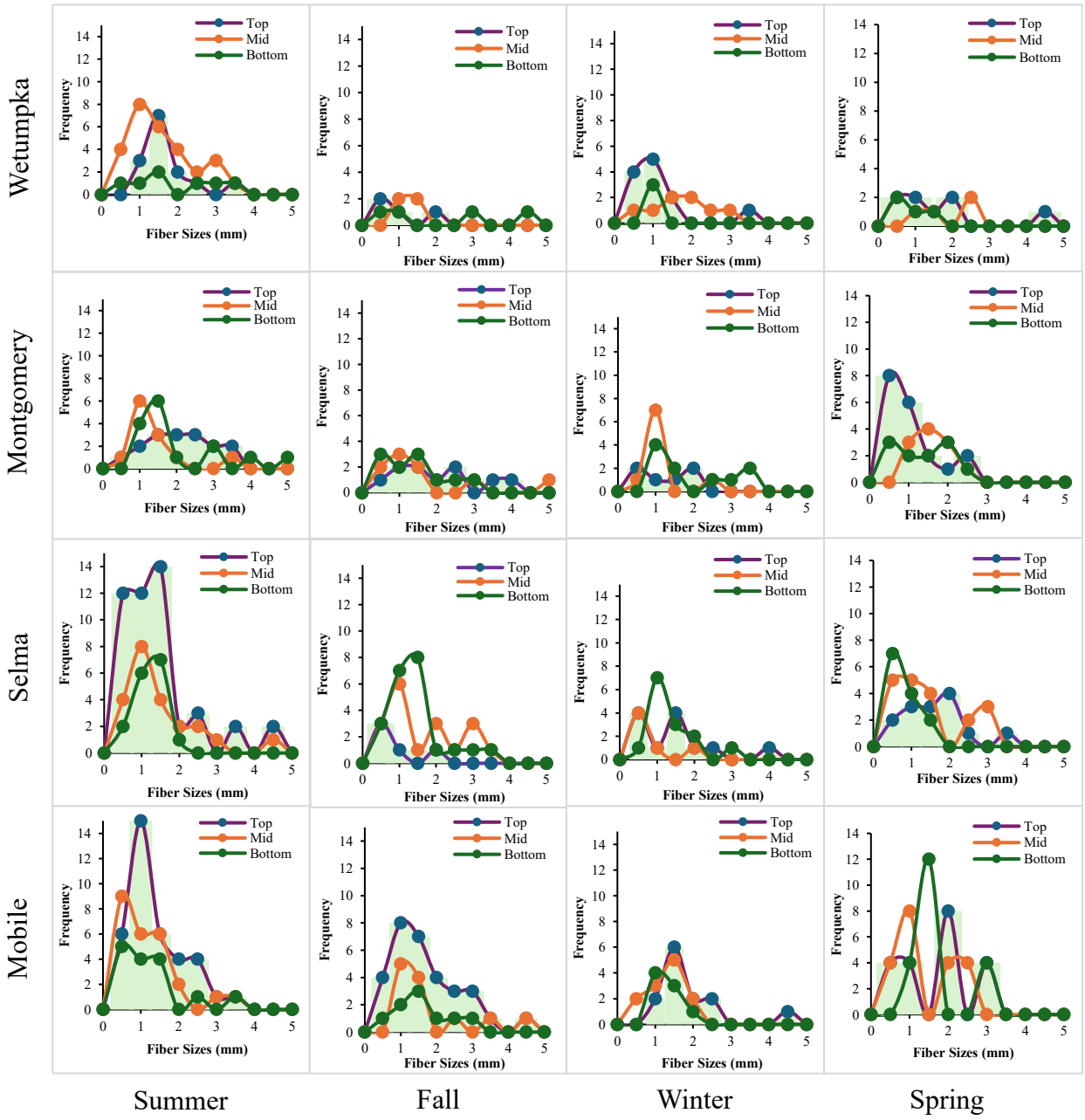

Figure A9: Size distribution of microplastics in below 38 µm sample fractions.

CHAPTER 3

3. EFFECTS OF CHRONIC EXPOSURE OF ENVIRONMENTALLY REALISTIC MICROPLASTICS ON *DAPHNIA MAGNA*: IMPORTANCE OF PARTICLE SIZE AND MORPHOLOGY AND IMPLICATIONS FOR RISK ASSESSMENTS

3.1. Abstract

Most ecotoxicity research with microplastics (MPs) in laboratories uses primary MPs, such as plastic microbeads. The present study aimed to determine the effects of three environmentally representative MPs: nylon and Kevlar microfibers and polystyrene cup fragments on *D. magna. Daphnia magna* was chronically exposed to each MP type using five test concentrations for 21 days to investigate MP uptake and effects on survivorship, growth, and reproduction. The results demonstrated that *D. magna* ingested MPs and the uptake concentration logarithmically increased with MP exposure concentration. Microplastics did not significantly affect the survival of *D. magna*. The reproduction rates in treatments of high concentrations (2.5, 25 mg/L) of nylon microfibers were significantly lower than the control. Dry weights of *D. magna* were found to be significantly lower when exposed to nylon microfibers at 0.064, 2.5, and 25 mg/L. Strong positive correlations between the ingested MPs and MPs in water were found. Significant negative correlations were found between MPs in water, ingested MPs and body size of *D. magna* in cup MP experiments. Particle size distribution analysis indicated that *D. magna* had a preferred size selection of MPs for ingestion. The effects of MPs on *D. magna* appeared to be influenced by particle size and surface morphology. More studies should be conducted to further understand the effects of MP types on different species of organisms and determine the relationship between MP uptake and effects, which is necessary for ecological risk assessment to support management of MP pollution.

Keywords – Microfibers, microplastic size distribution, *Daphnia magna*, reproduction rate, microplastic ingestion.

3.2. Introduction

Plastic production has increased dramatically since the 1950s, with millions of tons of plastic produced globally to date. It has been estimated that the annual plastic discharge into the aquatic ecosystem will reach 53 million tons by 2030 (Borrelle et al., 2020). Microplastics (plastics with 3-dimensional size ≤ 5 mm, MPs) constitute a significant portion of the plastic discharge. Previous research found various types of MP effects on aquatic life, with sublethal effects being more commonly reported than lethal effects. This includes effects on behavior, morphology, life history, biological and physical processes at molecular and cellular levels (Barboza et al., 2018; Barrick et al., 2025a; De Felice et al., 2019; Guilhermino et al., 2018; Magni et al., 2019; Murphy & Quinn, 2018; Rehse et al., 2016; Yu et al., 2018).

However, many ecotoxicological studies focused on short-term exposures to MPs using primary MPs (MPs produced by manufacturers for industrial application purposes) such as plastic microbeads (Aljaibachi & Callaghan, 2018; Canniff & Hoang, 2018; Hoang & Felix-Kim, 2020; Rehse et al., 2016). In the natural environment, the dominant MPs are fragmented particles of large plastic items, called secondary MPs. As a result, environmental MPs are diverse in shape and size and not as uniform as primary MPs (Hashmi et al., 2025; Kefer et al., 2022; Matavos-Aramyan, 2024). Microfibers (fibers with length ≤ 5 mm) have been reported to be the dominant MPs in the natural ecosystem, coming from the production, use, and discards of textile fiber materials at the end of their use (Henry et al., 2019; Periyasamy & Tehrani-Bagha, 2022). Effluents of wastewater treatment plants and surface runoff are believed to be important sources of microfibers (Sadia et al., 2022; Yaseen et al., 2022).

Living organisms in the natural environment are exposed to secondary MPs for longer times than primary MPs. The non-uniform morphology of environmental MPs can influence ingestion and excretion by living organisms leading to differences in uptake and effects compared to primary MPs that have uniform shape and smooth surface. For example, Canniff and Hoang (2018) found high ingestion of plastic microbeads by *D. magna* but no effect on survival and reproduction of the organisms was observed. Conversely, Barrick et al. (2024) found effects on *D. magna* survival when exposed to nylon microfibers with the same size and concentration as plastic microbeads used by Canniff and Hoang (2018). Therefore, ecotoxicology research using environmentally relevant exposure times (i.e. chronic as opposed to acute) and environmentally realistic MPs (i.e. secondary as opposed to primary) would provide a better understanding of MP impacts in the natural ecosystem.

Daphnia magna is a cladoceran species that serves as a model organism used for toxicological studies as it is considered to be an important link between primary producers and higher trophic levels (Miner et al., 2012). Preferential habitat for this species includes shallow water ecosystems that are now vulnerable to anthropogenic disturbance including chemical and MP pollution (Guilhermino et al., 2018). They have a short life cycle with a lifetime of approximately 1.5 months. They can mature and produce neonates in as little as 7 days. These biological characteristics make them a suitable model organism for chronic exposure studies to determine reproductive and growth effects of contaminants. Studies have reported the ingestion of a wide range of MPs by *D. magna* (Aljaibachi & Callaghan, 2018; Colomer et al., 2019; Frydkjær et al., 2017) including microfibers (Barrick et al., 2025; Jemec et al., 2016; Kim et al., 2021), microbeads and fragmented MPs (Canniff and Hoang 2028; Barrick et al., 2024; Funke et al., 2024; Schrank et al., 2019; Schür et al., 2023). Microplastics can be excreted but

excretion is dependent on the shape and morphology of particles. Incomplete excretion can result in MP accumulation in the digestive system of organisms and cause toxic effects to *D. magna* (Na et al., 2021). According to Barrick et al. (2024, 2025), the ingestion and excretion of secondary MPs by *D. magna* during a 48-h exposure was dependent on the plastic types. An uptake rate of 15.35 particles/h was found for MPs made from a single use plastic cup (cup MPs) while a rate of 0.95 particles/h was observed for MPs made from a single use plastic fork (fork MPs). The elimination rate of cup MPs was reported to be 1.77 particles/h while it was 0.27 particles/h for fork MPs.

To better understand the environmental impacts of MPs on aquatic species, it is important to conduct research using environmentally realistic MPs. With MPs being persistent in the natural environment, results of long-time exposure studies are more relevant for ecological risk assessment to support decision and policy making for management of plastic pollution. The present study aimed at investigating the effects on *D. magna* caused by chronic exposure of two different microfiber types (nylon and Kevlar) and cup MPs. The effect endpoints included survival, reproduction, body size, MP uptake and size distribution, and relationships between the measurement endpoints.

3.3. Materials and methods

3.3.1. Preparation and characterization of microplastics

Microfibers (nylon and Kevlar) were provided by Goonvean Fibers Ltd. Initial characterization by the manufacturer indicated that fiber lengths ranged from 0.1 mm to 0.25 mm. The reported microfiber density for Kevlar and nylon was 1.44 and 1.35 g/cm^3, respectively. No additive chemicals were incorporated into the material during the synthesis of the microfibers except a lubricant coating on their surfaces. To remove this coating, microfibers were treated with 1 g/L of

an anionic tenside solution (100g fibers/ 2L) for 30 min at 60°C (Barrick et al., 2025). After the removal of the surfactant, the microfibers were separated from the solution through warm water filtration. The microfibers were then dried in an oven at 60°C temperature overnight for later use (Barrick et al., 2025). Cup MPs were prepared from a single use plastic cup that was purchased from a grocery store. The cup was first cut into 1 x 1 cm pieces using a scissor, submerged in liquid nitrogen for approximately five min, and fed into a Pulversiette 14™ miller (Fristch) where highspeed sheering fractionated plastic samples. Samples were sieved through a filter to achieve a size range < 120 μm.

3.3.2. Quantification of microplastics

Microplastic size and concentrations in mg/L and MPs/L were measured. Homogenous microplastic solutions of each MP type were prepared by mixing 0.5 mg MPs with 5 mL of ethanol (70%) to achieve a concentration of 0.1 mg/mL. After mixing, the 0.1 mL solution was collected and filtered through a 1 μm Sartorius grid filter (cellulose nitrate) using a vacuum filtration system. The procedure was carried out in triplicate for each MP type to count and size MPs on the filters using an upright Nikon microscope (Eclipse C*i*-L Plus) with a Ds-F*i* 3 camera and NIS-Elements imaging software. The number of cup MPs (or microfibers)/mg were calculated to determine the conversion factor, which was used to prepare MP concentrations (in cup MPs (or microfibers)/L) for *D. magna* exposure experiments. The conversion factors for nylon microfiber, Kevlar microfiber, and cup MP were 23,733, 34,133, and 78,226 MPs/mg, respectively (Table B1).

3.3.3. Preparation of moderately hard water

Moderately hard water (MHW) was produced by dissolving equivalent amounts of mineral salts in reverse osmosis (RO) water using the USEPA Test Method for aquatic toxicology testing (Biesinger et al., 2002). Sodium bicarbonate ($NaHCO_3$) at a concentration of 90 mg/L, calcium

sulfate dihydrate ($CaSO_4.2H_2O$) and magnesium sulfate ($MgSO_4$) at a concentration of 60 mg/L each, and potassium chloride (KCl) at a concentration of 4 mg/L were dissolved in RO water. Temperature and pH of test water were measured daily during the experiment, and the results (average ± standard deviation) were 20.54°C $\pm$ 0.77 and 7.66 ± 0.35 respectively. Hardness and alkalinity were measured at the start and the end of experiments using titration methods (Eaton et al., 2005a, 2005b). The average and standard deviation of hardness and alkalinity were 91.33 ± 11.2 mg/L and 57.33 ± 7 mg/L as $CaCO_3$, respectively. These water quality characteristics are in the water quality range of the US EPA MHW (Biesinger et al., 2002).

3.3.4. Preparation of microplastic concentrations

Five test concentrations (defined as T_1, T_2, T_3, T_4, T_5 with T_1 having the lowest MP concentration and T_5 having the highest MP concentration) were prepared by mixing an equivalent amount of each MP type with MHW to achieve MP concentrations of 0.0064, 0.064, 0.25, 2.5, and 25 mg/L. An amount of 0.1, 1, and 10 mg of each MP type was added in each test chamber containing 400 mL MHW and mixed for three minutes using a glass rod to make 0.25 (T_3), 2.5 (T_4), and 25 (T_5) mg/L, respectively. Due to the accuracy limitations of our balance, MP concentrations of T_1 and T_2 were prepared using a dilution method. An amount of 0.1 mg of each MP type was mixed with 1 mL MHW to make a working MP concentration of 100 mg/L. An aliquot of 0.256 mL of this working MP solution was collected and mixed with 400 mL MHW to make a MP concentration of 0.064 mg/L for T_1. Similarly, a working MP concentration of 10 mg/L was prepared by mixing 0.1 mg MP with 10 mL MHW and 0.256 mL of the working MP solution was collected and mix with 400 mL MHW to make a MP concentration of 0.0064 mg/L for T_2. These concentrations are equivalent to 150, 2000, 6000, 59,000, and 593,000 nylon microfibers/L, 220, 2200, 8500, 85,300, and 853,000 Kevlar microfibers/L, and 500, 5000, 19,560, 195,560, and 1,955,650 cup MPs/L,

respectively (Table B2). The conversion factors used to convert MP concentration in mg/L to MPs/L were 23,733, 34,133, and 78,226 MPs/mg for nylon microfibers, Kevlar microfibers, and cup MPs, respectively (Table S1). Lower test concentrations (e.g., 0.0064, 0.064, 0.25 mg/L) are considered environmentally relevant (Beiras & Schönemann, 2020; Chen et al., 2023; Hashmi et al., 2025; Sun et al., 2021; Xiong et al., 2023; Zhao et al., 2014).

Microfibers often make clumps before adding them to the test chamber. To ensure their separation, the microfibers in water were mixed for approximately three minutes using a 1 mL pipette to squeeze water on the clumps until they are separated. Before introducing *D. magna* into the test chamber, a 1 mL subsample was collected from the center of each beaker at the middle of the water column to measure the number of microfibers or cup MPs in test water. A second 1 mL sample was collected in the middle of the experiment period (day 10) and a third 1 mL sample was collected at the end of the experiment on day 21 for MP analysis (Table S2). Samples were filtered onto a 1.0 μm Sartorius grid filter using a vacuum filtration system. The length of each microfiber and width and length of each cup MPs were measured using a LAXCO Stereo Microscope and an upright Nikon microscope (Eclipse C*i*-L Plus) with a Ds-F*i* 3 camera and NIS-Elements imaging software. To facilitate the measurement, a Nikon D-LEDI light source and quad band pass fluorescence cube (DAPI, GFP, TRITC, and CY5) was used to illuminate microfibers on the filter paper to improve detection.

3.3.5. Organism culture

An in-house culture of *D. magna* was maintained at the Ecotoxicology and Risk Assessment Laboratory of Auburn University following the USEPA Method for Toxicology Testing (Biesinger et al., 2002) to produce organisms for experiments. Twenty-five adult *D. magna* were kept in 1 L glass beakers containing 800 mL MHW. The water was changed three times per week on Monday,

Wednesday, and Friday. The temperature of the culture was maintained at 20.54 ± 0.8°C with a light:dark photoperiod of 16:8 hours. Organisms were fed daily with 6 ml *Raphidocelis subcapitata* at 3×10^7 cells/mL and 2 mL yeast, cereal leaves, and tetramine (YCT) (Aquatic Biosystems Inc.) Neonates *D. magna* (less than 24-h-old) were used to initiate experiments.

3.3.6. Exposure of microplastics to *Daphnia magna*

After preparing MP concentrations as described in section 2.4, ten < 24-h-old *D. magna* were added to each treatment beaker. Four replicates per treatment were used along with four replicates of control water (called Ctl) with no MPs (microfibers or cup MPs) added. Beakers were covered with a plexiglass sheet for the entire test duration to avoid potential contamination from an ambient environment. Freshwater with equivalent MP concentrations were prepared for water change every two days during the experiment. *Daphnia magna* were fed with algae (*Raphidocelis subcapitata*) at 3×10^7 cells/mL and YCT daily based on their age. The feeding rate increased when the organisms were bigger (Table B3).

The number of neonates and adults were counted daily. Dead adults (if observed) during the experiment were removed from the test chambers, collected in a glass vial, and frozen at -20°C until analysis of MP uptake. Neonates produced during the experiments were counted and removed from the test chambers daily. Living adult organisms at the end of 21-d experiments were also collected and stored in a glass vial at -20°C for later analysis of MP uptake.

Before analysis of MP uptake, the body sizes of each individual adult organism of each treatment replicate were measured. The length and width of the organism were measured using a LAXCO Stereomicroscope to compare significant differences between treatments and control. The dry and wet weights of all living adult *D. magna* in each treatment and control replicate were measured. Aluminum weight pans were preheated in an oven for 24 hours at 70°C and placed in the desiccator

to cool down at laboratory temperature. The initial weight of the pre-dried pan was measured on a Toledo microbalance with the accuracy of 0.01mg. After adding the adults, the final weight was taken again. After determining the wet weight, the organisms were kept in the oven to dry at 70°C for 24 hours. After 24 hours of drying, dry weights were measured using the same balance.

3.3.7. Digestion of *Daphnia magna*

Adult *D. magna* stored in the glass vials were allowed to thaw at laboratory temperature and rinsed three times with MilliQ-water® to wash off MPs adherence on the organism's surface. After rinsing, 1.5 mL of 30 % H_2O_2 was added to each vial and placed on a hotplate to undergo digestion at 70°C for 24 h (Barrick et al., 2025). After 24 h digestion, five mL of MilliQ-water® were added to dilute the digested solution prior to filtration. The diluted solution was poured onto a 1.0 µm Sartorius grid filter. The vials were rinsed with MilliQ-water® three times to ensure collection of all the MPs ingested by the organisms present on the filters. Counting and sizing MPs for each treatment replicate was conducted using a microscope as described above to determine MP ingestion by *D. magna*.

3.3.8. Data analysis

Survivorship for each treatment replicate was calculated using equation 1. The treatment average was calculated for comparison of statistically significant differences with the control.

$$Survivorship\ (\%) = \left(\frac{Number\ of\ living\ adult\ D.magna\ at\ the\ end\ of\ the\ experiment - number\ of\ dead\ adult\ D.magna}{Number\ of\ living\ adult\ D.\ magna}\right) * 100 \quad (1)$$

Wet weight per organism was calculated using equation 2. Dry weights were calculated using equation 3. The treatment average was calculated for comparison of statistically significant differences with the control.

$$Wet\ weight\ (mg/organism) =$$

$$\frac{[(weight\ of\ the\ pan + wet\ weight\ of\ living\ adult\ D.\ magna\ in\ a\ treatment\ replicate) - (weight\ of\ an\ empty\ pan)]}{Number\ of\ living\ adult\ D.\ magna\ in\ a\ treatment\ replicate}$$

(2)

$$Dry\ weight\ (mg/organism) =$$

$$\frac{[(weight\ of\ the\ pan + dry\ weight\ of\ living\ adult\ D.\ magna\ in\ a\ treatment\ replicate) - (weight\ of\ an\ empty\ pan)]}{Number\ of\ living\ adult\ D.\ magnain\ a\ treatment\ replicate}$$

(3)

The number of neonates produced per organisms daily were calculated using equation 4.

$$Neonates/organism = \left(\frac{Neonates\ observed\ on\ day\ n}{Number\ of\ living\ D.\ magna\ on\ day\ n-1}\right) \quad (4)$$

The total number neonates/organism produced in a treatment replicate during the 21-day experiment was calculated. The average total number neonates/organism produced in each treatment was calculated and used to compare differences between the treatment and the control to determine the effects of MP exposure on reproduction of *D. magna*.

Size (length) of exposed MPs in water and ingested MPs were analyzed for each MP type using a log-logistic distribution method for individual MPs to understand the size selection for ingestion by organisms and comparison between plastic types. The lengths were ranked from the shortest to the longest, and a centile was calculated for each MP using equation 1 (Hoang & Rand, 2015):

$$Centile = Rank\ \times \frac{100}{(n\ +1)} \quad (5)$$

where n is the total number of MPs. The 10^{th}, 50^{th}, and 90^{th} cumulative percentiles of MP length were calculated. The 10^{th} and 90^{th} percentiles are usually used to represent effect and exposure benchmarks in ecological risk assessment, respectively, and represent the size range of 80% MPs in the present study.

3.3.9. Statistical analysis

Statistical Analysis System (SAS) – Academics on Demand was used for the statistical analyses. Shapiro-Wilk's test was used to test for normal distribution of data. ANOVA with Dunnet test was used for pairwise comparison between the control and treatments that met the normal distribution and homogenous variance assumptions. For data that were not normally distributed, non-parametric analysis was used for significant comparison of the control and treatments. Statistical significance was set at a p-value ≤ 0.05. The relationship between MP uptake and MP concentrations in water was determined using a logarithmic model. To obtain a linear relationship, MP concentrations in water were transformed using the natural logarithmic function. The Pearson correlation method was used to analyze the correlations between the measurement endpoints.

3.3.10. Quality assurance

The laboratory bench was swept before processing the samples. A cotton laboratory coat was worn for the entire time while processing and analyzing the samples. Environmental contamination during these experiments was minimized by using glassware. All the glassware was rinsed three times with acetone and MilliQ-water® before starting the experiment. During digestion of samples, glassware was covered with aluminum foil to minimize potential contamination of airborne MPs deposition, whereas all the beakers were covered with a plexiglass sheet for the entire test duration. The pipette tip used for collecting water samples from suspension was replaced between each test replicate to avoid cross-contamination. Since the presence of ambient MPs in the laboratory is affirmed by recent studies, blank filters were placed on the bench during filtration to determine the background MPs concentrations in the laboratory.

3.4. Results

3.4.1. Survivorship

Daphnia magna survivorship remained between 88% to 100% for nylon microfiber, Kevlar microfiber, and cup MPs experiments. No statistically significant impact on mortality was observed for all three MPs (Fig. 3.1).

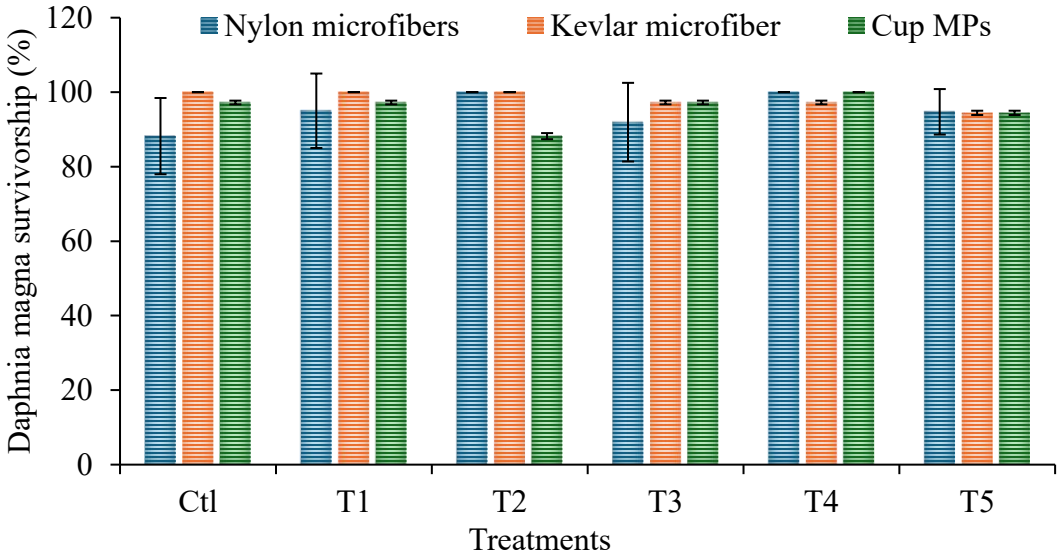

Figure 3.1. *Daphnia magna* survivorship after 21 days of exposure to MPs (A: nylon microfiber, B: Kevlar microfiber, C: cup MP). Data are the treatment average. Error bars represent standard deviations. No significant differences were observed between control and treatments.

3.4.2. Reproduction of *Daphnia magna*

The number of neonates produced per organism were calculated for all three experiments (Fig. 2). In the nylon microfiber experiment, on average, a total of 154 $\pm$ 10 neonates were produced per *D. magna* in control, 142 ± 17 in T_1, 137 ± 4 in T_2, 144 ± 7 in T_3, 138 ± 5 in T_4, and 142 ± 6 in T_5. The average total neonates/organism in treatments T_2, T_4, and T_5 were significantly lower than that of the control (p = 0.04, 0.04, and 0.05 respectively Fig. 2A). In the Kevlar microfiber experiment, the average total neonates/organism were 58 ± 7 in control, 65 ± 10 in T_1, 58 ± 7 in T_2, 68 ± 13 in T_3, 61± 14 in T_4, and 60 ± 5 in T_5. There was no statistically significant difference between the

control and any treatment (Fig. 2B). For the cup MP experiment, 66 ± 4 neonates/organism were observed in control, 66 ± 12 in T_1, 64 ± 7 in T_2, 64 ± 8 in T_3, 70 ± 9 in T_4, and 56 ± 13 neonates/organism in T_5 were found. No statistically significant difference between the treatments and control was observed in the cup MP experiment (Fig. 2C).

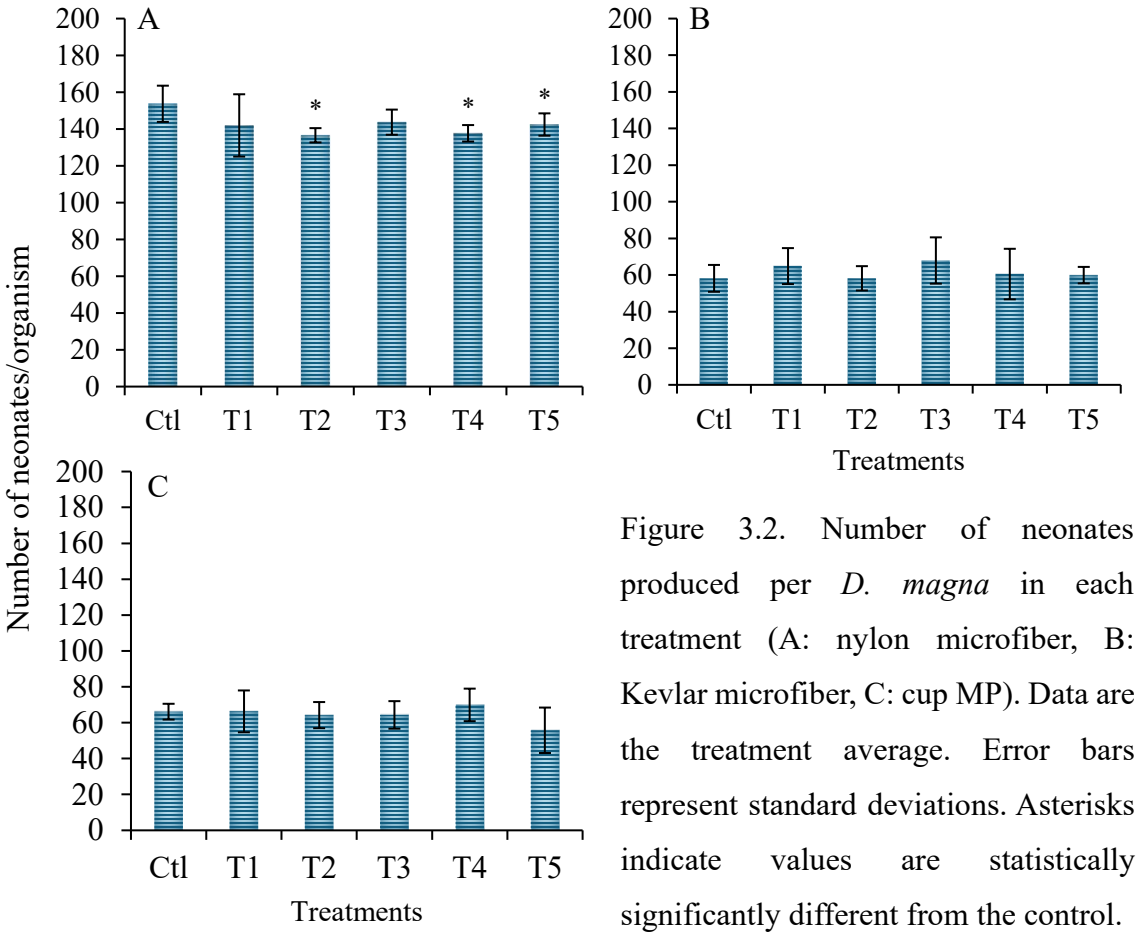

Figure 3.2. Number of neonates produced per *D. magna* in each treatment (A: nylon microfiber, B: Kevlar microfiber, C: cup MP). Data are the treatment average. Error bars represent standard deviations. Asterisks indicate values are statistically significantly different from the control.

3.4.3. Growth of *Daphnia magna*

The wet and dry weights per *D. magna* were calculated for each experiment. In the nylon microfiber experiment, the average wet weight ranged from 8.45 to 10.45 mg/organism and the average dry weight ranged from 0.77 to 0.95 mg/organism (Fig. 3A). Those ranges of wet and dry weight measurements were 6.31-8.12 mg/organism and 0.48-0.54 mg/organism for the Kevlar

microfiber experiment (Fig. 3B) and 6.41-6.81 and 0.63-0.68 mg/organism for the cup MP experiment, respectively (Fig. 3C).

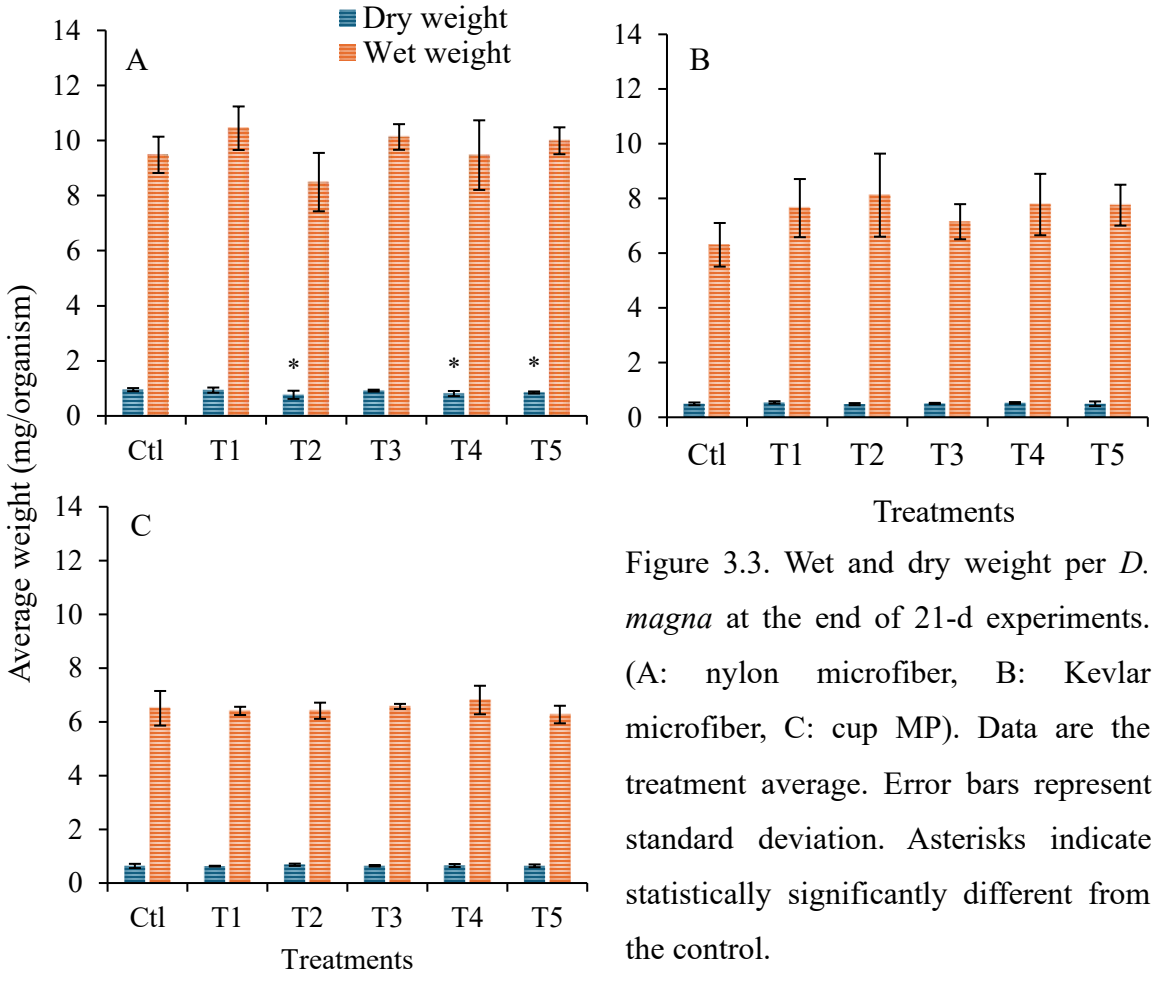

Figure 3.3. Wet and dry weight per *D. magna* at the end of 21-d experiments. (A: nylon microfiber, B: Kevlar microfiber, C: cup MP). Data are the treatment average. Error bars represent standard deviation. Asterisks indicate statistically significantly different from the control.

For dry weight, results of the nylon microfiber experiment, a statistically significant difference was observed between the control and T_2 ($p = 0.01$), T_4 ($p = 0.009$), and T_5 ($p = 0.05$). However, no statistically significant difference between dry weights of treatments and control was found for the Kevlar microfiber and cup MP experiments. No statistically significant difference between wet weights of treatments and the control in all three experiments was detected.

The length and width of each *D. magna* in each treatment was measured at the end of the experiment (Fig. 4). The average length ranges for the control and treatments of the nylon microfiber experiment were 5.18-5.37 mm (Fig 4A). For the Kevlar microfiber and cup MP

experiments, the average lengths were 4.09-4.19 (Fig 4B) mm and 3.563-3.93 mm, respectively (Fig 4C). The average ranges of widths were 2.6-2.82 mm for the nylon microfiber experiment (Fig 4A), 2.63-2.71 mm for the Kevlar microfiber experiment (Fig 4B), and 2.31-2.47 mm for the cup MP experiment (Fig 4C).

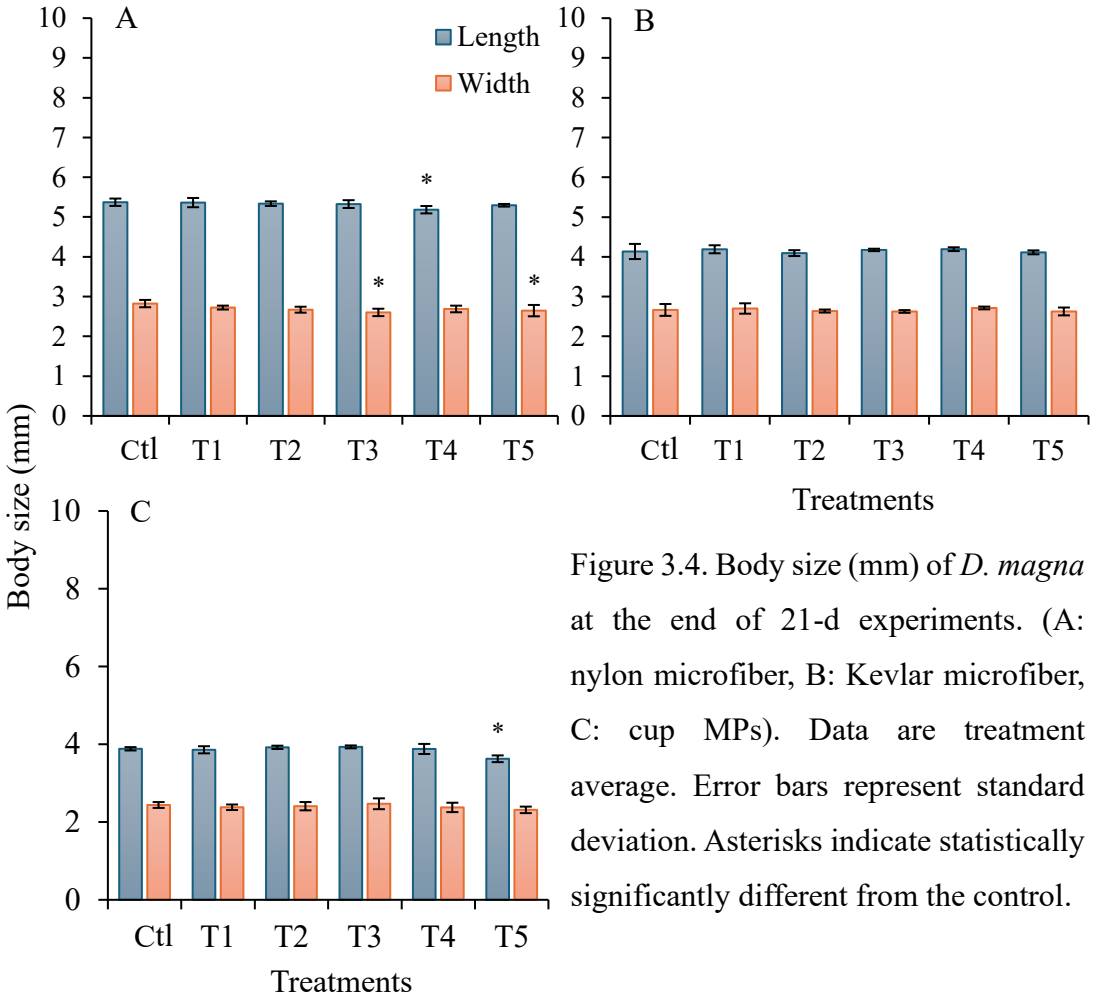

Figure 3.4. Body size (mm) of *D. magna* at the end of 21-d experiments. (A: nylon microfiber, B: Kevlar microfiber, C: cup MPs). Data are treatment average. Error bars represent standard deviation. Asterisks indicate statistically significantly different from the control.

There was a statistically significant difference between the length of control and T_4 of the nylon microfiber experiment (p = 0.03). For the cup MP experiment, a statistically significant difference was found between the length of control and T_5 (p = 0.03). For width results, statistically significant differences were found between the control and T_3 (p = 0.01), and T_5 (p = *0.05*).

However, no significant difference for width data was detected between the control and other five treatments in the cup MP and Kevlar microfiber experiments.

3.4.4. Microplastics in water and ingestion by *Daphnia magna*

Results of analysis of MPs in exposure water showed that overall MP concentrations were lower before than after mixing water except for some treatments at which the MP concentrations after mixing water were lower than those before mixing water, such as treatment T_1 of nylon microfiber and T_3 of cup MPs (Table S2). With cup MPs, a thin layer of MPs was found on the surface of the exposure water whereas no floating nylon and Kevlar microfibers were observed in the exposure water. These results suggest that MPs, especially nylon and Kevlar microfibers sedimented during the 48-h water change intervals. This is explainable because their densities are higher than the water density. For the nylon microfiber experiment, on average, 0.23, 0.49, 0.58, 0.48, 1.68, 3.82 microfibers were found per alive *D. magna* in control, T_1, T_2, T_3, T_4, and T_5, respectively, whereas 1.5 microfiber/organism was found in T_1, 1 microfiber/organism was found in T_2, 3 microfibers/organism were found in T_3, and 8 microfibers/organism were found in T_5, in dead *D. magna* (Table S2). In the Kevlar microfiber experiment, no microfiber was found in control and 0.93, 1.68, 5.38, 6.13, and 10.06 microfibers/organism were found in T_1, T_2, T_3, T_4, and T_5 in alive *D. magna,* respectively. In dead *D. magna,* no microfiber was found in organisms in control, T_1, and T_2, whereas 51 microfibers/organism were found in T_3, 120 microfibers/organism were found in T_4, and 83 microfibers/organism were found in T_5 (Table S2). However, the highest uptake was observed in the cup MP experiment. The control, T_1, and T_2 had 0.10, 0.09, and 0.15 cup MPs/organism whereas, 6.65, 28.61, and 49.86 cup MPs/organism were found in T_3, T_4, and T_5 in alive *D. magna*, respectively. In dead *D. magna*, 4 cup MPs/organism were found in the control, 8

cup MPs/organism were found in T_1, 19.25 cup MPs/organism were found in T_2, 109 cup MPs/organism were found in T_3, and 224 cup MPs/organism were found in T_5 (Table S2).

The relationship between MP uptake and MPs in water was analyzed. Since *D. magna* ingested MPs in the water column, at the water surface, and on the bottom of the exposure chamber (Barrick et al., 2025, observation of the present study), the nominal MP concentrations were used for the MP uptake and water MP relationship analysis. The analysis results showed that MP uptake by *D. magna* was related with MP concentration in water. The relationship followed a logarithmic model. The slope of the relationship increased in the order of nylon microfiber, Kevlar microfiber, and cup MP (Fig. 5). The statistical comparison of the slopes showed that there were significant differences between the slopes (p = 0.016 for nylon and Kevlar microfibers, p = 0.003 for nylon microfibers and cup MPs, p = 0.007 for Kevlar microfiber and cup MPs).

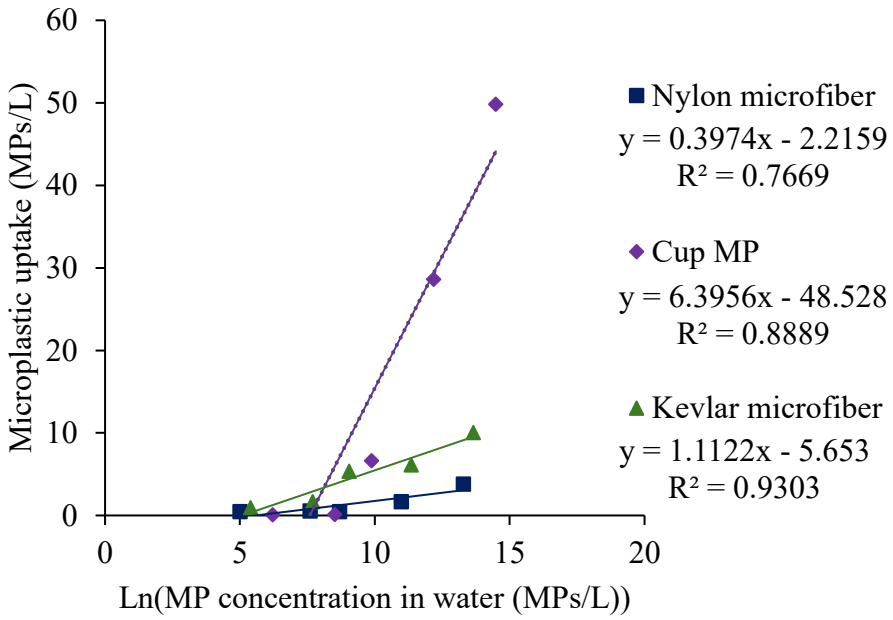

Figure 3.5. Relationship between MP uptake by *D. magna* and MP concentration in water.

Strong positive correlations between the concentrations of MPs (microfibers and cup MPs) ingested in and MPs (microfibers and cup MPs) in water of all experiments were found (Table

3.1). These results indicate that MP uptake by *D. magna* increased with increasing MP concentration in water. The correlations between MPs ingested, MPs in water and the length and width and overall size of *D. magna* in the cup MPs experiments were negative suggesting that MPs in water and MPs ingested have potential effect on the body size of *D. magna*.

Table 3.1. Correlations between biological measurement endpoints and MPs in water.

	Length	Width	Overall size (L*W)[a]	Weight/ organism	Neonates/ organism	MPs ingested (alive)	MPs ingested (dead)	Water MPs (BM)[b]	Water MPs (AM)[c]	Nominal conc.[d]
Nylon microfibers										
Length	1.000									
Width	-0.113	1.000								
Overall size (L*W)[a]	0.558	0.760	1.000							
Weight/organism	0.438	-0.080	0.250	1.000						
Neonates/organism	0.409	-0.440	-0.087	0.865	1.000					
MPs ingested (alive)	-0.361	-0.091	-0.350	-0.223	0.157	1.000				
MPs ingested (dead)	-0.912	-0.336	-0.512	-0.007	0.476	0.958*	1.000			
Water MPs (BM)[b]	-0.082	-0.200	-0.260	-0.085	0.331	0.956*	0.969*	1.000		
Water MPs (AM)[c]	-0.091	-0.187	-0.255	-0.091	0.321	0.960*	0.966*	1.000*	1.000	
Nominal conc.[d]	-0.110	-0.181	-0.263	-0.099	0.312	0.965*	0.966*	0.999*	1.000*	1.000
Kevlar microfibers										

Length	1.000									
Width	0.728	1.000								
Overall size (L*W)[a]	0.887*	0.962*	1.000							
Weight/organism	0.845	0.784	0.858	1.000						
Neonates/organism	0.639	-0.014	0.248	0.450	1.000					
MPs ingested (alive)	-0.112	-0.300	-0.257	-0.336	-0.148	1.000				
MPs ingested (dead)	0.289	0.845	0.702	0.692	-0.803	0.099	1.000			
Water MPs (BM)[b]	-0.319	-0.295	-0.342	-0.290	-0.397	0.890*	0.223	1.000		
Water MPs (AM)[c]	-0.367	-0.370	-0.411	-0.315	-0.368	0.868	0.121	0.995*	1.000	
Nominal conc.[d]	-0.430	-0.445	-0.487	-0.350	-0.357	0.830	0.032	0.979*	0.994*	1.000

Cup MPs

Length	1.000				
Width	0.878	1.000			
Overall size (L*W)[a]	0.971*	0.760	1.000		
Weight/organism	0.342	0.180	0.275	1.000	
Neonates/organism	0.804	0.504	0.677	0.101	1.000

MPs ingested (alive)	-0.846	-0.743	-0.825	-0.234	-0.548	1.000				
MPs ingested (dead)	-0.802	-0.490	-0.667	-0.302	-0.924	0.943*	1.000			
Water MPs (BM)[b]	-0.901*	-0.700	-0.831	-0.282	-0.720	0.969*	0.966*	1.000		
Water MPs (AM)[c]	-0.939*	-0.807	-0.905*	-0.227	-0.714	0.972*	0.909	0.985*	1.000	
Nominal conc.[d]	-0.975*	-0.793	-0.915*	-0.232	-0.854	0.890*	0.896	0.955*	0.971*	1.000

[a](L*W) = (Length*Width)

[b]BM = Before mixing

[c]AM = After mixing

[d]Conc. = concentration

*Indicates statistically significant at $p < 0.05$.

Results of the cumulative size distribution of MPs are shown in Fig. 3.6.

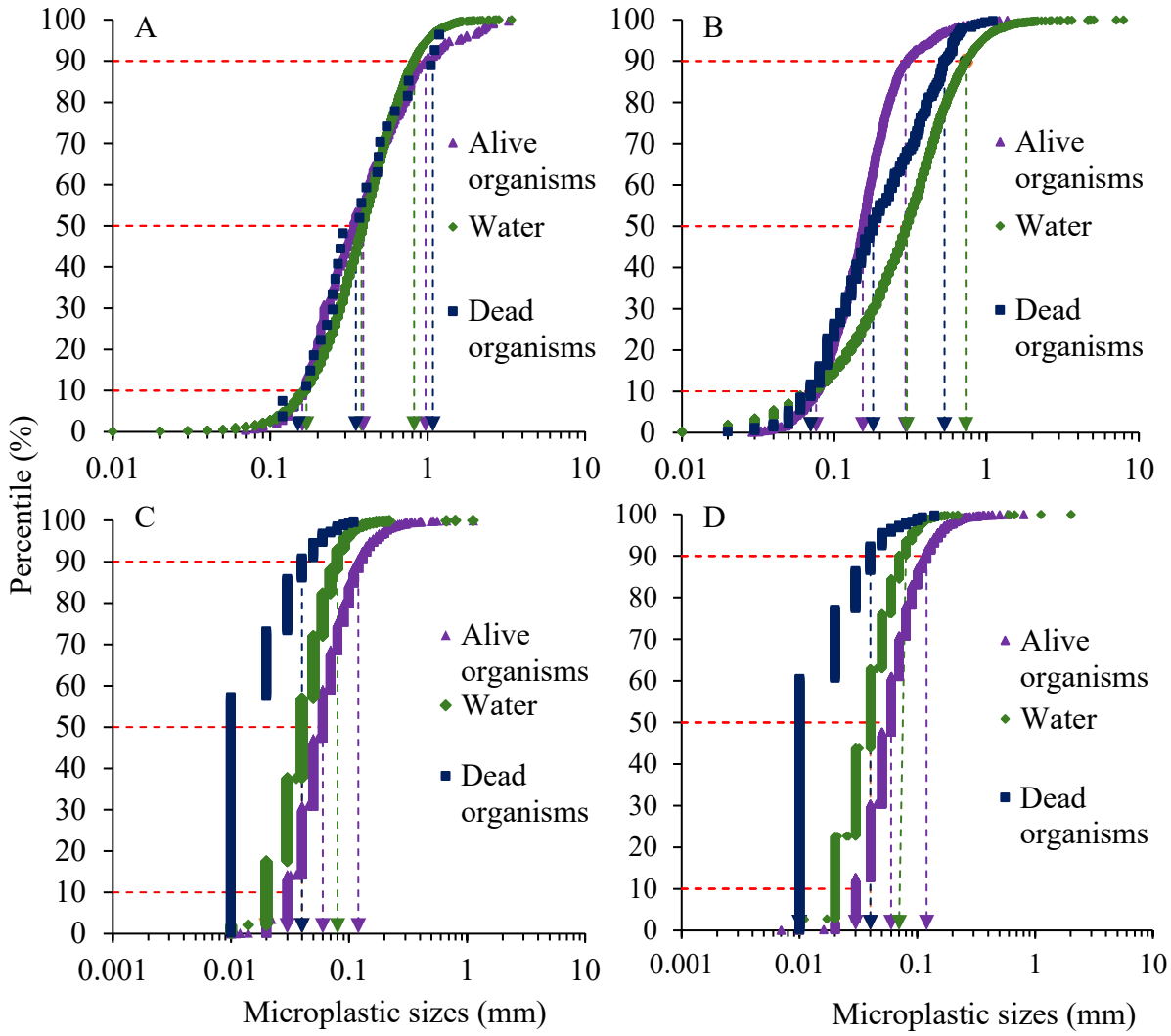

Figure 3.6. Cumulative frequency distribution of the particle size of MPs in water and MPs ingested by alive and dead *D. magna* (dash lines with arrow represent particle size of MPs corresponding to the 10th, 50th, and 90th percentiles, A: length of nylon microfibers, B: length of Kevlar microfibers, C: length of cup MPs, D: width of cup MPs.)

The 10th percentile size for nylon microfibers ingested by the *D. magna* was 0.16 mm. The 50th percentile size was 0.34 mm, and the 90th percentile size was 0.97 mm. In water, those respective percentiles were 0.17, 0.39, and 0.82 mm whereas in dead *D. magna*, these percentiles were 0.15,

0.35, and 1.08 mm, respectively (Fig. 3.6A, Table 3.2). For Kevlar microfibers, the 10^{th}, 50^{th}, and 90^{th} percentile size of ingested microfibers was 0.08, 0.15, and 0.29 mm, respectively. Those respective percentiles of MPs in water were 0.07, 0.30, and 0.73 mm and in dead *D. magna* were 0.07, 0.18, and 0.53 mm (Fig. 3.6B, Table 3.2). Cup MPs were smaller than nylon and Kevlar microfibers with the 10^{th}, 50^{th}, and 90^{th} percentiles of the length of ingested MPs by alive *D. magna* and MPs in water being 0.03, 0.06, and 0.12 mm and 0.02, 0.04, and 0.08 mm respectively. However, these percentiles were 0.01, 0.01, and 0.04 mm in dead *D. magna*, respectively (Fig. 3.6C, Table 3.2). The width of cup MPs was similar to the length of cup MPs. The 10^{th}, 50^{th}, and 90^{th} percentiles of the width of ingested MPs by alive *D. magna* and MPs in water were 0.03, 0.07, and 0.12 mm and 0.02, 0.04, and 0.07 mm, respectively (Fig. 3.6D, Table 3.2). These relative percentiles of the length and width of cup MPs in dead *D. magna* were 0.01, 0.01, and 0.04 mm (Fig. 3.6C, D, Table 3.2).

Table 3.2. Size distribution of MPs ingested by *D. magna* (alive and dead) and MPs in water.

Microplastic type	Exposure/ Ingestion	Length range* (mm)	Length corresponding to percentiles (mm)			Width range* (mm)	Width corresponding to percentiles (mm)		
			50th	10th	90th		50th	10th	90th
Nylon microfiber	Microplastics in water	0.01-3.41 (5264)	0.39	0.17	0.82	0.020-0.022 (20)	N/A	N/A	N/A
	Microplastics in alive *D. magna*	0.07-3.27 (282)	0.34	0.16	0.97	N/M	N/A	N/A	N/A
	Microplastics in dead *D. magna*	0.12-1.19 (26)	0.35	0.15	1.08	N/M	N/A	N/A	N/A
Kevlar microfiber	Microplastics in water	0.009-4.86 (5989)	0.30	0.07	0.73	0.040-0.042 (20)	N/A	N/A	N/A
	Microplastics in alive *D. magna*	0.03-1.37 (1007)	0.15	0.08	0.29	N/M	N/A	N/A	N/A
	Microplastics in dead *D. magna*	0.02-1.1 (340)	0.18	0.07	0.53	N/M	N/A	N/A	N/A

Cup MP	Microplastics in water	0.01-1.12 (3018)	0.04	0.02	0.08	0.01-2.02 (3018)	0.04	0.02	0.07
	Microplastics in alive *D. magna*	0.01-1.13 (3049)	0.06	0.03	0.12	0.007-0.08 (3049)	0.07	0.03	0.12
	Microplastics in dead *D. magna*	0.01-0.11 (401)	0.01	0.01	0.04	0.01-0.14 (401)	0.01	0.01	0.04

*Numbers in parentheses are the total number of MPs measured. N/A: not applicable. N/M: not measured.

3.5. Discussion

3.5.1. Microplastic uptake and size selection for ingestion -importance for bioavailability and risk assessments

The present study found that *D. magna* ingested MPs. The ingestion followed a logarithmic model with MP uptake concentration increasing with MP exposure concentration (Fig. 3.5). The slope of the relationship between the MP uptake and MP exposure concentration increased in the order of nylon microfiber, Kevlar microfiber, and cup MP. This relative increase in the slope reflects a relative increase in MP ingestion by the number of MPs per organism based on the number of MPs per litter of water and can be explained by the respectively relative decrease in the particle size of MPs. Ninety percent of nylon microfibers had sizes of ≤ 0.82 mm, whereas it was ≤ 0.68 mm and 0.12 mm for Kevlar microfibers and cup MPs, respectively (Fig. 3.6, Table 3.2).

Previous research has reported that MP uptake is dependent on the particle size (Cho et al., 2024; Mohamed Nor et al., 2021; Mondellini et al., 2024). With a given gut space, organisms can ingest a higher number of smaller MPs or fewer MPs with larger sizes (Canniff and Hoang 2018, Hoang and Fel-Kim 2020, Barrick et al., 2024). The ingested MP concentration by alive *D. magna* was positively correlated with water MP concentration for all MP types (coefficients > 0.96, Table 1). These results are in agreement with a previous acute exposure study conducted by Barrick et al. (2024) with the same type of MP. However, the MP uptake in the present study was up to ten times higher than the uptake found by Barrick et al. (2024). These differences demonstrate that chronic exposure resulted in higher MP uptake than acute exposure. Since MPs are persistent in the natural environment, aquatic organisms would experience chronic exposure in the natural ecosystem and might ingest more MPs in the natural ecosystem. This will raise concern about MP transfer through the food chain and food web in the natural ecosystem. Among the three MP types, the uptake

(MPs/organism) of cup MPs by alive *D. magna* was the highest following by the uptake of Kevlar microfibers and nylon microfibers. Approximately, the numbers of MPs/organism were ten times higher for cup MPs than nylon microfibers. It is not clear whether these differences in the MP uptake are due to the organism's preference of color, shape, and/or size of MPs. Cup MPs were red, Kevlar microfibers were pale yellow, whereas nylon microfibers were transparent white. It is important to mention that the results of the size distribution analysis of MPs found that cup MPs were the smallest with most particles (80%) having a length between 0.02 and 0.08 mm and a width between 0.02 and 0.07 mm (Fig. 3.6C, D, Table 3.2), whereas nylon microfibers had the largest size with 80% particles were between 0.17 and 0.82 mm (Fig. 3.6, Table 3.2), which is about ten times larger than cup MPs. The size of nylon microfibers *D. magna* ingested (80% in 0.16 - 0.97 mm range) was similar to the size of the particles in water. These results suggest that caution should be taken when interpreting MP uptake by organisms. An organism with a defined gut space can ingest a higher number of small MPs or fewer number of large MPs that occupied a similar gut volume of its digestive system. Therefore, when comparing MP uptake by organisms, particle size must be taken into consideration, especially for risk assessment that are based on MP uptake.

Comparing the uptake of Kevlar and nylon microfibers by alive *D. magna*, the size ranges of both microfibers in water are considered similar. *Daphnia magna* ingested Kevlar microfibers at a size range of approximately four times shorter than the size range of ingested nylon microfibers. Results of morphological analysis of the microfibers showed that Kevlar microfibers had a rough and irregular end structure with fibrils that might be harder for *D. magna* to ingest than nylon microfibers with smooth ends and surface (Barrick et al., 2025). Moreover, based on the surface morphology of the nylon and Kevlar microfibers, the present study suggests that the smoother

surface morphology of nylon microfibers enables *D. magna* to ingest them easier than Kevlar microfibers. While MPs ingested by alive *D. magna* has a size similar to (for nylon microfibers) or smaller (for Kevlar microfibers) than the size of MPs in water, the size of ingested cup MPs by alive *D. magna* was larger than the size of cup MPs in water (Fig. 3.6C, D, Table 3.2). These differences would be due to ununiform distribution of cup MPs in the exposure water with larger MPs being inundated while smaller size MPs were floating on the water surface due to the surface tension effects. This ununiform distribution of cup MPs in water was also observed by Barrick et al. (2025). Regarding the size of particles in alive and dead *D. magna*, cup MPs in dead *D. magna* were approximately three times smaller than those in alive *D. magna*. Although there was no clear relationship between mortality and MP uptake, dead *D. magna* ingested more MPs with smaller size than did alive *D. magna*. The findings of the present study indicate that *D. magna* appeared to have a particle size and shape preference for ingestion. This is important and should be taken into consideration for risk assessment for MPs involving MP uptake and effects.

3.5.2. Biological effects of microplastics

The present study evaluated the toxicity potential of chronic exposure to two types of microfibers (nylon and Kevlar) and cup MPs to *D. magna*. Overall, 5 ± 4.6 % mortality was observed for nylon microfiber exposure, 2.1 ± 2.3 % for Kevlar microfiber exposure, and 4 ± 4.04 % for cup MP exposure. However, none of these mortalities were statistically significantly different from the mortality of control. Similar results have been reported by other chronic exposure studies with plastic microbeads (Canniff & Hoang, 2018; Huang et al., 2022). Interestingly, Barrick et al. (2025) found significant mortality effects of acute exposure of the same nylon microfibers on *D. magna*. There is no clear explanation for these different effects on the survival of the organisms. The only difference between the two studies was feeding. The organisms were not fed during the acute

exposure of Barrick et al. (2025) while they were fed with algae and YCT in the present study. Algae has been reported to colonize on the surface of MPs and together with the colonization of other microorganisms, layers of biofilm are developed on MP surface that serve as a food source for *D. magna* and also make MPs be smoother for excretion, preventing their potential causing physical damages to the digestive system of the organisms, such as poking through the digestive tract (Amariei et al., 2022; Barrick et al., 2025; Canniff & Hoang, 2018; Rummel et al., 2017; Verdú et al., 2023).

Among the three MP types, only nylon had an effect on reproduction of *D. magna* (Fig. 3.2). The reproductive rates of all treatments, except treatments T_1 and T_3, were significantly lower than the reproductive rate of control. Comparing the MP uptake by the two particle types, the uptake in the number of MPs/organism was lower for nylon microfibers than for cup MPs. When taking the particle size into consideration, the total amount of MP uptake of each type are considered similar as discussed in the above section. In addition, a previous study using chronic exposure of plastic microbeads with a size within the range of the present study found no significant effects on reproduction of *D. magna* (Canniff & Hoang, 2018). Therefore, the difference in reproductive effects suggest an influence of particle type on biological effects of MPs. Nylon microfibers are long and can be harder to excrete and would stay longer in the digestive system of the organisms. More research is needed to understand the potential influence of the particle morphology of MPs on living organisms (An et al., 2021; Frydkjær et al., 2017; Schwarzer et al., 2022; Yokota & Mehlrose, 2020).

Overall, the three MP types did not markedly affect the growth of *D. magna*. However, for cup MPs, the length and the overall size (production of length and width) of *D. magna* were significantly negatively correlated with microplastic in water and microplastics ingested (Table

3.1). The width data also showed negative correlations with MPs in water and MPs ingested, although the correlations were slightly above the significant level (p = 0.06). The negative correlations were also evidenced for nylon microfibers but were not statistically significant. These negative correlations indicate that when MP concentrations in water and in *D. magna* were increased the organism's length and width and overall size will decrease. The results showed the potential impacts of MPs on *D. magna's* growth, especially with cup MPs. The effect can be related to the gut space occupying by MPs. Organisms will have less gut space for food if MPs are present in their digestive system. This would decrease energy intake for growth and development. More research should be conducted to determine the retention time of MPs in organisms and the potential impact of MPs on energy intake and biological performance of living organisms.

3.6. Conclusions and suggestions

The present study evaluated the effect of three different MPs (nylon and Kevlar microfibers, cup MPs) on *D. magna* using chronic exposure method. Results showed no significant effects on the survival of the organisms in all experiments. *Daphnia magna* ingested MPs and the ingestion followed a logarithmic model. Among the three MP types, the MP uptake (MPs/organism) was the highest with cup MPs and the lowest with Kevlar microfibers. Reproduction of *D. magna* was affected by nylon microfibers but not by Kevlar microfibers and cup MPs. The differences in the MP uptake and reproductive effects are believed to be related to particle morphology and size. *Daphnia magna* appeared to have a preferred size and shape selection for MP ingestion. Evidence for MP effects on *D. magna's* growth was shown, especially for cup MPs. The findings suggest that particle size and morphology and measurement units should be taken into consideration for interpreting MP effects involving MP ingestion, especially for risk assessment of MP exposure and

effects. More research should be conducted to understand the details of the influence of particle size and surface morphology on their biological effects.

3.7. References

1. Aljaibachi, R., & Callaghan, A. (2018). Impact of polystyrene microplastics on Daphnia magna mortality and reproduction in relation to food availability. *PeerJ*, *2018*(4), e4601. https://doi.org/10.7717/PEERJ.4601/SUPP-32
2. Amariei, G., Rosal, R., Fernández-Piñas, F., & Koelmans, A. A. (2022). Negative food dilution and positive biofilm carrier effects of microplastic ingestion by D. magna cause tipping points at the population level. *Environmental Pollution*, *294*, 118622. https://doi.org/10.1016/J.ENVPOL.2021.118622
3. An, R., Wang, X., Yang, L., Zhang, J., Wang, N., Xu, F., Hou, Y., Zhang, H., & Zhang, L. (2021). Polystyrene microplastics cause granulosa cells apoptosis and fibrosis in ovary through oxidative stress in rats. *Toxicology*, *449*. https://doi.org/10.1016/j.tox.2020.152665
4. Barboza, L. G. A., Vieira, L. R., & Guilhermino, L. (2018). Single and combined effects of microplastics and mercury on juveniles of the European seabass (Dicentrarchus labrax): Changes in behavioural responses and reduction of swimming velocity and resistance time. *Environmental Pollution*, *236*, 1014–1019. https://doi.org/10.1016/J.ENVPOL.2017.12.082
5. Barrick, A., Boardwine, A. J., & Hoang, T. C. (2024). Accumulation, depuration, and potential effects of environmentally representative microplastics towards Daphnia magna. *Science of The Total Environment*, *950*, 175384. https://doi.org/10.1016/J.SCITOTENV.2024.175384
6. Barrick, A., Boardwine, A. J., Nguyen, N. H. A., Sevcu, A., Novotna, J., & Hoang, T. C. (2025). Acute toxicity of natural and synthetic clothing fibers towards Daphnia magna:

Influence of fiber type and morphology. *Science of The Total Environment*, *967*, 178751. https://doi.org/10.1016/J.SCITOTENV.2025.178751

7. Beiras, R., & Schönemann, A. M. (2020). Currently monitored microplastics pose negligible ecological risk to the global ocean. *Scientific Reports*, *10*(1). https://doi.org/10.1038/S41598-020-79304-Z;SUBJMETA=158,172,4081,704;KWRD=ECOLOGY,ENVIRONMENTAL+IMPACT
8. Canniff, P. M., & Hoang, T. C. (2018). Microplastic ingestion by Daphnia magna and its enhancement on algal growth. *Science of The Total Environment*, *633*, 500–507. https://doi.org/10.1016/J.SCITOTENV.2018.03.176
9. Chen, H. L., Selvam, S. B., Ting, K. N., & Gibbins, C. N. (2023). Microplastic concentrations in river water and bed sediments in a tropical river: implications for water quality monitoring. *Environmental Monitoring and Assessment*, *195*(2), 1–10. https://doi.org/10.1007/S10661-022-10856-5/FIGURES/4
10. Cho, Y., Seo, E. U., Hwang, K. S., Kim, H., Choi, J., & Kim, H. N. (2024). Evaluation of size-dependent uptake, transport and cytotoxicity of polystyrene microplastic in a blood-brain barrier (BBB) model. *Nano Convergence*, *11*(1), 40. https://doi.org/10.1186/S40580-024-00448-Z
11. Colomer, J., Müller, M. F., Barcelona, A., & Serra, T. (2019). Mediated food and hydrodynamics on the ingestion of microplastics by Daphnia magna. *Environmental Pollution*, *251*, 434–441. https://doi.org/10.1016/J.ENVPOL.2019.05.034
12. De Felice, B., Sabatini, V., Antenucci, S., Gattoni, G., Santo, N., Bacchetta, R., Ortenzi, M. A., & Parolini, M. (2019). Polystyrene microplastics ingestion induced behavioral effects to

the cladoceran Daphnia magna. *Chemosphere*, *231*, 423–431. https://doi.org/10.1016/J.CHEMOSPHERE.2019.05.115

13. Eaton, A., Clesceri, L., Rice, E., & Greenberg, A. (2005a). *Standard Methods for the Examination of Water and Wastewater* (M. Franson, Ed.; 21st Edition). American Public Health Association.
14. Eaton, A., Clesceri, L., Rice, E., & Greenberg, A. (2005b). *Standard Methods for the Examination of Water and Wastewater* (M. Franson, Ed.; 21st Edition). American Public Health Association.
15. Frydkjær, C. K., Iversen, N., & Roslev, P. (2017). Ingestion and Egestion of Microplastics by the Cladoceran Daphnia magna: Effects of Regular and Irregular Shaped Plastic and Sorbed Phenanthrene. *Bulletin of Environmental Contamination and Toxicology*, *99*(6), 655–661. https://doi.org/10.1007/S00128-017-2186-3/TABLES/1
16. Funke, E., Webb, L., & Wolinska, J. (2024). The effect of microplastics on Daphnia fitness – Systematic review and meta-analysis. *Freshwater Biology*, *69*(4), 461–476. https://doi.org/10.1111/FWB.14228;WEBSITE:WEBSITE:PERICLES;JOURNAL:JOURNAL:13652427;WGROUP:STRING:PUBLICATION
17. Guilhermino, L., Vieira, L. R., Ribeiro, D., Tavares, A. S., Cardoso, V., Alves, A., & Almeida, J. M. (2018). Uptake and effects of the antimicrobial florfenicol, microplastics and their mixtures on freshwater exotic invasive bivalve Corbicula fluminea. *Science of The Total Environment*, *622–623*, 1131–1142. https://doi.org/10.1016/J.SCITOTENV.2017.12.020
18. Hashmi, S. I., Barrick, A., & Hoang, T. C. (2025). Discovery of microplastics in the Alabama River: Distributions, transport and loads, sources, and relative comparison with world rivers.

Journal of Hazardous Materials, *495*, 138813. https://doi.org/10.1016/J.JHAZMAT.2025.-138813

19. Henry, B., Laitala, K., & Klepp, I. G. (2019). Microfibres from apparel and home textiles: Prospects for including microplastics in environmental sustainability assessment. *Science of The Total Environment*, *652*, 483–494. https://doi.org/10.1016/J.SCITOTENV.2018.10.166
20. Hoang, T. C., & Felix-Kim, M. (2020). Microplastic consumption and excretion by fathead minnows (Pimephales promelas): Influence of particles size and body shape of fish. *Science of The Total Environment*, *704*, 135433. https://doi.org/10.1016/J.SCITOTENV.2019.135433
21. Hoang, T. C., & Rand, G. M. (2015). Mosquito control insecticides: A probabilistic ecological risk assessment on drift exposures of naled, dichlorvos (naled metabolite) and permethrin to adult butterflies. *Science of The Total Environment*, *502*, 252–265. https://doi.org/10.1016-/J.SCITOTENV.2014.09.027
22. Huang, C. H., Chu, T. W., Kuo, C. H., Hong, M. C., Chen, Y. Y., & Chen, B. (2022). Effects of Microplastics on Reproduction and Growth of Freshwater Live Feeds Daphnia magna. *Fishes 2022, Vol. 7, Page 181*, *7*(4), 181. https://doi.org/10.3390/FISHES7040181
23. Jemec, A., Horvat, P., Kunej, U., Bele, M., & Kržan, A. (2016). Uptake and effects of microplastic textile fibers on freshwater crustacean Daphnia magna. *Environmental Pollution*, *219*, 201–209. https://doi.org/10.1016/J.ENVPOL.2016.10.037
24. K. Biesinger, L. William, & W. Schalie. (2002). *PROCEDURES FOR CONDUCTING "DAPHNIA MAGNA" TOXICITY BIOASSAYS. USER'S GUIDE*. https://cfpub.epa.gov/si/si-_public_record_Report.cfm?Lab=NERL&dirEntryID=34106#:~:text=Description:,there%20was%20no%20significant%20effect.

25. Kefer, S., Friedenauer, T., & Langowski, H. C. (2022). Characterisation of different manufactured plastic microparticles and their comparison to environmental microplastics. *Powder Technology*, *412*, 117960. https://doi.org/10.1016/J.POWTEC.2022.117960

26. Kim, D., Kim, H., & An, Y. J. (2021). Effects of synthetic and natural microfibers on Daphnia magna–Are they dependent on microfiber type? *Aquatic Toxicology*, *240*, 105968. https://doi.org/10.1016/J.AQUATOX.2021.105968

27. Magni, S., Della Torre, C., Garrone, G., D'Amato, A., Parenti, C. C., & Binelli, A. (2019). First evidence of protein modulation by polystyrene microplastics in a freshwater biological model. *Environmental Pollution*, *250*, 407–415. https://doi.org/10.1016/J.ENVPOL.2019.-04.088

28. Matavos-Aramyan, S. (2024). Addressing the microplastic crisis: A multifaceted approach to removal and regulation. *Environmental Advances*, *17*, 100579. https://doi.org/10.1016/J.-ENVADV.2024.100579

29. Miner, B. E., de Meester, L., Pfrender, M. E., Lampert, W., & Hairston, N. G. (2012). Linking genes to communities and ecosystems: Daphnia as an ecogenomic model. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1735), 1873–1882. https://doi.org/10.1098/RSPB-.2011.2404;PAGEGROUP:STRING:PUBLICATION

30. Mohamed Nor, N. H., Kooi, M., Diepens, N. J., & Koelmans, A. A. (2021). Lifetime Accumulation of Microplastic in Children and Adults. *Environmental Science and Technology*, *55*(8), 5084–5096. https://doi.org/10.1021/ACS.EST.0C07384/SUPPL_FILE-/ES0C07384_LIVESLIDES.MP4

31. Mondellini, S., Schwarzer, M., Völkl, M., Jasinski, J., Jérôme, V., Scheibel, T., Laforsch, C., & Freitag, R. (2024). Size dependent uptake and trophic transfer of polystyrene microplastics

in unicellular freshwater eukaryotes. *Science of The Total Environment*, *929*, 172470. https://doi.org/10.1016/J.SCITOTENV.2024.172470

32. Murphy, F., & Quinn, B. (2018). The effects of microplastic on freshwater Hydra attenuata feeding, morphology & reproduction. *Environmental Pollution*, *234*, 487–494. https://doi.org-/10.1016/J.ENVPOL.2017.11.029
33. Na, J., Song, J., Achar, J. C., & Jung, J. (2021). Synergistic effect of microplastic fragments and benzophenone-3 additives on lethal and sublethal Daphnia magna toxicity. *Journal of Hazardous Materials*, *402*. https://doi.org/10.1016/j.jhazmat.2020.123845
34. Periyasamy, A. P., & Tehrani-Bagha, A. (2022). A review on microplastic emission from textile materials and its reduction techniques. *Polymer Degradation and Stability*, *199*, 109901. https://doi.org/10.1016/J.POLYMDEGRADSTAB.2022.109901
35. Rehse, S., Kloas, W., & Zarfl, C. (2016). Short-term exposure with high concentrations of pristine microplastic particles leads to immobilisation of Daphnia magna. *Chemosphere*, *153*, 91–99. https://doi.org/10.1016/J.CHEMOSPHERE.2016.02.133
36. Rummel, C. D., Jahnke, A., Gorokhova, E., Kühnel, D., & Schmitt-Jansen, M. (2017). Impacts of biofilm formation on the fate and potential effects of microplastic in the aquatic environment. *Environmental Science and Technology Letters*, *4*(7), 258–267. https://doi.org/10.1021/ACS.ESTLETT.7B00164/ASSET/IMAGES/LARGE/EZ-2017-00164X_0001.JPEG
37. Sadia, M., Mahmood, A., Ibrahim, M., Irshad, M. K., Quddusi, A. H. A., Bokhari, A., Mubashir, M., Chuah, L. F., & Show, P. L. (2022). Microplastics pollution from wastewater treatment plants: A critical review on challenges, detection, sustainable removal techniques

and circular economy. *Environmental Technology & Innovation*, *28*, 102946. https://doi.org/10.1016/J.ETI.2022.102946

38. Schrank, I., Trotter, B., Dummert, J., Scholz-Böttcher, B. M., Löder, M. G. J., & Laforsch, C. (2019). Effects of microplastic particles and leaching additive on the life history and morphology of Daphnia magna. *Environmental Pollution*, *255*(Pt 2). https://doi.org/10.1016/j.envpol.2019.113233
39. Schür, C., Beck, J., Lambert, S., Scherer, C., Oehlmann, J., & Wagner, M. (2023). Effects of microplastics mixed with natural particles on Daphnia magna populations. *Science of The Total Environment*, *903*, 166521. https://doi.org/10.1016/J.SCITOTENV.2023.166521
40. Schwarzer, M., Brehm, J., Vollmer, M., Jasinski, J., Xu, C., Zainuddin, S., Fröhlich, T., Schott, M., Greiner, A., Scheibel, T., & Laforsch, C. (2022). Shape, size, and polymer dependent effects of microplastics on Daphnia magna. *Journal of Hazardous Materials*, *426*, 128136. https://doi.org/10.1016/J.JHAZMAT.2021.128136
41. Sun, T., Zhan, J., Li, F., Ji, C., & Wu, H. (2021). Environmentally relevant concentrations of microplastics influence the locomotor activity of aquatic biota. *Journal of Hazardous Materials*, *414*, 125581. https://doi.org/10.1016/J.JHAZMAT.2021.125581
42. Verdú, I., Amariei, G., Rueda-Varela, C., González-Pleiter, M., Leganés, F., Rosal, R., & Fernández-Piñas, F. (2023). Biofilm formation strongly influences the vector transport of triclosan-loaded polyethylene microplastics. *Science of The Total Environment*, *859*, 160231. https://doi.org/10.1016/J.SCITOTENV.2022.160231
43. Xiong, F., Liu, J., Xu, K., Huang, J., Wang, D., Li, F., Wang, S., Zhang, J., Pu, Y., & Sun, R. (2023). Microplastics induce neurotoxicity in aquatic animals at environmentally realistic

concentrations: A meta-analysis. *Environmental Pollution*, *318*, 120939. https://doi.org/10.1016/J.ENVPOL.2022.120939

44. Yaseen, A., Assad, I., Sofi, M. S., Hashmi, M. Z., & Bhat, S. U. (2022). A global review of microplastics in wastewater treatment plants: Understanding their occurrence, fate and impact. *Environmental Research*, *212*, 113258. https://doi.org/10.1016/J.ENVRES.2022.-113258
45. Yokota, K., & Mehlrose, M. (2020). Lake Phytoplankton Assemblage Altered by Irregularly Shaped PLA Body Wash Microplastics but Not by PS Calibration Beads. *Water 2020, Vol. 12, Page 2650*, *12*(9), 2650. https://doi.org/10.3390/W12092650
46. Yu, P., Liu, Z., Wu, D., Chen, M., Lv, W., & Zhao, Y. (2018). Accumulation of polystyrene microplastics in juvenile Eriocheir sinensis and oxidative stress effects in the liver. *Aquatic Toxicology*, *200*, 28–36. https://doi.org/10.1016/J.AQUATOX.2018.04.015
47. Zhao, S., Zhu, L., Wang, T., & Li, D. (2014). Suspended microplastics in the surface water of the Yangtze Estuary System, China: First observations on occurrence, distribution. *Marine Pollution Bulletin*, *86*(1–2), 562–568. https://doi.org/10.1016/J.MARPOLBUL.2014.06.032

Appendix B

Table B1. Microplastic concentrations in number of particles/mg and mg/mL.

Microplastic types	Replicate	mg/mL	mL	Microfibers counted	Cup MPs (or microfibers)/mg	Average ± standard deviation cup MPs (or microfibers)/mg
Nylon	1	0.1	0.1	217	21,700	
Nylon	2	0.1	0.1	247	24,700	23,733 ± 1762
Nylon	3	0.1	0.1	248	24,800	
Kevlar	1	0.1	0.1	340	34,000	
Kevlar	2	0.1	0.1	363	36,300	34,133 ± 2103
Kevlar	3	0.1	0.1	321	32,100	
Cup	1	0.1	0.1	698	69,760	78,226 ± 7604
Cup	2	0.1	0.1	804	80,442	
Cup	3	0.1	0.1	845	84,475	

Table B2. Concentration of MPs and biological measurement endpoints.

Treatments	Nominal concentration (MPs/L)	MPs in water (mg/L)	MPs in water (BM) (MPs/L)	MPs in water (AM) (MPs/L)	Length of *D. magna* (mm)	Width of *D. magna* (mm)	Overall size (L*W) (mm^2)	Weight/ organism (mg/ organism)	Neonates/ organism	MPs ingested (alive) (MPs/organism)	MPs ingested (dead) (MPs/organism)
Nylon microfiber											
Ctl	0	0	0 ± 0	2 ± 2	5.37 ± 0.09	2.82 ± 0.09	15.14	0.95 ± 0.06	153.69 ± 9.84	0.23 ± 0.23	N/A
T_1	152	0.0064	1167 ± 1010	667 ± 382	5.36 ± 0.12	2.72 ± 0.02	14.61	0.94 ± 0.09	141.93 ± 16.94	0.49 ± 0.23	1.50 ± 0.00
T_2	1519	0.064	1333 ± 1876	1333 ± 804	5.34 ± 0.06	2.67 ± 0.03	14.25	0.77 ± 0.15	136.58 ± 3.88	0.58 ± 0.10	1.00 ± 0.00
T_3	5933	0.25	1833 ± 1507	2833 ± 804	5.33 ± 0.10	2.60 ± 0.00	13.86	0.92 ± 0.03	143.70 ± 6.83	0.48 ± 0.27	3.00 ± 0.00
T_4	59,333	2.5	3250 ± 2222	16,833 ± 3413	5.18 ± 0.09	2.69 ± 0.02	13.94	0.81 ± 0.09	137.65 ± 4.47	1.68 ± 0.68	N/A
T_5	593,325	25	29,083 ± 3574	201,583 ± 16633	5.30 ± 0.03	2.65 + 0.01	14.02	0.85 ± 0.04	142.43 ± 6.06	3.82 ± 2.25	8.00 ± 0.00
Kevlar microfiber											
Ctl	0	0	0 ± 0	2 ± 2	4.13 ± 0.19	2.66 ± 0.15	10.99	0.49 ± 0.05	58.15 ± 7.33	0.00 ± 0.00	N/A
T_1	219	0.0064	1083 ± 382	4500 ± 5879	4.18 ± 0.10	2.69 ± 0.13	11.23	0.54 ± 0.04	64.85 ± 9.83	0.93 ± 1.16	N/A
T_2	2185	0.064	1167 ± 1182	1667 ± 1909	4.09 ± 0.07	2.64 ± 0.04	10.79	0.48 ± 0.04	58.18 ± 6.64	1.68 ± 0.33	N/A
T_3	8533	0.25	2667 ± 3224	13,167 ± 4369	4.17 ± 0.03	2.63 ± 0.03	10.96	0.50 ± 0.02	67.90 ± 12.64	5.38 ± 1.94	51.00 ± 0.00
T_4	85,333	2.5	15,667 ± 19,348	72,500 ± 33,477	4.19 ± 0.05	2.72 ± 0.04	11.38	0.52 ± 0.03	60.49 ± 13.81	6.13 ± 2.15	120.00 ± 0.00
T_5	853,325	25	49,000 ± 59,043	339,583 ± 53,622	4.11 ± 0.05	2.62 ± 0.10	10.76	0.49 ± 0.09	59.91 ± 4.49	10.06 ± 4.93	82.50 ± 113.84
Cup MP											
Ctl	0	0	3 ± 3	0 ± 0	3.88 ± 0.04	2.44 ± 0.07	9.47	0.64 ± 0.08	66.15 ± 4.40	0.10 ± 0.14	4.00 ± 0.00
T_1	501	0.0064	1167 ± 289	1583 ± 629	3.86 ± 0.09	2.38 ± 0.07	9.18	0.63 ± 0.01	66.32 ± 11.67	0.09 ± 0.15	8.00 ± 0.00
T_2	5006	0.064	1167 ± 289	2333 ± 2309	3.92 ± 0.04	2.41 ± 0.11	9.44	0.68 ± 0.04	64.23 ± 7.29	0.15 ± 0.12	19.25 ± 2.48

T_3	19,557	0.25	8917 ± 6578	7250 ± 1521	3.93 ± 0.04	2.47 ± 0.14	9.72	0.64 ± 0.03	64.34 ± 7.67	6.65 ± 2.98	109.00 ± 0.00
T_4	195,566	2.5	14,583 ± 1258	47,667 ± 26,572	3.88 ± 0.13	2.38 ± 0.12	9.22	0.65 ± 0.06	69.93 ± 9.06	28.61 ± 13.68	N/A
T_5	1,955,651	25	38,083 ± 5210	137,472 ± 16,686	3.63 ± 0.09	2.31 ± 0.08	8.39	0.64 ± 0.05	55.78 ± 12.67	49.86 ± 21.62	224.00 ± 0.00

* The conversion factors used for converting MP concentrations from mg/L to MPs/L were 23,733 MPs/mg for nylon microfiber, 34,133 MPs/mg for Kevlar microfiber, and 78,226 MPs/mg for cup MPs (Table S1).

N/A: not applicable because of no mortality.

Table B3. Nourishment rations of *algae* and YCT fed to *D. magna* during chronic exposure to MPs.

Day	Algae (mL/beaker/day)	YCT (mL/beaker/day)
0	0.2	0.1
1,2	0.4	0.2
3	0.6 ml	0.2 ml
4,5	1 ml	0.4 ml
6-13	2 ml	1 ml
14-20	3 ml	1.5 ml
21*	N/F	N/F

*N/F: not fed because the experiment was terminated

CHAPTER 4

1. PRELIMINARY RISK ASSESSMENT FOR MICROPLASTICS IN THE ALABAMA RIVER

This chapter aims to discuss the potential impact of MPs on aquatic organisms based on the results of research in chapter 2 and chapter 3. Chapter 2 found MPs in the Alabama River with different types and sizes. The concentration varied by site and season. Results of chapter 3 research highlighted the potential effect of MPs on reproduction and growth of *D. magna.* The growth and reproduction of *D. magna* were negatively correlated with MPs in water and MPs the organisms ingested. The ingestion of MPs was dependent on particle size and plastic types. To understand the potential impact of MPs in the Alabama River on aquatic organisms, comparison of the size of MPs in the river with the size of MPs aquatic organisms ingested is necessary. Although *D. magna* doesn't represent the aquatic life of the Alabama River, they play an important role in aquatic ecosystems, especially in shallow habitat along the river edge. This initial risk assessment analyzed the size distribution of MPs in the Alabama River found in chapter 2 and MPs *D. magna* ingested in chapter 3 to determine the bioavailability of MPs in the Alabama River to *D. magna*. The particle size distribution method used in chapters 2 and 3 was used for analysis in this chapter. Since the ingestion and effects of MPs on *D. magna* were different for microfibers and non-microfibers, risk analysis in this chapter was conducted for microfibers and non-microfibers in the river. The probability of MPs in the Alabama River having sizes smaller than the 90^{th} percentile of the size of MPs *D. magna* ingested was calculated for different scenarios regarding the sites and seasons studied in chapter 2. The potential risk is expected to be higher for a scenario with a higher probability than others with lower probabilities. Fig. 4.1 and Fig. 4.2 show the cumulative

frequency distribution of microplastic size by season (A), site (B), and all sites and seasons (C) for fibers and non-fibers respectively.

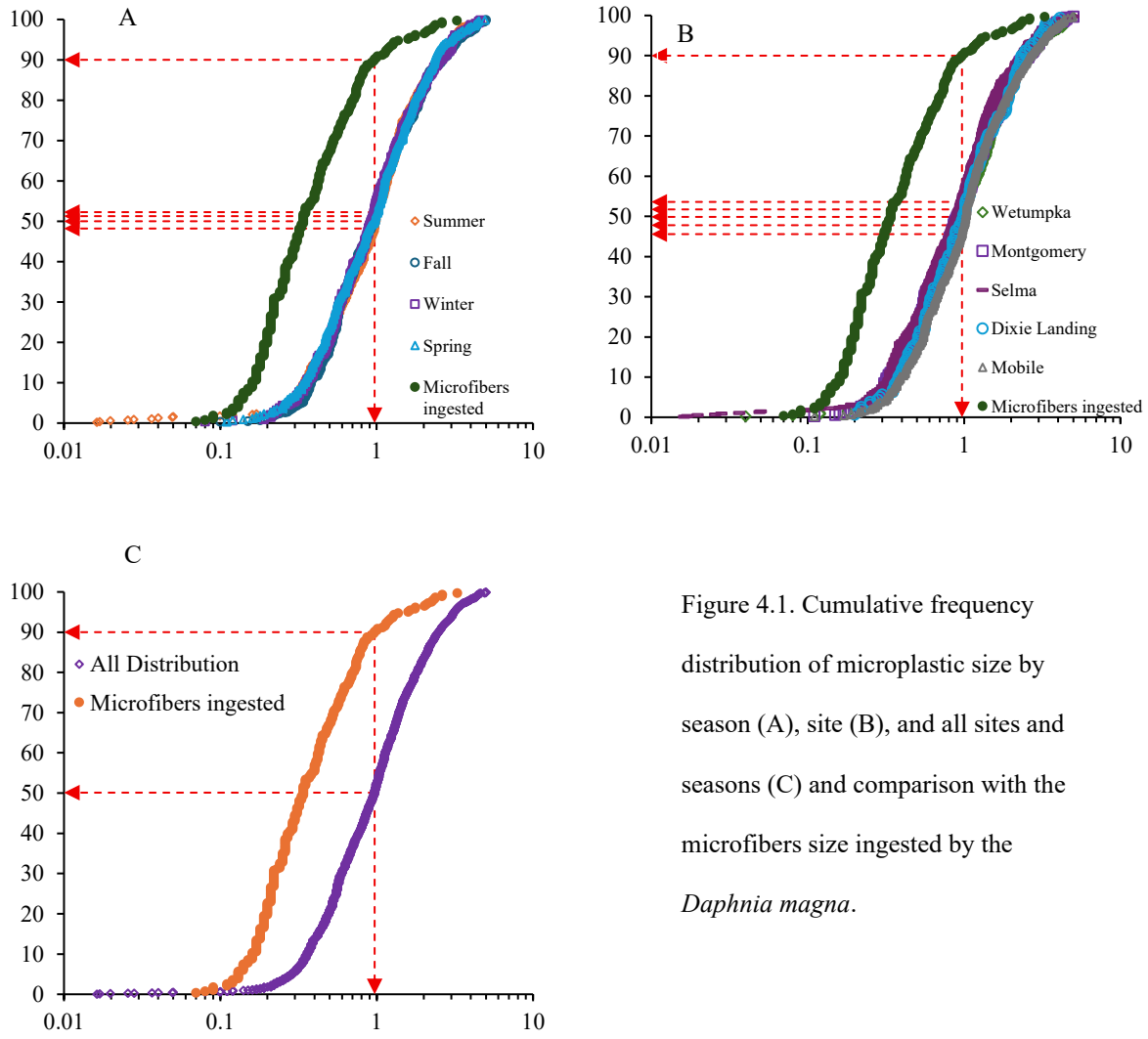

Figure 4.1. Cumulative frequency distribution of microplastic size by season (A), site (B), and all sites and seasons (C) and comparison with the microfibers size ingested by the *Daphnia magna*.

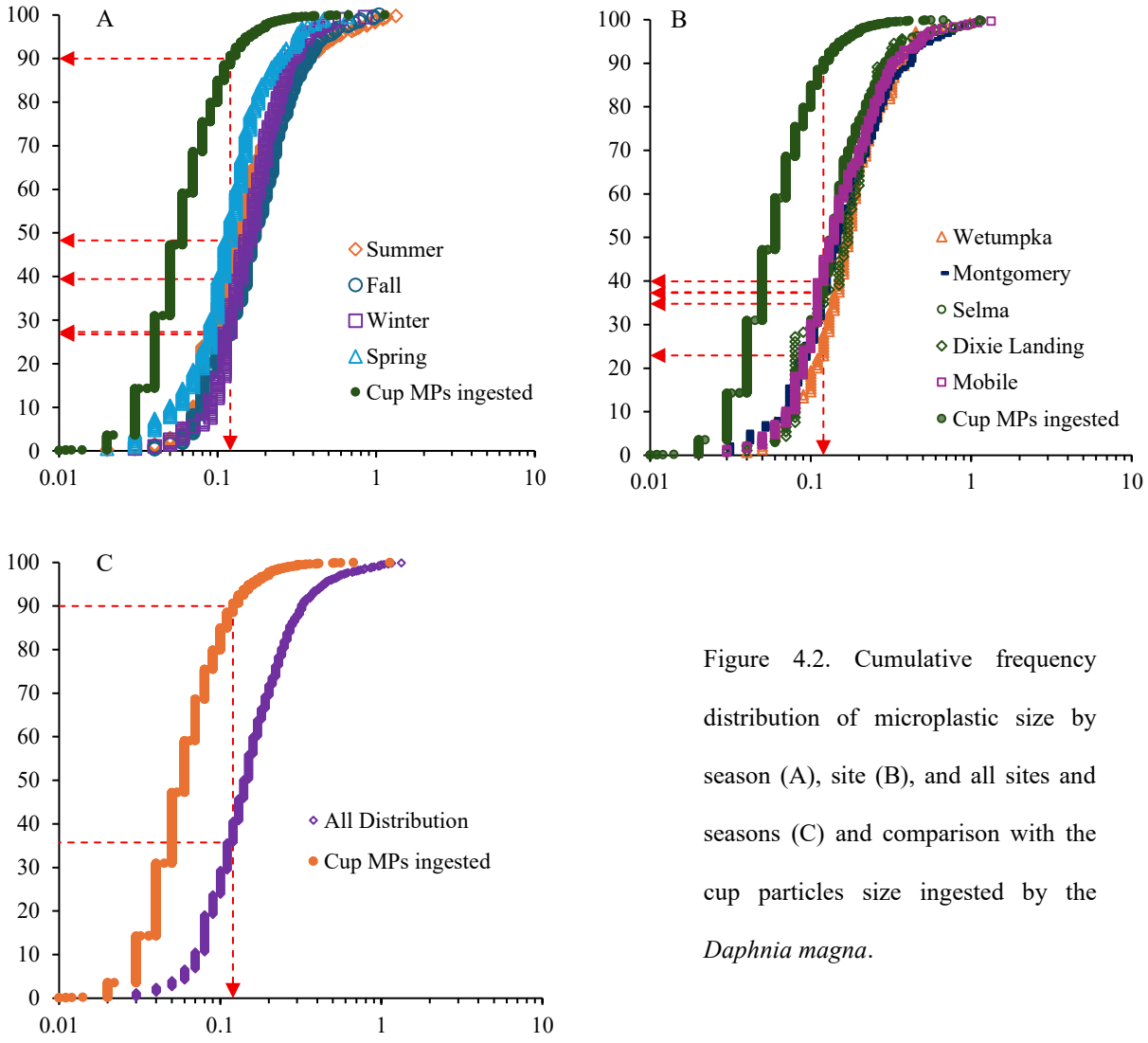

Figure 4.2. Cumulative frequency distribution of microplastic size by season (A), site (B), and all sites and seasons (C) and comparison with the cup particles size ingested by the *Daphnia magna*.

The size distributions of MPs are illustrated in Fig. C1 (for fibers, Appendix C) and Fig. C2 (for non-fibers, Appendix C). Fig. C3 and Fig. C4 show the size distributions of MPs by season for fibers and non-fibers, respectively. The probabilities of MPs in the Alabama River within the 90th percentile of size of MPs ingested by *D. magna* are presented in Table 4.1.

Table 4.1. Probability of MPs in the Alabama River with sizes within the 90th of the size of ingested MPs.

Site	Season	Probability of MPs in the Alabama River with sizes within the 90th of the size of ingested MPs	
		Microfibers	Non-microfibers
Wetumpka	Spring	40.78	40.11
	Summer	48.21	21.88
	Fall	57.83	09.52
	Winter	55.21	28.00
Montgomery	Spring	56.00	55.56
	Summer	44.09	40.43
	Fall	50.47	25.00
	Winter	57.50	36.36
Selma	Spring	58.33	42.55
	Summer	51.75	42.94
	Fall	51.50	30.30
	Winter	57.91	19.57
Dixie Landing	Spring	49.78	NA
	Summer	36.96	09.76
	Fall	55.50	75.00
	Winter	50.40	17.65
Mobile	Spring	43.70	53.97
	Summer	50.97	50.83
	Fall	44.98	11.29
	Winter	39.29	35.71
Wetumpka	All seasons	49.87	22.92
Montgomery	All seasons	51.74	37.44
Selma	All seasons	53.81	37.27
Dixie Landing	All seasons	48.43	34.78
Mobile	All seasons	45.58	39.93
All sites	Summer	48.19	39.44
All sites	Spring	50.00	48.28
All sites	Fall	51.27	26.74
All sites	Winter	52.26	27.27
All sites	All seasons	50.11	35.74

*NA data were not available because non-microfiber particles are not found in the samples.

There was a difference in the MP size distribution by season and site of the Alabama River. The site with the highest percentage of microfibers that were within the ingestible size range of *D. magna* was Selma in the spring (58.33%). Dixie Landing in the summer appeared to have longer microfibers with 36.96% being within the ingestible size range of *D. magna*. Interestingly, Dixie Landing in the fall had the smallest non-microfibers. Up to 75% of non-microfibers found in Dixie Landing in the fall were smaller than the ingestible MPs by *D. magna*. Most non-microfibers found in Wetumpka in the fall were larger than the ingestible MPs *D. magna*. Only 9.52% non-

microfibers would be ingestible by *D. magna*. Overall, 50 % of the microfibers and almost 36 % of the microplastic particles present in the Alabama River were found to be in the ingestible size range of *D. magna*. Less variation between sites and seasons was noted for microfibers than for non- microfibers. Regardless of the sites, 48 % microfibers and 40 % non-microfibers in summer, 51 % of the microfibers and 27 % of non-microfibers in fall, 52 % microfibers and 27 % non-microfiber in winter, and 50 % microfibers and 48 % non-microfibers in spring were in the ingestible size range of *D. magna*. Selma site seems to be polluted with smaller size microfibers than other sites in the Alabama River as the probability always remained above 50% at this site with the highest probability of 58% during spring season. Whereas the least probability among the ingestible size was noted to be 37% at Dixie Landing site in summer season.

Compared to microfibers, non-microfibers were found to be lower in concentration with no MPs detected at Dixie Landing site during spring season. In fall season, the highest probability of ingestible MP particles size was also recorded at the same site. However, compared to the probabilities for microfibers, non-microfibers probabilities were lower at most of the sites due to higher prevalence of microfibers compared to the non-microfibers in river ecosystems.

Overall, the results of this initial risk analysis showed that MPs in the Alabama River are highly available for small and sensitive aquatic organisms to ingest. The ingestion of MPs poses potential risks to aquatic life through the direct impacts on their growth and reproduction and indirect impacts via transfer of MPs through the food chain and food web. It is imperative to conduct more research with different plastic types and different aquatic species to gather more data for detailed risk assessments to support development of policies and regulations for ensuring a safer ecosystem and habitat for aquatic species and for human beings.

APPENDIX C

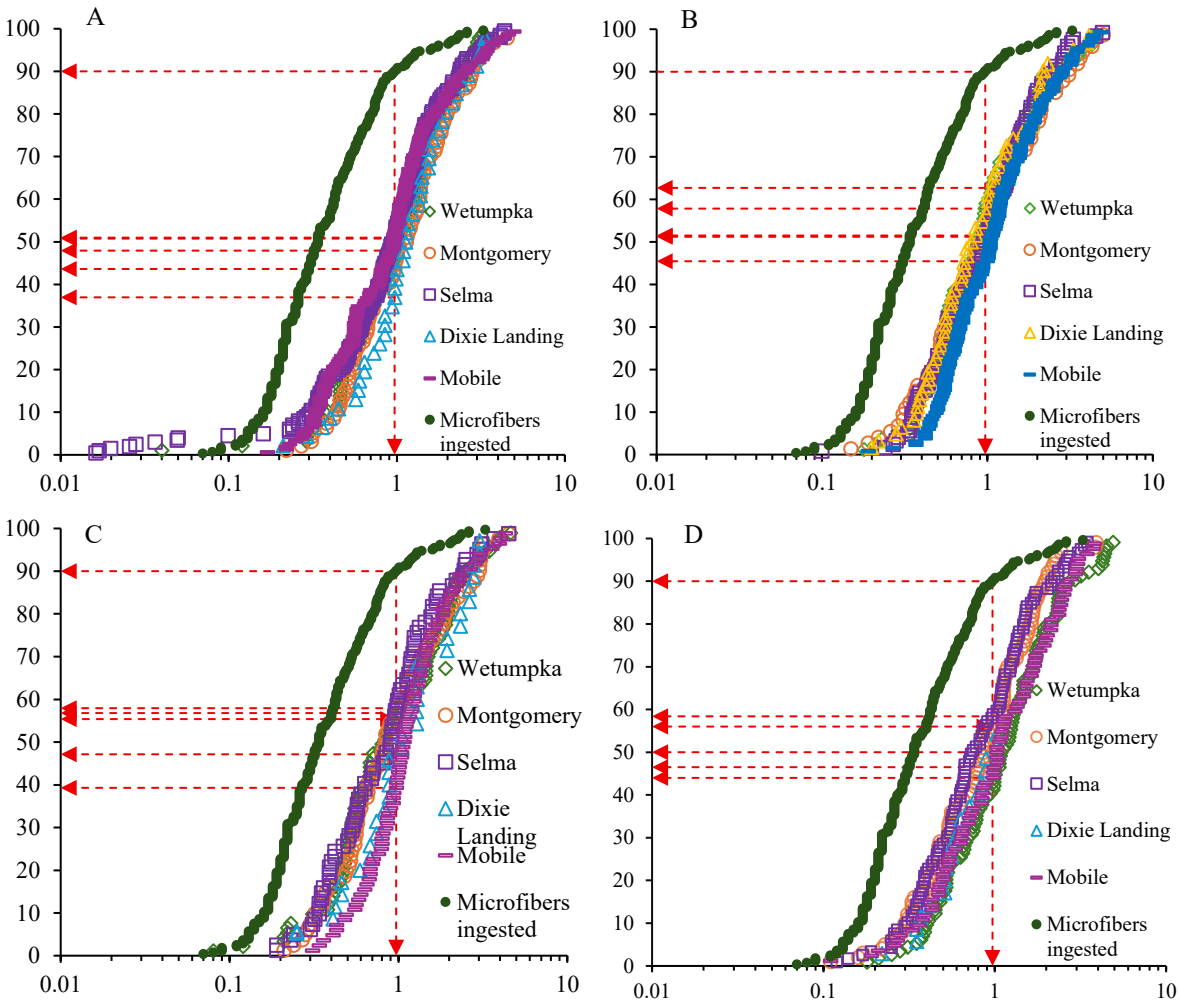

Fig. C1. Cumulative frequency distribution of microplastic size by site and comparison with ingested microfibers by *Daphnia magna* (A: summer, B: fall, C: winter, D: spring).

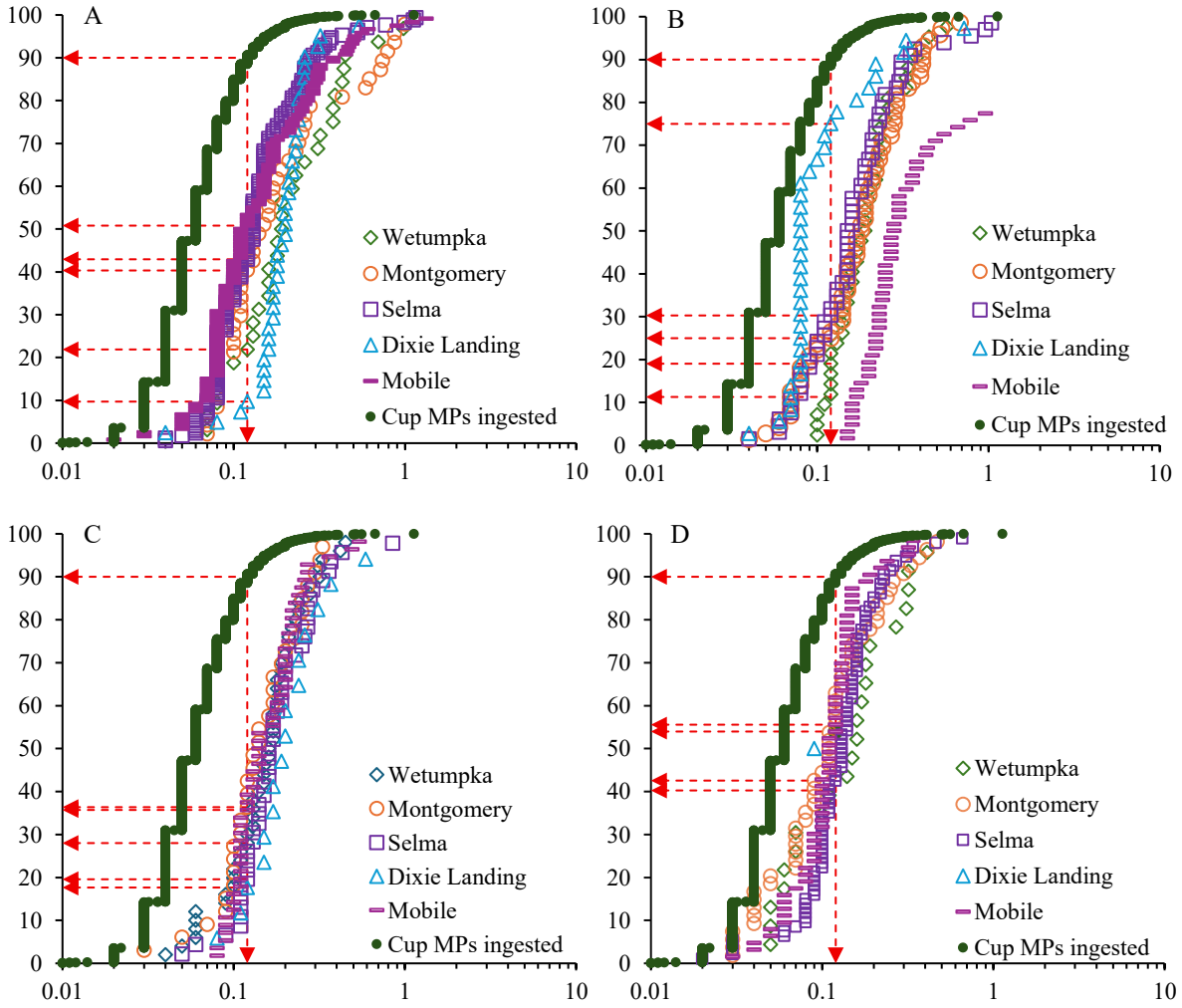

Fig. C2. Cumulative frequency distribution of microplastic fragments (non-fiber) size by site and comparison with ingested cup MP particles by *Daphnia magna* (A: summer, B: fall, C: winter, D: spring).

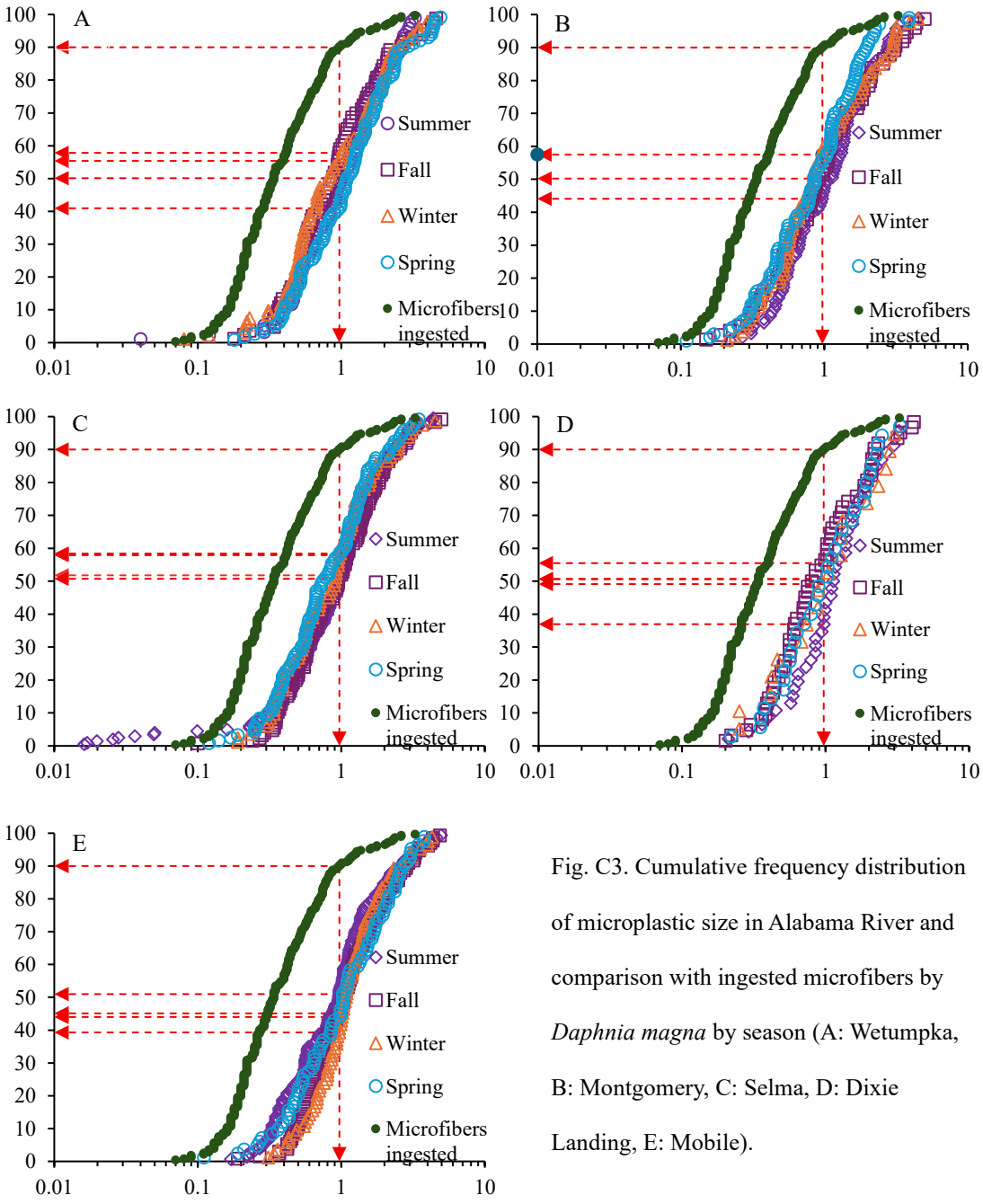

Fig. C3. Cumulative frequency distribution of microplastic size in Alabama River and comparison with ingested microfibers by *Daphnia magna* by season (A: Wetumpka, B: Montgomery, C: Selma, D: Dixie Landing, E: Mobile).

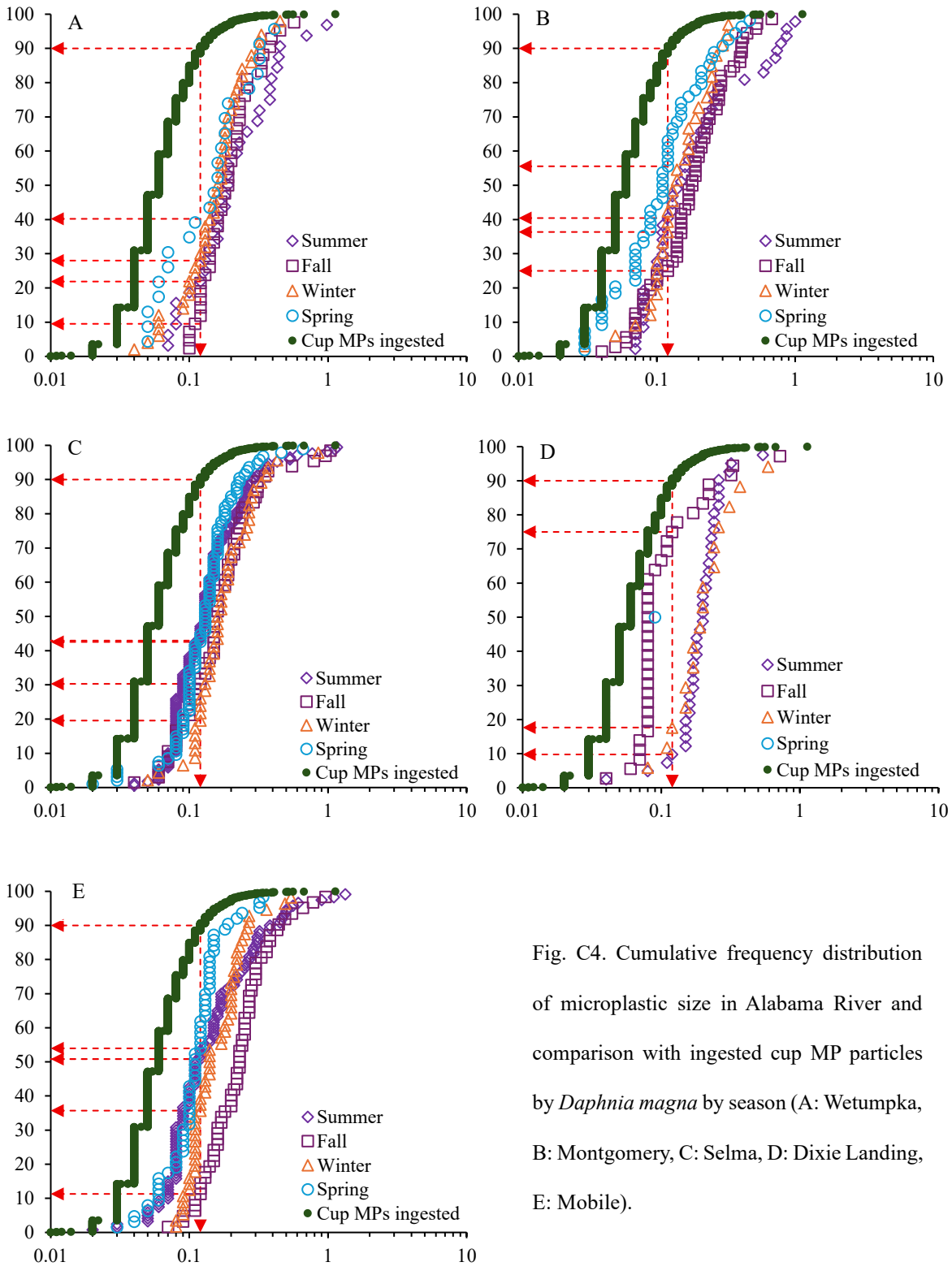

Fig. C4. Cumulative frequency distribution of microplastic size in Alabama River and comparison with ingested cup MP particles by *Daphnia magna* by season (A: Wetumpka, B: Montgomery, C: Selma, D: Dixie Landing, E: Mobile).