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# Drivers of Microparticles Abundance in Freshwater Fish and Macroinvertibrates

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# DRIVERS OF MICROPARTICLES ABUNDANCE IN FRESHWATER FISH AND MACROINVERTEBRATES

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ABSTRACT

Anthropogenic microparticles (MP) (i.e., particles < 5 mm) are defined as materials of manufactured origin found in the environment. They are widespread and a rapidly emerging global contaminant. MP include microplastics (< 5 mm), as well as other microfibers or particles, which can be synthetic, semi-synthetic and non-synthetic. Rivers are a critical source of plastics to oceans, and MP within rivers are retained in part though biological processes, including ingestion. MP ingestion by freshwater biota varies by proximity to point sources (e.g., wastewater treatment plants; WWTP), overall environmental concentrations, and trophic level and functional feeding groups, but few studies have examined these factors together. In our first study we examined fish collected in the North Shore Channel of the Chicago River to determine MP abundance within organisms. We then compared trophic position (i.e., $\delta^{15}$N) and proximity to a WWTP. With all species combined, MP concentration were not significantly different across study sites, but stable isotopes increased downstream. Considered individually, MP abundance relative to WWTP proximity varied by species, which we attributed to habitat preference and mobility. In a second project, we collected macroinvertebrates from three different watersheds in North America and compared the abundance of MP in macroinvertebrates to concentration within the environment (i.e., water column, surface, and benthic). Functional feeding groups were a significant predictor in the amount of microparticles found in macroinvertebrates. Additionally, the concentration of water column microparticles showed a positive relationship with all macroinvertebrate's microparticle abundance. Results will aid in understanding the ecological impacts of MPs on aquatic food webs and the pathways in which they can enter.

# CHAPTER I

# INTRODUCTION

## *Plastic in the environment*

Plastic pollution is inescapable in our modern world. Plastic pollution is ubiquitous at a global scale, including deep ocean, desert, tundra, tropical, and mountain habitats (MacLeod et al 2021). Research on plastic pollution has often focused on marine environments, as this was where conspicuous accumulations of plastic in the open ocean and on isolated coastlines were first documented. As the research on plastic pollution developed, many studies suggested that a large proportion of plastic found in the ocean was derived from freshwaters (Weiss et al 2021). Thus, plastic pollution is likely moving into freshwater ecosystems, and eventually to oceans, but there has been less study of plastic *within* freshwaters such as rivers and streams (van Emmerik et al 2022).

Plastic pollution is categorized by size. Macroplastics are plastic particles greater than 5 mm and microplastics are considered particles between 1 um and 5 mm (Hartmann et al 2019). Microplastics are a major focus of research because they are pervasive in aquatic environments, easily moved, and interact with organisms. Sources of microplastics to freshwaters include stormwater, agricultural runoff, wastewater treatment plants (WWTP), direct littering, atmospheric deposition, and fragmentation of other larger plastics (Hoellein and Rochman 2021, van Emmerik et al 2022). “Anthropogenic microparticles” are a broader category of pollutants that include microplastics and are defined as particles 1 um -5 mm and are materials found in environment of manufactured origin (Adams et al. 2021). Microparticles can be synthetic, semi-

synthetic or non-synthetic (Adams et al 2021, Collard et al 2018). This can include plastics, but also other materials such as textiles manufactured from natural materials (e.g., cotton, cellulose, silk, and wool), many of which are highly processed and contain additional pollutants including dyes, flame retardants, and antimicrobial compounds (Henry et al 2019). Anthropogenic microparticles are widespread and rapidly emerging contaminants found at a global scale (Rochman et al. 2016). Sources of microparticles are similar to sources of microplastics as described above. Urban landscapes have high densities of point and non-point sources of microparticles, and microparticles concentration in rivers is positively related to urban land use (Grbić *et al* 2020, Ballatore et al 2021).

A major point source pollutant of microplastics and microparticles in urban rivers are WWTPs (McCormick *et al.* 2016, Edo *et al.* 2020). Microparticles in treatment plants come from the fragmentation of textiles in washing machines, microplastics in personal care products (i.e., soap, toothpaste, and makeup), and plastic litter which enters sewers from littering. A high volume of microparticles enter WWTP daily. Fibers and microbeads are the most common shape of microparticles found (Bakaraki Turan et al 2021). The infrastructure of WWTPs is not designed to intercept microparticle pollution, however WWTPs can remove up to 99% of microplastics from raw sewage compared to untreated effluent (Carr *et al.* 2016, Bakaraki Turan et al 2021). The microparticles are typically captured in WWTP settling basins. Despite particle retention within WWTPs, due to the large volume of particles that enter a plant daily, the particles that leave the treatment plant and enter waterways create detectable differences in microparticles up and downstream of the release site. McCormick et al (2016) found that in 5 rivers in the Chicago region concentrations of microplastics were significantly greater directly downstream of the effluent release site compared to upstream. This greater exposure of

microplastics to organisms that live near the effluent release site may increase the abundance of microplastics found within their digestive tracts (Ziajahromi *et al*. 2016, Garcia *et al.* 2021).

*Microparticle interaction with organisms*

Once in the environment, plastic pollution can interact with aquatic organisms. The relative amount of microparticles in the environment is likely positively related to the amount of microparticles found in organisms that live in that environment. However, studies comparing these two factors have found this relationship ambiguous. For example, microplastics in macroinvertebrates in small streams were not directly linked to the environmental concentration (Simmerman and Wasik 2019, Windsor *et al.* 2019). Similarly, measurements of microplastics in fish were unrelated to microplastic concentrations in larger rivers (Simmerman and Wasik 2019, Garcia *et al.* 2021, and McNeish *et al.* 2018), and concentration of microplastics in dreissenid mussels were not related to environmental concentrations at multiple Lake Michigan sites near Milwaukee, Wisconsin (Hoellein *et al.* 2021). One factor to explain this disparity may be that microparticle concentrations in the environment are often considered a single value instead of the habitats where the organisms of interest are most likely to live and feed. For example, the concentration of microparticles in the water column can be significantly different than concentrations at the surface or in the benthos when taken at the same time and location. Microparticle distribution in benthic habitats of streams is heterogeneous, with greater abundance in depositional sites relative to erosional habitats such as cobbles (Vincent and Hoellein 2021). Also, the density of different polymer types lends some to be more buoyant and float on the surface while others my sink more quickly (Lenaker et al 2019). Because organisms feed or inhabit distinct locations in a stream, the concentration of microparticles they are exposed to within these varying locations will likely be reflected in the concentration of microparticles

found in their digestive tracts. However, no previous research has considered spatial heterogeneity of microparticles in the environment relative to microparticle abundance within stream organisms.

In addition to environmental exposure, microplastic concentrations within stream fish and macroinvertebrates is likely affected by trophic level, with those higher in the food web showing greater potential for microplastic ingestion (Krause *et al.* 2021). While microparticles can enter the food web at any trophic level via direct ingestion, organisms at higher trophic levels might also be enriched with microparticles due to bioaccumulation via predation (Krause *et al.* 2021). Trophic level can be assessed using different techniques. Methods like database comparison (e.g., fishbase), gut contents, and waste analyses consider a "snapshot" of an organism's food web interactions at any one given time. Individuals within a species can show variation in their trophic level, however this is obscured by species-wide estimates of categories such as predator, omnivore, or herbivore (Garcia *et al.* 2021). Stable isotopes can be used as a tool to quantify trophic niche by analyses of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$). This method allows quantification of diet reflecting an individual's activity over several weeks. Stable isotopes analysis are individual-based assessments, and valuable to reveal variability in trophic levels that can occur within a species (Garcia *et al.* 2021).

*Microparticles ecological effect on fish and macroinvertebrates*

Ingestion of microplastics and microparticles has been documented in fish across many species (Hossain and Olden 2022), with negative effects on fish including consumption rates, growth, reproduction, and survival (Foley et al 2018, Galafassi et al 2021, Hossain and Olden 2022). Freshwater fish have ecological and economical importance, including as human food sources and drivers of primary and secondary production of rivers (Welcomme 1985). Fish

provide other ecosystems services such as recycling of nutrients, control of pathogens and nuisance algae, and support of recreational activities (Holmlund and Hammer 1999). Microparticles are a pollutant of concern to these organisms. Understanding the factors that influence interaction and consumption of microparticles by fish is important to help inform mitigation and conservation strategies. For example, combining measurements of microparticles in fish digestive tracts, along with simultaneous measurements of gut contents and stable isotopes, could help illustrate the role of trophic level on microparticle abundance in fish.

Aquatic macroinvertebrates also ingest and interact with microplastics and microparticles. Once particles are ingested by macroinvertebrates, they can cause reduced feeding, reproduction, growth, and survival (Gago et al 2020, Garcia et al 2021). Microplastics can reduce the mobility of macroinvertebrates if attached to a limb, which can also increase chances of being preyed upon (Aljaibachi and Callaghan 2018, Nantege et al 2023). In lab experiments, increased microplastics exposure reduced feeding rates of *Sericostoma pyrenacium* (Trichoptera) larvae (Lopez-Rojo et al. 2020). Similarly, when the amphipod *Gammarus pulex* was exposed to chronic levels of microplastics, growth rates and reproduction decreased due to their consumption of plastics (Redondo-Hasselerharn et al 2018). Most analyses of microparticles' impacts on macroinvertebrates have been examined in the laboratory. Thus, field-based studies on the presence of microparticles found within macroinvertebrates of varying functional feeding groups are needed to offer insight into how microparticles effect *in situ* food web dynamics.

Once microparticles are ingested by aquatic organisms they have the potential to be transferred in the food web, however, measurements of microparticles across multiple species in freshwater ecosystems is lacking. Some preliminary evidence has linked trophic level with

microparticle abundance in freshwater fish and macroinvertebrates. Microplastics can bioaccumulate in higher trophic levels due to trophic transfer (Cuthbert et al 2019). High abundance of microplastics has been found in predators compared to omnivores and detritivores, suggesting microplastic trophic transfer (McNeish *et al.* 2018). The pathways microplastics take into the food web via direct consumption or trophic transfer depend both on the organism's feeding type and environmental concentration of microplastics (Garcia *et al.* 2020). More studies are needed to assess the role of trophic level on microparticle abundance in organisms and will benefit from including multiple methods for estimating trophic level across a range of fish and macroinvertebrate species.

*Thesis objectives*

Microparticle concentrations within riverine organisms is driven by a combination of factors including the relative abundance of microparticles in the environment, habitat preference, position in the food web, and proximity to a point source. However, no previous research has assessed the role of multiple factors on microplastic abundance in stream macroinvertebrates and fish at multiple spatial scales. I measured microparticle concentrations in multiple aquatic habitats (i.e., surface, water column, and benthic) and in aquatic organisms that represent a range of habitat preference and trophic levels, conducted across multiple sites in multiple watersheds. My thesis covers two research projects examining the drivers of microparticles abundance in freshwater fish and macroinvertebrates: 1) I used fish from the Chicago River to examine microparticle abundance in proximity to a known point source pollutant and trophic position, and 2) I collected macroinvertebrates from three watersheds and determined their microparticle abundance compared to environmental microparticle concentrations from the sites they were

collected. Results from these projects will inform our understanding of how microparticles enter and move in aquatic food webs.

# CHAPTER II

# MICROPLASTICS IN FISH RELATIVE TO POINT SOURCE PROXIMITY AND TROPHIC LEVEL IN AN URBAN RIVER

## Introduction

Microplastics (particles < 5 mm) are pervasive in aquatic environments globally (Walker 2021, Du et al 2020, Li et al 2020). In freshwater ecosystems, sources of microplastics to the environment include stormwater, agricultural runoff, wastewater treatment plants (WWTP), direct littering, and atmospheric deposition (Hoellein and Rochman 2021). Urban landscapes have high densities of point and non-point sources of microplastics, and microplastics concentrations in rivers is positively related to urban land use (Li et al 2023, Kunz et al 2023, Grbić *et al* 2020). Understating the sources, fate, and biological interactions of microplastics in urban waterways is an important step in conservation of critical freshwater resources and organisms.

Fish consume microplastics in freshwater ecosystems (Scherer *et al.* 2017, Hou *et al.* 2021). Organisms may intentionally consume microplastics if mistaken for food, or unintentionally consume microplastics via ingestion of plastics within their prey (Wang et al. 2020, Foley *et al.* 2018). Physical effects of ingestions may result in internal injuries to fish and feelings of fullness that reduce foraging (Wright et al 2013). A review of literature which studied the biological effects (e.g., consumption, growth, reproduction, and survival) of microplastic ingestion by freshwater fish revealed a range of potential impacts, including neutral and negative impacts that are variable across taxa and trophic levels (Galafassi et al 2021, Wootton et al 2021,

Wang et al 2020, Foley *et al.* 2018). Negative impacts on health include sublethal problems related to ingestion and feeding behavior (Galafassi et al 2021, Wang et al 2020) and growth (Pannetier et al 2020).

WWTP are a major point source of microplastic pollution in urban rivers (McCormick *et al.* 2016, Edo *et al.* 2020). Microplastics are present in wastewater due to fragmentation of plastic textiles in washing machines, microplastics in personal care products (i.e., soap, toothpaste, and makeup), and plastic litter from the environment which enters street sewers from littering (Hamidian et al 2021, Yaseen et al 2022). Although WWTP are not designed to capture microplastic pollution, studies suggest they can remove up to 99% of microplastics from raw sewage compared to untreated effluent (Carr *et al.* 2016). Despite their relative efficiency in microplastic removal, WWTPs release treated effluent into rivers which contains microplastics. For example, concentrations of microplastics were significantly greater directly downstream of effluent release sites in 5 rivers in the Chicago region (McCormick *et al.* 2016). It follows then that organisms near effluent release sites are exposed to higher concentrations of microplastics from WWTP effluent and may have higher abundance of microplastics in their digestive tracts (Ziajahromi *et al*. 2016, Garcia *et al.* 2021, Park et al 2020).

In addition to environmental exposure, microplastic concentrations within stream fish may be affected by trophic level. Every trophic level has the potential for direct microplastic ingestion into the food web, which can lead to biomagnification of plastics in top predators of the food web (Krause *et al.* 2021). Preliminary evidence of positive links between trophic level and microplastic abundance in freshwater fish are mixed. Some data showed microplastic abundance in fish from higher trophic levels was greater than those in lower tropic levels (McNeish et al. 2018, Costa et al. (2023). In contrast, some evidence suggests omnivorous fish have more

microplastics in them (Garcia et al 2020). It remains unclear whether trophic position or foraging behavior contributes more to microplastic ingestion.

Assessing fish trophic level and feeding habits is done by several different techniques. Common approaches include using database records for species trophic levels (Froese and Pauly 2017) and analyses of gut or feces contents. However, database records represent a broad average for a species, and gut contents only consider a "snapshot" of an organism's food web interactions at a given moment in time (Garcia *et al.* 2021). Stable isotopes can be used as a tool to quantify trophic niche whereby carbon ($\delta^{13}C$) helps determine sources of primary production, and nitrogen ($\delta^{15}N$) aides in quantifying trophic levels away from such sources (Alp & Cucherousset 2022). This method allows quantification of diet reflecting an individual's activity over several weeks to months (Hette-Tronquart, 2019). Stable isotopes analyses are individual-based assessments, and valuable to reveal variability in trophic levels and foraging habits that can occur within a species. To date, few studies assess how microplastic abundance in fish responds to estimated trophic levels via stable isotope analysis despite the known benefits of including multiple methods for estimating trophic level and species diets (Garcia et al. 2021).

Microplastic concentrations within freshwater fish is driven by a combination of many factors, including the relative abundance of microplastics in the environment, position in the food web, and proximity to a point source. However, previous studies have not assessed the role of multiple factors on microplastic abundance in urban stream fish simultaneously. We measured microplastic concentrations, estimated trophic position via $\delta^{15}N$, and assessed stomach contents for fish of varying functional groups in a heavily modified urban river to better understand factors driving microplastic infiltration into aquatic food webs. We predict higher microplastic concentration at the WWTP effluent site and several kilometers downstream site compared to an

upstream site. We also predicted that microplastic abundance would differ among functional groups, trophic positions (i.e., $\delta^{15}N$), and stomach contents.

**Methods**

*Study site*

The Chicago River is part of the Chicago area waterway system (CAWS), which is a network of waterbodies engineered to manage storm and wastewater. Between 1889 and 1910 a system of locks and dams was constructed to regulate water movement from Lake Michigan into the Chicago River. The North Shore Channel (NSC) is part of CAWS, created to help drain Chicago's northern communities as well as pull Lake Michigan water towards the Chicago River to increase its flow. The Terrence J. O'Brien water reclamation plant processes about 966 million liters of water per day which is dispatched into the NSC near Touhy Avenue in Evanston, IL, approximately 7.4 km downstream of Lake Michigan. The O'Brien WWTP is known to be a point source of microplastics (McCormick et al. 2014, McCormick et al. 2016).

For our study we chose three locations surrounding the T.J. O'Brien WWTP: upstream, at the effluent site, and downstream. Our upstream (Up) site is approximately 2 km upstream from the effluent release site, at the Dempster Street bridge crossing. Water at this location is largely from Lake Michigan and is low in microplastics (McCormick et al. 2016). We also selected a site 115 m downstream of the WWTP effluent released site (WWTP), where the NSC crosses Touhy Avenue in Chicago, IL. Finally, our downstream ("Down") site was the intersection of the NSC with Diversey Avenue, in Chicago, approximately 10 km downstream of the WWTP. This site has water from Lake Michigan, WWTP effluent, and water from the North Branch Chicago River watershed (Hoellein et al. 2017, Vincent and Hoellein 2021).

*Fish specimen collection*

Fish specimens were collected during an annual monitoring program conducted by the Metropolitan Water Reclamation District of Great Chicago (MWRD). Fish are collected by MWRD through the 'Ambient Water Quality Monitoring' program which continuously measures the health of the waterways which receive WWTP effluent from MWRD plants. MWRD collects fish via pulsed-Direct Current (120 pulse sec $^{-1}$ targeting 12-14 amps) electrofishing at several sites throughout the CAWS. In 2019, large numbers of small fishes were captured from multiple locations throughout the CAWS. To facilitate accurate identification and measurement, a majority of the individuals were euthanized (MS-222; Tricaine-S: 0.26 g/L) and preserved (Carosafe, propylene glycol; Carolina Biological Supply, Burlington, NC, USA). Collection and handling protocols were consistent with accepted methods from the American Fisheries Society (Midway et al. 2022) and the American Veterinary Medical Association (Leary et al. 2013). From these stored samples we selected 6 species (*Lepomis macrochirus*, *Neogobius melanostomus*, *Fundulus notatus, Pimephales notatus*, *Notemigonus crysoleucas*, and *Dorosoma cepedianum*) with large enough sample sizes across our 3 sites of interest (ideally, $n = 5$ individuals per species per site) (Table 1). These species spanned a gradient of trophic levels and feeding guilds (Table 1).

*Microplastics analysis*

We examined microplastics in fish gut contents following similar procedures in previous studies (McNeish et al. 2018, Hou et al. 2021, Hou et al. 2022). First, each preserved fish and materials used for dissection (i.e., enamel tray, scalpels, dissecting scissors, forceps, and ruler) were rinsed with DI water that was pre-filtered through a 363 µm mesh (hereafter: DI water). We measured fish wet weight (g) and standard length (mm) (i.e., from the tip of the snout to the

caudal fin). We used a scalpel or dissecting scissors to cut from the mouth to the urogenital opening along the ventral side of the fish, exposing the entire digestive tract. We removed the digestive tract and placed it in a pre-cleaned glass mason jar and immediately covered it with a metal lid. We recorded the amount of time elapsed during the dissection (i.e., from when the fish was removed from its container to the time the digestive tract was placed in the glass jar) to conduct timed accounts for contamination (see below). Between dissections, we rinsed scalpels, forceps, dissecting scissors, and tray with DI water, and we changed gloves to prevent contamination (McNeish et al. 2018, Hou et al. 2021, Hou et al. 2022).

After dissections, fish digestive tracts were dried, digested, and filtered (Hou et al. 2022). The glass jars containing the samples were covered with aluminum foil and placed in a drying oven at 40ºC overnight or until the sample was dry (1,320 Economy Oven, VMR, Radnor, Pennsylvania, USA). After cooling to room temperature, we added 20 mL of 30% hydrogen peroxide and 20 mL of 0.05 Fe (II) solution (0.05 mol $L^{-1}$ $FeSO_4$ + 3 mL $H_2SO_4$) and placed jars on a rotation table for at least 24 hours to break down organic material without impacting the recovery of microplastics (Hoellein et al. 2021, McNeish *et al.* 2018, Lusher et al. 2017, Munno et al. 2018). After the 24-hour shaking at room temperature we added 20 ml of $H_2O_2$ and placed samples in a 40ºC oven. When the digestive tract organic material was no longer visible, the remaining solution was filtered with a vacuum onto a gridded cellulose fiber filter (0.45 um pore size: Whatman, Pittsburgh, Pennsylvania, USA). We transferred the filter onto a 20 mL aluminum pan (Thermo Fisher Scientific Incubator, Marietta, Ohio, USA) and covered it with aluminum foil (Hou et al. 2022 and Hoellein *et al.* 2021). The pan and filter were placed in the drying oven dried at ~45ºC overnight. Using a dissecting microscope (x25-30 magnification; model ASZ30L3, Bausch & Lomb, Rochester, New York, USA), we counted all microplastics,

and categorized by shape (fiber, fragment, film, foam) and color, and measured length and width (Hou et al. 2021). Samples were assessed by two independent researchers and if counts did not agree (by +/- 3 microplastics) a third independent researcher counted the sample (Hou et al. 2021). We recorded the lowest value from the researchers' assessment.

We prepared microplastics for micro- Fourier transform infrared (μFT-IR) identification. We placed a thin layer of Skin Tac (Torbot Group Inc.), which is a rosin-based adhesive, on a pre-cleaned glass microscope slide (Thaysen *et al*. 2020). We placed particles on the rosin individually using forceps. The first 3 particles of each color-shape-combination was selected from each slide (e.g., the first three clear fibers, the first three black fibers, and so on). If a filter did not have 3 particles of a particular color-shape-combination, then all were placed on the slide. We drew circles around each particle using a fine tip marker, and left the slides covered with a box while the rosin dried. Then we placed glass cover slips over the particles, secured the edges with tape, and stored slides securely until polymer identification. For the polymer identification we used a micro-FT-IR spectrometer (Spotlight 200i, Perkin Elmer, Waltham, MA, USA) in attenuated total reflectance (ATR) mode via a 100 μm diameter germanium crystal. Spectrum results from 16 scans were saved under micro-ATR mode across wavelengths from 650 $cm^{-1}$ to 4000 $cm^{-1}$. Results were compared to a reference library and known standards using Spectrum 10 Software (Perkin Elmer) with a target match between samples and standards at 0.53-0.95. Background scans were taken before each analysis scan of a particle.

*Accounting for contaminations*

All laboratory surfaces were wiped with a cellulose sponge (JINCLEAN; model nCratch10, Amazon.com) soaked in DI water prior to digestion. All researchers wore yellow polypropylene-coated smocks (Kleenguard A70, Fisher Scientific, Pittsburgh, PA, USA) to

reduce a contamination from clothing. We chose yellow as it is rarely found in our environmental microplastic samples (McNeish et al. 2018, Hou et al. 2021, Hoellein et al. 2021). We washed and rinsed all glassware DI water prior to use and stored with aluminum foil covers.

We accounted for contamination by running laboratory controls across a gradient of timed exposures. Contamination can occur as dust settles onto the enamel tray during dissection, and fish dissection times were variable depending on size and species. Interspersed among the dissections we conducted a series of contamination measurements by setting our clean enamel tray for a defined amount of time: 3, 5, and 7 min. After the allotted time we rinsed the tray with filtered DI water and poured it into a glass mason jar (n=18). Control samples underwent the same drying, digestions, filtering, counting, and picking procedure as described above for our fish samples. To determine the amount of potential contamination our fish samples incurred, we calculated microplastic counts as No/$cm^2$/min (enamel tray area was 778 $cm^2$). We considered the area of potential contamination of the fish during dissection to be the area accounted for by fish size and placement of tools (e.g., 120 $cm^2$ Gizzard Shad, Golden Shiner, Bluntnose minnow, and Blackstripe Minnow). For Bluegill and Round Goby, the potential contamination area was larger (198.5 $cm^2$) to account for the workspace needed for gut content analysis. When calculating potential contamination for these species, the given time for each dissection and estimated area of the fish was used to determine the contamination value. We rounded up to the nearest integer to generate conservative estimates for particle subtraction. Contamination results showed that for Gizzard Shad, Golden Shiner, Bluntnose minnow, and Blackstripe Minnow two (2) microplastic fibers were subtracted from the sample total to determine final counts. Bluegill and Round Goby samples had three (3) microplastic fibers subtracted to determine their final counts.

*Stable isotopes*

We measured s $\delta^{15}$N from fish tissue taken from the nape of each fish, for Blackstripe Topminnow the peduncle was used instead due to the smaller size of this species. We rinsed the fish with DI water and removed the tissue using a cleaned scalpel. The tissue samples were rinsed with DI water and individually stored in microcentrifuge tubes with ethanol until later processing. Tissue samples were dried at 60 ˚C for 24 to 48 hours, until mass of samples did not vary between ~2 hours of drying. We ground the samples to a fine powder using a mortar and pestle, rinsed with DI water between samples. Stable isotopes were analyzed at the Boston University Stale Isotope Laboratory using a GV Instruments IsoPrime Isotope Ratio Mass Spectrometer (Wythenshave, United Kingdom) interfaced through a GV Instruments Diluter and Ref Gas box to an Elementar vario ISOTOPE Cube elemental Analyzer (Ronkonkoma, NY, USA). Data were then corrected to the international standards of atmospheric N for nitrogen.

*Stomach contents*

We examined gut contents to quantify fish diet using Bluegill and Round Goby. Of the 6 study species, gut contents for these two species were most amenable to taxonomic identification via dissecting scope, whereas the other 4 taxa were either too small or deemed the stomach contents would not be visually enumerable (i.e., detritus, biofilm, vegetation, etc). For the analysis we dissected the fish's digestive tract from mouth to anus. We separated the stomach and placed it in a clean enamel tray, while the remaining digestive tract was placed into a pre-cleaned glass jar. We cut open the stomach, removed the contents, and visually identified organisms to the lowest taxonomic group under a dissecting microscope. We classified 7 prey taxa in Round Goby and Bluegill stomachs. These were *Bosmina*, chydorids, chironomids, Naididae, copepod, amphipods and 'other/unknown'. After identification, we added the stomach

contents to the glass jar containing the remainder of the digestive tract, which were subject to microplastic analysis. We recorded the total time elapsed from dissection to completion of gut content analysis to account for contamination.

*Data analysis*

We compared microplastics and $\delta^{15}N$ between sites and species using R statistical software (2022.07.1+554) (R Core Team 2020) by constructing general linear mixed models (GLMM) first with microplastics as a dependent variable, and then with $\delta^{15}N$ as the dependent variable. Initial inspection of data indicated high variability of microplastic counts among species, and this heteroscedasticity was accounted for within the model that was built using the glmmTMB package. For both models the interaction between species and locations was of primary interest. To account for differences in microplastic counts related to the size of individual we included mass as a covariate (not necessary for $\delta^{15}N$ analysis). Finally, we conducted a third model to examine any potential connection between microplastics and $\delta^{15}N$. We checked residuals and model fit using the DHARMa package as well as with *AIC()* and *AICc()* to verify our final model had the best fit and to account for sample size. Negative binomial regression models were used with microplastics whereas normal linear (Gaussian) regression models were used with $\delta^{15}N$. The *emmeans* package was used to estimate means and 95% confidence intervals which we then displayed using ggplot2.

We used non-metric multidimensional scaling (nMDS) to visualize patterns in gut content and polymer compositions among sites and species. First, we converted count data to relative proportions for each individual (i.e., percentage). We used *metaMSD* function ("vegan" package; Oksanen et al. 2019) to perform nMDS and determined a square root transformation followed by a Wisconsin standardization would aide in nMDS fitting. These transformations

help to decrease the influence of highly abundant counts (species or microplastics) and increase the ability to display differences between communities. To determine significant differences between sites and species we performed a perMANOVA using adonis2() ("RVAideMemoire" package).

**Results:**

*Microplastics and stable isotopes among sites and species*

We examined microplastics and stable isotopes in 76 fish from 3 sites: upstream (N=28), WWTP (N=27), and downstream (N=21) (Table 1). All individuals contained microplastics in their digestive tracts (Table 1). The best-fitted model for microplastics in fish indicated that counts differed between locations within and among species (interaction: $\chi2=33.00$, $p < 0.001$, Table 2). Although the model accounted for variation related to the mass of the fish, no effect was found ($\chi2= 0.108$, $p = 0.742$, Table 2). The interaction is explained by trends found with Bluegill and Blackstripe Topminnow, as the four other species showed no differences in microplastic among sites (Gizzard Shad, Golden Shiner, Bluntnose Minnow, and Round Goby; Figure 1). Bluegill (*Lepomis macrochirus*) showed higher concentrations at the WWTP compared to the downstream site (p-value<0.01; Figure 1). In contrast, Blackstripe Topminnow had higher concentrations of microplastics upstream and downstream compared to at the WWTP (p-value<0.01, p-value<0.05, respectively; Figure 1).

Stable isotope analysis revealed differences in $\delta^{15}N$ among sites and species. The best-fitted model for $\delta^{15}N$ values in fish had species and location as significant interacting predictors ($\chi2=61.737$, p-value<0.001; Table 2). While Gizzard Shad showed no difference in $\delta^{15}N$ values among the 3 sites (Figure 2) and the sample size of Blackstripe Topminnow was too low to be included, significant differences among sites existed for the other species. Golden Shiner and

Bluntnose Minnow both had significantly lower $\delta^{15}N$ values upstream compared to the WWTP and downstream (p-values <0.001). Bluegill had a significantly higher $\delta^{15}N$ value downstream compared to upstream at WWTP (p-values <0.05; (Figure 2). Round Goby showed significant differences between all sites with downstream highest and upstream lowest (Figure 2).

*Relationship between microplastics and $\delta^{15}N$*

Generalized linear models demonstrated no relationship between $\delta^{15}N$ and microplastics concentration (Table 4). Although both $\delta^{15}N$ and microplastics differed by both location and species, these patterns do not relate to each other and exhibit different site and species interactions (Table 2). Results suggest independence between $\delta^{15}N$ and microplastics across the environmental gradients included in this analysis.

*Gut contents*

Gut contents were generally different between Bluegill and Round Goby (PERMANOVA, r= 0.116, p-value=0.032; Table 5; Figure 3). Within each species PERMANOVA indicated differences among locations although this was not visually apparent in the nMDS plots of the data (r=0.319, p-value<0.001; Table 5; Figure 3). What was evident in nMDS plots is that gut content composition upstream and downstream were much more variable than those from fish found at the WWTP, which could have led to the significant PERMANOVA result as it is known to be sensitive to heteroscedasticity.

*Characterization of microplastics in environmental samples and controls*

Microplastics shape, color, and polymer appeared similar across the 3 sites when displayed via nMDS plots (Figure 4). Accordingly, PERMANOVA test revealed no significant differences across species (F=1.15, p-value=0.323; Table 6) or sites (F=1.14, p-value=0.347; Table 6). By shape, fibers dominated all microplastics found within the fish (99.5% of all

counted microplastics), while only 5 fragments were found out of 1,070 total microplastic particles. We scanned 560 microplastics particles. Of those particles, 204 gave us acceptable scan results. We identified 268 particles as cellulose, 61 as rayon, 60 as polyester (Figure 8). Of all the particles collected in the fish, 214 were clear, 152 were blue, and 58 were red (Figure 9).

We assessed microplastics in control samples using the same processes as the environmental samples, and compared their composition by shape, color, and polymer type. Fibers dominated the particles found on control filters (n=299); one fragment was found in 3 of the control samples. By polymer type, the most common material type was cellulose (n=69), polyethylene (n=3), and rayon (n=12) (Figure 8). By color, the most common colors were clear (n=181), blue (n=65), and red (n=19) (Figure 9).

**Discussion**

Our findings show that microplastic concentrations in fish from heavily modified urban environments are highly variable, being influenced by a combination of geographic proximity to wastewater effluent (i.e., microplastic exposure), species functional group, and actual diet composition. While proximity to a point source of microplastics (i.e., WWTP) was a primary driver of $\delta^{15}$N values of fish tissues, its capacity to explain microplastic abundance across fish species was mixed, and thus $\delta^{15}$N estimation of trophic position generally did not predict microplastic abundance. In addition to site and trophic level, many controlling factors act simultaneously to drive microplastic dynamics in freshwater food webs, including egestion rates, movement, and particle characteristics. Our general conclusion is that location, and by proxy magnitude of exposure, was not the primary factor determining microplastic abundance in the digestive tract of these organisms.

*Microplastics in fish: high variation among sites*

We attribute the lack of spatial patterns in microplastic abundance in fish to differences in movement and foraging behaviors, as well as variation in particle ingestion and egestion rates. The only species to exhibit patterns of ingested microplastics which mirrored known differences in exposure quantities in this study was Bluegill. Round Goby also showed a geographic trend similar to that of Bluegill, but high variability among individuals led to a non-statistically significant difference among locations. In contrast to our results, Park et al. (2020) measured significantly lower microplastics from fish collected upstream of a WWTP relative to downstream. Similarly, microplastics in trout were significantly higher within and downstream of the city of River Falls (Wisconsin, U.S.A.) relative to rural sites upstream (Simmerman and Wasik 2019). In the Milwaukee River, Hoellein et al. (2021) found high variation in microplastics of dreissenid mussels, with no significant difference in microplastic concentrations for organisms found near a WWTP effluent site compared to other locations within the same waterway.

The collective patterns in our data suggest that the study species are likely to reside in the general area of collection for more than a day (i.e., stomach contents were distinct among locations) and up to a few weeks (i.e., isotopes were distinct among locations), even though microplastics were largely not different among sites. Stomach contents of fish is generally thought to reflect a period of < 48 hours, and for some smaller fish egestion can occur on the order of a few hours (Ory et al 2018). Geographic patterns in stomach contents of the two species we analyzed suggests limited movement among the locations from which they were sampled. Bluegills movement can be seasonally variable. When tracked in September (when our fish were also collected) their average movement rate has been estimated to be 30 m/h (Paukert et al 2004).

Round Gobies are territorial, and tend to stay in one place, usually in a shelter of rocks which they defend, and will move quickly when away from such refuge (Hayes 2008). Isotopes are thought to reflect dietary uptake on the order of weeks to months. We recorded distinct spatial patterns in isotope values for 5 of the 6 species studied, suggesting the individuals resided in the general proximity of where they were found for several days to weeks. The exception to these trends was Gizzard Shad, whose isotopic signatures were not geographically distinct. The high similarity of isotopic values of Gizzard Shad suggest they likely do move among the sites from which we collected them whereas the other species remained in relative proximity to their collection site.

In addition to movement, how organisms capture and ingest food could affect the amount and types of ingested microplastics. For example, Bluegill and Round Goby are invertivores with benthic (or benthopelagic) feeding habits. They both also showed clear differences in gut contents among the 3 sites, which likely reflect the prey communities available given the known differences in environmental conditions at each site. This may have contributed to the pattern of higher microplastics at the WWTP site relative to the other sites for Bluegill, and a similar trend for Round Goby. Overall, the feeding mode and territorial aspects of these two species life history may contribute to the pattern of microplastics we documented according to their collection sites.

Life history characteristics that trend towards more movement and foraging habits for Bluegill and Round Goby offer some explanation for the microplastics data, but the remaining four species did not support our hypothesis regarding drivers of microplastic concentrations in fish. Three of the species showed no pattern in microplastics relative to WWTP proximity, while Blackstripe Topminnow individuals collected at the WWTP showed significantly lower values

than upstream. These four species had lower estimated trophic positions (i.e., 2.4-3.2) than Bluegill and Round Goby. We were unable to identify the materials in their gut contents, so we don't know if their diet varied among sites as we documented for Bluegill and Round Gobies. Finally, these species tend to spend more time in pelagic or demersal zones than Round Goby or Bluegill. The typical movement patterns of these four species are more in open water, moving to avoid predators or follow food sources (Carlander 1969, Chivers and Brown 1996). It appears variables other than $\delta^{15}N$ values were driving microplastic dynamics in these taxa, such as movement, foraging behaviors, or feeding mechanisms (i.e., gill rakers) that we did not quantify.

Egestion rates and timing may have affected microplastic concentrations in the collected fish. Like stomach content analyses of diet compositions, our microplastic analysis represents a "snapshot" of an individual's recent patterns of ingestion and egestion (Hou et al 2022, Farrell and Nelson 2013). For example, an individual fish collected for this study may have just evacuated their gut contents, but not eaten their next meal, prior to being collected, which would enhance variability in the microplastic abundance data. Microplastics can show distinct egestion rates from general food items, which vary by fish species and microplastic size (Roch et al. 2021). Studies on egestion rates of microplastics particles in freshwater fish are relatively limited but include a few similar taxa to those included in this study. For example, Hou et al (2022) found the mean residence time of microplastic fibers in digestive tracts of Round Gobies collected in the Chicago area was about 24 hours, suggesting that microplastics found in an individual's gut represent the microplastics consumed in the previous day. Roch et al (2021) fed Rainbow Trout and Common Carp different-sized microplastics (0.02 – 1 mm polymethylmethacrylate fragments) and showed that trout preferentially egested larger particles compared to smaller ones, but carp showed no preference in microplastics egestion based on

size. In addition, variation in microplastic ingestion based on size or color can occur, which would decouple patterns of microplastic in the environment relative to those within the fish (Xiong et al. 2019).

*Fish $\delta^{15}N$ values: impacted by site and trophic level*

The stark differences in $\delta^{15}N$ between upstream and downstream of WWTP across many of our fish is likely an effect of the effluent itself and not diet. Our isotopic results followed known patterns whereby WWTP effluent has enriched $\delta^{15}N$ values which manifest throughout the downstream aquatic food web (Kendall 1998). Fish upstream had lower $\delta^{15}N$ compared to fish at the WWTP and downstream illustrating the immediate influence of the WWTP effluent on overall $\delta^{15}N$ values even some 10 km downstream. Typically, $\delta^{15}N$ values in organisms shift in accordance with trophic level in a food chain. Our fish primarily consumed secondary consumers throughout each of the locations we sampled, which would not have induced such a large jump in $\delta^{15}N$ values.

Among trophic levels, we predicted higher $\delta^{15}N$ in fish known to consume secondary consumers relative to those consuming primary consumers or producers, due to isotopic fractionation of $\delta^{15}N$ in animals. The data were consistent with the prediction within each of the sites, as $\delta^{15}N$ was highest for Bluegill and Round Goby compared to Golden Shiner and Bluntnose Minnow, which are thought to consume detritus, biofilms, and zooplankton. However, we note $\delta^{15}N$ showed the least differences among fish species at the WWTP site, which is likely due to the overarching influence of wastewater on stable isotope values for these individuals. One exception to this overall trend was results for Gizzard Shad, which did not always show lowest $\delta^{15}N$ among fish species at each site despite being primarily detritivores (De Brabandere et al 2009, Yako et al 1996). At the downstream site, Gizzard Shad $\delta^{15}N$ values showed the

expected pattern (i.e., lower than Bluegill and Round Goby), however, at the upstream site, Gizzard Shad $\delta^{15}$N values were the same or higher than the two tertiary consumers. Given that isotope and microplastic consumption was rather similar across all sites for Gizzard Shad, and that they are known to swim long distances to forage on zooplankton blooms or filter feed (Drenner et al. 1984), it's likely that movement among our sites was high enough that $\delta^{15}$N values did not stabilize to reflect any one locale like other fish exhibited.

Other studies have shown elevated $\delta^{15}$N values in fish near WWTP effluent (Loomer et al 2015, Morrissey et al 2013, Hoffman et al. 2012). For example, fish found by effluent loading sites had significantly enriched $\delta^{15}$N values and overall poorer health than those found outside the impacted area in the Maroochy Estuary (Queensland, Australia) (Schlacher et al 2007). In a study across urban rivers in South Wales (United Kingdom), macroinvertebrate communities showed elevated $\delta^{15}$N values in wastewater affected sites, suggesting that wastewater-derived nitrogen was incorporated into the food web (Morrissey et al 2013). For species which are known to have smaller daily to weekly movement distances, our data are generally consistent with these patterns, however for species which may move longer distances over the course of a week or month, isotopic values may blend among the locations leading to no geographic pattern among our sites being evident. (i.e., Gizzard Shad).

*Fish microplastics and $\delta^{15}$N were unrelated*

We predicted a positive relationship between $\delta^{15}$N and microplastics in fish (Krause et al. 2021). We expected this because higher trophic level fish could consume microplastics incidentally from the environment, as well as bioaccumulate from prey. Trophic transfer of microplastics is possible and demonstrated elsewhere (Au et al 2017). However, we found no evidence to support this pattern with our data. We interpret the lack of statistical correlation

between $\delta^{15}$N and microplastics as a function of differences in their processing during digestion, and the temporal scale of their influence. Isotopic fractionation allows for food web inferences to be drawn from a fairly broad scale over a period of weeks (e.g., benthic vs pelagic, or trophic level comparisons) but have limited capacity for revealing narrow changes in diet (e.g., a switch between prey with similar niches) over short time scales. In contrast, the microplastics measured in this study are likely not subject to long term retention in the digestive tract (Hou et al. 2022) and are indicative of feeding behaviors only from the previous day. However, we note that a relationship between $\delta^{15}$N and microplastics may occur for particles larger and smaller than the range we measured, as they are more likely to bioaccumulate. Larger plastic particles might become trapped in a fish's digestive system. Smaller particles, especially < 10 um or below, can be assimilated by fish and moved to different tissues (Campanale et 2020). Assessment of a greater range of particle sizes may show a correlation with $\delta^{15}$N values as it pertains to increasing trophic level's accumulation of microparticles through direct consumption and trophic transfer.

We set up our study to cover a wide range of variation in potential $\delta^{15}$N values and microplastics across fish, as we expected this large gradient would help illustrate any connections between the metrics. Our study suggests microplastic infiltration into food webs is more nuanced than site-based exposure amounts or trophic level indicators. Individuals' movement and foraging behaviors affect microplastic consumption in ways that were not captured by grouping species in known functional or trophic groupings, nor through estimating bioaccumulation through $\delta^{15}$N trophic positioning. We suggest further research consider more individuals collected over time, coincide with population studies that consider fish range and

movement, and conduct measurements of microplastics and stable isotopes in basal food resources along with analyses in fish.

Other studies have examined stable isotopes, food web dynamics, and microplastics in fish and found variable results when assessing relationships among the factors. For example, Setälä et al. (2014) found polystyrene microplastics which were fed to zooplankton were found in their mysid shrimp predators, and Ferrell and Nelson (2013) found trophic transfer of microplastics between mussels and crab predators. However, studies examining microplastics with stable isotopes (as an indicator of food web position) *in situ* have produced mixed results (Au et al. 2017), likely due to issues related to the timeframe of reference of isotopes combined with individual variation in behavior not replicated in laboratory feeding trials. No relationship was found between trophic position of different deep-sea fish in Monterey Bay canyon relative to the amount of microplastics found within them (Hamilton et al. 2021). Trophic position ($\delta^{15}$N) was not related to microplastics concentration in fish collected in the Garonne River (Garcia et al 2021). In contrast, Andolina et al (2022) found that stable isotopes values were positively related to microplastic ingestion of fish in the Mediterranean Sea.

*Gut contents show spatial variation in diet among sites*

Our analysis of gut contents in Bluegill and Round Goby showed clear compositional differences between sites, suggesting that diets for both species were driven by prey availability at each location. Chironomids were the dominant organism identified from fish guts at the WWTP site. Chironomids can tolerant ecosystems with high nutrient pollution, which is what we expect to find at an effluent release site (Hamdhani et al. 2020). At the downstream site, gut content composition was strongly influenced by the presence of amphipods, which are common in the CAW and considered relatively tolerant to urban conditions (Cook and Hoellein 2016).

Our upstream site consists largely of water directly from Lake Michigan. This source of water is likely the reason that the abundance of copepods was a strong environmental vector and key species in the diet for both species at the upstream site. As such, it appears that although diet composition is rather distinct among sites, and isotope values suggest many of the species reside within each site for a few days or more, it was surprising to see little differences in microplastic composition or abundances among the sites.

*Microplastics in fish: context for concentration and polymer types*

The range of microplastics recorded in fish guts were similar to previous measurements, an average of 13.7 microplastics per individual across all sites (range=1.3-29.2 particles/fish). Microplastics from a variety of fishes in the Milwaukee, Muskegon, and St. Joseph Rivers (tributaries of southern Lake Michigan) ranged from a mean of 0-16 microplastics/individual across taxa (McNeish et al. 2018). In particular, Round Goby has been included in several recent studies and published values provide context for these data. Round Gobies collected in Great Lake's harbors had means > 20 (no/fish) (McNeish et al. 2018, Munno et al. 2021) suggesting urbanization may lead to greater exposure and consumption of microplastics. Round gobies in our study mirror this idea with microplastic abundances being lowest at our upstream site (~2 no/fish) and higher downstream of the WWTP (5 – 10 no/fish). Together we add to the evidence that proximity to urbanization increases the microplastic loads ingested by organisms.

We found a variety of polymer shapes and types, which was consistent with the composition of particles found in digestive tracts of freshwater fish from previous studies in the region. In this study, 99% (n=1056) of the particles were fibers and 1% (n=5) were fragments, with other shapes not encountered. Most of the particles (49%) identified were cellulose (n=200) followed by rayon (16%, n=62) and polyester (9%, n=40). The total amount of microplastic

particles (acrylic, nylon, polybutylene, polyester, polyethylene, polypropylene, polystyrene) found within our fish was 113 (30% of total particles). Other studies showed fibers dominating microparticle shapes found, for example, only 1-3% of microplastics were fragments in fish collected in Milwaukee and St. Joseph Rivers and the rest were fibers (97-100%) (McNeish et al. 2018). This pattern is also true for fish collected in Lake Michigan at Calumet Park where all particles were fibers and over 80% of identified particles were anthropogenic (unknown or cellulosic) or cellulosic (Hou et al 2021).

*Conclusions and future work*

Our aim was to quantify the spatial and ecological dynamics of microplastics and $\delta^{15}$N by sampling multiple freshwater fish species, with the objective to understand mechanisms which affect their values, and to determine if there was any connection between the two metrics. Our results indicate the combination of exposure and species life history affect microplastic abundance in the digestive tracts of freshwater fish. We found no relationship between stable isotope analysis and microplastics suggesting that trophic position was not a good indicator of microplastic abundance. Furthermore, our gut content analysis revealed different community structures at our sites, despite their relatively close geographic proximity. In follow up studies, researchers should consider that urban waterways can show high heterogeneity in environmental conditions across small spatial scales, and that fish life history and movement are likely key drivers of microplastic dynamics. We suggest further investigation to consider distribution of microplastics across small geographic ranges. In addition, insights will be gained from studies that are comprehensive at the food web scale and can generate long-term data sets of microplastics found in urban fish.

# CHAPTER III

# MICROPARTICLE ABUNDANCE IN MACROINVERTEBRATES RELATED TO FUNCTIONAL FEEDING GROUPS AND WATER COLUMN MICROPARTICLE CONCENTRATIONS

## Introduction

Anthropogenic microparticles (particles < 5 mm), defined as materials of manufactured origin found in the environment (Adams et al. 2021), are widespread and rapidly emerging contaminants found at a global scale (Rochman et al. 2016). Anthropogenic microparticles include microplastics (< 5 mm), as well as other microfibers or particles which can be synthetic, semi-synthetic and non-synthetic (Adams et al 2021, Collard et al 2018). Sources of anthropogenic microparticles in these freshwater ecosystems included treated effluent wastewater treatment plants (WWTP), agricultural/urban runoff, direct littering, and atmospheric deposition (Wang et al 2022, Hoellein and Rochman 2021). Once in a waterway, microparticles can be consumed by organisms including macroinvertebrates, fish, and birds (Rochman et al 2016, Hou et al. 2021). Macroinvertebrates intentionally consuming particles by mistaking them as food items, or unintentionally consuming them through passive filter feeding or ingesting an organism that has microparticles inside them (Provencher et al 2019, Chae et al 2018).

Microparticle pollution is commonplace within the bodies of freshwater macroinvertebrates (Nantege et al 2023) and may be an important entry point for microparticles in aquatic food webs. Microplastics within macroinvertebrate tissues can negatively affect the nutritional absorption, growth, moulting, reproduction, and survival (Nantege et al. 2023,

Ockenden et al 2022, Foley et al 2018). Because plastic pollution is expected to increase in the future (Geyer et al. 2017), it is likely to have more pronounced impacts on individuals, communities, and macroinvertebrate-mediated ecosystem processes (i.e., decomposing, nutrient cycling) in the years ahead (Nantege et al. 2023).

Understanding factors which drive the number and type of microparticles found within freshwater macroinvertebrates is an emerging field of study. The amount of microparticles in organisms is likely positively related to the amount in their environment. However, studies comparing microplastic concentrations in the environment to abundance within organisms have generated equivocal results (Parker et al 2022, Windsor et al 2019). One reason for this lack of clarity may be that previous work has considered microplastic concentration in the environment as single value, rather than quantifying microplastic concentrations across multiple aquatic habitats where organisms may be more likely to encounter them (i.e., water surface, sediment, different benthic substrate types) (Parker et al 2022, Garcia et al 2021). The distribution of microplastics in a river is heterogeneous, with greater abundance in depositional sites relative to erosional habitats such as cobbles (Vincent and Hoellein 2021). Similarly, the distribution and range of stream organisms are variable. Since organisms feed or inhabit distinct locations in a stream, the concentration of microparticles they are exposed to will likely be reflected in the concentration of microparticles found in their digestive tracts. However, no previous research has considered spatial heterogeneity of microparticles in the environment to microparticles abundance within organisms.

Microparticle concentrations within macroinvertebrate is influenced by multiple factors, including habitat, size, and functional feeding group (FFG). Common FFGs for macroinvertebrates include shredders, grazers, filterers, collectors, pierces, suckers, and

predators. We expect FFG can affect the abundance of microparticles in macroinvertebrates. For example, predators are likely to have high concentrations compared to other FFGs because they can directly consume microparticles and indirectly consume them through trophic transfer. In addition, measuring microparticles in different FFGs may offer greater clarity on links between environmental concentrations and FFG type. For example, filterer's microparticle concentration is most likely to be related to microparticle concentrations in the water column, rather than concentrations in benthic or surface water habitats. No previous research has assessed the role of environmental and biological factors (e.g., environmental concentration and FFG) on microparticle abundance in stream macroinvertebrates.

In this study we aimed to quantify relationships between microparticle concentrations in macroinvertebrates relative to habitat-specific environmental concentrations and macroinvertebrate biology and ecology, including FFG and size. We predicted there would be a positive relationship between overall microparticle concentrations in macroinvertebrates and microparticle concentrations in the environment, with predators having the highest concentration of any FFG. We also predicted a positive relationship between individual FFGs and the habitats they occupy and forage in (i.e., collectors and water column, filterers and water column, gatherers and water column, shredders and benthos).

## Methods

### *Study sites*

The three watersheds in our study are the North Branch of the Chicago River (Illinois, USA), the Don River (Ontario, Canada), and the Ipswich River (Massachusetts, USA) (Figure 9). The Chicago and Don Rivers flow through an urbanization gradient. At the headwaters the surrounding land is forest or wetland. Further downstream, the landscape is suburban and urban.

In contrast, the Ipswich River is more urban at headwater sites and more forested with wetlands downstream before it meets the Atlantic Ocean. Each river has multiple point sources (e.g., wastewater treatment plants) and non-point sources (e.g., storm run-off, direct littering). We measured microparticle concentrations at 4 locations in each river (i.e., 2 headwater sites, 1 site near the middle of the watershed, and 1 site at the base of the watershed). We collected macroinvertebrates at the same locations to measure microparticle concentration in their digestive systems.

*Habitat-specific collection of microparticles in streams*

We collected samples at 3 habitats in the stream: surface water, water column, and the benthic zone. For surface water, a 100-micron mesh net that was pre-rinsed with filtered DI water was deployed at the surface of the water for 5-20 min. To quantify the volume of water moving through the net, we measured the net width, the depth of the water in the net, the water velocity, and the deployment time. The collected material from the net was moved into a pre-cleaned glass jar using filtered DI water. For the water column, we used a peristaltic pump with 0.95 cm diameter tygon tubing (Geotech, Crest Hill, IL) to run 30 L of river water over a 100-um sieve. The sieve was covered with foil to prevent contamination during pumping. All material on the 100-um sieve was rinsed with pre-filtered DI water into a pre-cleaned glass mason jar. For the benthic zone collection, we inserted a 'stove pipe' (i.e., a metal trash can with the bottom removed, height=1 m, diameter=0.46 m) into the sediment (Vincent and Hoellein 2021). We measured the water depth at 5 locations within the area sampled by the opening of the stove pipe, then disturbed the sediment to suspend and evenly distribute the particles within the water. A pre-rinsed glass, 2 L bottle was placed into the stove pipe to collect a subsample. All glass jars

were placed in a cooler in the field, transported back to the laboratory within 4-6 hours, and then placed in a refrigerator (4$^{o}$C) until later processing for microparticles.

*Macroinvertebrate sampling*

We collected live macroinvertebrates at multiple habitat types in the benthic substrate (rocks, fine and coarse organic matter) at each study site. An environmental habitat assessment was completed first at each study site to help identify microhabitats within the stream's collections reach. We did this to pinpoint the ideal sample location in each stream that would generate the most diversity of macroinvertebrates collected. Once assessment was complete, researchers determined 3 locations within the reach to collect macroinvertebrates. Samples were collected using a 500-micron kick net, where a flat bottom net was placed downstream while a collector stands just upstream and disturbs the habitat by kicking around rocks, sediment, leaves, or litter for about 1 minute. This causes macroinvertebrates to get swept downstream into the net (Lenat et al. 1988). After each collection the macroinvertebrates and excess vegetation was emptied into a jar and preserved with 70% ethanol to be transported to the lab.

*Laboratory processing: Microparticles in water and sediment*

Water and sediment samples were first sieved to remove particles smaller than the size range of limit used for our analysis (i.e., <100 μm). We rinsed material from each glass container over a 100 μm sieve using pre-filtered DI water. For water column and surface water samples, the material collected in the sieve was rinsed with hydrogen peroxide (30% in water), transferred into a clean glass beaker, covered with aluminum foil, and then we proceeded with peroxide digestion (see below). In contrast, the benthic samples were subject to density separation prior to peroxide oxidation.

For the density separation we used a calcium chloride solution ($CaCl_2$) solution. We combined 800 g of $CaCl_2$ into 2 L beaker filled with 1 L of filtered DI water. This solution was heated and stirred until the salt was dissolved (~30 min). We let the solution cool to room temperature and verified the density (1.4 g/ml) with a clean hydrometer. If the density was below 1.4 g/ml then we added up to 50 g of $CaCl_2$ reheated, stirred, cooled, and checked density. This process was repeated as needed until the density was 1.4 g/ml. The final solution was filtered with a cellulose filter (0.45 um pore size: Whatman, Pittsburgh, Pennsylvania, USA) and stored at room temperature. To begin salinity separation, we poured the contents of the jars over a 100 μm sieve. Next, we rinsed the sediment of the benthic samples into a clean 400 mL beaker using $CaCl_2$ solution (Way et al 2022). We added 250 mL of $CaCl_2$ and stirred the samples to suspend all the material and left the beaker untouched for 2 hours. A clean metal spoon was used to decant the top liquid and floating particles into a separate, clean 1 L beaker. An additional 250 mL of $CaCl_2$ was added, stirred, and the sample sat for 2 hours. The surface materials were again removed via spoon into the 1 L beaker. We discarded the sediment at the bottom of the separation beaker. The material in the 1 L beaker containing the $CaCl_2$ and microparticles was stirred with a clean metal stirring rod and left overnight. The following day we rinsed everything from this beaker over a 100-um sieve using DI water to rinse away the salt. The material on the 100 μm sieve was rinsed with hydrogen peroxide in a clean glass beaker, and samples were next subject to peroxide oxidation.

Surface, net, and benthic samples were processed using wet peroxide oxidation (De Frond 2022). We added hydrogen peroxide to each beaker to reach a final total volume of 100 ml. Beakers covered with aluminum foil were placed in an oven at 40$^{o}$C for 24-48 hours (1,320 Economy Oven, VMR, Radnor, Pennsylvania, USA). Afterwards, the solution was poured over

three stacked sieves, 500 µm on the top, followed by a 250 µm sieve, and then a 100 µm sieve on the bottom. Additional DI water was used to rinse the jars over the sieves. The contents captured on each level of the stalked sieves were individually transferred to a new clean beaker with DI water used to rinse the contents into the beaker. This separation divided each sample into three size classes. Each sample was vacuum filtered onto a gridded cellulose fiber filter (0.45 um pore size: Whatman, Pittsburgh, Pennsylvania, USA). We rinsed the filters with DI water then transferred the filter onto a 20 mL aluminum pan (Thermo Fisher Scientific Incubator, Marietta, Ohio, USA) and covered it with aluminum foil (Hou *et al*. 2022 and Hoellein *et al.* 2021). The pan and filter were placed in the drying oven dried at ~45ºC overnight until microplastic counting and polymer identification (see below).

*Laboratory processing: Macroinvertebrates*

Macroinvertebrates sample jars were emptied over a 100 µm sieve and rinsed with DI water. We emptied the sieved contents onto a clean enamel tray to pick the macroinvertebrates out of the sample. Each was placed in 20- or 50-ml glass vials containing 70% ethanol. We identified individuals to the lowest possible taxonomic level and categorized them into known functional feeding groups (FFG) (e.g., shredders, collectors, predators, and scrapers) (Merritt et al. 2019). Diptera were identified to family, and worms (Oigochaetes) were identified to order (no leeches were collected). The remaining macroinvertebrates were identified to genus when possible. If genus could not be determined (e.g., due to missing body parts) the organisms were identified to family. A randomly selected subset of individuals from each FFG at each site (N=16 sites) were measured to nearest mm and then placed in clean glass jars in preparation for peroxide oxidation. For predators, we placed individuals in jars due to their large relative size. All other invertebrates were grouped with 5 individuals in each glass beaker (Hoellein et al.

2021). Individual organisms were broken up by pulling the head capsule off the organism to aid in the digestion process. We followed the same wet peroxide oxidation and sample filtration and storage processes as noted for surface and water column samples above.

*Microparticles: Counting and polymer identification*

We conducted particle counting using a dissecting microscope (x25-30 magnification; model ASZ30L3, Bausch & Lomb, Rochester, New York, USA). For each filter, we counted all microplastics, and categorized by shape (i.e., fiber, fragment, film, fiber bundle, foam, pellet) and color (Kotar et al 2022, Hou et al. 2021). Once filters were counted, they were covered with aluminum foil and carefully stored until we returned to remove a subset of particles for polymer identification.

A subset of each category of microparticles from each filter were removed for polymer identification. We used a shape and color-based selection process for subsampling. We selected the first 5 particles of each color shape combination from each filter (e.g., the first 5 blue fibers, the first five 5 black fragments). If there were less than 5 of a color shape combination, we collected all of the particles. A thin layer of Skin Tac (Torbot Group Inc. Warwick, RI) was applied to a pre-cleaned glass microscope slide and the selected particles were placed on the adhesive. A circle was drawn around each particle and then the whole slide was left to dry under a box to avoid contamination. Once dried (24-48 hours) a glass cover slip was placed over the particles and stored until polymer identification.

For the polymer identification process, we used a micro- fourier-transform infrared spectroscopy (FTIR) (Spotlight 200i, Perkin Elmer, Waltham, MA, USA) in automated total reflectance (ATR) mode. Spectrum results were completed across wavelengths 450 $cm^{-1}$ to 4000 $cm^{-1}$ and compiled for 16 individual scans per particle. Results were compared to a reference

library and known standards using Spectrum 10 Software (Perkin Elmer) with a target match between samples and standards at 0.51-0.95. The particle length and width were determined based on the picture of the particle on the micro-FTIR spectrometer camera.

*Microparticles: Accounting for contamination*

We used multiple steps to account for contamination in the field and lab. All laboratory surfaces were wiped with a cellulose sponge (JINCLEAN; model nCratch10) soaked in DI water prior to digestion. All researchers wore yellow polypropylene-coated smocks (Kleenguard A70, Fisher Scientific, Pittsburgh, PA, USA) to reduce contamination from clothing. We chose yellow as it is rarely found in our environmental microplastic samples (McNeish et al. 2018, Hou et al. 2021, Hoellein et al. 2021). We washed and rinsed all glassware with pre-filtered DI water prior to use and stored with aluminum foil covers.

Controls were run separately for our environmental and macroinvertebrate samples. Environmental controls were taken in the field at each site for surface, water column, and benthic samples. Surface water and water column samples were taken using the same methods described above in the field, but using pre-filtered DI water, and then processed for microplastics in the lab. Benthic samples were taken by opening and closing clean glass mason jars at the sites, then following the same laboratory procedures above. Macroinvertebrate controls were run in the lab. Clean empty glass beakers were used and treated as though they had individuals in them for the digestion, filtration, and microplastics quantification processes. We accounted for contamination according to mean values from the controls for the sample type (water column, surface, benthic, and macroinvertebrate). We used a color and shape-based control correction. For instance, if our control had an average of 1 clear fiber, then we would subtract 1 clear fiber for each corresponding test sample. Any control correction that resulted in a negative value in the sample

was set to zero. We accounted for water column microparticle in benthic samples by determining the ratio of the volume of the trash can used to sample and the subsample of the collected benthic sample. This ratio was applied to our benthic count data to subtract the microparticles that would have been in the water column of the trash can prior to disturbing and suspending the microparticles in the benthos. A color control correction was done for these samples as well.

Final concentrations from each of the sample types were expressed based on the control-corrected counts and the sample characteristics. For surface water and water column samples, the number of particles was expressed relative to the volume of the sample ($No/m^3$). For benthic samples, we calculated the total number of particles trapped in the stove pipe based on the volume and particle count of the subsample, and the volume of the stove pipe. We expressed concentration as the total number of particles relative to the benthic area covered by the stove pipe. Finally, for macroinvertebrates, we expressed the number of particles measured per individual.

*Data analysis*

For this project we focused on quantifying drivers of microparticles in macroinvertebrates, so, we did not analyze patterns in microparticle concentrations in the environment among sites, as that is the focus of separate work. We used generalized linear models (GLM) to determine the relationships between microparticle concentration in macroinvertebrates relative to watershed (i.e., Chicago, Don, and Ipswich), tributary position (i.e., headwater, mid-watershed, or downstream), FFG, length of macroinvertebrates, and environmental microparticle concentrations (i.e., surface water, water column, and benthic). We conducted GLM approaches using packages in the R statistical software (2022.07.1+554) (R

Core Team 2020) as completed in previous analyses (Hou et al 2021, Hou et al. 2022, Hall et al. 2018, Nix et al. 2018).

First, we assessed the distribution of the dataset for microparticles in macroinvertebrates. Units for microparticle concentration in macroinvertebrates for building our model were in No./5 individuals since integer data are required for models to run and most of the invertebrates were processed for microparticles in groups of 5. We found a negative binomial (NB) distribution was the best for this pooled data set via *model.sel*() ([MuMln package]; Barton, 2020), and Akaike's information criterion, determined by comparing against other distributions (Gaussian, Poisson, Zero-inflated negative binomial and zero-inflated Poisson) (Table 8).

After determining the best distribution, a series of NB GLM analyses (*glmmTMB*(), [glmmTMB package]; Brooks et al. 2007) were constructed with all variables as fixed effects in the models. We first explored all univariate models, and the most significant factor from the univariate models was selected as the first factor in testing our multivariate models. Additional factors were added in our multivariate models to explore all possible significant model outcomes. All univariate and additive multivariate possible model combinations were explored (24 models total + Null model). The overall best model was determined by comparing model weights ($w_i$) and $AIC_c$ against all other models (Table 9). Model residuals (extracted via *simulateResiduals*() [DHARMa package]; Hartig, 2021) were found to have no significant outliers or dispersion, had no significant deviations from uniformity, and had homogenous variance. An ANOVA Type II was used to determine if the best fitting model's main effects were significant (ANOVA [car package]; Fox et al., 2022). Post-hoc pairwise comparison between sample location and FFG were conducted via Tukey's honestly significant difference tests to determine if microparticle patterns were different between factors (*pairs*() [emmeans package]: Lenth, 2022). Our best

fitting model was checked for collinearity and all variables had a variation inflation factor <2 and therefore considered not to be collinear (*check_collinearity*() [performance package]; Lüdecke et al., 2021).

We used non-metric multidimensional scaling (nMDS) to visualize patterns in microparticle compositions among locations and species. First, we converted polymer type data to relative proportions for each individual. We used *metaMSD* function ("vegan" package; Oksanen et al. 2019) to perform nMDS and determined a square root transformation followed by a Wisconsin standardization would aide in nMDS fitting. These transformations help to decrease the influence of highly abundant counts (microparticles) and increase the ability to display differences between communities. To determine specific significant differences between watershed, locations, and FFG we performed a *perMANOVA* using adonis2() ("RVAideMemoire" package).

**Results**

*Macroinvertebrates*

A total of 3404 individuals were identified across our 3 watersheds and 12 locations. Those individuals were identified to be members of 11 orders, 32 families and 33 genera. Organisms that were identified within the FFGs of piercers or scrapers were excluded from our analysis (n=8) because their mode of feeling does not reflect the environmental conditions that we sampled from. The FFGs included in our analysis were collectors, shredders, filterers, gatherers, and predators (Table 10).

*Microparticles in macroinvertebrates*

Microparticle concentrations in macroinvertebrates were variable among sites and watersheds. The site with the highest abundance of microparticle (No./individual) was York

Mills (YM) in the Don River watershed, and on the low end, several sites showed zero microparticles/individuals. Because variation among individual sites was high, comparing mean values across watersheds and watershed position showed similar mean abundance (No./individual) (Figure 10).

Generalized linear models demonstrated that FFG and water column microparticle concentrations were the strongest explanatory variables across best-fitting and competing models for predicting microparticles in macroinvertebrates (Table 9). The best fitted model included FFG and water column microparticle as explanatory variables (Figure 11, Figure 12, Table 9), with both factors being significant predictors (Table 11).

We repeated generalized linear mixed models for each individual FFG using the same set of explanatory variables. Three of the FFGs (filterers, gatherers, and predators) did not have enough data points to sustain individual model generation, however, the datasets for shredders and collectors were large enough to be examined. The competing models analyzed for shredders showed no explanatory variables predicted microparticle abundance, as competing models were not significantly different than null models (Table 10). In contrast, competing models for collectors revealed that water column micro*fiber* concentration and water column microparticle concentrations were explanatory variables across competing models (Figure 13, Table 10). The best-fitting model including water column microfiber concentrations had a weight of 100%, while the competing model with the explanatory variable of water column microparticle concentrations had a weight of 78% (Table 10). Water column microfiber and microparticle concentrations co-vary and therefore were not tested in competing models together. Water column microfiber concentrations was the strongest significant predictor of microparticle abundance in collectors (Table 11).

*Characterization of microparticles in environmental samples, macroinvertebrates, and controls*

We visually identified 580 microparticles in all macroinvertebrate samples. Most of our microparticles were fibers (n=513, 88%), and few were fragments (n=36, 6%). No other microparticle shapes were found. Using our subsampling approach for particle polymer identification, 549 were removed from filters and placed on glass slides for analyses via μ-FTIR. Unfortunately, 108 microparticles were lost during transfer or storage, leaving n=441 particles that were identified (86% of the total removed). Of these 441 particles, the most common found across all macroinvertebrate samples were cellulose (n=296; 67%), followed by polybutylene (n=21, 5%) and polyester (n=21, 5%). We found 61 particles (14%) that showed no matches to our database, which we report as 'unknown' but consider anthropogenic based on particle processing and visual identification processes. The most common colors were clear (n=231, 40 %), blue (n=110, 19 %), and black (n=95, 16%).

Our macroinvertebrate microparticles shape, color and polymer were similar across watersheds, location, and FFGs. We used NMDS to visualize the patterns according to each grouping (Figure 14). In addition, perMANOVA test revealed no significant differences across watershed (F=1.61, p-value=0.143), location (F=0.961, p-value=0.53) and FFG (F=0.471, p-value=0.918).

In our Chicago environmental samples, we found a total of 2,100 microparticles. Of those particles 1,415 were picked for polymer identification. By shape, fibers were the most dominant across all sites (n=1802, 86%), followed by fragments (n=168, 8%). During our polymer identification, some particles were lost (n=134, 9%) or were scanned and came back as unknown (n=203, 14%). The most commonly scanned particle was cellulose (n=548, 38%). The most common microplastics were polyester (n=138, 10%), polyethylene (n=123, 9%) and

polybutylene (n=83, 6%). Polymer identification is currently underway for samples from the Don and Ipswich Rivers.

We assessed contamination in our control samples using the same laboratory processes as our environmental and macroinvertebrate samples. In our macroinvertebrate control samples 30 particles were identified. All particles were fiber except 1 fragment. Most of the microparticles were identified as cellulose (n=23), two were identified as microplastics (acrylic and polybutylene), one was unknown, and the rest were lost (n=4). The most common colors were clear and blue (n=8), followed by black (n=4), and grey (n=4). In the control samples for the Chicago River environmental data collection, 708 microparticles were identified. By shape, fibers were the most dominant (n=648, 92%), followed by film (n=49, 7%).

**Discussion**

Microparticle abundance in macroinvertebrates collected from three watersheds, and among sites from different positions within each watershed, was most strongly related to FFG, followed by water column microparticle concentrations. We expected microparticle abundance in the environment would be most strongly affected by the number of particles found within organisms, however, particles are unevenly distributed in the environment and their consumption by invertebrates is mediated by their diet and habitat, which is consolidated within the FFG categories. These data represent an important basis for understanding the key factors that drive microparticle abundance within macroinvertebrates, and thereby the movement of microparticles within freshwater food webs.

*Factors which affect microparticle abundance in macroinvertebrates*

Our data clearly showed that microparticle concentration in macroinvertebrates was most strongly related to FFG. Of the five FFG represented in this analysis (i.e., shredder, gatherer,

collector, filterer, and predator), predators had significantly greater microparticles (No/individual) compared to all other FFG. In addition, shredders had significantly less microparticles (No./individuals) compared to all FFGs besides gatherers. This pattern supports our hypothesis that predators would have the highest concentration of microparticles compared to other FFGs. This pattern could be due to predators having higher direct consumption of microparticles (i.e., exposure from taking in water) in addition to consuming prey that contain microparticles (Lusher et al 2017, Garcia et al 2021). Our data did not discriminate between the two mechanisms, although quantifying those pathways separately would be a valuable area for future research.

Differences among the non-predator trophic levels, including low abundance of microparticles in shredders, also suggest that ingestion pathways impact microparticles within freshwater macroinvertebrates among non-predator taxa. Collectors and filterers had significantly higher microparticles than shredders, while gatherers were intermediate. In our study the patterns may be attributed to the variety of types of taxonomic groups in each category. Filterers were *Hydropsychidae* and gatherers were *Elmidae, Caenis,* and *Baetis*. In contrast, collectors were all *Chironomidae*, while shredders were amphipods and isopods. Thus, the number of taxa within each FFG is modest, although typical of urban streams given the environmental pressures which reduce macroinvertebrate species richness. Thus, differences we found among FFGs may be influenced by differences in habitat or feeding method among these individual taxa, rather than FFG broadly speaking. We also note the abundance of microparticles in all four groups was much lower than in predators, so the scale of variation in microparticle abundance across the non-predator FFGs was low overall. More research is needed to link the

specific feeding strategies of different taxa to the abundance and microparticles in freshwater ecosystems.

Our data are consistent with previous research which shows ingestion of microparticles by macroinvertebrates occurs globally (Nantege et al. 2023), with some suggestion that higher trophic levels show greater concentrations in other studies, depending on feeding mode (Cuthbert et al 2019). However, documenting the uptake mechanisms and possible bioaccumulation of microparticles in freshwater food webs is challenging (Krause et al 2020). Cuthbert et al (2019) observed microplastics were transferred from prey (*Culex pipiens)* to predator (*Chaoborus flavicans*) via consumption in a laboratory study. Similarly, Chae et al (2018) demonstrated microplastic transfer in a 4 species food chain in the laboratory (*Chlamydomonas reinhardtii, Daphnia magna, Oryzias sinensis*, and *Zacco temminckii*). Ockendon et al. (unpublished data) found a piercing/sucking predator (backswimmers) did not take up nanoplastics from its Daphnia prey, because nanoplastics were located in the Daphnia gut, which was not pierced by the predator. In contrast, the authors noted that engulfing predators (Damselfly) did take up nanoplastics from *Daphnia* prey because they ingested the entire organism. Garcia et al. (2021) suggested that direct consumption of microplastics in macroinvertebrates could represent the main pathway for microplastics to enter an aquatic food web, rather than bioaccumulation. We are not aware of other studies which have documented a difference in microparticle concentrations among freshwater macroinvertebrate FFGs collected *in situ* as shown in this study. More analyses are needed to understand the pathways of microparticles through food webs via trophic level and feeding mode. We suggest combinations of laboratory and field research will be required.

Microparticle abundance in macroinvertebrates (no/individual) was also positively related to water column microparticle concentrations (particles/$m^3$) (Figure 12). This supports our hypothesis that environmental concentrations of microparticles affects the abundance of microparticles by macroinvertebrates. That is, macroinvertebrates found in environments with higher microparticles concentrations will have a greater chance of consuming microparticles. We note the explanatory power of water column concentration was lower than for FFGs, suggesting that while potential exposure from environmental concentrations was important in determining microparticle abundance in invertebrates, FFG was the primary factor.

The relationship between environmental microparticle concentrations and organismal microparticle concentrations has been quantified in previous publications and shown equivocal results. In a laboratory experiment, Schell et al. (2022) found that macroinvertebrates (*Hyalella azteca* and *Asellus aquaticus*) ingested more polyester fibers from the water column than microplastics in the sediment, despite their putative role as shredders. This result matches our model's output which showed water column concentrations were related to macroinvertebrate concentrations and benthic concentrations were not. However, other studies have found no significant relationship between microplastic pollution in the environment and abundance of microplastics in organisms (Garcia et al 2021). It is possible that documenting a relationship between water column and macroinvertebrate concentrations may be affected by temporal variation of both parameters. Water column concentrations can be subject to abrupt changes in concentrations during stormwater runoff (Windsor et al 2019). Similarly, egestion of microparticles from invertebrates may decouple concentrations within their digestive tracts from concentrations in the environment at the time of collection. The amount of replication and the duration of study needed to uncover connections between environmental compartments and

concentrations in biota is still not clear. Our study benefited from a wide gradient of environmental conditions across the 12 study sites which helped document the positive connection. Additional research may also benefit from incorporating large spatial and temporal scales of variation.

We expected that body size could be related to microparticle abundance, where larger organisms would have the capacity for holding more particles. In our study, microparticle abundance showed a similar *trend* as body size, as mean predator body size was 11 mm, collectors were 6 mm, gatherers were 5 mm, filterers were 6 mm, and shredders were 6 mm. We included body length as a factor in the analysis, however, it was not a significant explanatory variable for microparticle concentration when considered with all FFGs combined or when FFGs were considered individually. Published research examining the relationship between microparticles and body size in macroinvertebrates have shown mixed results. Abundance of microplastics increased with body size and trophic position in macroinvertebrates (Garcia et al. 2021). This was attributed to a larger organism's ability to retain particles within them. Larger organisms also have the ability to ingest particles that are bigger (Schell et al 2022). However, Nantege et al. (2023) showed smaller organisms had more microplastics in their body compared to larger organisms, despite the differences in the size of ingested particles. Overall, our data did not show a role for body size as a driver of microparticle abundance, despite its careful documentation in the analysis. However, we suggest future studies incorporate body size as a possible explanatory factor and may benefit from including more specific size metrics such as wet weight, dry mass, or ash free dry mass of the study subjects.

Last, we note the pattern in microparticles among FFGs may be related to methodological artifacts of how the organisms were processed. For predators, we processed organisms

individually. This was possible given their larger size. For the other FFGs, we proceeded organisms in groups of 5 individuals of the same species. We then expressed concentration as the number of microparticles per number of individuals in the sample. Thus, for non-predator organisms, the data point combined results among 5 individuals and did not represent the range of concentrations across each individual. For instance, one organism in that group of 5 could have zero microparticles and another 10, but our results would show the abundance of microparticles in the group rather than individuals. We minimized the impact of this methodological constraint by repeating the analysis on multiple groups of 5 individuals for each of the FFGs. However, we acknowledge the mean and variation in microparticle abundance in non-predator FFGs may be affected by this approach relative to the dataset for predators, where individual-based variation remained intact.

*Microparticle concentration for individual FFGs*

While assessing individual FFGs we predicted that microparticle concentrations would be related to the habitat microparticle concentrations where each was most likely to feed. We successfully tested shredders and collectors as individual datasets. Both were largely benthic feeders, so we expected microparticle abundance in each would be most strongly related to benthic concentrations. Our results did not support this predication.

Shredders showed no relationship to any of the measured environmental variables. Shredders in our study were *Gammarus pulex* and Isopoda. *Gammarus pulex* are a tolerant, scavenging crustacean often found in shallow streams with muddy and/or stony bottoms (Inglis 1980). Similarly, aquatic isopods are common in streams with low water quality and rich in organic matter (Missouri Department of Conservation). Both can be omnivorous in diet but are often classified as shredders since they feed on decaying plant material. Overall, microparticle

concentrations were lowest in shredders compared to other FFGs, likely contributing to the lack of relationship to environmental concentrations. We note that other macroinvertebrate shredding taxa (e.g., caddisfly and stonefly) which are not as common in urban streams due to their environmental tolerance, could offer additional insight into how shredders are influenced by environmental microparticle concentrations. For example, Ockenden et al (unpublished) suggested that microplastics have the potential to disrupt caddisfly larvae behavior and feeding rate. This is important because it can disrupt ecological processes since shredders are important for leaf litter decompositions in aquatic environments.

In contrast to shredders, microparticle concentrations in collectors showed a positive relationship with water column microfiber concentrations. All collectors were *Chironomidae* larvae, which are non-selective generalist feeders (Merritt et al 2019). *Chironomus* are highly tolerant to adverse environmental conditions and are widespread globally in freshwater environments (Merritt et al 2019). They are common prey item for many predatory aquatic insects and are therefore important base food web organism**.** The positive relationship with water column microfibers, rather than benthic microparticles, suggest their ingestion pathway may not be determined by sediment dynamics as strongly as possible water column-based food sources. Like with shredders, collector species which are common in less polluted sites may also offer insight into the role of collecting FFG on microparticle abundance in invertebrates, but none were observed in our study sites.

Previous studies have suggested that collectors like *Chironomus* could be used as a 'bioindicator' species for microplastics at the site they are collected (Akindele et al. 2020). Our results offer some support for this concept because our model related microfiber abundance in the water column to abundance found in *Chironomus*. Silva et al (2021) also found a positive

relationship between environmental concentrations and microplastics ingested by *Chironomus*. In addition, *Chironomus riparius* that ingested microplastics showed delayed imagoes emergence and decreased larval growth (Silva et al. 2019). Their indiscriminate feeding style allows them to ingest microparticles at a rate that is reflective of the environment's concentration (Nantege et al. 2023). Overall, these data suggest a potential role for midges to act as an overall indicator of microparticles in the environment and can justify the additional research required to continue to document potential connections across a diversity of environmental conditions.

*Conclusions and future work*

The goal of this project was to understand the factors that drive microparticle concentrations in stream macroinvertebrates. Our results indicated the primary drivers were FFG, followed by water column microparticle concentrations. When isolating individual FFGs we saw a similar pattern, where microparticles in collectors increased with increasing microfiber concentrations in the water column. This data makes a key contribution to this growing field of study and supports the need for many follow-up studies in subsequent research. For example, we were unable to document the connection between each FFG and each environmental factor due to the low number of individuals for some FFG. Thus, we suggest future studies should aim to increase the sample size of all FFGs to reach further conclusions. Our study was conducted in three watersheds in North America, two of which were heavily urbanized. Future studies could benefit from including watersheds that span a wider range of urbanization gradients and longer time scales. Laboratory studies and *in situ* data collection should be directed at understanding the pathway for microparticle ingestion (i.e., trophic transfer and direct ingestion). Finally, we suggest more research is needed on the capacity for microplastic abundance in

macroinvertebrates to affect critical ecosystem processes such as secondary production, organic matter decomposition, and food web dynamic

# APPENDIX

# TABLES AND FIGURES

Table 1. Summary of all fish used in study including species characteristics, the number of individuals at each location (No.) and size range (minimum to maximum). Up = upstream, Down = downstream, and WWTP = at the effluent release site of the wastewater treatment plant (WWTP).

| Taxa | Common Name | Functional Feeding Group & Habitat | Trophic Fraction | Location | N | Size (g) | Mean MP (No/ind.) |
|---|---|---|---|---|---|---|---|
| *Dorosoma cepedianum* | Gizzard Shad (GZ) | Detritivore/ Planktivore Pelagic | 2.4 | Up | 5 | 7-13.7 | 18.2 |
| | | | | WWTP | 5 | 7.6-8.9 | 14.4 |
| | | | | Down | 1 | 1.43 | 3.0 |
| *Notemigonus crysoleucas* | Golden Shiner (GS) | Invertivore Demersal | 2.7 | Up | 5 | 0.32-0.64 | 5.4 |
| | | | | WWTP | 5 | 0.46-0.71 | 5.8 |
| | | | | Down | 4 | 0.12-0.22 | 1.3 |
| *Pimephales notatus* | Bluntnose Minnow (BN) | Omnivore Demersal | 2.7 | Up | 5 | 1.5-2.1 | 9.2 |
| | | | | WWTP | 5 | 2.3-3.1 | 4.2 |
| | | | | Down | 5 | 0.10-0.30 | 6.6 |
| *Galaxiella nigrostriata* | Blackstripe Minnow (BS) | Invertivore Benthopelagic | 2.9 | Up | 4 | 0.01-0.19 | 7.0 |
| | | | | WWTP | 3 | 0.04-0.13 | 1.33 |
| | | | | Down | 1 | 0.04 | 7.0 |
| *Lepomis macrochirus* | Bluegill (BG) | Invertivore Benthopelagic | 3.2 | Up | 5 | 1.1-1.5 | 17.4 |
| | | | | WWTP | 5 | 0.9-1.4 | 29.2 |
| | | | | Down | 5 | 0.5-0.66 | 6.4 |
| *Neogobius melanostomus* | Round Goby (RG) | Zoobenthivore Benthic | 3.3 | Up | 4 | 8.6-13.9 | 2.3 |
| | | | | WWTP | 4 | 0.27-1.7 | 10.3 |
| | | | | Down | 5 | 0.16-1.49 | 5.2 |

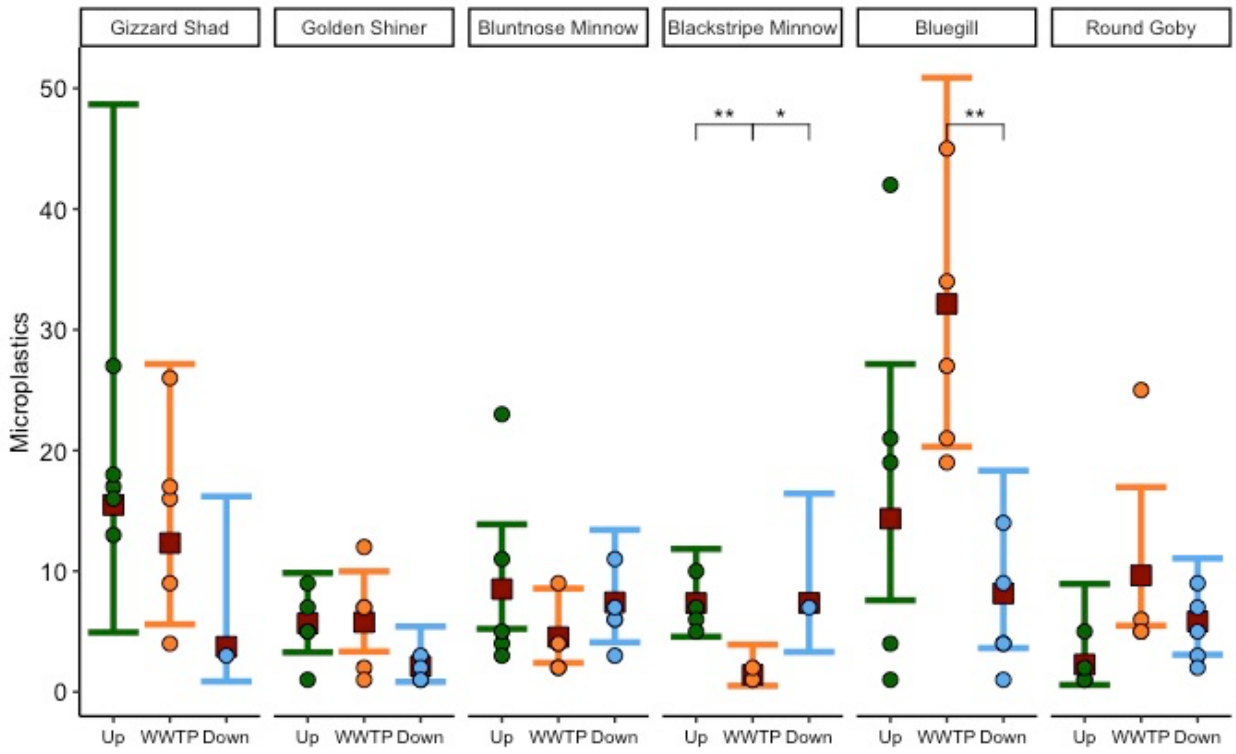


Figure 1. Microplastic in fish taxa increasing from lowest trophic fraction to highest (left to right), collected upstream (Up), downstream (Down) and at the effluent release site of the wastewater treatment plant (WWTP). Vertical lines indicate 95% confidence intervals, red boxes indicated the estimated marginal mean number of microplastics of a given species at a location. Raw data is displayed with filled in circles. Asterisks indicate significance values (* =p-value <0.05, **=p-value <0.01)

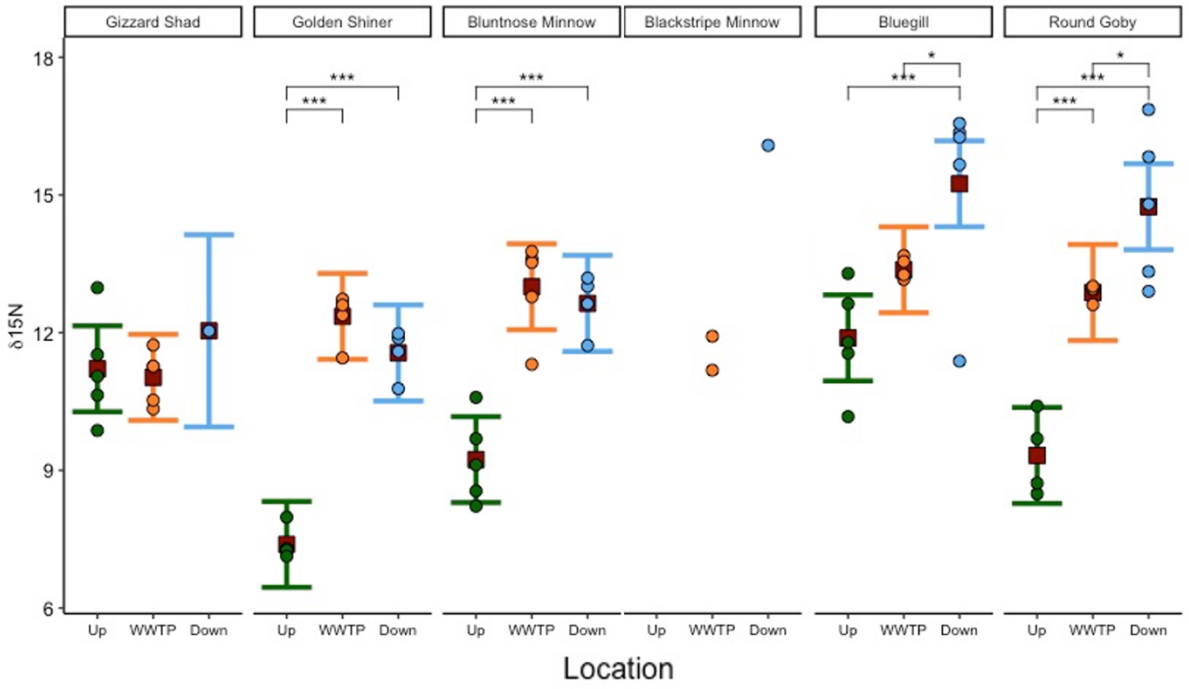


Figure 2. Stable isotopes in fish taxa increasing from lowest trophic fraction to highest (left to right), collected upstream (Up), downstream (Down) and at the effluent release site of the wastewater treatment plant (WWTP). Vertical lines indicate 95% confidence intervals, red boxes indicated the estimated marginal mean number of microplastics of a given species at a location. Raw data is displayed with filled in circles. Asterisks indicate significance values (* =p-value <0.05, **=p-value <0.01, ***=p-value<0.001)

Table 2. Type II Analysis of Deviance table of best fitting models to explain microplastics and $\delta^{15}N$ values in fish with fixed effects (species, location, and species + location) and random effects (mass). Significant values ($p<0.05$) are in bold.

| | | Chi Square | df | p-value |
|---|---|---|---|---|
| Microplastics | | | | |
| | Species | 48.401 | 5 | **<0.001** |
| | Location | 5.637 | 2 | 0.060 |
| | Mass | 0.108 | 1 | 0.742 |
| | Species: Location | 33.921 | 10 | **<0.001** |
| $\delta^{15}N$ | | | | |
| | Species | 83.720 | 4 | **<0.001** |
| | Location | 183.700 | 2 | **<0.001** |
| | Location: Species | 61.737 | 8 | **<0.001** |
| Microplastics | | | | |
| | $\delta^{15}N$ | 0.8013 | 1 | 0.371 |
| | Species | 31.9397 | 6 | **<0.001** |
| | Location | 4.3138 | 2 | 0.116 |
| Microplastics | | | | |
| | $\delta^{15}N$ | 7.2729 | 1 | **0.007** |
| | Location | 11.7317 | 2 | **0.002** |
| | $\delta^{15}N$: Location | 6.2363 | 2 | **0.044** |

Table 3. Model selection results evaluating the best and competing models for two dependent variables: microplastics and $\delta^{15}N$ values, explained by the interactions between (indicated by *) species and location and sometimes with mass as a random effect (indicated by 1| notation commonly used in Program R). Abbreviations: AICc = Akaike's information criterion correction for sample sizes; Neg. = negative, BS = Blackstripe Minnow.

| Model | Dispersion | Model Type | df | AICc |
|---|---|---|---|---|
| *Dependent variable: Microplastic* | | | | |
| Species * Location | | Gaussian | 19 | 541.9959 |
| Species * Location + (1\|Mass) | | Gaussian | 20 | 545.6467 |
| Species * Location | | Poisson | 18 | 550.3651 |
| Species * Location + (1\|Mass) | | Poisson | 19 | 553.7807 |
| Species * Location + (1\|Mass) | | Neg. Binomial | 20 | 490.073 |
| Species * Location + (1\|Mass) | Species | Neg. Binomial | 25 | 494.565 |
| *Dependent variable- $\delta^{15}N$* | | | | |
| Location | | Gaussian | 4 | 276.0775 |
| Location | Location | Gaussian | 6 | 268.4627 |
| Location + Species | | Gaussian | 9 | 255.5746 |
| Location * Species | | Gaussian | 16 | 221.6649 |
| Location * Species (BS removed) | | Gaussian | 16 | 221.6649 |

Table 4. Model selection results evaluating the best and competing models for microplastics counts using $\delta^{15}N$ values, location, species and their interactions (indicated by *). Random effects indicated by (1|variable). Abbreviations: AICc = Akaike's information criterion correction for sample sizes; Neg. = negative.

| Model | Dispersion | Model Type | df | AICc |
|---|---|---|---|---|
| *Dependent variable: Microplastic* | | | | |
| $\delta^{15}N$ | Species | Neg. Binomial | 3 | 468.6659 |
| $\delta^{15}N$ + Location | Species | Neg. Binomial | 10 | 472.3670 |
| $\delta^{15}N$ * Location | Species | Neg. Binomial | 12 | 473.4492 |
| $\delta^{15}N$ * Species | Species | Neg. Binomial | 18 | 465.2663 |
| $\delta^{15}N$ + Species + Location | Species | Neg. Binomial | 15 | 461.4646 |
| $\delta^{15}N$ * Species + Location. | Species | Neg. Binomial | 20 | 465.9898 |
| $\delta^{15}N$* Location + (1 \| Species) | Species | Neg. Binomial | 13 | 471.9236 |

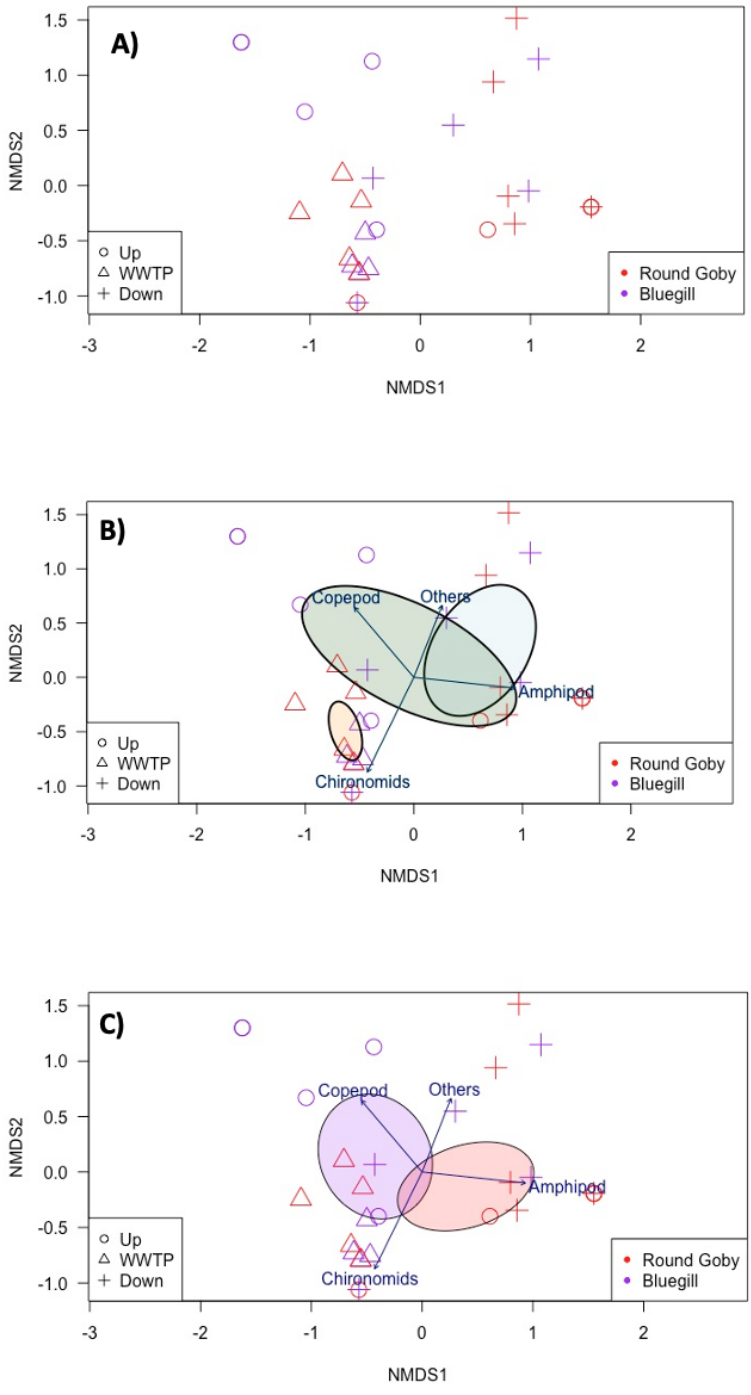


Figure 3. (A) NMDS ordination of Bluegill and Round Goby gut content analysis, (B) NMDS with 95% confidence intervals for location (Upstream=dark green, WWTP=orange, Downstream=light blue) and species, and (C) NMDS with 95% confidence intervals for species (Bluegill=purple, Round Goby=red).

Table 5. PERMANOVA comparison for fish gut content analysis between species and location. Significant values ($p<0.05$) are in bold.

| PERMANOVA | F-value | Significance | |
|---|---|---|---|
| Location | **6.1338** | **<0.001** | |
| Species | **2.9959** | **0.41** | |
| | | | |
| Pairwise Comparison | | WWTP | Down |
| | Down | **0.003** | ---- |
| | Up | 0.117 | 0.291 |

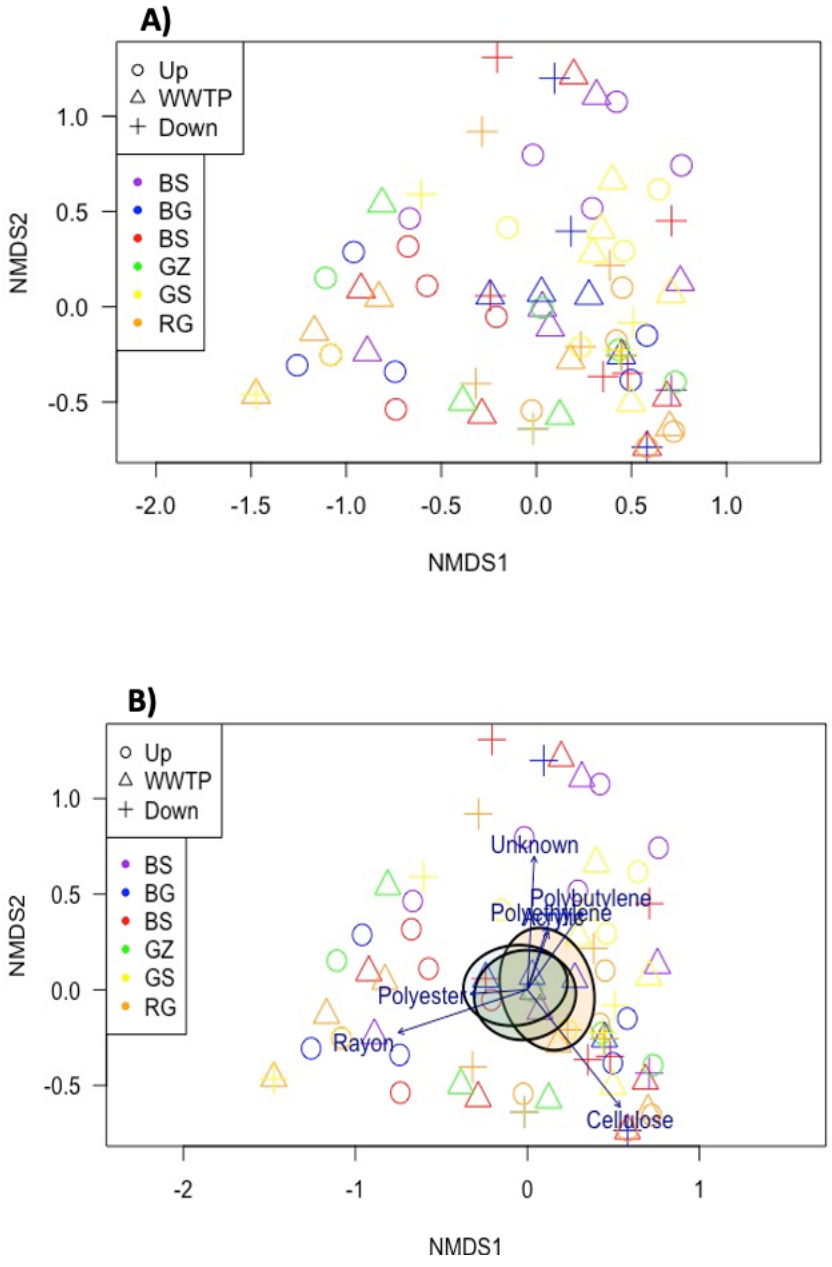


Figure 4. A) NMDS ordination of microplastics analysis across species, and B) NMDS with 95% confidence intervals for location (Upstream=dark green, WWTP=orange, Downstream=light blue).

Table 6. PERMANOVA comparison for polymer identification analysis between species and location. Significant values ($p<0.05$) are in bold.

| PERMANOVA | F-value | Significance | |
|---|---|---|---|
| Location | 1.1402 | 0.3469 | |
| Species | 1.1556 | 0.3239 | |
| | | | |
| Pairwise Comparison | | WWTP | Down |
| | Down | 0.86 | ---- |
| | Up | 0.86 | 0.86 |

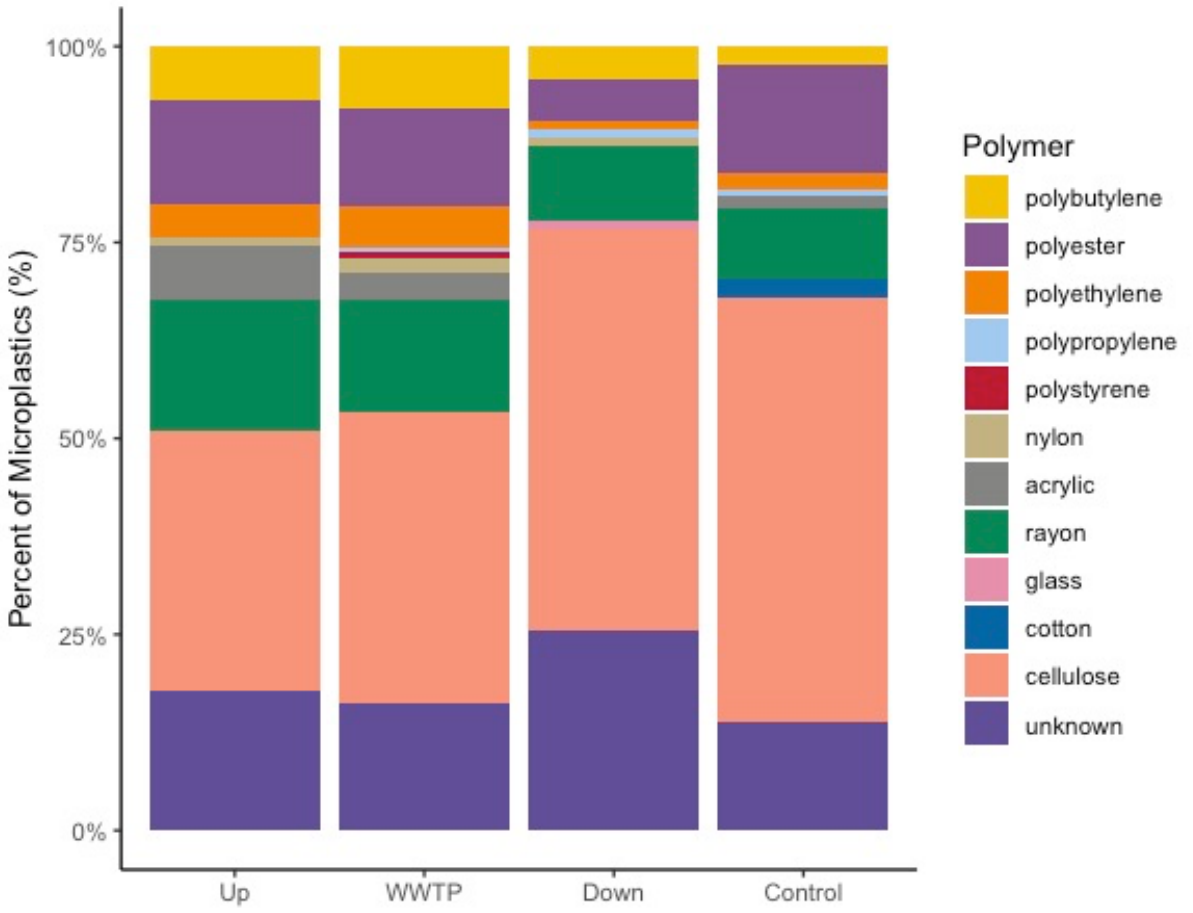


Figure 5. Composition of microplastics polymer type from fish collected upstream (Up), downstream (Down), at the effluent release site of the wastewater treatment plant (WWTP) and laboratory controls (Control).

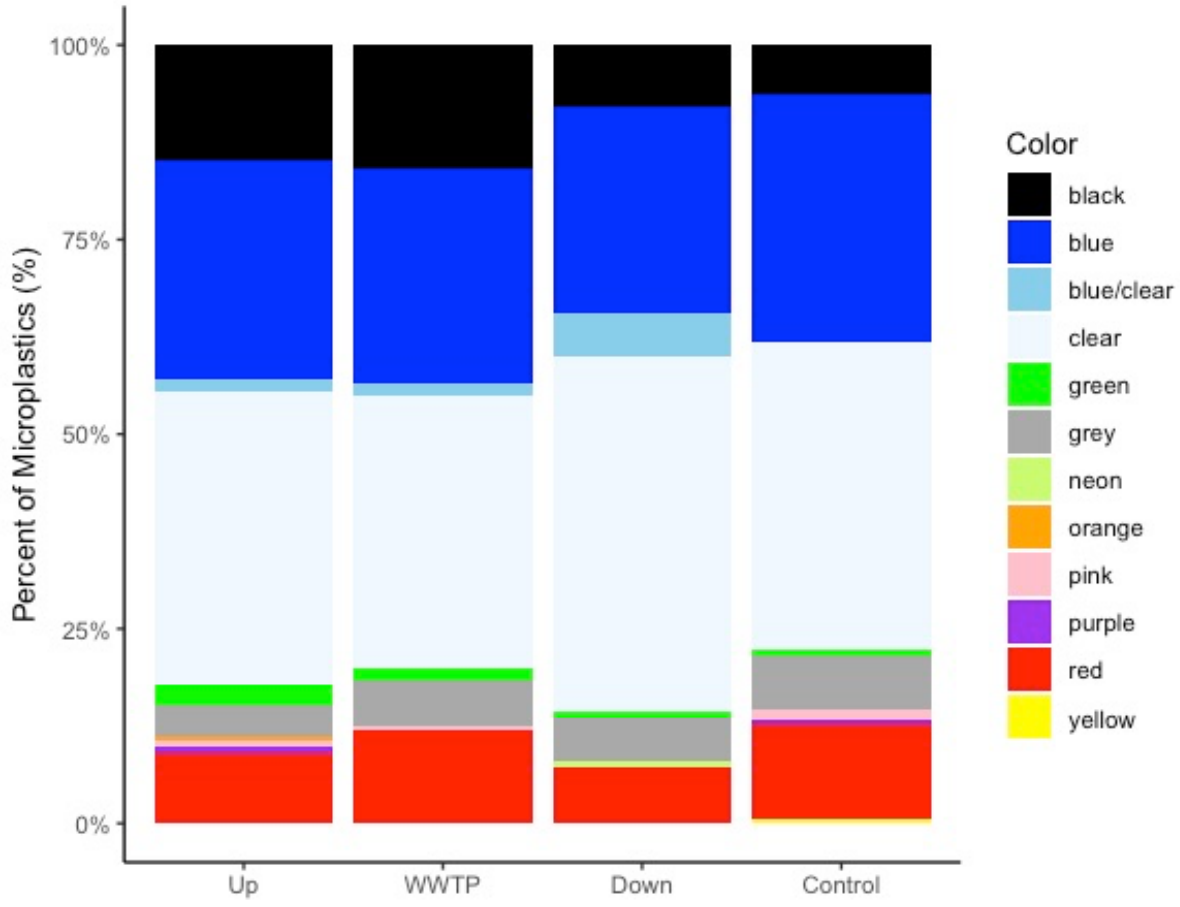


Figure 6. Composition of microplastics colors from fish collected upstream (Up), downstream (Down), at the effluent release site of the wastewater treatment plant (WWTP) and laboratory controls (Control).

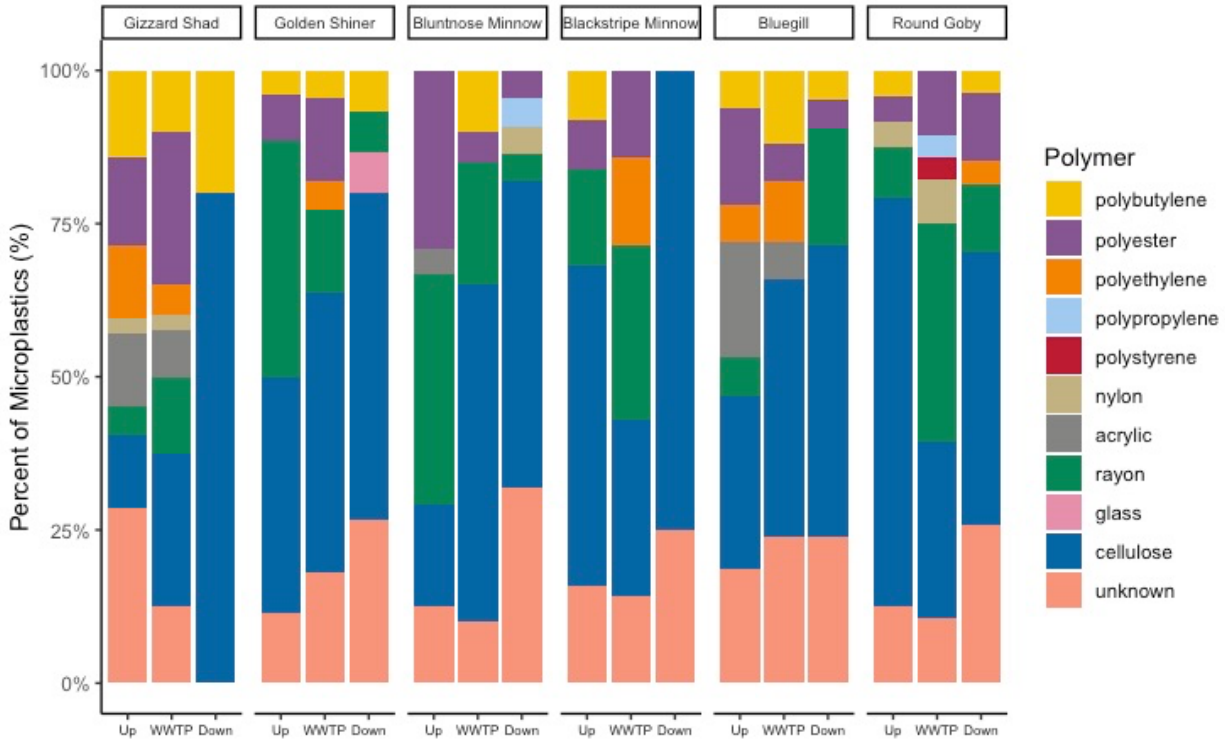


Figure 7. Composition of microplastics polymer types between fish taxa increasing from lowest trophic fraction to highest left to right), collected upstream (Up), downstream (Down) and at the effluent release site of the wastewater treatment plant (WWTP).

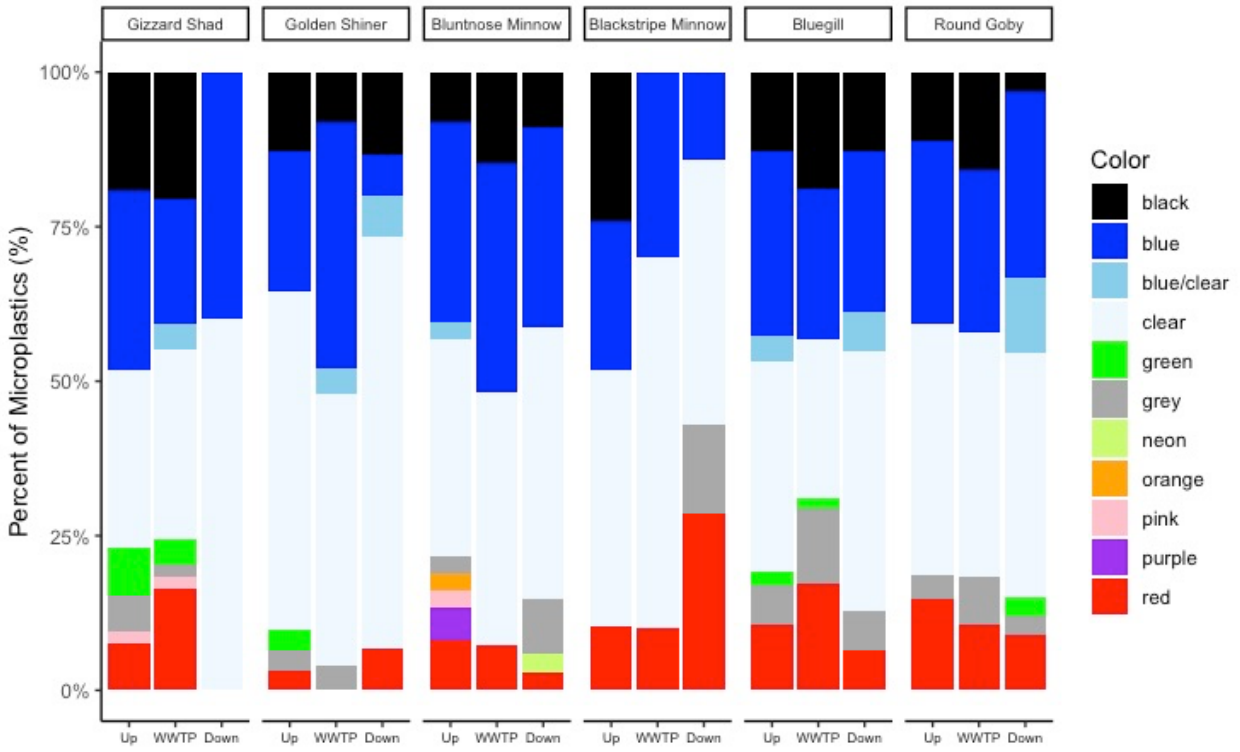


Figure 8. Composition of microplastics colors between fish taxa increasing from lowest trophic fraction to highest left to right), collected upstream (Up), downstream (Down) and at the effluent release site of the wastewater treatment plant (WWTP).

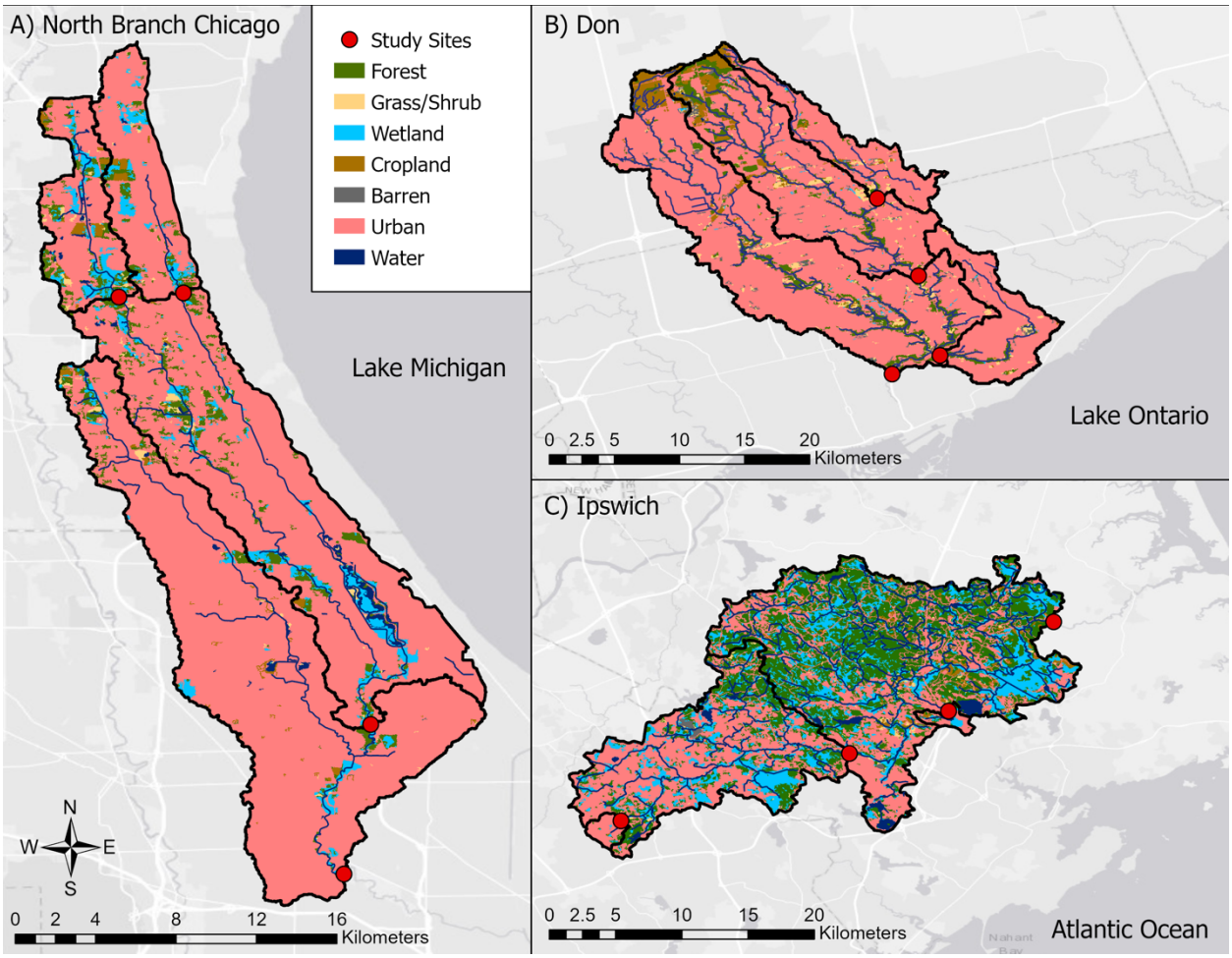


Figure 9. Land use of A) North Branch of the Chicago River, B) Don River, and C) Ipswich River.

Table 7. Summary of all macroinvertebrates used in our analysis including the taxonomical group, functional feeding group (FFG), sites they were collected, and the total number organisms found across all sites.

| Taxonomic Group | FFG | Chicago | | | | Don | | | | | Ipswich | | | No. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Chironomidae* | Collector | x | x | x | x | x | x | x | x | x | x | x | x | 175 |
| *Gammarus pulex* | Shredder | x | x | x | x | | | | x | | x | x | x | 120 |
| Isopoda | Shredder | | | x | x | x | | x | x | | | | | 30 |
| *Hydropsychidae* | Filterers | x | | x | x | x | x | x | x | | | x | x | 45 |
| *Caenis* | Gatherer | | x | | | | | | | | | | | 5 |
| *Baetis* | Gatherer | | | | | | | | | | | x | x | 15 |
| *Clepelmis* | Gatherer | | | | | x | x | | x | | | | | 10 |
| *Basiaeschna janata* | Predator | x | | | | | | | | | | | | 1 |
| *Amphiagrion* | Predator | | x | x | x | | | | | | | | | 3 |
| *Remenus* | Predator | | | | | | | | | | | x | | 2 |
| *Climacia* | Predator | | | | | | | | | | | | x | 2 |
| Perlodidae | Predator | | | | | | | | | | x | | | 1 |
| Empididae | Predator | | | | | | | | x | | | | | 1 |
| *Amphiagrion* | Predator | | | | | | x | | | | | | | 1 |
| *Limnoporus* | Predator | | | | | | | x | | | | | | 1 |

Table 8. Akaike's information criterion corrected values for sample sizes (AICc) for the statistical distribution of microparticles abundance in macroinvertebrates (No./individual), Shredders (No./individual), and Collectors (No./individual). Distributions tested included negative binomial (NB), zero-inflated negative binomial (ZINB), Gaussian, zero-inflated Poisson (ZIP), and Poisson.

| Distribution | AICc | ΔAICc | $w_i$ |
|---|---|---|---|
| *All Macroinvertebrates Model* | | | |
| NB | 541.4 | 0.00 | 0.745 |
| ZINB | 543.5 | 2.14 | 0.255 |
| Gaussian | 645.5 | 104.17 | <0.0001 |
| ZIP | 894.2 | 352.79 | <0.0001 |
| Poisson | 895.4 | 354.03 | <0.0001 |
| *Shredder Model* | | | |
| Poisson | 122.4 | 0.00 | 0.536 |
| NB | 124.2 | 1.86 | 0.211 |
| ZIP | 124.7 | 2.30 | 0.170 |
| ZINB | 126.7 | 4.34 | 0.061 |
| Gaussian | 128.7 | 6.39 | 0.022 |
| *Collector Model* | | | |
| Poisson | 160.7 | 0.00 | 0.678 |
| NB | 162.7 | 2.01 | 0.248 |
| Gaussian | 165.1 | 4.43 | 0.074 |

Table 9. Model selection results evaluating the best and competing models for microparticles abundance in macroinvertebrates (No./individual) explained by experimental variables and macroinvertebrate length. Null models were included as reference regardless of if the null was or was not a competing model. Abbreviations: LL is the log-likelihood ratio; AICc is Akaike's information criterion corrected for samples size; ΔAICc is the difference from the best fitting model; $w_i$ is the AICc weight; MP is Microparticles; FFG is functional feeding group; Wcol is the microparticle concentration in the water column; Position is the relative location in each watershed (i.e., headwater, branch, main stem); Surf is microparticle concentration in the surface water; Location is each unique sample site.

| | df | LL | AICc | ΔAICc | $w_i$ |
|---|---|---|---|---|---|
| *All Macroinvertebrates Model* | | | | | |
| MP + FFG + Wcol | 7 | -206.09 | 427.55 | 0.00 | 0.4734 |
| MP + FFG + Wcol + Position | 9 | -205.12 | 430.49 | 2.94 | 0.1090 |
| MP + FFG + Wcol Fibers | 7 | -207.58 | 430.52 | 2.96 | 0.1075 |
| MP + FFG | 6 | -209.46 | 431.93 | 4.38 | 0.0530 |
| MP + FFG + Wcol + Watershed | 9 | -205.97 | 432.19 | 4.63 | 0.0467 |
| MP + FFG + Watershed | 8 | -207.30 | 432.39 | 4.83 | 0.0423 |
| MP+ FFG + Wcol Fibers + Position | 9 | -206.18 | 432.61 | 5.06 | 0.0378 |
| MP + FFG + Surface | 7 | -209.02 | 433.41 | 5.85 | 0.0254 |
| MP + FFG + Length | 7 | -209.02 | 433.41 | 5.86 | 0.0253 |
| MP + FFG + Benthic Fibers | 7 | -209.32 | 434.00 | 6.45 | 0.0188 |
| MP + FFG + Surf Fibers | 7 | -209.33 | 434.02 | 6.47 | 0.0187 |
| MP + FFG + Benthic | 7 | -209.40 | 434.17 | 6.62 | 0.0173 |
| MP + FFG + Wcol Fiber + Watershed | 9 | -207.14 | 434.53 | 6.98 | 0.0145 |
| MP + FFG + Position | 8 | -208.77 | 435.31 | 7.76 | 0.0098 |
| MP + FFG + Location | 17 | -199.38 | 441.25 | 13.70 | 0.0005 |
| MP + Length | 3 | -262.75 | 531.77 | 104.22 | 0.0000 |
| MP + Surf Fibers | 3 | -266.95 | 540.17 | 112.62 | 0.0000 |
| Null | 2 | -268.62 | 541.38 | 113.82 | 0.0000 |
| MP + Benthic Fibers | 3 | -267.74 | 541.76 | 114.21 | 0.0000 |
| MP +Wcol Fibers | 3 | -267.93 | 542.14 | 114.59 | 0.0000 |
| MP + Surf | 3 | -267.97 | 542.22 | 114.66 | 0.0000 |
| MP + Wcol | 3 | -268.08 | 542.44 | 114.88 | 0.0000 |
| MP + Benthic | 3 | -268.17 | 542.63 | 115.07 | 0.0000 |
| MP + Watershed | 4 | -268.23 | 544.94 | 117.39 | 0.0000 |
| MP + Position | 4 | -268.60 | 545.66 | 118.11 | 0.0000 |
| MP + Location | 13 | -264.78 | 560.36 | 132.80 | 0.0000 |

Table 10. Model selection results evaluating the best and competing models for microparticles abundance in Shredders (No./individual), and Collectors (No./individual) explained by experimental variables and macroinvertebrate length. Null models were included as reference regardless of if the null was or was not a competing model. Abbreviations: LL is the log-likelihood ratio; AICc is Akaike's information criterion corrected for samples size; ΔAICc is the difference from the best fitting model; $w_i$ is the AICc weight; MP is Microparticles; FFG is functional feeding group; Wcol is the microparticle concentration in the water column; Position is the relative location in each watershed (i.e., headwater, branch, main stem); Surf is microparticles concentration in the surface water; Location is each unique sample site.

| | df | LL | AICc | ΔAICc | $w_i$ |
|---|---|---|---|---|---|
| *Shredders Model* | | | | | |
| Null | 1 | -60.11 | 112.40 | 0.00 | 0.2620 |
| MP + Surf | 2 | -59.91 | 124.30 | 1.91 | 0.1010 |
| MP + Wcol Fibers | 2 | -60.04 | 124.50 | 2.18 | 0.0890 |
| MP + Length | 2 | -60.07 | 124.60 | 2.23 | 0.0870 |
| MP + Wcol | 2 | -60.07 | 124.60 | 2.24 | 0.0860 |
| MP + Surf Fibers | 2 | -60.08 | 124.60 | 2.25 | 0.0860 |
| MP + Position | 2 | -60.10 | 124.70 | 2.30 | 0.0840 |
| MP + Benthic | 2 | -60.11 | 124.70 | 2.30 | 0.0840 |
| MP + Benthic Fibers | 2 | -60.11 | 124.70 | 2.30 | 0.0840 |
| MP + Watershed | 3 | -59.70 | 126.30 | 3.97 | 0.0360 |
| MP + Location | 11 | -57.96 | 152.60 | 30.24 | 0.0000 |
| *Collectors Model* | | | | | |
| MP + Wcol Fibers | 2 | -73.59 | 151.6 | 0 | 0.2400 |
| MP + Wcol | 2 | -73.669 | 151.7 | 0.16 | 0.2200 |
| MP + Wcol + Ben Fibers | 3 | -73.319 | 153.4 | 1.86 | 0.0950 |
| MP + Wcol + Position | 3 | -73.349 | 153.5 | 1.92 | 0.0920 |
| MP + Wcol + Surf | 3 | -73.478 | 153.7 | 2.17 | 0.0810 |
| MP + Wcol + Benthic | 3 | -73.568 | 153.9 | 2.35 | 0.0740 |
| MP + Wcol + Length | 3 | -73.658 | 154.1 | 2.53 | 0.0680 |
| MP + Wcol + Surf Fibers | 3 | -73.667 | 154.1 | 2.55 | 0.0670 |
| MP+ Watershed | 3 | -74.632 | 156 | 4.48 | 0.0260 |
| MP + Wcol + Watershed | 4 | -73.389 | 156.1 | 4.56 | 0.0250 |
| Null | 1 | -79.276 | 160.7 | 9.12 | 0.0030 |
| MP + Surf | 2 | -78.448 | 161.3 | 9.72 | 0.0020 |
| MP + Position | 2 | -78.523 | 161.45 | 9.86 | 0.0020 |
| MP + Length | 2 | -78.675 | 161.7 | 10.17 | 0.0010 |
| MP + Surf Fiber | 2 | -79.792 | 162 | 10.4 | 0.0010 |
| MP + Benthic Fibers | 2 | -79.14 | 162.7 | 11.1 | 0.0010 |
| MP + Benthic | 2 | -79.274 | 162.9 | 11.37 | 0.0010 |

| | | | | | |
|---|---|---|---|---|---|
| MP + Location | 12 | -66.134 | 170.5 | 118.89 | 0.0000 |
| MP + Wcol + Location | 12 | -66.134 | 170.5 | 18.89 | 0.0000 |

Table 11. Type II Analysis of Variance Deviance table of best fitting models with significant factors explaining microparticle abundance in macroinvertebrates or collectors. Significant values ($p < 0.05$) are in bold.

| | | Likelihood Ratio | |
|---|---|---|---|
| Parameter | *df* | $X^2$-Value | P-Value |
| *All Macroinvertebrate model* | | | |
| FFG | 4 | 306.69 | **<0.001** |
| Water Column | 1 | 7.22 | **0.007** |
| *Collectors Model* | | | |
| Water Column Fibers | 1 | 11.37 | **<0.001** |

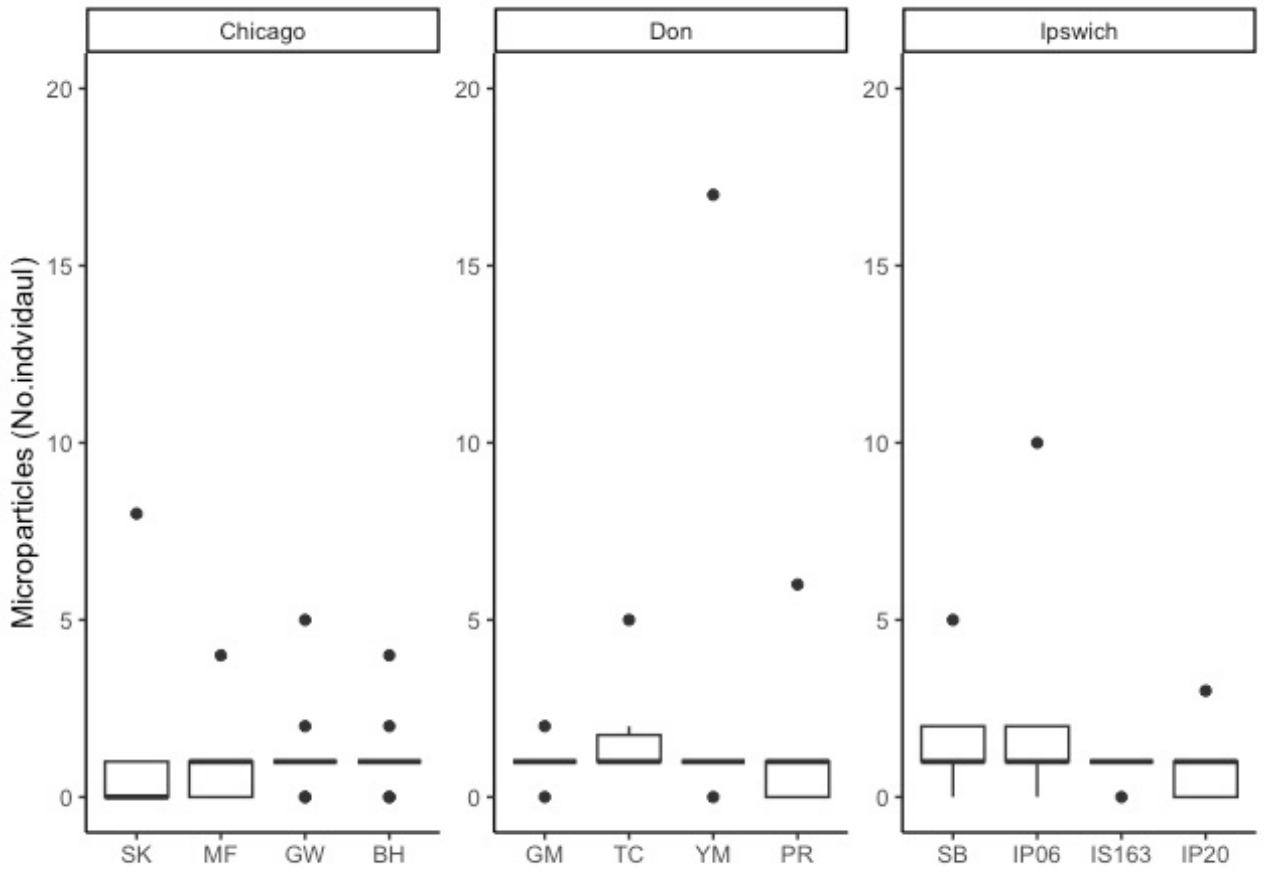


Figure 10. Microparticles in macroinvertebrates collected across all study sites. Vertical lines indicate 95% confidence intervals, solid black lines indicate the estimated marginal mean number of microparticles of macroinvertebrates found at a location. The black dots represent outliers.

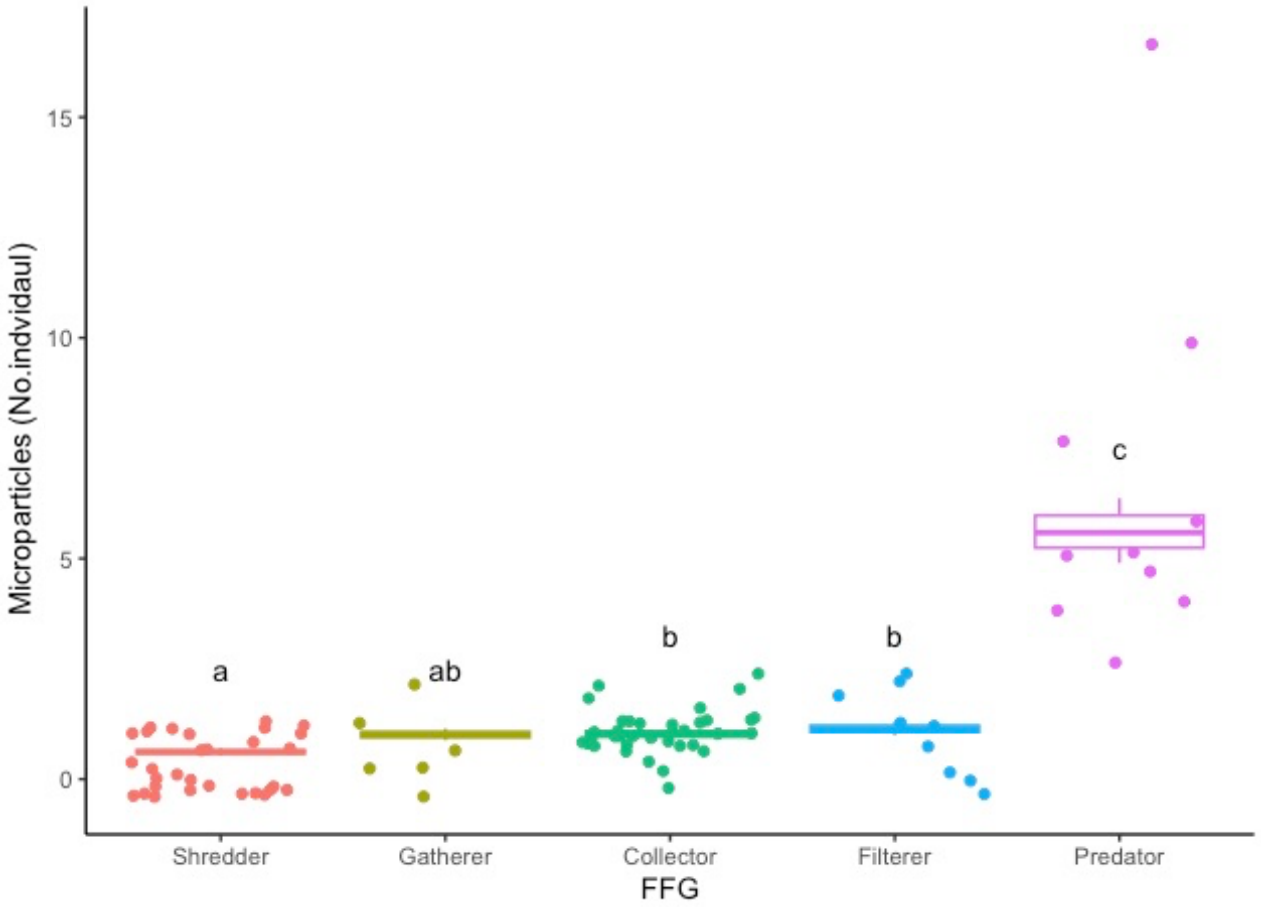


Figure 11. Microparticles found in macroinvertebrates compared to functional feeding groups (FFG). Points represent raw data values of microparticles per individual. Box and whisker are outputs from our best fitting predictive model. Vertical lines indicate 95% confidence intervals, upper and lower end of the boxes represent the quartiles, and the midline is the estimated marginal mean number of microparticles of a given FFG.

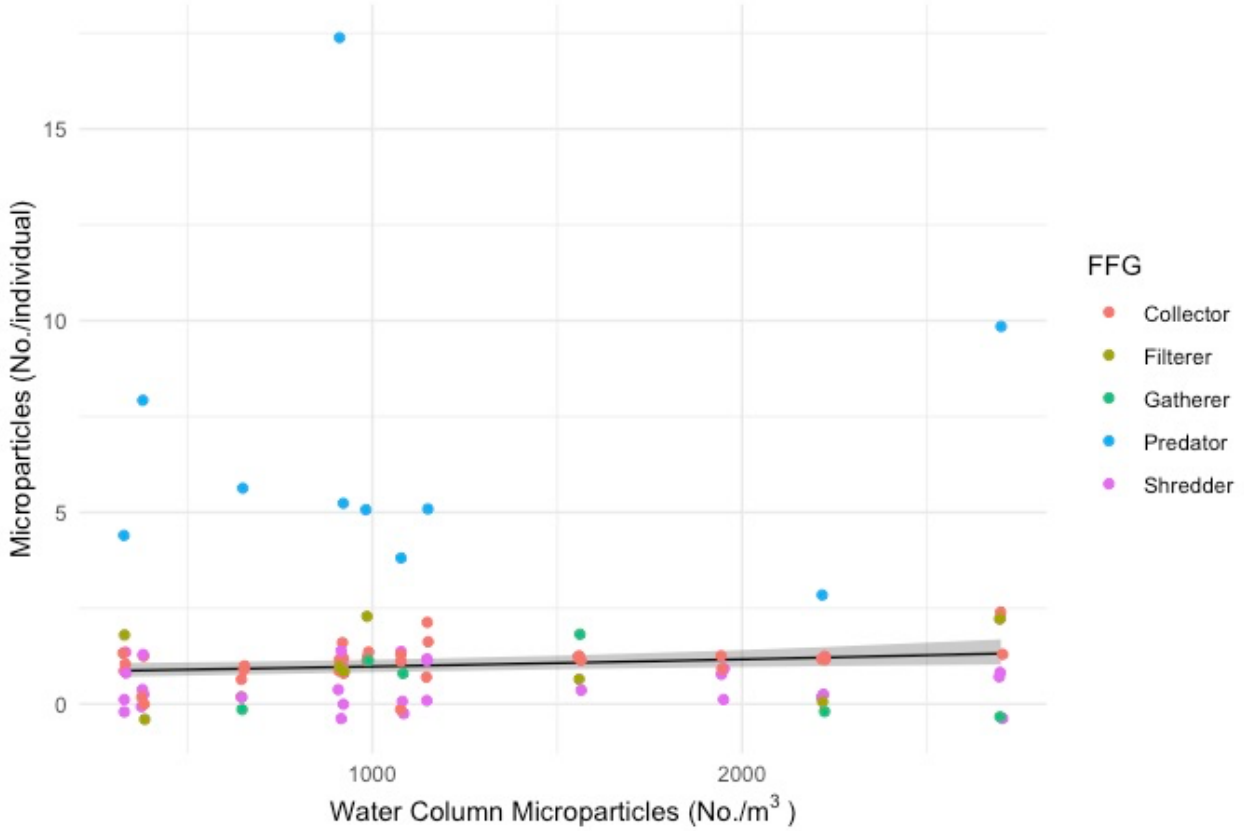


Figure 12. Microparticles found in macroinvertebrates compared to water column microparticles concentrations (No./$m^3$). Points represent raw data values of microparticles per individual. The linear regression line is an output function from our best fitting predictive model estimated the marginal mean number of microparticles in an individual macroinvertebrate at any given water column concertation of microparticle (No./$m^3$). Gray shading indicates 95% confidence intervals of our best fitted predicted model.

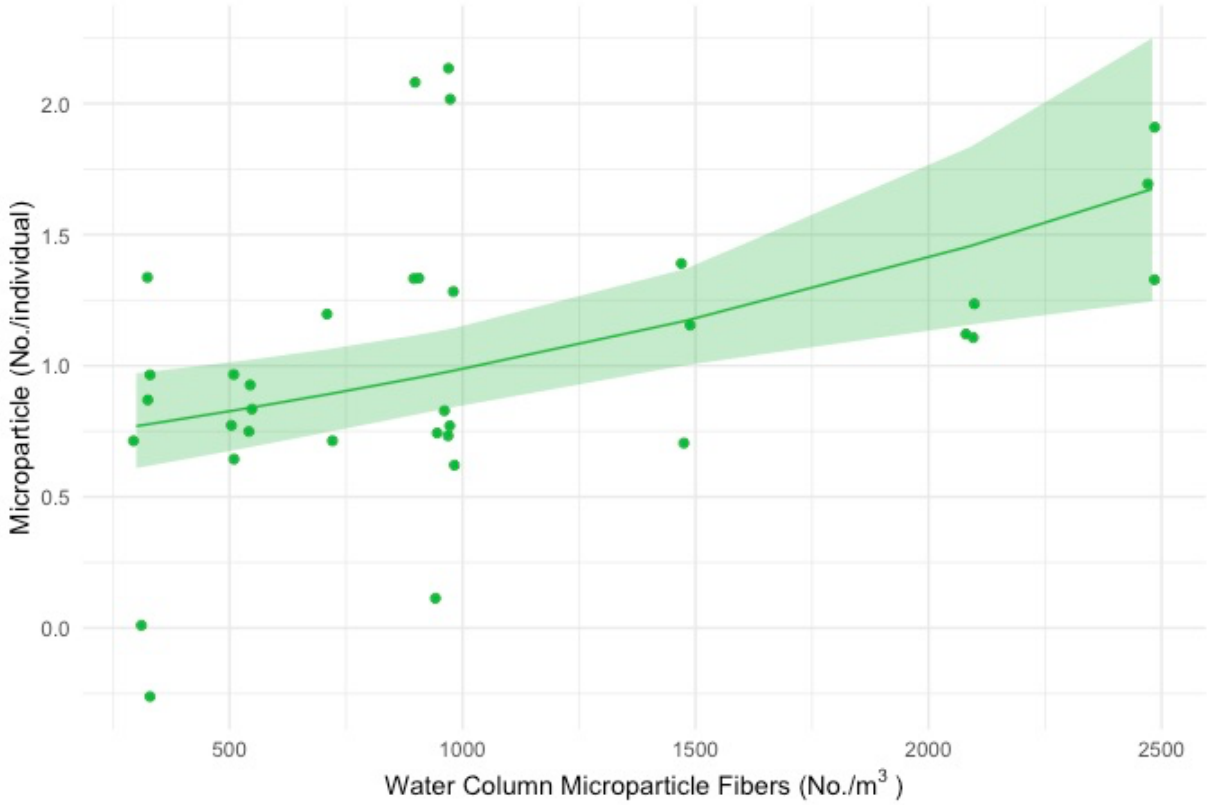


Figure 13. Microparticles found in Collectors (No./ individual) compared to water column microplastics fiber concentrations (No./ $m^3$). Points represent raw data values of microparticles per individual. The linear regression line is an output function from our best fitting predictive model estimated the marginal mean number of microparticles in an individual Collectors at any given water column concertation of microparticle fibers (No./$m^3$). Gray shading indicates 95% confidence intervals of our best fitted predicted model.

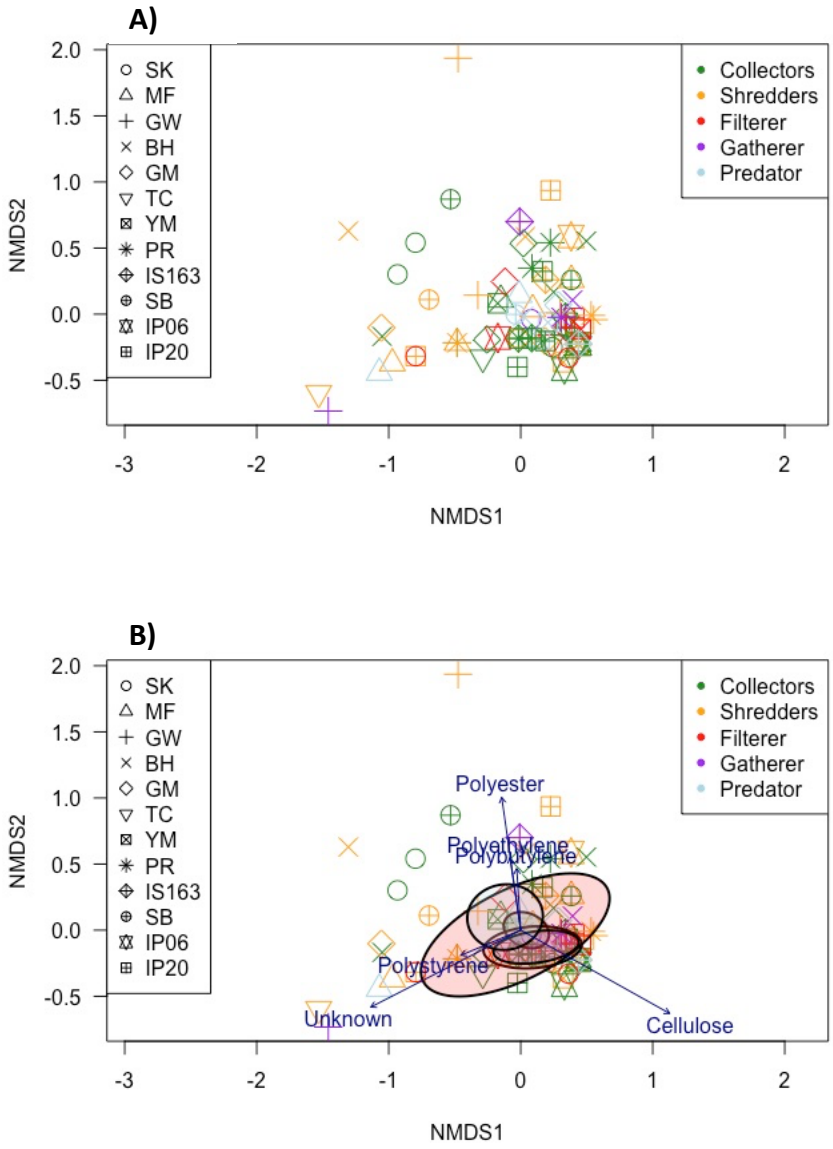


Figure 14. A) Non-metric multidimensional scaling (NMDS) ordination of microparticles analysis in macroinvertebrates among sites and functional feeding groups (FFG), and B) NMDS with 95% confidence intervals for FFG illustrated as ellipses (Collectors=green, Shredders=orange, Filterers= red, Gatherers=purple, Predators=light blue).

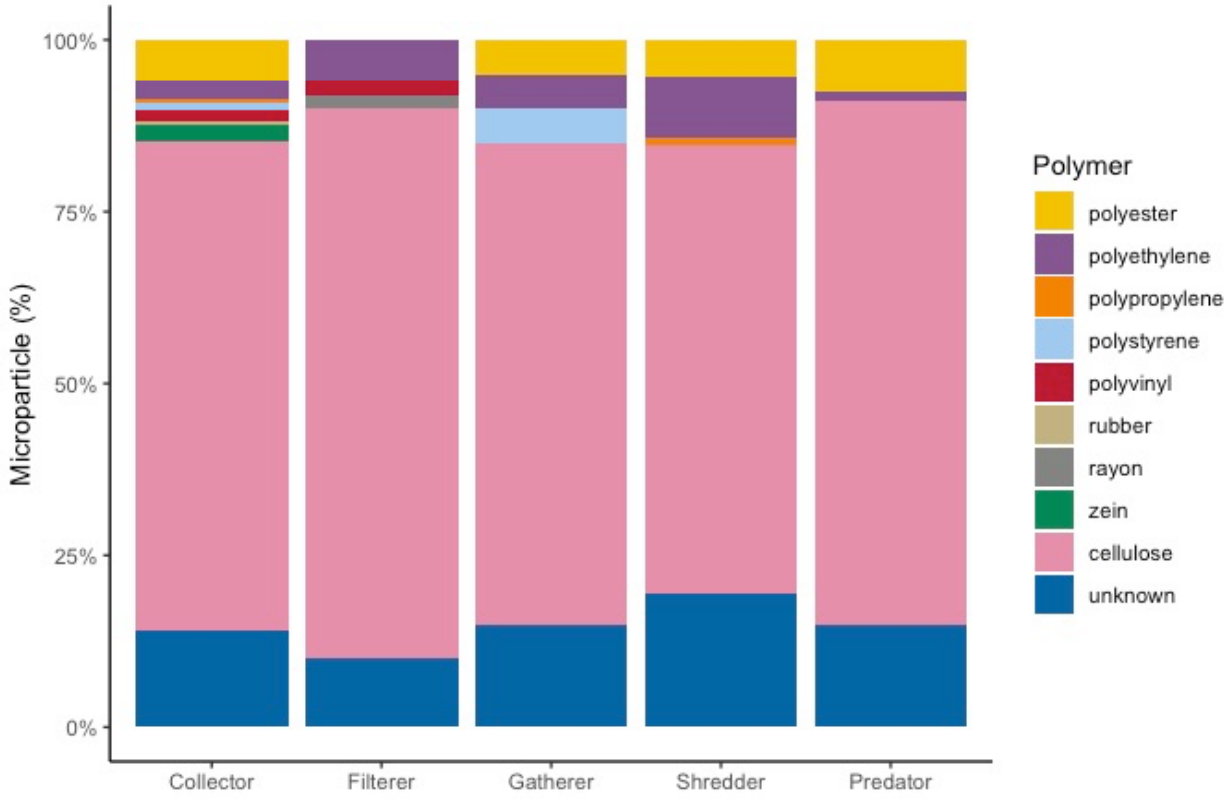


Figure 15. Composition of microparticles polymer type from macroinvertebrate functional feeding groups collected across all study sites.

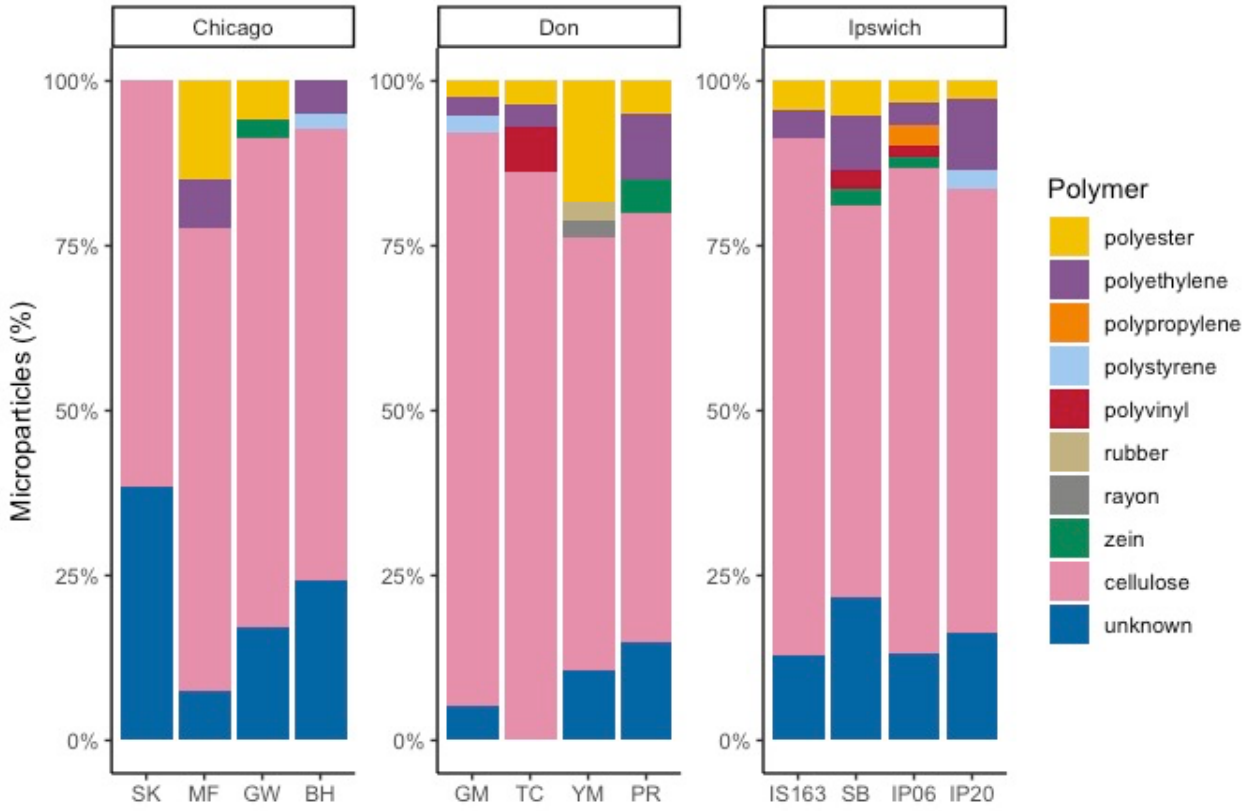


Figure 16. Composition of microparticles polymer type from macroinvertebrate collected across at each study sites.

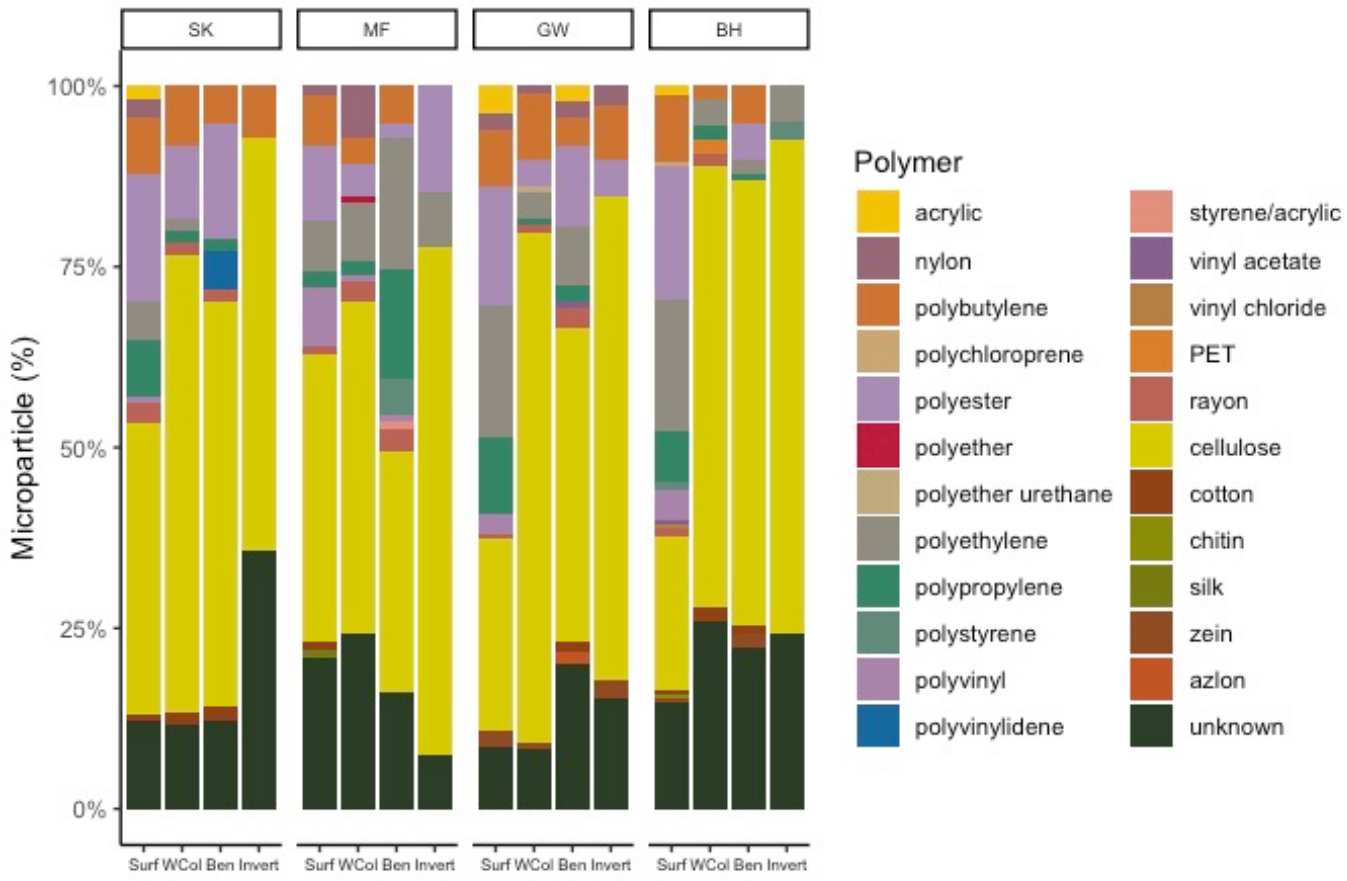


Figure 17. Composition of microparticle polymer type from environmental samples collected at 4 sites in our Chicago watershed.

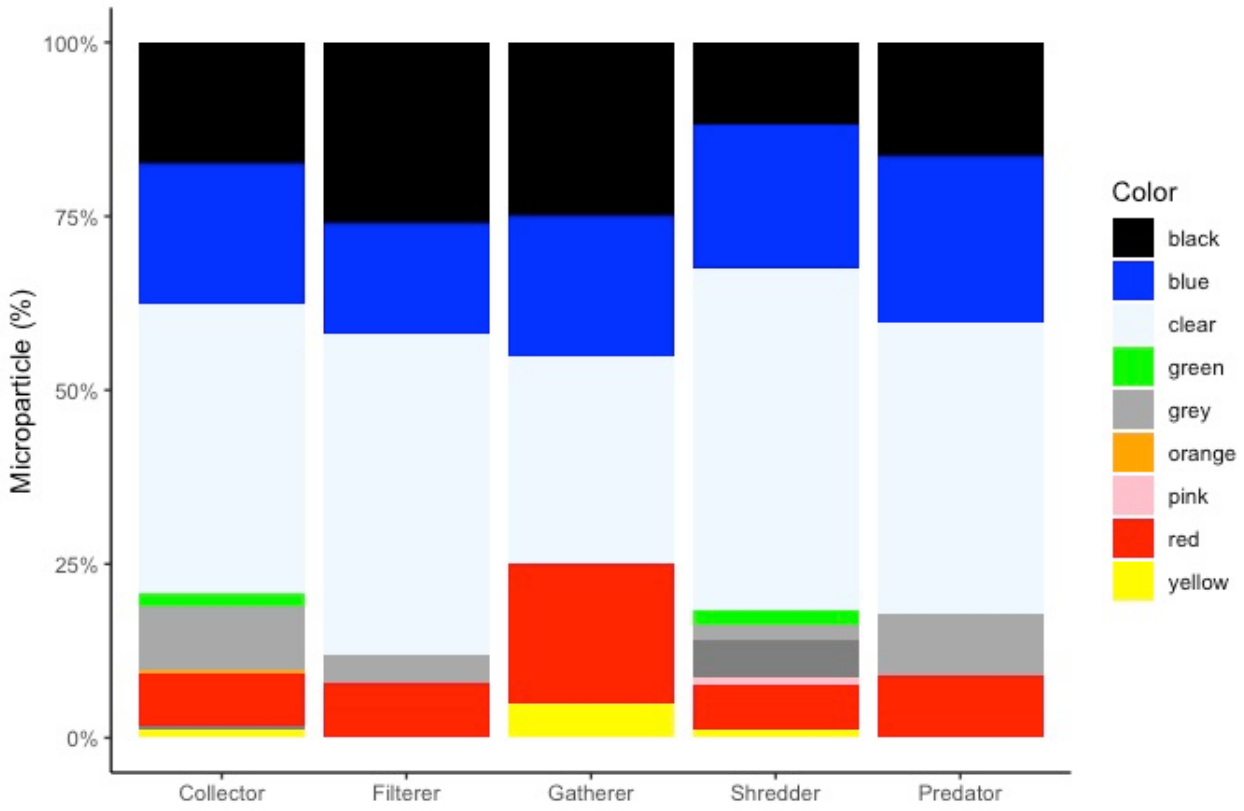


Figure 18. Composition of microparticles colors from macroinvertebrate functional feeding groups collected across all study sites.

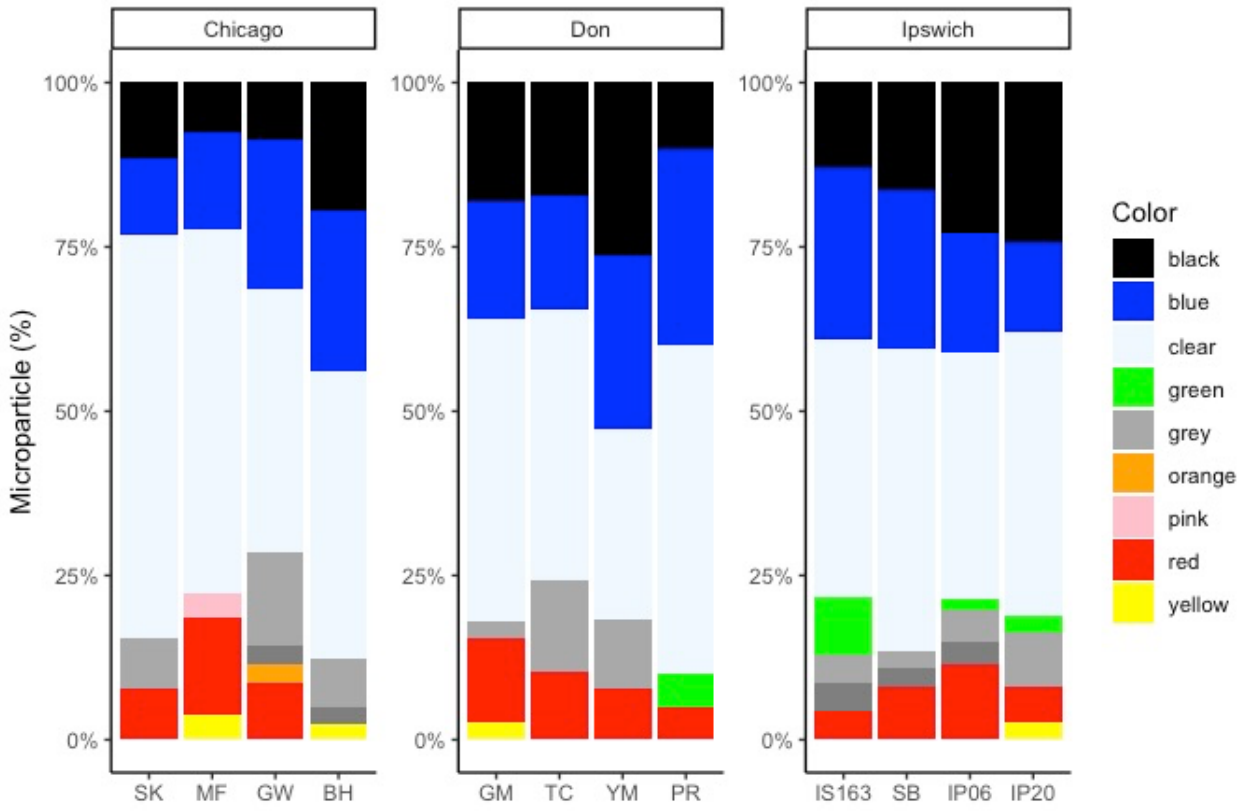


Figure 19. Composition of microparticles color from macroinvertebrate collected across at each study sites.

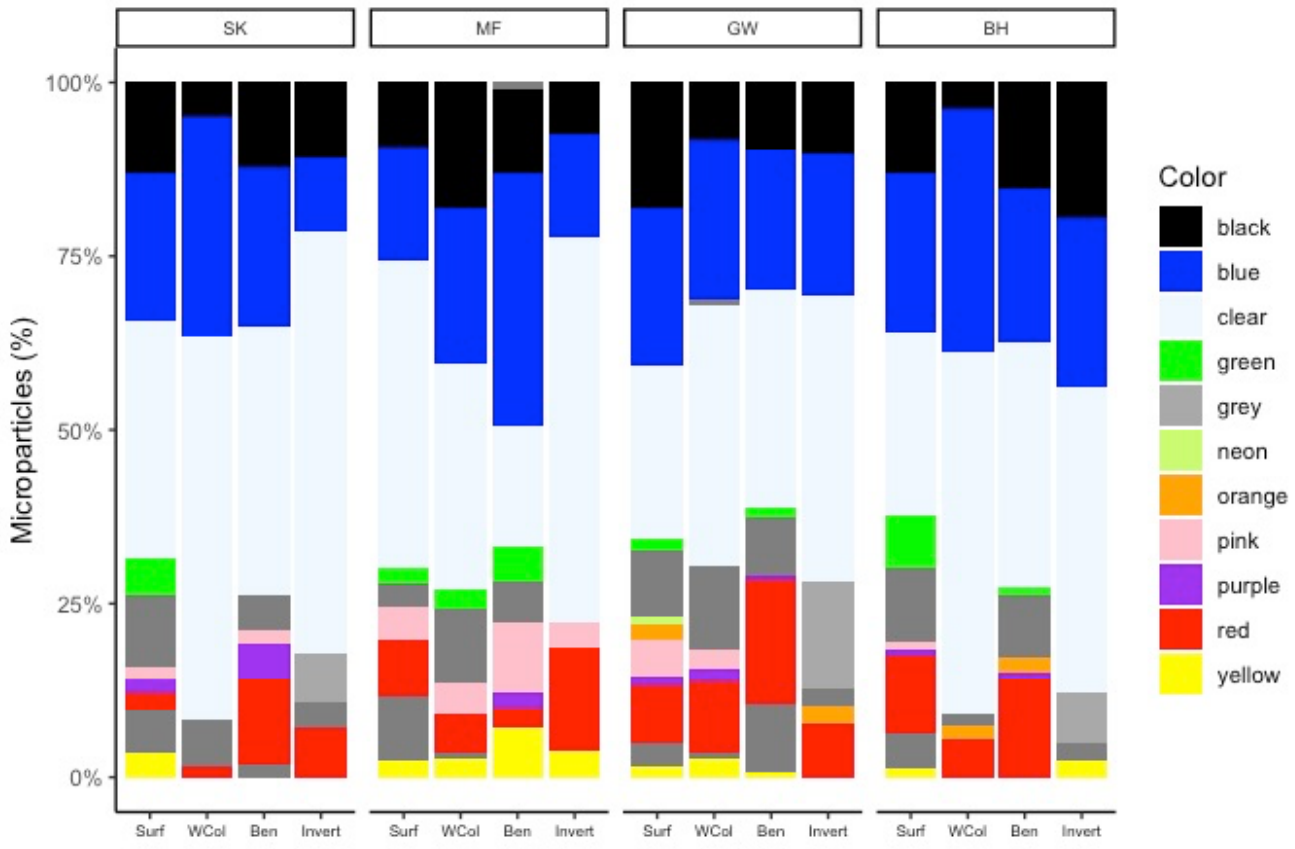


Figure 20. Composition of microparticle colors from environmental samples collected at 4 sites in our Chicago watershed.

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# VITA

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