Fishing for Plastics
Assessing Microplastics in Freshwater Fish: A Critical Review

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Abstract

Microplastics (MP), plastic fragments that do not exceed the size of 5mm, are found polluting terrestrial, marine, and freshwater environment causing global concern. Microplastics are transported from terrestrial sources through the improper disposal of plastic products, leachates from landfills, and from incomplete removal from wastewater treatment plants. Rivers transfer MPs to the marine environments but also start to accumulate in freshwater systems. Much of the research done on MPs is predominantly focused on the marine environment, over the past decade only 13% of studies investigated the occurrence in freshwaters and fewer describe their presence in freshwater organisms. Microplastic pollution in freshwater fish is a globally growing interest, with only a few current studies. Between these studies there are discrepancies in methodologies specifically in sampling, the preparation of the sample, and the expression of results. This lack of coherence hampers the comparison between studies resulting in the failure to comprehend the full extent of MP pollution. The importance of choosing sampling sites efficiently through GIS will help the field narrow its questions faster with less resources expended. This review focuses on MP ingestion by freshwater fish and aims to provide an overview of the existing data, recommend ways to harmonize methodologies, and create a map that outlines parameters that aid in focusing study areas in NYS.

Introduction

Plastic products are valued for their durability, low production cost, versatility, and long life (Hammer et al., 2012). Although plastics' valuable features make exceptional materials, there are growing global concerns over its invasive dispersal into terrestrial and aquatic environments. Annual global plastic production exceeded 320 million tons in 2016; a large portion of this increasing production serves only ephemeral purpose, after which it is quickly discarded. A small portion of plastic is recycled or incinerated, but the majority finds its way to landfills or is discarded into the natural environment, where it makes up 10% of global municipal waste (Lebreton et al., 2019)(Barnes et al., 2010). While plastics in general pose risks, it’s less invisible MPs that are the cause for alarm (Andrady, 2011).

Despite the recognition that MPs accumulate in freshwater ecosystems there has been little monitoring of, or research examining, the presence of MPs in freshwater ecosystems (Wagner & Lambert, 2017). Rather, research to date has been predominantly focused on the marine environment. The imbalance is understandable given the high economic value of marine resources and the corresponding funding allocated to marine research (Collard et al., 2019). Resulting in 13% of all MP studies investigate freshwater (Wagner & Lambert, 2017) and still fewer specifically describe the presence of MPs in freshwater organisms. Of these freshwater studies, many used only small samples sizes. This shows that we lack the data density to understand the spatial and temporal drivers of MP contamination in freshwater and their implications for the health of these critical ecosystems (Ryan et al., 2009)(Fig 1).

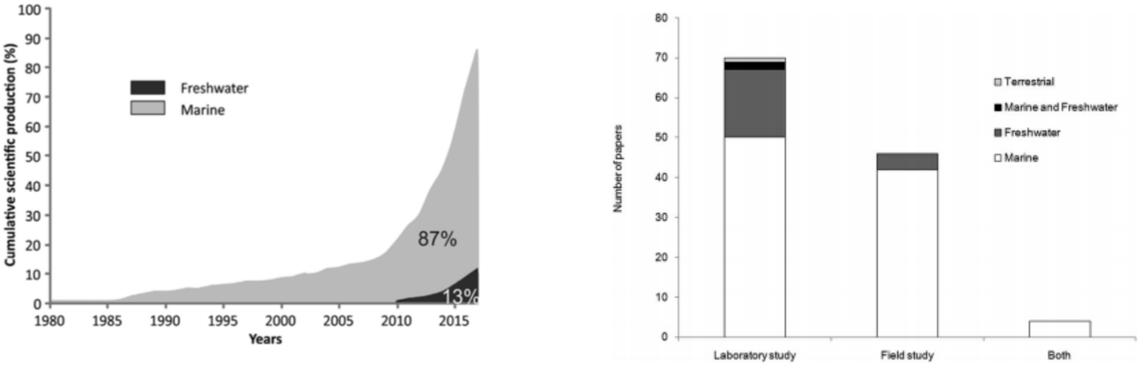

Figure 1 A. Publication trend of papers researching MPs in aquatic organisms (Blettler et al., 2018). B. Comparison of number of studies on marine organisms versus freshwater organisms in the field and the lab. (Lusher et al 2016).

MPs are defined as synthetic organic polymer fragments that do not exceed the size of 5mm (Derraik, 2002) Physical, biological, and chemical processes can degrade the structural durability of plastics (Cole et al., 2011). MPs can be categorized into either primary (orginal size is less than 5mm) or secondary (mechanically degraded from larger materials) MPs (Thompson et al., 2004). Primary MPs are often found in consumer textiles, medicines, and facial/body scrubs. Secondary MPs are created through the mechanical breakdown. The majority of MPs found are secondary MPs. The occurrence of secondary MPs will increase as long as there is an input of plastic waste (Cole et al., 2011) (Fig 2).

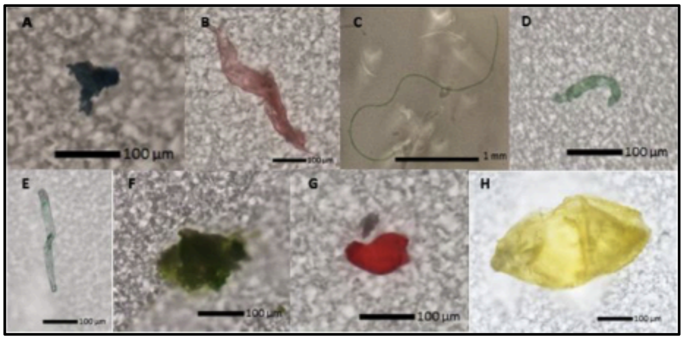

Figure 2 Identified MPs found in Belgium. A. foam, B. film, C. fiber, D. film, E. fiber, F. foam, G. fragment, H. pellet (Slootmaekers et al. 2019)

Microplastics from terrestrial sources contribute to 80% of marine litter through improper disposal of plastics, leachates from landfills, and from WWTP (Cole et al., 2011; Thompson et al., 2004). Microplastics are present on beaches, surface waters, throughout the water column, and even found in the remote waters of the both the Arctic and Antarctic (Barnes et al., 2010). The great pacific garbage patch, the accumulation of plastic litter in the North Pacific gyre, is widely used to publicize the magnitude of plastic pollution in the ocean. The great pacific garbage patch has an estimated 79,000 tons of plastics floating inside a 1.6 million km^2 area and found that MPs account for 8% of the mass (Lebreton et al. 2018). Marine species coming in contact with and ingesting plastics can cause internal and external wounds, skin lesions, sores, suffer from blocked GI tract and can absorb toxins from the environment. Research on MPs in the marine environment are accountable for 63.5% off all published articles in the following journals: *Marine Pollution Bulletin* (30.3%)*, Environmental Science & Technology* (17.5%)*, Environmental Pollution* (9.2%)*, and Marine Environmental Research* (5.5%) (Barboza & Gimenez, 2015). This large body of MPs focused marine research chronicles the adverse effects they have on aquatic organisms and provides a template for similar work in fresh water environments.

Current freshwater studies focus on the accumulation and sources of MP contamination. Landscape features in a watershed influence MP transportation throughout rivers however, few studies have analyzed how the landscape influences the interactions with aquatic organisms. Landscape features that act as sinks such as, dams, lakes, and low-velocity zones encourage the deposition and accumulation of MPs. WWT effluent has also been observed as one of the major point-sources of MP pollution (McNeish et al., 2018). Wastewater effluent deposits MPs into rivers which travels into oceans. The debris that does not end up in the ocean starts to accumulate in freshwater systems (Free et al., 2014). Microplastics deposited in fast moving and dynamic freshwater systems like rivers can eventually reach the ocean, however, a more pressing concern is when MPs come to rest in static freshwaters (e.g. lakes or ponds) because they can more easily accumulate (Klein et al., 2018).

The large discrepancy in methodologies observed in the literature shows the important need for more concise measurements, visual sorting, species selection, and sample size. The inconsistency in methodology results in different conclusions on what species/waterbodies are most effected. For instance, benthic and demersal feeders were observed to have higher MP concentrations (Jabeen et al., 2017; Murphy et al., 2017) while, a different study found lower MP concentrations in these species (Rummel et al., 2016). This review aims to harmonize methodologies, compare MP contamination in fish species, and provide parameters that aid in focusing study areas.

Environmental Harms

The negative effects plastics have on the physical, chemical, and biological environment is cause for global concern. Organisms that are exposed to plastics face physical harms such as entanglement and ingestion. Large plastic debris, known as macroplastics, are the concern for entanglement and resulting in injury and death. Because of the disposal of industrial fishing nets, entanglement is more commonly seen in oceans among large animals where it can result drowning, suffocation, or starvation. The presence of macroplastics in the marine environment have been found to result in injury and death of marine birds, mammals, fish, and reptiles (Derraik, 2002). Microplastic ingestion by contrast, is common in both marine and freshwater species with aquatic species consuming MPs directly or indirectly by consuming individuals from lower trophic levels (McNeish et al., 2018). MP ingestion causes severe impacts on aquatic species and can form a negative feedback loop where ingestion alters feeding behaviors. In marine environments, increased MP digestion is negatively correlated with food consumption (Spear et al., 1995), and because plastics are made from durable polymeric compounds such as polystyrene, ingested MPs are recalcitrant to digestion, often blocking the gastrointestinal tract (Carpenter et al., 1972)**.** Although direct consumptions happens, the majority of fish ingest MPs through predation, leading to bioaccumulation at higher trophic levels (McNeish et al., 2018). Along with this physical accumulation, impacted organisms are exposed to a range of associated chemicals.

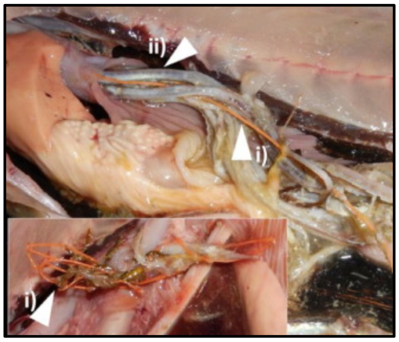

Image 1. Mackerel caught at Helgoland, showing evidence of ingesting micro-fibers (Rummel et al., 2016)

Plastics leach toxic plasticizers such as phthalates and BPA which are known endocrine disruptors that pose a great health risk to aquatic organisms (Hammer et al., 2012). Further, these same plastics can also harbor heavy metals (e.g. chromium, cadmium, lead) that are used in their production as colorants, stabilizers, and or plasticizers (Ernst et al., 2000). And, because they absorb hydrophobic pollutants like PCBs and DDT, MPs expose freshwater organisms to a wide range of additional environmental pollutants (Hammer et al., 2012). Laboratory based studies have demonstrated the potential chemical hazards imposed by MPs on freshwater organisms (Lei et al., 2018; Lu et al., 2016; Ma et al., 2016). Study conducted by (Ma et al., 2016) researched the sorption of phenanthrene in *Dephnia magna* however, showed physical damage and high toxicity (Ma et al., 2016) **.** Other freshwater studies focused on the accumulation of MPs in larval and adult zebrafish, the ingestion of MPs resulted in intestinal damage, changes in locomotion and metabolic processes (Lei et al., 2018; Lu et al., 2016.

Plastics are also effected by biofilms that are microbial communities that can attach to and grow on surfaces. Biofilms make surfaces less hydrophobic while also increasing the density of plastic allowing lighter pieces to sink to benthic zones making the surface less hydrophobic

(Carr et al., 2016). The interaction between plastics and biofilms provides insight into the sorption of toxic and persistent organic pollutants (Jingyi Li et al., 2018).

Table 1. Summary table adapted from Collard et al. 2019 describing MP ingestion by freshwater fish and studies methodologies. AP = anthropogenic particle, PL = plastic particle Ox. = oxidizers, Rn = Raman spectroscopy, FTIR = Fourier transform infrared spectroscopy

No. sp	n/sp.	Country	Water body	Targeted particles (AP/PL)	Extracted particle size (um)	Visual sorting	Digestion agent	Spectro-scopic analyses	Procedural blanks	Reference
16	1-63	Brazil	River	PL	?	X	-	FTIR	-	(Andrade et al., 2019)
2	20	Tanzania	Lake	PL	>500	X	Hydroxides	FTIR	-	(Biginagwa et al., 2016)
5	10-75	Canada	River	PL	>5	X	oxidizers	-	X	(Campbell et al., 2017)
1	60	France	River	AP & PL	>5	-	oxidizers	Rn	X	(Collard et al., 2018)
4	10	Switzerland	Lake	PL	?	X	-	-	-	(Faure et al., 2015)
3	41	Switzerland	Lake & Sea	PL	?	X	-	-	-	(Faure et al., 2012)
1	64	England	River	AP & PL	>1.2	X	oxidizers	Rn	-	(Horton et al., 2018)
2	96	U.S.A	Lake	PL	?	X	Hydroxides & Acid	-	X	(Hurt et al., 2020)
6	20-40	China	Lake	PL	>5	X	oxidizers	FTIR	X	(Jabeen et al., 2017)
2	10 and 66	England	River & Estuary	PL	?	X	-	FTIR	-	(McGoran et al., 2017)
11	1-17	U.S.A	River	PL	>0.45	X	oxidizers	FTIR	X	(McNeish et al., 2018)
11	87	Argentina	River	PL	?	X	oxidizers	-	-	(Pazos et al., 2017)
2	318 and 118	U.S.A	River	Pl	>53	X	-	-	-	(Peters & Bratton, 2016)
44	1-67	U.S.A	River & Estuary	PL	?	X	-	FTIR	-	(Phillips, n.d.)
22	-	Germany	River & Lake	PL	>20	X	hydroxides & acid	-	X	(Roch & Brinker, 2017).
1	186	France	River	PL	>1.2	X	-	-	-	(Sanchez et al., 2014)
6	60	Malaysia	River	PL	?	X	-	-	-	(Sarijan et al., 2019)
1	48	Brazil	River	PL	>63	X	-	-	-	(Silva-Cavalcanti et al., 2017)
1	78	Belgium	River	PL	>8	X	oxidizers	Rn & FTIR	X	(Slootmaekers et al., 2019)

1	180	Australia	Wetland	PL	>20	X	Hydroxides	FTIR	X	(Su et al., 2019)
1	10	China	Lake	PL	>200	X	Hydroxides	Rn	X	(Xiong et al., 2018)
1	11	China	Lake	PL	>8	X	Hydroxides & oxidizers	Rn	X	(Yuan et al., 2019)
13	1-6	China	River	PL	>1.2	X	Hydroxides	Rn	-	(Zhang et al., 2017)

able 2. Summary table adapted from Collard et al. 2019 on plastic characteristics from gut contents of freshwater fish

Species (n)	**Location**	**Item per individual**	**Item/g Gut Contents**	**Contaminated Individuals (%)**	**Mean plastic size of range (mm)**	**Spectro-scopic identification**	**References**
16 species	Xingu River, Brazil	-	-	26.7	1-15	yes	(Andrade et al., 2019)
Lates niloticus *Oreochromis niloticus*	Lake Victoria, Tanzania	-	-	20 20	-	yes	(Biginagwa et al., 2016)
Exos lucius *Catostomus commersoni* *Notropis atheirnoides* *Pimephales promelas (34)* *Eucalia inconstans*	Wascana Creek, Canada	-	-	73.5	-	no	(Campbell et al., 2017)
Squalius cephalus (60)	Seine River, France	0.16	0.16	15	2.67	yes	(Collard et al., 2018)
Alburus alburnus *Perca fluviatilis* *Rutilus rutilus (10)* *Leuciscus leuciscus*	Lake Geneva, Switzerland	3.1 0 0 0.3	-	7.5	-	no	(Faure et al., 2015)
Abramis brama (2) *Esox lucius (21)* *Rutilus rutilus (18)*	Lake Geneva, Switzerland & The Mediterranean Sea	-	-	-	-	no	(Faure et al., 2012)
Rutilus rutilus (64)	River Thames, England	0.69	-	32.8	-	yes	(Horton et al., 2018)
Dorosoma cepedianum (72) *Micropterus salmoides (24)*	Evergreen Lake, USA Lake Bloomington, USA	1–49	-	100	-	no	(Hurt et al., 2020)
6 species	Taihu Lake, China	2.4	3.4	95.7	0.4-24.8	yes	(Jabeen et al., 2017)
Platycephalus indicus *Osmerus eperlanus*	Thames Estuary, England	-	-	85 20	-	yes	(McGoran et al., 2017)
17 species	Several rivers, USA	~13	-	85	<1.5	yes	(McNeish et al., 2018)
11 species	Rio de La Plata, Argentina	19.2	-	100	0.06-4.7	no	(Pazos et al., 2017)

Lepomic macrochirus (318) *Lepomis megalotis (118)*	Brazos River, Texas	-	-	45	-	no	(Peters & Bratton, 2016)
44 species	Several rivers, Texas	-	-	8.2	-	yes	(Phillips, n.d.)
22 species	lake & rivers, Germany	0.2	-	18.8	0.889	no	(Roch & Brinker, 2017).
Gobio gobio (186)	Several rivers, France	-	-	12	-	no	(Sanchez et al., 2014)
Anabas testudineus (13) *Clarias gariepinus (21)* *Cyclocheilichthys apogon (2)* *Oreochromis mossambicus (18)* *Oxyeleotris marmorata (1)* *Pangasius hypophthalmus (5)*	Skudai River, Malaysia	0.38 0.33 0.50 1.61 2.00 4.00	-	23.08 19.05 50 55.56 100 100	-	no	(Sarijan et al., 2019)
Hoplosternum littorale	Pajeu River, Brazil	3.6	-	83	<'1-12	no	(Silva-Cavalcanti et al., 2017)
Gobio gobio (78)	Several rives, Belgium	-	-	9	0.67	yes	(Slootmaekers et al., 2019)
Gambusia holbrooki	Melbourne Area, Australia	0.6	-	19.4	0.09−4.86	yes	(Su et al., 2019)
Gymnocypris przewalskii	Qinghai Lake, China	5.4	-	-	-	yes	(Xiong et al., 2018)
Carassius auratus (11)	Poyang Lake, China	-	-	91	0.1-1	yes	(Yuan et al., 2019)
13 species	Xiangxi River, China	-	-	25.7	0.3-1.8	yes	(Zhang et al., 2017)

Measuring Microplastics in Freshwater Fish

A central limitation of the current body of work is the failure to establish a common set of repeatable, reliable, and targeted metrics for quantifying microplastics in freshwater organisms. As a group, past studies suffer from variable quality control, assurance, and detection methodologies that hamper reliable comparisons. Future work should focus on measurement quality with attention paid to the risk of fiber contamination. (Hermsen et al., 2018) review standards of quality for MP measurement. They outline steps including wearing 100% cotton clothing, always wearing gloves, working under a hood, covering samples when not being inspected, and using non-plastic materials when possible. They further suggest validation and isolation techniques, including intentional "spiking" of tissue samples by deliberately adding MPs on both blank and tissue samples to calculate percent recovery.

Detection methodology is also an important factor limiting the comparability of data. Initially, studies relied on simple visual sorting (Sanchez et al., 2014), (Phillips, n.d.), (Faure et al., 2015), (Peters & Bratton, 2016), (McGoran et al., 2017), (Silva-Cavalcanti et al., 2017), (Sarijan et al., 2019)Although this method is valued for keeping dietary remains intact, visual underrepresents the presence of smaller MPs (Vandermeersch et al., 2015). Enzymatic digestion treatments are currently being tested but their higher costs may prove prohibitive for widespread application (Courtene-Jones et al., 2017; Löder et al., 2017). Chemical treatments using acids, bases, and oxidizers are also possible; however, the use of acids is discouraged because it can degrade sensitive types of plastic (Claessens et al., 2013). Bases by contrast, such as those used in alkaline hydrolysis, are effective at decomposing soft tissue, but leave most MPs intact (Hurley et al., 2017). Oxidizers are preferred because they decompose both soft tissue and hard skeletal parts (Jabeen et al., 2017; McNeish et al., 2018; Pazos et al., 2017; Windsor et al., 2019).

Based on a series of quality assurance tests, wet peroxide oxidation (WPO) is the current preferred sample preparation method. This digestion technique using 30% H_2O_2, most commonly in the presence of a Fe(II) catalyst (Fenton's reagent) at a temperature of 65 °C (O'Connor, 2019). This method can bleach some particles, resulting in misreported colors, but is a robust method for estimating both the amount and type of microplastic (O'Connor et al., n.d.) More importantly, spiked microfiber tests indicated WPO oxidation produced a 95% recovery rate so long as it was not heated for more than 48 hours (Jiana Li et al., 2016).

Sample analysis following digestion commonly employs Fourier-transform infrared spectroscopy (FTIR) (Biginagwa et al., 2016; McGoran et al., 2017; Phillips, n.d.; Slootmaekers et al., 2019) or Raman spectroscopy (Horton et al., 2017; Slootmaekers et al., 2019). These methods are time consuming but provide definitive confirmation of plastic type. An emerging technique, Nile Red (NR) dye, is easier and similarly effective, suggesting it could replace more labor intensive alternatives while also improving accuracy and efficiency. Plastics exposed to Nile red dye fluoresce under blue light and orange filter, making for easy sorting. Further, because the technique exposes clear color variations between plastic types, detailed sorting and analysis is possible (Maes et al., 2017).

Mapping Microplastics in Freshwater Fish

Defining the source of MPs in the freshwater environment is necessary for identifying sites of high and low concern for research. Each year, 1.15-2.41 million tons of plastics enter the oceans via rivers (L. Lebreton et al., 2018). The largest source (80%) of MP in rivers is attributed to effluent from wastewater treatment plants (WWTP), likely because MPs can pass through treated effluent (Cole et al., 2011)Carr et al., 2016; Fendall & Sewell, 2009; Mason et al., 2016). The combination of inappropriate waste management and high population density are positively correlated to high plastic loads in rivers (Baldwin et al., 2016; Free et al., 2014). The most prevalent type of plastic in WWTP effluent is polyethylene terephthalate (PET), meaning concentrations of PET are higher in the vicinity of WWTP (McCormick et al., 2014; Rodrigues et al., 2018). Although lake sediments closer to MP sources are believed to have higher MP concentrations (Ballent et al., 2016; Su et al., 2016; Yuan et al., 2019), the size of the waterbody has no relation to MP concentration factors observed (Dris et al., 2015; Eriksen et al., 2013; Gasperi et al., 2014; Lechner et al., 2014). 80% of MP pollution in freshwater systems comes from WWTP (Cole et al., 2011). The spatial relationship between WWTP and MP pollution is based on whether the fish were sampled either up or downstream from the WWTP (Campbell et al., 2017). While the MP in rivers eventually ends up in the ocean, the static waters of lakes trap MP (Jingyi Li et al., 2018). Therefore, lakes close to WWTP are at a high risk for bioaccumulation. As for the biota, species feeding habits are still being disputed in the literature. Benthic and demersal feeders have been seen to have higher MP concentrations (Jabeen et al., 2017; Murphy et al., 2017) and some saw the opposite (Rummel et al., 2016).

Although freshwater fish are comparatively understudied in the MP field, the body of research is growing. Here we propose a geospatial sampling framework for stratifying future freshwater MP research. The watershed's degree of urbanization and WWTP in the vicinity are known MP risk indicators. Methodology and reporting should follow the recommendations made by O'Connor (O'Connor et al., n.d.). We present a sample map of New York State to identify waterbodies with high MP contamination likelihood.

We defined urbanization as the watershed percent urban land use, watershed population density, and watershed percent impervious surface (Baldwin et al., 2016). To choose research sites efficiently GIS techniques can be applied to prioritize sites close to WWTP and in drainage basins with high population density and impervious surface percentage. The idea is to test the relationship between those factors and MP concentrations. Sampling every water body in the United States is not necessary or valuable, instead maps using data of known contamination factors can help narrow the research needed to prove that relationship.

In QGIS using the NYS Shoreline the HUC250K drainage basins were clipped (U.S. Geological Survey, 2020). Then download NLCD Land Cover Data (MRLC, 2016). The classes of interest are 23 and 24, developed, medium and high intensity. Using the r.reclass tool in the GRASS plugin everything was either classified as 0 (undeveloped) or 1 (developed). The same was done for impervious surface data (MRLC, 2016). After reviewing the data, the choice was made to only use impervious surface as it covered more area than land cover and population density was unattainable. Figure 3 shows the overlap of impervious surface and land cover.

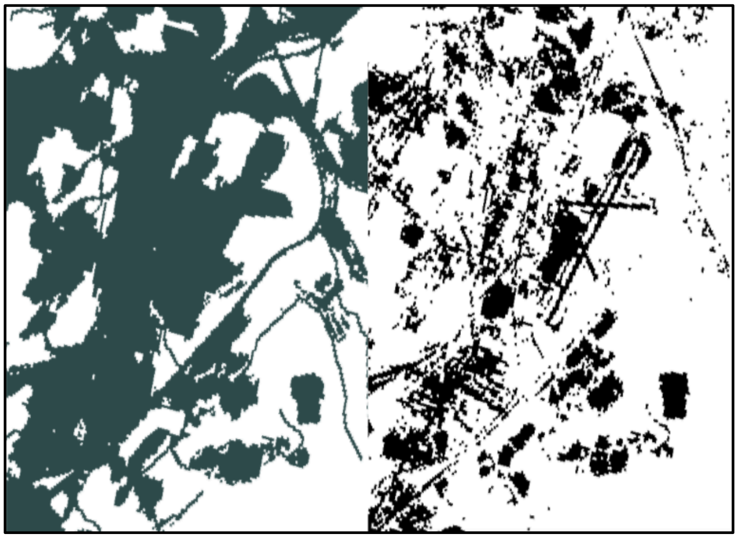

Figure 3. The left is impervious surface and the right is categories 23 and 24 in the National Land Cover Data

Zonal statistics calculates impervious surface and urban land cover per drainage basin. Once it was counted per drainage basin then divided by the area to normalize it for its size. An example would be if a drainage basin had 2,000 pixels of impervious surface, the effect would be larger if it was 1,000 square miles rather than 10,000 square miles. For this example, the top 5 drainage basins for impervious surface were selected. In NYS they are East Branch Delaware, Mohawk, Indian, Black, and Upper Hudson (Figure 4).

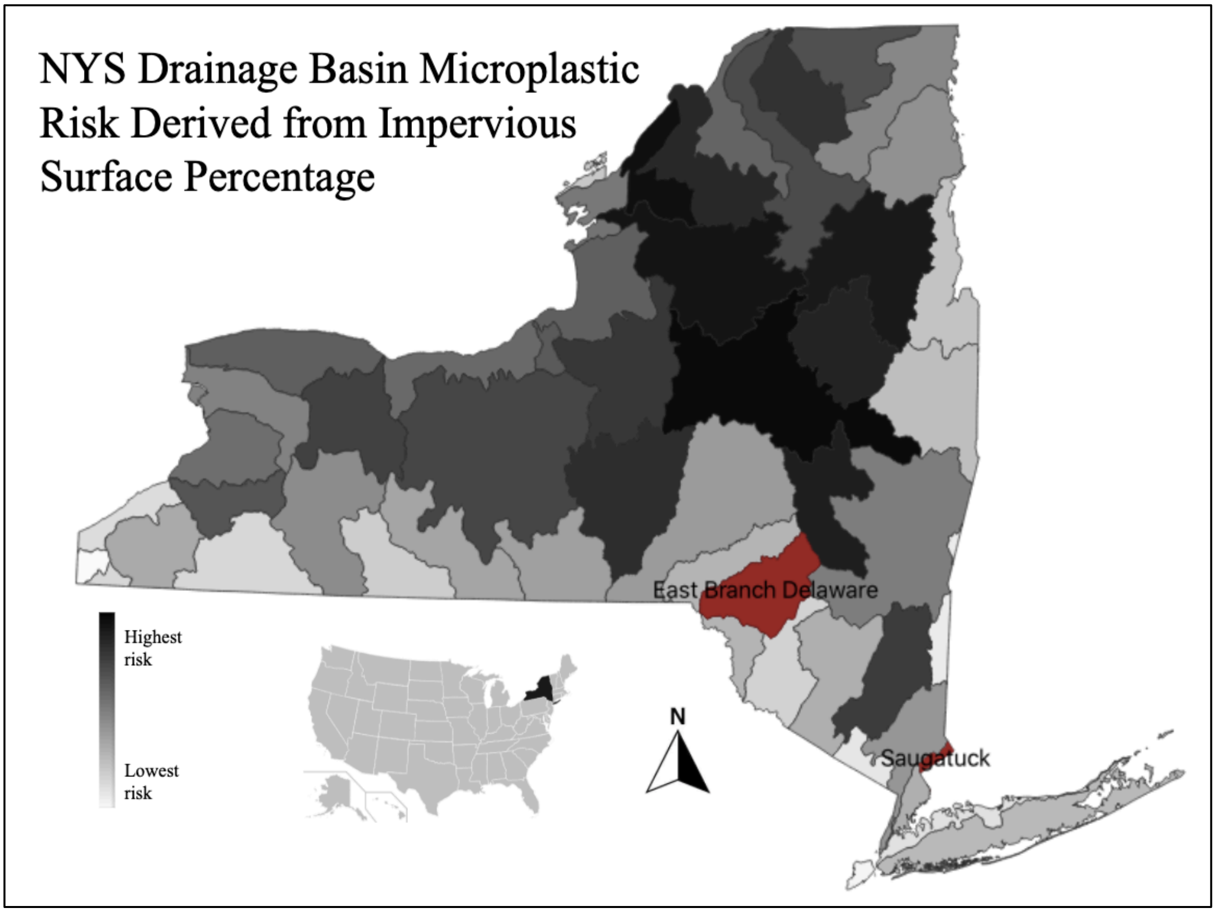

Figure 4. Impervious surface per drainage basin was calculated to show which drainage basins are most at risk from plastic pollution in NYS. The impervious surface value was divided by the area of the basin to account for concentration. East Branch Delaware is the highest risk basin and Saugatuck is the third lowest basin of concern.

The WWTP portion of risk factors was included by creating 1,500 foot buffers around every WWTP (Environmental Protection Agency, 2020) and running the intersection tool with the hydrology of NYS (U.S. Geological Survey, 2020). Wastewater treatment plants dispose of their effluent in the closest waterbody. After looking at Google Maps it was decided for NYS all plants investigated were within 1,000 feet of a river or lake, so 1,500 feet was chosen to ensure nothing was missed. To narrow the search WWTP water bodies in the East Delaware Branch were investigated. The select by location tool was used to locate waterbodies within the East Branch Delaware area also identified as risk areas through proximity to WWTP. This yielded 7 waterbodies worth investigating; Beaver Kill, Catskill State Park Ponds, Lily Ponds, and 4 other unnamed ponds (Figure 4a). The lowest risk drainage basin in NYS is French. It contained no WWTP risk water bodies. The second lowest risk was Sandy Hook-Staten Island but all water affected was coastal so the third lowest risk was examined, Saugatuck. The same procedure was done to yield 7 waterbodies at low risk for MP pollution; Byram River, Mianus River, Wampus Pond, an unnamed lake, and 3 unnamed ponds (Figure 4b). For comparisons to be derived we suggest finding species overlap in high and low risk drainage basins to compare waterbodies.

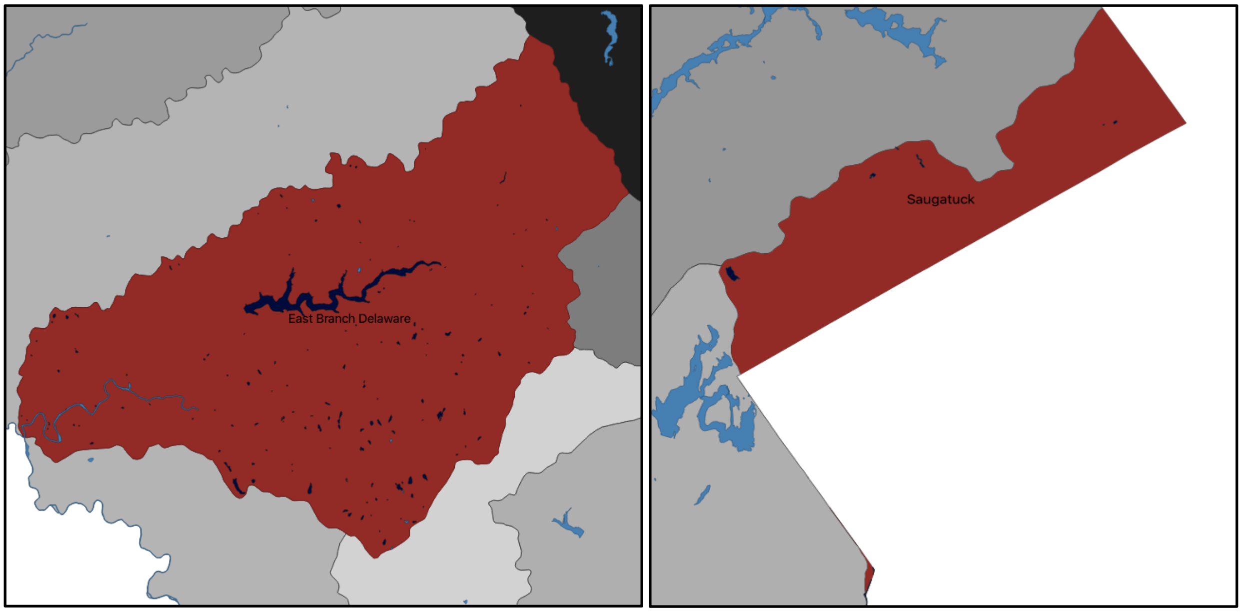

Figure 5 A. The most at risk drainage basin (by impervious surface) with waterbodies likely affected by WWTP in navy. B. Third least at risk drainage basin with waterbodies affected by WWTP in navy.

Identifying waterbodies at either end of the spectrum is important to organize distributive sampling campaigns. Our research started off gathering fish guts from fishermen in ice fishing competitions. Given the funding, a large campaign can be done at target waterbodies to assess the accuracy of these trends and modeling. Integrating an app for fishermen to log the exact GPS coordinates of their catch with the fish length and species before they send in the guts would be useful. The field of MP research in freshwater biota is new and a more largescale sampling campaign would help fill in the gaps greatly.

Conclusion

MPs are being deposited in terrestrial, freshwater, and marine environments. For the past couple of decades' research has been focused on MP impacts on the marine environment. Only recently has there been a push to change the imbalance in research between marine and freshwater studies. These studies that do exist provide critical insight into the abundance and accumulation of MPs in fish and the indirect and direct sources into freshwater systems. However, across these studies there are many inconsistencies among methodologies that results in different conclusions. It is known that WPO is the preferred sample preparation method but 47.82% of studies used either a different technique or had no sample preparation (Table 1). Also, to get accurate MP identification the use of FTIR or Rn analysis is required but 43.47% of studies relied simply on visual identification (Table 1). Now that there is growing interest in MPs in freshwater organisms, harmonized methodologies are required to make proper comparisons between studies. The major source for freshwater aquatic systems is known as WWTP effluent. As the literature grows we urge scientists in the field to consider study sites based on known sources and their proximity. Science supporting this present so legislation to require filtration upgrades to catch MPs in effluent is encouraged. Like other reviews, the call for methodology uniformity is clear. The choice of research site should be tied to recommendations made by the map in places of both high and low probability of contamination. (1) the sample size for fish gut samples need to be greater than 50. All the studies show a large discrepancy in sample size, ranging from only 10 to over 300 fish guts (Table 1). (2) there needs to be stricter definitions on

MP size that should be measured. The observed particle size used in many studies ranged from 0.45um to over 500um (Table 2). This measurement should be standardized to 1um. (3) Spectroscopy, Rn or FTIR, should be used to identify the MPs. Visual sorting and identification is still important to measure the MPs but that must be followed by Rn or FTIR analysis (Collard et al., 2019). Overall, the field of MP in freshwater fish guts is understudied and requires more attention.

As the need for freshwater continues to rise there is an obvious need for improved treatment at WWTPs so less MPs are being transported through rivers by the effluent released. Future studies need to focus on both the MP contamination of the ecosystem and fish that are ingesting the MPs. This type of study will allow scientist to determine the correct indicator species that exposes the health of the system (Collard et al., 2019). Overall, the field of MP in freshwater fish is understudied and requires more attention. The plethora of studies done on the marine environment shows how far reaching and how impactful MP pollution is. The current increase in MP research in freshwater fish is finally providing information on how MPs are affecting environments that are closer to home. Globally freshwater is becoming a limiting resource and pollution is threatening the availability of freshwater uses (Eerkes-Medrano et al., 2015). Collaborative efforts to increase research and to create harmonious methodologies will be required to better understand MPs as an evolving threat in freshwater environments.

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References

Andrade, M. C., Winemiller, K. O., Barbosa, P. S., Fortunati, A., Chelazzi, D., Cincinelli, A., & Giarrizzo, T. (2019). First account of plastic pollution impacting freshwater fishes in the Amazon: Ingestion of plastic debris by piranhas and other serrasalmids with diverse feeding habits. *Environmental Pollution (Barking, Essex: 1987)*, *244*, 766–773. https://doi.org/10.1016/j.envpol.2018.10.088

Andrady, A. L. (2011). Microplastics in the marine environment. *Marine Pollution Bulletin*, *62*(8), 1596–1605. https://doi.org/10.1016/j.marpolbul.2011.05.030

Baldwin, A. K., Corsi, S. R., & Mason, S. A. (2016). Plastic Debris in 29 Great Lakes Tributaries: Relations to Watershed Attributes and Hydrology. *Environmental Science & Technology*, *50*(19), 10377–10385. https://doi.org/10.1021/acs.est.6b02917

Ballent, A., Corcoran, P. L., Madden, O., Helm, P. A., & Longstaffe, F. J. (2016). Sources and sinks of microplastics in Canadian Lake Ontario nearshore, tributary and beach sediments. *Marine Pollution Bulletin*, *110*(1), 383–395. https://doi.org/10.1016/j.marpolbul.2016.06.037

Barboza, L. G. A., & Gimenez, B. C. G. (2015). Microplastics in the marine environment: Current trends and future perspectives. *Marine Pollution Bulletin*, *97*(1), 5–12. https://doi.org/10.1016/j.marpolbul.2015.06.008

Barnes, D. K. A., Walters, A., & Gonçalves, L. (2010). Macroplastics at sea around Antarctica. *Marine Environmental Research*, *70*(2), 250–252. https://doi.org/10.1016/j.marenvres.2010.05.006

Biginagwa, F. J., Mayoma, B. S., Shashoua, Y., Syberg, K., & Khan, F. R. (2016). First evidence of microplastics in the African Great Lakes: Recovery from Lake Victoria Nile perch and Nile tilapia. *Journal of Great Lakes Research*, *42*(1), 146–149. https://doi.org/10.1016/j.jglr.2015.10.012

Blettler, M. C. M., Abrial, E., Khan, F. R., Sivri, N., & Espinola, L. A. (2018). Freshwater plastic pollution: Recognizing research biases and identifying knowledge gaps. *Water Research*, *143*, 416–424. https://doi.org/10.1016/j.watres.2018.06.015

Campbell, S. H., Williamson, P. R., & Hall, B. D. (2017). Microplastics in the gastrointestinal tracts of fish and the water from an urban prairie creek. *FACETS*, *2*(1), 395–409. https://doi.org/10.1139/facets-2017-0008

Carpenter, E. J., Anderson, S. J., Harvey, G. R., Miklas, H. P., & Peck, B. B. (1972). Polystyrene spherules in coastal waters. *Science (New York, N.Y.)*, *178*(4062), 749–750. https://doi.org/10.1126/science.178.4062.749

Carr, S. A., Liu, J., & Tesoro, A. G. (2016). Transport and fate of microplastic particles in wastewater treatment plants. *Water Research*, *91*, 174–182. https://doi.org/10.1016/j.watres.2016.01.002

Claessens, M., Van Cauwenberghe, L., Vandegehuchte, M. B., & Janssen, C. R. (2013). New techniques for the detection of microplastics in sediments and field collected organisms. *Marine Pollution Bulletin*, *70*(1), 227–233. https://doi.org/10.1016/j.marpolbul.2013.03.009

Cole, M., Lindeque, P., Halsband, C., & Galloway, T. S. (2011). Microplastics as contaminants in the marine environment: A review. *Marine Pollution Bulletin*, *62*(12), 2588–2597. https://doi.org/10.1016/j.marpolbul.2011.09.025

Collard, F., Gasperi, J., Gabrielsen, G. W., & Tassin, B. (2019). Plastic Particle Ingestion by Wild Freshwater Fish: A Critical Review. *Environmental Science & Technology*. https://doi.org/10.1021/acs.est.9b03083

Collard, F., Gasperi, J., Gilbert, B., Eppe, G., Azimi, S., Rocher, V., & Tassin, B. (2018). Anthropogenic particles in the stomach contents and liver of the freshwater fish Squalius cephalus. *Science of The Total Environment*, *643*, 1257–1264. https://doi.org/10.1016/j.scitotenv.2018.06.313

Courtene-Jones, W., Quinn, B., Murphy, F., Gary, S. F., & Narayanaswamy, B. E. (2017). Optimisation of enzymatic digestion and validation of specimen preservation methods for the analysis of ingested microplastics. *Analytical Methods*, *9*(9), 1437–1445. https://doi.org/10.1039/C6AY02343F

Derraik, J. G. B. (2002). The pollution of the marine environment by plastic debris: A review. *Marine Pollution Bulletin*, *44*(9), 842–852. https://doi.org/10.1016/S0025-326X(02)00220-5

Dris, R., Imhof, H., Sanchez, W., Gasperi, J., Galgani, F., Tassin, B., & Laforsch, C. (2015). Beyond the ocean: Contamination of freshwater ecosystems with (micro-)plastic particles. *Environmental Chemistry*, *12*(5), 539–550. https://doi.org/10.1071/EN14172

Eerkes-Medrano, D., Thompson, R. C., & Aldridge, D. C. (2015). Microplastics in freshwater systems: A review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. *Water Research*, *75*, 63–82. https://doi.org/10.1016/j.watres.2015.02.012

Eriksen, M., Maximenko, N., Thiel, M., Cummins, A., Lattin, G., Wilson, S., Hafner, J., Zellers, A., & Rifman, S. (2013). Plastic pollution in the South Pacific subtropical gyre. *Marine Pollution Bulletin*, *68*(1–2), 71–76. https://doi.org/10.1016/j.marpolbul.2012.12.021

Ernst, T., Popp, R., & van Eldik, R. (2000). Quantification of heavy metals for the recycling of waste plastics from electrotechnical applications. *Talanta*, *53*(2), 347–357. https://doi.org/10.1016/S0039-9140(00)00491-4

Faure, F., Corbaz, M., Baecher, H., & Felippe, L. (2012). Pollution due to plastics and microplastics in Lake Geneva and in the Mediterranean Sea. *ARCHIVES DES SCIENCES*, 7.

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

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

Free, C. M., Jensen, O. P., Mason, S. A., Eriksen, M., Williamson, N. J., & Boldgiv, B. (2014). High-levels of microplastic pollution in a large, remote, mountain lake. *Marine Pollution Bulletin*, *85*(1), 156–163. https://doi.org/10.1016/j.marpolbul.2014.06.001

Gasperi, J., Dris, R., Bonin, T., Rocher, V., & Tassin, B. (2014). Assessment of floating plastic debris in surface water along the Seine River. *Environmental Pollution (Barking, Essex: 1987)*, *195*, 163–166. https://doi.org/10.1016/j.envpol.2014.09.001

Hammer, J., Kraak, M. H. S., & Parsons, J. R. (2012). Plastics in the marine environment: The dark side of a modern gift. *Reviews of Environmental Contamination and Toxicology*, *220*, 1–44. https://doi.org/10.1007/978-1-4614-3414-6_1

Hermsen, E., Mintenig, S. M., Besseling, E., & Koelmans, A. A. (2018). Quality Criteria for the Analysis of Microplastic in Biota Samples: A Critical Review. *Environmental Science & Technology*, *52*(18), 10230–10240. https://doi.org/10.1021/acs.est.8b01611

Horton, A. A., Jürgens, M. D., Lahive, E., van Bodegom, P. M., & Vijver, M. G. (2018). The influence of exposure and physiology on microplastic ingestion by the freshwater fish Rutilus rutilus (roach) in the River Thames, UK. *Environmental Pollution*, *236*, 188–194. https://doi.org/10.1016/j.envpol.2018.01.044

Horton, A. A., Walton, A., Spurgeon, D. J., Lahive, E., & Svendsen, C. (2017). Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities. *Science of The Total Environment*, *586*, 127–141. https://doi.org/10.1016/j.scitotenv.2017.01.190

Hurley, R. R., Woodward, J. C., & Rothwell, J. J. (2017). Ingestion of Microplastics by Freshwater Tubifex Worms. *Environmental Science & Technology*, *51*(21), 12844–12851. https://doi.org/10.1021/acs.est.7b03567

Hurt, R., O'Reilly, C. M., & Perry, W. L. (2020). Microplastic prevalence in two fish species in two U.S. reservoirs. *Limnology and Oceanography Letters*, *5*(1), 147–153. https://doi.org/10.1002/lol2.10140

Jabeen, K., Su, L., Li, J., Yang, D., Tong, C., Mu, J., & Shi, H. (2017). Microplastics and mesoplastics in fish from coastal and fresh waters of China. *Environmental Pollution*, *221*, 141–149. https://doi.org/10.1016/j.envpol.2016.11.055

Klein, S., Dimzon, I. K., Eubeler, J., & Knepper, T. P. (2018). Analysis, Occurrence, and Degradation of Microplastics in the Aqueous Environment. In M. Wagner & S. Lambert (Eds.), *Freshwater Microplastics: Emerging Environmental Contaminants?* (pp. 51–67). Springer International Publishing. https://doi.org/10.1007/978-3-319-61615-5_3

Lebreton, L., Slat, B., Ferrari, F., Sainte-Rose, B., Aitken, J., Marthouse, R., Hajbane, S., Cunsolo, S., Schwarz, A., Levivier, A., Noble, K., Debeljak, P., Maral, H., Schoeneich-Argent, R., Brambini, R., & Reisser, J. (2018). Evidence that the Great Pacific Garbage Patch is rapidly accumulating plastic. *Scientific Reports*, *8*(1), 1–15. https://doi.org/10.1038/s41598-018-22939-w

Lebreton, Laurent, Egger, M., & Slat, B. (2019). A global mass budget for positively buoyant macroplastic debris in the ocean. *Scientific Reports*, *9*(1), 1–10. https://doi.org/10.1038/s41598-019-49413-5

Lechner, A., Keckeis, H., Lumesberger-Loisl, F., Zens, B., Krusch, R., Tritthart, M., Glas, M., & Schludermann, E. (2014). The Danube so colourful: A potpourri of plastic litter outnumbers fish larvae in Europe's second largest river. *Environmental Pollution (Barking, Essex: 1987)*, *188*, 177–181. https://doi.org/10.1016/j.envpol.2014.02.006

Lei, L., Wu, S., Lu, S., Liu, M., Song, Y., Fu, Z., Shi, H., Raley-Susman, K. M., & He, D. (2018). Microplastic particles cause intestinal damage and other adverse effects in zebrafish Danio rerio and nematode Caenorhabditis elegans. *Science of The Total Environment*, *619–620*, 1–8. https://doi.org/10.1016/j.scitotenv.2017.11.103

Li, Jiana, Qu, X., Su, L., Zhang, W., Yang, D., Kolandhasamy, P., Li, D., & Shi, H. (2016). Microplastics in mussels along the coastal waters of China. *Environmental Pollution (Barking, Essex: 1987)*, *214*, 177–184. https://doi.org/10.1016/j.envpol.2016.04.012

Li, Jingyi, Liu, H., & Paul Chen, J. (2018). Microplastics in freshwater systems: A review on occurrence, environmental effects, and methods for microplastics detection. *Water Research*, *137*, 362–374. https://doi.org/10.1016/j.watres.2017.12.056

Löder, M. G. J., Imhof, H. K., Ladehoff, M., Löschel, L. A., Lorenz, C., Mintenig, S., Piehl, S., Primpke, S., Schrank, I., Laforsch, C., & Gerdts, G. (2017). Enzymatic Purification of Microplastics in Environmental Samples. *Environmental Science & Technology*, *51*(24), 14283–14292. https://doi.org/10.1021/acs.est.7b03055

Lu, Y., Zhang, Y., Deng, Y., Jiang, W., Zhao, Y., Geng, J., Ding, L., & Ren, H. (2016). Uptake and Accumulation of Polystyrene Microplastics in Zebrafish (Danio rerio) and Toxic Effects in Liver. *Environmental Science & Technology*, *50*(7), 4054–4060. https://doi.org/10.1021/acs.est.6b00183

Ma, Y., Huang, A., Cao, S., Sun, F., Wang, L., Guo, H., & Ji, R. (2016). Effects of nanoplastics and microplastics on toxicity, bioaccumulation, and environmental fate of phenanthrene in fresh water. *Environmental Pollution*, *219*, 166–173. https://doi.org/10.1016/j.envpol.2016.10.061

Maes, T., Jessop, R., Wellner, N., Haupt, K., & Mayes, A. G. (2017). A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile Red. *Scientific Reports*, *7*(1), 1–10. https://doi.org/10.1038/srep44501

Mason, S. A., Garneau, D., Sutton, R., Chu, Y., Ehmann, K., Barnes, J., Fink, P., Papazissimos, D., & Rogers, D. L. (2016). Microplastic pollution is widely detected in US municipal wastewater treatment plant effluent. *Environmental Pollution (Barking, Essex: 1987)*, *218*, 1045–1054. https://doi.org/10.1016/j.envpol.2016.08.056

McCormick, A., Hoellein, T. J., Mason, S. A., Schluep, J., & Kelly, J. J. (2014). Microplastic is an Abundant and Distinct Microbial Habitat in an Urban River. *Environmental Science & Technology*, *48*(20), 11863–11871. https://doi.org/10.1021/es503610r

McGoran, A. R., Clark, P. F., & Morritt, D. (2017). Presence of microplastic in the digestive tracts of European flounder, Platichthys flesus, and European smelt, Osmerus eperlanus, from the River Thames. *Environmental Pollution*, *220*, 744–751. https://doi.org/10.1016/j.envpol.2016.09.078

McNeish, R. E., Kim, L. H., Barrett, H. A., Mason, S. A., Kelly, J. J., & Hoellein, T. J. (2018). Microplastic in riverine fish is connected to species traits. *Scientific Reports*, *8*(1), 11639. https://doi.org/10.1038/s41598-018-29980-9

Murphy, F., Russell, M., Ewins, C., & Quinn, B. (2017). The uptake of macroplastic & microplastic by demersal & pelagic fish in the Northeast Atlantic around Scotland. *Marine Pollution Bulletin*, *122*(1–2), 353–359. https://doi.org/10.1016/j.marpolbul.2017.06.073

O'Connor, J. D., Mahon, A. M., Ramsperger, A. F. R. M., Trotter, B., Redondo-Hasselerharm, P. E., Koelmans, A. A., Lally, H. T., & Murphy, S. (n.d.). Microplastics in Freshwater Biota: A Critical Review of Isolation, Characterization, and Assessment Methods. *Global Challenges*, *n/a*(n/a), 1800118. https://doi.org/10.1002/gch2.201800118

Pazos, R., Maiztegui, T., Colautti, D., Paracampo, A., & Gómez, N. (2017). Microplastics in gut contents of coastal freshwater fish from Río de la Plata estuary. *Marine Pollution Bulletin*, *122*. https://doi.org/10.1016/j.marpolbul.2017.06.007

Peters, C. A., & Bratton, S. P. (2016). Urbanization is a major influence on microplastic ingestion by sunfish in the Brazos River Basin, Central Texas, USA. *Environmental Pollution*, *210*, 380–387. https://doi.org/10.1016/j.envpol.2016.01.018

Phillips, M. B. (n.d.). *THE OCCURRENCE AND AMOUNT OF MICROPLASTICS INGESTED BY FISHES IN THE WATERSHEDS OF THE GULF OF MEXICO*. 34.

Roch, S., & Brinker, A. (2017). Rapid and Efficient Method for the Detection of Microplastic in the Gastrointestinal Tract of Fishes. *Environmental Science & Technology*, *51*(8), 4522–4530. https://doi.org/10.1021/acs.est.7b00364

Rodrigues, M. O., Abrantes, N., Gonçalves, F. J. M., Nogueira, H., Marques, J. C., & Gonçalves, A. M. M. (2018). Spatial and temporal distribution of microplastics in water and sediments of a freshwater system (Antuã River, Portugal). *The Science of the Total Environment*, *633*, 1549–1559. https://doi.org/10.1016/j.scitotenv.2018.03.233

Rummel, C. D., Löder, M. G. J., Fricke, N. F., Lang, T., Griebeler, E.-M., Janke, M., & Gerdts, G. (2016). Plastic ingestion by pelagic and demersal fish from the North Sea and Baltic Sea. *Marine Pollution Bulletin*, *102*(1), 134–141. https://doi.org/10.1016/j.marpolbul.2015.11.043

Ryan, P. G., Moore, C. J., van Franeker, J. A., & Moloney, C. L. (2009). Monitoring the abundance of plastic debris in the marine environment. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1526), 1999–2012. https://doi.org/10.1098/rstb.2008.0207

Sanchez, W., Bender, C., & Porcher, J.-M. (2014). Wild gudgeons (Gobio gobio) from French rivers are contaminated by microplastics: Preliminary study and first evidence. *Environmental Research*, *128*, 98–100. https://doi.org/10.1016/j.envres.2013.11.004

Sarijan, S., Azman, S., Said, M., & Lee, M. H. (2019). Ingestion of Microplastics by Commercial Fish in Skudai River, Malaysia. *EnvironmentAsia*, *12*, 75–84. https://doi.org/10.14456/ea.2019.47

Silva-Cavalcanti, J. S., Silva, J. D. B., França, E. J. de, Araújo, M. C. B. de, & Gusmão, F. (2017). Microplastics ingestion by a common tropical freshwater fishing resource. *Environmental Pollution*, *221*, 218–226. https://doi.org/10.1016/j.envpol.2016.11.068

Slootmaekers, B., Catarci Carteny, C., Belpaire, C., Saverwyns, S., Fremout, W., Blust, R., & Bervoets, L. (2019). Microplastic contamination in gudgeons (Gobio gobio) from Flemish rivers (Belgium). *Environmental Pollution*, *244*, 675–684. https://doi.org/10.1016/j.envpol.2018.09.136

Spear, L. B., Ainley, D. G., & Ribic, C. A. (1995). Incidence of plastic in seabirds from the tropical pacific, 1984–1991: Relation with distribution of species, sex, age, season, year and body weight. *Marine Environmental Research*, *40*(2), 123–146. https://doi.org/10.1016/0141-1136(94)00140-K

Su, L., Nan, B., Hassell, K. L., Craig, N. J., & Pettigrove, V. (2019). Microplastics biomonitoring in Australian urban wetlands using a common noxious fish (Gambusia holbrooki). *Chemosphere*, *228*, 65–74. https://doi.org/10.1016/j.chemosphere.2019.04.114

Su, L., Xue, Y., Li, L., Yang, D., Kolandhasamy, P., Li, D., & Shi, H. (2016). Microplastics in Taihu Lake, China. *Environmental Pollution (Barking, Essex: 1987)*, *216*, 711–719. https://doi.org/10.1016/j.envpol.2016.06.036

Thompson, R. C., Olsen, Y., Mitchell, R. P., Davis, A., Rowland, S. J., John, A. W. G., McGonigle, D., & Russell, A. E. (2004). Lost at sea: Where is all the plastic? *Science (New York, N.Y.)*, *304*(5672), 838. https://doi.org/10.1126/science.1094559

Vandermeersch, G., Van Cauwenberghe, L., Janssen, C. R., Marques, A., Granby, K., Fait, G., Kotterman, M. J. J., Diogène, J., Bekaert, K., Robbens, J., & Devriese, L. (2015). A critical view on microplastic quantification in aquatic organisms. *Environmental Research*, *143*, 46–55. https://doi.org/10.1016/j.envres.2015.07.016

Wagner, M., & Lambert, S. (2017). *Freshwater Microplastics: Emerging Environmental Contaminants?* https://doi.org/10.1007/978-3-319-61615-5

Windsor, F. M., Tilley, R. M., Tyler, C. R., & Ormerod, S. J. (2019). Microplastic ingestion by riverine macroinvertebrates. *Science of The Total Environment*, *646*, 68–74. https://doi.org/10.1016/j.scitotenv.2018.07.271

Xiong, X., Zhang, K., Chen, X., Shi, H., Luo, Z., & Wu, C. (2018). Sources and distribution of microplastics in China's largest inland lake – Qinghai Lake. *Environmental Pollution*, *235*, 899–906. https://doi.org/10.1016/j.envpol.2017.12.081

Yuan, W., Liu, X., Wang, W., Di, M., & Wang, J. (2019). Microplastic abundance, distribution and composition in water, sediments, and wild fish from Poyang Lake, China. *Ecotoxicology and Environmental Safety*, *170*, 180–187. https://doi.org/10.1016/j.ecoenv.2018.11.126

Zhang, K., Xiong, X., Hu, H., Wu, C., Bi, Y., Wu, Y., Zhou, B., Lam, P. K. S., & Liu, J. (2017). Occurrence and Characteristics of Microplastic Pollution in Xiangxi Bay of Three Gorges Reservoir, China. *Environmental Science & Technology*, *51*(7), 3794–3801. https://doi.org/10.1021/acs.est.7b00369