Microplastics in the Mississippi River and Mississippi Sound

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Summary

Microplastics (MPs) are a diverse suite of contaminants composed primarily of synthetic polymers that are less than 5 mm in diameter. Their occurrence in the aquatic environment is well documented, with higher concentrations found in areas where plastic litter accumulates. Given their small size their ingestion and impact on aquatic life poses a serious threat, particularly for filter-feeding organisms like oysters. Yet, surprisingly little is known about the concentrations, types, sizes, and loadings of MPs in the Mississippi River and its major tributaries, and along oyster reefs in the Mississippi Sound. This study was conducted to improve our capacity to characterize MP pollution and to collect preliminary data on MPs in the system to lay the groundwork for a more comprehensive study.

Microplastic pollution is an emerging science, and accurately collecting, detecting, quantifying, and identifying MPs in water and biota is challenging. In this study, we developed field and analytical methods to reliably measure MPs, focusing primarily on the smaller size fraction (<500 µm). Our "one pot" method prepares water samples in the same vessel (Mason jars) that they are collected in right up until the MPs are transferred onto filters or spectroscopic windows for analyses, minimizing contamination, degradation, and losses. Because it uses inexpensive and easily assembled materials, it can be adapted for MP surveys worldwide. We applied it to grab samples collected from the Mississippi River, its major tributaries, and the Mississippi Sound Estuary. MPs (>~25 µm) were quantified using fluorescence microscopy, and a subset of samples were identified by µFTIR Imaging spectroscopy or laser direct infrared analysis (LDIR). Key findings include:

- Average concentrations of MPs in the Mississippi River system ranged from ~14 MPs/L in the Tennessee River to ~83 in the Ohio River during low-flow (summer) conditions;
- Concentrations were generally lower during flooding, likely due to dilution;
- Morphology of the MPs was dominated by fragments (~85%), fibers (~8%), and beads (~7%);
- Particle count increased with decreasing size, and most of the MPs (>60%) were in the lowest size fraction measured (~25–90 µm);
- Polyester, polyethylene, polypropylene, and polyacrylate were the primary MP type;
- Samples collected from sites near population centers tend to have higher MP concentrations;
- Ohio and Missouri rivers have the highest tributary loadings, Tennessee and Yazoo rivers the lowest, reflecting both flows and MP concentrations;
- Loads of MPs tended to increase down river and ranged from ~87 to ~129 trillion MPs/day near New Orleans, suggesting that receiving waters in the Gulf of Mexico (nGoM) likely act as a sink for plastic pollution that is continually funneled through the Mississippi River;
- Concentrations of MPs in the Mississippi Sound ranged from ~12 to 381 particles/L (higher than the rivers) and tended to decrease at sites impacted by freshwater intrusion during flood events;
- >50% of the MPs in the Sound were in the lower size fraction (~25–90 µm), consisted mostly of fragments (~84%), fibers (~11%), and beads (~5%); the most identified plastic was polyester, followed by acrylates/polyurethanes, polyamide, polypropylene, polyethylene and polyacetal.

Other project goals were to train students on this emerging water issue, help educate the public on MP pollution and its impacts, and submit a competitive grant proposal based on early findings. To that end, this work was the basis for a Ph.D. in analytical chemistry as well as multiple undergraduate student research projects. We visited local middle schools, hosted visits from high-school science classes, and presented our work to the public at a Science Café. We hosted scientists from the USGS who came to learn our new method for MP sampling and analysis and to discuss future collaboration. We also presented research at state, national, and international scientific meetings, and Dr. Cizdziel co-chaired a special symposium on MP pollution at an international (SETAC) meeting. Because of setbacks due to hurricanes and Covid-19, research on MPs in oysters and interactions of MPs with mercury was slowed. Thus, this report focuses on two key parts: (1) Method development and MPs in the Mississippi River System and (2) MPs in the Mississippi Sound. A USGS 104g grant continuing and expanding this research is ongoing and will provide additional insight into the prevalence, characteristics, and impacts of MPs along the Mississippi River and Mississippi Coast.

Contents

INTRODUCTION

Led by consumer products, the worldwide demand for plastic continues to grow with global production at nearly 350 megatons in 2017 (Plastics Europe 2018). Unfortunately, careless discarding of plastic and mishandling of the plastic waste stream has resulted in widespread plastic pollution, including the infamous oceanic garbage patches (Lebreton 2018). Further, plastics in the environment weather and degrade as a result of ultraviolet radiation, microorganisms, temperature changes, and mechanical forces (e.g. wave action), yielding smaller and smaller particles called micro- and nano-plastics. Here, we focus on MPs, which have been described as "any synthetic solid particle of polymeric matrix, with regular or irregular shape and with size ranging from 1 μm to 5 mm, of either primary or secondary manufacturing origin, which are insoluble in water" (Frias 2019).

The occurrence of MPs in the aquatic environment is well documented, with higher concentrations generally found near population centers (Li 2018). MPs have also been detected in remote areas, including the Arctic Ocean (Lusher 2015), deep-sea sediments (Free 2014), and mountain lakes (Cauwenberghe 2013). Given their small size and ubiquitous nature in lakes, rivers, and oceans, their ingestion and impact on aquatic life poses a serious threat, particularly for small suspension-feeding organisms (Auta 2017). Moreover, MPs have been shown to be substrates (vectors) for other contaminants, including persistent organic pollutants such as dichloro-diphenyl-trichloroethane (DDT), both in laboratory studies and in field studies (Teuten 2009; Costa 2017; Tourinho 2019).

Unfortunately, there are often wildly different estimates reported for MP abundances in natural water, even from the same waterbodies, making meaningful comparisons difficult and hindering the utility of real-world MP surveys (Lusher 2017; Lenz 2018; Jiang 2018). Some of these disparate results may be due to inherent variability at the sites, but part of the problem may be the different sampling, sample preparation, and analytical methods used. On one hand, the wide range of approaches to MP analyses is not surprising given that MPs (1) are a diverse class of contaminant encompassing a wide variety of sizes, morphologies, and chemical and physical properties (Rochman 2019), (2) partition into different environmental compartments depending on size, density, biofouling, and other factors (Hartmann 2019), and (3) have only recently (in the last decade) caught the attention of the larger scientific community. On the other hand, MP analytical methods need to become more harmonized to increase the quality and comparability of experimental data.

Two common ways to sample plastic debris suspended in water is through use of a surface or subsurface tow net or by collecting a known volume of water at a specific location (bulk water sampling). Nets are typically used in investigating large areas with results being reported in particles/m3, whereas bulk water sampling is more accurate as a snapshot and is often reported in particles/L. A major drawback to sampling with a net is that it fails to capture particles smaller than the mesh opening (typically 333-μm), and these smaller particles tend to be the most abundant. In contrast, bulk water sampling captures all size fractions of particles in the water. Another advantage of bulk water sampling is the elimination of contamination from sampling equipment such as nylon nets and ropes. However, trawling with a net or bulk water sampling should be considered complementary techniques, covering different parts of the overall MP pollution (Tamminga 2019).

When using a net, the plastics caught in the cod end are typically rinsed out into a container for later processing in the laboratory. Determining the volume of water passing through the net or being pumped through collection sieves is important to accurately calculate MP concentrations. At some point the net and bulk sampling methods converge with the samples being filtered through a sieve or series of sieves to isolate particulates by size fraction(s). Larger particles can be removed by tweezers and analyzed by FTIR or other means. If the remaining solids collected on the sieves or filters are organic-rich they are typically subjected to either enzymatic digestion (Cole 2014) or wet peroxide oxidation, the latter sometimes in the presence of a Fe(II) catalyst (Tagg 2017), to digest labile organic matter and "clean" the plastic surfaces. A final filtering step is used to concentrate the MPs which can then be examined directly on a filter by conventional light microscopy (Masura 2015), stained with Nile Red dye and examined by fluorescence microscopy (Erni-Cassola 2017), or transferred to a spectroscopic window/slide or a suitable filter for chemical imaging by Focal Plane Array (FPA)-µFTIR or Raman spectroscopy (Loder 2015; Tagg 2015; Olesen 2017; Wolff 2019). Note that prior to chemical imaging the MPs on the filter are transferred (dispersed) into a solvent such as ethanol (often by sonication) before an aliquot is applied on the spectroscopic window and dried (Liu 2019).

The above sample preparation scheme can be hampered by multiple sieving and transfer steps, which increase the likelihood of contamination and losses, while decreasing throughput. Regardless of the method used, it is imperative that it minimizes contamination, losses, and degradation of MPs to obtain meaningful (reliable and reproducible) data.

Here, we present a novel low-cost and efficient bulk water sampling method for the analysis of MPs in water. The single-pot method prepares samples in the same vessel (Mason canning jars) that they are collected in right up to the point where they are transferred onto filters for analysis (Figure 1). We compare the new method to conventional sieving, demonstrating that it lowers contamination, losses, and carryover between samples and improves recoveries. The method is particularly useful for the analyses of smaller MPs that can't be easily manipulated with tweezers or are too small to be seen with the naked eye. These smaller size fractions are also more prone to contamination and require µ-spectroscopic imaging to identify the plastics.

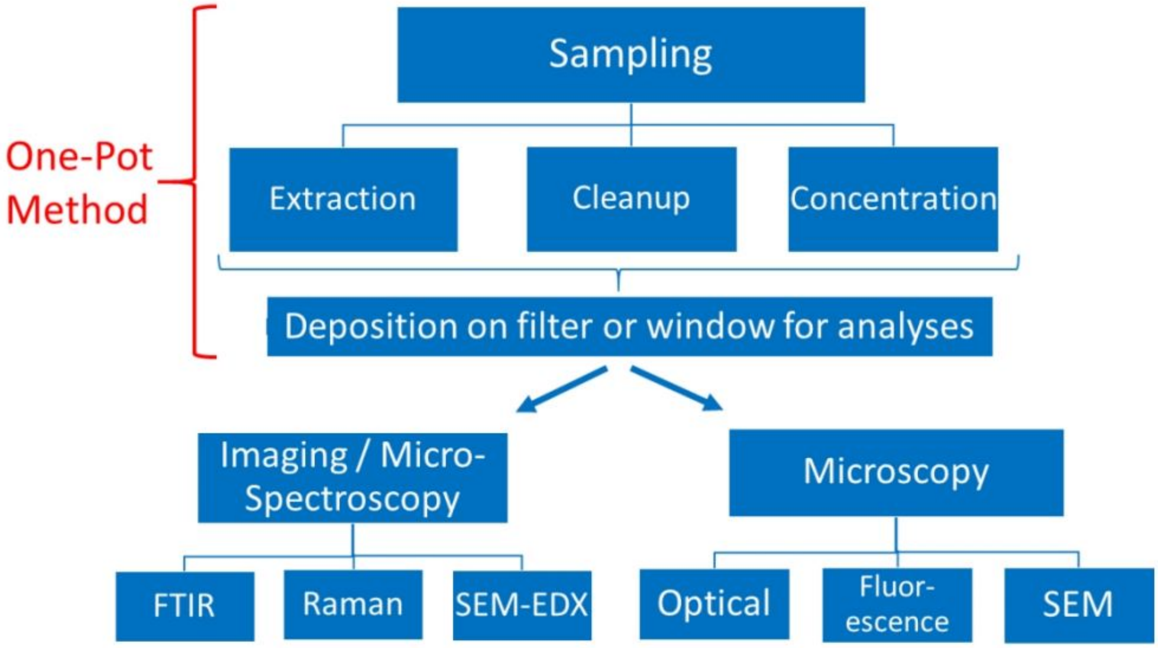


Figure 1. Flow-chart showing the general scheme for characterizing microplastic pollution < 1mm in size, including the portion covered by the single-pot method. The single-pot method prepares samples in the same vessel (Mason jars) that they are collected in right up until they are transferred onto filters or windows for final spectroscopic or microscopic analysis.

Further, we applied the single-pot method to samples collected along a large transect of the main stem of the Mississippi River and in several of its major tributaries during historic flooding in the spring of 2019 and during more normal flow conditions later that summer. We quantified the MPs (down to ~30 µm in size) using Nile Red fluorescence detection and identified MPs in a small subset of samples using µFTIR-Imaging. Here, we report preliminary results for the concentrations, river loads, shapes, size distribution, and chemical composition of MPs in this important large-scale riverine network.

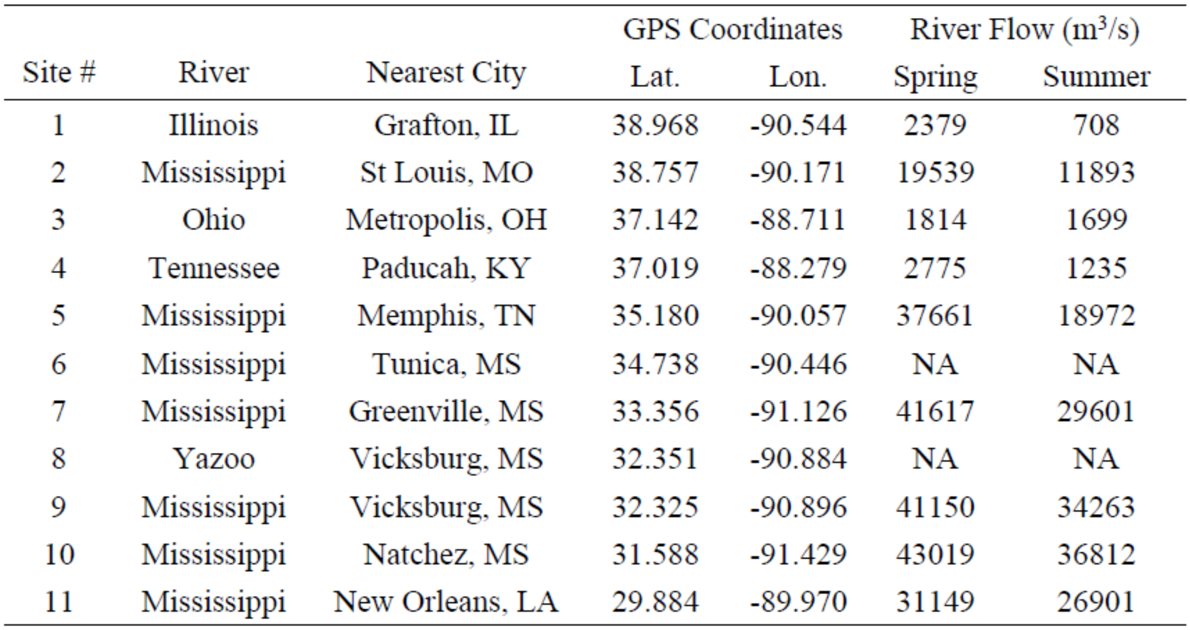
METHODS

Study Site: Mississippi River System

To understand the occurrence, distribution, types, and sources of MP pollution in the Mississippi River system requires an extensive spatial and temporal study. The Mississippi River Basin, the largest drainage basin in the United States, consists of an intricate system of waterways, tributaries, and commercial routes. In this study, we collected surface water from 11 sites, 7 on the main stem of the Mississippi River extending from the northern-most site near St. Louis to the southern-most site near New Orleans, and 4 from its major tributaries, including the Illinois, Ohio, Tennessee and Yazoo Rivers (Figure 2). Specific sampling locations (GPS coordinates) and river flow information are provided in Table 1. Samples were collected during major (historic) flooding in May 2019 and during more normal flow conditions (post-flood) the following August. Thus, this work represents "snapshots" of the system during these two different seasons and flow regimes. The Missouri River and the Mississippi River at Tunica and Greenville were inaccessible due to high waters and flood damage at sampling sites.

Table 1. Sampling location and river flows for the Mississippi River and major tributaries

Sites are listed north to south, with site numbers depicted in Figure 1



Site #	River	Nearest City	GPS Coordinates		River Flow (m^3/s)	
			Lat.	Lon.	Spring	Summer
1	Illinois	Grafton, IL	38.968	-90.544	2379	708
2	Mississippi	St Louis, MO	38.757	-90.171	19539	11893
3	Ohio	Metropolis, OH	37.142	-88.711	1814	1699
4	Tennessee	Paducah, KY	37.019	-88.279	2775	1235
5	Mississippi	Memphis, TN	35.180	-90.057	37661	18972
6	Mississippi	Tunica, MS	34.738	-90.446	NA	NA
7	Mississippi	Greenville, MS	33.356	-91.126	41617	29601
8	Yazoo	Vicksburg, MS	32.351	-90.884	NA	NA
9	Mississippi	Vicksburg, MS	32.325	-90.896	41150	34263
10	Mississippi	Natchez, MS	31.588	-91.429	43019	36812
11	Mississippi	New Orleans, LA	29.884	-89.970	31149	26901

NA = Not Available

Figure 2. Map showing the locations in the Mississippi River (dark circles) and in major tributaries (open circles) that were sampled during the spring and summer of 2019. Numbers are Major cities and state borders are shown for reference. Site numbers are listed north to south, with corresponding GPS locations and river flow data in Table 1.

Study Site: Mississippi Sound

The Mississippi Sound is a 145 km sound located along the coasts of Mississippi and Alabama in the northern Gulf of Mexico. It has significant commercial and ecological importance to the area and is known for harvesting of shellfish. However, oyster populations, particularly in the western portion of the sound, have been curtailed in recent years due to pollution (e.g. oil spills) and weather events, including hurricanes and flooding. In this study, we collected water samples from ten sites, four of which were directly above oyster reefs, with the remainder spread throughout the Mississippi Sound (Figure 3). Specific sampling locations (GPS coordinates) and certain water quality measurements are provided in Table 2. Samples were collected in the spring and summer of 2019 during a period of major (historic) flooding on the Mississippi River, as well as in September of 2019 and January and February of 2020 during more normal salinity conditions (post-flood). During periods of flooding on the lower Mississippi River, its waters are diverted through the Bonnet Carre Spillway, preventing flooding downstream in the city of New Orleans. These floodwaters spill into Lake Ponchartrain, then Lake Borgne, and from there enter the western end of the MS Sound.

Figure 3. Map showing the sampling locations (circles) in the Mississippi Sound with direction of freshwater inflows from the Bonnet Carre Spillway and the Pearl River (red arrows). Sample site numbers increase from west to east. Sampling sites 2, 4, 6, and 8 (red stars) were at oyster reefs. Red circles represent sample sites collected by the Mississippi Department of Environmental Quality and blue by the University of Mississippi. Site 10 is off the map about 40 km to the east in Alabama waters. The inset shows the general location in the USA.

Table 2. Geographic coordinates for sites where water was collected along the Mississippi Gulf Coast for microplastic analyses. Sites are listed west to east, with site numbers depicted in Figure 3.

Site #	Site Name	Reef Site	GPS Coordinates		Depth (m)	Sampling Dates	
			Lat.	Lon.		Open Spillway	Closed Spillway
1	St. Joe's Pass	No	30.1068	-89.5528	3.7	Jul. (2019)	Jan. (2020)
2	Waveland Reef	Yes	30.2730	-89.3702	2.6	Apr. (2019)	Sept. (2019)
3	Bay St. Louis	No	30.3510	-89.3547	1.3	Apr. (2019)	Sept. (2019)
4	St. Stanislaus Reef	Yes	30.3023	-89.3272	1.9	Apr. (2019)	Sept. (2019)
5	TNC Bay St Louis	No	30.3451	-89.2949	1.5	Apr. (2019)	Sept. (2019)
6	Henderson Pt.	Yes	30.2926	-89.2711	3.0	Apr. (2019)	Sept. (2019)
7	Pass Christian	No	30.2850	-89.2371	3.8	Jul. (2019)	Jan. (2020)
8	Kittiwake Reef	Yes	30.3324	-89.1652	2.3	Apr. (2019)	Sept. (2019)
9	Biloxi Bay	No	30.3753	-88.8306	1.4	Jul. (2019)	Jan. (2020)
10	Middle Bay	No	30.3749	-88.3992	1.2	Jul. (2019)	Jan. (2020)

Sampling the Mississippi River system using the single-pot method

Two different sampling approaches were explored: high-volume (~360 L) field-filtered sampling and low-volume (~1 L) grab sampling (Figure 4). The high-volume method utilized a water transfer pump to sample ~360 liters of water over 5 minutes, which was passed through the 100 µm cod-end of a plankton net. The cod-end was then rinsed into a large stainless-steel bowl and the contents rinsed into a quart-sized (946 mL) Mason jar. Slightly larger 1 L volume Mason jars are available outside of the United States. Hereafter, we report abundances of MPs/L, adjusting for any differences in volume. The high-volume sampling approach was logistically challenging, time-consuming, and often yielded high amounts of particulate matter, particularly for river waters which are often turbid. High concentrations of particulates can lead to clogged filters and potential counting errors due to overlapping particles, necessitating the need for density separation. Thus, in this study we used the grab sample method which provided sufficient volumes for detecting MPs above blank levels. However, the high-volume method may be suitable for sampling in areas where MP abundances are especially low and the water has less particulate matter.

Figure 4. River water passing through the 100 µm cod-end net using the high-volume sampling method (left); intake chamber and hose for the high-volume sampling method (middle); and low volume grab-sampling with a ~ 1 L Mason jar.

Grab samples were collected from shore at the water surface directly into 946 mL Mason (canning) jars using a pole (Figure 4). The Mason jars are available from most major grocery or home hardware stores and can be readily be attached to a variety of sampling equipment for collection of water samples from the shore of spillways, rivers or lakes, or off boats or docks. Larger size Mason jars can also be used but are more difficult to handle with the added weight and volume. For other sampling scenarios, such as deep-water samples collected using a Rosette sampler, the water can simply be transferred (poured) into the Mason jar. Once full the Mason jar is tightly capped and placed in a crate or cooler for transport, although the samples do not need to be kept cold. However, if the samples are to be stored for extended periods (>1 month) before processing, they can be preserved with addition of isopropanol (1:1 v/v). In this study, we did not preserve samples as they were processed within 3 weeks of collection, instead keeping them in their original container (full) until sample preparation.

Sampling the waters of the Mississippi Sound

In four separate sampling campaigns we obtained bulk water (grab) samples from the Mississippi Sound during periods when the Bonnet Carre Spillway was open and dumping freshwater into the Sound and when it had been closed for extended periods. Our research group at the University of Mississippi (UM) collected samples during April (open spillway) and September (closed spillway) of 2019 from sites 2, 3, 4, 5, 6, 8, and the Mississippi Department of Environmental Quality (MDEQ) obtained samples in July of 2019 (open spillway) and January of 2020 (closed spillway) from sites 1, 7, 9 and 10 (Figure 3 and Table 2). Grab samples were collected just below the surface into glass Mason (canning) jars (946 mL) and tightly capped using metal lids. MDEQ also collected samples <1 m above the bottom using a 4 L Van Dorn sampler, transferring the water to Mason jars (946 mL) immediately after collection. The samples were placed in coolers and shipped to the laboratory at UM for processing. Note: Mason jars are available from most major grocery or home hardware stores. We used jars that have a 946 mL capacity, but slightly larger (1 L) jars are readily available outside of the USA. Hereafter, we report abundances of MPs/L, adjusting for differences in volume.

Sample preparation using the single-pot method

Mason jars containing water from the Mississippi River and its tributaries and from the Mississippi Sound were placed in a clean laminar flow hood located in a HEPA-filtered clean room. The lids were removed and replaced with lids that were cut with a round 57 mm diameter opening and outfitted with a round 84 mm diameter 200 x 600 mesh (~30 µm) screen made of Monel, a nickel copper alloy (Unique Wire Weaving Co. Inc. Hillside, NJ, USA) (Figure 5). Unlike stainless steel, the Monel screen (filter cloth) is flexible enough to mold into the lid’s seal when tightened to prevent leaks, yet strong enough to be cleaned and reused. Next, the jar was turned upside down and a jet of air was used to break the surface tension and allow the water out of the jar through the screen. Any source of clean compressed gas will work, including canned compressed air. This process can be done at a sink or into a bucket in the clean hood. Once the water was removed the lid was opened and the screen carefully rinsed back into the Mason jar using a Teflon squeeze bottle with ultrapure water (purified, deionized, and 0.2 µm-filtered; Milli-Q, Millipore, Burlington, MA, USA). While this washing step should be thorough to quantitatively transfer MPs off the filter back into the container/solution, the volume of water should be kept to < ~100 mL to avoid dilution of reactants during the subsequent digestion step.

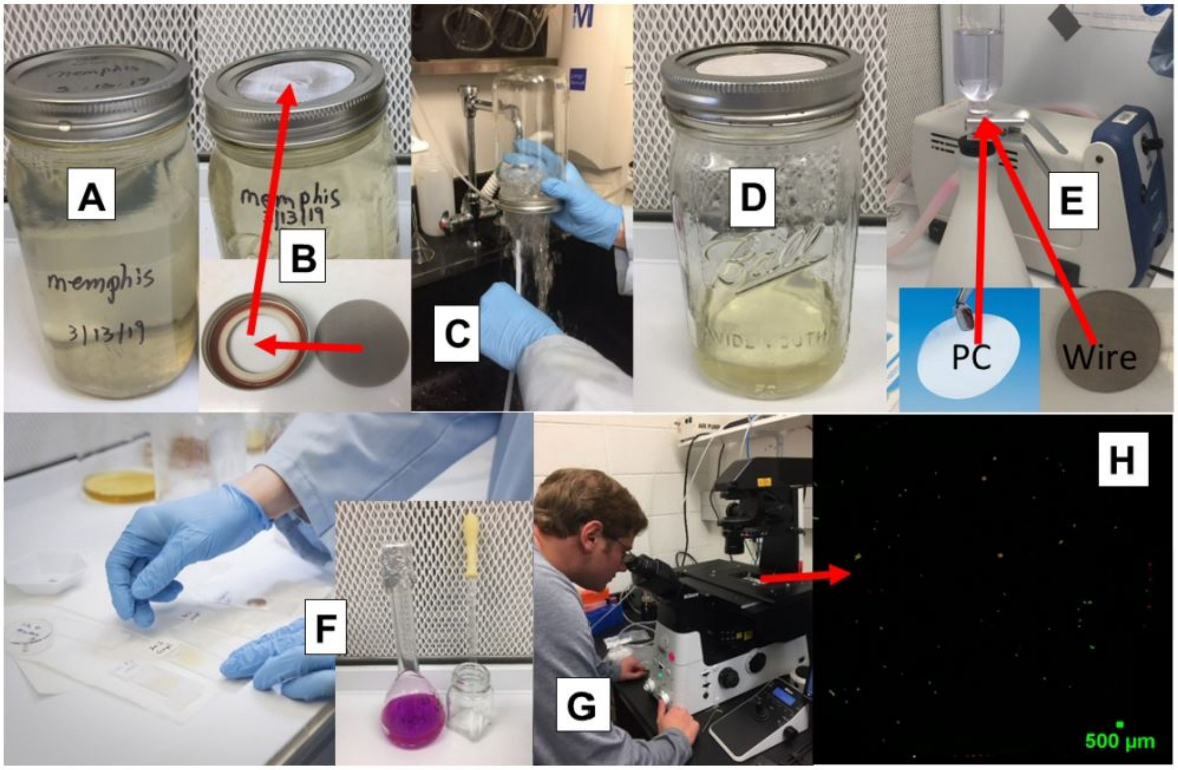


Figure 5. Single-pot sample preparation and analytical scheme used in this study. Sample collected in the field in a Mason jar (A). Replacement of the solid lid with a 30 µm mesh Monel screen cover (B). Injecting a stream of clean air to pass the water through the screen (C). Digestion of organic matter using H2O2 in the presence of an iron catalyst after rinsing the screen back into the Mason jar (D). The digested solution is filtered through the screen cover and rinsed back into the jar as before. This final rinse solution then filtered through either a 25-mm diameter 10 µm pore size polycarbonate (PC) filters for fluorescence microscopy or a 30 µm wire (Monel) screen for micro-spectroscopic imaging (E). The PC filters are stained with Nile red dye and placed on microscope slides (F) and analyzed by fluorescence microscopy (G). False color image obtained using fluorescence microscopy showing putative microplastics (stained with Nile red dye) from a typical sample collected from the Mississippi River (H).

Next, a wet peroxide oxidation was used to remove the natural organic material which can interfere in the analyses. Using a peroxide oxidation avoids both strong acids and heat which can damage the MPs (Claessens 2013; Erni-Cassola 2017; Munno 2017). Specifically, 20 mL of 30% H_2O_2 and 20 mL of 0.05 M Fe(II) solution (Fenton's reagent) was added directly to each sample in the Mason jars. The lid was placed on but not screwed down to avoid pressurization. Note: the Monel screen cover can also be used as it will allow CO_2 to escape. Typically, the mixture bubbled and became amber colored, but as the reaction proceeds to completion the color shifted to a pale yellow. Depending on the amount of organic material remaining, additional aliquots of H_2O_2 were added until the digestion was complete. Note: larger debris that is clearly not plastic such as pieces of wood can be removed with tweezers. Following digestion, the 30 µm mesh Monel screen/lid combination was secured back on the Mason jar and the solution was forced out with pressured air as before. Then the screen was carefully removed and rinsed 3 times back into the Mason jar.

Finally, the samples were vacuum filtered using an all glass filtration apparatus to concentrate the MPs for analysis. Here, we used two different filters, a 25 mm diameter, 10 µm pore size, polycarbonate track-etched filter (PC filter) (Sterlitech Corporation Kent, WA, USA) for fluorescence microscopy, and a 25 mm diameter, ~30 µm pore size, 200 x 600 mesh Monel wire screen for µFTIR-Imaging.

Enumeration of microplastics using fluorescence microscopy

To detect MPs and assess their size distribution and morphology, fluorescence microscopy was used after staining the plastic particles with Nile Red dye fluorochrome (Erni-Cassola 2017). Briefly, Nile Red dye solution (10 µg/mL in methanol) was added directly (dropwise) to the PC filter containing putative MPs until the filter was covered with the dye solution and left to dry in the laminar flow hood for ~15 min. Note: the dye should be applied gently to the filter to avoid displacing particles off the filter. Then the 25 mm PC filters were carefully placed between a microscope slide and coverslip and taped together to ensure that objects on the filter were secure. The slides were stored in a glass petri dish in the clean room until analysis.

Putative MPs were detected and counted using a Nikon Ti2 Eclipse Fluorescence Microscope. The specific microscope, camera, and counting settings used for this work are provided in Table 3. The procedure was a four step process that took ~20 minutes/sample: (1) images were collected across the entire filter area and stitched together into one larger image, (2) automatic image thresholding was performed to convert the image into a binary image, (3) the built in object count software was run, (4) the MPs data were sorted based on size or morphology. We counted and categorized uniformly fluorescing objects in the shape of fibers, fragments (particles with sharp edges), or beads (circular objects); objects that were clearly not plastic or that had biological features such as spines or striations were not counted. If in doubt we did not count the object as plastic, making our estimates conservative.

Table 3. Fluorescence microscopy settings and parameters

Components	Instrument Settings
Microscope	Nikon Ti2 Eclipse
Optics	Plan Apo λ 4x
Filter Set	FITC (470 nm excitation)
Dichroic Mirror	505 nm lp
Emission Filter	535 nm bp
Excitation Source	Lumencor Spectra, 470 nm
Modality	Wide-field fluorescence
Camera	pco.edge
Camera Specs	Aperture: 0.2; Exposure: 100 ms; Readout: 110 MHz; 16 bit
Image Processing	Nikon Software
Counting Range	100 µm^2 and larger

Fluorescence microscopy provides a cost-effective, high-throughput way to detect MPs with images that can be processed for size and morphology. However, it is worth pointing out some of its limitations. One limitation is that sample preparation (digestion) may leave some non-plastic particles intact that may also fluoresce (e.g. chitin), although adjusting fluorescence parameters and visual inspection can be used to minimize false positives (Erni-Cassola 2017). Plastics can also be negatively affected by certain digestion techniques, especially if done at elevated temperature or under acidic or basic conditions, potentially impacting their size and causing discoloration (Nuelle 2014). Another limitation is that MPs made of tire rubber don’t readily fluoresce (Erni-Cassola 2017). Despite these limitations, Nile Red combined with fluorescence microscopy is a powerful technique that is increasingly being used to assess MP contamination in a variety of matrices (Maes 2017; Hengstmann 2019; Scircle 2019).

Identification of MPs in select samples or river water by FPA-µFTIR-Imaging

To assess suitability of the single-pot method for FPA-µFTIR-Imaging and to identify the major type of MPs in a subset of water samples, we used an Agilent Cary 620 FTIR microscope coupled to an Agilent Cary 670 FTIR spectrometer at Aalborg University, Denmark. µFTIR-Imaging is currently considered the most suitable technique to analyze small MP (< 500 µm) without pre-sorting and providing unbiased results (Loder 2015; Primpke 2018; Vianello 2019). Water samples were prepared as before but filtered onto 25 mm Monel screens. These screens were individually placed into 25 mL glass scintillation vials and submerged in 2.5 mL of 50% ethanol. The vials were sealed and placed in an ultrasonic bath for 5 minutes, after which the Monel screens were removed and rinsed with 2.5 mL of 50% ethanol. The 5 mL sample was split and a 2.5 mL portion was shipped to Denmark for FPA-µFTIR-Imaging. There, aliquots of known volume were deposited onto a zinc selenide (ZnSe) windows held in compression cells (PIKE Technologies, Fitchburg, WI, USA) using a capillary glass pipette (micro-classic, Brand GmbH, Germany), and dried at 55˚C on a heating plate overnight. The windows were then placed onto the FTIR-microscope stage. The system uses a 128 x 128 pixel Focal Plane Array (FPA) detector capable of simultaneously acquiring 16,384 spatially resolved spectra over an area of 704 x 704 microns/tile using 15x IR Cassegrain, which provides a pixel size of 5.5 µm. The analysis was performed by scanning the entire active area of the windows, which is approximatively 78.5 mm^2. The instrument operated in transmission mode with an active spectral range from 850 to 3750 cm^{-1}, collecting 120 co-scans for the background (also collected on a ZnSe window) and 30 co-scans for the samples at a spectral resolution of 8 cm^{-1} and a beam attenuation of 50%. The scan time was ~4 hours.

Data analysis was carried out using siMPle, a software developed by Aalborg University (AAU) and Alfred Wegener Institute (AWI) (siMPle, 2019); a paper detailing the software was under review at the time of submission. Much like its predecessor software MPhunter (Primpke 2018), SiMPLe allows for automatically detecting the particles on the scanned surface, correlating the raw spectra, the 1st and 2nd derivative of all sample spectra to a custom-built database containing more than 100 reference spectra (polymers, paints and resins, and non-synthetic materials). Moreover, siMPle automatically measures the size of the particles and can also provide a mass estimation using the area, the density and an estimated thickness of the identified particles (Liu 2019).

Identification of MPs in seawater by Laser Direct Infrared (LDIR) analysis

To identify the major types and size fraction of plastics in a subset of seawater samples, we used the Agilent 8700 LDIR analyzer at Agilent Technologies Application Laboratory in Wood Dale, IL, USA. For LDIR analysis, we prepared seawater samples as before (see Sample preparation using the single-pot method), except we did the final filtering using a wire mesh screen with ~25 µm openings. The screen was then submerged with 50% ethanol in a glass vial and subjected to ultrasonication for ~2 min. The screen was then removed with forceps and the vial sealed and shipped overnight to Agilent. There, the samples were deposited on MirrIR low-e Kevley microscope slides. The solvent was allowed to evaporate, leaving behind the MPs adhering to the slide.

The LDIR analyzer was run in trans-reflectance mode, where the system directs IR laser light through the sample (particle) and the light is then reflected back off the reflective slide through the particle as it exits. As noted, the analyzer first scans the slide to locate each particle using IR light at 1,800 cm-1, a frequency at which little or no absorption occurs but instead the light is scattered when encountering a particle. The system utilizes image analysis techniques to determine the boundary of the particle and hence the dimensions. In addition, a full spectrum covering the range of the instrument is collected of each particle and compared to the spectral library in real time. The particle is identified based on this comparison to a spectral database built into the software. Validation of this method was achieved through the use of a blind sample consisting of a blank spiked with MPs composed of polyethylene, polyamide (nylon), polypropylene, and polystyrene, and each of these types of MPs were identified in the analyses. All data analysis and processing was done in real time using the Agilent Clarity Software.

Blanks, replicates, and spike recovery tests

Many of the early papers on MP pollution do not include sufficient quality assurance measurements to demonstrate the reliability of the data. Such measurements are especially important when developing and validating a new method. Here, we evaluate our single-pot method with multiple blanks, replicates, and spike recovery measurements. To assess contamination, we prepared blanks to assess various aspects of the sample processing including total procedural blanks. To assess precision, we split samples and compared each half both to each other and to other un-split samples from the same site. To assess accuracy, we spiked samples with known amounts of MPs, processed the samples, and determined recoveries.

For the recovery experiments, 50 bright red acrylonitrile butadiene styrene (ABS) particles were added to DI water (filtered through 10 µm filters) in Mason jars. Twelve samples for each of two size categories (250-500 µm and 500-1000 µm) were spiked. Within each of these size fractions, 6 samples were processed with the single-pot method, as described earlier, and 6 using the conventional sieving method. In the conventional method, samples from the jar were transferred into the standard test sieve (20.5 cm diameter; Advantech, New Berlin, WI, USA) and washed with 0.45 µm filtered water. The particles on the sieve were then rinsed back into Mason jar. All samples were then processed the same as real samples, except Nile Red dye was not added to the final filters. The filters were sandwiched between microscope slides and securely taped. Recoveries were determined by visual counting using a magnifying glass and tweezers.

Recovery tests for smaller particles that are difficult to manipulate or that are invisible to the naked eye is challenging. Here, we used MPs in the 125-250 µm size range that were generated from weathered plastic (high density polyethylene) found in the environment (Sardis Lake, Mississippi, USA). The weathered fragments were cut to smaller pieces and ground into MPs using a cryomill (SPEX Certiprep, Metuchen, NJ, USA). The particles were then sorted into different size fractions using standard sieves (Gilson Company, Inc.). A few mg of the 125-250 µm size fraction were dispersed in 0.05% (v/v) sodium dodecyl sulfate (SDS) in ultrapure water and a 0.5 ml aliquot of the solution was pipetted into ~100 mL of ultrapure water which was then filtered and stained with Nile Red dye as described earlier. These filters were examined by microscopy and the number of particles in the 125-250 µm size fraction ranged from 34 to 54, which overlaps the amount we measured for this size category in 1 L of river water. The filters were carefully placed into small glass covered petri dishes, brought back to the clean MPs laboratory, and thoroughly rinsed into Mason jars in the clean hood. These samples, along with blanks, were then processed as before and re-examined by fluorescence microscopy to determine recoveries.

RESULTS AND DISCUSSION

Part I: Method Development and Microplastics in the Mississippi River System

Laboratory and reagent blanks

Using fluorescence microscopy, we analyzed a variety of blanks to assess contamination and carryover between samples. Reagent blanks (n=3) consisting of Nile Red dye solution deposited directly on the filters yielded 2 or fewer MPs. Similarly, air blanks prepared by leaving a filter exposed in the laminar flow while preparing other samples (~1 day) were also negligible. Full procedural (method) blanks using ultrapure water (purified, deionized, and filtered through a 0.22 µm membrane with a Milli-Q A10 system) as the sample and for rinsing yielded blanks that averaged 35 ± 4 total putative MPs (n=9; ±1 standard error), with most being in the lowest size fraction (30-90 µm) (Figure 6). Method blanks were always less than samples from the Mississippi River and its tributaries. We emphasize that it is important to include multiple blanks in each batch of samples because contamination can vary from batch to batch. Steps to lower blanks should include avoiding plastic (e.g. pipette tips), filtering reagents, avoiding clothing that can readily shed fibers, and working in a clean laboratory environment.

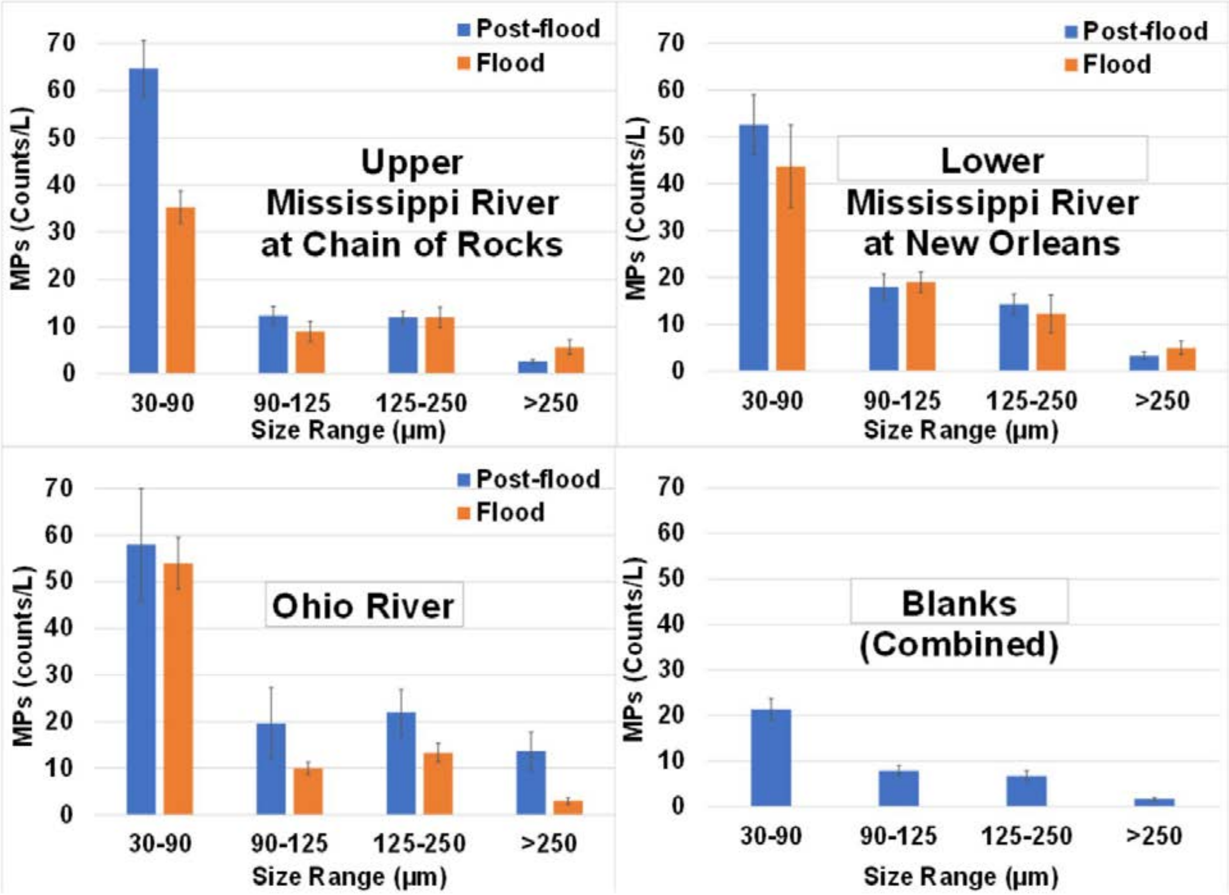


Figure 6. Microplastics as a function of particle size in method blanks and samples from the Mississippi River near New Orleans, LA and the Ohio River near Fort Massac, IL determined using fluorescence microscopy. The same y axis is used to aid comparison. Error bars are ±1 standard deviation.

Recovery and replicate/split sample experiments

Average recoveries for MPs spiked into water ranged from 97% – 116% (Table 4). For the larger particles (250-500 µm and 500-1000 µm), mean recoveries for the single-pot method were 97% for both size fractions, compared to 90% and 93% for the conventional method, demonstrating a modest improvement. For smaller MPs (125-250 µm), the mean recovery was 116% (range 105-130%). These results are assuring, particularly for the smallest size fraction, considering the experimental challenges in detecting, spiking, and quantifying them.

Table 4. Recoveries for water spiked with microplastics using conventional (open) and one-pot (closed) sieving

			% Recovery: Mean (range)	
Counting Method	n	Size fraction (µm)	Conventional Method	One-pot Method
Visual	6	500-1000	93 (90-98)	97 (94-100)
Visual	6	250-500	88 (82-98)	97 (92-100)
Fluorescence	3	125-250	NA	116 (105-130)

Given the inhomogeneity of natural water samples and the challenges of isolating and quantifying smaller MPs, there will always be some variability between samples. Reproducibility for real-world samples is presented in the *Microplastics in the Mississippi River and several of its major tributaries* section of this paper. Here we highlight a comparison between two river samples collected moments apart, with one analyzed "whole" (~1 L) and the other split into two equal halves (~0.5 L each). Results showed that the split samples had a similar number of MP particles (145 and 160) compared to 242 particles in the 1 L (whole) sample. On the one hand, this shows that that the numbers of MPs in the split samples decreased nearly in half as expected. On the other hand, because the split samples had 60% and 66% of the total number of MPs detected in the un-split sample, it emphasizes the importance of thoroughly cleaning equipment and screens that are to be reused to minimize contamination and carryover between samples.

Variations and remarks on the single-pot method

The single-pot method could be a useful tool in the growing practice of citizen science owing to the relative ease of sample collection and availability of materials used. The use of citizen science in assessing MP pollution is an exciting opportunity to get communities involved in science and increase environmental stewardship in local waterways. There are a few published examples of citizen science MP studies and this single-pot method is a suitable starting point for such studies, particularly since these studies almost always rely on grab samples (Bosker 2017; Barrows 2018). Whereas fluorescence microscopy and µFTIR-Imaging is likely beyond the scope of most citizen science-based MP surveys, the single-pot method's screens/filters can be photographed and/or assessed using magnifying glasses or optical microscopy, with larger MPs removed with tweezers for further study.

Indeed, the single-pot method is versatile and can be adapted as needed by researchers. For example, besides the high-volume sampling method described earlier, other variations include adding pre-filtering or density separation for turbid water with higher silt and sand content. A 1-mm (or other mesh size) screen can be added to the Mason jar (as before) and the water simply poured into a second clean mason jar. This allows rapid removal of larger debris that can be present in samples collected from turbulent waters. The filter can be inspected for larger MPs and the water (containing MPs less than the screen opening size) can be processed in the new (second) Mason jar as before. In the current study, however, we found this unnecessary and kept all analyses to a single pot.

Another variation allows sampling larger volumes of water in the field by adding the screen top (instead of the closed cover), inverting the setup, and displacing the water with a stream of air such as from a bicycle tire pump. Then temporarily removing the cover/screen, refilling the jar without over-spilling, and placing the screen/cover back on. This process can be repeated multiple times as desired accumulating MPs from the water if done carefully and if the total volume is kept track of.

We also found that Mason jar metal covers tended to rust over time and while the Monel screen doesn't readily corrode, the rust from the covers can transfer or stain the Monel screen. Thus, it is best to replace the covers periodically before rust becomes a problem.

Microplastics in the Mississippi River and several of its major tributaries

While several studies have examined MP pollution in the northern Gulf of Mexico (Wessel 2016; Di Mauro 2017), studies on MPs in the Mississippi River system are scarce. Martin used micro-attenuated total reflectance FTIR to quantify and characterize the Mississippi River's contribution of MP debris to the Gulf of Mexico (Martin 2018). The authors report a total of 7,600 suspect MPs were quantified from 24 samples (an average of ~11.6 ± 3.8 MPs/L) by removing them from filters using tweezers and using micro-attenuated total reflectance-FTIR for confirmation.

Here, we used the single-pot method with fluorescence microscopy and µFTIR-Imaging to quantify and identify MPs in water samples collected from the Mississippi River and its' tributaries during flood and post-flood conditions. Concentrations and loads of MPs in the Mississippi River system are given below in Table 5. Concentrations (MPs/L) ranged from 14 in the Tennessee River during flooding to 83 in the Ohio River during low-flow (summer) conditions. Loads of MPs tended to increase down the Mississippi River, ranging from ~87 to ~129 trillion MPs/day near New Orleans close to the river's mouth. These levels are higher than those reported by Martin, however our analysis includes smaller MPs that cannot be removed with tweezers for quantification (Martin 2018). We note that concentrations of smaller MPs (~5-333 µm) in the open ocean were recently reported on the order of 10^2-10^3 particles/L, with even higher concentrations (and variability) near shore (Brandon 2019).

Whereas flooding decreased concentrations of MPs, likely due to dilution, its impact on overall loads varied between sites. It should be noted that sampling during the flood typically required collection from low-lying areas adjacent to the river channel. Also, flooded samples were more likely to include invertebrate exoskeletons that can, to some extent, adsorb the Nile Red dye. However, their bio-structure is readily identified and they were excluded from the final MP count. The prevalence of this type of debris was atypical and wasn't nearly as prevalent in samples collected during non-flooded river conditions. The size distribution of MPs was relatively uniform between flood and post-flood samples (Figure 6). However, differences and trends in size distribution may be masked by the uncertainty in the data. Overall, the MPs consisted primarily of fragments (~85%), followed by fibers (~8%) and beads (~7%). This distribution of morphology was remarkably consistent between sites and during the two flow regimes.

Other trends observed in the data were (1) the Mississippi River generally had higher concentrations of MPs/L than samples from its tributaries (except for the Ohio River), and (2) the loading of MPs tended to increase downriver with highest loads in the lower Mississippi River near its mouth. How these trends are influenced by seasons and certain other factors such proximity to wastewater treatment plant outflows are under investigation.

Select water samples were also processed and analyzed by µFTIR-Imaging to show that the single-pot method is amenable to the technique. A detailed comparison of MPs abundances determined by fluorescence microscopy and FPA-µFTIR-Imaging is beyond the scope of this pilot project. Here, we focused on using µFTIR-Imaging to identify the major type of MPs in select samples from the Mississippi River. Again, we found that river samples yielded higher MPs counts than blanks, with the most common polymer types being polyester, polyethylene, polypropylene, polyacrylate, and polyurethane. For example, we detected 47 particles in a sample from the upper Mississippi River near St. Louis, with 34 particles identified as polyester and 6 as polypropylene, and 62 particles in a sample from the lower Mississippi River near New Orleans, with 38 identified as polyester and 17 as polypropylene. Additional µFTIR-Imaging of samples is needed to fully characterize spatial and temporal MP trends in the system.

Table 5. Concentrations and loads of microplastics (>30 µm) in the Mississippi River and its tributaries during the spring and summer of 2019 determined using the one-pot method and fluorescence microscopy[a]

Site #	River	Site	Spring (flooding)			Summer (post-flood)		
			n	Mean (Range) (Particles/L)	Loads (Counts x 10^{12}/day)	n	Mean (Range) (Particles/L)	Loads (Counts x 10^{12}/day)
1	Illinois	Grafton, IL	3	35 (0-62)	7.21	3	56 (34-67)	3.38
2	Mississippi	St. Louis, MO	3	28 (1-43)	46.69	3	60 (34-85)	61.22
3	Ohio	Metropolis, IL	3	47 (40-51)	7.34	3	83 (30-172)	12.02
4	Tennessee	Paducah, KY	2	14 (12-15)	3.32	3	35 (32-38)	3.74
5	Mississippi	Memphis, TN	3	33 (9-60)	107.80	3	28 (0-77)	17.44
6	Mississippi	Tunica, MS		NA	NA		NA	NA
7	Mississippi	Greenville, MS	4	15 (10-27)	53.55		NA	NA
8	Yazoo	Vicksburg, MS	4	45 (17-78)	NA	2	73 (28-118)	NA
9	Mississippi	Vicksburg, MS	3	18 (4-30)	64.30	3	103 (71-151)	306.86
10	Mississippi	Natchez, MS	3	24 (19-29)	90.31	3	52 (27-87)	148.87
11	Mississippi	New Orleans, LA	4	38 (0-109)	86.54	3	56 (23-85)	128.57

[a] Data is blank-subtracted. NA = Not Available (site inaccessible or flow data unavailable).

Part II. Microplastics in the Mississippi Sound

We found no significant difference (t-Test, α =0.05, unequal variances) between the samples collected just below the surface (n=48) and those collected near the bottom (n=28) during the same sampling event and across all sampling sites. The lack of difference by depth is not surprising given the shallow waters (often <3 m deep) and mixing by wave action. Hereafter we do not distinguish samples by depth.

Spatial and temporal trends of MPs in the Mississippi Sound

Microplastic concentrations from all sites and sampling campaigns are summarized in Table 6. Average concentrations ranged from 30 to 192 particles/L across the sites, with individual samples ranging 12 to 381 particles/L. It is not unusual to observe such a wide range of MP concentrations, especially when counting particles as low as 30 µm in bulk water grab samples. Indeed, sampling methods will affect the counts and distribution of MPs measured (Stock et al. 2019). For example, nets are typically used in investigating large areas with results being reported in particles/m^3, whereas bulk water sampling, done here, can be provide a snapshot at a given site and is generally reported in particles/L. A major drawback to sampling with a net is that it fails to capture particles smaller than the mesh opening (typically 333-µm), and these smaller particles tend to be the most abundant (Song et al. 2014). Conversely, the probability of capturing larger particles in grab samples is lower compared to trawling with a net.

Using the average concentration of MPs determined throughout the study (129 ± 93 MPs/L) and the average volume of water an adult oyster filters daily (189 liters) (Newell 2004), we estimate oysters may be exposed to nearly 24,000 MPs daily (range ~5600 to ~36,000). We stress that this is only an estimate and that MP concentrations and filtering rates will vary depending on site-specific conditions, oyster species, and other factors. Moreover, whether the MPs are actually entrained in oyster tissues likely depends on their size and morphologies and whether they are ingested or just make contact with the tissues (see *microplastic morphologies* below). Recent studies on oyster ingestion of MPs have shown that oysters are ingesting and retaining between 0.6 and 16.5 MPs per individual (Li et al. 2018; Teng et al. 2019; Baechler et al. 2019). These studies also show that oysters nearer to urban centers often contained higher concentrations of MPs. Given the prevalence of commercial fishing, oil drilling, and shipping ports in the nGoM, oysters along the Gulf Coast could be accumulating a considerable amount of MPs.

Despite the inherent variability in MP concentrations, we observed a moderate positive correlation (r = 0.62) between MP concentration and salinity (the latter used as a proxy for lack of freshwater intrusion) when the spillway was open (Figure 7). The correlation (r-value) improves to 0.87 after removing sites with only 2 data points (sites 1 and 5). There was no such trend when the spillway was closed suggesting a link between freshwater inflows and MP concentrations, essentially a dilution effect during high river discharges. Indeed, for the majority of the sites MP concentrations were higher when the spillway was closed (Table 6). Further, the MP concentrations in the seawater were typically higher than what we observed for the Mississippi River and its tributaries (Scircle et al. 2020). We conclude that seawater along the Mississippi Gulf Coast has higher MP abundances (when including MPs sizes down to ~30 µm) than the river supplying new loads of MPs to the coastal area. This finding that the estuaries have higher concentrations of MPs than their riverine inputs has been observed elsewhere (Newell 2004). This is not surprising as the river is continually flushed of plastics and the estuary serves as a sink for these plastics, and with time the plastics mechanically and photolytically degrade to smaller and smaller particles.

Table 6. Concentrations of microplastics (>~25 µm - 5 mm) along the Mississippi Gulf Coast during freshwater inflows and the summer and fall of 2019 and winter of 2020.[a]

Site #	Location / Name	Open Spillway				Closed Spillway			
		n	Mean (Range) (MPs/L)	SD	Salinity (ppth)[b]	n	Mean (Range) (MPs/L)	SD	Salinity (ppth)[b]
1	St. Joe's Pass	2	196 (12 – 381)	NA	0.39	1	309	NA	NA
2	Waveland Reef	5	73 (55 – 153)	50	0.32	2	174 (39 – 309)	NA	16.4
3	Bay St. Louis	4	49 (18 – 80)	25	0.67	3	50 (0 – 116)	60	NA
4	St. Stanislaus Reef	4	36 (18 – 65)	20	0.40	5	121 (34 – 199)	76	16.8
5	TNC Bay St. Louis	2	69 (38 – 100)	NA	0.54	3	39 (15 – 73)	30	15.3
6	Henderson Pt. Reef	3	30 (20 – 50)	17	3.14	5	139 (20 – 198)	76	17.2
7	Pass Christian	12	178 (55 – 328)	22	8.1	3	176 (124 - 202)	44	4.7
8	Kittiwake Reef	4	121 (22 – 196)	82	4.21	-	NA	NA	18.9
9	Biloxi Bay	8	179 (64 – 278)	82	13.5	3	94 (55 – 116)	34	9.3
10	Middle Bay	6	192 (39 – 326)	10	19	3	141 (41 - 263)	11	17

[a] Data is blank-subtracted; NA = Not Available. [b] ppth = part per thousand; oysters require at least 8 ppth salinity to grow (Virginia Dept. of Environmental Quality).

Because a high-degree of spatial variability is common in MP studies (Zhao et al. 2019; Goldstein et al. 2013), especially when counting smaller size fractions like in this study, it can be difficult to observe trends, particularly where there are no major point sources. Here, except for during intense freshwater inputs in the western portion of the Sound during the spring and summer, MP concentrations did not show any distinct trend based in open water areas based on longitude. However, sites 3 and 5, both located inside Bay St. Louis, tended to have lower concentrations compared to adjacent open waters of the Sound. For example, when the spillway was closed (i.e., "normal" conditions) the average concentration inside the Bay was 54 ± 18 MPs/L (n=5; ±1 SE) while just outside the Bay levels were 145 ± 18 MPs/L (n=13; ±1 SE) (see also Table 6). The reason for this is unclear and requires further study, but the circulation, mixing, and flow inside Bay St. Louis is certainly different than the other coastal open water sites. Additionally, the bay has its own freshwater input coming from the Wolf River, which could further contribute to the lower concentrations observed. Interestingly, the size distributions of MPs in samples were generally similar between periods when the spillway was open and when it was closed, with the smallest size fraction, 20 – 90 µm, being the most common and comprising ~70% of counted particles.

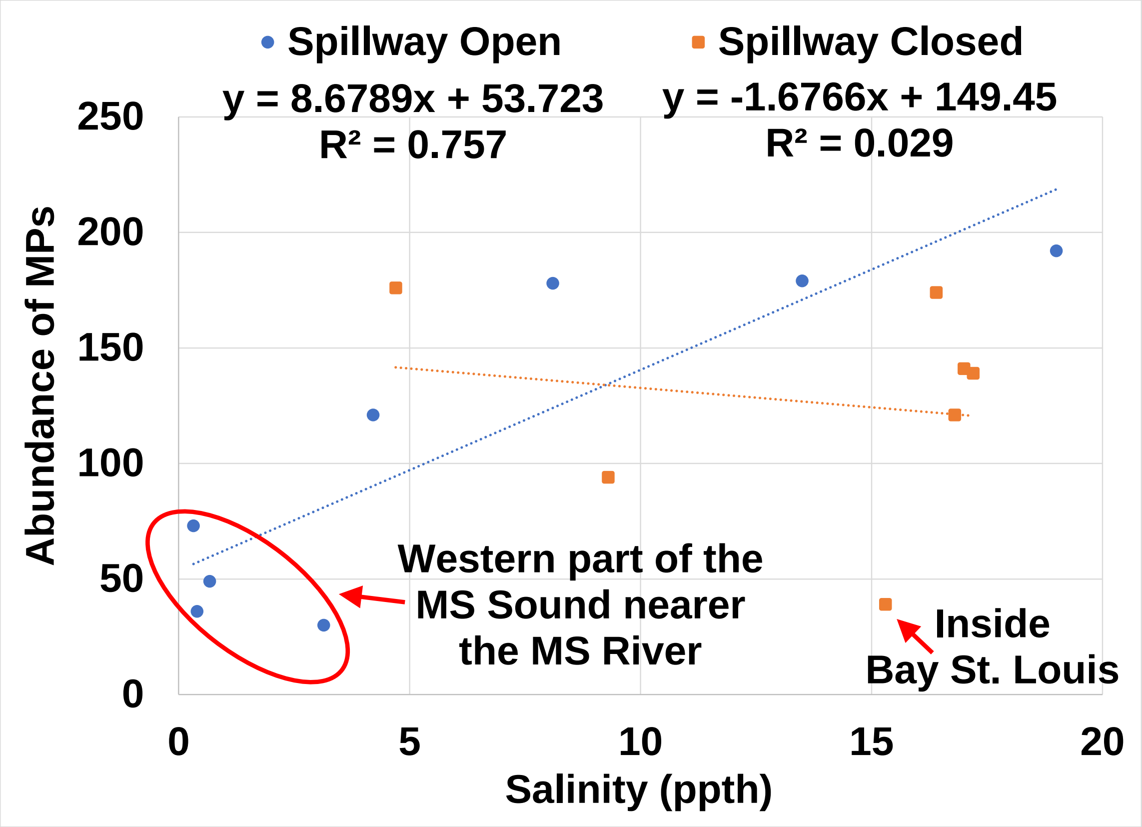


Figure 7. Salinity versus microplastic abundances in water from sites across the Mississippi Sound showing a moderate correlation during freshwater intrusion from flooding (spillway open). All sites were from open water areas in the Gulf, except for Bay St. Louis. Data are means of three or more measurements. ppth = parts per thousand.

Microplastic morphologies

The microplastic morphologies determined by fluorescence microscopy were generally consistent from site to site (Figure 8). The distribution of morphologies were similar whether the water was gathered in Bay St. Louis or at a reef in the Mississippi Sound. Fragments made up the majority of MPs counted (77-88%), followed by fibers (7-15%), and beads (1-8%). This contrasts with studies that have predominantly found anthropogenic fibers in oyster tissue (Keisling et al. 2020; Rochman et al. 2015; Baechler et al. 2019). However, most of these studies employ optical microscopy for detection instead of fluorescence microscopy, targeting larger microplastics. A study that used µFT-IR analyses for MP contamination in bivalves found that most of the MPs are indeed fragments (Phuong et al. 2018). But it is also possible that fibers may be disproportionally retained because of their shape. Regardless, the connection between levels of MP pollution at reefs and the concentrations of MPs ingested by oysters may not exhibit a linear relationship and requires further scrutiny.

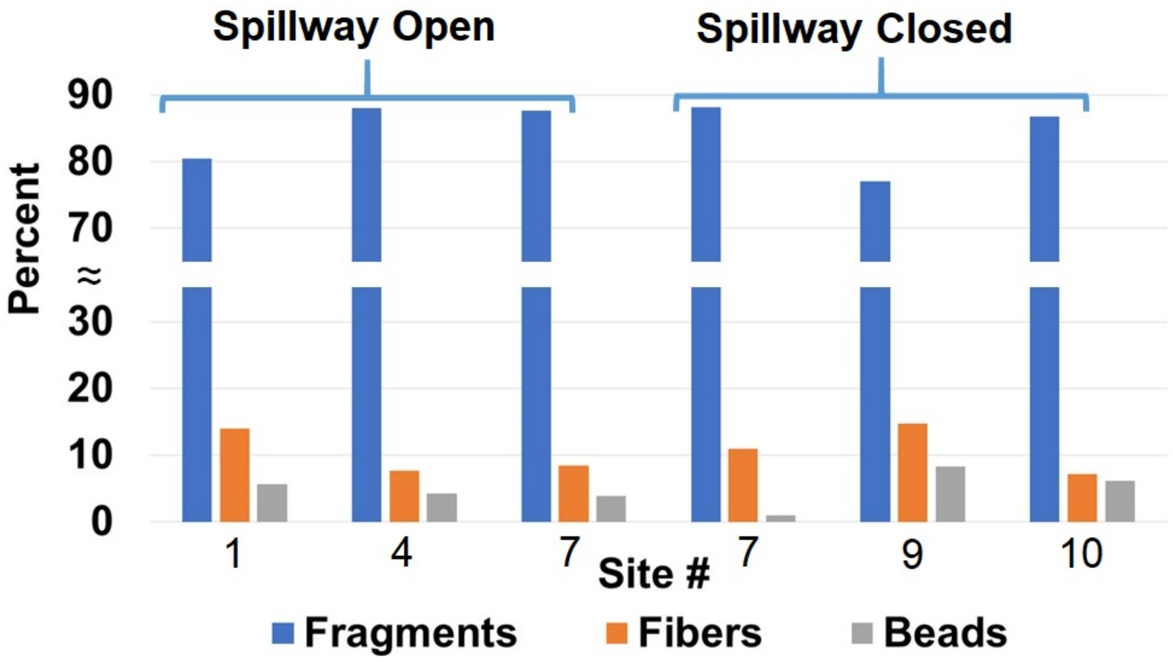


Figure 8. Morphologies of microplastics in water from select sites in the Mississippi Sound. Site number information is given in Table 6.

Identification and quantification of microplastics using LDIR

LDIR analysis was applied to identify putative MPs for a selection of samples from sites 1, 7, 9 and 10 (see Figure 1 for locations and Table 3 for LDIR results). The most identified plastic throughout the samples was polyester, followed by acrylates/polyurethanes, and polyamide, respectively. Other plastics identified were polypropylene, polyethylene, polyacetal, and polytetrafluoroethylene (PTFE). Whereas polyesters and polyamides are typically used in synthetic fibers, all of the identified plastics are commonly encountered in similar studies (e.g. Han et al. 2020).

LDIR data was also used to quantify the abundance of MPs (see MP counts in Table 3, third column) and their size distribution in the same analytical run (Figure **9**). The LDIR data followed the previously discussed trend with lower numbers of particles in the water when the spillway was open compared to when it was closed. The data also showed a general increase in the overall number of MPs at sites further to the west nearer the mouth of the Mississippi River, a major source of plastic pollution (Table **6**). However, given the limited number of samples that were analyzed by LDIR we hesitate to make a detailed comparison with fluorescence microscopy. Nevertheless, it's worth noting that while the absolute numbers of particles detected by the two techniques were in the same magnitude of each other (usually between a hundred and a thousand particles), LDIR tended to detect more particles. It should be emphasized that unlike fluorescence microscopy that involves non-targeted staining of particles and assumes fluorescing particles are plastic, LDIR produces spectra on individual particles confirming their identity. Moreover, regardless of the technique used to identify particles, there are those that remain unidentified; and they could be mixtures of polymers or polymers that have adhering particles or biofilms that complicate the spectra and decrease the probability of a library match. Thus, the fluorescence counts reported herein can be considered conservative.

Overall, we show that Agilent's LDIR analyzer is a powerful new automated analytical tool to rapidly detect and characterize MP pollution. A detailed comparison between LDIR and other chemical imaging techniques used for microplastic analyses will be the subject of a future report.

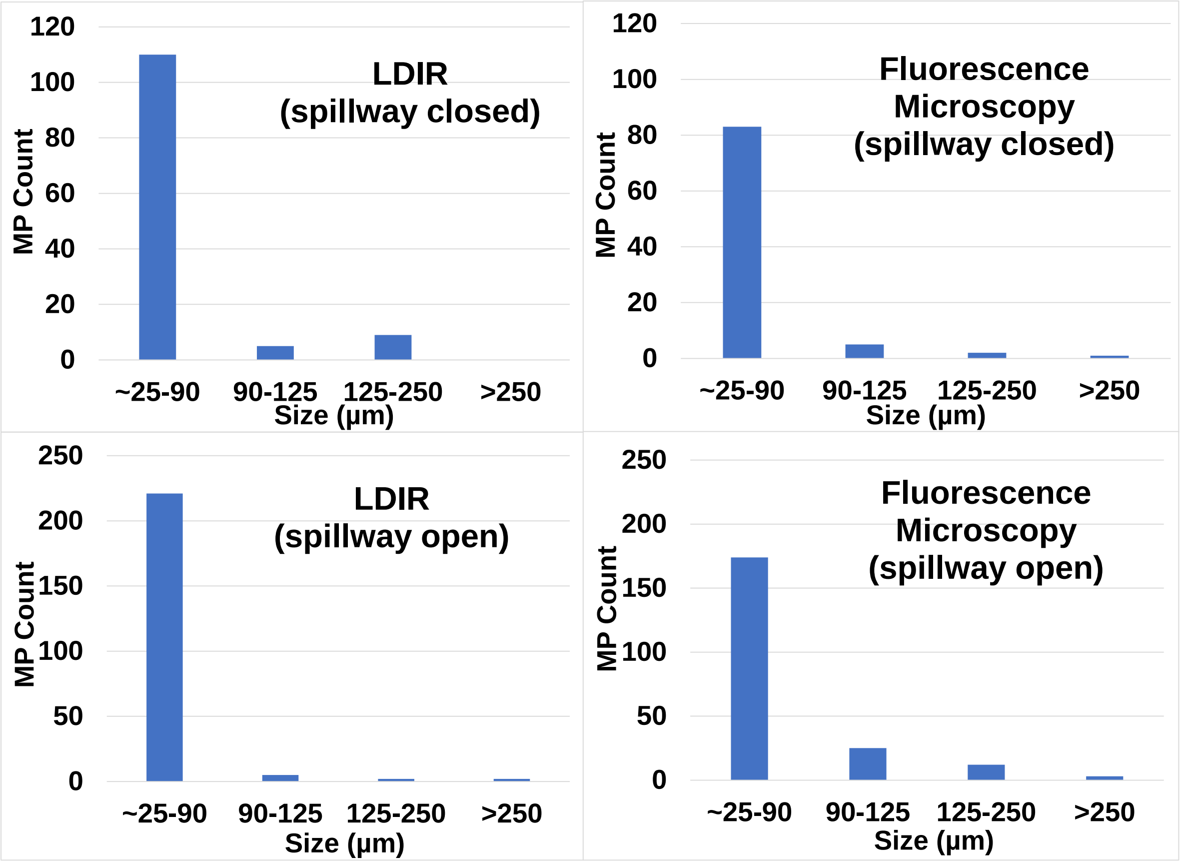


Figure 9. Particle size distribution for microplastics from 1 L of seawater collected in the Mississippi Sound and determined by LDIR (left) and fluorescence microscopy (right). Note the different scale for MP counts for spillway open (top, indicating freshwater intrusion) and spillway closed (bottom, representing normal salinity conditions).

CONCLUSIONS

This study demonstrated that MPs, primarily fragments and fibers, are prevalent in the Mississippi River System and at oyster reefs along the Gulf Coast, and that the Mississippi River is a major source of the synthetic particles to the northern GoM.

Part I. Method Development and MPs in the Mississippi River System.We developed and validated a versatile single-pot method for collection and preparation of natural water for MP analyses. The method reduces sample preparation time, while minimizing outside contamination and carry-over between samples, and is amenable to multiple analytical techniques for detection and characterization, including optical and fluorescence microscopy and imaging techniques that center on smaller MPs (<333 µm), including µFTIR and µRaman. The method was applied to assess MP contamination in the Mississippi River system, the largest drainage basin in the USA, during both flooded and low-flow conditions. Concentrations of MPs were lower during flooding. Loading of MPs generally increased as the Mississippi River approached its terminus at the Gulf of Mexico and suggests that the waters of the northern Gulf of Mexico likely act as a sink for plastic pollution that is continually funneled through the Mississippi River. Given the availability of the materials and ease of use, the single-pot approach can potentially harmonize sample collection and preparation for many MP surveys worldwide, especially those involving volunteers from the community and schools.

Part II. MPs in the Mississippi Sound. We assessed MP pollution in water at oyster reefs along the Mississippi Gulf Coast when: (1) historic flooding of the Mississippi River caused the Bonnet Carre Spillway to remain open for a record period of time causing major freshwater intrusion to the area and deleterious impacts to the species, and (2) the spillway was closed and normal salinity conditions resumed. Microplastics (>~25 µm–5 mm) were isolated using a single-pot method that prepared samples in the same vessel (Mason jars) that they are collected in right up until the MPs were transferred onto filters for analyses. The MPs were quantified using Nile Red fluorescence detection and identified using laser direct infrared (LDIR) analysis. Concentrations ranged from ~12 to 381 particles/L, and tended to decrease at sites impacted by major freshwater intrusion. With the spillway open, average MP concentrations were positively correlated with salinity (r=0.87, p=0.05) for sites with 3 or more samples examined. However, the dilution effect on MP abundances was temporary and oyster yields suffered from the extended periods of lower salinity. There were no significant changes in the relative distribution of MPs during the freshwater intrusions; most of the MPs (>50%) were in the lower size fraction (~25–90 µm), consisted mostly of fragments (~84%), followed by fibers (~11%) and beads (~5%). The most identified plastic was polyester, followed by acrylates/polyurethanes, polyamide, polypropylene, polyethylene and polyacetal. Overall, this work provides much-needed empirical data on the abundances, morphologies, and types of MPs that oysters in the Mississippi Sound are exposed to, although how much of these MPs are ingested and their impacts to the organisms deserves further scrutiny. This work is believed to be the first major application of LDIR to the analysis of microplastics in natural waters.

Ongoing research includes (1) additional identification of MPs isolated from the Mississippi River system and in the northern GoM by µ-FTIR imaging, (2) examining the interactions of mercury with virgin and weathered MPs, (3) evaluating temporal patterns in MP pollution at key sample sites, and (4) measurements of MPs in oyster tissues from the Mississippi Coast.

Dissemination of Research Results

Publications

- Scircle A. and Cizdziel J.* (2019) "Detecting and Quantifying Microplastics in Bottled Water using Fluorescence Microscopy: A New Experiment for Instrumental Analysis and Environmental Chemistry Courses", J. Chem. Ed. https://doi.org/10.1021/acs.jchemed.9b00593

- Scircle A., Missling K., Cizdziel J.* (2020) "Single-Pot Method for Collection and Preparation of Natural Water for Microplastic Analyses: Microplastics in the Mississippi River System During and After Historic Flooding in 2019" *Environ Toxicol Chem*. https://doi.org/10.1002/etc.4698

- Scircle A., Cizdziel J.* (2020) "Occurrence of Microplastic Pollution at Oyster Reefs and Other Coastal Sites in the Mississippi Sound, USA: Impacts of Freshwater Inflows from Flooding" Toxics, 8, 35. https://doi.org/10.3390/toxics8020035

- Scircle A. (2020) "Microplastic pollution in the Mississippi River and the Mississippi Sound Estuary: concentrations, sizes, types, loadings, and method development", [Doctoral Dissertation, University of Mississippi]. ProQuest Dissertations.

Presentations at Scientific Meetings

Cizdziel J., Scircle A. "Spatial Study of Microplastics in the Mississippi River Basin", Society for Environmental Toxicology and Chemistry (SETAC) Europe 29th Annual Meeting, Helsinki, Finland, 26-30 May 2019.

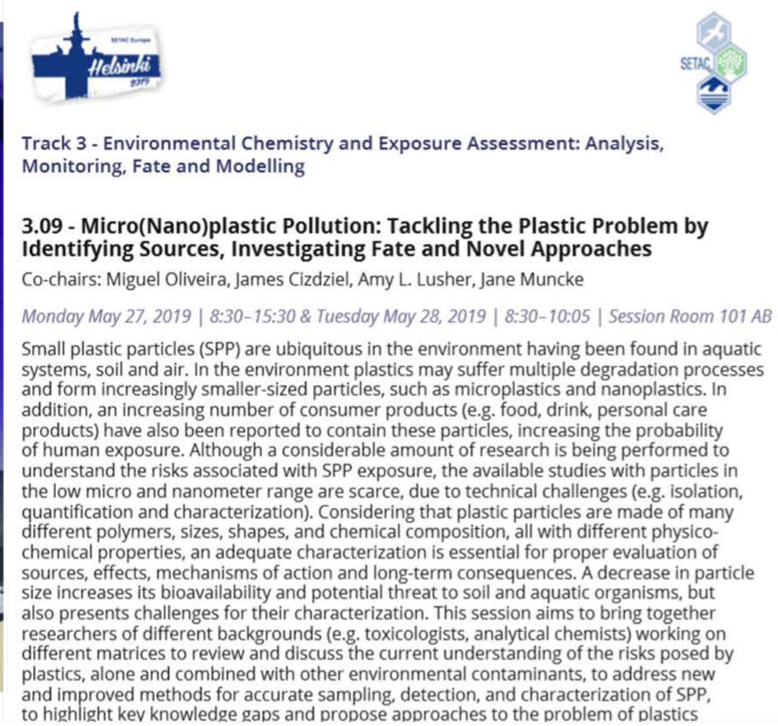
Scircle A., Cizdziel J. "One-Pot Method for Collection and Preparation of Microplastic Samples for Detection and Characterization Purposes", Mississippi Water Resources Conference, Jackson, Mississippi (April 2-3, 2019).

Cizdziel J., Scircle A. "Microplastics in the Mississippi River System", Mississippi Water Resources Conference, Jackson, Mississippi (April 2-3, 2019).

Cizdziel J., Scircle A. "Microplastics in the Mississippi River Basin", Society for Environmental Toxicology and Chemistry (SETAC) North America 39th Annual Meeting, Sacramento, CA, 4-8 November 2018.

Scircle A. Cizdziel J. "One Pot Method for Collection and Preparation of Water and Sediment Samples for Detection and Characterization of Microplastics", Society for Environmental Toxicology and Chemistry (SETAC) North America Annual Meeting, Toronto, Canada, Nov. 2019.

Amy Lusher James Cizdziel Miguel Oliveira



Track 3 - Environmental Chemistry and Exposure Assessment: Analysis, Monitoring, Fate and Modelling

3.09 - Micro(Nano)plastic Pollution: Tackling the Plastic Problem by Identifying Sources, Investigating Fate and Novel Approaches

Co-chairs: Miguel Oliveira, James Cizdziel, Amy L. Lusher, Jane Muncke

Monday May 27, 2019 | 8:30–15:30 & Tuesday May 28, 2019 | 8:30–10:05 | Session Room 101 AB

Small plastic particles (SPP) are ubiquitous in the environment having been found in aquatic systems, soil and air. In the environment plastics may suffer multiple degradation processes and form increasingly smaller-sized particles, such as microplastics and nanoplastics. In addition, an increasing number of consumer products (e.g. food, drink, personal care products) have also been reported to contain these particles, increasing the probability of human exposure. Although a considerable amount of research is being performed to understand the risks associated with SPP exposure, the available studies with particles in the low micro and nanometer range are scarce, due to technical challenges (e.g. isolation, quantification and characterization). Considering that plastic particles are made of many different polymers, sizes, shapes, and chemical composition, all with different physico-chemical properties, an adequate characterization is essential for proper evaluation of sources, effects, mechanisms of action and long-term consequences. A decrease in particle size increases its bioavailability and potential threat to soil and aquatic organisms, but also presents challenges for their characterization. This session aims to bring together researchers of different backgrounds (e.g. toxicologists, analytical chemists) working on different matrices to review and discuss the current understanding of the risks posed by plastics, alone and combined with other environmental contaminants, to address new and improved methods for accurate sampling, detection, and characterization of SPP, to highlight key knowledge gaps and propose approaches to the problem of plastics

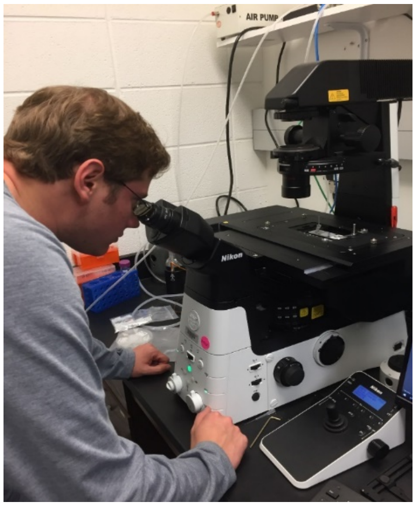
Meeting with USGS Colleagues

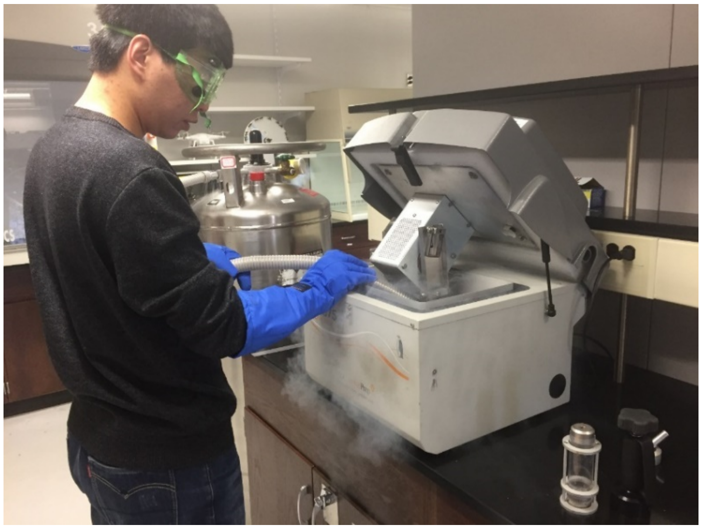
We hosted scientists from the USGS Washington Water Science Center's Microplastics Laboratory who came to learn about our new field and analytical methods. Our discussions, including field methods, quality assurance methods, and confirmatory methods like µFTIR and Raman, were extremely valuable and will help in the development of a future workshop on MPs.

Student Involvement/Education

This project contributed toward a Ph.D. in analytical chemistry and provided a number of research opportunities for undergraduate students. Below is a list of completed student projects.

- "Microplastic pollution in the Mississippi River and the Mississippi Sound Estuary: concentrations, sizes, types, loadings, and method development" (A. Scircle, Ph.D. 12/2020)
- "Recovery tests for a novel one-pot method for analysis of microplastics from natural waters" (Libby Li, undergraduate thesis, 5/2019)
- "Quantitative analysis and characterization of microplastics in the Mississippi River" (H. Horton, undergraduate thesis, 5/2019)
- "Preparation and characterization of virgin and weathered microplastics using cyromilling and scanning electron microscopy" (D. Behrend, undergraduate thesis, 12/2019)





Photos:
Austin Scircle using the fluorescence microscope (top left).

Danielle Behrend sieving cryomilled microplastics (top middle).

Libby Li examining microplastics for recovery experiments (top right).

Zhiqiang Gao adding liquid nitrogen to cryomill for preparing microplastics (bottom).

Outreach

Dr. Cizdziel and graduate student Austin Scircle at Lafayette Middle School discussing microplastic pollution to a student assembly.

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Disclaimer

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