

## Video Article

# Sampling, Sorting, and Characterizing Microplastics in Aquatic Environments with High Suspended Sediment Loads and Large Floating Debris

Katherine M. Martin<sup>1</sup>, Elizabeth A. Hasenmueller<sup>2</sup>, John R. White<sup>3</sup>, Lisa G. Chambers<sup>4</sup>, Jeremy L. Conkle<sup>1</sup><sup>1</sup>Department of Physical and Environmental Sciences, Texas A&M University-Corpus Christi<sup>2</sup>Department of Earth and Atmospheric Sciences, Saint Louis University<sup>3</sup>Department of Oceanography and Coastal Sciences, Louisiana State University<sup>4</sup>Department of Biology, University of Central FloridaCorrespondence to: Jeremy L. Conkle at [jeremy.conkle@tamucc.edu](mailto:jeremy.conkle@tamucc.edu)URL: <https://www.jove.com/video/57969>DOI: [doi:10.3791/57969](https://doi.org/10.3791/57969)

Keywords: Environmental Sciences, Issue 137, Microplastics, microfibers, marine debris, river sampling, freshwater sampling, water filtration

Date Published: 7/28/2018

Citation: Martin, K.M., Hasenmueller, E.A., White, J.R., Chambers, L.G., Conkle, J.L. Sampling, Sorting, and Characterizing Microplastics in Aquatic Environments with High Suspended Sediment Loads and Large Floating Debris. *J. Vis. Exp.* (137), e57969, doi:10.3791/57969 (2018).

## Abstract

The ubiquitous presence of plastic debris in the ocean is widely recognized by the public, scientific communities, and government agencies. However, only recently have microplastics in freshwater systems, such as rivers and lakes, been quantified. Microplastic sampling at the surface usually consists of deploying drift nets behind either a stationary or moving boat, which limits the sampling to environments with low levels of suspended sediments and floating or submerged debris. Previous studies that employed drift nets to collect microplastic debris typically used nets with  $\geq 300 \mu\text{m}$  mesh size, allowing plastic debris (particles and fibers) below this size to pass through the net and elude quantification. The protocol detailed here enables: 1) sample collection in environments with high suspended loads and floating or submerged debris and 2) the capture and quantification of microplastic particles and fibers  $< 300 \mu\text{m}$ . Water samples were collected using a peristaltic pump in low-density polyethylene (PE) containers to be stored before filtering and analysis in the lab. Filtration was done with a custom-made microplastic filtration device containing detachable union joints that housed nylon mesh sieves and mixed cellulose ester membrane filters. Mesh sieves and membrane filters were examined with a stereomicroscope to quantify and separate microplastic particulates and fibers. These materials were then examined using a micro-attenuated total reflectance Fourier transform infrared spectrometer (micro ATR-FTIR) to determine microplastic polymer type. Recovery was measured by spiking samples using blue PE particulates and green nylon fibers; percent recovery was determined to be 100% for particulates and 92% for fibers. This protocol will guide similar studies on microplastics in high velocity rivers with high concentrations of sediment. With simple modifications to the peristaltic pump and filtration device, users can collect and analyze various sample volumes and particulate sizes.

## Video Link

The video component of this article can be found at <https://www.jove.com/video/57969/>

## Introduction

Plastic was first observed in the ocean as early as the 1930s<sup>1</sup>. Recent estimates of marine plastic debris range from over 243,000 metric tons (MT) of plastic on the ocean's surface to 4.8-12.7 million MT of plastic entering the ocean from terrestrial sources annually<sup>2,3</sup>. Early studies on marine plastic debris focused on macroplastics ( $> 5 \text{ mm}$  diameter) as they are easily visible and quantifiable. However, it was recently discovered that macroplastics represent  $< 10\%$  of plastic debris, by count, in the ocean, indicating that the overwhelming majority of plastic debris is microplastic ( $< 5 \text{ mm}$  diameter)<sup>2</sup>.

Microplastics are categorized into two groups: primary and secondary microplastics. Primary microplastics consist of plastics that are manufactured at a diameter  $< 5 \text{ mm}$  and include nurdles, the raw pellets used to make consumer products, microbeads used as exfoliants in personal care products (e.g., facial wash, body scrub, toothpaste), and abrasives or lubricants in industry. Secondary microplastics are created within the environment as larger plastic debris is fragmented by photolysis, abrasion, and microbial decomposition<sup>4,5</sup>. Synthetic fibers are also secondary microplastics and are a growing concern. A single garment can release  $> 1,900$  fibers per wash in a domestic washing machine<sup>6</sup>. These microfibers, as well as microbeads from personal care products, are washed down drains and into the sewer system before entering wastewater treatment plants. Murphy (2016) found that a wastewater treatment plant serving a population of 650,000 reduced the microplastic concentration by 98.4% from influent to effluent, yet 65 million microplastics remained in effluent and sludge each day<sup>7</sup>. Even with high percentages of microplastics being removed during the treatment processes, millions, possibly billions, of microplastics pass through wastewater treatment plants daily and enter surface waters in effluent<sup>6,8,9,10,11</sup>.

Due to their environmental release, microplastics have been found in the digestive and respiratory tissues of marine organisms across all trophic levels<sup>12,13,14,15</sup>. Their impact after uptake is variable, with some studies not observing harm, while others demonstrate numerous effects such as physical and chemical tissue damage<sup>4,6,14,15</sup>. Due to these discoveries, interest in this field has increased over the past five decades.

However, only recently have studies begun to quantify plastic debris, particularly microplastics, in freshwater systems, such as rivers and lakes, or assess the effect on organisms dwelling in these habitats<sup>12,16,17,18</sup>. Rivers are a major source of plastic debris found in the ocean as they receive wastewater effluent and surface water runoff that contain microplastics and macroplastics.

The protocol detailed here can be used to collect microplastic samples where drift nets are not feasible; specifically, in aquatic environments with high concentrations of suspended sediments and large floating debris like the Mississippi River. The Mississippi River watershed is one of the world's largest and has a population of >90 million people, likely making it one of the largest sources of plastic debris to the ocean<sup>19,20</sup>. Each year, the Mississippi River discharges an average of 735 km<sup>3</sup> of freshwater into the Gulf of Mexico, along with high concentrations of suspended sediments (~60 to >800 mg/L) and large debris<sup>13,21</sup>. Water samples were collected at two depths (*i.e.*, surface and 0.6-depth) at various locations along the Mississippi River and its tributaries in translucent 1 L low-density polyethylene (PE) containers using a peristaltic pump. In the lab, samples were filtered using nylon mesh sieves and mixed cellulose ester membrane filters simultaneously with a custom-made 63.5 mm (2.5 in) polyvinyl chloride (PVC) cylinder with union joints to insert the sieves and filters<sup>22</sup>. The inclusion of PVC unions in the filtration device allows for filtration by as many or as few particle size classes as desired. Additionally, it can be used to capture microplastic debris down to sub-micron sizes using membrane filters when studying synthetic fibers. Once filtered, samples were dried and suspected plastics were identified and sorted from the mesh sieves and membrane filters under a stereomicroscope. Suspected plastics were then examined using micro-attenuated total reflectance Fourier transform infrared spectroscopy (micro ATR-FTIR) to eliminate non-synthetic materials or determine polymer type. Considering the size of microplastic particulates and fibers, contamination is commonplace. Sources of contamination include atmospheric deposition, clothing, field and lab equipment, as well as deionized (DI) water sources. Multiple steps are included throughout the protocol to reduce contamination from various sources while conducting all stages of the study.

## Protocol

### 1. Water Sample Collection

1. Collect water samples and water quality data of interest by boat where the river is well-mixed, ideally at locations where river stage or discharge is known (*e.g.*, United States Geological Survey (USGS) gauging stations).<sup>20</sup> To assure that the water is well-mixed, guide the boat using a handheld meter immersed in the river to where conductivity stays relatively constant.
2. At the sampling sites, record location coordinates and depth. To find the 0.6-depth, simply multiply the total depth by 0.6. Measure water quality parameters of interest (*e.g.*, turbidity, temperature, conductivity, pH, and dissolved oxygen (DO)) using a handheld meter. To measure the parameters, pump sample water from the desired depth into a wide-mouth container using the peristaltic pump and immediately take the measurements (step 1.5).
3. Use a peristaltic pump with tubing to obtain samples from the surface and 0.6-depth. Attach the correct tubing length to the pump for the given depth.
  1. Due to the strong currents in river systems, attach a 6.4 mm welded chain to the pump tubing using zip ties to help weight the tubing. At the end of the chain, place a weight or cement block to further weight the chain and tubing assembly.  
**Caution:** Do not attach the weight or cement block directly to the pump tubing.
4. Place the effluent end of the tubing over the boat's edge, away from clothing that could shed fibers. Slowly lower the influent end of the tubing to the desired depth (*i.e.*, the surface or 0.6-depth). Then, run the pump in reverse to purge the tubing with air for at least 30 s. After air purging, reverse the pump direction and rinse the tubing with sample water from the desired depth while allowing the water to drain off the boat or into a waste container. Stop the pump after the tubing has been rinsed for at least 30 s.
5. Rinse the container used for water quality measurements three times with sample water, dumping the rinse water each time. Once rinsed, fill the container with sample water and measure the water quality parameters of interest using a handheld meter (step 1.2).
6. Collect a microplastic subsample by placing the tubing effluent into a labeled, 1 L container that has been pre-rinsed with at least 250 mL of DI water three times. Then, rinse the container three additional times with the sample water, discarding the rinse water each time. Once the microplastic container is rinsed, fill it with the sample.
7. Using the same peristaltic pump method outlined in step 1.6, collect a subsample for total suspended solids (TSS) in a labeled, 250 mL bottle that has been pre-rinsed with at least 100 mL of DI water three times. Rinse the bottle three more times with sample water, discarding the rinse water each time. Once the TSS container is rinsed, fill it with the sample.
8. Collect field triplicates and blanks at least once per day in the field, in the same manner described in steps 1.6-1.7, for quality assurance/quality control (QA/QC) purposes. To collect a blank, bring two 1 L containers of DI water to the field. After purging the pump tubing with air, open the first container of DI water and rinse the pump tubing using the method described in step 1.4. Once the tubing is rinsed, open the second container of DI water and pump it into an empty 1 L container and a 250 mL bottle for microplastic and TSS blanks, respectively.
9. Store the microplastic and TSS subsamples on ice until returning to the lab, where they will be stored at -20°C until they are processed.  
**Caution:** Make sure to leave some head space in the sample containers so that they are not damaged due to ice expansion when freezing.  
**Note:** The protocol can be paused here.

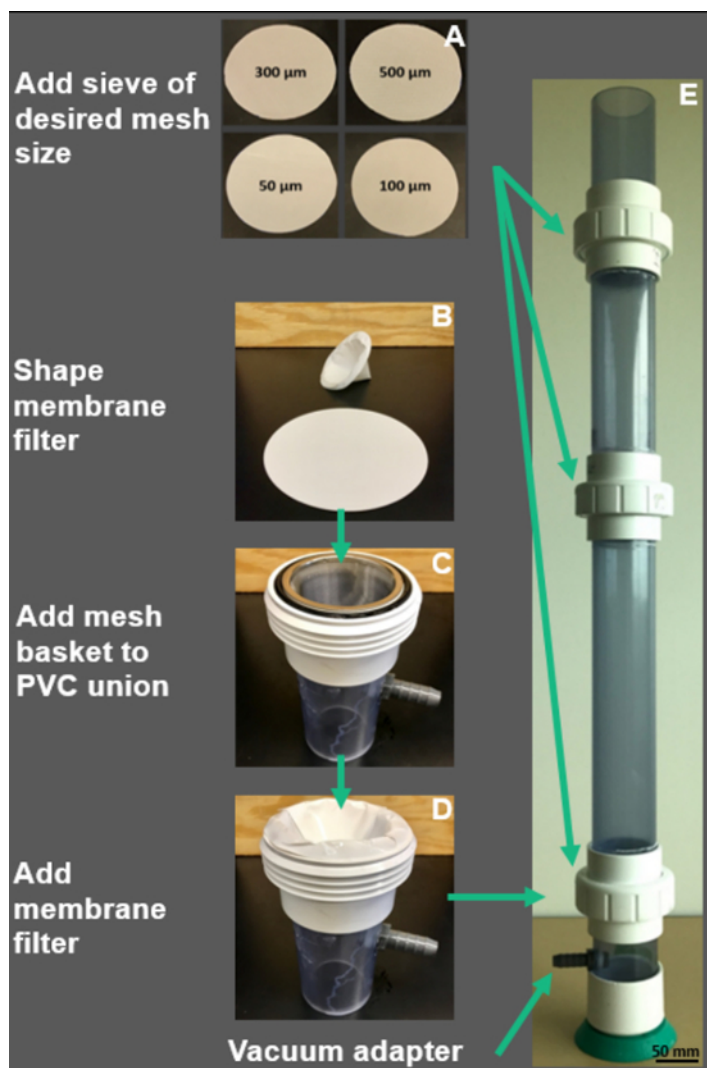
### 2. TSS Determination

1. Use United States Environmental Protection Agency (USEPA) method 160.2 to determine TSS with the 250 mL subsamples collected in the field<sup>23</sup>. Compare the calculated TSS values with the total plastics found.

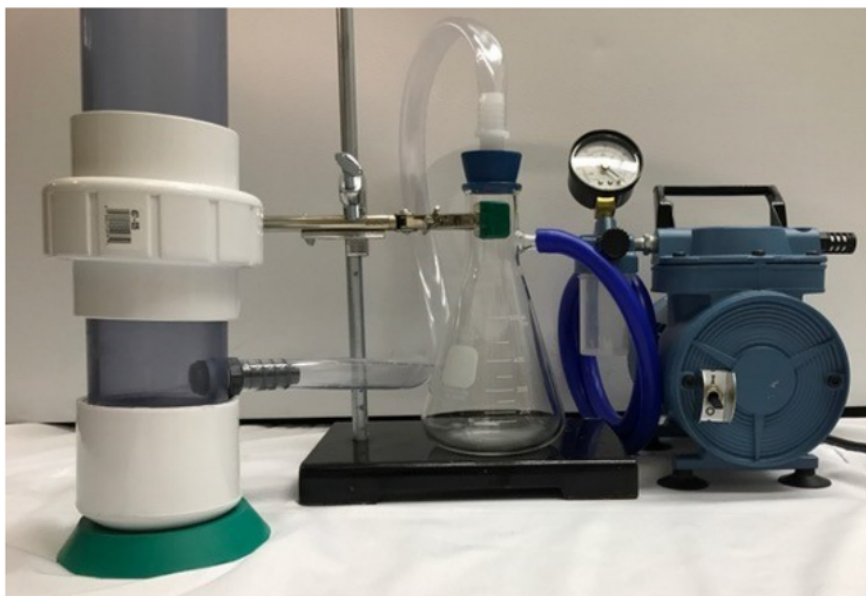
### 3. Microplastic Filtration Device Assembly

1. Thoroughly rinse the filtration device and nylon mesh sieves (**Figure 1**) three times with at least 250 mL of DI water. Place mesh sieves of desired pore sizes (*e.g.*, 50 µm, 100 µm, 300 µm, 500 µm) into each union joint with pore size decreasing from the top to the bottom of the filtration device (**Figure 1A**). Seal each union joint tightly to prevent leaking.

2. Fold the mixed cellulose ester membrane filter(s) (142 mm diameter) of desired pore size(s) (e.g., 0.45  $\mu\text{m}$ ) into a cone shape and place it into the filtration device:
  - Note:** Folding the membrane filter will provide more surface area to prevent clogging of the filter.
  - 1. Wet the membrane filter with DI water. While damp, fold the membrane filter into a cone shape with a diameter that fits into the filtration device. Also, fold a small lip along the edge of the cone so that it fits over the top of the union joint (**Figure 1B**).  
**Caution:** The membrane filter must be wet before folding to prevent tearing.
  - 2. Place the stainless steel mesh basket into the last union joint (**Figure 1C**). Carefully place the cone-shaped membrane filter into the basket (**Figure 1D**). Fold the lip of the membrane filter over the edge of the union joint.  
**Note:** The mesh basket will support the filter and reduce breakage once a vacuum has been applied.
3. Place a mesh sieve with the smallest desired pore size (e.g., 50  $\mu\text{m}$ ) on top of the membrane filter in the last union joint seen in **Figure 1**.  
**Note:** This will provide extra support to hold the membrane filter in place during filtration.
4. Once all union joints are sealed tightly, attach the hose from the top of the filtering flask to the base of the filtration device. Then attach the hose from the side of the filtering flask to the vacuum pump as illustrated in **Figure 2**.



**Figure 1: Assembly of the filtration device.** (A) The filtration device is assembled by placing mesh sieves of desired pore size into the upper union joints. (B) The mixed cellulose ester membrane filter(s) must be folded into a cone-shaped to fit the diameter of the filtration device; the cone should include a small lip to fit over the edge of the union joint to secure the filter in place. (C) A mesh basket is placed into the union to add stability to the membrane filter. (D) The folded membrane filter is added to the mesh basket and the smallest mesh sieve size is placed over the top of the membrane filter. (E) The fully assembled filtration device. [Please click here to view a larger version of this figure.](#)



**Figure 2: Assembly of the filtering flask and pump.** A filtering flask is attached to the filtration device vacuum adapter using a clear vinyl tubing. The filtering flask is then attached to the vacuum pump. [Please click here to view a larger version of this figure.](#)

## 4. Sample Filtration

1. Collect equipment blanks prior to filtration each time the device is assembled. Thoroughly rinse the device three times with at least 250 mL of DI water before the blank is collected. These blanks are collected using the steps outlined in steps 4.2 - 4.4.
2. Turn on the vacuum pump. Ensure that the pressure of the vacuum pump **does not exceed 127 mm Hg**, or the membrane filter could tear. **Caution:** Depending on the flow rate of sample filtration, pressure could increase inside the filtration device if sediment clogs the mesh sieves or membrane filters. This could potentially lead to a rupture in the membrane filter before reaching a reading of 127 mm Hg. For this reason, watch the pressure closely as it may need to be adjusted below 127 mm Hg on a sample by sample basis.
3. Use a 500 mL graduated cylinder, triple rinsed with at least 250 mL of DI water, to measure the total volume of the sample. Record the volume and transfer the sample from the graduated cylinder to the filtration device. **Caution:** Depending on the size of the water sample and the filtering flask, the filtering flask may need to be emptied multiple times during sample filtration.
  1. To empty the filtering flask, turn off the pump and detach the two hoses from the flask. Empty the flask into a separate waste container. **Caution:** Keep the filtered sample water until the entire sample has been filtered and it is confirmed that the membrane filter is intact.
  2. To continue the filtration cycle, reattach the hoses to the filtering flask, as outlined in step 3.4, and turn on the pump.
4. Once the entire sample has been filtered, rinse the sample container and graduated cylinder three times with at least 250 mL of DI water. After each rinse, filter the water used to rinse the container and graduated cylinder to ensure all particulates have been filtered.

## 5. Microplastic Filtration Device Disassembly

1. Rinse the walls of the filtration device three times with at least 250 mL of DI water to ensure that all particulates have been filtered and none remain on the filtration device.
2. Turn off the vacuum pump, then carefully unscrew and detach the first union. Turn the pump back on and use a DI water wash bottle to rinse the edges of the union joint. Wash particulates at the edges of the mesh sieve into the center to ensure that they are all collected.
3. Turn the pump off and remove the mesh sieve carefully with clean forceps, making sure not to touch the particulates on the surface of the mesh sieve. Place the mesh sieve into a covered Petri dish and dry it at 60 °C for 24 h. Once dry, samples can be stored until analysis can begin.
4. Repeat steps 5.1 - 5.3 for each union joint housing a mesh sieve.
5. For the last union joint that houses a mesh sieve and membrane filter, repeat steps 5.1-5.3 for the mesh sieve. **Caution:** Be careful when rinsing the mesh sieve, as sample can be lost if rinsed under the membrane filter.
6. Turn the vacuum pump on and rinse the edges of the membrane filter using a DI water wash bottle. Wash particulates at the edges of the membrane filter into the center to ensure the full sample is filtered. Before removing the membrane filter, ensure that all water has passed through it and that no water is pooling on its surface. **Caution:** Again, be careful when rinsing the membrane filter as sample can be lost if rinsed under it.
7. Carefully remove and unfold the membrane filter with the forceps. Place the membrane filter into a Petri dish or foil envelope appropriate for its diameter. **Note:** The membrane filter must be damp while being handled to prevent tearing.
8. Dry the covered membrane filter in the oven at 60 °C for 24 h. Once dry, store samples until analysis can begin. **Note:** The protocol can be paused here.

## 6. Particulate Analysis

1. Leave the mesh sieve or membrane filter in the Petri dish and remove only the lid to begin examining the sample for microplastics. This will ensure that if any particulates fall off the mesh sieve or membrane filter they will remain in the Petri dish, which can be analyzed after all particulates are removed from the mesh sieve or membrane filter.
2. Examine the mesh sieve or membrane filter under a stereomicroscope (14 - 90X magnification) to identify suspected plastic particulates and fibers. Use the following criteria when identifying suspected plastics: no cellular structure, fibers are equal thickness throughout, and particles are not shiny<sup>24</sup>.
3. Remove all suspected plastics from the mesh sieve or membrane filter and place them into a collection vial containing 70% ethanol. Record the color and shape (e.g., particulate, fiber, film, etc.) of each suspected plastic.
4. Once all suspected plastics are removed from the mesh sieve or membrane filter and quantified, examine both the lid and bottom of the Petri dish following steps 6.2 - 6.3.
5. After the mesh sieve or membrane filter and Petri dish have been examined and all suspected plastics removed and quantified, place the particulates or fibers from the collection vial onto a 12-slot aluminum coated slide for analysis using a micro ATR-FTIR.

**Note:** It is not always feasible to test every suspected plastic on the micro ATR-FTIR. Therefore, "strategically choose" the amount that will address the goals of the study and anomalies in the suspected plastics (e.g., a high number of similar fibers or particles)<sup>25</sup>. In a general sense, test as many suspected plastics as possible, but no less than 20%.

1. Once suspected plastics are analyzed using micro ATR-FTIR, use spectral databases to determine if a given sample is plastic and, if so, determine the plastic's polymer type.

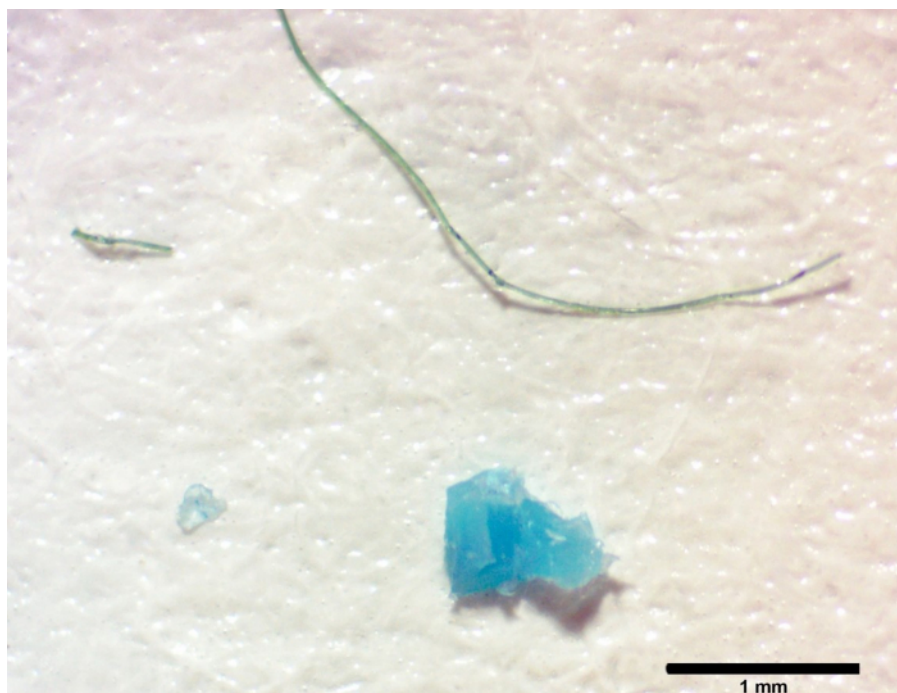
### Representative Results

To validate the recovery rates of this protocol, three samples ( $V_1$ - $V_3$ ) from Oso Bay, Corpus Christi, Texas (adjacent to the Texas A&M University Corpus Christi Campus), were spiked with 10 blue PE particulates (ranging from 50-100  $\mu\text{m}$  in diameter) and 50 green nylon fibers of various lengths (**Figure 3**). Sample TSS was calculated (Section 2) and then the samples were filtered using the methods outlined in Sections 3-5. The blue PE particulates and green nylon fibers were then separated and quantified (**Table 1**). Other fibers and particulates were observed on the mesh sieves and membrane filters, likely derived from the Oso Bay water sample. On average, 100% of the PE particulates and 92% of the nylon fibers were recovered. A loss of fibers may be due to a small amount of sample loss during filtration or incorrect identification.

An equipment blank was collected from the filtration device by filtering 1000 mL of DI water. This blank was analyzed using 100  $\mu\text{m}$  and 50  $\mu\text{m}$  mesh sieves and a 0.45  $\mu\text{m}$  membrane filter. A total of 7 fibers (blue and clear) were found in the equipment blank. This contamination could have been from the filtration device, laboratory equipment, atmospheric deposition, or DI water. However, the fibers were not similar to the blue PE particulates and green nylon fibers used to spike the samples.

This protocol was created to process samples from the Mississippi River watershed, including the Mississippi River mainstem and the Missouri River. Preliminary analyses from the Mississippi River and Missouri River had an average TSS of 63 mg/L. While the TSS values of Oso Bay are typically below those observed in the Mississippi River watershed, sediment was intentionally disturbed prior to water collection to simulate higher suspended sediment concentrations that might be encountered in large river systems. The average TSS in the Oso Bay samples was 1,865 mg/L, which is ~30 times higher than the TSS calculated for the Mississippi River and Missouri River samples. The turbid Oso Bay samples suggest successful filtration for samples with a TSS of up to ~1,800 mg/L using the techniques outlined here.





**Figure 3: Particulates and fibers used for percent recovery validation.** Image of two blue PE particulates and two green nylon fibers in a range of sizes used to spike the validation samples from Oso Bay in Corpus Christi, Texas. [Please click here to view a larger version of this figure.](#)

Sample	TSS (g/L)	0.45 μm		50 μm		100 μm		Total		% Recovered	
		Fibers	Particulates	Fibers	Particulates	Fibers	Particulates	Fibers	Particulates	Fibers	Particulates
V1	4.663	1	0	18	0	31	10	50	10	100	100
V2		0	0	21	0	28	10	49	10	98	100
V3		0	0	27	0	14	10	41	10	82	100

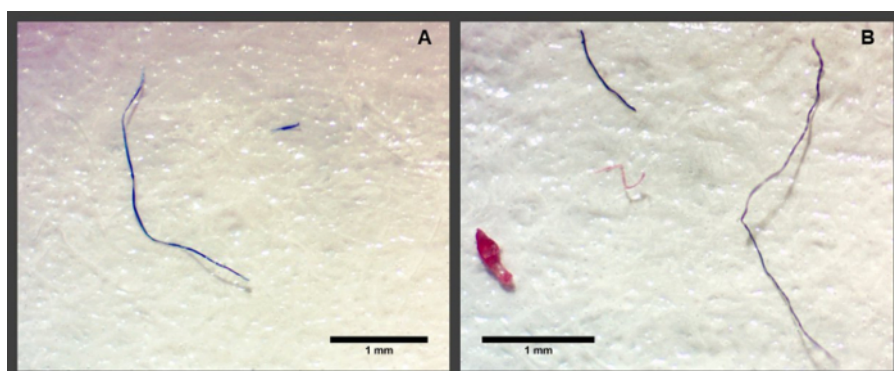
**Table 1: Results from validation samples.** A set number of blue PE particulates and green nylon fibers were added to samples taken from Oso Bay in Corpus Christi, Texas, to validate the filtration device and analysis protocol. Three microplastic validation samples (V<sub>1</sub>-V<sub>3</sub>) and one TSS sample were taken at the same location at the bank of Oso Bay. The fibers and particulates were quantified for each pore size and a total was calculated for each validation sample. Using the known amount of fibers and particulates used to spike the samples and the total recovered from each sample, the percent recovery was calculated.

The protocol was also designed to sample rivers from two depths: the surface (the river depth with the highest velocity) and 0.6-depth (the river depth with approximately average velocity for the entire water column). Samples from the Mississippi River and Missouri River (**Table 2**) were collected and analyzed as described above. To examine the effect of depth on microplastic concentration, the first and second samples were taken at the same location (*i.e.*, the Mississippi River at Alton, Illinois) but at different depths. To examine the possible effect of sampling location on microplastic loading, the first and third samples were taken at the same depth but at different locations (*i.e.*, the Mississippi River at Alton, Illinois, and the Missouri River above Saint Louis, Missouri). Examples of the fibers and particulates found in the preliminary Mississippi River basin samples are shown in **Figure 4**.

Location	USGS Gauging Station	Depth	Turbidity	TSS	Fibers	Particles	Fibers	Particles	Fibers	Particles	Fibers	Particles	Total	Fiber/Particle Ratio
					0.45 mm		50 mm		100 mm		Total			
					m	NTU	g/L	#/L						
MS; Alton, IL	USGS 05587498	0	38.3	0.063	80	0	126	1	54	1	260	2	262	130
MS; Alton, IL	USGS 05587498	20.1	61.4	0.090	191	0	151	5	195	1	537	6	543	90
MO; Columbia Bottom, MO	USGS 06935965	0	30.8	0.036	122	4	57	0	37	0	216	4	220	54

MS = Mississippi River; MO = Missouri River

**Table 2: Mississippi River watershed sample collection and analysis data.** Preliminary samples were collected near USGS gauging stations at the Mississippi River and Missouri River. Depth (m), turbidity (NTU), and TSS (mg/L) were measured for each site. Samples were filtered and analyzed following this protocol. Fibers and particulates were quantified for 50 µm and 100 µm pore size mesh sieves as well as a 0.45 µm membrane filter. Due to a lack of materials collected on a 500 µm mesh sieve, this size is excluded from the results presented.



**Figure 4: Example particulates and fibers found in preliminary samples from the Mississippi River watershed.** Images of fibers and particulates quantified in a sample (Table 2) taken from the surface of the Mississippi River at Alton, Illinois. (A) Image of two blue fibers that range in size on a 0.45 µm membrane filter. (B) Image of a red particulate and various fibers found on a 50 µm mesh sieve, showing the range in color, size, and shape of the microplastics found in the Mississippi River watershed. [Please click here to view a larger version of this figure.](#)

## Discussion

Microplastic collection using drift nets is the conventional method in environments like the ocean where both sediment and plastic concentrations are low, thus requiring large sample volumes. However, drift nets are not always practical or safe in rivers with high sediment loads and large floating or submerged debris. Additionally, it is not feasible to use a drift net when attempting to thoroughly capture and quantify microplastic materials, particularly fibers, as most nets used for plastic surveys have mesh sizes  $\geq 300 \mu\text{m}$ . The protocol described in this paper allows for sampling in waterbodies containing high sediment loads while also permitting the capture of microplastics  $< 300 \mu\text{m}$  in diameter. The method and associated filtering device are versatile and can be adapted to specific project needs. Furthermore, data obtained with this protocol will help develop mitigation strategies to improve water quality and measure the effectiveness of these strategies, such as the recent microbead ban<sup>26</sup>.

This method enables control of sample collection depth, volume input, and separation of microplastics into size classes while accounting for multiple sources of contamination. Employing a peristaltic pump permits the user to collect samples at any desired depth by adjusting the length of the pump tubing. Users can easily control the sample volume with the use of the filtration device, while the detachable union fittings allow for adjustments in filter material and pore sizes to accommodate variable diameters and concentrations of plastic. We found that a 1 L sample size was ideal for quantifying microplastics in the Mississippi River watershed for several reasons. First, within 1 L of water, we found that there were several hundred suspected fibers and particles. Second, the high sediment masses in samples with volumes larger than 1 L slowed filtering substantially. Third, longer filtering times could potentially lead to greater lab contamination. The filtration device and the ability to easily adapt it to differing project needs facilitate the collection and analysis of microplastic debris at sub-micron sizes, which is particularly helpful when studying synthetic fibers.

The inclusion of union joints eases the removal of mesh sieves or membrane filters between filtration cycles but requires that joints be sealed firmly and carefully to ensure mesh sieves and membrane filters are seated properly, which prevents the loss of sample (Sections 3 and 5). To prevent tearing or cracking, the membrane filter needs to be damp before handling it, but dry before microscope analysis. Rupturing can occur in the membrane filter before the pump pressure reaches 127 mm Hg (steps 4.2), especially in samples with high sediment volume. Therefore, the pressure must be watched carefully and adjust as needed.

Though the protocol for using the filtration device alleviates problems associated with deploying drift nets such as clogging of the net with suspended sediments, it increases sample processing in the lab, which increases the chances for contamination. To reduce or eliminate potential contamination from sample handling, all equipment must be thoroughly rinsed with sufficient quantities of DI water three times and blanks must be taken from each device (e.g., peristaltic pump, filtration device, collection container) throughout sample collection, processing, and analysis.

Each environment and equipment blank will then be filtered and analyzed using the protocol outlined in Sections 4-6. The use of an ultra-pure water filtration system could reduce potential contamination from DI water used for rinsing and blanks.

In the lab, at least 20% of the samples should be analyzed by two individuals to ensure consistent plastic identification. During filtration and analysis in the lab, open Petri dishes can serve as lab blanks and be placed in designated areas for the duration of the analysis period. Each lab blank will then be analyzed using the protocol in Section 6. To prevent contamination from atmospheric deposition, cover all equipment with aluminum foil after washing with DI water.

The use of a peristaltic pump and custom-made microplastic filtration device in this protocol allows users to collect samples in environments containing high concentrations of suspended sediments. Additionally, this method allows users to capture and quantify microplastic debris <300 µm, specifically microfibrers. The percent recovery for this protocol was measured to be 100% and 92% for PE particulates and nylon fibers, respectively, showing relatively high recovery rates. Preliminary samples were taken in the Mississippi River watershed also using this protocol where 1 L samples averaged >200 microplastics ranging in size (0.45-500 µm), shape, and color. This protocol will guide similar studies on the fate, effects, and sources of microplastics.

## Disclosures

The authors have nothing to disclose.

## Acknowledgements

The project for which this protocol was established was funded by the National Oceanic and Atmospheric Administration (NOAA) Marine Debris Program (# NA16NO29990029). We thank Miles Corcoran at the National Great Rivers Research and Education Center (NGRREC) in Alton, Illinois, for help with site selection and boat operation. Field and lab work was completed with the help of Camille Buckley, Michael Abegg, Josiah Wray, and Rebecca Wagner.

## References

1. Fowler, C. W. Marine debris and northern fur seals: A case study. *Marine Pollution Bulletin*. **18** 326-335 (2015).
2. Eriksen, M. *et al.* Plastic pollution in the world's oceans: More than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. *PLoS One*. **9** (12), e111913 (2014).
3. Jambeck, J. R. *et al.* Marine pollution. Plastic waste inputs from land into the ocean. *Science*. **347** (6223), 768-771 (2015).
4. Andrady, A. L. Microplastics in the marine environment. *Marine Pollution Bulletin*. **62** (8), 1596-1605 (2011).
5. Cole, M., Lindeque, P., Halsband, C., & Galloway, T. S. Microplastics as contaminants in the marine environment: a review. *Marine Pollution Bulletin*. **62** (12), 2588-2597 (2011).
6. Browne, M. A. *et al.* Accumulation of microplastic on shorelines worldwide: Sources and sinks. *Environmental Science & Technology*. **45** (21), 9175-9179 (2011).
7. Murphy, F., Ewins, C., Carbonnier, F., & Quinn, B. Wastewater treatment works (WwTW) as a source of microplastics in the aquatic environment. *Environmental Science & Technology*. **50** (11), 5800-5808 (2016).
8. Zubris, K. A., & Richards, B. K. Synthetic fibers as an indicator of land application of sludge. *Environmental Pollution*. **138** (2), 201-211 (2005).
9. Fendall, L. S., & Sewell, M. A. Contributing to marine pollution by washing your face: Microplastics in facial cleansers. *Marine Pollution Bulletin*. **58** (8), 1225-1228 (2009).
10. Gregory, M. R. Plastic 'scrubbers' in hand cleansers: A further (and minor) source for marine pollution identified. *Marine Pollution Bulletin*. **32** (12), 867-871 (1996).
11. Bayo, J., Olmos, S., López-Castellanos, J., Alcolea, A. Microplastics and microfibrers in the sludge of a municipal wastewater treatment plant. *International Journal of Sustainable Development and Planning*. **11** 812-821 (2016).
12. McCormick, A., Hoellein, T. J., Mason, S. A., Schlupe, J., & Kelly, J. J. Microplastic is an abundant and distinct microbial habitat in an urban river. *Environmental Science & Technology*. **48** (20), 11863-11871 (2014).
13. Farrell, P., & Nelson, K. Trophic level transfer of microplastic: *Mytilus edulis* (L.) to *Carcinus maenas* (L.). *Environmental Pollution*. **177** 1-3 (2013).
14. Rochman, C. M. *et al.* Scientific evidence supports a ban on microbeads. *Environmental Science & Technology*. **49** (18), 10759-10761 (2015).
15. Taylor, M. L., Gwinnett, C., Robinson, L. F., & Woodall, L. C. Plastic microfibre ingestion by deep-sea organisms. *Scientific Reports*. **6** 33997 (2016).
16. Mani, T., Hauk, A., Walter, U., & Burkhardt-Holm, P. Microplastics profile along the Rhine River. *Scientific Reports*. **5** 17988 (2015).
17. Morritt, D., Stefanoudis, P. V., Pearce, D., Crimmen, O. A., & Clark, P. F. Plastic in the Thames: a river runs through it. *Marine Pollution Bulletin*. **78** (1-2), 196-200 (2014).
18. *National Park Servies*. <https://www.nps.gov/miss/riverfacts.htm> (2017).
19. *United States Census Bureau*. <https://www.census.gov/geo/maps-data/data/tiger-data.html> (2010).
20. United States Geological Survey (USGS). *National Water Information System: Web Interface*. <<https://waterdata.usgs.gov/nwis/rt>> (2016).
21. Grimes, C. B. Fishery Production and the Mississippi River. *Fisheries*. **28** (8), 17-26 (2001).
22. Talvitie, J. *et al.* Do wastewater treatment plants act as a potential point source of microplastics? Preliminary study in the coastal Gulf of Finland, Baltic Sea. *Water Science and Technology*. **72** (9), 1495-1504 (2015).
23. United States Environmental Protection Agency (USEPA). *Method 160.2: Residue, Non-filterable*. (Gravimetric, Dried at 103-105C). (1971).
24. Nor, N. H., & Obbard, J. P. Microplastics in Singapore's coastal mangrove ecosystems. *Marine Pollution Bulletin*. **79** (1-2), 278-283 (2014).
25. Woodall, L. C., Gwinnett, C., Packer, M., Thompson, R. C., Robinson, L. F., & Paterson, G. L. Using a forensic science approach to minimize environmental contamination and to identify microfibrers in marine sediments. *Marine Pollution Bulletin*. **95** (1), 40-46 (2015).



26. S. 1424 - 114th Congress. *Microbead-Free Waters Act of 2015*. <[www.congress.gov](http://www.congress.gov)> (2015).