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Microplastic sampling, sorting and characterization in aquatic environments with high suspended sediment loads and large floating debris

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Corresponding Author:	Elizabeth Hasenmueller Saint Louis University Saint Louis, Missouri UNITED STATES
Corresponding Author's Institution:	Saint Louis University
Corresponding Author E-Mail:	hasenmuellerea@slu.edu
First Author:	Katherine M Martin
Other Authors:	Katherine M Martin
	John R White
	Lisa G. Chambers
	Jeremy L Conkle
Author Comments:	Please find attached our revised research article title: Microplastic sampling, sorting and characterization in aquatic environments with high suspended sediment loads and large floating debris. Most microplastic sampling occurs at the surface of marine environments and consists of trawling. This may not be possible in high sediment load systems with large floating debris, particularly major rivers. Trawling also limits the size of plastic debris captured to the mesh diameter used (typically 300 µm). This manuscript addresses collection and analysis of samples from aquatic environments with high suspended solid loads and allows for the capture and quantification of microplastic particles and fibers <300 µm. We have done our best to address reviewer and editor comment and hope that our responses meet JoVE's high standard. We welcome any and all additional comments. Thank you again for the opportunity to submit to JoVE.
Additional Information:	
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Cover Letter

TEXAS A&M UNIVERSITY
CORPUS CHRISTI

DEPARTMENT OF PHYSICAL & ENVIRONMENTAL SCIENCES
COLLEGE OF SCIENCE & ENGINEERING
6300 OCEAN DR. UNIT 5802
CORPUS CHRISTI, TEXAS 78412-5802
O: 361.825.2681 F: 361.825.2135

April 16, 2018

TO: Editor of the Journal of Visualized Experiments

RE: Manuscript Re-submission

Dear JoVE Environmental Science Editorial Staff,

Please find attached our updated re-submission titled: **Microplastic sampling, sorting and characterization in aquatic environments with high suspended sediment loads and large floating debris**. Most microplastic sampling occurs at the surface of marine environments and consists of trawling. This may not be possible in high sediment load systems with large floating debris, particularly major rivers. Trawling also limits the size of plastic debris captured to the mesh diameter used (typically 300 µm). This manuscript addresses collection and analysis of samples from aquatic environments with high suspended solid loads and allows for the capture and quantification of microplastic particles and fibers <300 µm.

Thank you for allowing us to revise our manuscript before a final decision was made. We hope that we have made corrections that meet the high standards of JoVE. Please let us know if you require any additional information or edits prior to your final decision.

Sincerely,

Jeremy L. Conkle
Assistant Professor
Department of Physical & Environmental Sciences
Texas A&M University Corpus Christi
jeremy.conkle@tamucc.edu
361.825.2862

TITLE:

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

AUTHORS & AFFILIATIONS:

Katherine M. Martin[1], Elizabeth A. Hasenmueller[2], John R. White[3], Lisa G. Chambers[4], Jeremy L. Conkle[1]

[1]Department of Physical and Environmental Sciences, Texas A&M University-Corpus Christi
[2]Department of Earth and Atmospheric Sciences, Saint Louis University
[3]Department of Oceanography and Coastal Sciences, Louisiana State University
[4]Department of Biology, University of Central Florida

Corresponding Author:

Jeremy L. Conkle (Jeremy.Conkle@tamucc.edu)
Tel: (361) 825-2862

Email Addresses of Co-authors:

Katherine M. Martin (Kmartin16@islander.tamucc.edu)
Elizabeth A. Hasenmueller (elizabeth.hasenmueller@slu.edu)
John R. White (jrwhite@lsu.edu)
Lisa G. Chambers (Lisa.Chambers@ucf.edu)

KEYWORDS:

Microplastics, microfibers, marine debris, river sampling, freshwater sampling, water filtration

SUMMARY:

Most microplastic research to date has occurred in marine systems where suspended solid levels are relatively low. Focus is now shifting to freshwater systems, which may feature high sediment loads and floating debris. This protocol addresses collecting and analyzing microplastic samples from aquatic environments that contain high suspended solid loads.

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 ≥300 µm 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 µm. 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.

INTRODUCTION:

Plastic was first observed in the ocean as early as the 1930s[1]. 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[2,3]. Early studies on marine plastic debris focused on macroplastics (>5 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 mm diameter)[2].

Microplastics are categorized into two groups: primary and secondary microplastics. Primary microplastics consist of plastics that are manufactured at a diameter <5 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[4,5]. 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[6]. These microfibers, as well as microbeads from personal care products, are washed down drains and into the sewer system before entering wastewater treatments 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[7]. 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[6,8-11].

Due to their environmental release, microplastics have been found in the digestive and respiratory tissues of marine organisms across all trophic levels[12-15]. Their impact after uptake is variable, with some studies not observing harm, while others demonstrate numerous effects such as physical and chemical tissue damage[4,6,14,15]. 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[12,16-18]. 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[19,20]. Each year, the Mississippi River discharges an average of 735 km^3 of freshwater into the Gulf of Mexico, along with high concentrations of suspended sediments (~60 to >800 mg/L) and large debris[13,21]. 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[22]. 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

1.1. 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).[20] 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.

1.2. 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).

1.3. 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.

1.3.1. 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.

1.4. 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.

1.5. 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).

1.6. 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.

1.7. 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.

1.8. 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.

1.9. 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

2.1. Use United States Environmental Protection Agency (USEPA) method 160.2 to determine TSS with the 250 mL subsamples collected in the field[23]. Compare the calculated TSS values with the

total plastics found.

3. Microplastic Filtration Device Assembly

3.1. 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.

3.2. Fold the mixed cellulose ester membrane filter(s) (142 mm diameter) of desired pore size(s) (*e.g.,* 0.45 µm) 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.

3.2.1. 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.

3.2.2. Place the stainless steel mesh basket into the 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.

3.3. Place a mesh sieve with the smallest desired pore size (*e.g.,* 50 µm) 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.

3.4. 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**.

[Insert **Figure 1** here]

[Insert **Figure 2** here]

4. Sample Filtration

4.1. 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.

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.

4.3. 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.

4.3.1. 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.

4.3.2. To continue the filtration cycle, reattach the hoses to the filtering flask, as outlined in step 3.4, and turn on the pump.

4.4. 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

5.1. 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.

5.2. 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.

5.3. 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.

5.4. Repeat steps 5.1 - 5.3 for each union joint housing a mesh sieve.

5.5. 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.

5.6. 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.

5.7. 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.

5.8. 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

6.1. 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.

6.2. 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[24].

6.3. 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.

6.4. 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.

6.5. 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)[25]. In a general sense, test as many suspected plastics as possible, but no less than 20%.

6.5.1. 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 µm 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 µm and 50 µm mesh sieves and a 0.45 µm 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.

[Insert **Figure 3** here]

[Insert **Table 1** here]

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 were collected and analyzed as described above (**Table 2**). 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**.

[Insert **Table 2** here]

[Insert **Figure 4** here]

FIGURE AND TABLE LEGENDS:

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.

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.

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.

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.

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_1-V_3) 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.

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.

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 ≥300 µm. The protocol described in this paper allows for sampling in waterbodies containing high sediment loads while also permitting the capture of microplastics <300 µm 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[26].

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 and prevent 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 qualities 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 microfibers. 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.

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DISCLOSURES:

The authors have nothing to disclose.

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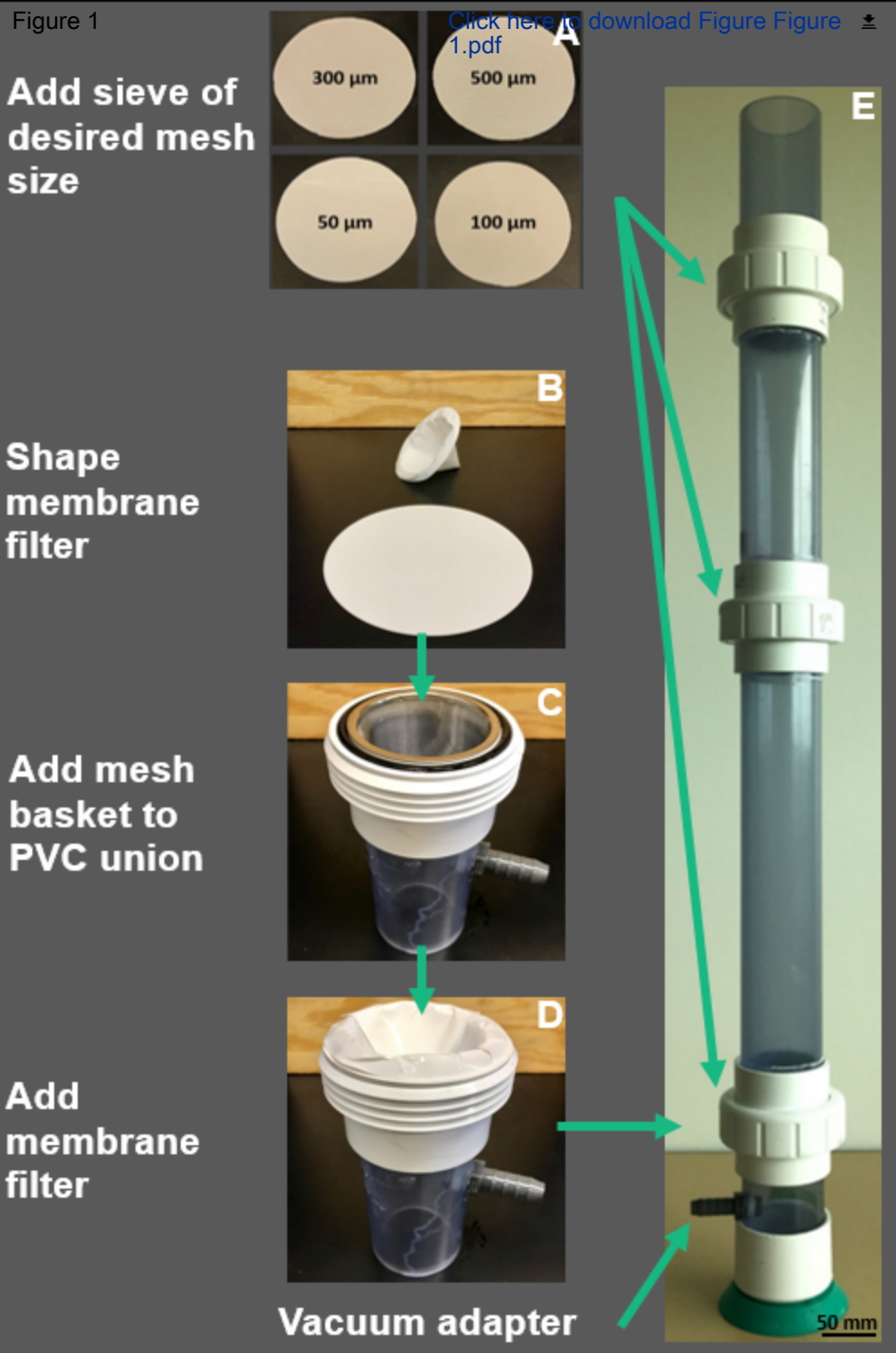

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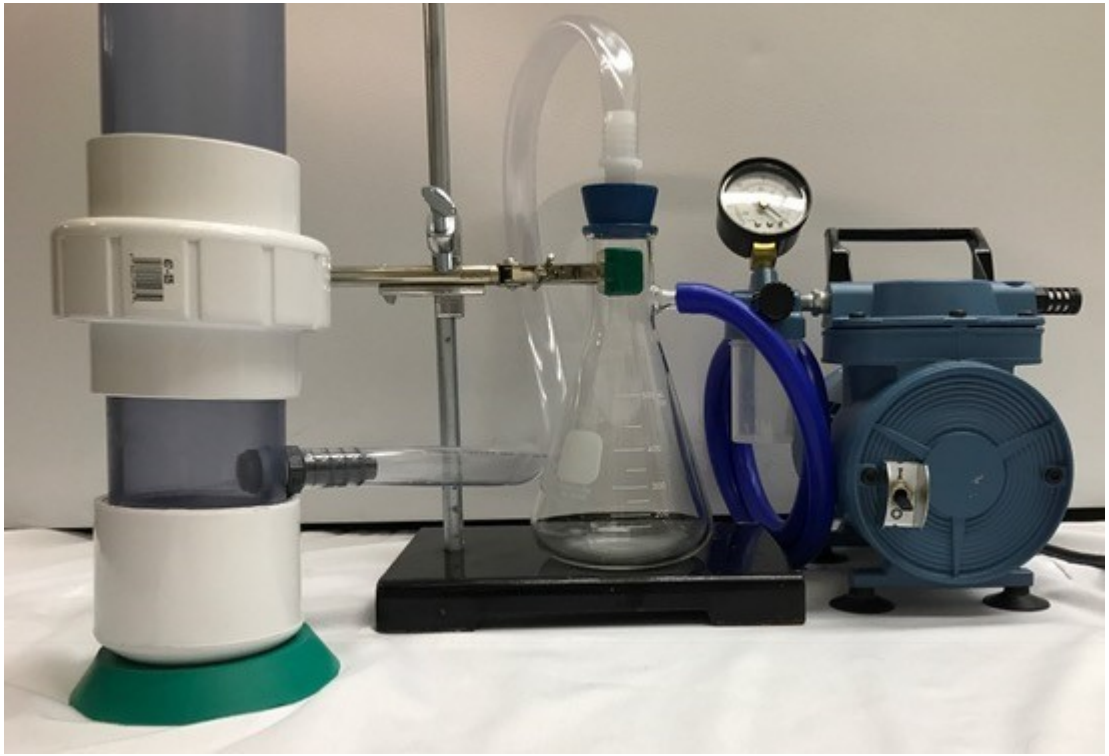

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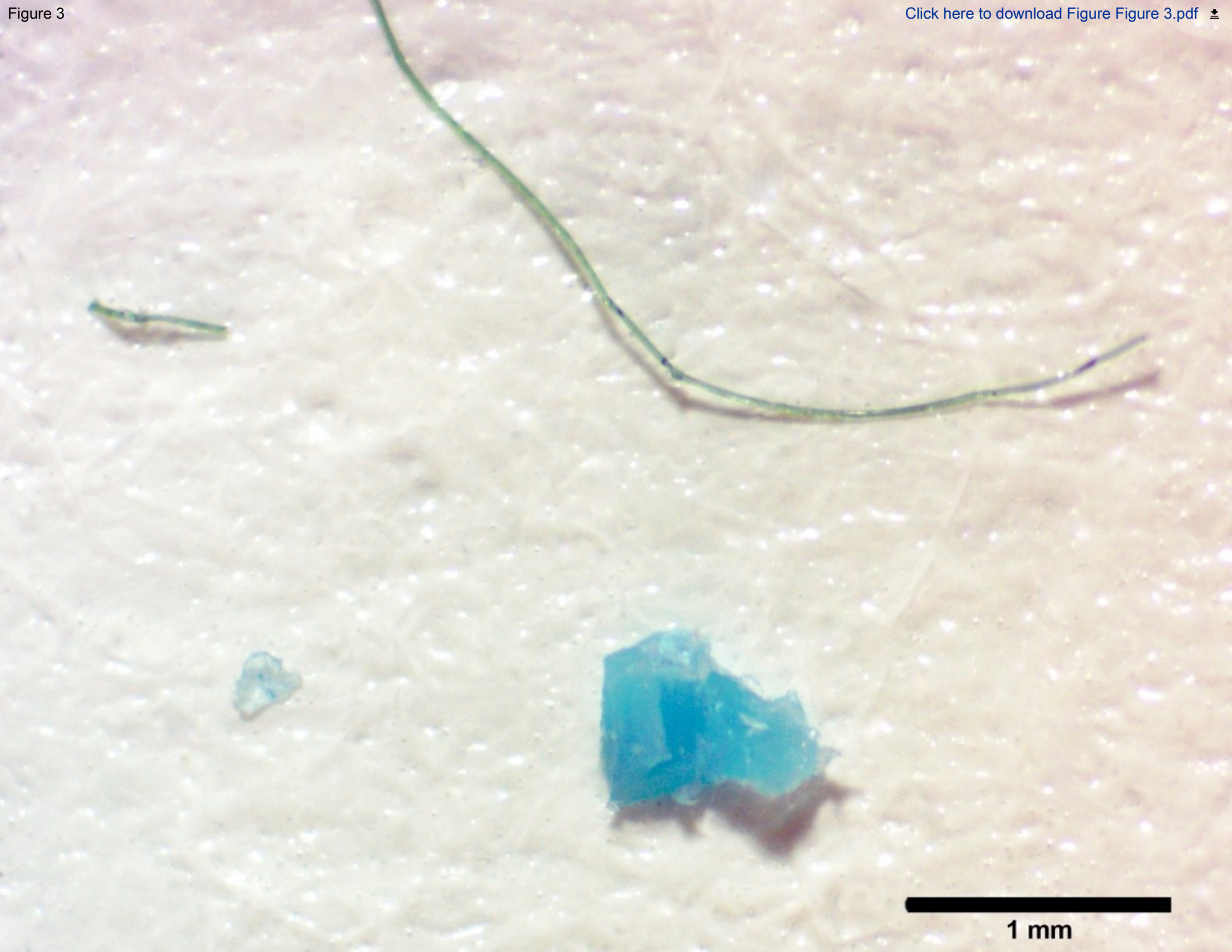

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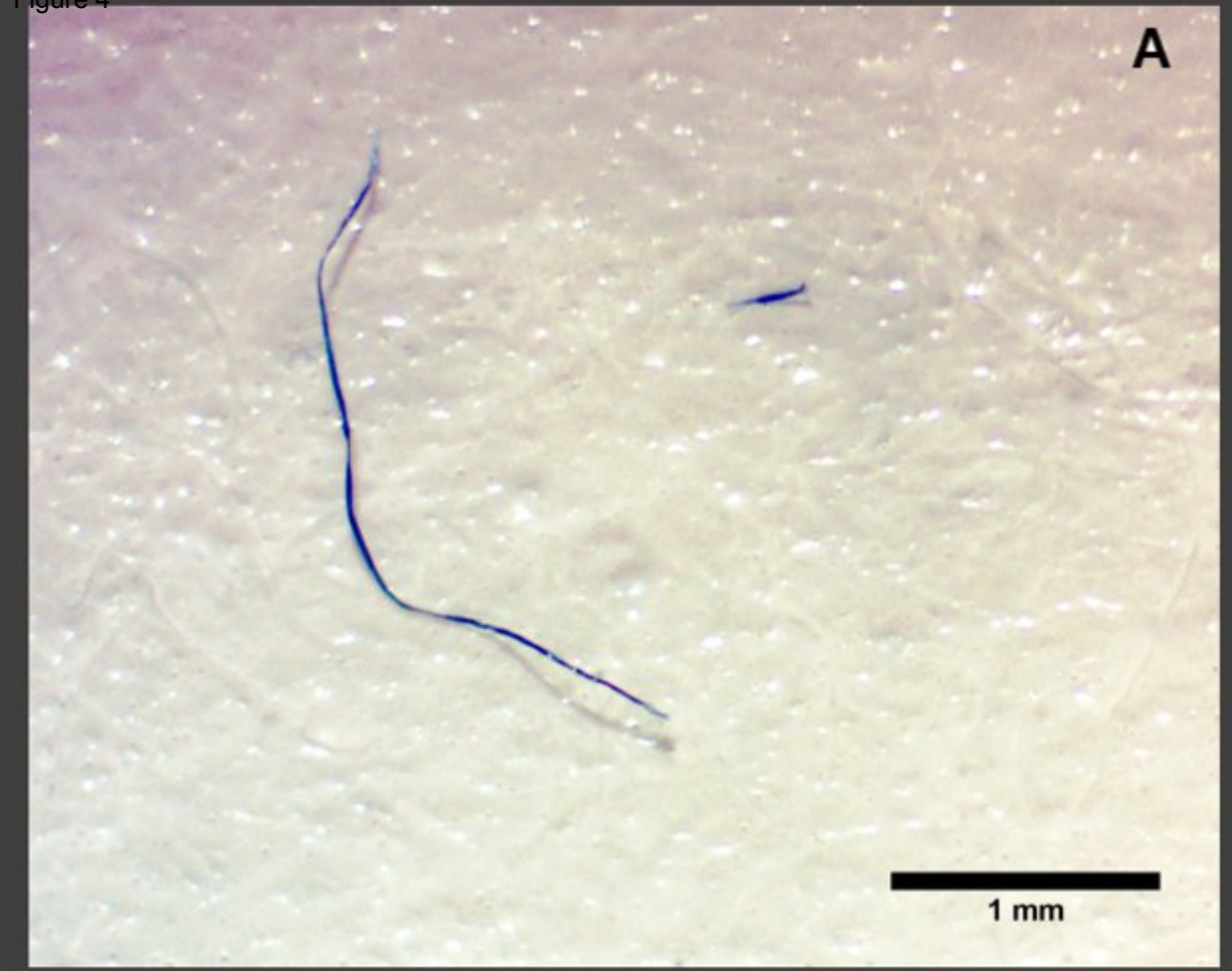

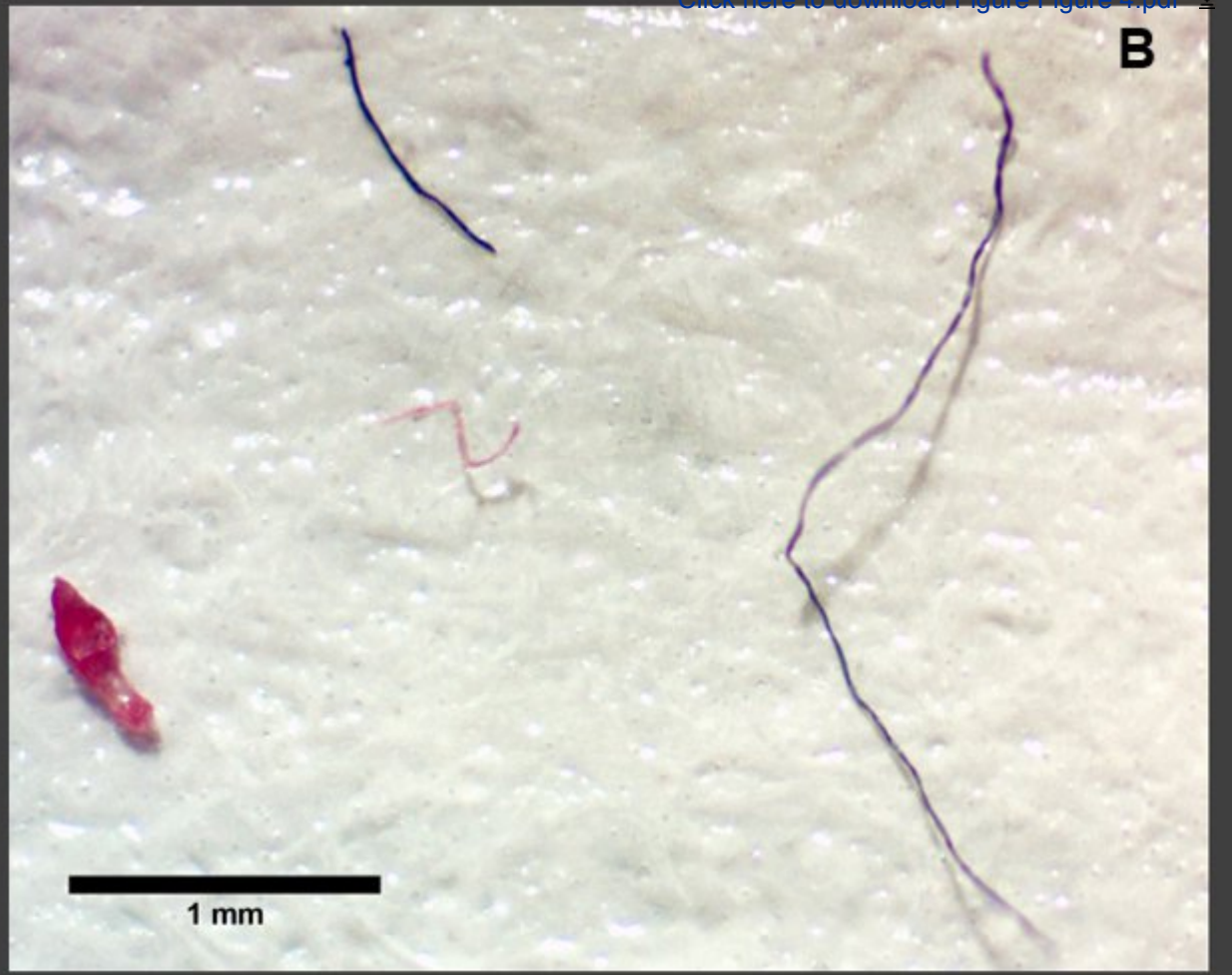

Table 1

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

ɔtal	% Recovered	
Particulates	Fibers	Particulates
10	100	100
10	98	100
10	82	100

Table 2

Location	USGS Gauging Station	Depth	Turbidity	TSS	Fibers 0.4
		m	NTU	g/L	
MS; Alton, IL	USGS 05587498	0	38.3	0.063	80
MS; Alton, IL	USGS 05587498	20.1	61.4	0.090	191
MO; Columbia Bottom, MO	USGS 06935965	0	30.8	0.036	122

MS = Mississippi River; MO = Missouri River

Particles	Fibers	Particles	Fibers	Particles	Fibers	Particles	Total	Fiber/ Particule Ratio
5 µm		50 µm		100 µm		Total		
			#/L					
0	126	1	54	1	260	2	262	130
0	151	5	195	1	537	6	543	90
4	57	0	37	0	216	4	220	54

Materials Table

Click here to download Materials Table 20180416 Materials.xlsx

Name of Material/ Equipment	Company	Catalog Number
1L Cubitainer Containers, Low-Density Polyethylene	VWR	89094-140
2-1/2" Clear Schedule 40 Rigid PVC Pipe	United States Plastic Corporation	34138
2-1/2" PVC SCH 40 Socket Union	Supply House	457-025
Nylon 6 Woven Mesh Sheet, Opaque Off-White, 12" Width, 12" Length, 500 microns Mesh Size, 38% Open Area (Pack of 5)	Small Parts via Amazon	CMN-0500-C/5PK-05
Nylon 6 Woven Mesh Sheet, Opaque White, 12" Width, 12" Length, 100 microns Mesh Size, 44% Open Area (Pack of 5)	Small Parts via Amazon	B0043D1TB4
Nylon 6 Woven Mesh Sheet, Opaque White, 12" Width, 12" Length, 50 microns Mesh Size, 37% Open Area (Pack of 5)	Small Parts via Amazon	B0043D1SGA
Mixed Cellulose Ester Membrane, 0.45um, 142mm, 25/pk	VWR	10034-914
Metal Mesh Basket Tea Leaves Strainer Teapot Filter 76mm Dia 3pcs	Uxcell via Amazon	a15071600ux0260
1/2" PVC Barbed Insert Male Adapter	Supply House	1436-005
1/2 in. O.D. x 3/8 in. I.D. x 10 ft. PVC Clear Vinyl Tube	Home Depot	702229

YSI Professional Plus Multiparameter Instrument with Quatro Cable	YSI	6050000
2100P Portable Turbidimeter	Hach	4650000
FEP-lined PE tubing	Geotech	87050529
Geopump Peristaltic Pump Series II	Geotech	91350123
MeiJi Techno EMZ-8TR Microscope	Microscope.com	EMZ8TR-PLS2
Nicolet iS10 FTIR Spectrometer	Thermo Electron North America	912A0607
Nicolet iN5 FTIR microscope	Thermo Electron North America	912A0895
Germanium (Ge) ATR	Thermo Electron North America	869-174400
Aluminum EZ-Spot Micro Mounts (Pkg of 5)	Thermo Electron North America	0042-545
Aluminum Coated Glass Sample Slides	Thermo Electron North America	0042-544

Comments/Description

Containers used to collect and store samples.

The PVC pipe used to make the device comes as an 2.43 m pipe. The pipe was then cut to the desired lengths for each section seperated by union joints. Section lengths were decided by predicting smaller pore sizes would clogg the device quicker. Longer sections were placed above the smaller pore sizes to collect and hold water to prevent needing to disassemble the device to change a filter while a sample remained in the device. For one filtration device one 18 in, one 12 in, and two 6 in peices are needed.

Union joints were glued to PVC pipe to house nylon sieves and mixed cellulose membranes.

Mesh sheets were cut into circles to match the diameter of the outer diameter of the PVC pipe. The edges were glued to esure no fraying would occur. The glue 's diamter should not extend into the inner diameter of the PVC so that it will not be affected during filtration.

Mesh sheets were cut into circles to match the diameter of the outer diameter of the PVC pipe. The edges were glued to esure no fraying would occur. The glue 's diamter should not extend into the inner diameter of the PVC so that it will not be affected during filtration.

Mesh sheets were cut into circles to match the diameter of the outer diameter of the PVC pipe. The edges were glued to esure no fraying would occur. The glue 's diamter should not extend into the inner diameter of the PVC so that it will not be affected during filtration.

Mixed cellulose membrane filter with 0.45 um was used as the last filter. A large diameter was used to allow the filter to be folded into a cone to increase surface area of the filter to prevent clogging.

The mesh basket used to provide extra support for the membrane filter to prevent tearing when pressure was applied by a vacuum pump.

A vacuum adapter was added to allow vacuum filtration in the case of slow filtration due to high sediment concentration.

Tubing used to connect the vacuum pump to the filtration device.

Handheld meter used to measure additional water quality parameters parameters (e.g., turbidity, temperature, conductivity, pH, and dissolved oxygen (DO)).

Handheld meter used to measure turbidity.
Tubing used with perestaltic pump to collect water samples from desired depths.

Pump used to collected water samples.

Microscope used analyze mesh sieves and membrane filters to quanitfy suspect microsplastics.

FTIR used to analyze suspect microplastics.

FTIR microscope used to analyze suspect microplastics.

Geranium ATR accessory used along with the Nicolet iN5 FTIR microscope to analyze suspect microplastic.

Microscope slides used along with the Nicolet iN5 FTIR microscope to analyze suspect microplastic.

Microscope slides used along with the Nicolet iN5 FTIR microscope to analyze suspect microplastic.

1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
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Author(s): Katherine M. Martin; Elizabeth A. Hasenmueller; John R. White, Lisa G. Chambers; Jeremy L. Conkle

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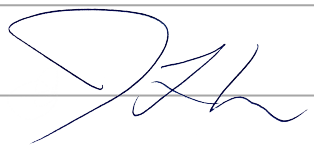
Signature:	 Date: **02/02/2018**

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We agree that this was not clear and have attempted to clarify this section. Line 127-132 now reads: ***"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).20 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."***

12. Protocol: 1.2: Please use the imperative tense for all the sentences in the protocol step. Please move the discussion part of this step to either Introduction or Discussion section.
We agree that the information presented on these lines should be moved out of the protocol and has been moved to the Representative Results. See Protocol 1.2 (Line 134-144).

13. Protocol: 1.3.1: Please use the imperative tense for all the sentences in the protocol step.
We agree with the editorial comments and have attempted to correct the tense of section 1.3.1. Line 150-153 now reads:
"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."

14. Protocol: 1.6: How much DI water is needed for "pre-rinse"?
We have added text to clarify (line 169-172) which now reads:
"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."

15. Protocol: 1.7, 1.8, 3.2.1, 3.2.2, 3.3, 4.1, 4.2, 6.2, 6.5.1: Please avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Please use the imperative tense for all the sentences in the protocol step.
Thanks for the more concise wording tips, all sections listed here have been changed accordingly.

16. Protocol: 2.1: If the actions are not fully described here and the reader is directed to an external reference, please do not highlight the step.
The highlighting has been removed from Protocol 2.1.

17. Protocol: 7: What are the actions here? Please clearly describe the actions in the imperative tense. Please move the discussion to the Discussion section.
We agree that there was no clear actions in Protocol 7. The section has since been moved to the Discussion section.

18. After revising the protocol, please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.
Completed.

19. Please include all relevant details that are required to perform the step in the highlighting. For example: If step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be highlighted.
Completed.

20. Figure 1: Please add a scale bar to the images.
We are unsure how to address the editor's comment. Is a scale bar needed on all images within the figure or only image E? A scale bar has been added to Figure 1E, but we are concerned that a scale bar would not be easily visible on the remaining images. Also, the perspective of the images in Figure 1B-D distorts the size of the filtration device in which the diameter at the top of the cylinder does not match the bottom.

21. If you are reusing figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [AUTHOR] et al.[REFERENCE]".
Completed.

22. Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials in separate columns in an xls/xlsx file. Please list all the materials, equipment, instrument, and software used in your work.
Completed.

Reviewers' comments:

Reviewer #1:
Manuscript Summary:
The manuscript does a great job detailing the experimental design of microplastic collection via pump apparatus. This is of interest due to its ability to correct for the underestimation of plastics recovered with trawl devices. One of the concerns with this paper is the use of plastic items throughout the protocol. While, the reviewers do account for this by having blank samples it does present a little worry in the investigation of microplastics. However, as long as scientist that implement this procedure include blanks, they should be able to account for possible microplastic pollution. The paper does a great job explaining each component and rationale for use. I think overall this will be a benefit for future microplastic collection studies in river systems and other aquatic environments. The apparatus also allows for the recovery of microplastics that are mostly not represented due to the size fractions in which they represent. A highlight of this paper is the use of this apparatus in an actual field setting. The data here is beneficial to later studies and also can be used to highlight the discrepancies between microplastics collections with trawls. There are many opportunities for this apparatus and will greatly enhance the field.

Minor Concerns:
Would it be possible to use sieves that are not nylon? Nylon being a plastic polymer, it may cause some concerns later down the line.
Yes, other materials can be substituted for the nylon mesh filters. This has been stated for the reader in lines 470-471. Nylon was used in this protocol based on pricing, durability, and availability.
"…while the detachable union fittings allow for adjustments in filter material and pore sizes to accommodate variable diameters and concentrations of plastic."

In the protocol, DI water is used thoroughly throughout, I would recommend filtering the DI water 2x to remove any possible microplastics that may be transferred via the tubing connected to the DI instrument. Has the lab done any analysis on DI water to ensure no contamination from that source?
Our lab has not done analysis specifically on our DI water though our equipment blanks do include possible contamination from DI water as seen in lines 120. We also mention the use of an ultra-pure water system if readers are concerned about contamination form DI water (lines 504-505).

Sampling time and sedimentation may cause some problems in clogging the pump, this was mentioned and should be taken into consideration for studies that plan to do longer sampling times greater than 1 hour. To correct for this, maybe using multiple pumps throughout the study?
We are unsure of what the reviewer is implying and how to address it. Is the reviewer suggesting more pumps in the case of one burning out or multiple pumps with different vacuum strength?

On site blanks should be used not just for the device but also atmospheric contamination that may occur, this would be beneficial as well for QA/QC purposes.
Site blanks address this as they follow the same procedures in which samples are collected, therefore, open for the same amount of time that an actual sample would be.

Reviewer #2:

Manuscript Summary:

This study presents methods for collecting, separating, and counting microplastic from high turbidity aquatic habitats. The authors present a nice set of images and directions for users to follow the process of field collection and laboratory analysis. I think this will make a nice contribution to a rapidly growing field of study. The authors could improve the manuscript by editing for clarity and brevity, and describing the potential sources of contamination in more organized fashion. I find the writing can suffer at places with sentences that are overly complex and repetitive. I have provided detailed and constructive comments to address these shortcomings below. Following revision according to the suggested edits, I support publication of this manuscript.

Major Concerns:

L 93. More deliberate statements on contamination are needed here and elsewhere. Something like "Contamination of environmental samples with microplastic is commonplace, especially for fibers. Contamination sources include atmospheric deposition (in the environment and the laboratory), clothing and equipment from researchers, as well as microplastic contamination in water sources, chemicals, and sampling devices. We include many steps in the protocol to reduce contamination from various sources, and urge researchers to be vigilant to contamination while conducting all stages of microplastic studies."
Thanks for the more concise wording tips. We have taken the reviewers suggestions and rewritten the sentences to include a more precise description of potential contamination. Lines 117-122 now read: ***"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."***

Minor Concerns

L23. What does 'dangerous' mean? Sharp? Heavy? Clarity is needed on this point.
We appreciate the comment but due to the limited word count of the short abstract we are unable to add detail about the "dangers". However in the long abstract and throughout the paper, dangerous is better defined. Line 33

L 30. Often river collection is by drift nets with an anchored boat (rather than trawling, which is often a net deployed behind a moving boat).
We agree that drift nets can be used with both anchored and moving boats. This has been corrected here and elsewhere when trawl or trawling was used. Line 40

L 42. Edit "…to determine microplastic polymer type. Recovery was measured for …."
Thanks for the more concise wording tips. The text on lines 55 has now been changed to
"…determine microplastic polymer type. Recovery was measured by spiking samples…"

L 45. Move the penultimate sentence in the abstract to the end.
Thank you for the suggestion. We agree it would also be good at the end of the section but based on the work that was completed and flow of the section we believe it is best suited where it is. However, we have added text at the end of the section to tie it to the penultimate sentence.

L 102. Edit "to determine polymer type."
Thanks for the more concise wording tips. The text on line 116-117 has now been changed to
"…to eliminate non-synthetic materials or determine polymer type."

L 109. In rivers, the 'center' is not always where the water is well mixed. This depends on a great number of factors affecting river geomorphology (i.e., meanders, infrastructure, subsurface features).
We agree with the reviewer and have attempted to improve the sentence. Protocol Section 1.1 (Lines 127-132) has been changed to:
"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).20 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."

L 109. The phrase "and/or" should be avoided here and elsewhere. Use "or" in this case.
Thanks for the more concise wording tips. The text on line 129 has now been changed to
"…river stage or discharge…"

L 113. Avoid the term 'flow velocity' (used twice in this sentence). The correct term here is velocity or water velocity (i.e., speed).
Thanks for the more concise wording tips. This text was also moved to the Representative Results section per editor suggestion, lines 388-390 has now been changed to
"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."

L 169. I recommend the authors point out that measurements of organic and inorganic components in the suspended sediments (i.e., ash-free dry mass) are also valuable here.
Thanks for the suggestions. We agree that it could be valuable to measure organic and inorganic components in the suspended sediments but we have described the protocol we used.

L 173. It strikes me that a description of the device is missing, and should be included as step 1. Then, step 2 should describe the assembly of the device.
After reading through the Journal's Instructions for Authors, all text in the protocol section of the article must be written in imperative tense preventing a description of the instrument in the protocol.

L 218. The authors state "transfer the sample from the container ot the filtration device and record the total volume." However, there is a graduated cylinder used in between the 'container' and the 'device' correct? The phrasing here is a little confusing.

We agree that the wording was awkward and have attempted to clarify it. The sentence now reads (Lines 252-254):
"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."

L 229. Here and elsewhere, do the authors measure contamination in DI water? Try as we might - and from many different sources - we find a few fibers in our DI sources. I recommend the authors suggest readers also test their Di water. In addition, we were able to reduce (but not eliminate) microfiber DI water contamination by wrapping the DI faucet outlet with small mesh.
The reviewer has a good point, we did not conduct a study to evaluate the contamination from the DI water. Due to journal restraints, we are unable discuss testing the DI water as it is not a part of our protocol. However we have added a comment about reducing DI water contamination by using ultra-pure water sources to the Discussion (Line 504-505).

L 229. The authors should note that all containers (including graduate cylinder, sample containers, and petri dishes) should be kept covered at all times - or as much as possible - to avoid contamination. We use foil for this, but I imagine parafilm would work as well.
We agree with the reviewers comment and have added it to the Discussion (Line 519-520). We also covered our equipment as much as possible.

L 243. "forceps" is more appropriate than tweezers here and elsewhere
Thanks for the more concise wording tips. The text on line 279 has now been changed to
"…carefully with clean forceps, making sure…"

L 250. I am having a hard time picturing what the authors mean by "under the end of the membrane filter lying below the mesh sieve." Could a picture be used for this?
We agree that the wording used to describe this step was confusing and have attempted to clarify. We do not think a picture would help to depict this, but it will be filmed for the journal. Lines 292-296 now reads:
"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 ponding on its surface. Caution: Again, be careful when rinsing the membrane filter as sample can be lost if rinsed under it."

L 258. Edit "foil envelope appropriate for the diameter of…"
Thanks for the more concise wording tips. The text on line 299-300 has now been changed to
"…foil envelope appropriate for its diameter."

L 271. What type of microscope and what magnification?
We agree that this was not clear and have added text to clarify. Line 313-314
"…filters under the stereomicroscope (14X-90X magnification) to identify…"

L 274. I disagree that 'homogenous color' throughout is an indicator limitation for microplastic. We find many microplastic fragments and fibers that have variable color patterns. In some cases, it seems the dye is leeching from the plastic, and in others the plastic has printed stripes or text.
We agree with the reviewer as non-homogeneous fibers have been seen in the samples. This has been removed from the sentence. Line 315-317

"…no cellular structure, fibers are equal thickness throughout, and particles are not shiny."

L 289. Edit "Same results are compared to spectral databases to determine plastic polymer type, or if the material is non-synthetic."
Thanks for the more concise wording tips. The text on lines 347-349 has now been changed to ***"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."***

L 308. Edit "To validate the recovery rates of this…"
Thanks for the more concise wording tips. The text on line 352 has now been changed to ***To validate the recovery rates of this protocol…"***

L 309. Edit "10 blue PE fragments…"
We do not know if the particulates found are fragmented from larger items so we do not feel it is more accurate to call them particulates. (Line 354)

L 312. Edit "Other fibers and fragments that we observed on the sieves and membrane filters were resident to Oso Bay."
Thanks for the more concise wording tips. Please see the comment above about refraining from using fragment in the place of particulate. The text on lines 357-360 has now been changed to ***"Other fibers and particulates that were observed on the mesh sieves and membrane filters, likely derived from the Oso Bay water sample."***

L315. Edit "…amount of sample loss during filtration or incorrect identification."
Thanks for the more concise wording tips. Please see the comment above about refraining from using fragment in the place of particulate. The text on lines 361-362 has now been changed to ***"A loss of fibers may be due to a small amount of sample loss during filtration or incorrect identification."***

L 322. Edit "…filtration device, laboratory equipment, or air, but was not similar to the blue fragments and green fibers used to spike the samples."
We agree that this was not clear and have added this accordingly. Line 367-369 now reads: ***"…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."***

L 325. Edit "This protocol was created to process samples from the Mississippi River watershed. Preliminary analyses form the Mississippi and Missouri Rivers had an average TSS of 0.063 g/L. validation samples were taken from Oso…
Thanks for the more concise wording tips. The text on lines 371-374 has now been changed to ***"This protocol was created to process samples from the Mississippi River watershed, including the Mississippi River main stem and Missouri River. Preliminary analyses from the Mississippi River and Missouri River had an average TSS of 63 mg/L."***

L 331. Edit: "Turbidity in the Mississippi and Missouri River samples suggest successful filtration for samples with at least 4.6 g/L TSS."
Thanks for the more concise wording tips. The text on lines 379-383 has now been changed to

"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 techniques outlined here."

Upon further review, it was found that the TSS was calculated incorrectly for the Oso Bay samples. This has also been changed.

L 338. Edit: "Samples from the Mississippi and Missouri Rivers were collected at the surface and at 0.6 m depth (table 2) and analyzed as described above. To examine the effect of depth on microplastic concentration, the first and third…"
Thanks for the more concise wording tips. The text on lines 390-396 has now been changed to ***"Samples from the Mississippi River and Missouri River were collected and analyzed as described above (Table 2). To examine the effect of depth on microplastic concentration, the first and second samples were taken at the same location (i.e. Mississippi River at Alton, IL) but at different depths."***

L 343. What does "different locations" mean in this sentence? Different rivers? Or different sites within the same general area?
We agree that "different locations" was vague and have attempted to clarify the sentence. Line 397-399 now reads:
"…different locations (i.e. Mississippi River at Alton, Illinois and the Missouri River above Saint Louis, Missouir)."

L 347. The table format was challenging as it was spread across several different pages on the pdf

L 393. Edit to "Microplastic collection in nets is the conventional method…"
Thanks for the more concise wording tips. The text on lines 451-452 has now been changed to ***"Microplastic collection using drift nets is the conventional method in environments…"***

L 397. See Barrows et al. 2017: Grab vs. neuston tow net: a microplastic sampling performance comparison and possible advances in the field. Analytical Methods. This paper has a robust comparison of net and grab samples, pertinent to the claims here.
The authors agree that the suggested paper has relevant information to our manuscript. However, we are not comparing the merits of tow nets vs grab samples. Rather we are stating that tow nets are not feasible under the environmental conditions of this study, nor would they capture microfibers, which is a major focus of this method. Therefore, we have decided not to include it as citation.

L 402. Edit "data obtained with this protocol will help develop mitigation and water quality improvement strategies. E
Thanks for the more concise wording tips. The text on lines 461-463 has now been changed to ***"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."***

L 406. This paragraph is lacking a topic sentence. Something is needed that sets up the details included below. Something like "This device allows for increases in control for collection depth, separation of microplastic among size classes, and high volumes that could reduce in the influence of contamination."
We agree with the reviewer that the start of this paragraph could be improved. Lines 465-466 now reads:

"This method enables control of sample collection depth, volume input, and separation of microplastics into size classes while accounting for multiple sources of contamination."

L 423. Edit "…sealed firmly but carefully to ensure sieves…"
Thanks for the more concise wording tips. The text on line 485 has now been changed to ***"…sealed firmly and carefully to ensure mesh sieves…"***

L 426. Edit "…analysis. Rupturing can occur in the membrane filter before the pump pressure reaches 127 mm Hg, especially in samples with high sediment volume. Pressure should be watched carefully and adjusted as needed." (end of paragraph)
Thanks for the more concise wording tips. The text on lines 490-495 has now been changed to ***"Rupturing can occur in the membrane filter before the pump pressure reaches 127 mm Hg (Section 4.2), especially in samples with high sediment volume. Therefore, the pressure must be watched carefully and adjust as needed."***

L 433-441. I am not convinced that this paragraph is needed, as the information is repetitive. Is the reason for including it to have a summary? It sounds much like an abstract. I suggest deleting here.
We agree that the last two paragraphs were repetitive and have combined the two in a way that better suits the article.