From: em.jove.14956.598468.098e62f1@editorialmanager.com <em.jove.14956.598468.098e62f1@editorialmanager.com> on behalf of Phillip Steindel <em@editorialmanager.com>

Sent: Monday, February 26, 2018 9:09 AM

To: Elizabeth Hasenmueller

Subject: Revisions required for your JoVE submission JoVE57969

CC: kmartin16@islander.tamucc.edu, jrwhite@lsu.edu, lisa.chambers@ucf.edu, jeremy.conkle@tamucc.edu

Dear Dr. Hasenmueller,

Your manuscript, JoVE57969 Microplastic sampling, sorting and characterization in aquatic environments with high suspended sediment loads and large floating debris, has been editorially and peer reviewed, and the following comments need to be addressed. Note that editorial comments address both requirements for video production and formatting of the article for publication. Please track the changes within the manuscript to identify all of the edits. After revising and uploading your submission, please also upload a separate rebuttal document that addresses each of the editorial and peer review comments individually.

**Your revision is due by Mar 19, 2018.**

To submit a revision, go to the JoVE submission site and log in as an author. You will find your submission under the heading "Submission Needing Revision".

Best,

Phillip Steindel, Ph.D.

Review Editor

JoVE

617.674.1888

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About JoVE

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Editorial comments:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

**Completed.**

2. Please provide an email address for each author on the first page.

**Completed.**

3. Please use SI units, e.g. please use “µL” instead of “µl”. Please leave a white space between the values and the units.

**Completed.**

4. Please define all abbreviations before use.

**Completed.**

5. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.

**Completed.**

6. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Any text that cannot be written in the imperative tense may be added as a “Note.” However, notes should be concise and used sparingly. Please include all safety procedures and use of hoods, etc.

**Completed.**

7. Please avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol.

**All uses of “could be,” “should be,” and “would be” in the Protocol have been addressed and changed accordingly.**

8. The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. The Protocol steps should contain only 2-3 actions per step and a maximum of 4 sentences per step.

**Completed.**

9. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action.

**Completed.**

10. We cannot film the steps that are not fully described. Please do not highlight those steps which actions are only explained in the external references. Please keep those steps in the protocol but not highlighted. Please attention that your manuscript after acceptance, will be the source for our script writers for the video production.

**Completed.**

11. Protocol: 1.1: How is that done? What is the container?

**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.**