**TITLE:** A Flow-through Exposure System for Evaluating Suspended Sediments Effects on Aquatic Life

**AUTHORS:** Suedel and Wilkens

**Editorial Comments:**

Your manuscript has been modified by your editor, please maintain the current formatting throughout the manuscript. Please use the updated manuscript located in your Editorial Manager account (under “File Inventory”) for all subsequent revisions.

**Response:** The updated manuscript provided by the Editor was used to modify it to address comments from both the editor and peer reviewers. All modifications were made in redline/strikeout as requested.

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

**Response:** The manuscript was proof-read for errors. Errors found in the numbering of references were fixed.

**Comment:** JoVE reference format requires that DOIs are included, when available, for all references listed in the article. This is helpful for readers to locate the included references and obtain more information. Please note that often DOIs are not listed with PubMed abstracts and as such, may not be properly included when citing directly from PubMed. In these cases, please manually include DOIs in reference information.

**Response:** DOIs were included where available for any new references added to address reviewer comments.

**Comment:** Step 5.3: Please remove the http link and move it to the reference section. Please provide the appropriate reference number here (instead of the link).

**Response:** The http link was moved to the reference section and an appropriate reference added to this section (USEPA 2016).

**Comment:** Formatting: Please split 1.1.1, 1.2.1, 1.3, 1.4.1, 2.3, 3.2, 4.7, 4.8, 5.5.6, 5.6.2.1 into two steps.

**Response:** Each of the steps listed were split into two steps and re-numbered accordingly.

**Comment:** Please reevaluate length when steps have been appropriates divided. (Please highlight 2.75 pages or less of text (which includes headings and spaces) to identify which steps should be visualized to tell the most cohesive story of your protocol steps. Please see JoVE’s instructions for authors for more clarification. Remember that the non-highlighted protocol steps will remain in the manuscript and therefore will still be available to the reader.)

**Response:** After making the revisions needed to address all comments, we revisited the length of the highlighted text to confirm that it does not exceed 2.75 pages in length. To meet the length requirement, we un-highlighted Sections 5.6.1 through 5.6.2.2.

**Comment:** Grammar: 2.2 – “laboratory buildings air compressor”

**Response:** The phrase “…laboratory buildings air compressor” was replaced with “…laboratory building’s air compressor.”

**Comment:** Additional detail is required:

-1.1.1 – What dimensions are used?

-1.3, 1.3.1 – How large is the hole? How is it cut?

-1.3.2 – How is the plug made? What are the dimensions?

-1.3.3 – How many 9 cm holes should be cut?

-2.3 – Do you mean “solenoid valve”? Please describe how parts are connected in the order in which they are connected. It is unclear what actions are performed or how we can film them.

-4.1 – Where should the sensor be positioned to accomplish this?

-4.2 – Please clarify the last sentence. Where should quick connects be installed here?

**Response:**

-1.1.1- Added dimensions as requested. Dimensions are also listed in the table of materials.

-1.3, 1.3.1, 1.3.2- We recently purchased 5 gal domed-bottom tanks to replace the carboy aquaria. The new tanks are superior to the original design; therefore, the directions have been updated to reflect the new tanks.

-1.3.3- The text states 10 total holes (1.4).

-2.3- Agree. This section was rewritten to better explain the process. Also modified 3.2, 3.3, 3.4 and 3.5.

-4.1- Text revised to clarify.

-4.2- Text revised to clarify.

**Comment:** Discussion: Please discuss any modifications/troubleshooting that can be performed.

**Response:** We added two paragraphs to the discussion section that describes appropriate modifications and troubleshooting that can be performed (to meet experimental objectives) while still maintaining the full capability of the technology. This included the flexibility of scaling the technology and certain components, including aquarium size, if a smaller or larger footprint is desired.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

**Comment:** This paper describes the development and operation of an automated laboratory system that can be used to test how aquatic species respond to carefully controlled suspended sediment dosages. The methodology is sound and the presentation is clear. This paper and more widespread use of the FLEES technology will benefit resource managers tasked with efficiently protecting vulnerable biological resources from suspended sediment impacts.

**Response:** We appreciate the comment.

Major Concerns:

N/A

Minor Concerns:

**Comment:** Line 53 - The term "environmental window" needs to be explained. Perhaps insert (seasonal dredging restriction) after the phrase.

**Response:** The parenthetical passage “(seasonal dredging restrictions)” was added to line 53 to address the comment.

**Comment:** Line 62 - "Spawning, migration, and physical disturbance" are not life stages. Dredging restrictions are most commonly imposed to protect early life history stages (eggs and larvae) and to keep migratory routes open for anadromous species.

**Response:** The text was revised to read “The life stages commonly cited are eggs and larvae for the purpose of keeping migration routes open for anadromous species.”

**Comment:** Line 404 - A brief review of what "existing methods" are would be helpful, either here or in the Introduction.

**Response:** We added two references (Clarke and Wilber 2000 and Wilber and Clarke 2001) to line 404 (now 474) to address the comment.

**Comment:** Line 412 - Consider including the biological responses that were assessed, for instance, mortality? Hatching success?

**Response:** The passage “(e.g., survival and growth)” was added to line 412 (now 481) to address the comment.

Additional Comments to Authors:

N/A

Reviewer #2:

Manuscript Summary:

**Comment:** Overall well written with great detail in the assembly instructions. Sometimes the details are too finite and need to be put into context so the reader doesn't get lost or lose interest. Take some time to explain the importance of the testing system, its strengths and weaknesses, and how this system improves the ability to test using this new apparatus compared to other techniques or standard methods. The software description can be simplified and summarized without discussion of programming details.

**Response:** The strengths and limitations of the approach are already included in the discussion section. Two references were added to the discussion to address a previous comment about improvements to existing methods. The software description was added to address a previous comment by the editor.

**Comment:** [Editorial recommendation: Please keep JoVE’s protocol requirements in mind as you address the above comments - the protocol must contain sufficient detail in order to enable users to accurately replicate your technique. We recommend NOT removing any details from the protocol text.]

**Response:** Agreed. No details were removed from the manuscript to address the comment.

Major Concerns:

**Comment:** Please include additional pictures for each step of the construction process. The reader will be lost without more schematics or pictures.

**Response:** We have added 3 photos of various components of the technology, namely the close-up of the inside of an aquarium (Fig 2), overview of a water bath (Fig 3), and slurry tank and diaphragm pump (Fig 4).

Minor Concerns:

**Comment:** Be more consistent with abbreviations and acronyms. Provide a few references or examples of recent testing applications? Who needs this technique as we don't know who you are speaking to.

**Response:** We made edits to an earlier version of the manuscript (per the editor) to address consistent use of abbreviations and acronyms. References to recent applications are found in the following references already cited in the text: Suedel et al. 2012; Suedel et al. 2014; Wilkens et al. 2015; Suedel et al. 2015. Who needs this technique was addressed by the addition of a sentence at the end of the last paragraph of the discussion: “This technology can be used by any investigator interested in answering questions about the effects of sediment suspended in surface water bodies on aquatic species.”

Additional Comments to Authors:

N/A