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

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KEYWORDS:
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SHORT ABSTRACT:
A robust and flexible flow-through exposure system designed to maintain sediment in suspension is presented. The system is used to investigate the effects of suspended sediment on various aquatic species and life stages in the laboratory.

LONG ABSTRACT:
This paper describes the Fish Larvae and Egg Exposure System (FLEES). The flow-through exposure system is used to investigate the effects of suspended sediment on various aquatic species and life stages in the laboratory by using pumps and automating delivery of sediment and water to simulate suspension of sediment. FLEES data are used to develop exposure-response curves between the effects on aquatic organisms and suspended sediment concentrations at the desired exposure duration. The effects data are used to evaluate management practices used to reduce the interactions between aquatic organisms and anthropogenic causes of suspended sediments. The FLEES is capable of generating total suspended solids (TSS) concentrations as low as 30 to as high as 800 mg/L, making this system an ideal choice for evaluating the effects of TSS resulting from many activities including simulating low ambient levels of TSS to evaluating sources of suspended sediments from dredging operations, vessel traffic, freshets, and storms.

INTRODUCTION:

Dredging operations use mechanical methods to remove bottom sediments from harbors and navigation channels. During removal, some portion of the disturbed sediment is suspended into the water column, potentially making this a source of physical stress to aquatic species. In addition to being suspended, the sediment may be transported away from the dredge by ambient conditions before settling out of the water column. The combination of these two mechanisms means that an aquatic organism occurring near an operating dredge may be exposed to suspended sediments and suffer adverse effects. To address such concerns, environmental windows (seasonal dredging restrictions) are routinely used as a management practice to reduce or eliminate risk of potentially harmful impacts of suspended sediments from dredging activities on aquatic resources[1,2].

Environmental windows are most commonly established to protect endangered, threatened or commercially valuable species such as the walleye (*Sander vitreus*) and eastern oyster (*Crassostrea virginica*)[3]. The supporting justification for imposing environmental windows often focuses on how dredge activities may potentially physically disturb (e.g., suspended sediment) an animal's ability to complete a specific part of its life history. The life stages commonly cited are eggs and larvae for keeping migration routes open for anadromous species[3]. However, there is limited information concerning species-specific biological effects relevant to suspended sediment[4,5] available to inform using environmental windows as a risk management tool.

For these reasons, the FLEES was designed, built, and used to simulate the suspension of sediment, and to determine its effects on early life stages of aquatic organisms. FLEES studies use fine-grained sediment particles (i.e., predominantly silts, clays, and fine sands) which are most likely to remain in suspension and migrate furthest from the source. The FLEES is capable of testing fish eggs and larvae, but it can also be retrofitted to accommodate other aquatic organisms, making it a unique capability. The resulting biological response data can then be used to assess the effects of suspended sediments. The following procedures provide an overview of how the technology can be constructed and operated to yield repeatable suspended sediment concentrations and effects data using various aquatic species.

PROTOCOL:

All FLEES experiments with vertebrates were performed under the appropriate Engineer Research and Development Center (ERDC) Environmental Laboratory Institutional Animal Care and Use Protocols.

1. FLEES modules, water bath, and aquaria

1.1) Obtain wood posts, studs and plywood for building the module. Construct the modules (in number and size) similar to a basic workbench to meet research objectives.

1.1.1) Cut the plywood (0.127 cm) for the top and shelf. Cut the posts (10.16 x 10.16 cm) for the legs. For the top, cut the studs (5.08 x 10.16), build a frame and fasten the plywood to the frame. Cut a notch at the top of each leg to create a ledge and bolt the top frame to the legs.

1.1.2) For the shelf, cut the studs (5.08 x 10.16), build a frame and fasten plywood (0.127 cm) to the frame. Cut a notch out of the legs 45 cm from the bottom and bolt the shelf frame to the legs. Ensure assembly is square and level.

1.2) Obtain water bath tanks from a fiberglass tank manufacturer specializing in aquaculture tanks. Fit into the module stand a tank no larger than 152 cm long x 91 cm wide x 61 cm high. Incorporate two 2.54 cm polyvinyl chloride (PVC) slip couplings on one end of the tank by fiber glassing the couplings flush with the inside of the tank bottom.

1.2.1) Place tank onto the constructed module stand with the tank drains facing the end of the stand water will drain (Figure 1). Mark on the plywood floor of the stand where the tank holes are located.

1.2.2) Push the tank back and using a 3.175 cm hole saw cut two holes in the plywood for the tank drains. Slide the tank back so the drains sit in the cut holes. Connect one of the tank drains to a floor drain and the other to a water chiller heat exchanger.

Note: This section assumes a sanitary sewer drain is already in place.

[Place Figure 1 here]

1.3) Obtain a 19 L domed bottom polyethylene tank (27.9 cm diameter x 36.2 cm high).

1.3.1) To construct an overflow drain, use a hole saw and cut a 2.54 cm diameter hole 5 cm from the top of the tank. Install a bulkhead fitting and an insert on the exterior of the bulkhead to serve as the overflow drain.

1.3.2) To construct the slurry/water inlet, use a hole saw and cut another 2.54 cm diameter hole 5 cm from the top of the aquarium. Install another bulkhead fitting and a threaded elbow hose barb (Figure 2).

[Place Figure 2 here]

1.3.3) To construct the pump outlet, use a hole saw and cut a 2.54 cm diameter hole through the middle of the tank bottom and install a bulkhead fitting. Thread the exterior side of the bulkhead with an elbow hose fitting.

1.3.4) To construct the pump inlet, use a hole saw and cut another 2.54 cm diameter hole located off center of the tank bottom and install a bulkhead fitting. Thread the exterior side of the bulkhead with an elbow hose fitting.

1.4) On the exterior side of the water bath tank, measure 9 cm from the bottom and draw a line along the length of the tank. Following the line and with a hole saw, cut a pair of 2.54 cm diameter holes along the length of the water bath for each aquarium (10 total holes; distribute evenly). Install bulkhead fittings.

1.5) Obtain magnetic drive pumps (maximum flow rate 28 L/min) for recirculating water in aquaria and suspending sediments. Mount the pumps to a stand that will fit under the water bath along the side containing the holes for connecting to the aquaria. Install an inline cord switch for each pump or wire the pumps to a switch box for power.

1.6) Thread the exterior side of the water bath tank bulkheads with hose barbs. Attach vinyl tubing to the pump inlet and outlet and connect it to the bulkheads going to the appropriate aquarium. Inside the water bath install a quick disconnect insert into the bulkhead.

1.7) Place the aquaria into the water bath in two rows; with three aquaria in one row arranged along the length of the water bath and the remaining aquaria in the second row (Figure 3).

[Place Figure 3 here]

1.8) Connect each aquarium to a pump. Attach vinyl tubing to the hose barbs installed on the bottom of the aquaria and attach to quick disconnect valve hose barb. Connect the quick disconnects between the pump and aquarium. Install a ball valve in this connection to isolate the pump for maintenance purposes.

1.9) Connect each aquarium's overflow drain to a common drain via vinyl tubing. Connect the common drain to the water bath drain.

1.10) Connect each aquariums slurry/water inlet to the slurry and water system installed on top of the module.

1.11) Mount two light-emitting diode fixtures, designed for aquarium use, approximately 60 cm above the aquaria in each module. Use a light controller (wirelessly connected to the lights) to vary the light intensity, light color, and light cycle (*e.g.*, 16 h light:8 h dark) to meet experimental requirements.

1.12) Install a timer in the laboratory to control ambient lighting.

2.   Slurry System

2.1) Place one 450 L cone-bottom polyethylene tank with cover and stand at the end of the last module in line (either end can be used) to serve as the slurry reservoir. Mount a small submersible pump inside the tank to create the sediment/water slurry. Install a water chiller heat exchanger adjacent to the tank to control slurry temperature. Using a hole saw, cut a 2.54 cm hole in the tank cover to provide access to a turbidity sensor for monitoring the slurry (Figure 4).

[Place Figure 4 here]

2.2) Mount an air-operated double diaphragm pump on a stand next to the slurry tank. Connect the slurry tank drain to the pump inlet. Incorporate a PVC tee (to direct slurry to the pump or to the laboratory drain) and valves to connect the tank to the pump to isolate the tank and the pump for maintenance. To power the pump, connect it to the laboratory building's air compressor.

2.3) To provide slurry to each aquarium upon demand, mount PVC pipe on top of the modules and create a recirculation line. At the point of use located farthest from the slurry reservoir, install a return line to transport unused slurry back to the reservoir. Use flexible PVC and union fittings to connect between modules.

2.5) Using a hole saw, cut 2.54 cm diameter holes in the top of the module to connect each solenoid valve to the appropriate aquarium.

2.6) Connect the PVC pipe mounted on top of module near the slurry tank to the air-operated pump using flexible PVC and union fittings. Connect the return line to the top of the slurry tank.

2.7) To adjust the amount of slurry introduced by the solenoid valve, install a water pressure regulator in the return line. Adjust to create the desired pressure.

3.   Water System

3.2) To provide water to each aquarium upon demand, mount PVC pipe on top of the modules and create a recirculation line. Mount the PVC pipe higher than the slurry recirculating pipe. At the point of use located farthest from the water reservoir, install a return line to transport unused water back to the reservoir. Used flexible PVC and union fittings to connect between modules.

3.3) Connect the water solenoid valves to the recirculating water pipe using tees, ball valves, and union fittings to isolate solenoids from the main PVC pipe for maintenance. Mount the solenoids behind and higher than the slurry solenoid valve. Connect the water solenoid valve to the slurry solenoid valve via vinyl tubing and hose barb fittings so that when the water solenoid turns on it will wash out remaining slurry from the line.

3.4) Connect the PVC pipe mounted on top of the module near the water reservoir to the water pump using flexible PVC and union fittings. Connect the return line to the top of the water reservoir.

3.5) To adjust the amount of water introduced by the solenoid valve, install a water pressure regulator in the return line. Adjust to create the desired pressure.

4.   Sensors, Data Acquisition, Instrument Control and Automation

4.5) Use a system-design platform and development environment to design a computer application for data acquisition, instrument control and automation[6]. With this program, design an application to integrate the OBS and solenoid valves for measuring turbidity and introducing slurry and water into each aquarium.

4.6) To create a variety of NTU exposure regimes, design the program to create individual profiles for each aquarium[6]. Create a tab and graphical user interface (GUI) for programing aquarium profiles. Label the tab 'Profiles'.

4.6.1) Program the software to control exposure duration in minutes for each aquarium. Incorporate a loop sequence to continuously repeat exposure duration instructions until a certain condition is reached such as length of time. Incorporate an iteration to control how many times the loop will repeat before ending or moving onto the next set of instructions.

4.6.2) Program the software to set an NTU level in each aquarium. Integrate the NTU level into the same loop/iteration sequence controlling exposure duration. Use this feature to create a variety of exposure regimes (e.g., continuous, pulsed, or no exposure) for specified durations.

4.6.3) Program the software to control the opening time of the water system solenoid valves for introducing water into each aquarium in seconds (e.g., 10 s for aquarium 1, 25 s for aquarium 2, etc.). Integrate the water solenoid opening times into the loop/iteration sequence controlling exposure duration and NTU levels.

4.6.4) Design the program to save all steps under 4.4 as a 'profile' for each aquarium. Include the capability to allow a user to recall saved profiles.

4.7) Create a new tab and GUI. Label the tab 'Profile Status'. Design GUI to display a live summary of the currently loaded profile including currently active loop sequence, test time elapsed, and test time remaining.

4.8) Create a new tab and GUI for setting water and slurry system solenoid valve values. Label the tab 'Valve Setup'.

4.8.1) Program a water valve cycle interval in seconds. Design this interval as a loop sequence used to set the time between events when all water system solenoids open consecutively (in step 4.4.3 the user programmed how long each valve will remain open). Program a water valve delay in seconds. Use the delay feature to set the time between valves opening (e.g., 2 s after previous valve closed next valve will open).

4.8.2) Program a slurry valve cycle interval in seconds. Design this interval as a loop sequence to set the time between events when all NTU levels measured by the OBS in each aquarium are checked against the NTU set in the aquarium profile. Check the valves and sensors consecutively. If an NTU in the aquarium is lower than the profile NTU setting then program the computer to open the slurry valve.

4.8.3) Program a slurry valve opening time in seconds. Use this feature to control how long a valve remains open if slurry is needed. Program a slurry valve delay in seconds. Use the delay to set the time between valve openings.

Note: Ensure the cycle interval (step 4.6.1 and 4.6.2) is long enough to allow for water and slurry introductions before next loop begins.

4.8.4) Create buttons to manually turn on/off each water and slurry system water valve.

4.9) Create a new tab and GUI for setting up the OBS sensors located in each aquarium and slurry tank (N=16). Label the tab 'OBS Setup'. Give each OBS a name.

4.9.1) Create a feature to enter data from the OBS manufacturers test certificate to calculate corrections for each OBS. Enter the standard NTU low (lowest NTU recorded) and standard NTU high (highest NTU recorded) as well as the voltage range for the low and high NTU.

4.10) Create a new tab and GUI to display real time NTU measurements and NTU setting for each aquarium, as well as water temperature in each water bath. Create a button to start/stop all profiles. Create the capability to individually pause or stop an individual profile.

4.10.1) Create a feature to log water temperature readings for each water bath, NTU settings and measurements for each aquarium and time stamp data into a spreadsheet. Label the tab 'Water Bath'

5.   Experimental Preparation

5.1) Collect sediment from an area routinely dredged to maintain navigation channel depth, in close proximity to species of concern, and is known to lack historical contamination. Collect the sediment using a grab sampler or similar (*e.g.*, Van Veen). Place sediment in 19 L plastic buckets and ship overnight on ice. Store sediments at 4 ºC until use.

5.2) Wet sieve sediment through a 1 cm screen to remove large debris; then sieve through a 450-micron stainless steel screen. Retain fine-grained (fine sands, silts, and clays) particles passing through the screen for experimental use.

5.3) Analyze sieved sediments for chemical contamination (*e.g.*, metals, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, etc. For analytical methods, see USEPA[7]). Characterize physicochemical parameters such as grain size distribution (percent sand silt, and clay), pH, salinity, organic carbon, and organic matter[8] to meet study requirements.

5.4) Identify the exposure duration (e.g., 72 h) and TSS concentrations (e.g., 0, 100, 250, and 500 mg/L) based on existing data or other information characterizing the suspended sediment of interest.

Note: Use TSS as the exposure concentration rather than NTU. TSS quantifies the mass of particles present in the water column and directly relates to physical and behavioral effects such as abrasion, loss of orientation and reduced feeding exhibited by some organisms.

5.5) Establish the NTU-TSS relationship for each FLEES aquarium.

5.5.1) Turn on all FLEES hardware used for data acquisition, instrument control and automation. Randomly assign TSS treatments to FLEES aquaria using a random number table or other appropriate method. In the profile GUI, create a profile for each aquarium to execute a 72 h (4,320 min) continuous exposure using the assigned TSS concentrations generated from the random number table.

5.5.2) Use professional judgment to initially program NTUs to meet TSS concentrations in each aquarium. For the control (0 mg/L TSS) set the NTU to 0; 100 mg/L TSS set the NTU to 100; 250 mg/L TSS set the NTU to 280; and 500 mg/L TSS set the NTU to 600.

Note: Each OBS probe will have slightly different NTU reading which is inherent to the manufacturing of the probe.

5.5.3) Set the opening time for water system solenoid valves to 10 s for each aquarium.

5.5.4) Save profile for each aquarium.

5.5.5) On the Valve Setup tab, program the water and slurry valve cycle interval. Set the water cycle interval for 600 s and water valve delay for 5 s. Set the slurry cycle interval for 180 s, opening for 3 s and delay for 1 s.

Note: With this program, in a 72 h test the NTUs in each aquarium will be checked 1,440 times by the computer to determine if additional slurry will be introduced and the water valves will open 432 times. Slurry valve openings are positively correlated with increasing NTUs. Typically, at 100 mg/L slurry valves open for an approximate total of 5% of the exposure duration or 72 openings; 250 mg/L ≈ 11% (158 openings); and 500 mg/L ≈ 35% (504 openings). To equal volume exchanges between aquaria adjust the opening time of the water solenoid valves for aquaria assigned lower NTUs. This will result in increased slurry valve openings at lower NTUs.

5.5.6) Fill the slurry tank with carbon filtered lab water. Start pump to recirculate water. In a separate container, use a mechanical mixer and homogenize test sediment.

5.5.7) After the sediment is homogenized, remove a small portion (≈500 ml) and introduce into the slurry tank using a graduated polypropylene beaker. Continue to introduce sediment until 1,000 NTU is achieved.

5.5.11) Compare averages obtained in Section 5.5.4 to the observed NTU measurements recorded for each aquarium. Reprogram the NTU limits until the desired TSS concentration is achieved (*e.g*., 600 NTU ≈ 500 mg/L TSS).

5.6) Determine mesh size of screen needed to contain animals within each aquarium.

5.6.1) For larger animals such as fish (*e.g*., > 3 cm) or shellfish place a screen on the bottom to separate animals from the pump opening. Install a screen insert in the aquarium's overflow drain bulkhead to prevent escape.

5.6.2) Contain small life stages such as fish eggs, larvae and fry in a chamber (10.16 cm diameter (i.d.) by 12.7 cm long (1,029 ml) made of PVC pipe) for submerging in a FLEES aquarium (Figure 5).

5.6.2.1) Cut three 8.25 cm wide by 9.52 cm long holes out of the side of the chamber. Install nylon screen cloth on the bottom of the chamber and on the holes cut of the side. Use a PVC cap as a removable lid to introduce and remove test animals.

5.6.2.2) Cut a circular hole in the cap sufficient for viewing test animals from above while leaving an edge for attaching a nylon screen cloth. Install all screens on the inside of the chamber to prevent organisms from coming into contact with the sharp PVC edges.

Note: Select a screen mesh size that contains test animals while enabling suspended test sediment to enter.

[Place Figure 5 here]

5.6.2.3) Completely submerge the chamber in the middle or to the side of an aquarium by suspending it vertically using three short lengths of rope (#18 white twisted mason line) and hooks constructed from electrical wire. Tie a Blake's hitch knot near each hook and adjust the length of the rope to level the chamber.

5.7) Determine how many aquarium tank volume exchanges are required per day to meet project and water quality objectives. Adjust the water pressure regulator (see Section 2.2) and solenoid opening time (e.g., open every 10 min for 10 s) to create the desired water flow rate. Fill water

baths with water and operate water chiller heat exchangers to confirm that test temperatures can be achieved and maintained.

6. Experimental Procedures

6.1.2) In the profile GUI, create a profile for each aquarium to execute a 72 h (4,320 min) continuous exposure using the same TSS assignments used in preparation. Use data obtained during experimental preparations to program NTUs to meet TSS concentrations in each aquarium. For the control (0 mg/L TSS) set the NTU to 0; 100 mg/L TSS set the NTU to 100; 250 mg/L TSS set the NTU to 280; and 500 mg/L TSS set the NTU to 600.

6.1.3) On the Valve Setup tab, use data obtained from preparation (Section 5.5.8) to program the water and slurry valve cycle interval. Set the water cycle interval for 600 s and water valve delay for 5 s. Set the slurry cycle interval for 180 s, opening for 3 s and delay for 1 s.

6.1.4) Introduce animals into aquaria using guidelines set forth in the approved animal care and use protocol. For eggs, transfer from the holding tank to an exposure chamber via a plastic transfer pipette. For larger fish, such as fingerling (2-8 cm total length), use a nylon aquarium net.

6.3) Maintain FLEES daily by topping off water and slurry reservoirs with test water and sediment.

Note: The frequency of slurry introductions is positively correlated with increasing NTU levels. Therefore, the amount of water and sediment used each day is dependent on programmed NTUs and desired volume exchanges. Typically, 25-50 gal may be used each day of either water or slurry.

6.3.1) Gently wipe OBS probes daily with a wet cloth to remove sediment build up on the sensor face. Check water chillers and pumps for normal operation. Collect concurrent TSS measurements daily to predict TSS for the remainder of the day based on NTU measurements recorded at specified intervals by the computer program.

6.4) Measure temperature, dissolved oxygen, pH (and other parameters depending on the species and other requirements) daily for each aquarium using a hand-held multi-probe water quality instrument designed for this purpose.

6.5) Terminate an experiment automatically by specifying exposure duration in each aquarium profile or manually by stopping all aquarium profiles.

6.6) Determine experimental endpoints to be measured such as hatching success, time to hatch, mortality, growth (length and weight), and gross morphology.

REPRESENTATIVE RESULTS:
A series of operational runs is performed before beginning an experiment to ensure that the FLEES is delivering the appropriate concentrations of sediments to each aquarium (Sections 5.5 and 6.2). Figure 6 illustrates how NTU concentrations are maintained in experimental aquaria to achieve target suspended sediment concentrations. In this example, FLEES evaluated whether suspended sediment could be maintained over a three-day period with the proposed test sediment, a duration of exposure typical for such experiments simulating typical exposures in the field. Each of the aquaria was set to consistently maintain a turbidity over the three-day period. Test sediments were introduced over a 4 h period on 30May2016. The 0 mg/L TSS concentration represented a control aquarium with no added sediment. At the 100, 250, and 500 mg/L target concentrations, aquaria consistently maintained turbidity of 90, 240, and 430 NTU, respectively, over the three-day experimental period (Figure 6). Sediment pulses reflective of programmed and timed additions of suspended sediment are visible at each concentration in the graph. The flexibility of the FLEES allows investigators to come within about 20-40 NTU of the target concentration. These data demonstrate the ability of the FLEES technology to maintain the desired suspended sediment concentration over time.

[Place Figure 6 here]

Similarly, Figure 7 shows the results of how TSS concentration can be maintained at considerably lower concentrations representing ambient conditions.

[Place Figure 7 here]

The FLEES is calibrated using test sediment to quantify the relationship between TSS and turbidity to ensure target TSS concentrations are achieved (Sections 5.4 and 5.5.2). Figure 8 illustrates the relationship between NTU and TSS for an example test sediment evaluated in the FLEES. In this example, the test sediment exhibited consistently higher NTU values relative to each corresponding TSS concentration evaluated. These data can then be used to calibrate the FLEES and report results in TSS for subsequent appropriate evaluation of effects.

[Place Figure 8 here]

Figure Legends:
Figure 1. Schematic diagram of the Fish Larvae and Egg Exposure System (FLEES). The FLEES is modular and so is transportable.

Figure 2. Polyethylene tank. A 19 L domed bottom polyethylene tank showing the overflow drain (top, with screen insert; 1.3.1; 5.6.1), slurry water inlet (right elbow; 1.3.2), pump outlet (center bottom; 1.3.3), pump inlet (off-center bottom; 1.3.4), OBS probe and clamp (4.1), and bottom screen (black ring on bottom; 5.6.1).

Figure 3. Water bath. Overview of a water bath with five aquaria arranged in two rows.

Figure 4. Slurry tank. Cone bottom slurry tank with cover and poly stand. Slurry water temperature is controlled by water chiller located on the floor left of the stand. The tank is connected to an air operated double-diaphragm pump (left foreground) to provide slurry to each aquarium (2.2).

Figure 5. **FLEES sub-chamber**. Overview of an exposure sub-chamber suspended in an aquarium with no added sediment (left). Fish larvae of the appropriate size can be contained within the sub-chamber to reduce the possibility of escape and injury (right).

Figure 6. Representative FLEES results at four suspended sediment concentrations. Nephelometric turbidity unit (NTU) data recorded by FLEES computer software at 5 min intervals over a 3 day period during an experiment designed to achieve 0, 100, 250 and 500 mg/L total suspended solids concentrations.

Figure 7. Representative FLEES results near ambient conditions. Nephelometric turbidity unit (NTU) data recorded by FLEES computer software at 5 min intervals over a 7 day period during an experiment designed to achieve 25 NTU, equivalent of 20 mg/L TSS for the test sediment.

Figure 8. The NTU-TSS relationship for an example test sediment. The targeted TSS treatment concentrations were 0 (control), 100, 250 and 500 mg/L. Error bars represent the SEM.

DISCUSSION:
The FLEES technology improves on existing methods[4,9] by maintaining and controlling suspended sediments over a wide range of exposure times and suspended sediment concentrations using an automated, computer controlled system. The technology is flexible such that it can be used to evaluate the effects of suspended sediments to multiple aquatic species and life stages of varying sizes from eggs to adults depending on the species. In the future, the technology is capable of assessing suspended sediment effects to submerged aquatic vegetation.

The FLEES has been used to successfully generate effects data (*e.g.,* survival and growth) for various fish such as walleye[5,10], Atlantic sturgeon[11] and oysters[12]. The FLEES performed as designed in evaluating the effects of different suspended sediments collected from various locations across the United States on aquatic species from both freshwater and marine environments. The portability and packaging of the technology also makes it conducive to field use.

The most critical steps in the methodology to ensure success are to: 1) calibrate the FLEES with every test sediment so the relationship between TSS and turbidity can be quantified, thereby enabling matching target TSS concentrations; 2) performing experiments based on TSS and not turbidity so that experimental results can be compared to appropriate regulatory criteria and standards; 3) use the appropriate double diaphragm pump so that the sediment/water slurry can be routed without ruining the pump; 4) use sediments considered chemically uncontaminated so that the physical effects of suspended sediment is not confounded by chemical contamination.

The technology measures turbidity as NTUs in each aquarium via OBS mounted in each aquarium, yet there are distinct and significant differences between the two measurements[9]. Suspended sediment measurements detect particles that have mass, and is often measured gravimetrically or with acoustic technologies. Turbidity is a measure of clarity that is measured as light scattered by material in a water sample by a nephelometer. While turbidity is an important factor in describing suspended sediments, it is influenced by sediment particle size, shape, and number and therefore can vary widely in the environment and from experiment to experiment when using different sediments. For experiments using the technology, TSS measurements (mg/L) are used to develop exposure concentrations because they quantify the mass of particles present in the water column and directly relate to effects in aquatic organisms[4]. TSS is also the unit of measure regulatory bodies use to set environmental windows thresholds for turbidity.

There are some limits to this technology in the maximum quantity of suspended sediment it can transport. Based on experiments conducted to date, TSS concentrations have been successfully maintained up to 600 mg/L. FLEES has continuously produced up to 600 NTUs for seven continuous days and 10-30 NTUs for 30 continuous days with minimal pump maintenance. While the FLEES can maintain TSS concentrations approaching 800 mg/L, concentrations exceeding this amount would require a slurry holding tank of increased capacity. Suspended sediment concentrations exceeding about 800 mg/L would also potentially result in larger particles dropping out of suspension, resulting in NTU readings that do not accurately reflect actual exposure. Nonetheless, concentrations up to 500 to 600 mg/L are considered the maximum concentration expected directly adjacent to an operating dredge, so greater concentrations are not relevant to most dredging operations[4].

Another limit to this technology is the sediment grain size distribution. Particle sizes greater than about 250 μm need to be sieved before use so that larger particles do not tax the pump or settle in the aquaria. Such limitations are not considered significant because it is the finer silt and clay particles that migrate farthest from the source and thus have the greatest potential to cause harm to aquatic animals. While the computer program can be programmed to yield continuous suspended sediment concentrations in each aquarium, it also can be programmed to vary or pulse depending on experimental objectives. In some cases, however, sediment with high percentage clay fraction may not settle sufficiently to adequately mimic a pulsed exposure.

The automation and programming features of the FLEES results in a system that can accurately and precisely maintain TSS levels as well as introduce clean water. Because of these features, FLEES can be easily modified to meet other experimental needs. For example, each FLEES aquarium can be treated as a slurry reservoir and from it slurry may be introduced into another exposure chamber outside of the aquarium. If larger exposure aquaria are desired then the system can be scaled up to meet these needs. FLEES tanks may also be retrofitted to study the effects of sedimentation.

FLEES is a durable system and requires minimal maintenance. Aquarium pump housings can be used multiple times before needing maintenance. The housing should be dissembled, cleaned an inspected after each experiment. Typically the impeller is the first part to fail followed eventually by the remainder of the housing. The air-operated double diaphragm pump is quite durable and

generally does not require inspection after each experiment; however, it is recommend, depending on use, that it be inspected at least annually. The pump manufacturer usually provides a repair kit for commonly worn parts. Clean water should be pumped through the slurry line after each experiment to remove remaining slurry and to clean out the solenoid valves. The remainder of FLEES, including aquaria, water baths and reservoirs should be cleaned following appropriate laboratory procedures.

The FLEES is designed for transport to other sites. The plumbing, electrical, and data connections between each module are made with unions or plugs so they may be easily disconnected for transport and reconnected at a new location. Once transported, the FLEES can utilize a local water source which may be required to meet specific experimental needs. This feature allows for testing of aquatic species that may otherwise be unachievable due to transport limitations or survivability in a laboratory environment.

This paper describes an automated laboratory system designed to evaluate the effects of suspended sediment on various aquatic species. The FLEES technology is capable of exposing aquatic organisms to TSS concentrations reflecting dredging operations, vessel traffic, freshets, and storms [14]. 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.

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Figure

Slurry Tank

120

100
96

84

72

60

48
GALLONS
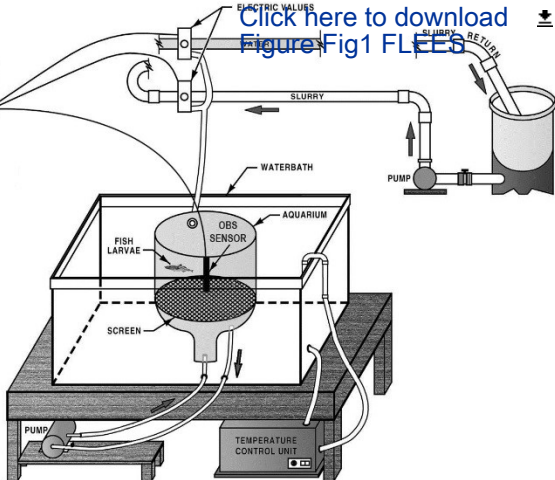
Figure

Parts List for one FLEES Module, Water Bath, and Aquarium

Description

post, wood - used to build module (cut to 78 in)
plywood, marine grade - fastened to wooden posts about 18 in off ground - for holding water bath (60 in x 42 in)
plywood - fastened on top of wooden posts - for holding pipes, solenoids and electrical (60 in x 42 in)
stud, wood - used to brace plywood and wooden posts (cut to fit)
tank, fiberglass - water bath with two drains: 1) to supply chiller; and 2) to drain water
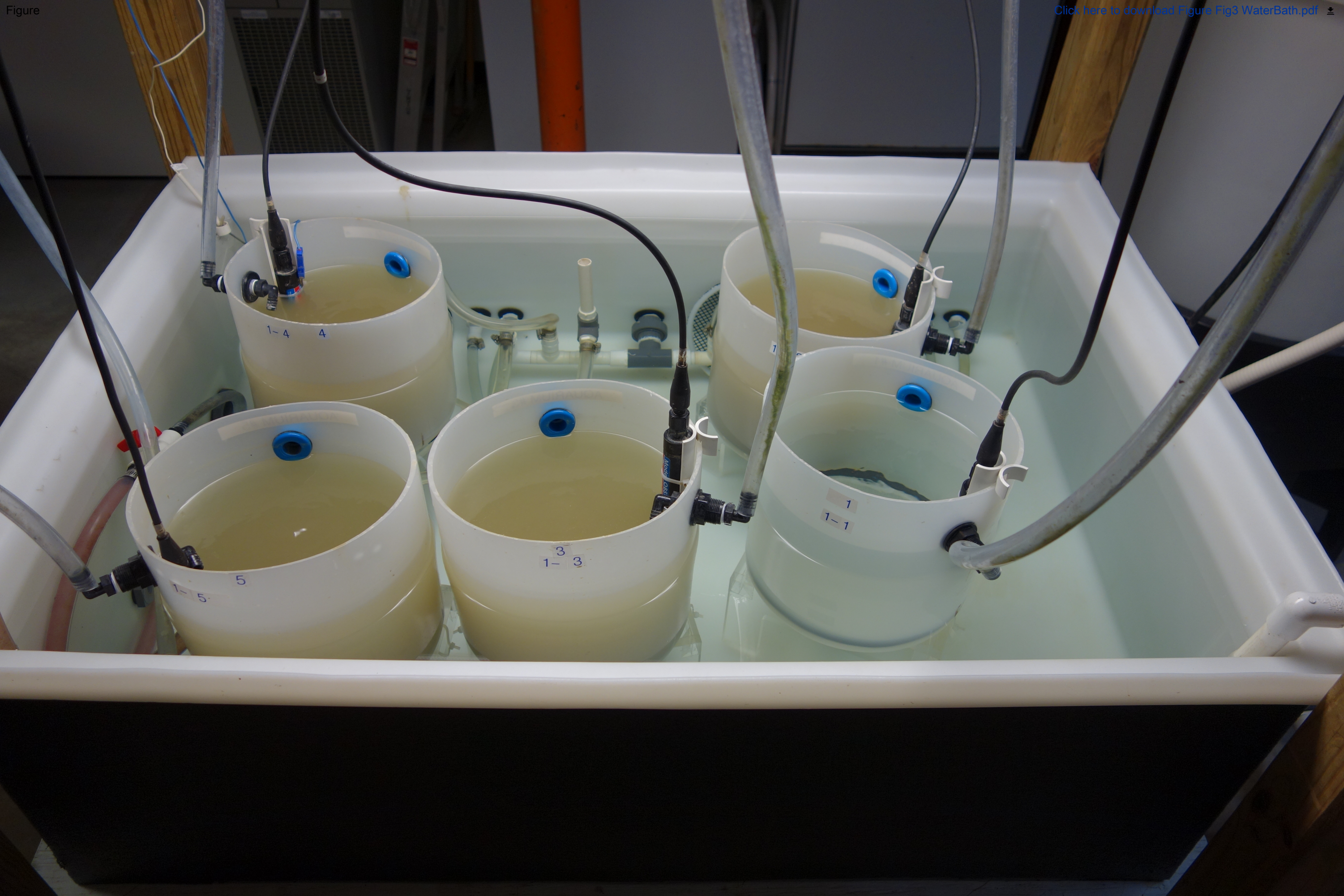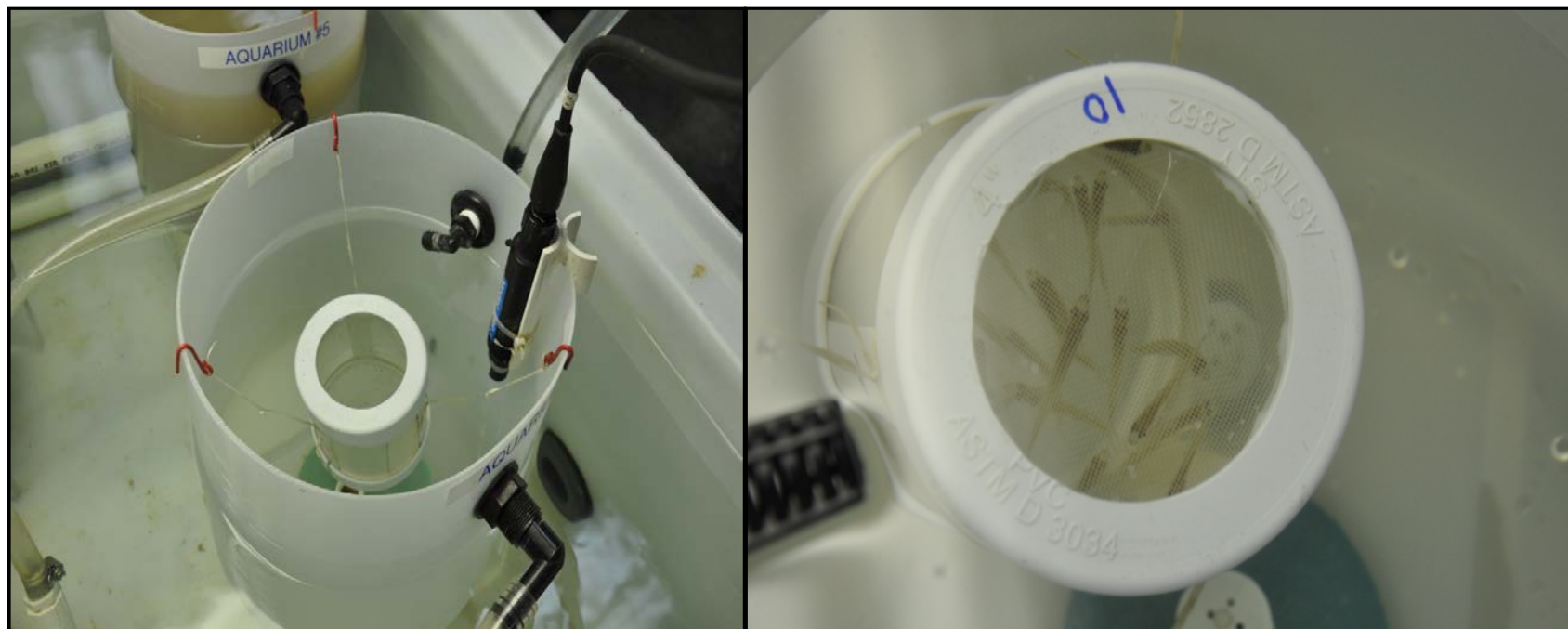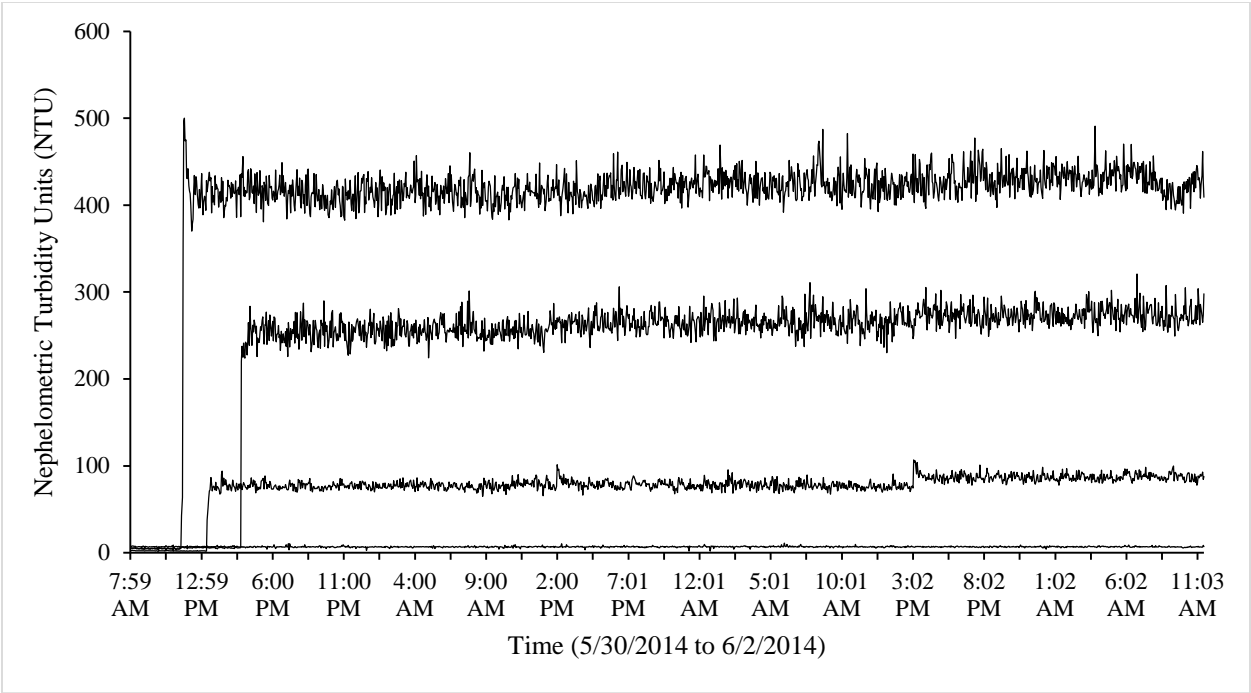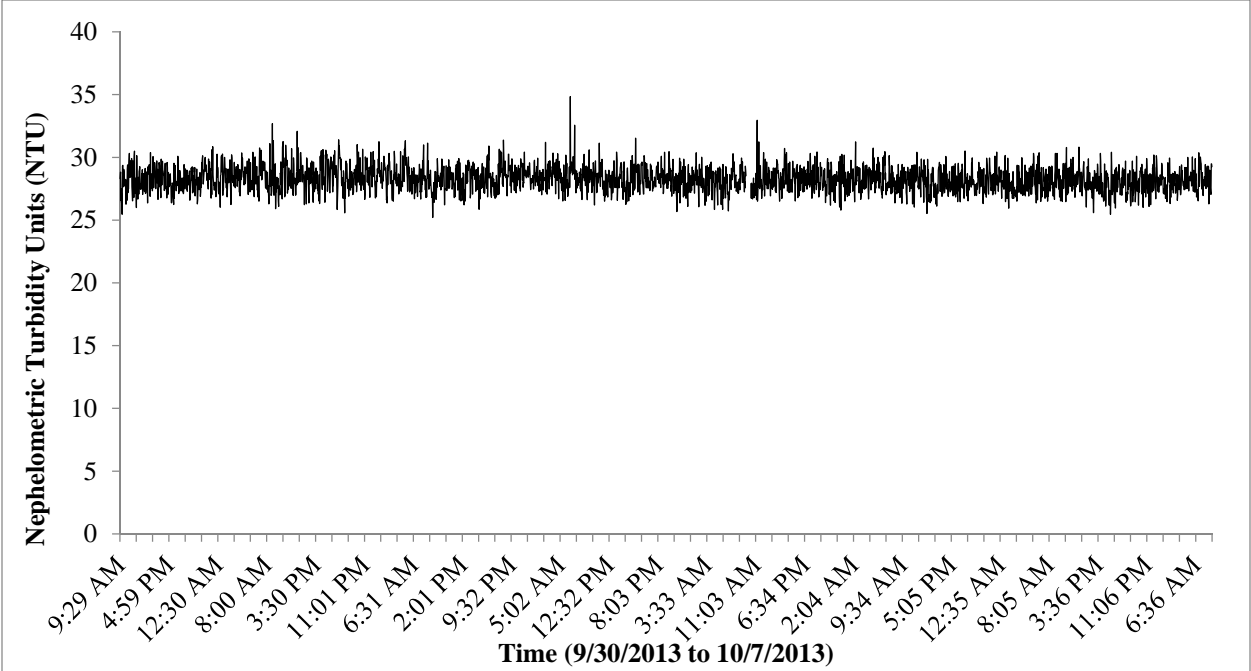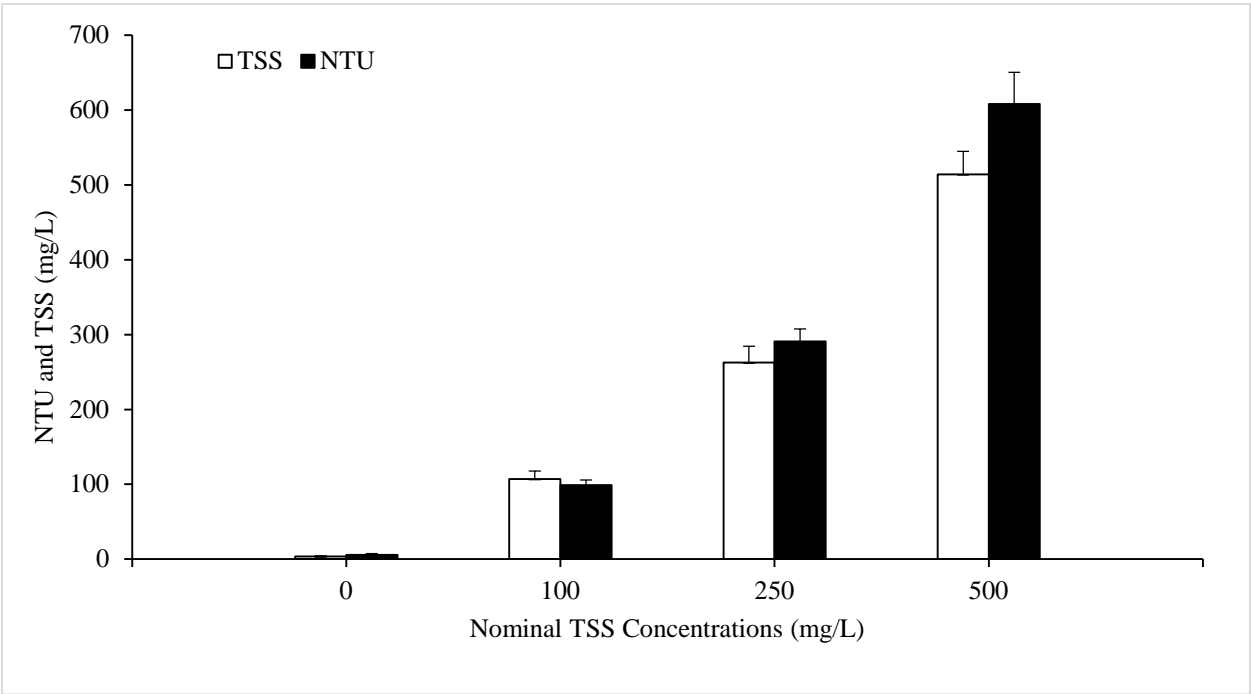chiller, water with self contained pump - for water bath; chiller sits under module
tank, domed bottom - FLEES aquaria - sit inside water bath
tank, stand - acrylic stand, 12 in x 12 in x 6 in - to hold aquaria
urethane, liquid- to seal carboy plug and prevent leaking
pump, magnetic drive - to suspend sediment in each aquarium
light, LED - installed over water bath
pipe, PVC schedule 40 - installed in drain of water bath to control water level
fittings, bulkhead - for aquaria/water bath connections to pumps, drains, water and slurry lines
fittings, quick-disconnect, male pipe threaded inserts - insert in tank bulkhead
fittings, quick-disconnect, valved hose barbs - connection between aquarium and insert in tank bulkhead
fittings, black HDPE threaded elbow - for aquaria vinyl tube connections to slurry/water line and pump
fittings, black HDPE threaded adapter - for connections between pump and tank bulkhead
tube, vinyl - connect slurry/water line to aquaria and to connect pumps to aquaria
tube, vinyl - connect to aquaria drains inserts and water bath drain
clamp, hose, stainless steel - to clamp vinyl tube to hose barbs

Parts list for Slurry System

Description

chiller, water with self contained pump - sits off to side of slurry tank
125 gallon open top cone bottom tank w/Stand - 42 in x 35 in - contains the water and sediment to make slurry
Cover for 125 gallon tank
valve, PVC - connect tank drain to pump - isolate for maintenance
pump, double diaphragm mounted on stand - used to recirculate slurry
sensor, optical backscatter - measure NTU in slurry tank
pipe, PVC Schedule 40 - to recirculate slurry
pipe, flexible PVC - fitted with union and used to connect to next module
union, PVC Schedle 80 Socket - connect slurry line with next module
solenoid, plastomatic (normally closed) - introduce slurry
fitting, PVC tee - connect slurry pipe with solenoid
fittings, 1 in PVC ball valve threaded - shut off for slurry delivery to solenoid/water lines
fittings, 1 in PVC union threaded - connect slurry solenoid to shut off valve
tube, vinyl - connection between water solenoid and slurry solenoid

Parts list for Water System

Description

chiller, water with self contained pump - sits off to side of reservoir
125 gallon open top cone bottom tank w/Stand - 42 in x 35 in - contains the water and sediment to make slurry
Cover for 125 gallon tank
valve, PVC - connect tank drain to water pump
pump, magnetic drive, in-line use - used to recirculate water to aquaria and chiller
solenoid, alco - introduce water
fittings, black HDPE reducer connector - connect 1/4 in hose water line from solenoid to 1/2 in hose
fittings, black HDPE tee - connect 1/2 in hose water line and slurry to aquaria
fittings, street elbow
fittings, PVC threaded pipe nipples - connect union fittings with solenoids and other connections
fittings, union threaded - connect slurry/water lines with next module
fittings, reducer bushing - connect to reducer tee in water line

fittings, threaded pipe nipples - connection between bushing and water solenoid

pipe, PVC - make connections between tank, pump and chiller

Parts list for Sensors, Data Acquisition Device, and Computer Software

Description

software, LabView

SCXI-1001 12-Slot Chassis, U.S. 120 VAC

SCXI 1100 - 32-Channel, ±10 V Analog Input Module

SCXI 1303 - Terminal block designed for high-accuracy thermocouple measurements

SCXI 1102B - 32-Channel Thermocouple/Voltage Input Module

SCXI 1161 - General-Purpose Relay Module

SCXI 1300 - General-Purpose Voltage Module

PCMCIA Card DAQCARD-AI-16E-4

sensor, optical backscatter - measure NTU in each aquarium

Quantity	Size	Company
4	4 in x 4 in x 8 ft	Local vendor
1	3/4 in x 4 ft x 8 ft	Local vendor
1	1/4 in x 4 ft x 8 ft	Local vendor
4	2 in x 4 in x 96 in	Local vendor
1	150-gal	Hydro Composites, LLC, Stockdale, TX, USA
1	1/2 hp	Remcor Products Co., Glendale Heights, IL, USA
5	19 L	United States Plastic Corp, Lima, OH, USA
5	custom	custom built by ERDC shops
5	epoxy kit	Forsch Polymer Corporation, Englewood, CO
5	28 liter per min	March Manufacturing Inc., Glenview, IL, USA
2	based on area to light	C2 Development, Inc., Ames, IA, USA
-	1 in	Local vendor
20	1/2 in FPT x FPT	Lifegard Aquatics, Cerritos, CA, USA
10	1/2 in MPT	Cole-Parmer, Vernon Hills, IL, USA
10	1/2 in	Cole-Parmer, Vernon Hills, IL, USA
20	1/2 NPT x 1/2 in Hose ID	United States Plastic Corp, Lima, OH, USA
10	1/2 NPT x 1/2 in Hose ID	United States Plastic Corp, Lima, OH, USA
25 ft	1/2 in ID	Local vendor
25 ft	5/8 in ID	Local vendor
40	#8	Local vendor

Quantity	Size	Company
1	1/2 hp	Remcor Products Co., Glendale Heights, IL, USA
1	125 gal	United States Plastic Corp, Lima, OH, USA
1	42 in x 35 in	United States Plastic Corp, Lima, OH, USA
2	1-1/2 in	local plumbing vendor
1		Wilden-pumps.co.uk & Air Pumping Ltd., Essex, UK
1	0-1000 NTU	Campbell Scientific, Logan, UT, USA
20 ft	1 in	local plumbing vendor
10 ft	1 in	local plumbing vendor
8	1/2 in	local plumbing vendor
5	1/2 in NPT threaded, 24 VAC	Plast-O-Matic Valves, Inc., Cedar Grove, NJ, USA
5	1 in x 1 in x 1 in slip x slip x FIPT	local plumbing vendor
7	1/2 in	local plumbing vendor
5	1/2 in	local plumbing vendor
50 ft	1/4" ID	Local vendor

Quantity	Size	Company
1	1/2 hp	Remcor Products Co., Glendale Heights, IL, USA
1	125 gal	United States Plastic Corp, Lima, OH, USA
1	42 in x 35 in	United States Plastic Corp, Lima, OH, USA
2	1 in	local plumbing vendor
1	1/12 hp	Little Giant, Fort Wayne, IN, USA
5	24 v, 1/4 in NIPT	discontinued; ASCO, Florham Park, NJ,USA for similar
5	1/2 in hose ID x 1/4 in hose ID	local plumbing vendor
5	1/2 in NPT x 1/2 in hose ID x 1/2 in hose ID	local plumbing vendor
5	1/2 in 90° MIPT x FIPT	local plumbing vendor
12	1/2 in	local plumbing vendor
6	1 in PVC	local plumbing vendor
5	1/2 in male by 1/4 in female FIPT	local plumbing vendor

Quantity	Size		Company
5	4 in long x 1/4 in		local plumbing vendor
5 ft	Schedule 40		local plumbing vendor

Quantity	Size	Company
1	N/A	National Instruments, Austin, Texas, USA
1	N/A	National Instruments, Austin, Texas, USA
1	N/A	National Instruments, Austin, Texas, USA
2	N/A	National Instruments, Austin, Texas, USA
1	N/A	National Instruments, Austin, Texas, USA
6	N/A	National Instruments, Austin, Texas, USA
1	N/A	National Instruments, Austin, Texas, USA
1	N/A	National Instruments, Austin, Texas, USA
5	0-1000 NTU	Campbell Scientific Inc., Logan, UT, USA

Catalog Number	Comments
N/A	
N/A	
N/A	
N/A	
FBT-226	
CFF-500	
5197	
N/A	
60A Liquid Urethane	
MDX-3-1/2 115 v	
Hydra 26	
N/A	
R270900	
EW-31303-36	
EW-31303-11	
62043	
62017	
N/A	
N/A	
N/A	

Catalog Number	Comments
CFF-500	
8586	
8935	
N/A	
P2/PPPP/WF/WF/PTV/400	contact distributor
OBS-3+	
N/A	
N/A	
N/A	
EASYMT4V12R24-PV	contact distributor
N/A	
N/A	
N/A	
N/A	

Catalog Number	Comments
CFF-500	
8586	
8935	
N/A	
3-MD-SC	
N/A	
N/A	
N/A	
N/A	
N/A	
N/A	
N/A	

N/A
N/A

Catalog Number	Comments
LabView 2015 Base	
776571-01	
776572-00	
777687-03	
776572-02B	
776572-61	
777687-00	
N/A - legacy	used cards available online
OBS-3+	

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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