Outdoor mesocosm experiments to improve understanding of risks to environmental health

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Abstract

Mesocosms are outdoor experimental systems designed to separate and test environmental responses, in this case, cumulative effects to field-collected periphyton and macroinvertebrate communities from multiple stressors. This experimental system produces valuable, highly reproducible data, and along with field monitoring, laboratory bioassays and modeling results, provides a strong weight-of-evidence approach for aquatic risk assessment. Through the control of confounding environmental variables, this type of experiment permits the separation of interactions between multiple stressors in complex effluents or due to shifting ambient conditions. The system described is modular and was originally developed to facilitate transport of the entire system to remote or industrial test sites (e.g., for *in situ* 21-d chronic level testing). However, this setup is also appropriate for long term installations with suitable provision for routine maintenance and losses associated with regular wear-and-tear on equipment. The following protocol details a basic stream mesocosm system setup (e.g., 4 replicate streams per treatment-level) which is the foundation for the more than a dozen stream mesocosm experiments conducted by the authors since the 1990s.

Introduction

Stream mesocosms are medium sized (1 to 1000-L) test systems designed to simulate real stream ecosystems. They fill a crucial gap from traditional bioassay techniques to identify, quantify and assess multiple stressors. Laboratory toxicity studies (e.g., LC50), can pinpoint toxicity concentrations, however, studies are traditionally done on single species, and experimental setup does not allow for the interaction of other environmental variables with the test organisms. Field studies can assess effects on aquatic communities, however, in the natural environment it is often difficult to determine cause, as the quantities and interactions between multiple stressors, environment variables and exposure pathways often interact and confound conventional approaches.

Mesocosms are a hybrid of field and laboratory techniques that allow for the control of stressors (single, or multiple), and of key environmental parameters, such as water temperature and water chemistry, light and diel temperature cycles, that occur naturally to create greater field relevance than laboratory tests. These types of studies tend to generate high quality biological data the results of which can then be used in applications such as environmental risk assessment. Some of the most significant whole-ecosystem experiments that have ever been conducted were only possible after extensive mesocosm studies (Schindler 1998). The system described below has tested the effects of the interactions of multiple stressors on small fish, benthic macroinvertebrate and periphyton communities and continues to be an important tool for environmental risk assessment (European Commission 2014) (**Fig. 1**).

Aquatic macroinvertebrate communities are ideal for assessing environmental degradation as they are highly sensitive, widespread and generally accessible for collection. Essential to ecosystem function, these organisms are also an integral part of the food web as they feed on leaf detritus and periphyton attached to cobble substrates. This is convenient, as

small cobbles are readily transported and inoculated into the artificial streams to serve as a natural and familiar food supply for introduced organisms. Macroinvertebrates also serve as important foodstuffs for many fish species.

Although some small fish studies have been conducted in similarly sized systems; larger organisms tend to require larger streams (upwards of 300-L) which are more expensive to run and more difficult to operate and transport. Typically, larger mesocosm installations sacrifice replication for large-scale, single-treatment mesocosms (e.g., marine mesocosms in Alexander et al. 2016). By contrast, the method described below favours replication over size. Our combined approach uses a weight-of-evidence technique to integrate findings from field and laboratory studies to offer insight in complex systems.

There are four stages in a mesocosm study:

study design – initial considerations before the experiment starts, including: what type of experiment (press or pulse), sub/lethal, stressor(s) to be tested, concentration(s) of interest, randomization required, schedule, substrate and benthic community sourcing and procurement of necessary equipment.

setup – getting the mesocosm ready for the experiment, including: site preparation, construction, mesocosm assembly, transport, establishment of treatments, cobble and gravel substrate inoculation, periphyton establishment as well as invertebrate community collection, subsampling, inoculation and establishment.

routine maintenance – maintaining and monitoring the mesocosms is required throughout the duration of the study. This includes: water sampling, routine flow checks, modifying flow depending on treatment regime and emergent insect collection, and

takedown – coordinating the end of the experiment, including: collection of final water samples, as well as preservation of benthic macroinvertebrates and periphyton communities. Clean up equipment and test site also occur during this stage.

The following procedure focuses on the setup stages of a mesocosm deployment including initial setup, power and water, substrate collection and macroinvertebrate inoculation. Since mesocosms are very customizable, variations in design and approach will alter system layout, required equipment and supplies. More complex study designs in **Associated Publications** are all based on the artificial stream setup described in this protocol. Next steps as well as suggestions for as to routine maintenance and takedown of this system are addressed in the **Troubleshooting** section.

Reagents

- Chemicals to be tested at appropriate concentrations for each stock tank e.g., $NaNO_3$ (s) (99.0 %; S8170-1 KG, Sigma-Aldrich, MO, USA) or NaH_2PO_4 (s) (98.0 %; S9638-1 KG, Sigma-Aldrich, MO, USA) for simulating different nutrient gradients

- Ethyl alcohol (95.0 %; #493511; Sigma-Aldrich, MO, USA) for preservation of benthic macroinvertebrate samples.

- Formalin (10%; F5304 or F5554; Sigma-Aldrich, MO, USA) or suitable alternative (e.g., propylene glycol; P4347; Sigma-Aldrich, MO, USA) preservative for periphyton samples.

Equipment

2.1 Initial setup

- Large open field, levelled (e.g., 10-m x 10-m or larger)

- Mesocosm tables, replicate artificial streams (n = 4 to 8 replicates per treatment level), tray with drain hole (n = 1 per treatment level), manifold (n = 1 per treatment level) and reservoir (n = 1 per treatment level).

2.2 Power and water

- Power supply (e.g., 200-amp panel)

- Water supply – well, river/lake or stock tanks to store delivered water. Water supply should be similar to that of test ecosystem.

- Metered diaphragm pumps such as PulsaFeeder Pulsar 25H (www.pulsafeeder.com) or gravity fed for maintaining chemical concentrations

- March Circulation pump (LC 3CP MD 115V, https://www.marchpump.com) with a box of standard 3 prong plug ends, to deliver treated water from the reservoir into the manifold under pressure.

- Flexible vinyl tubing (various sizes, dependent on pumps selected)
- Chemicals/effluent to be tested see **Reagents**.
- Detailed equipment lists for power and water supply can be found in **Supplementary Files**

2.3 Substrate and benthic macroinvertebrate community collection

- Waders
- Shovels to field collect gravel
- Sieves (2 mm, 4 mm and 9.5 mm) to separate gravel into fine gravel (2-4 mm) and coarse gravel (4-30 mm)
- Field collected cobblestones (5-8 cm, 5 stones per replicate stream)
- Fine forceps
- Hand-held macroinvertebrate sampling nets of known surface area (see Fig. 1a)
- Sample jars suitable for transporting live benthic macroinvertebrates (e.g., medium square Ziploc® containers)
- Indelible marker or wax crayon to mark subsampler set and replicate
- Air stones for each sample jar
- Large coolers to transport substrates, periphyton and collected benthic macroinvertebrate to test site

2.4 Deployment, routine maintenance and experimental takedown

- Large graduated cylinder (1000-ml or larger) to measure flow rate
- Water quality multimeter (e.g., YSI ProDSS E-528-6262870-1, Hoskin Scientific, Oakville, Ontario, Canada)
- Scalpels or toothbrushes for collection of periphyton community from cobbles
- Small sample jars suitable for preserving frozen periphyton community samples (20-ml Polyethylene vials #58500-20, Kimble Chase Glass Co., Vineland, NJ, USA)

- Portable freezer (Engel fridge/freezer MT35F-U1, Sawafuji Electric Co. Ltd., Tokyo, Japan)
- Large sample jars suitable for preserving benthic macroinvertebrate community samples (e.g., 1 L pre-cleaned HDPE wide mouth jars #05-719-738, Fisher Scientific, New Hampshire, USA)
- Preservative for benthic macroinvertebrates see **Reagents**
- Fine forceps

Procedure

3.1 Initial design and setup

Study site selection is based primarily on how accessible the test site is (e.g., availability of a level field of suitable size). Availability and quality of a water supply as well as the potential access to power are important considerations. Security of the study site can also be a factor because mesocosm studies typically run for extended periods (3 or more weeks). Thus, safe access to the site for the setup, experiment and takedown periods is required. Distance from the test site to a suitable location for collection of the test organisms and substrates should also be considered well in advance of deployment. Reconnaissance trip(s) to find the appropriate collection site well before the experiment is highly advisable. All biological collections should be at a reference area with representative diversity of organisms (Fig. 1a).

Our modular mesocosm approach (**Fig. 2**), was originally designed to be transported to test sites with groups of up to eight (8) replicate streams arranged on treatment tables (Fig. 2a). These treatments were initially attached to supporting wooden pallets for easy transport by forklift (see also Culp et al., 2003 in Assoc. Publications). Early studies using this method focused on effluent gradients from pulp mills and metal mining to assist in the determination of underlying causes of toxicity in these industrial effluents (Environment Canada 2010; 2012). Subsequent experiments have looked at gradients of priority and emerging contaminants to determine safe levels of these substances in the environment or to unravel confounding results due to natural gradients such as nutrient masking of effects in field applications (Alexander et al. 2013).

Each replicate stream has a planar area of 0.065 m^2 and a 10-L volume. Each cylinder independently simulates environmental flows while the shorter cylindrical design reduces edge effects on stream periphyton and macroinvertebrate communities (**Fig. 3**). The rounded edges also allow for reduced wall growth, increased animal movement and better contaminant mixing. Rectangular tanks create backflows and unusual eddies that are widely thought to be more stressful for higher densities or more active organisms. The implications of scale to environmental problems using mesocosms is reviewed in

Petersen et al. (2009). Often, however, the weaknesses of the system appear to be directly linked to weaknesses in the study design. This issue is not unique to mesocosm studies.

3.2 Power and Water

River or treatment water is fed into the polyethylene table reservoir before it's drawn towards the circulation (March) pump (**Fig. 4**). The water flows through the lower valve of the table reservoir into the March pump where it is pumped into the treatment manifold intake. The second manifold intake is connected to one of the upper valves on the table reservoir which is closed to create system pressure. From the treatment manifold, the water is pushed through the lower hose barbs into each of the stream mesocosms. The water then enters the artificial stream, fills up the cylindrical basin while a motorized stirrer rotates internal paddles to generate instream flow conditions similar to 2^{nd} to 4^{th} order streams. Water then overflows the basin emptying onto the tray where it then drains back into the table reservoir via two cylindrical funnels built into the tray (Fig. 4). Water is exchanged in each replicate stream is typically exchanged every 7-min. The tray discharge then either returns to the reservoir for continued recirculation while the reservoir is completely exchanged every 1-4 hours depending on the temperature needs of the study design. Waste water (either from the table or the reservoir tank) is transported through solid black corrugated loom tubing to a separate wastewater holding tank. Wastewater is then passed through carbon filters (e.g., Culligan Inc., activated carbon filter cylinder, Moncton, NB, Canada) to remove contaminants before any water is discharged to the environment.

3.3 Substrate and benthic macroinvertebrate community collection

Prior to initiating the experiment, benthic substrates are introduced into each replicate stream. Inoculating each stream with a mixture of gravel (fine and coarse) and cobblestones creates an ideal substrate for benthic macroinvertebrates. Gravel and cobble should be collected from a pristine site (e.g., un-impacted location). Mixture of substrate sizes should also reflect the simulated riverine habitat to be tested and preferably be collected near (e.g., downstream) of the benthic macroinvertebrate collection site. Cobble should also be gently hand washed to remove attached invertebrates while maintaining the preexisting periphyton community. This procedure establishes a lotic substrate consisting of a 2-3 cm layer of gravel plus surface cobblestones covered with periphyton similar to the original habitat of the benthic community examined.

3.3.1 Collection of substrates

Use shovels for gravel substrates collection. Fine and coarse gravel are shoveled, sieved and transported to the mesocosm test site. Transfer should be done relatively quickly, as the biofilm on each rock is alive and vulnerable to desiccation or compositional shifts with changing water temperature.

Each replicate stream receives the same volume of fine and coarse gravel of the volume and size measurements below (see Fig. 1c). Mix gently by hand to evenly distribute the amounts of fine and coarse gravel in each replicate stream (Fig. 1d, 3b, 4).

Each replicate stream receives:

250 mL of >2mm to <4 mm of fine gravel

750 mL of >4 mm to <9.5 mm of coarse gravel

Thus, to inoculate 32 streams with substrate:

8-L total, of >2mm to <4 mm of fine gravel is needed (250 ml x 32)

24-L total of >4 mm to <9.5 mm of coarse gravel is needed (750 ml x 32)

3.3.2 Collection of cobble substrates

Typically, in a separate trip, cobbles (5-8 cm in diameter) are collected for each artificial stream. Cobbles will be carefully selected from the stream bottom, gently hand rinsed, and inspected to remove visible invertebrates with a minimum disturbance of the biofilm. Place cobble in a cooler maintaining original rock orientation with algae covered side facing up. Cobble should be covered in water during transport to maintain periphyton community.

We recommend 5 cobbles (approximately 5-8 cm diameter) be placed in each replicate stream on top of the gravel substrate. Systematic allocation of cobbles to each stream is recommended to minimize size bias. Allow 3-5 days for periphyton colonization, dependent on ambient temperatures before inoculating macroinvertebrates to prevent starvation.

3.3.3 Collection and inoculation of macroinvertebrates

The benthic macroinvertebrate assemblage should be collected from a single riffle (e.g., < 10 x 10 m, water depth < 30 cm). Collecting the benthic community from a small defined area reduces the high inherent variability in diversity and abundance. The collection site should be near, preferably upstream of the gravel and cobble collection site (without being in the disturbance zone) as the organisms will already be acclimated to nearby substrate and its biofilm. In our research, we have at times used the same collection site for several years further increasing our understanding of that system and the organisms that reside there as well as facilitating comparisons between experiments over time.

The benthic macroinvertebrate assemblage should also be collected using a sampling device with a known area to facilitate an overstocking of ~10-20% in the event of any mortality due to handling or transport of organisms to the test site (Fig. 1b). We have used U-nets (area = 0.06 m^2) for this purpose and have developed a subsampling procedure to inoculate as similar a benthic macroinvertebrate assemblage into each replicate stream at the test site as possible (Fig. 1). This subsampling process has been described in detail elsewhere (e.g., Alexander et al. 2013; see also Associated Publications). In brief, the subsampling procedure consists of collecting a series of U-nets by a team of samplers working together (Fig. 1a). The number of U-nets is determined by the need to overstock by ~10% to offset any mortality of macrobenthos in the transport from the river to the test site. For example, if 100 streams are to be inoculated, 110 U-nets would be required. Both the number of streams to be inoculated and how many subsamples can easily be 'split' in the subsampler alter the number of subsamples required (e.g., see 4-way pie plate subsamples in Fig. 1b). As the number of streams used in an experiment depends on the replication required *a priori* power analyses is highly recommended to determine whether expected effects would be detectable given the variability in the subsampled community. Please also note, other types of nets can also be used for collections but the relative density of organisms in the test system should not be greater than 20% overstocked compared to the riverine community.

Once macroinvertebrates are collected, sample containers must be kept cool and transported to the mesocosm site as quickly as possible. Minimizing water temperature differences between the river water and the artificial stream water is highly recommended. Once the macroinvertebrates are at the test site, they should be transferred into individual artificial streams using a randomized pattern. It is important to make note of which artificial stream receives which subsample to help with later data interpretation. Turning off the motorized stirrer (Fig 1c,d; 3a,b) also helps invertebrates to settle into the substrate unimpeded during the inoculation transfer. Once macroinvertebrates are introduced, attach emergence traps (e.g., 400 Nitex© mesh, Aquatic Ecosystems Inc., Apopka, FL, U.S.A.) or other barriers to prevent invertebrate escapees (e.g., Fig. 2a). Introduce treatments only after all of the above is functioning as expected. Generally, allow ~2 days for macroinvertebrates to settle before initiating treatment.

Troubleshooting

4.1 Initial setup

- Unpack, assemble and connect piping and electrical components. Confirm assembled parts connect and/or operate as expected.

- Turn on water and verify stream functionality and adjust tubing and electrical as needed.

- Deploy temperature loggers or other equipment. Check that data is logging correctly and that parameter values are as expected prior to initiating the experimental treatments.

- Allow streams to run for an extended period (e.g., at least a half day) to confirm proper functioning. Check water levels in each stream and / or reservoir (if using) and adjust or re-calibrate flow rates to desired levels.

- Allow the system to run for a couple of days once substrates are in place to support the growth of the periphyton community prior to the inoculation with macroinvertebrates.

- Attach emergence traps once macroinvertebrates are introduced (e.g., 400 Nitex© mesh, Aquatic Ecosystems Inc., Apopka, FL, U.S.A.).

- Introduce treatments only after all of the above is functioning as expected.

4.2 Routine maintenance

- System should be checked daily and detailed notes made.

- Flow rate and temperature should be checked daily. Pump settings may need to be regularly adjusted in order to maintain required flow rates over the course of the experiment.

- Inspect for visual differences between streams (e.g., algal growth) should also be noted in a field notebook or on the daily log sheet.

- Take water samples periodically to confirm desired concentrations as well as routine measurements such as major ions, pH, turbidity etc.

Collect emergent insects.

4.3 Experimental takedown

- Any experimental treatments (e.g., contaminants) should be stopped during takedown to protect personnel from accidental exposure.

- Experimental takedown should be completed in one day which may require coordination of a large team (e.g., 6-12 persons) working together to collect water, periphyton, and macroinvertebrate community samples.

- Disassembling and cleaning should be completed as soon as possible and preferably during the experimental takedown.

Time Taken

Anticipated Results

Mesocosm experiments tend to reduce uncertainty of cause through the high-level control of multiple stressors, yet allow for the aquatic community to be exposed to natural environmental fluctuations allowing for meaningful, reproducible data. The advantages of our method include increased control compared to field studies while simulating natural processes all without harming natural systems. Mesocosm data is often easier to collect, process and interpret as each treatment-level is sufficiently replicated to identify trends. Some estimates from previous studies suggest that our statistical power for large effect sizes are greater than 90% (e.g., Beta > 0.9, alpha < 0.05). However, the cost of these experiments often dictate that these approaches are only employed where a high level of certainty is required. To date, this approach has been used to support the development of guidelines for mixtures of pesticides and tease apart interacting responses of real communities to complex effluents in combination with environmental gradients (see Associated Publications).

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Figures

Figure 1

Field collection of aquatic macroinvertebrate community (a), pie plate subsampler of benthic macroinvertebrates (b), adding substrates to replicate streams (c), inoculated replicate stream ready for treatment dosing (d).

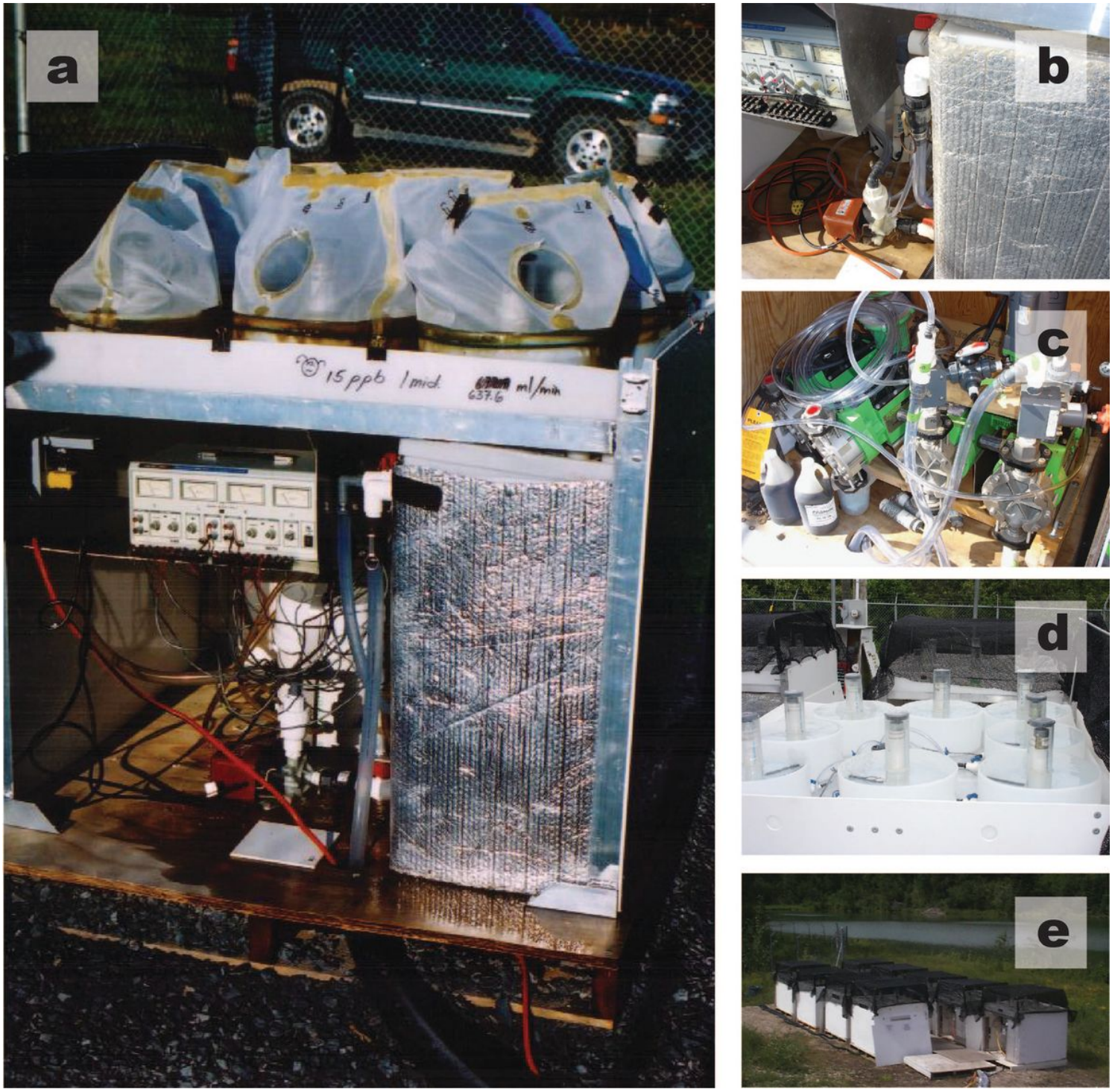

Figure 2

Overview of stream mesocosm equipment. Stream mesocosm table on forklift pallet (a). Closeup of stream reservoir and power supply (b). Dual Pulsar Pump (PulsaFeeder Pulsar 25H, www.pulsafeeder.com) with injector heads (c). Multi-table setup prior to inoculation (d). Nine tables riverside in northeastern Alberta (e).

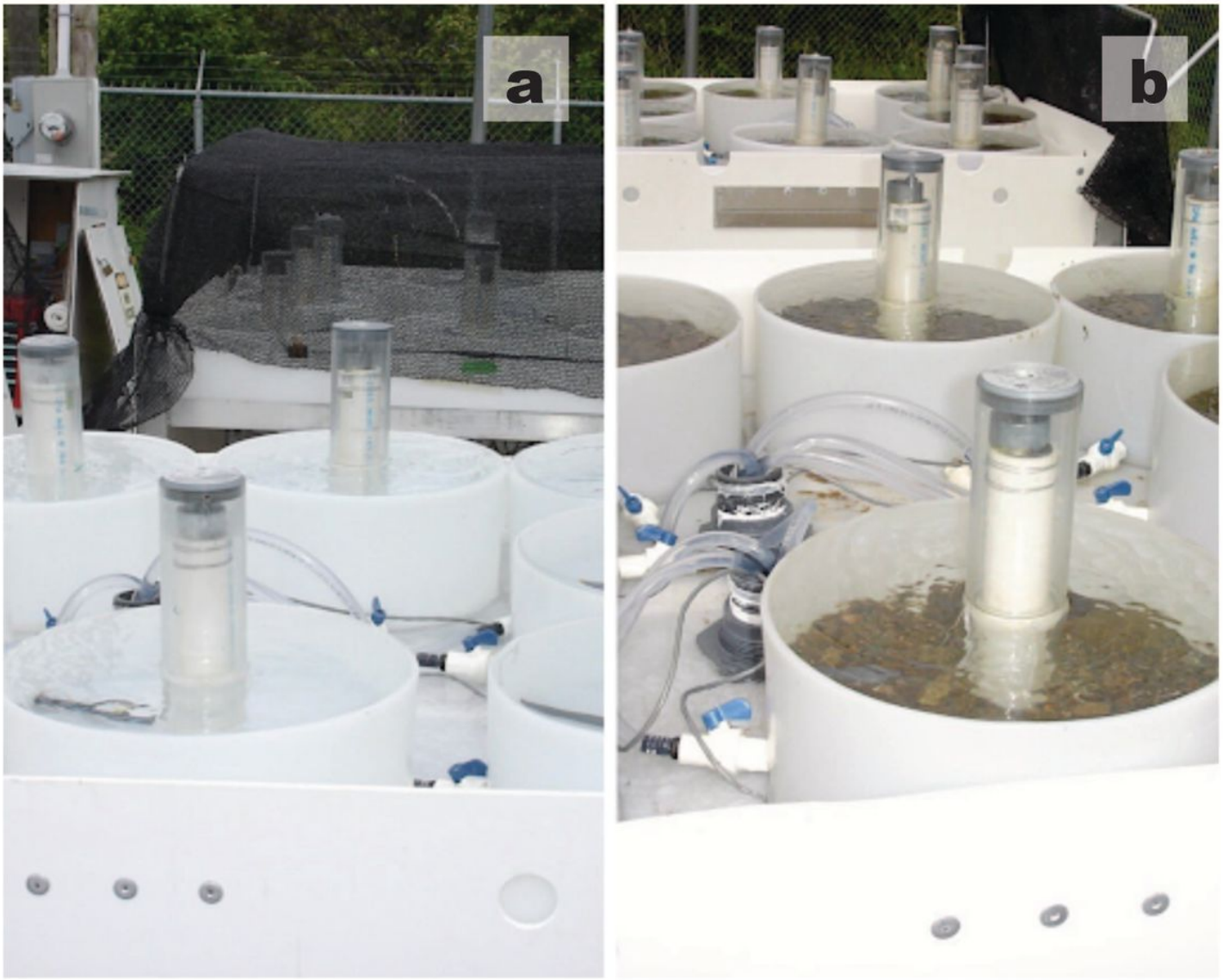

Figure 3

Modular stream table approach with eight (8) replicate streams; No experiment running and prior to substrate and invertebrate inoculation (a), Experiment running with substrates and inocula (b).

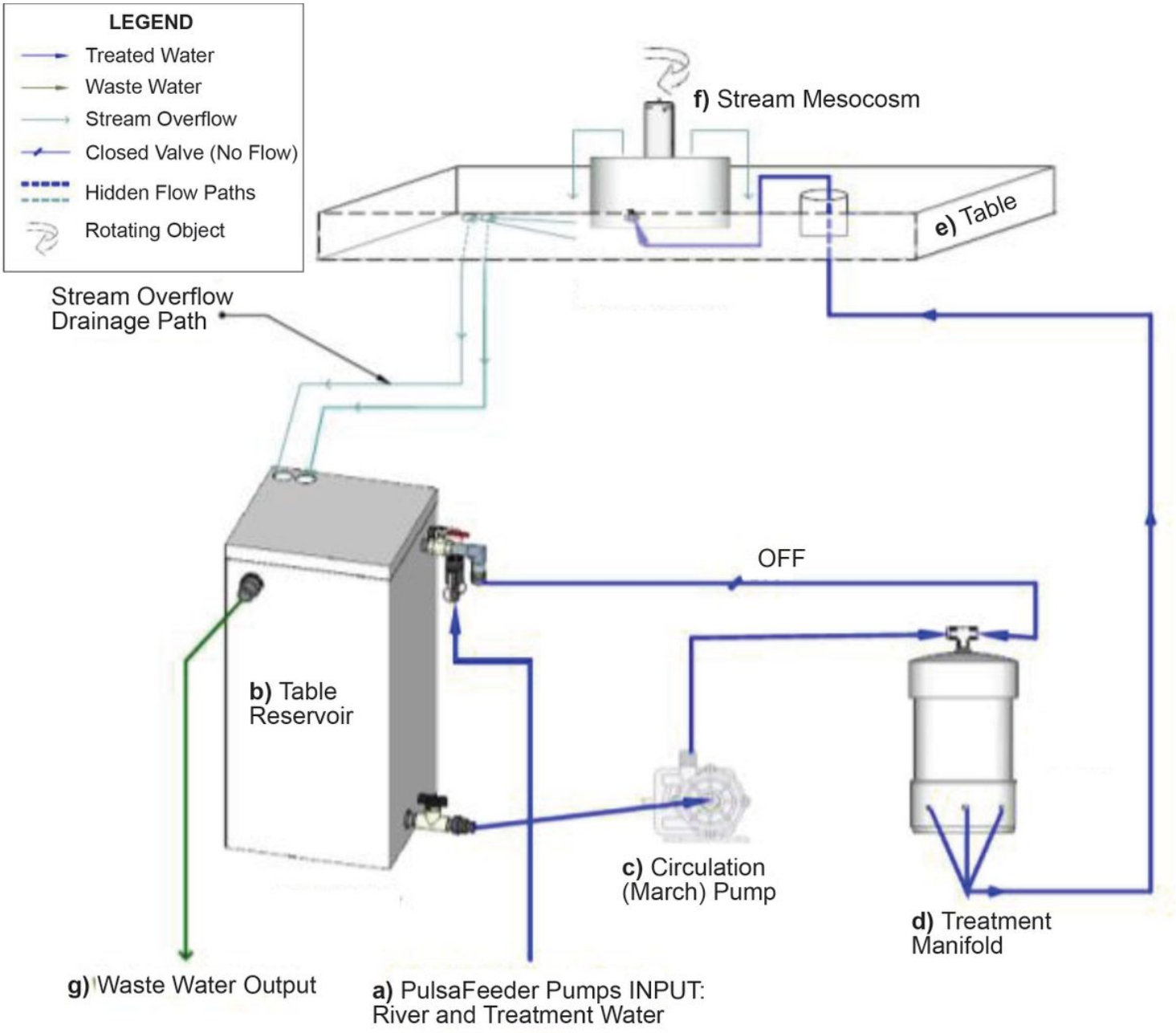

Figure 4

Detailed schematic and process diagram of a mesocosm table with a single replicate artificial stream. The flow of water is as follows: source water is fed into (a) the polyethylene table reservoir (b) then drawn from the lower valve towards the circulation (March) pump (c). Water is then pumped into the treatment manifold intake (d) and pushed through the lower hose barbs onto the table above (e) and into each replicate stream (f). Water then overflows the basin emptying back onto the table tray (e) where it can then drain back into the table reservoir (a) via two cylindrical funnels within the floor of the tray. Water can be recirculated from the reservoir or discharged as waste (g). In a typical setup water is completely exchanged from the table reservoir every 1 to 4 hours depending on the temperature needs of the study. Water exchange in each replicate stream is much faster and can be in the range of every 7 minutes (see Associated Publications). Additional schematics can be found here: Scaled drawings.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFilesformesocosmsv2.docx