Artificial stream channels for ecological experiments:

Design, use, and example applications

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Abstract

Artificial streams are effective tools for ecological research, as they offer unique opportunities to address significant issues in stream ecology. However, the current literature lacks a detailed primer on the construction and use of artificial streams for ecological inquiry, which limits opportunities for investigators to incorporate these powerful tools into their research. Our objectives are to provide a starting point for those interested in constructing and using replicated artificial stream mesocosm experiments and to encourage their use in future research on stream ecosystems. First, we discuss the advantages and potential applications of artificial streams to address key questions in stream ecology. Next, we provide detailed design plans for a recirculating artificial stream array, with advice on construction, arrangement, and maintenance. Finally, we provide guidance on strategies for experimental design, sampling, and data analysis based on examples from recent research, as well as a list of "lessons learned" from our experience using artificial streams. This overview of constructing and using artificial streams can help support the creative development of new ecological insights and experimental approaches for robust measurements of factors driving complex interactions in stream ecosystems.

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Introduction

The use of mesocosms has a long history in aquatic research because they enable the implementation of controlled, manipulative experiments, usually outdoors, to address basic ecological phenomena such as species interactions, community dynamics, and ecosystem processes (Graney 1993; McIntire 1993). Odum (1984) described mesocosms as a valuable experimental approach for revealing basic properties of the whole ecosystem that can bridge the reductionist and holistic approaches of population and ecosystem ecologists, respectively. We define artificial streams as mesocosms that have flowing water, which is the defining characteristic of lotic ecosystems. Artificial streams provide a middle ground between microcosms (e.g., small bottles or aquaria) and whole-stream experiments in the field (Lamberti and Steinman 1993; Matthews et al. 2006). Other significant advantages of artificial streams include the ability to replicate treatments, to control a wide range of variables, and to experimentally amend streams with a range of anthropogenic contaminants or organisms, which may not be permissible or ethical in field experiments.

Replication is a considerable challenge for conducting manipulative field experiments in situ, and a major advantage of artificial stream experiments is that treatments can be replicated. Even streams in close physical proximity (e.g., located in the same sub-watershed) can vary in key physicochemical, structural, and biological characteristics (Hoellein et al. 2007, 2013; Entrekin et al. 2007, 2008), making it nearly impossible to achieve replication of selected attributes in field experiments. In fact, many manipulative stream studies have faced significant challenges during peer review because of the issue of pseudoreplication (Hurlbert et al. 1984). Work-around approaches such as comparing a stream before and after manipulation are complicated by spatial and temporal variability in environmental conditions and require large

amounts of data for statistical evaluation (Roberts et al. 2007, Entrekin et al. 2009).

Manipulations conducted at larger scales (e.g., reach, ecosystem) are often used to obtain more

realism at the expense of replication (Carpenter et al. 1987). One of the main advantages of

artificial streams is the ability to replicate and provide robust experimental controls by

establishing multiple artificial streams running in parallel in a single location (i.e., an artificial

stream array; Fig. 1). Artificial stream arrays permit researchers to use individual artificial

streams as experimental units, and thus replicate treatments and controls, improving statistical

power to detect treatment effects that are not attainable in whole-ecosystem manipulations.

Artificial streams enable manipulation of many fundamental drivers of ecological

processes, such as temperature (e.g., exploration of Q_{10} relationships), light (e.g., construction of

irradiance curves), nutrient stoichiometry (Stelzer and Lamberti 2001), velocity (e.g., Cook

2014), biotic community composition (e.g., Cardinale 2011), and disturbance (e.g., Peterson and

Stevenson 1992; Holomuzki and Biggs 2000), all of which are challenging to manipulate

effectively, realistically, or consistently in natural stream channels. Additionally, interactive

effects (i.e., synergistic or antagonistic) can be difficult to separate in situ because of

confounding factors. Artificial streams enable research on the individual and combined effects of

multiple variables simultaneously, such as the combined influence of light and nutrients on

species composition. Artificial streams also allow researchers to evaluate mechanistic links

between cause and effect in aquatic ecology. For example, ecological processes can be examined

under various conditions of climate change and drought (Ledger et al. 2012). Finally, artificial

streams make it relatively easy to manipulate organismal population densities without risk to the

ecosystem (e.g., abundances of predators and/or prey, introduction of non-native or invasive

species), and one can label and monitor individual organisms over time. Whole-ecosystem

manipulations of temperature, drought conditions, or community composition may be logistically challenging or unethical (e.g., introduction of invasive species), although in situ experimental enclosures have been effectively used (Lamberti and Resh 1983; Chick et al. 2008; Schofield et al. 2008).

Another valuable application of artificial stream approaches is the investigation of the ecosystem effects of a wide range of anthropogenic contaminants, which are a growing concern in stream ecology (Malmqvist and Rundle 2002; Rosi-Marshall and Royer 2012). Anthropogenic contaminants include those that have been widely documented in streams, such as pharmaceuticals and personal care products (Kolpin et al. 2002), pesticides (Wauchope 1978), metals (Rice 1999), and nutrients (Alexander and Smith 2006). In addition, there are contaminants of concern whose environmental concentrations and potential ecological effects have not been well-documented, such as genetically-engineered toxins (Rosi-Marshall et al. 2007), nanomaterials (e.g., carbon nanotubes, metals, and metal oxides; Klaine et al. 2008; Bernhardt et al. 2010; Tong et al. 2015), microplastics (McCormick et al. 2014), and anthropogenic litter (McCormick and Hoellein 2016). The effects of anthropogenic contaminants on stream ecosystems can be difficult to measure in situ because of low concentrations, co-occurrence of additional chemical and physical stressors, and the ethical and legal limitations of contaminant release at field sites, especially for long-term studies. Indeed, artificial streams may be the ideal method to explore the effects of contaminants on stream ecosystem processes or stream organisms without causing environmental damage (Kosinski and Merkle 1984; Relyea and Diecks 2008; Griffiths 2011; Rosi-Marshall and Royer 2012). For example, researchers can add potentially toxic contaminants to artificial streams including trace metals (Clements et al. 1989a, 2013), engineered nanomaterials (Kulacki et al. 2012; Ozaki et al. 2016; Binh et al.

2016), antimicrobials (Drury et al. 2013), pesticides (Relyea and Diecks 2008), pharmaceuticals (Hoppe et al. 2012; Lee et al. 2016; Richmond et al. 2016), or anthropogenic litter (e.g., plastic; Hoellein et al. 2014), and measure the effects on organismal physiology, ecological processes, or species interactions. These compounds can be added singly or in mixtures to assess antagonistic or synergistic effects, and concentrations of contaminants can be carefully controlled to assess dose-response relationships. Finally, artificial streams allow researchers to assess the potential ecological impacts of contaminants before they reach the environment. With careful consideration of transferability of the experimental results to nature and coupled with additional verification in the field (see 'considerations for scaling up' below), artificial streams offer opportunities to predict ecological consequences and potentially provide guidance to industry and regulators in the development and use of these contaminants.

Stream design and construction

Construction material: fiberglass or PVC canvas

We use an artificial stream design (McIntire et al. 1964; Steinman and McIntire 1986) that recirculates water with a paddle wheel to simulate the flowing water of a stream (Fig. 1). In contrast to flow-through experimental streams (e.g., Matthews et al. 2006), recirculating artificial streams can be used to address research questions that benefit from a closed-system approach (described below). These artificial streams are large mesocosms with a centerpiece that forms a raceway around which the water circulates. Streams can be constructed from a variety of materials, depending upon researchers' needs for permanence and replication. For permanent installations, we use streams made of fiberglass, constructed from a mold as replicate units. We recommend purchasing and saving the mold for future manufacture of additional streams. In Fig.

2, we provide the measurements for stream channel arrays at our institutions (i.e., Cary Institute of Ecosystem Studies, Millbrook, NY, Loyola University Chicago, Chicago, IL and University of Florida, Gainesville, FL), but the dimensions can be modified to suit the available space. Stream channels can be placed on the ground or on tables/frames. Streams placed on the ground, buried in the ground, or enclosed in thermal jackets will maintain more consistent temperatures than streams exposed to the air, so researchers may consider using insulation for thermal consistency. Our stream arrays have 5-6 paddle wheels attached to a single metal rod that is turned by a motor to recirculate water in multiple streams at the same time (Table 1; Fig. 1C). We note that continuous operation of motors required for long experiments requires motor maintenance and may necessitate spare motors in case of malfunction. We add water to the streams with a hose, and remove the water with a vacuum pump with a hose attachment covered with mesh netting on the inlet to prevent removal of organisms and rocks. However, a standpipe with plumbing could be installed at the time of manufacture to simplify water replacement or to make a modified flow-through system; the incoming flow rate and total volume will determine the turnover time of the water in the stream. If the use of a standpipe is desirable, it is also necessary to have a metered water source (see 'water source and maintenance' below). Although it would be relatively straightforward to install drains or standpipes into the streams, we keep them as "closed" systems with no drains to reduce the risk of leaks.

For a temporary artificial stream installation, we have constructed stream channels from polyvinyl chloride (PVC) canvas (Table 1), commonly used as the base layer of tents, which is waterproof, inert, and flexible. PVC streams are mobile, and advantageous when space is only temporarily available or for experiments in multiple locations. We construct our PVC streams by cutting an oval shape for the bottom of the stream floor and two long straight pieces to form the

inner and outer sides of the streams. We cut the pieces with an overlap of 5 cm (2 inches) and use a heat gun to melt the inner and outer sides to the bottom piece of PVC canvas to form the stream. Because PVC canvas is very flexible, the streams require supports to hold the sides of the channels upright when filled. We use thin-gauge, stainless steel sheet metal for the supports. We cut the metal into strips the same height as the stream walls and attach supports to the outside of the PVC canvas with plastic clothespins for rigidity (Fig. 3D). Incomplete sealing of the seams can cause leaks, which are sealed with silicon, liquid tape, or a heat gun and a PVC canvas patch. We recommend filling streams and searching for leaks before experiments begin.

Paddle wheel design and motor speed

We use motor-driven paddle wheels to move the water in both fiberglass and PVC streams. We align 5-6 individual paddle wheels connected by a central rod to the motor, with each individual wheel containing 5 paddles (Fig 2B). The central rod should be strong and straight, as a bend will significantly alter the turning of the wheels. The rod should be aligned so that each paddle wheel is immersed in water to the same depth in each stream, typically 3-6 cm. We use sealed bearings attached to supports between every other stream to hold and align the central rod (Fig. 3). A single motor (Grainger Dayton DC Gearmotor 22rpm 90V, Lake Forest, Illinois, USA) is capable of a maximum of 22 rotations per minute (rpm) under full load with 280 inches per pound of torque (~323 cm per kilogram), and can recirculate water in up to 5-6 experimental streams without excessive torsion of the central rod or physical strain on the motor. We use a handheld laser photo tachometer (Electronic Specialties 332 Pro Laser No-Contact Photo Tachometer, Genoa City, Wisconsin, USA) to measure motor speed and ensure consistency between separate rows within the stream array. We constructed a wooden box stand

to place the tachometer directly in front of the motor rod, to which we applied reflective tape. To measure motor speed, we point the tachometer laser beam at the reflective tape (Fig. 3B). We typically use speeds of 15-20 rpm, though this range varies by stream, motor design, and paddle wheel dimensions. Speeds below 10 rpm can damage the motor, while speeds above 20 rpm may cause the paddle wheels to splash water out of streams. The maximum water velocity we could obtain without splashing was 0.7-0.8 m s^{-1} (with a depth of 10 cm and volume of 60 L). Faster water velocity could be used, but may require less water volume, taller channel sides, deeper immersion of paddle wheels, or installation of baffles in front of the paddlewheels. We recommend checking motor speed regularly, especially when multiple motors are in use simultaneously. Regular side-by-side visual comparison of paddle wheel movement can also help detect speed alterations, so visibility of the paddle wheels is important. If shade cloth for manipulating light levels or mesh netting (see section on invertebrate colonization below) is used to cover the streams, a frame or other structure should be constructed around the paddle wheels to prevent objects from getting caught (Fig.1A-B). In our experience, motor failure is typically caused by deterioration of bushings, which can be easily repaired. Of course, care should be taken to ensure that any objects placed in the channels do not get trapped by the paddles, which could damage the paddle or the motor.

Location for artificial stream arrays

When deciding where to build an experimental stream array, we recommend considering both operational and biological factors. Experimental stream arrays can require a considerable amount of space. Indoor spaces allow for control of environmental variables, including light, temperature, and rainfall, and are likely to have continuous power, water sources, and plumbing

options. Greenhouses work well for stream arrays, but controlling temperature by opening windows may allow animals to colonize from outside. Moreover, high levels of light intensity and solar heating might require shading of streams or windows. Cold temperatures may inhibit some stream experiments in non-heated greenhouses. In New York, for example, we do not typically use the facility from December through February. Other indoor spaces may be suitable if artificial sunlight is provided, and may help buffer temperature variation. Finally, we note that evaporation can increase relative humidity in indoor facilities and alter environmental conditions. A strong ventilation system, if feasible, may be used to control humidity levels.

Outdoor artificial stream arrays will be influenced by the surrounding environment. These realistic environmental conditions (i.e., changes in light, temperature, precipitation over time) may be considered advantageous for some research questions, but may cause challenges for others. While flow-through artificial streams require an angled stream bed, recirculating arrays should be placed on level ground to maintain constant depth and velocity among replicate streams. In outdoor facilities, we use electricity from the electrical grid, and batteries or a small generator for backup power during outages. Although it is possible to provide electricity for a stream array with solar power, the size and cost of solar panels and batteries depend on hours of direct sunlight and may be prohibitive.

Light Source Considerations

Regardless of location, arrays should have uniform light coverage because small variations in light may affect productivity and limit detection of treatment effects, especially in areas with plant canopies. A randomized block design for treatments is highly recommended and may be able to mitigate confounding effects of light variability. We recommend deploying light meters throughout the array, or using a fluorescent dye with a predictable degradation rate with

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exposure to solar radiation (Bechtold et al. 2012), to measure spatial variation in light in the stream array. Measurements should be conducted prior to an experiment to ensure similar light regimes among treatments. Alternatively, but less desirably, light meters can be deployed during an experiment for post hoc standardization of light-dependent endpoints.

Water source and maintenance

We recommend locations with a source of readily available water that does not require transport. Chlorinated tap water should not be used unless degassed for 24 hours or treated to remove chlorine. Treated tap water may also have softening agents that could affect aquatic organisms. We have used river water that was collected in a tank and then pumped into streams, but the amount of suspended sediment, nutrients, and biological propagules from river water will vary with changing environmental conditions. Groundwater may be used, although researchers should consider concentrations of nutrients, oxygen, and potential contamination from urban runoff (e.g., road salt). Groundwater that is low in nutrients or oxygen may require nutrient supplementation or aeration, although operation of the paddle wheels can aerate the stream within minutes. Frequent water source testing to account for seasonal changes in groundwater may also be needed. Primary water chemistry variables to monitor include dissolved oxygen, conductivity, nitrate, ammonium, phosphate, and dissolved organic carbon, but selection of variables will depend on the research questions and water source.

The volume of water added to each stream will depend on stream design. For our design, 40-70 L of water provides sufficient contact with the paddle wheels, provides sufficient freeboard for placing objects like rocks in the streambed, and prevents the paddle wheels from splashing water over the sides. We use aquarium-safe silicone sealant to attach a small metal

ruler inside the stream to record the water depth, including displacement from adding stream substrates, and to monitor the water level and account for evaporative losses by adding water over the course of an experiment; we turn off motors while measuring water levels to increase accuracy. In addition, we add tape or other markings to indicate desired water levels in case the rulers detach. Evaporative losses under summer conditions can be high and daily water additions are necessary. If the water source is low in dissolved oxygen or has other characteristics that might be disruptive when modeling continuous metabolism, the timing of the addition of water should be considered and carefully recorded.

We recommend conducting water changes to account for losses of added solutes by degradation or sorption. The frequency of water changes will depend on the experiment. If there is a colonization period prior to the beginning of the experiment, we typically conduct 50% water changes at least once a week to replenish nutrients. A full water change (>80%) can be done a few days before the experiment starts to ensure uniform conditions across replicate streams. However, if there are organisms in the streams, we recommend limiting water changes to 50% to limit stress from low water levels or rapid temperature shifts. High nutrient demand can quickly deplete available nutrient concentrations in the streams, in which case nutrient amendments may be needed to maintain aquatic productivity. For investigations of solute, contaminant or particle fate, we have also conducted experiments where only the water lost by evaporation or sampling was replenished to maintain a consistent concentration in the water column throughout the duration of the experiment. Water changes generally preclude modelling of continuous metabolism on the day of the water change, so frequency of desired water changes needs to be timed with consideration of planned measurements.

At the conclusion of stream experiments or during water exchanges, disposal of water (including contaminants) and associated organisms should be considered. If researchers are concerned with invasive or non-native organisms, a 10% bleach solution can be used to kill organisms from the streams. However, we caution the use of bleach in some circumstances when unknown interactions between bleach residue and treatments (e.g., pharmaceuticals) may be a relevant concern. Removal of contaminants may require water treatment. For example, we have used an activated carbon trickle system to remove organic compounds from stream water. Careful water removal, cleaning, and rinsing is needed between experiments to avoid carryover and potential inter-stream variation in water chemistry and biology (see 'cleaning the streams' below).

Experimental design

The design of artificial stream experiments will change with research questions and objectives. In this section, we present general recommendations regarding replication, experiment duration, water chemistry, substrata, biota, and statistical analysis. We describe these considerations and our experience with elements of experimental design to support the planning and execution of successful artificial stream experiments in the future.

Replication and duration

The first consideration for experimental design is the number of streams required. Although maximizing the number of streams increases replication and potential treatments, it also increases demands on maintenance that require considerable effort, including water replacement, monitoring, and maintaining experimental conditions. Researchers must balance the number of streams with the number of measurements made in each stream. Thus, statistical

power should be considered prior to starting an experiment, although post-hoc power analyses have been conducted (Lee et al. 2016) (see 'statistical analyses' below). As a starting point, we recommend that experiments should include at least 4 replicate streams for each treatment, as 4 replicates are often needed to obtain sufficient replication for statistical significance, and will give researchers a 'buffer' of N=3 per treatment should a stream fail. For permutation-based statistics such as analysis of molecular variance (AMOVA) or permutational multivariate analysis of variance (PERMANOVA), it is impossible to obtain $p < 0.05$ when testing the difference between two treatments with only three replicates per treatment because there are only 10 distinct permutations possible (lowest $p = 0.091$), whereas 4 replicates per treatment has 35 permutations possible (lowest $p = 0.028$; Clarke and Warwick 2001; Fitzpatrick 2009). Note that if researchers take multiple measurements within each stream (e.g., sediment organic matter from several locations in the stream), these measurements should not be considered replicates. Rather, the stream is the experimental unit. Our second recommendation is that researchers use a randomized block design to assign treatments to streams. Environmental variables (e.g., light, temperature) can vary across a stream array, even if the array is located indoors, and using randomized blocks for treatments across the array will limit the differences within the array to confound treatment effects. Additionally, randomizing the position of treatments in the array ensures that if a motor fails, multiple replicates of a given treatment are not compromised (i.e., in a random order, place one replicate of each treatment on each motor).

The duration of artificial stream experiments depends on research objectives, but we recommend that experiments last from several weeks to several months depending on the activities of organisms of interest (Table 2). For example, aquatic insect emergence can decline after two weeks, so we find that a 14 day study is appropriate for those studies. In contrast, we

have conducted experiments over 1-4 days to study relatively rapid biogeochemical processes (Dutton et al. 2018), and up to 5 months to study leaf breakdown (Cook and Hoellein 2016). Each experiment will involve significant sampling and maintenance, and a longer experiment is not always better. Also, biofilm development on the channel surfaces over time may be undesirable. We recommend analyzing data as they are collected to guide the experiment's duration. One benefit of artificial streams is that they provide more realistic conditions than chambers, so running artificial stream experiments longer than typical chamber experiments is recommended. Finally, we suggest researchers consider including a pre-treatment period for colonization and acclimation of biotic communities before initiating any experimental treatments (see 'biofilm inoculation and colonization' below), unless assessing colonization or succession is the objective of the research.

Water column conditions

Protocols for maintaining or manipulating stream water conditions are crucial in the design of artificial stream experiments. Maintaining constant concentrations of solutes in the water column presents a considerable challenge in closed systems because of biological uptake, decomposition, sorption, and evaporation. For manipulation or amendment of nutrients (e.g., nitrogen or phosphorus) in artificial streams, strategies include a continuous nutrient drip, slow-release nutrient diffusing substrates (Tank and Dodds 2003; Tank et al. 2006), or repeated pulse additions to account for nutrient uptake (Ozaki et al. 2016; Binh et al. 2016) (but see 'biofilm inoculation and colonization' below). For experiments that require the addition of compounds with unknown biological or chemical reactivity (e.g., anthropogenic contaminants), knowledge of breakdown rates and fates (e.g., sorption to sediments) may be required. We have added

compounds of interest to a target concentration both in pulses only at the start of an experiment (Drury et al. 2013; Ozaki et al. 2016), and in repeated pulses at regular intervals (Hoppe et al. 2012; Binh et al. 2016). When using these approaches, we strongly recommend frequent analysis of water and sediment to manage targets for solute concentrations.

Substrata

The abundance, type, and relative distribution of substrata in artificial streams should be established to suit the research questions. We have used a wide variety of substrates in our experiments including cobbles, gravel, pebbles, clay tiles, sand, leaves, detritus, and fine sediment. Physical variables such as substratum surface structure and its influence on flow heterogeneity can influence biofilm colonization and productivity, as well as macroinvertebrate development, movement, and predation (Corkum et al. 1977; Brusven and Rose 1981). Artificial substrata such as ceramic tiles can reduce the complexity and may be desirable for their uniformity or ease of sampling (Lamberti and Resh 1983), but also reduce the realism compared to the benthos in natural streams. Artificial substrata can be embedded among natural rocks and used as effective sampling units for endpoints that may require uniform sampling areas (e.g., chlorophyll *a* or benthic organic matter). Sorption and leaching dynamics of materials between stream water and substrata can influence solute concentrations (e.g., phosphorus sorbs to clay, hydrophobic compounds sorb to organic materials), so preliminary testing of sorption dynamics may be warranted (Hoppe et al. 2012). We once found that certain rocks containing carbonate minerals sorbed pharmaceuticals, whereas those composed of silicate minerals did not, so we have used the latter. We recommend discarding substrata after experiments to avoid contamination of future studies, but certain materials (e.g., chemically inert rocks) may be reused

after they are thoroughly cleaned. If the experimental design includes the removal of some substrata during the experiment (e.g., for analysis of biofilms), we recommend replacing the material that has been removed with sterile material of similar size and shape to avoid alterations in stream hydrodynamics.

Biofilm inoculation and colonization

The source and timing of biofilm inoculation and colonization are critical when planning experiments in artificial streams. One approach is to import intact biofilms into the artificial streams on substrata incubated in natural streams, such as rocks, tiles, or organic matter (e.g., "conditioned" leaves). Because transporting biofilms from a real stream to artificial streams may shift communities and processes in response to the altered environmental conditions (Hoellein et al. 2014), we recommend an acclimation period prior to the initiation of any experimental treatments. In addition, it is critical to include untreated control streams in the experimental design to discriminate treatment effects from the effects of placing biofilms into the artificial streams.

Another approach is to import biofilm-forming organisms from the field by collecting stream water, sediment, organic matter, or periphyton, and to allow these organisms to colonize the substrates in artificial streams (Bowling et al. 1980; Drury et al. 2013; Ozaki et al. 2016; Binh et al. 2016). Any of these materials can be homogenized into a uniform inoculum by blending and adding directly to the artificial streams. The inoculum can be sieved to minimize additions of macroconsumers, although microscopic consumers and eggs of aquatic larvae will remain. After allowing the inoculum to become well-mixed into the water column, a 24 h settling period with no water flow may promote attachment of cells to substrata. We typically allow 4-6

weeks for biofilm colonization of artificial streams using this approach to allow sufficient accumulation of biomass, although in warmer climates, sufficient biomass can be present in 1-2 weeks (Drury et al. 2013; Hoellein et al. 2014; Ozaki et al. 2016; Binh et al. 2016). Pre-treatment conditions should be measured to ensure the colonization period has not allowed divergence of individual streams outside of variability deemed acceptable for the research questions posed. While nutrient amendments can increase biofilm production and decrease colonization time, nutrient enrichment can also change community succession and diversity (Stevenson et al. 1991; Hillebrand and Sommer 2000). If mimicking natural community composition is a research priority, nutrient amendment should not exceed levels found in situ. For studies that include leaf litter, we use either leaves that are conditioned in a natural stream or senesced, dry leaves combined with an inoculum of bacteria and fungi from a nearby stream (Hoppe et al. 2012). Because dried leaves will initially leach a significant amount of dissolved organic matter (DOM) in the first 24-48 h, we recommend a pre-leaching step if the high DOM pulse is undesirable. For experiments mimicking forested, headwater streams where allochthonous detritus fuels ecosystem processes, we recommend shading to inhibit algal growth on organic substrates that could complicate measurements of heterotrophic processes or communities (e.g., organic matter decomposition rates).

Invertebrate colonization

We use several strategies for adding macroinvertebrates to artificial streams depending on the research question, but we have generally had success with macroinvertebrates that are entirely aquatic and omnivorous (e.g., *Gammarus* spp., *Asellus aquaticus*, and *Corbicula fluminea*). In one experiment, we added 50 *G. fasciatus* to each stream and recovered thousands

of individuals after 82 days (Hoppe et al. 2012). For macroinvertebrates that inhabit biofilms, we transfer hard substrates with biofilms colonized in natural streams to the artificial streams. For example, baskets with rocks can be allowed to colonize with invertebrates for 4–6 weeks in a nearby stream, and the baskets can be transferred to artificial streams (Clements et al. 1989b, Richmond et al 2016). We have maintained representative invertebrate communities using leaf pack colonization, where leaf packs are left in the field for 2-3 weeks (Richmond et al. in review). If adult insects can enter the artificial streams, we recommend covering the streams with fine mesh netting to prevent unequal invertebrate colonization or terrestrial insect subsidies that will compromise the experimental design. Additionally, the use of mesh netting to cover the streams is useful for measuring invertebrate emergence. Invertebrates can be added to artificial streams while housed in growth chambers or sediment trays (e.g., for bivalves) (Rosi-Marshall 2004; Rosi-Marshall and Meyer 2004; Vaughn et al. 2004; Hoppe et al. 2012; Turek 2013).

Statistics

A valuable attribute of artificial stream experiments is that replication supports robust statistical analyses not often possible with ecosystem-scale experiments. We recommend that the statistical approach be selected during the planning phase. Some statistical methods for analysis of artificial stream data include analysis of variance (ANOVA), repeated measures ANOVA, mixed-effects models, linear regression, permutational multivariate ANOVA and non-metric multidimensional scaling (nMDS) for community composition. Mixed-effects models have been useful for explicitly including variation among days and among streams as fixed or random factors, thus increasing the power of the statistical model (Richmond et al. 2016; Lee et al. 2016). When planning the statistical analyses, each stream should be considered an experimental

replicate. For example, taking three samples out of one stream and treating each of them as replicates violates assumptions of independence. However, taking multiple randomly distributed samples from each stream and physically combining them or averaging the data into a single value for the stream accounts for within-stream variation (e.g., distance from paddle wheel, microhabitat differences on curves versus the straightaway) without violating assumptions of independence. Variability can be reduced by sampling only the straight sections of the streams and not the curves, as these locations differ in velocity and sediment depth, which may influence the response variables.

Data collection

Microbial communities

Microbes can inhabit the water column (i.e., seston), the sediment, and solid surfaces (i.e., biofilms) within artificial streams, and any of these communities can be analyzed depending on the research objectives. We have measured biofilm abundance, community composition, and metabolism as response variables in artificial stream experiments. We recommend scraping a portion of substratum and analyzing the resulting slurry for a variety of metrics, including ash-free dry mass for standing stock, chlorophyll *a* for algal biomass (Steinman et al. 2006), microscopic observation of bacteria and algae (Kepner and Pratt 1994; Lowe and LaLiberte 2006; Julius and Theriot 2010), extracellular enzyme activity (Bell et al. 2013), and DNA-based molecular analyses of microbial community composition (Zimmerman et al. 2014). For example, we have used high-throughput sequencing analysis of 16S rRNA gene amplicons to assess bacterial community composition in artificial streams (Drury et al. 2013; Ozaki et al. 2016; Binh

et al. 2016; Lee et al. 2016). We have conducted all of the above analyses using water column samples to characterize the seston as well.

Biofilm metabolism can be measured as oxygen production (gross primary production) or uptake (community respiration) by biofilms in the artificial streams during light and dark conditions, respectively (Bott 2006). We have conducted biofilm metabolism measurements by placing a portion of substrate (e.g., a rock or tile with biofilm) into a transparent container (e.g., glass jar) filled with artificial stream water and sealed with no air bubbles, and measuring the dissolved oxygen (DO) concentrations in the water before and after incubation for a known period of time (usually 2-4 h), under both light and dark conditions within the artificial streams. The change in DO concentration multiplied by the volume of water in the container provides a measure of oxygen production or consumption. We recommend using 'blanks' (containers with only stream water) to correct for changes in oxygen concentrations resulting from abiotic processes or the activity of organisms in the water column of the artificial streams. We note that these 'blanks' actually represent seston communities and can be used as a response variable as well. For a true biological control, some form of biocide (e.g., mercuric chloride) could be added to incubation containers (not the stream channels) to test for physical/chemical changes in the absence of any biological activity (Brock 1978). We have also measured denitrification enzyme activity in artificial stream samples by a modified version of the standard acetylene inhibition method (Ozaki et al. 2016).

Invertebrates

We measure a suite of invertebrate dynamics in artificial streams, including drift, emergence, behavior, growth rates and diet. To measure invertebrate drift, we use a small drift

net (pore size ≤ 250 μm) fitted to the width and maximum water level of the stream channels (Fig. 4A). Drift nets fit tightly within the stream channel, and are held in place with a small tile or rock, and placed in the streams for 2-24 h (Clements et al. 1989b). To measure invertebrate emergence, we have trapped emerged invertebrates using fitted nets around each individual stream and captured insects using either a mouth siphon attached to a sampling vial or a modified dry hand vacuum (e.g., Craftsman Model No. 315.115710, Hoffman Estates, Illinois, USA). We modified the hand vacuum by removing the front housing of the vacuum and fitting the filter opening with a PVC tube (Richmond et al. 2016). We use a second PVC tube that fits into the first tube, but is easily removable, and cover the vacuum-side opening of the inner tube with fine mesh netting. After emerged invertebrates are vacuumed into the mesh netting, we cap the other end of the inner tube and while the vacuum is running, remove the tube, and immediately wash the interior of the tube through the mesh end with ethanol to preserve specimens (Fig. 4B). Insects can also be aspirated from the interior tube and frozen or dried, negating the need to use a preservative, which can be problematic for contaminant analysis.

Artificial streams can also be used in studies of behavior, growth, and diet of macroinvertebrates. Species interactions or behaviors can be easily monitored using artificial streams. For example, we used artificial streams to examine burial rate and horizontal movements of an invasive Asian clam (*Coribicula fluminea*) in response to substrate type and conspecific crowding (Turek 2013). We used small plastic trays with different substrata, placed marked clams into the trays, recorded their initial locations using a grid, and placed the trays into the streams. We recorded initial behavior using mounted video cameras, and recorded clam locations after one week in the streams. Chambers can be used to investigate predator-prey interactions or to measure individual growth rates (Hoppe et al. 2012). To measure predator-prey

interactions or conduct growth chamber experiments, small plastic Nylon mesh tea infusers (Toby Tea Boys, Plymouth Tea Co., Chatham, MA, USA) or stainless steel tea infusers (Mesh Wonder Ball, Harold Import, Inc., Lakewood, NJ, USA) can be added to the artificial streams to house individual invertebrates. All chambers should be securely fastened (e.g., with a suction cup) and submerged within the stream channel and a small rock or tile with biofilm can be added to the chamber as substratum or a food source for invertebrates. Artificial streams can also be used to study diet and assimilation of aquatic insects fed distinct food items. Aquatic insects can be collected from the streams and analyzed for gut contents or stable isotope signatures (Rosi-Marshall et al. 2016). Adult insects can be collected upon emergence and analyzed for stable isotope ratios, although their largely reduced gut precludes gut content analysis at this stage. When collecting insects for analysis of stable isotope signatures, care must be taken to allow sufficient time for tissue turnover, which may vary by taxa. In experiments using contaminants such as pharmaceuticals, insects can also be collected for tissue analysis to determine bioaccumulation. Researchers will need to use care when selecting preservative agents to use (if any) to avoid interference with measurements in the collected tissues.

Degradation and fate studies

Degradation, sorption, or deposition rates of environmentally relevant materials (e.g., organic pollutants, pharmaceuticals, proteins, microplastics, environmental DNA (eDNA), etc.) are often obtained from microcosm studies, which often do not represent realistic field conditions. Dosing studies performed in artificial stream arrays can be useful in constraining decay rates (e.g., environmental protein, Griffiths et al. 2017), and examining mechanisms of compound fate via retention or sorption, consumption, or physical breakdown. In this type of

experiment, artificial streams are an excellent middle ground between microcosms and natural systems by providing a closed system with enough manipulability to discern how biological and physical factors influence dynamics and fate. For fate or degradation studies, we recommend including a "blank" reference stream, treated with only dosed water, to account for any sorption that occurs on the fiberglass of the stream, or loss to volatilization.

Whole-system functional measures

We measure several metrics of ecosystem function in artificial streams, including whole-stream metabolism (i.e., gross primary production and community respiration), net ecosystem production, nutrient uptake, whole-stream nitrogen dynamics (i.e., denitrification and nitrogen fixation), leaf litter decomposition, bioaccumulation, and secondary production. For measurements of ecosystem metabolism, we deploy data-logging sensors in each stream to record dissolved oxygen and temperature at short intervals (i.e., 5-10 min, using miniDOTs, Precision Measurement Engineering, Inc. Vista, CA, USA) paired with PAR or PAR-calibrated light sensors (Odyssey, Dataflow Systems Pty Ltd., Christchurch, New Zealand; HOBO). We measured reaeration using a conservative gas such as propane or sulfur hexafluoride (SF_6; Kilpatrick et al. 1989; Wanninkhof et al. 1990), via the time it takes sub-saturated ground water to become saturated following the approach of Hall et al. (2015), or estimated reaeration using inverse-modeling approaches and Bayesian estimation (Grace et al. 2015).

We measure net primary production as the biomass of microbes colonizing each stream that has accrued over time, which has the benefits of requiring no removal of specimens during the experiment, and sampling occurs entirely at the end. After quantifying the nutrient content and character of suspended fine particles, we fill the streams to their original volume, and then

sample the water column to estimate seston ash-free dry mass, chlorophyll *a* concentration, species composition, and water chemistry. Then, we remove most of the stream water with a pump head that is covered with mesh to prevent loss of small invertebrates. We then scrub the streambed and all substrates to extract biofilms and invertebrates from the whole stream, taking care to scrub in a gentle manner to preserve integrity of invertebrate specimens. With a wet/dry vacuum cleaner, we transfer the resulting slurry to a bucket. After measuring the total volume of the slurry, we take a subsample to estimate biofilm ash-free dry mass, chlorophyll *a* concentration, species composition, and water chemistry. We then pass the resulting slurry through a sieve (mesh size will depend on research question) to extract invertebrates. We use the ash-free dry mass of seston and biofilm subsamples to estimate net primary production for each stream.

We have measured nutrient uptake and leaf litter decomposition using methods identical to those used in the field, or by modifying methods to account for recirculation. Nutrient uptake in artificial streams can be measured with isotope tracer additions, or by using short-term nutrient additions (Tank et al. 2006). For the latter, we enrich artificial streams with nutrients to 20-50 μg L^{-1} above background concentrations, wait until the stream is well-mixed, and estimate nutrient removal over time (where time is used to estimate total distance), rather than distance as is done in flow-through systems or natural streams. By sampling artificial stream water chemistry at multiple time points after enriching the stream (e.g., every half hour for 2 hours), we can estimate a first-order decay rate (k; h^{-1}), which can be converted to standard nutrient spiraling metrics of uptake velocity (v_f) or areal uptake (U) (Tank et al. 2006). Dyes or conservative tracers can be used to ensure complete mixing. We have also used a Submersible Ultraviolet Nitrate Analyzer (SUNA, Satlantic, Halifax, Canada) to continuously measure nitrate

concentrations in the artificial streams, which can provide insight into diel nitrate dynamics, or to use pulse-based approaches to measuring nutrient spiraling (e.g., TASCC; Covino et al. 2010). We note that the recirculating nature of our streams makes pulse-based approaches difficult and we have not tested these approaches in our streams.

We have measured leaf litter decomposition in artificial streams using the litterbag approach (Cook 2014), where a known volume of naturally-senesced, air-dried leaves are incubated in mesh bags and periodically removed to measure rates of mass loss, although we made one modification to this method for artificial streams. Because leaf decomposition rates may be influenced by the disruption of stream flow by the physical presence of litterbags and by the amount of organic matter standing stock, we kept the number of litterbags in the stream the same at all times by adding 'spare' bags of leaves that were at the same stage of decomposition as the bags we removed. This required setting up extra streams at the start of the experiment to supply the replacement litter bags.

Artificial streams can also provide an ideal system for measuring isotope uptake and turnover, as well as biomagnification and bioaccumulation. Artificial streams can be stocked with organisms that have been raised on a common diet and given a food resource with known isotopic signature or elemental concentration. Subsets of the organisms can then be sampled on a routine basis to determine rates of uptake and turnover or bioaccumulation. These measurements are more precise than in situ rates because researchers can control the availability of other food resources, and sample all resources available on a small spatial scale.

Macroinvertebrate community and population dynamics can be measured in artificial streams that have been supplemented with these organisms (see above for colonization and sampling approaches). We have had success with numerous aquatic invertebrate taxa by either

adding a specific number of individuals or adding chambers colonized with invertebrates

collected from natural streams. Depending on the research question, numerous endpoints that

relate to whole-system measures of invertebrate populations can be measured, such as biomass

production and population dynamics (Hoppe et al. 2012) (Cook and Hoellein 2016) and

community structure (Richmond et al. 2016).

Cleaning the streams after experiments

After removing all biomass and objects from the streams, we recommend cleaning

streams quickly, within hours of termination, so that residues such as calcium do not dry and

become difficult to remove. For fiberglass streams, we fill the streams with a dilute solution of

vinegar and water (approximately 1:10 vinegar:water) to cover all surfaces that were submerged

during the experiment. We allow streams to soak for 24 h in the vinegar solution while running

the paddlewheels. To remove calcium residue, we use a spray bottle to apply a 10% or greater

solution of vinegar while gently scraping with a plastic spatula. After scrubbing all residues from

the stream surface and paddlewheel, we vacuum the vinegar solution, thoroughly rinse the

streams with clean water, and vacuum the streams dry. In the case of experiments using

chemicals, an additional wipe down with 100% ethanol is performed to remove any remaining

chemical residues. Allowing the streams to dry completely in sunlight is a good additional

measure for removing microbial or chemical residues. If fiberglass stream surfaces become

scratched or uneven enough to warrant concerns about sorption of chemicals or microhabitat

variability, resurfacing of the streams should be considered.

Considerations for scaling up artificial stream results to in situ conditions

The benefits of artificial stream arrays are many, but researchers should carefully consider their limitations before attempting to build a facility of their own and while designing experiments. Primary considerations when building an experimental stream array include the logistical concerns such as the cost of building and maintaining the facility, as well as the labor required to manage the experiment and take measurements from the streams. The cost of building an artificial stream facility will depend on the construction materials and type of housing. The cost of maintaining the facility after construction can be relatively low, especially for permanent, indoor artificial streams. While experiments are running, the artificial streams require frequent, regular supervision to ensure motors do not fail, objects do not fall into or out of the streams, that there are no leaks, and nets, shade cloths, and other objects do not get caught in the paddle wheels.

An additional consideration is whether artificial streams sufficiently represent an adequate scale to answer a given research question. Although the artificial streams described in this manuscript are mesocosms for testing processes related to the structure and function of benthic assemblages, they cannot adequately model larger streams or rivers with planktonic communities, hydrogeomorphic dynamics of a deep water column with multiple photic zones, interactions between the hyporheic zone and stream water, or species interactions of higher trophic levels (e.g., fishes or other large predators that may quickly deplete their prey). Flow-through artificial stream channels have been successfully used in other studies (e.g., Wipfli et al. 1998; Connolly and Pearson 2007), and the research planned will influence decisions about whether to use flow-through or recirculating designs. Flow-through designs typically require a large source for water and have often been constructed stream-side or using groundwater or reservoir sources (Aubeneau et al. 2014). While our experiences have shown that stream

organisms can acclimate and carry out their life cycles in the recirculating artificial streams described herein, limited natural habitats in artificial streams inevitably place unrealistic selective pressures on these communities. Although these artificial streams have been able to support studies of diverse ecological processes, they are not designed to exactly mimic natural streams and results should be interpreted with these limitations in mind. The many advantages of replicability, manipulability, and range of use make artificial stream experiments valuable in understanding potential direct and indirect effects that are not easily investigated in natural systems.

Conclusion

There are many considerations when constructing and running experiments using artificial streams, including mechanical logistics, experimental design, and relationships to *in situ* ecological dynamics (Fig. 5). We present a synthesis of design elements and options that have proven successful, along with important 'lessons learned' (Table 3). There is a tremendous amount of flexibility and creativity that researchers can apply to the approaches described here.

Artificial stream arrays allow researchers to pose exciting new questions about stream ecosystems that push the boundaries of traditional ecological studies. While researchers should carefully consider elements of the artificial streams which may limit transferability to real world conditions, artificial streams can answer questions about a wide range of challenging environmental issues that cannot be addressed the same way in situ. For example, we study the effects of multiple stressors on a range of taxonomic groups in urban streams, the influence of various forms of animal resource subsidies on biogeochemistry and whole ecosystem processes, and the occurrence and longevity of environmental DNA. Artificial streams and other mesocosm

designs make it possible to conduct controlled, manipulative experiments are powerful research

tools that produce information needed to support evidence-based environmental management and

decision-making (Higgins et al. 2011; Bilotta et al. 2014). We encourage researchers to use this

practical guide to navigate the logistical and empirical considerations necessary when building

and using an artificial stream array, and hope that this review can help support the creative

development of new insights and experimental approaches for robust measurements of factors

driving the complex interactions in stream ecosystems.

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1   **Table 1.** Materials used in construction of artificial streams and collection of experimental measures, with example manufacturer and

2   part number information.

Category	Item	Purpose	Example manufacturer	Part #
Stream motor and paddlewheel assembly	Gearmotor, 22 RPM, 90 vdc	Turning the paddle wheel to generate stream flow	Grainger	4Z130
	DC Motor Speed Controller	Controlling paddlewheel speed	Grainger	4Z527
	5/8 Shaft Coupler	Connecting a shaft to the motor	Grainger	6X072
	3/4 Shaft Coupler	Connecting a shaft to the motor	Grainger	4X191
	Buna-N Insert	Joining couplers	Grainger	1X407
	Tachometer	Measuring rotation speed of motors		
International (240 V) or battery (12 V) usage	750 W step up/down transformer	Converting 240 V to 110 V for the DC speed controller	Grainger	30C519
	Fuse holder	Connecting fuses between converter and speed controllers	Grainger	1DD10

	Fuses (3 amp, 90 V DC)	Fuses between converter and speed controllers	Grainger	1CM05
	800 Watt inverter	Converting 12 V DC to 120 V AC for battery use with motors	Grainger	1YAY6
Stream channel construction	Fiberglass	Artificial stream channels	Local supplier	
	PVC Canvas	Artificial stream channels	Local supplier	
Experimental measurements	Toby Tea Boys or Mesh Wonder Ball	Macroinvertebrate growth chambers	Plymouth Tea Co. or Harold Import, Inc.	

1

1   **Table 2.** Recommended duration of artificial stream experiments

Ecological endpoint	Recommended duration	Justification and reference if available
metabolism or nutrient uptake	2-7 weeks	Hoellein et al. 2014, Ozaki et al. 2015, Lee et al. 2016
biofilm biomass (AFDM, chl *a*)	2-4 weeks	Lee et al. 2016, Subalusky et al. 2019
microbial/algal composition	2-5 weeks	Drury et al. 2013, Ozaki et al. 2015
antibacterial resistance	2-5 weeks	Drury et al. 2013
leaf litter decomposition	3-7 weeks	Cook 2014, Richmond et al. 2019
invertebrate biomass	3-12 weeks	Hoppe et al. 2012, Richmond et al. 2016
invertebrate emergence	2 weeks	If reproduction is low, populations will decline after 2 weeks; Richmond et al. 2016, Richmond et al. 2019
invertebrate growth rate	1-2 weeks	If contaminant concentration is high or taxon is sensitive (e.g., mayflies), mortality is likely in 1 or 2 weeks.
invertebrate composition	3 weeks	Richmond et al. 2016, Richmond et al. 2019
particle degradation	10 days	A. Shogren et al. 2019
biogeochemical cycling	1-5 days	C. Dutton et al. 2018, Robson et al. 2020, Reisinger et al. 2021

2

1   **Table 3.** Lessons learned from artificial stream experiments

1. Our artificial streams are not capable of reaching very high flow velocities, which may allow
   the growth of algae and other organisms that prefer low flow. When paddle wheels were set
   to higher speeds, water can splash out of the streams and affect the volume of water and
   concentration of contaminants in the streams. Manual scouring of biofilms may be sufficient
   for mimicking a high flow event.

2. Motors may fail for a variety of reasons, including but not limited to short circuits or buildup
   in the bushings inside the motors. Maintenance of the motors should be performed before
   every experiment and spare motors should always be available during an experiment.

3. If multiple motors are used to power paddle wheels, multiple replicates of a treatment should
   not be positioned on the same motor because motor failure could compromise multiple
   replicates of a treatment.

4. Temporary artificial stream installations constructed from PVC canvas should be filled with
   water to check for leaks before beginning an experiment.

5. If modeling continuous metabolism, the timing of water additions or water changes should be
   recorded to remove these time periods from the models or should be timed for periods when
   data for modeling are not critical.

6. Streams should be covered with fine mesh netting to prevent unwanted entry of
   invertebrates or debris. A sturdy and rigid frame should be constructed around the paddle
   wheels to prevent netting and other objects from becoming entangled (refer to Fig. 1A). A
   long elastic band around the whole stream and/or binderclips can be used to secure netting
   (refer to Fig. 1B). We monitor the streams and adjust netting twice daily during experiments.
   Spare netting material is also recommended.

7. Invertebrates are sensitive to oxygen levels in the streams. When transporting invertebrates from the field and into artificial streams, they should be stored in plenty of stream water (not tap or deionized water) and air should be pumped into the water if they require storage for more than a few minutes. If artificial streams have been filled with groundwater, the paddle wheels should be turned on to aerate the streams for several minutes; dissolved oxygen should be >60% saturation before adding invertebrates.

8. Temperature of the water may pose a problem for sensitive organisms in artificial streams, especially streams that are positioned above the ground. However, we found that invertebrates are resilient to high temperature as long as the temperature changes are gradual and not too extreme (e.g., not >35°C). Invertebrates should be allowed to acclimate to the temperature of the facility before being added to the artificial streams.

9. If testing the ecological effects of chemical compounds, substrata added to the artificial streams should be chemically inert. We tested the sorption properties of several types of landscaping river rock before adding them to the artificial streams.

10. If high concentrations of chemical compounds have been used in the streams, chemical residues may remain and affect future experiments. Before starting a new experiment, the streams should be thoroughly wiped with 100% ethanol and dried completely, ideally under full sunlight, to remove as much residue as possible.

1

1 **Figure Legends**

2 **Fig. 1.** Photographs of artificial stream arrays A-B) made with fiberglass stream channels and

3 housed in a greenhouse at the Cary Institute of Ecosystem Studies, Millbrook, NY, and C) made

4 with PVC canvas and temporarily arranged outdoors at the Mara River Water Users'

5 Association, Mulot, Kenya.

6

7 **Fig. 2.** Engineering drawings showing a) specific dimensions for the construction of a stream

8 channel (1.69 m length × 0.16 m depth) and b) the paddlewheel (130 mm length × 140 mm
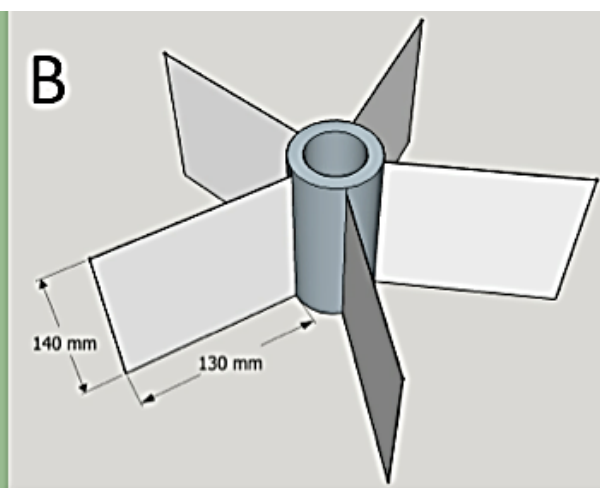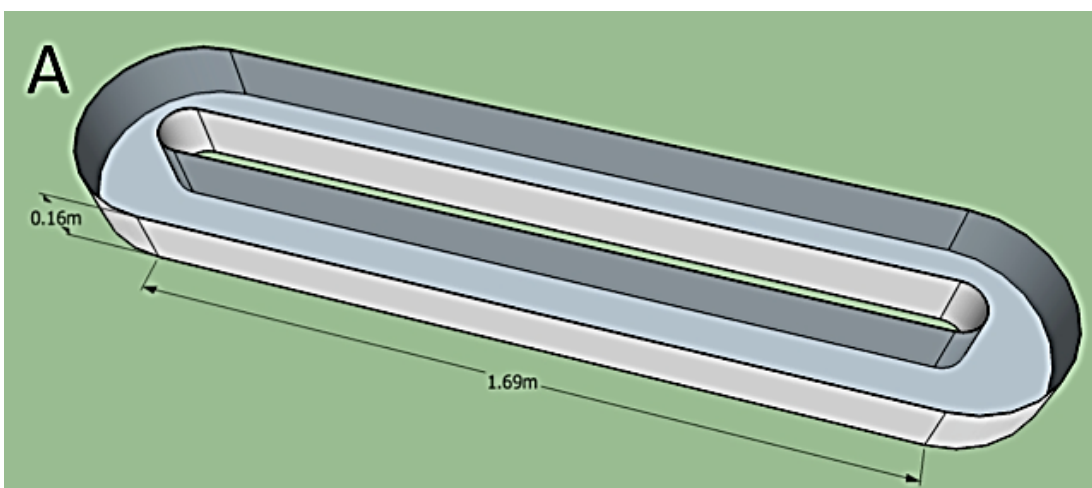
9 width).

10

11 **Fig. 3.** Photographs of individual components of artificial stream arrays: A) paddle wheel; B)

12 wooden box stand holding a tachometer for measuring paddle wheel speed; C) sealed bearings

13 attached to the central rod connected to the paddle wheels in Millbrook, NY; and D) paddle

14 wheel and rod and support system in Mulot, Kenya.

15

16 **Fig. 4.** Photographs of tools for measuring invertebrate responses: A) drift net for collecting

17 drifting aquatic macroinvertebrates inside an artificial stream channel and B) modified vacuum

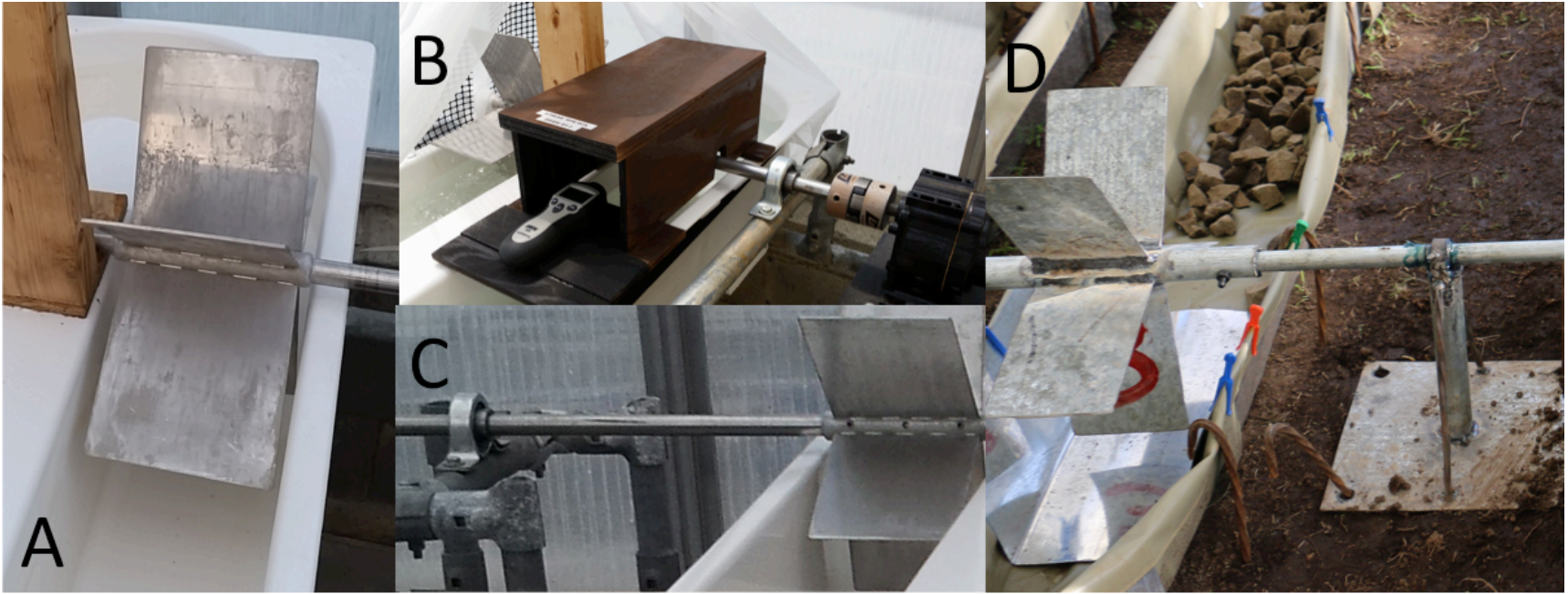18 and insert for collecting emerged macroinvertebrates.

19

20 **Fig. 5.** The anatomy of an artificial stream experiment showing key considerations when

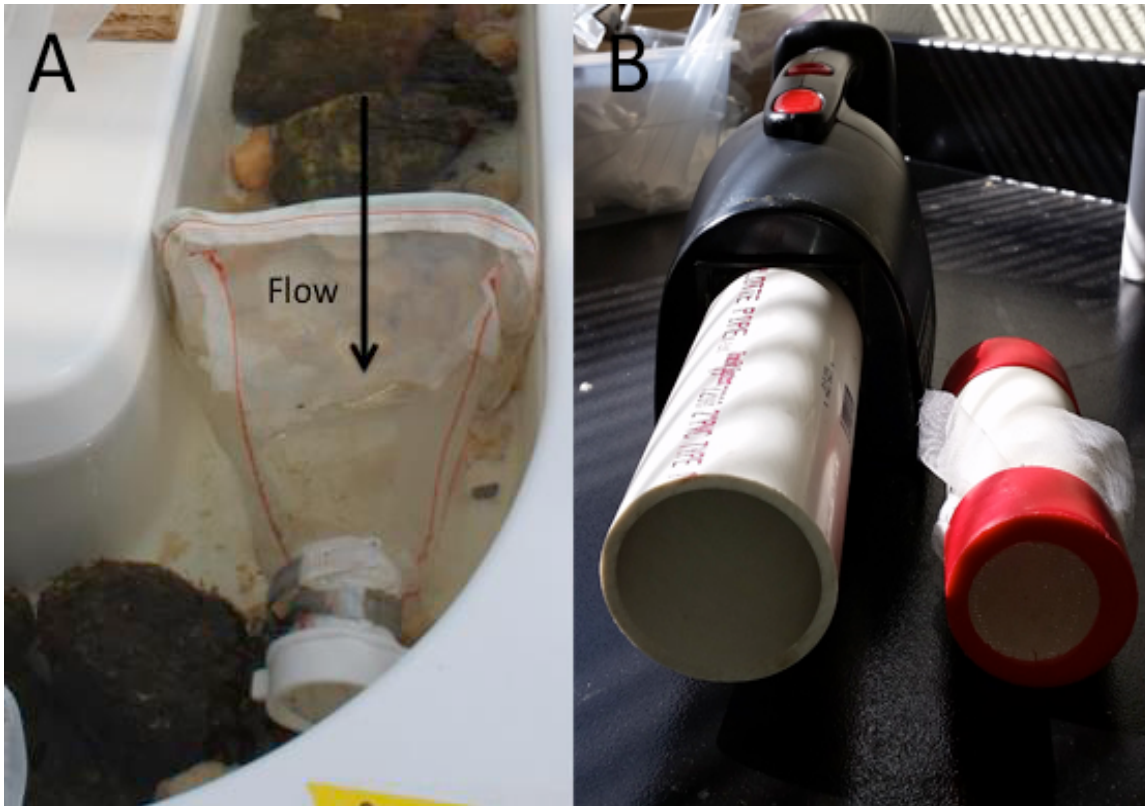21 constructing and undertaking experiments using artificial streams.

1

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1

Anatomy of an Artificial Stream Experiment

Streams
- Recirculating or flow-through
- Indoor or outdoor facility
- Shape and materials

Water
- Source
- Volume
- Replacement

Flow Mechanism
- Materials
- Configuration
- Speed

Substrates
- Source
- Composition and shape
- Configuration in stream

Organisms
- Biofilm
- Invertebrates
- Vertebrates

Treatments
- Nutrients or subsidies
- Contaminants
- Other stressors

1