Mesocosm experiments

Mesocosms should be set in an open field. Try to avoid obvious environment discontinuities; the most obvious of these will be related to shading and exposure to the sun.

Most of the time, you will want to block the experiment. Environmental gradients are inevitable. Block effects are ubiquitous in our experiments.

Remove any old labels from the mesocosms. Make sure holes in the 0.28 m^2 plastic tubs are well-covered with fiberglass screening. The standpipes on the 1.35 m^2 fiberglass tanks should be covered with screening.

Label the mesocosms. Use label tape; do not write directly on the mesocosm. Each gets a unique number (no letters, no codes). Include as little information about the treatment as possible, to help you remain blind as far as possible. On the other hand, clear labels can help reduce errors in handling the experiment later on.

At the same time as you label the mesocosms, set up an Excel file that contains the experimental design. The first column is the unique mesocosm number, subsequent columns will have the block number, and information about treatments. Hereafter, all data collected during the experiment will require only the unique mesocosm number. The design file can be merged with other data files to ensure that treatment information is matched with the data you have collected.

Place mesocosms at even intervals on level pads. This can take some work if the ground is uneven, but it is important if you want each mesocosm to hold the same amount of water and experience similar exposure to the sun. It also produces an experimental array that appears neat and ordered, which creates the impression for passersby that you're doing real science!

It is good to place mesocosms close together (reducing environmental variation), but be sure to leave isles for tending them and enough space between for the lids.

Fill mesocosms with regular tap water. We will call this day 0. Let the water sit overnight (until day 1) before adding anything else.

Put a lid on the mesocosm immediately after filling. We use tough green 43% shade cloth.

There are different styles of mesocosm experiments. The following comments apply if you are attempting to establish self-sustaining outdoor mesocosms, in which animals will not have to be fed or otherwise attended.

- Fill mesocosms three weeks before the tadpoles are anticipated (in early spring) or 1-2 weeks early (in summer). Filling mesocosms too early can be catastrophic, due to invasion by filamentous algae. If you think your mesocosms were filled a bit too early, you might consider introducing an herbivore that is not a part of your experiment (snails or tadpoles). This is never desirable, because later on these animals will compete with your experimental subjects.
- Add dried leaf litter to each mesocosm on day 1. The usual amounts are 40-60 g per mesocosm (for 0.28 m^2 plastic tubs) and 400-500 g (for 1.35 m^2 fiberglass tanks).
- I usually stir the leaves on day 2, to help them sink faster. This is not necessary in wet weather. If the leaves float they will block sunlight and thereby delay the early development of the mesocosm.
- I often add rabbit chow (the pellet kind) to each mesocosm on day 1, to provide additional nutrients to jump-start the ecosystem. Two grams in the 0.28 m^2 plastic tubs and 10 g in the 1.35 m^2 fiberglass tanks.
- Add pond water to each mesocosm on two different days. This might be about day 2-3 and day 4-5. Adding pond water is important for two reasons: (1) it includes all the bacteria, fungi, rotifers, ciliates, and other microorganisms that are essential for a healthy freshwater

ecosystem, and (2) it includes zooplankton. Without zooplankton, mesocosms will become cloudy (anoxic) and the animals you care about will die.

* Collect water from a nearby pond. Take along two car boys, a 6-L plastic bin, a zooplankton-mesh hand net from a nearby pet shop, and a pair of waders. Walk through the pond sweeping the net back and forth, stopping occasionally to deposit its contents into the bin.
* Do not allow your net to pass through or near vegetation or the bottom of the pond. If you accidentally pick up some detritus, reject that sweep and start again. In the end, your zooplankton sample should look clean: lots of animals, no scum, no grass blades, nothing that could harbor predators or their eggs.
* Top up the car boys with clean water from the pond.
* Allmendteich is a good pond (47.48325, 8.5426; Swiss coordinates 683.2 259.73).
* When you get back home, look through your collection to ensure that no small predators were accidentally brought home. Put all the pond water together in an 80-L tub, diluted as necessary with aged water, to give a healthy quantity of water. Generally, you want as many litres of water as you have mesocosms in your experiment -- that way, each mesocosm will receive four cupfuls.
* Mix thoroughly, fill sets of 200-ml plastic cups, and add them to the mesocosms. Repeat this process, trying to put at least 3-4 cups into each mesocosm. Go through the array of mesocosms in a different sequence each time.
* Repeat the process of adding zooplankton on two different days.

If your mesocosm experiment does not have leaves and zooplankton, then the method is more like a laboratory experiment. Animals can be introduced as early as day 1. You will not need to feed the tadpoles as much as you do in the laboratory, because mesocosms contain some nutrients from the water supply and rainfall, and they are exposed to sunlight. Watch closely for anoxic conditions; adding zooplankton on about day 3 would be a good idea.

Counting out the experimental animals is easy, but it is important to do it correctly and carefully. The goal is to get the right number of individuals into each mesocosm, and to get comparable samples of your animals into each mesocosm. Randomization is usually not possible at this step, because thousands of animals can be involved, but assigning animals haphazardly can effectively eliminate bias.

* Lay out bins on a table, a few more than there are mesocosms in the experiment. Each of these corresponds to one of the outdoor mesocosms. Add some aged water to each bin.
* Decide how many tadpoles from each clutch (or other source container) will go into each mesocosm.
* Bring in the first clutch, and set it on the table. Sit down and get comfortable.
* Count out the tadpoles in groups of 2-5. These are numbers than can be counted quickly and accurately. Suck up a group in a turkey baster, deposit it into a small cup, and put that cup next to one of the bins. Repeat this process, setting cups next to bins in haphazard order, until each bin has one small cup sitting next to it. Now repeat the process, placing a second cup next to each bin in haphazard order until there are two small cups next to each bin. Repeat as necessary until the first clutch has contributed the correct number of tadpoles to each bin.
* Now deposit the small cups into their respective bins. Count all the tadpoles as you do this, so that every cup full of tadpoles is counted at least twice. You will be surprised by how many mistakes you discover.
* Now go get the second clutch and repeat the process described above.

* If you make a mistake, you have to begin again. There is no way to return to a clutch later and collect a new sample from it that is comparable to the one you obtained from the steps described above.
* After every bin has been filled, randomly choose which are the extras and which go into the experiment. Set aside the extras. Take the other bins outside, and float each one in a mesocosm. Check that every mesocosm has one bin floating in it. Release the tadpoles.
* If you drop a bin, those extras that you counted out will come in handy. Otherwise, they can be used as a sample of animals at the outset of the experiment.

If the experiment ends before metamorphosis, mesocosms must be drained and all animals removed, counted, and weighed.

* Remove all the leaves. It is impossible to be certain you have caught all the tadpoles if some of the leaves still remain in the mesocosm.
* Break tadpoles into small groups to be counted. It is difficult to count tadpoles in groups of more than about 5-10.
* Weigh the survivors quickly, because they lose weight quickly after being removed from the mesocosm.

If the experiment extends through metamorphosis, you will need to catch and remove metamorphosing individuals at least once a day once emergence has started. Tadpoles should be removed at stage 42, because otherwise they can drown in the mesocosms. Large nets with a deep pocket are useful for this.

Metamorphs should be held until tail resorption is complete and mass has stabilized (stage 45 or 46). They should be stored out of the sun, in a place that does not get too hot. Small containers with tight-fitting lids are good for holding metas. The container should contain only a few ml of water, and should be tilted so that the froglets can easily crawl up out of the water even if their legs are weak.

Metamorphs can be held in groups, but you need to be careful because they are very vulnerable at this stage to bacterial or fungal infections.

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