Lab II-1

Building and Observing Microcosms

Equipment and Materials

You'll need the following items to complete this lab session. (The standard kit for this book, available from *www.thehomescientist.com*, includes the items listed in the first group.)

Materials from Kit

- □ Goggles

Materials You Provide

- □ Gloves
- □ Bag, brown paper
- □ Camera (optional)
- □ Eggshell and yolk (see text)
- □ Funnel (or aluminum foil to make your own)
- □ Jars, wide-mouth (see text)
- □ Mixing bowl or similar container
- □ Newspaper
- □ Pond water/sediment/vegetation (see text)
- □ Shredder and/or scissors
- □ Soft drink bottles, 500 mL or 1L
- □ Trowel, ladle, or other large scoop
- □ Water, spring or boiled tap

Background

Microcosms are simplified artificial ecosystems that are used to simulate and observe the behavior of natural ecosystems under controlled conditions. Open or closed microcosms provide an experimental area for ecologists to study natural ecological processes. Microcosm studies can be very useful to study the effects of disturbance or to determine the ecological role of key species. A Winogradsky column is an example of a microbial microcosm.

Most of the other lab sessions in this book are organized into related groups, may be performed in any order you wish, and many (not all) can be completed over the course of one lab period to at most several lab periods over a week or two. Unfortunately—unlike chemistry, physics, and most other sciences—biology labs can't all be broken down into self-contained single lab sessions. Living things run on their own schedules. Life cycles are what they are, and we're often powerless to speed them up or slow them down.

Building and observing microcosms is an excellent example of one of these on-its-own-schedule activities. There is much to be learned by doing this activity, but pursuing it properly requires

frequent and detailed observations over a period of weeks to months. Accordingly, rather than attempt to distribute these activities out over many lab session groups, we decided to consolidate them in this group of lab sessions, which we'll begin at the start of the semester and continue throughout the semester.

Some of the individual sessions in this group will be repeated periodically throughout the semester, beginning early and continuing. For example, we'll observe microscopically the populations of our pond water microcosms throughout the semester, noting changes that occur in the types and mix of different organisms as the microcosm life cycle progresses from juvenile to mature to senescent. Our microcosms will also serve as resources for some later topics. For example, we'll observe succession in the microcosms, which properly belongs in ecology, but requires extensive and repeated observations over the course of many weeks. Similarly, we can use our microcosms as a source of live protists when we do the lab session on protists.

In this lab session we'll build two types of microcosms based on water, sediment, and plant life gathered from a pond or stream. We'll then observe these microcosms periodically over the course of several weeks, noting changes that occur through the life cycles of the various organisms present.

The first type of microcosm resembles a standard aquarium. We'll use wide-mouth jars filled with a relatively thin layer of sediment, with pond water and aquatic plants above it.

> We actually used square nut jars from Costco, but any similar clear, colorless glass or plastic wide-mouth jars of about 1 to 2 liters capacity suffice. Other possibilities include canning jars, pickle jars, large peanut butter jars, and so on.
>
> Before using them, wash the jars and lids thoroughly with dish-washing detergent, rinse thoroughly in tap water, and allow them to dry. Do not use soap, which leaves a film that is toxic to many microorganisms.

These aquaria will provide the opportunity to observe a wide diversity of microlife, especially protists. We'll build several of these microcosms initially, some of which we'll modify later to observe the effects of environment changes on the populations present.

The second type of microcosm, called a *Winogradsky column*, is a tall column that contains a thick layer of pond sediment and pond water. We'll build several Winogradsky columns using 500 mL or 1 L soft drink bottles, with different columns containing different nutrients, and observe the changes in these columns over the course of several months. Although many types of lifeforms will be present initially, the real purpose of the Winogradsky column is to produce a mixed bacterial culture and observe the different types of bacteria that thrive in different micro-environments within the columns.

The Winogradsky column has been used for decades in biology classes to demonstrate the metabolic diversity of prokaryotes (bacteria and archaea). All organisms require a carbon source and an energy source to live and grow. Some organisms, called *autotrophs*, obtain carbon from atmospheric carbon dioxide, while others, called *heterotrophs*, obtain carbon from the organic compounds such as carbohydrates that are produced by autotrophs. Similarly, some organisms, called *phototrophs*, obtain energy directly from sunlight via photosynthesis, while others, called *chemotrophs*, obtain energy from breaking down chemical compounds.

Different organisms use all four possible combinations of these methods to obtain carbon and energy:

- *Photoautotrophs* obtain carbon from carbon dioxide and energy from sunlight.

- *Chemoautotrophs* obtain carbon from carbon dioxide, but cannot obtain energy from sunlight, and so must obtain it by breaking down chemical compounds.
- *Photoheterotrophs* obtain energy from sunlight, but cannot obtain carbon from carbon dioxide, and so must obtain it from carbohydrates or other organic compounds.
- *Chemoheterotrophs* obtain both carbon and energy by breaking down chemical compounds.

> In fact some organisms use different methods depending on the environment they happen to find themselves in. For example, the bacterium *Rhodospirillum rubrum*, which we'll examine in a later lab session, is chemotrophic under aerobic conditions but phototrophic under anaerobic conditions.

All four of these strategies are represented in a typical Winogradsky column, which also provides a visible example of how different organisms occupy different ecological niches, according to how they obtain carbon and energy.

A Winogradsky column, from top to bottom, may include the following ecological micro-niches:

Aerobic water layer
: The cellulose present initially causes a microbial bloom, which quickly exhausts the oxygen in the sediment and most of the water column, leaving only the top centimeter or so of the water column aerated. Aerobic species survive and flourish only in this part of the column.

  The surface of this layer is populated by sheathed bacteria. The remainder of the aerobic water layer is populated by cyanobacteria (formerly called blue-green algae), which are the only photosynthetic bacteria species. In some columns, cyanobacteria may bloom, oxygenating the entire water column and even the top portion of the sediment column.

Anaerobic water layer
: Because oxygen diffuses very slowly in water, most of the water column is anaerobic (oxygen-depleted). Species such as purple non-sulfur bacteria thrive in this layer.

Anaerobic boundary layer
: This boundary layer between the anaerobic sediment layer beneath it and the anaerobic water layer above it is populated by green sulfur bacteria just above the sediment and purple sulfur bacteria just above the green sulfur bacteria. These bacteria consume the hydrogen sulfide gas produced in the anaerobic sediment layer beneath, converting sulfide ions to sulfate ions. The bacteria are visible as a thin purplish layer lying just above a thin greenish layer, which in turn lies just above the sediment.

Anaerobic sediment layer
: This layer, populated by anaerobic sulfate-reducing species such as *Clostridum* and *Desulfovibrio*, consumes the sulfate ions produced by the sulfur bacteria in the layer above it and releases hydrogen sulfide gas, which in turn feeds the sulfur bacteria.

Each Winogradsky column is unique, even if it started with the same combination of water, sediment, and nutrients, so we'll make several Winogradsky columns and observe and compare them as they mature.

Procedure II-1-1: Gathering materials

Field trip!

The raw materials we need for our microcosms are simple, and can all be obtained from the nearest pond or stream: water, sediment, and aquatic plant life. You can obtain these specimens

at any time of year, although it is best if the source is not frozen over.

> Even in winter, it's possible in most areas to find accessible pond or stream water. In very cold weather, the populations of the organisms present will usually be much lower than during the other seasons, and the mix of species present may differ. Those populations should rebound quickly once you build your microcosm and allow it to sit at room temperature for a day or two.

The amounts needed depend on the number and size of microcosms you intend to build. For example, if you intend to build six aquarium microcosms in 1.25-liter peanut butter jars and four Winogradsky columns in 500 mL soft drink bottles, you will need about ten liters of water (clean 2-liter soft drink bottles make convenient collection vessels), perhaps three liters of sediment, and enough aquatic plant life to provide substantial and diverse plant populations in each of the aquarium microcosms. It's better to get too much raw material than not enough.

> Use extreme caution when you obtain and handle pond or stream water, sediment, and plant life. Most of the organisms found in typical ponds and streams are harmless to humans, but more than a few are human pathogens. Always wear gloves and goggles when obtaining or handling specimens, and always wash thoroughly with soap and water after doing so. It's also a good idea to spray or rinse the contaminated exteriors of sealed collection containers with disinfectant before taking them home.

When collecting the plants and sediment, try to avoid collecting rocks, sticks, and other large objects. (Well, actually, a snail or two can't hurt, but if you include a snail in one of your aquaria make sure also to include lots of vegetation to make sure the poor snail doesn't suffocate.)

Try to obtain as many types of plants as possible, including those rooted to the bottom, floating in the water, and lying on the bottom. If there is visible pond scum (algae) present, obtain some of it as well. If possible, keep the plants segregated by type, using small jars or zip seal plastic bags to contain them. Collect sufficient examples of each type of plant to provide populations of it in each of your aquarium microcosms. On each collection container, note that plant's environment (for example, free-floating, rooted in the sediment, and so on.)

Build your microcosms as soon as possible after you collect the specimens. Even if you allow the plant and sediment specimens to dry out, most of the types of organisms present will survive, but our goal is to reproduce as closely as possible the original ecosystem present in the pond or stream, so the less delay the better.

> Retain some mixed pond or stream sediment and vegetation for later use. Spread the material in a thin layer on newspaper and allow it to dry thoroughly in the shade. (Avoid direct sunlight.) Store the material in a labeled paper bag.

Procedure II-1-2: Building aquarium microcosms

Insofar as is possible, our goal is to make each of our aquarium microcosms identical, using the same amount of sediment, the same amount of water, and the same amount and mix of aquatic plant life.

1. Put on your gloves and goggles.
2. Arrange all of your wide mouth jars on your work surface, side by side.
3. Transfer sufficient pond or stream sediment to each jar to fill it about one quarter full. Try to keep the levels in all jars the same.

4. If your plant specimens include examples that you found rooted to or lying on the bottom, place those plant specimens accordingly in each of your containers, trying to keep the quantities and mix the same in each of your containers.
5. Transfer sufficient pond or stream water to each jar to fill it about 5 cm from the top. Again, try to keep the water levels as similar as possible.
6. Transfer examples of any free-floating plants, algae, and other non-anchored flora to the containers, again trying to keep the populations and mixes the same between containers.
7. Replace the lids, seal them tightly, and then spray or drench the containers thoroughly with disinfectant to kill any organisms on the exteriors of the containers.
8. Transfer all of the containers to an area where they can remain undisturbed and will be exposed all day to bright daylight, but not to direct sunlight. If necessary, you can put the containers under a plant-grow light to ensure they receive adequate light for long periods every day. It's important that the containers be maintained at about room temperature, so avoid areas where they might be exposed to large temperature variations.
9. Remove and discard your gloves and wash your hands thoroughly with soap and water.

Procedure II-1-3: Building Winogradsky column microcosms

Just as with the aquarium microcosms, our goal is to make each of our Winogradsky column microcosms as similar as possible, other than differences in added nutrients. With the exception of one control Winogradsky column, which contains only unenriched sediment, each of the other Winogradsky columns begins with sufficient enriched sediment to fill the column roughly half full. To that, we'll add add about half as much unenriched sediment to fill the column to a total of about three quarters. Finally, we'll fill the column with pond or stream water, leaving a small air gap.

> We assume that you're making four Winogradsky column microcosms, each in a 1-liter soda bottle, which will require a total of about three liters of sediment and sufficient pond or stream water to nearly fill the bottle. If you're making a different number or size of columns, adjust the quantities accordingly.

1. Put on your gloves and goggles.
2. All but the first Winogradsky column will contain sediment supplemented with shredded newspaper (cellulose) to provide a carbon source. One double-width sheet of newsprint will provide sufficient cellulose for those three columns. Shred or cut the newspaper into small pieces.

> We ran newspaper through a confetti-shredding office shredder, but you can instead use a strip shredder and then cut the strips into smaller pieces with scissors. If you're very patient, you can just use scissors.

3. Transfer enough sediment to half fill three bottles to a mixing bowl or similar container. Stir in the paper bits to mix them throughout the sediment. Retain the remaining sediment for use later.
4. Label one bottle “paper only”. Transfer sufficient cellulose-enriched sediment to that bottle to fill it about half full and set the bottle aside.
5. Add a raw egg yolk to the sediment remaining in the mixing bowl. Mix the egg yolk thoroughly into the sediment. Egg yolk provides a sulfur source.

6. Label a second bottle “paper + yolk”. Transfer enough of the cellulose and egg yolk enriched sediment to fill that bottle to the same level as the first bottle and set the bottle aside.
7. Crush the egg shell into small pieces and mix them into the sediment remaining in the mixing bowl. Eggshell provides a calcium source.
8. Label a third bottle “paper + yolk + shell”. Transfer enough of the cellulose, egg yolk, and egg shell enriched sediment to fill that bottle to the same level as the first and second bottles and set the bottle aside. Discard any remaining enriched sediment.
9. Carefully transfer enough unenriched sediment to the first bottle to fill about half the remaining space. Do so gently to avoid mixing the sediment layers. Your goal is to have distinct layers of sediment, with the enriched sediment on the bottom, covered by a layer of unenriched sediment. Repeat for the remaining two bottles, bringing the sediment to the same level in all three.
10. Label a fourth bottle “unenriched”. Transfer sufficient unenciched sediment to that bottle to bring the level to the same as the other three bottles.
11. Disturbing the sediment layers as little as possible, carefully transfer sufficient pond or stream water to each bottle to fill it, leaving a small air gap at the top of the bottle.
12. Replace the lids on all four bottles, seal them tightly, and then spray or drench the containers thoroughly with disinfectant to kill any organisms on the exteriors of the containers.
13. Transfer all of the bottles to an area where they can remain undisturbed and will be exposed all day to bright daylight, but not to direct sunlight. If necessary, you can put the bottles under a plant-grow light to ensure they receive adequate light for long periods every day. It's important that the bottles be maintained at about room temperature, so avoid areas where they might be exposed to large temperature variations.
14. Remove and discard your gloves and wash your hands thoroughly with soap and water.

Procedure II-1-4: Observing Winogradsky column microcosms

Observing a Winogradsky column microcosm is a long-term project. Some visible changes occur soon after you build the microcosm, but the major changes take place gradually over weeks and months.

1. For the first week after you build your Winogradsky column microcosms, observe each of them daily. Note any change in the appearance of the layers. If you have a camera, shoot images of each of the microcosms and file those images by date and microcosm makeup.
2. From day 8 until day 30, observe the microcosms every two to three days. After day 30, begin observing the microcosms weekly. Again, note any visible changes and shoot images to record them.

The exact contents of these microcosms is unknown, so it's possible they contain pathogenic organisms. When you have finished observing your Winogradsky column microcosms dispose of them by incineration or by submerging them in a bucket of chlorine bleach solution before removing the cap and mixing the contents of the microcosm with the bleach solution.

Advanced students can use these microcosms as sources of different bacteria species to culture—for example, the *Rhodospirillum* rubrum we use in a later lab session—but it's much less risky simply to buy pure cultures of the species you need rather than attempting to culture them from wild sources. **Do not open any of these**

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microcosms unless you are confident that you are competent to work with possible pathogenic organisms.

Review Questions

??? Production: Please strip the answers (formatted as comments) from the final layout. RBT

1. We emphasize safety in collecting and handling pond or stream specimens because of the possible presence of pathogens. Using the Internet or other resources, determine two common pathogens in each of the three classes: viruses, bacteria, and protozoa.

   Answers may vary, but two common viral pathogens found in source water are Enteroviruses and Novoviruses. Among bacteria, two common pathogens are Campylobacter and the 0157:H7 strain of E. coli. Among protozoa, the most common human pathogens found in source water are Cryptosporidium and Giardia.

2. We built Winogradsky columns with sediment enriched by carbon, carbon+sulfur, and carbon+sulfur+calcium. Using the Internet or other resources, suggest several other elements we might have used to enrich the sediment.

   Answers may vary, but nitrogen, phosphorus, and/or potassium are good candidates. We might also choose to build Winogradsky columns that are enriched with respect to various micronutrients, including copper, iron, magnesium, manganese, molybdenum, and zinc.