Chapter 7

The Use of Aquatic Research Microecosystem in the Biology Teaching Laboratory

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Introduction

One objective of this laboratory exercise is to allow students to sample and explore changes in various successional stages of an ecosystem without the high cost to the school of transporting students to the ecosystem, and without the high cost to the ecosystem of damage done by a large number of student samplers. Another objective is to allow students to compare differences between control (or natural) microecosystems and experimental microecosystems disrupted by some type of treatment. Students may also compare different microecosystems by using inoculants from different natural systems. At the conclusion of this exercise students should understand the concepts of succession, diversity and energy utilization. They should also have a better grasp of the processes and interactions of photosynthesis and respiration.

Senior high school students taking 2nd year biology or college students in introductory biology classes can use this laboratory with the staggered inoculation/unified testing approach (from now on referred to as the *basic* method). Upper level college students taking ecology courses can use this laboratory with the unified inoculation/staggered testing approach (the *advanced* method). The student time required for the former is about 1 to 2 hours, while the latter requires a larger amount of student time spread over the whole period the student is attending the laboratory.

The preparatory time for the *basic* method is about 1 to 2 hours/week for the first 2 weeks, and 2 to 4 hours/week for the last two weeks, with a 2 or 3 hour set up time in order to do the testing of the microecosystem. The *advanced* requires about 9 to 10 hours preparation at the beginning of the experiment and some monitoring of the experimental area throughout the length of the experiment.

This laboratory exercise is written to follow the Introductory Biology Laboratory process and use the basic method to study the importance of various inoculants, various growth media and a variable or disturbance on the growth of microecosystems.

Student Materials

In the early days of space exploration two techniques were proposed for providing life support for astronauts on long trips into space. One was the hardware, or abiotic, technique in which all the life-support materials were bottled up, used once, and discarded by the astronauts. The other was the biotic approach in which living organisms would recycle the wastes from the astronauts and in turn provide life-support material to the astronauts. Obviously, plants were the key to this second method. Astronauts produce carbon dioxide and nitrogenous wastes and need oxygen and food. Plants use carbon

dioxide and nitrogenous wastes and produce oxygen and edible plant matter. Aquatic algae were the logical plants because of their high productivity and small space requirements. However, pure algal cultures proved to be terribly unstable and susceptible to disease. A modified system using a stable group of different algae, bacteria and small protist organisms as found in sewage ponds appeared to be a useful alternative to pure algal cultures.

Long-term space voyages have never materialized and so biotic life support systems have never been tried. However the research into these systems provided a tool, the microecosystem, for use in studying ecological processes and principles here on earth. Growth media have been perfected (Taub and Dollar 1964) and several microecosystems have been developed (Beyers 1963; Cooke 1967; Gordon et al. 1968; Ferens and Beyers 1972).

One of these ecological processes is ecological succession. All ecosystems begin as barren areas (rock or water) and progress through stages of colonization by different plants and animals which are adapted to living in each stage. Each group of organisms changes the abiotic environment of the ecosystem, and these changes allow new organisms with different adaptations to colonize the same area. Eventually these changes produce a diverse and stable group of organisms which live in a given ecosystem, and these organisms maintain themselves through the years with little change. The process of change from barren to complex is called succession, and the end ecosystem is called a climax. Apparently the climax system has reached a limiting point in the physical environment; perhaps there is a limit to energy (sunshine, organic matter, etc.), or nutrients (water, minerals, etc.), or space. Each organism is adapted to best survive in this highly diverse system.

Succession in nature is usually rather slow. It takes years for an old field to become a forest. However, it is often possible to see what has happened or what will happen to a given part of an ecosystem by looking at an environmental gradient extending from the most barren area to the most complex area of an ecosystem. In our old field example (in the Southeast), you can envision what the center of the field will look like as succession proceeds by walking from the center of the field into the nearest forested area. As you go along you will pass through areas of annual broad-leaf plants, perennial grasses, young bushy trees (persimmon and plum) and pine trees, and finally to a young broadleaf tree forest. An even better example of succession visible in space occurs on South Carolina's barrier islands. The ocean current is gradually moving these islands southward by removing sand from the northern ends of the islands, carrying this sand past the island and depositing it at the southern end of the island. Thus, the one end of the island (the southern) is always new or barren, and the oldest (northern) area is just ready to fall into the sea. By moving from south to north you will move from sea oat grass areas, to scrub oaks and vines, to pines and water oaks and perhaps to pure oak forest.

Another way to see succession in action is to use the microecosystems mentioned above, because succession occurs quite rapidly in them. Once a bit of an old microecosystem has been placed in a new barren area (an Erlenmeyer flask full of growth medium) the organisms grow, become numerous, die out and are replaced by other organisms in a relatively short time. It is possible to merely watch succession occur, to compare successional stages between different microecosystems, or to examine how some disturbance can affect the process of succession.

Instructors' Materials

As previously noted, this exercise may be conducted in one of two ways: staggered inoculation/unified testing (the *basic* method) or unified inoculation/staggered testing (the *advanced* method). In this exercise we will use the basic method, but the variations for implementation of the advanced method will be noted.

In the basic method the microecosystems are started by placing 100 ml of growth medium in 250 ml Erlenmeyer flasks (200 ml of growth medium in 500 ml Erlenmeyer is also appropriate) and sterilizing them in an autoclave. Then an inoculant of 1 or 2 ml from a mature ecosystem is introduced into each flask. The experimental microecosystems can then be treated with some variable to evaluate the effect of this disturbance on the microecosystem. This process can be repeated at intervals of increasing frequency until you have microecosystems ranging in age from 2 days to 4 weeks, with a distribution of about 2 days old, 6 days old, 10 days old, 14 days old, 21 days old and 28 days old. By using this basic method students can look at many stages of change in one laboratory session.

The advanced method lends itself to long-term student projects where students take responsibility for starting the total number of microecosystems at one time, perhaps treating half of them with some experimental variable, and then sacrificing microecosystems at intervals to evaluate changes between intervals and/or changes between treated and non-treated microecosystems at various intervals.

Obviously one basic assumption of either method is that all the identical microecosystems behave in identical ways. From much research this appears to be true. A microecosystem sacrificed at some time interval can be assumed to have gone through all the stages found in earlier sacrificed microecosystems if they were all treated identically.

Growth Medium

Many types of solutions can theoretically be used as growth media. However, many of these are toxic to parts of the community which are to be grown

in them and therefore are of limited value. Also some growth media contain inorganic material only and promote autotrophic (plant first) succession, while others contain organic material also and promote heterotrophic (animal first) succession. Appendix A details the three most common growth media used in microecosystem growth studies by the Biology Program at Clemson. Rapid Grow solution is the easiest to make, promotes autotrophic growth, and is probably toxic to some of the protozoans. Hay culture medium is also easy to make, promotes heterotrophic succession and is safe for most organisms. Taub and Dollar solutions are difficult to make, since you need to round up all the chemicals required and must weigh amounts accurately, but they provide the most complete and safest growth media available. Taub and Dollar media promote autotrophic succession unless the Proteose Peptone supplement is added, in which case heterotrophic succession results.

This exercise is designed to examine mircoecosystems grown with two of these three growth media: the Rapid Grow growth medium and the heterotrophic Taub and Dollar growth medium. One hundred milliliters of Taub and Dollar growth medium can be placed in each of four 250 ml Erlenmeyer flasks, and 100 ml of Rapid Grow growth solution can be placed in each of two of the same type of flask. (See Table 7.1.)

Inoculants

The most successful inoculants come from somewhat stressed aquatic systems with really abundant species growing in high nutrient situations. These are usually sewage treatment ponds, dairy farm ponds, and slightly polluted farm run-off streams and lakes. City park ponds, horticultural garden ponds and farm ponds also provide decent inoculants. A scoop of soil from an earthworm culture, or a small amount of crumbled hay may also be used. Once microecosystems are established an old system is usually used to provide inoculants for the new systems when needed. Students may be encouraged to bring in suitable samples and to start their own microecosystems. One of the most successful microecosystems used in student projects came from an old Vitalis Hair Oil bottle found full of water and algae and stuck to the bottom of a temporary pond at an abandoned airfield.

Table 7.1

Number	*Growth Medium*	*Inoculant*	*Treatment*
1	Taub & Dollar	Texas	—
2	Taub & Dollar	Texas	Yes
3	Taub & Dollar	Pendleton	—
4	Taub & Dollar	Pendleton	Yes
5	Rapid Growth	Texas	—
6	Rapid Growth	Pendleton	—

This exercise is designed to examine microecosystems grown from two different inoculants. One came from a sewage oxidation pond in Austin, Texas in 1959 and has been grown in the laboratory for many years. The other inoculant came from a sewage holding pond in Pendleton, South Carolina in 1980 and has proven to grow well on the same growth medium as the Texas microecosystem. Both of these inoculants were placed into both Rapid Grow and Taub & Dollar growth medium flasks (Table 7.1).

Disturbances

Over the years many different substances have been introduced into microecosystems to investigate the effect of these substances on a complete ecosystem instead of on only one species. These substances include ionizing radiation (Ferens and Beyers 1972); crude oil (Dolan, unpublished data); mercury; defoliants (such as 2,4 D and 2,4,5 T) and pesticides (all from Wagner, unpublished data). Living organisms, including daphnia, *Heterandria formosa* minnows, and *Gambusia affinis* minnows, have also been used as disturbances in aquatic microecosystems.

In this exercise the experimental microecosystems could be treated with some variable from above as an example of a typical disturbance in a microecosystem. Thus, altogether there could be six different kinds of microecosystems to look at as shown in detail in Table 7.1.

Monitoring of the Microecosystems

Changes in the microecosystems are usually measured by three methods: (1) Microscopic observation can be made of the number of species and individuals seen in a sample from the microecosystem. (2) The pH of the microecosystem can be measured over a 24 hour period. The pH change observed in 24 hours relates to the rate of photosynthesis and respiration in the microecosystem. (3) Measurement of the biomass or weight of living organisms in the microecosystem is done by filtering the contents of the flask and weighing the material trapped on the filter paper after drying to constant weight.

Data Sheet

Age (days)	*Species Present*	*Numbers*	*Autotroph/Heterotroph*
2			
4			
10			
14			
21			
28			

Usually the observation method, or perhaps observation and biomass methods, can be used with the *basic* method of studying the microecosystem, while the pH measurement is usually used with the *advanced* approach to studying the microecosystems.

1. *Microscopic observation.* Swirl the youngest microecosystem to stir the contents completely. Take a sample with a pasteur pipette from the *center* of the flask and put two drops on a microscope slide. Cover slip the slide and examine on scanning, low and high power. Identify the organisms present either as species 1, 2, 3 etc. or by name, using illustrations provided as your instructor requests, and record the data on the data sheet. Also indicate if the organism is an autotroph (possesses chlorophyll) or a heterotroph (no chlorophyll present) and if the organism is rare (only 1 or 2 seen), common (frequently seen), or abundant (almost too many to count). Repeat the process for the other microecosystems in a series, putting the data on the data sheet.

Usually you will work with only one or two successional sets of microecosystems. Other groups in your laboratory will examine the other microecosystems, and your instructor will help you compare the data at the end of the laboratory.

What do these numbers tell us about succession? Both the numbers of species and numbers of individuals should start out low and increase through time, with individual numbers increasing rapidly and numbers of species increasing more slowly. At first only a few species do well in the "barren" ecosystem, but they become very numerous. Then, as the environment changes, more species become evident and each shares the space available for a given number of individuals.

Do your data reflect this hypothesis? Why or why not?

Which did you see more of first—autotroph or heterotroph? Why?

Comparison of species in different microecosystems can be done by adding up the numbers of species seen in any one flask and putting that value in the following table.

Comparison of Species

Microecosystem Number	*Age (days)* 2	6	10	14	21	28
1						
2						
3						
4						
5						
6						

Comparison of numbers in different microecosystems can be done by assigning numerical values to the descriptions. Thus rare would rate as 1, common as 2, and abundant as 3. Add the ratings for each stage or flask and put that value in the following table.

What do these values tell you about the difference or similarities of the various microecosystems?

If they are different, why are they different?

Graph the values from these tables on graph paper if it will allow you to see the differences among the microecosystems better.

In talking to the other groups in the laboratory and in looking at their samples, do you find that the species differ in the different microecosystems? in the different treatments? Why or why not?

Why are some organisms adapted to living in the early or "barren" stages of ecosystem succession when by their efforts they change the system and are out-competed by other organisms in the new environment?

2. *pH measurement.* The amount of carbon dioxide dissolved in the water affects the pH of the microecosystem by the production of carbonic acid. The photosynthesis of the microecosystems in the light uses CO_2, and therefore the pH gradually rises during the daylight. At night the microecosystem respires and releases CO_2 which lowers the pH of the microecosystem as carbonic acid forms in the water.

By placing a pH electrode into a microecosystem and hooking a continuous recorder to the pH meter a 24 hour pH curve can be produced (Fig. 7.1). This curve is directly related to CO_2 gain or loss to the water of the microecosystem. This water can be titrated with CO_2–saturated water, and a curve of the amount of CO_2 gain and loss to the water may be produced for the 24 hour period. This method is detailed in Beyers (1970). A simple but accurate approximation of CO_2 gain and loss may be made by graphing pH units gained and lost for the 24 hours (as in Fig. 7.2) and weighing the area of graph paper

Comparison of Numbers

Microecosystems Number	*Age (days)* 2	6	10	14	21	28
1						
2						
3						
4						
5						
6						

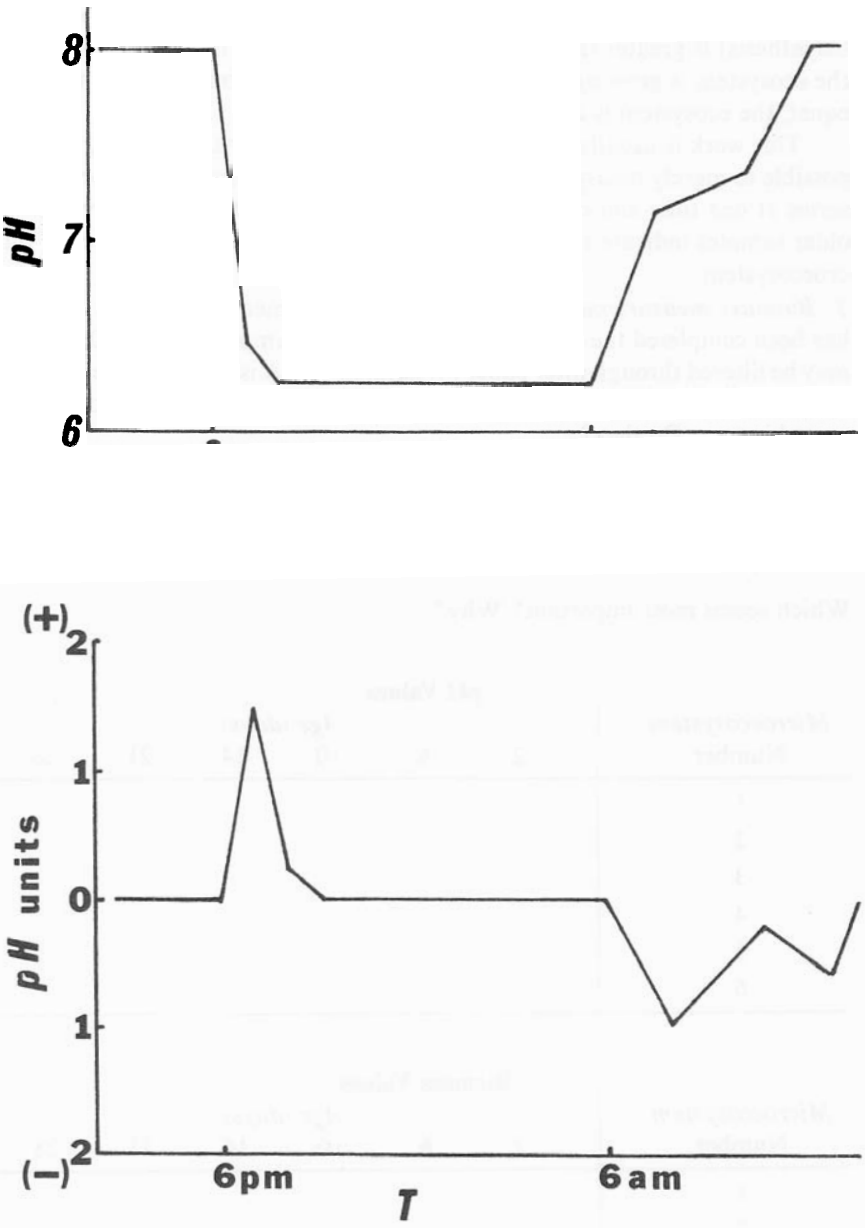

Figure 7.2. 24-Hour pH unit (CO_2) gain or loss for a microecosystem. + indicates CO_2 gain; − indicates CO_2 loss.

for either gain or loss during the period. If the area (weight) of CO_2 loss (photosynthesis) is greater than the area (weight) of CO_2 gain (respiration), then the ecosystem is growing. If it is reversed, the ecosystem is dying. If they are equal, the ecosystem is at equilibrium or climax.

This work is usually done only for the advanced testing method, but it is possible to merely measure pH of all the microecosystems in one successional series at one time and compare the values. In daylight higher pH values in older samples indicate more photosynthesis, and thus a more productive microecosystem.

3. *Biomass measurement.* After the pH measurement and species analysis has been completed the entire microecosystem or sample thereof (50 ml etc.) may be filtered through filter paper (Whatman GF/A is best) and the retained material weighed after air- or oven-drying. Older microecosystems should have more biomass. Do they?

Do various treatments differ? If so why?

Overall do your results indicate differences between growth media, inoculation and/or treatments?

Which seems most important? Why?

pH Values

Microecosystem Number	*Age (days)* 2	6	10	14	21	28
1						
2						
3						
4						
5						
6						

Biomass Values

Microecosystem Number	*Age (days)* 2	6	10	14	21	28
1						
2						
3						
4						
5						
6						

6	

6	

References

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APPENDIX A

Taub and Dollar One-Half-Strength Solution (All in Distilled Water)

Solution	Salt	gm/l stock	ml stock/l working solution
B	$MgSO_4 \cdot 7H_2O$	24.65	1.0
C	KH_2PO_4	13.80	1.0
	NaOH	2.80	
D	$CaCl_2$	11.70	10.0
	(or $CaCl_2 \cdot 2H_2O$)	(14.70)	
F	EDTA	26.10	0.062
	NaOH	10.70	
	$FeSO_4 \cdot 7H_2O$	24.90	
G	H_3BO_3	1.85	0.50
	$ZnSO_4 \cdot 7H_2O$	0.28	
	$MnCl_2 \cdot 4H_2O$	1.98	
	$NaMoO_4 \cdot 2H_2O$	0.242	
	$CuSO_4 \cdot 5H_2O$	0.049	
	$Co(NO_3)_2 \cdot 6H_2O$	0.291	
Supplements (both are used for heterotrophic succession)			
	NaCl	5.84	15.0
	Proteose Peptone (American Scientific Products)		.5gm/liter
	NaCl	5.84	15.0
	Proteose Peptone (American Scientific Products)		.5gm/liter
	NaCl	5.84	15.0
	Proteose Peptone (American Scientific Products)		.5gm/liter

Rapid Grow Solution

1 gm Rapid Grow per liter of distilled water. Available from garden supply dealers.

Hay Culture Solutions

50 gm hay boiled for 15 min in 750 ml distilled water

Filter through cheese cloth or filter floss.

Increase volume to 1 liter for stock solution.

Working solution: 100 ml stock per 900 ml distilled water.