

Chapter 13

Eutrophication - A Project Lab for Multi-Section Lab Courses

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Introduction

Background

Students at Georgia Southern University are required to successfully complete an environmental science course and its associated lab course as part of the core curriculum. Currently, there are four environmental courses and labs offered: Environmental Biology and Environmental Biology Laboratory; Environmental Chemistry and Environmental Chemistry Laboratory; Environmental Geology and Environmental Geology Laboratory; and Environmental Physics and Environmental Physics Laboratory. Of the four science disciplines, the Department of Biology is the only department with the facilities to accommodate large numbers of students. Thus, both the lecture and lab are open to over 900 students per semester. There are five lecture sections and 36 lab sections offered each semester; each lab section enrolls 25 students. Students are encouraged but not required to take the lecture and laboratory component at the same time.

The laboratory course consists of both project based labs and stand-alone labs. During a normal semester, there are two projects going simultaneously. Projects are set up during the second lab of the semester and maintained throughout the remainder of the semester. Students are required to make weekly measurements and observations prior to beginning the stand-alone lab for that week. All the labs easily tie into each other and to the projects. Students, working in groups, are required to present a poster detailing one of the projects and their results at the end of the semester. Much of the laboratory instrumentation and equipment was purchased through an NSF-ILI grant awarded in July 1998.

General Information

Eutrophication is a process whereby nutrient-rich water supports prolific growth of algae and/or other aquatic surface plants (such as duckweed, water hyacinth, water fern, mosquito fern, and water lettuce). This process occurs naturally as sediment is moved from streams to ponds, or erosion takes place along the banks of waterways (Botkin and Keller 2000; Miller 2001). However, this process can be speeded up considerably by human activities. Nutrients, mostly in the form of nitrate and phosphate, are introduced via effluents from sewage treatment plants, runoff from fertilizers or animal waste, and erosion of topsoil (Miller 2001). As temperatures warm, surface plants and algae experience abundant growth. Dissolved oxygen is depleted at both the surface and the bottom of the waterway when the dense algae or plants die and fall to the bottom and are decomposed by aerobic bacteria. As the oxygen levels decrease, animal life begins to die off, eventually leading to fish kills.

Eutrophication has become a major problem in coastal waterways. Dr. Jane Lubchenco of Oregon State University, testifying before the House of Representatives in May of 1999, stated that there are at least 50 of these dead zones located around the world. She further described some 1600 square miles of low or no oxygen in the Gulf of Mexico, making it the largest area so affected in the Western Hemisphere. States such as Florida are particularly vulnerable due to its expansive coastline combined with numerous golf courses whose upkeep requires substantial quantities of fertilizers. Additionally, agricultural industries such as poultry farms that are located close to waterways are increasing the speed of eutrophication. As their liquid waste is sprayed on adjacent fields, nutrients from the waste are being washed into bodies of water and causing major algal blooms.

Objectives

- Provide students with a simulation of the process of eutrophication using both aquatic environments and terrestrial environments.
- Provide the students with an opportunity to become familiar with various water quality testing instruments.
- Provide students with a collaborative learning environment.

Time Considerations

Initial setup and discussion of this project takes less than two hours (we set up two projects at once in a two-hour lab). The most time consuming aspect of setup is instruction on how to use the various pieces of equipment. The first ten minutes of each lab thereafter is devoted to students collecting weekly data and recording that data. The project can be run between 7 and 12 weeks, but enough time should be set aside at the end of the project for discussion and a poster session (or other form of assessment). Upon breaking down the project, students test the soil of the terrestrial component to determine pH, and concentrations of nitrogen, phosphorous, and potassium.

The following timetable is recommended:

- one lab period for setup and initial discussion
- 10-15 minutes per lab period for data collection and recording
- one lab period for breakdown of the project to include soil quality testing and discussion of results
- one lab period for the poster session

Materials

Initial Setup

- Three 5-, 10-, or 25-gallon aquariums
- Dissolved oxygen meter – one per classroom. Recommended models are Hanna Handheld Waterproof DO Meter (pricey but reliable) or YSI Model 550 DO Meter; both can be purchased from Fisher Scientific (800-766-7000), YSI 550 also from Forestry Suppliers (800-647-5368).
- Conductivity meter – one per classroom. Recommended models Corning Model 311 or Corning Model 316; both can be purchased from Fisher Scientific.
- pH meter – one per classroom. Recommended models any bench top (more durable than most portables). We use Denver Basic or Fisher Acumet (same instrument); Denver can be purchased from many vendors including Fisher.
- Buffer solutions – pH 4.0 and 10.0
- Top loading electronic balance – one per classroom
- Spectrophotometer – one per classroom. Recommended model ThermoSpectronic Genesys 20; can be purchased from several vendors including Fisher Scientific.
- Cuvettes, Kim wipes, and plastic transfer pipettes for spectrophotometer
- Thermometer, spirit-filled – one per classroom. Many of the meters used also measure temperature so you may omit this.
- 100-ml Graduated cylinder – 3 per classroom. We use plastic.
- 1-Liter Flasks – 3 per classroom. One flask will contain tap water, one will contain a moderate concentration of fertilizer solution (1g/liter of tap water), and one will contain a high concentration of fertilizer solution (5g/liter of tap water)
- 250-ml Beaker – 2 per classroom. One to be filled with deionized water for pH electrode and one empty to get water samples to test pH.
- Masking tape to label plant pots
- 6-inch plant pots – two per lab group
- Potting soil or potting soil and topsoil mixture. If potting soil is peat-like, add topsoil (50/50) to allow soil-testing capsules to work.
- Radish seeds – 3 seeds per pot
- Fertilizer – use Miracle Grow or something like Miracle Grow. We purchase anything near 15-30-15, which can be purchased at a local plant store or farm store.
- *Elodea* or *Anachris* – 15 to 20 stems per classroom. This will be divided up between the three aquariums.
- 500-ml Graduated cylinder – one per classroom for adding fertilizer solutions to aquariums.
- Water Quality Test Kits – one per classroom for nitrate, nitrite, and phosphorous. We use LaMotte kits purchased from Carolina Biological (800-334-5551).
- Suggested additions (from the workshop): duckweed, water hyacinth, or any other surface plant that will have abundant growth when given excess nutrients

Weekly Requirements

- Dissolved oxygen meter
- Conductivity meter
- pH meter, beaker of deionized water, 4.0 and 10.0 buffers, and empty 250 ml beaker
- Spectrophotometer, cuvettes, Kim wipes, plastic transfer pipettes for samples
- Thermometer
- 500-ml graduated cylinder
- Rulers – 1 per group
- 1-Liter flask – three per classroom (one containing tap water, one with moderate concentration of fertilizer, one with high concentration of fertilizer)

Breaking Down

- Dissolved oxygen meter
- Conductivity meter
- pH meter, beaker of deionized water, 4.0 and 10.0 buffers, and empty 250 ml beaker
- Spectrophotometer, cuvettes, Kimwipes, plastic transfer pipettes for samples
- Thermometer
- Rulers – 1 per group
- Plastic basin or bucket – 3 per lab room (one for soil from control plants, one for moderate fertilizer, one for high fertilizer)
- Plastic spoons – 2 or 3 per group
- Soil test kits – 1 per group. We recommend Rapitest® which can be purchased through Carolina Biological (800-334-5551), Wards Biology (800-962-2660), and Nasco Science (800-558-9595) or others

Notes for the Instructor**I. Procedures for Setup and Tips**

1. Fill all three aquariums to within 6-8 cm from the top with tap water and let sit overnight to remove any chlorine (or use InstaChlor or other chlorine removers).
2. Since all aquariums are filled from the same source (tap water), only one tank needs to be tested for nitrate, nitrite, and phosphate levels. This will help reduce costs. Additionally, if you have several lab sections, you may want to have the first lab in that classroom do the testing and leave the numbers on the board for subsequent classes.
3. The same is true for weighing of *Elodea* or any other aquatic plant you use. The first lab in the classroom can weigh and leave the weights for subsequent classes.
4. I recommend having no more than five students in a group. Each group should be assigned the weekly task of collected specific data. Our classrooms are equipped with five benches seating five students each. Bench one tests for dissolved oxygen, bench two test conductivity, bench three tests pH, bench four tests optical density, and bench five measures temperature and adds the appropriate fertilizer solution. Assignments for your students will depend on your lab configuration.
5. Each student group should plant radish seeds in two pots. This gives plenty of replicates in the case of plant death or no germination. Once plants have germinated, students should choose the strongest stem and weed out the others to cut down on competition for nutrients and water. This will insure the best growing conditions. Plants should be kept on a light cycle (we use a 10-hour light: 14-hour dark cycle) to promote growth. Watch for plants drying out in between lab meetings. (When our air conditioning goes out, we have to give a supplemental watering.

Rather than trying to figure out which plant receives which treatment, we give them all tap water. This infrequent occurrence should not significantly change the results.)

6. It is important that students know why they are doing this project. Since they work on one piece of equipment, they can usually tell you why they are looking at their particular component but may not be able to tell you about other components. We usually discuss what they are finding and what it means during the brief time they are doing their weekly observations to keep it fresh in their mind.

Procedures for Breakdown and Tips

1. Students should make a final measurement of their plants and put the soil in the appropriately labeled basin or tray: control, moderate fertilizer, and high fertilizer. Masking tape should be removed from the pots and plants either discarded or taken with them.
2. Students should test the soil for pH, nitrogen, potassium, and phosphate using a soil test kit. Results for each treatment should be averaged.
3. Water test kits should be used by the first lab to test each aquarium for nitrate, nitrite, and phosphate concentrations. You will need to calculate dilution factors prior to lab as the concentrations of both the moderate and high fertilizer tanks will be too high for the tests to detect.
4. *Elodea* and other plant materials should be weighed. The *Elodea* from the high fertilizer tank will often disintegrate as students try to remove it from the aquarium. Final weights and percent decomposition should be shared with subsequent labs.
5. Students will get class averages for radish plant heights, either height over time, or total growth for the two treatments and the control. Initial and final data should be obtained for *Elodea* and results from water test kits. All other weekly data should be graphed over time.

Student Outline

Introduction

Aquatic environments are susceptible to many forms of pollution. Some pollutants, such as acid rain, present immediate, observable harm to the environment, while others deliver more subtle, long-lasting effects. One such effect is the process of *eutrophication*, resulting from continuous pollution in the form of agricultural run-offs or sewage outflows (human or animal). Both fertilizers and sewage are high in *nitrogen* and *phosphorus*, two nutrients essential for plant growth in any environment. In aquatic systems, these nutrients typically are in low concentrations, limiting the numbers of algae and aquatic plants. When concentrations are increased to very high levels, eutrophication occurs.

The first stage of eutrophication is an *algal bloom*. Algal populations experience exponential growth in response to heavy nutrient loads in this situation. Algal blooms appear as a thick green film near the surface of the water. This prevents sunlight from reaching other algae and aquatic plants below the surface, causing them to die. The large amounts of dead plant material are then decomposed by bacteria that use oxygen and produce carbon dioxide while feeding on the plant matter. High rates of decomposition result in *critically low oxygen levels and high levels of carbon dioxide*, both of which are detrimental to aquatic animals. The result of eutrophication is typically a large number of dead organisms, most noticeably a fish kill.

In this lab, we will observe the effects of three fertilizer concentrations (control, moderate, and high) on terrestrial plants and aquatic environments. Over the course of several weeks, we will measure the growth of terrestrial and aquatic plants as well as water quality of the aquatic environments.

What effect do you think fertilizer concentrations will have on the terrestrial plants?

What effect(s) will the fertilizer concentrations have on the aquatic environments?

Procedure

Terrestrial Environment

1. *Each bench* should obtain two pots from their instructor and *completely* fill them with soil from the bucket labeled “potting soil-compost mixture.” On a piece of masking tape, write your *name*, your *TA’s name*, your *lab day and time*, and your *treatment*. Place the tape on the outside of the pot.
2. Place your pot in the tray on the plant rack assigned to you by your instructor. Water your plant with *100ml of water* only. No fertilizer will be added to any treatments the first week to allow for germination of your plant. Each week thereafter you will water your plant with your *assigned treatment*.
3. Plant *two* seeds in each pot by making two depressions 1-2 cm deep, placing a seed in each, and then covering them with soil. One of the two plants will be thinned out; the other used for the experiment. *Using more than two seeds will result in crowding of your plants and will eventually kill them.*

Aquatic Environment

The first lab of the week will do the following:

1. Obtain some *Elodea* (an aquatic plant) from your instructor. Place it on several layers of paper towel to absorb the water. Divide it into 3 fairly even bunches.
2. Determine the mass of one bunch of the *Elodea* using the electronic balance and record it in row labeled “Control” in Table 1. Place the *Elodea* in the aquarium labeled “Control.”
3. Repeat with the second bunch of *Elodea*; record the weight in the row labeled “Moderate” and place it in the aquarium with the same label. Repeat with the third bunch and record the weight in the row labeled “High” and place it in the aquarium with the same label. *Be sure to put the*

weights of all three bunches of Elodea on the board to be used by the remainder of the labs for the rest of the week.

4. Measure the nitrate, nitrite, and phosphorous of the aquatic environments using the test kits provided. Test the water from only one tank, as there has not been anything added to any of the tanks yet. Record the nutrient levels in Table 2. *Be sure to record this information on the board to be used by labs for the rest of the week.*

All Labs will do the following each week:

1. Measure the *water quality variable assigned to your bench for all treatments* with the appropriate instrument. Record them in the appropriate table (3,4, or 5) depending on the treatment. Your instructor will demonstrate the use of the water quality meters. *Your bench will be responsible for measuring this variable each week for all three treatments throughout the semester.* The following are water quality variables for each bench:

Bench 1 will test for dissolved oxygen for each tank

Bench 2 will test the conductivity for each tank

Bench 3 will test the pH for each tank

Bench 4 will test the optical density for each tank

Bench 5 will record the temperature for each tank and will water each tank with 250 ml of the appropriate fertilizer solution

2. Each week, the plant height for each pot *for your bench* needs to be measured. Record that information in Table 3. After measuring, each plant should be watered with 75 ml of the appropriate fertilizer solution. *Water your plants while they are on the plant tray, not on your bench.*

Final Week:

The first lab of the week will use the water quality test kits and record their data on the board. This information will be shared with the remainder of the labs for the week. You will also weigh the Elodea remaining in each tank and record this data on the board.

All labs will complete the following:

1. Collect all necessary data and record it in Table 3, 4, or 5.
2. Record any qualitative information that you think might be a result of fertilizer concentration (e.g., color of water, health of *Elodea*, health of radish, etc.).

Data Tables

Table 1. Average mass of Elodea watered with one of three concentrations of fertilizer.

Treatment	Initial mass (g)	Final mass (g)	change (g)	percent change (%)
control				
moderate				
high				

Table 2. Nutrient concentration of an aquatic environment watered with one of three fertilizer treatments.

Nutrient	Control			Moderate			High		
	Initial Conc. (ppm)	Final Conc. (ppm)	Change (ppm)	Initial Conc. (ppm)	Final Conc. (ppm)	Change (ppm)	Initial Conc. (ppm)	Final Conc. (ppm)	Change (ppm)

Table 3. Plant and water quality measurements made on terrestrial and aquatic environments over eight weeks with **no** fertilizer added.

Week	Plant height (cm)	Oxygen (ppm)	Conductivity ($\mu\text{S}/\text{cm}$)	pH	Optical density	Temperature ($^{\circ}\text{C}$)
1						
2						
3						
4						
5						
6						
7						
8						

Table 4. Plant and water quality measurements made on terrestrial and aquatic environments over eight weeks with **moderate** fertilizer added.

Week	Plant height (cm)	Oxygen (ppm)	Conductivity ($\mu\text{S}/\text{cm}$)	pH	Optical density	Temperature ($^{\circ}\text{C}$)
1						
2						
3						
4						
5						
6						
7						
8						

Table 5. Plant and water quality measurements made on terrestrial and aquatic environments over eight weeks with **high** fertilizer added.

Week	Plant height (cm)	Oxygen (ppm)	Conductivity ($\mu\text{S}/\text{cm}$)	pH	Optical density	Temperature ($^{\circ}\text{C}$)
1						
2						
3						
4						
5						
6						
7						
8						

Questions for Thought

Under which treatment did the terrestrial plants grow best? Under which treatment did the aquatic plants grow best? If there was a difference, was it due to treatment?

Which treatment caused the largest change in water quality (using both the weekly data and the initial and final water nutrient measurements)?

Were there differences in effects between terrestrial and aquatic environments for any individual treatments? If so, why?

Acknowledgments

I would like to thank the Graduate Teaching Assistants from the Department of Biology at Georgia Southern University. Their suggestions for improvement over the last two years has helped develop this project lab into the success it is today. Further, I would like to thank the Department of Biology and the Georgia Southern University Office of Research and Development for the providing funds that allowed me to travel to ABLE.

Literature Cited

- Botkin, D. and E. Keller. 2000. Water pollution and treatment. Pages 414-438, *in* Environmental science: earth as a living planet. John Wiley & Sons, 649 pages.
- Luchenco, Jane. 1999. <http://www.house.gov/resources/106cong/fisheries/99may06/lubchenco.htm>
- Miller, G.T., Jr. 2001. Water resources and water pollution. Pages 287-325, *in* Environmental science. Eighth edition. Brooks/Cole, 549 pages.

Appendix A – Poster Requirements

Environmental Biology Laboratory Poster Presentations-General Requirements

Poster sessions will be held during your regularly scheduled lab period. **Attendance is mandatory or you will get a zero for your poster grade.**

Grading: Posters will be graded in three parts:

- 1) Evaluation by your peers - 10 of the 50 points available (20%) will be given by your classmates. Each student will be given one evaluation form per poster. You will be evaluated on the following criteria:
 - a. General appearance - is it neat and well laid out?
 - b. Content - does it contain all the required elements?
 - c. Clarity - does it make sense?
 - d. Thoroughness - have you **completely** covered
 1. how you did the experiment
 2. what your results were
 3. why you got those results
 4. what those results mean
 - e. Group understanding - can you answer questions from your peers and instructor about your project?
- 2) Within-group evaluations - 10 points (20%) will be given by your partners to evaluate your contribution and participation in the project. This provides those students who have shared equally in both the project and the poster to get full points and those who have not done their share to lose points. This evaluation will be on a separate form from the peer evaluation.
- 3) Lab Instructor evaluation - 30 total points from your lab instructor . Your lab instructor will grade your poster as a group, not an individual, so each person in the group will receive the same grade on content but not necessarily on participation. Posters will be evaluated using the same criteria as the peer evaluations above but in more detail. For example, content will be graded on the presence of all the required elements to include properly completed tables and/or graphs, etc.

Required Elements

Size: Posters should be standard poster size.

ALL SECTIONS, TO INCLUDE GRAPHS AND TABLES, MUST BE COMPUTER GENERATED.

Title for the poster.

Authors - list all of the people in your group **alphabetically by last name**, whether you think they participated fully or not.

Double space **each** of the following sections and use **12-point font** on all text:

Introduction:

Write a brief (**one paragraph**) introduction to include why you did the experiment (what importance does it have in the environment), and a broad explanation of what you did (not how you did it).

Hypothesis or hypotheses: state your hypothesis or hypotheses in a brief but complete sentence.

Procedures:

In paragraph form, explain how you did it (hint: much of this is in your lab manual; summarize in **your own words**). Include both control and treatment when applicable.

Results:

Appropriate tables and figures (computer generated!). Each table and figure should be self-explanatory.

Conclusions and Discussion:

Did the results support the hypothesis? Why or why not? Support this with your results. Be *very* thorough here. Include any possible sources of error and how they may have influenced your results.

How do you think your test system reflects what is going on in the environment?

Acknowledgments - include **only** people who actually participated and/or helped you on your project.

Peer Evaluation Form

Your Social Security No: _____

Assign the appropriate number of points for **each** category for **each** poster.

- 1. General Appearance:** Is the poster neat and well laid out? **2 pts. total**
- 2. Content:** Does it contain all of the required elements? (Title, Authors, Introduction, Hypothesis, Prediction, Procedures, Results, Conclusion, and Acknowledgments) **1 pt. total**
- 3. Clarity:** Does the poster make sense? **2 pts. total**
- 4. Thoroughness:** Does the poster completely cover a) how the experiment was done; b) what the results were; c) why they got the results they did; d) what those results mean? **3 pts. total**
- 5. Group Understanding:** Were group members able to answer questions from you and the instructor? **2 pts. total**

Authors of Poster: _____

1. 2. 3. 4. 5. **Total Points Earned:**

Authors of Poster: _____

1. 2. 3. 4. 5. **Total Points Earned:**

Authors of Poster: _____

1. 2. 3. 4. 5. **Total Points Earned:**

Authors of Poster: _____

1. 2. 3. 4. 5. **Total Points Earned:**

Authors of Poster: _____

1. 2. 3. 4. 5. **Total Points Earned:**

Within Group Evaluations

Your Social Security No. _____

Group Member's Name: _____

Points for Participation in Data Collection (Circle One):

1 2 3 4 5 6

Points for Participation in Poster Construction (Circle One):

1 2 3 4