Promoting Student Involvement with Environmental Laboratory Experiments in a General Microbiology Course

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This is a descriptive study of a series of laboratory exercises on environmental microbiology carried out by students in a general microbiology course during eight of the twelve weeks of the semester. The revised laboratory component is predicated upon seawater and sediment samples collected by student pairs using marine sampling equipment on a field trip aboard a research vessel. Two longitudinal studies were performed: assay for antibiotic production from isolated actinomycetes and construction and observation of Winogradsky columns. Two additional experiments: culturing microalgae and water testing for coliforms also used the samples collected by the students. The advantages of long-term, challenging laboratory experiences actively involving the students in group process, self-direction, and scientific practices are discussed. Also considered are development of laboratory skills, scientific competencies, and students' self-confidence in carrying out such environmental investigations. Plans for future assessment of student learning are presented.

Throughout the United States, curriculum reform in the sciences is underway in introductory biology courses to promote students overcoming their fear of biology and emphasizing science as a process (14). Several years ago, curriculum recommendations for undergraduate microbiology courses were set forth by the American Society for Microbiology (ASM) (2). In response to the ASM's core themes and concepts, the author revised the General Microbiology course offered to biology majors in the Department of Biological Sciences at Kingsborough Community College of The City University of New York, located in Brooklyn, New York. The underlying theme of this revised course was a new focus on the diversity of microbes, particularly the beneficial roles of microbes as well as their positive activities and interactions vital to sustaining life on the planet. Historically, some college microbiology courses tended to emphasize microbes involved in disease, thereby conveying a possible misconception that microbes are viewed solely in a potentially deleterious way. This emphasis has contributed to a microbe "phobia" among science students at various levels of education. While there is no doubt damaging microbes are quite significant and warrant study, the science of microbiology is far more comprehensive. Many microbes interact with plants, humans, and other animals and contribute to the environment in innumerable, sometimes neglected, but highly beneficial ways.

Kingsborough Community College (KCC) is an urban community college. Its 71-acre campus is on a peninsula bordered by the waters of Sheepshead Bay and Jamaica Bay. The college has several vessels available for class trips, for science courses, and for faculty and students to carry out scientific investigations in the proximal marine areas and within easy access to the campus. Students can take biology courses

within the Department of Biological Sciences leading to an Associate in Science in Biology in preparation for further upper-division education. To complete this Associate in Science degree program, students are required to take a one-year General Biology sequence followed by two advanced biology courses of their choice along with other science and general education requirements. The advanced biology laboratory courses most recently offered include: Comparative Anatomy, Developmental Biology, General Microbiology, Genetics, Invertebrate Biology, and Marine Biology. Each of these 12-week courses is offered once a year, enrolls a maximum of 24 students, and consists of a 3-hour laboratory and 3-hour lecture-discussion session. For the most part, the bulk of the students have limited experiences in conducting laboratory experiments, going aboard vessels, and using marine sampling equipment. Many have little knowledge about the marine environment and the diversity of the microbial world in different environments.

This paper is a descriptive study of a series of laboratory exercises on environmental microbiology that students conduct throughout eight of the twelve weeks of the General Microbiology course. The revised labs incorporate a field trip to collect environmental samples, seawater and sediment, using the research vessels and marine sampling equipment available at KCC. The importance of field trips in biology majors' courses, such as botany and ecology, has previously been reported (1, 12).

The revised 3-hour laboratory component of the course has been designed so students will be actively engaged in laboratory experiences. Research literature supports the idea that student involvement leads to learners who are motivated and more involved in the learning process (7). To many students, microbiology lab is the more dynamic portion of a microbiology course. The many aspects of microbes, their anatomy, biochemistry, and potential use in other fields of study are made apparent by laboratory experiments the students actually carry out. At KCC, the instructor emphasizes the importance of microbes by using environmental examples.

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Experiments on isolation of antibiotic-producing microbes and oil degradation by microbes emphasize microbial diversity. These experiments also illustrate the variety of biochemically produced compounds and enzymes that are used industrially. Students also study the environmental problem of algae overgrowth as a result of the addition of nitrates and phosphate to water. So that the disease potential of microbes is not ignored, students cultivate tomato plants and infect them with either tobacco mosaic virus or *Agrobacterium tumefaciens*. Students observe the development of tumors in plants infected with *A. tumefaciens* and realize the potential of this bacterium to be used to transfer genes to plants. In another genetic study, students transform *Escherichia coli* with a plasmid carrying the *lux* genes from *Vibrio harveyi* responsible for producing bioluminescence. Each experiment had an environmental theme yet demonstrated various roles and activities of microbes. The rationale was to have

students become aware of two important biological topics, their environment and the microbial world, and the delicate balance involved in sustaining both. The laboratory experiments for the entire course, including those just mentioned plus the environmental activities this paper will focus on, are listed in Table 1 along with the time frame in which they were performed.

MATERIALS AND METHODS

Field trip for collection of water and sediment samples.

To begin the environmental microbiology exercises, the laboratory experience of the fourth week of the semester, usually in late March to early April, involved the students going on a boat trip in Jamaica Bay. The ship's captain and crew and the class instructor provided thorough safety instructions and shipboard procedures. The students were given instruction about marine safety procedures prior to boarding the vessel,

TABLE 1. Laboratory experiments and time frame to conduct experiments^a

Lab activity	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12
Microscopy												
Staining												
Pure culture techniques												
Bacterial growth, population counts, and types of media												
Field trip: water and sediment collection												
Winogradsky column												
Isolation of antibiotic producers and testing for antibiotic production												
Culturing microalgae												
Biochemical testing to identify unknown bacteria												
Inhibition of microbial growth, antiseptics, disinfectants, antibiotics, and ultraviolet light												
Algal bloom study												
Fungi, protozoa, and slime molds												
Viral plaque assay												
Bioremediation: oil spills and cellulose degradation												
Colilert water analysis												
Mutualism, commensalism and antagonism (TMV and <i>A. tumefaciens</i>)												
Food microbiology												
Genetic transfer of <i>lux</i> gene												

^aThe table shows the laboratory activities performed during the 12-week semester. The darkened boxes designate the weeks in which the experiments were being performed. Note: the use of prior techniques, such as aseptic transfer or pure culture techniques, are not marked on the table.

and all personnel on the vessel were required to wear life jackets from boarding of the vessel until the vessel returned to shore. While on this trip, an introduction to the ecology of this region, which includes the largest national urban park, The Gateway National Recreational Area, was given. The prime objective of this lab was for each pair of students to become familiar with the basic collection equipment and to collect a water and sediment sample at depths of 15 to 25 feet. With the assistance of their instructor, students learned how to use sampling equipment: a LaMotte water sampler, a benthic box, and a VanVeen sampler. Beyond these collections, some students enthusiastically volunteered to collect samples from their backyards, vacant land in their neighborhoods, and local ponds and beaches. The additional sites provided a wide range of samples representing different microbial communities on which the students could conduct future experiments. After collection of enough sediment to fill a 1 gallon Ziploc bag, students classified their sediments as mud, clay, silt, or sand based on the color and texture. White to tan colored sediment with a gritty texture when rubbed against a ceramic tile is sand. Clay is usually chocolate brown in color with a smooth texture. Silt is usually chocolate brown in color with a gritty texture. Mud is usually black in color and is a combination of smooth and gritty textures. Upon returning to the laboratory, the students stored all their samples in a refrigerator.

Actinomycete isolation and testing for antibiotic production. Students prepared dilutions (10^{-1} to 10^{-4}) of 1 gram of their sediments in sterile water and plated them on appropriate media. To isolate actinomycetes, bacteria well known for their ability to produce antibiotics, actinomycete isolation medium, chitin agar, and starch-casein agar were used (Table 2). All media were supplemented with 15 µg/ml of nystatin and 15 µg/ml of cycloheximide to inhibit fungal growth. These plates were incubated for 4 weeks at 28°C. After the 4 weeks of incubation, actinomycete isolation plates were observed. Each student pair purified colonies that appeared to be actinomycetes on glucose peptone agar plates, using previously learned pure culture techniques. All plates were incubated at 28°C for 1 week. In the fifth week, these purified colonies were examined macroscopically and microscopically. Based on these observations, colonies preliminarily identified as actinomycetes were then grown in two types of fermentation broth, yeast extract and glucose peptone broth, for 1 week. Actinomycetes were tested for antibiotic production against a variety of gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*),

gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Serratia marcescens*), yeast (*Candida albicans*), and filamentous fungi (*Aspergillus niger*) by an agar diffusion assay similar to that carried out for Kirby-Bauer testing of antibiotics. Glucose peptone agar plates were swabbed with the test organisms. Then, disks saturated with the 1-week-old actinomycete culture broths were placed on the seeded plates. Bacterial plates were incubated at 37°C for 24 to 48 hours; fungal plates were incubated at 28°C for 72 to 96 hours. After incubation, the students examined plates for zones of inhibition and measured them in millimeters. The number of actinomycetes capable of producing antibiotics was determined. This longitudinal study took 8 weeks for the students to complete.

Winogradsky column. During the 4-week incubation period previously mentioned, the collected sediment and water were continually used to set up other experiments. Student pairs prepared a Winogradsky column using their sediment and water samples. Plastic fluorescent light containers were cut to 12 inches in length and used for their columns. A rubber stopper was placed at one end, the column was filled with sediment and overlaid with their water sample, and plastic wrap was used to seal the opposite end. Students recorded ongoing changes in color and gas production taking place in the Winogradsky column over a 6-week

TABLE 2. Components of media used for Actinomycete isolation and testing for antibiotic production

Medium	Component	
Actinomycete isolation medium (AIM) (DIFCO)	AIM	22.0 g
	Glycerol	5 g
	d H ₂ O	1,000 ml
Chitin agar	Agar	20.0 g
	Chitin	4.0 g
	K ₂ HPO ₄	0.7 g
	MgSO ₄ ·7 H ₂ O	0.5 g
	KH ₂ PO ₄	0.3 g
	FeSO ₄ ·7 H ₂ O	0.01 g
	MnCl ₂ ·4 H ₂ O	0.0001 g
	ZnSO ₄ ·7 H ₂ O	0.0001 g
	d H ₂ O	1,000 ml
Starch casein agar	Agar	18.0 g
	Starch	10.0 g
	Casein	1.0 g
	d H ₂ O	1,000 ml
Glucose peptone agar	Glucose	50.0 g
	Agar	18.0 g
	Peptone	15.0 g
	d H ₂ O	1,000 ml
Yeast extract broth	Malt extract	10.0 g
	Yeast extract	4.0 g
	Glucose	4.0 g
	d H ₂ O	1,000 ml
Glucose peptone broth	Glucose	50.0 g
	Peptone	15.0 g
	d H ₂ O	1,000 ml

longitudinal period and submitted a written report, including labeled drawings of their column, for each of the 6 weeks.

Culturing microalgae. Water and sediment were also used to culture microalgae. Sediment was placed in plastic petri dishes and the water sample was added. Coverslips were then placed on top of the water-sediment layer and exposed to sunlight for 1 week. Students examined their sediment each day to ensure their cultures were not drying. If drying was evidenced, more water sample was added. After 1 week, the coverslips were placed on glass slides and examined with a microscope for microalgae.

Water testing for coliforms. The water samples were tested using the Colilert water testing system (Idexx Laboratories) to determine the presence of total and fecal coliforms. The Colilert testing system consists of a sterile 100-ml plastic bottle and Idexx Colilert reagent powder. The students put 100 ml of their water sample in the sterile bottle and then added the Idexx Colilert reagent. The bottle was sealed, shaken to dissolve the powder, and incubated at 37°C for 24 hours. After incubation the students observed the color of the water in the bottle, with yellow indicating the presence of coliforms. If the water in the bottle was yellow, it was then exposed to long-wavelength ultraviolet light. If the water fluoresced, fecal coliforms were present.

Detailed procedures for all of these exercises were provided to the students in *Laboratory Exercises in Microbiology* (11). The students used this manual throughout the course and were asked to perform the experiments following the procedures in the lab manual. Since minimal time was taken during the lab to state the procedures, students worked on their experiments for most of the 3-hour lab periods.

RESULTS AND DISCUSSION

Field trips provide educational experiences for students well beyond any limitations of traditional classroom work. The importance of field trips in biology majors' courses, such as botany and ecology, has previously been reported (1, 12). Hupper et al. (4) reported that teaching students about their environment using field trips empowered students, leading to the development of citizens concerned about environmental issues. For the past 5 years Kingsborough's General Microbiology classes have been taken out on a research vessel to collect water and sediment samples. During this time, there have been at least two students each year who had never been aboard a boat. The students initially were surprised when they were informed they would be going on a field trip in a microbiology course and carrying out experiments actively involving them in collecting specimens with marine sampling equipment. Based on their other laboratory experiences, most students assumed that all lab materials would be prepared for them. When the reasons for the field trip were discussed, students began to realize the extent of the field of microbiology to include ecology, soil and water chemistry, and marine science.

While there are many colleges offering General Microbiology courses that are not as fortunate to have vessels available, with some effort and planning, comparable field expe-

riences involving marine and/or aquatic environments can be instituted. It is usually possible to rent safe, reliable vessels on an hourly basis. Many of the experiments described in this paper could also be carried out using soil samples collected from gardens, backyards, vacant land lots, fields, and farmland. Sediment and water specimens could also be obtained from local ponds, lakes, streams, rivers, and beaches.

The field trip during the fourth week of the semester was an early experience in the course that required students to work in pairs and begin to undertake learning activities using a team approach. This fostered students getting better acquainted with one another, learning to work with another person in meeting the goals of the experiments, and sharing responsibilities for discussing their observations and data. Despite some initial uncertainty by students, all student pairs eventually were able to effectively collect water and sediment samples after multiple trials, and this provided a sense of success. These early lab successes built further confidence and a strong desire to more actively participate in the upcoming lab experiments and other educational experiences. Also, it was noted that when students developed competencies in carrying out procedures and using scientific equipment they began to view themselves as more complete and engaged individuals. The value of conducting such investigations as a researcher is evidenced at all levels of education and equally so with teachers participating in courses with inquiry-based laboratories (6).

Students isolated actinomycetes from their marine sediments and tested their isolates for antibiotic production. They used pure culture techniques learned in previous labs. In so doing, the importance of learning and applying these techniques was reinforced, as suggested in the ASM Undergraduate Microbiology Curriculum Recommendations (2). For the students to finally determine whether they isolated any antibiotic-producing actinomycetes took 8 weeks. Throughout this longitudinal study, students remained interested and were enthusiastic about continuing their work each week on this particular project. Each group determined the number of antibiotic-producing actinomycetes they isolated, and this information was pooled to determine the results for the entire class. Usually, the number of antibiotic-producing cultures was approximately 10% of all the isolated actinomycetes. Upon the class discussion of these data, students gained a greater understanding of the arduous efforts frequently required in developing novel antibiotics.

There were three other experiments in which students utilized the environmental samples they collected: the Winogradsky column, cultivating microalgae, and water testing using the Colilert method. The Winogradsky columns were a 6-week longitudinal study conducted by the students. Each student submitted a report that included a description of the events that took place in the column and their explanations for changes in the column's appearance each week. The six drawings provided by the students were expected to be in color and labeled with the different microbes found within various locations in the column. Many students prepared computerized colored drawings. These drawings not only

reflected their understanding of the microbial succession which occurred in the column but excellent computer skills as well.

Cultivating microalgae required the students to come to lab each day. The students were self-directed, responsible for their work, and made daily visits to the lab with little difficulty. Once the students observed their algal slides, they realized the overwhelming presence of a diverse population of algae in their water samples taken from an urban environment. Prior to carrying out this experiment, many students verbalized their belief that urban waters were undoubtedly highly polluted and probably incapable of sustaining organisms, such as algae, which they typically associated with clean waters.

When the Colilert water testing system was used to determine the presence of coliforms, once again students were surprised to observe their water samples were devoid of coliforms. It was noted that in recent years, concerted efforts were undertaken in the New York Harbor by a variety of federal, state, and city agencies, and the current absence of coliforms in the samples might be the result of these more vigorous environmental measures. Other possible explanations were that water samples were collected during the months of March or early April when cold temperatures inhibit coliform growth or the levels of coliforms might be so low they were not detected.

Each of the experiments in the environmental microbiology section of the course required each student to submit a written project. The Winogradsky column project was described earlier in this paper. For the isolation of antibiotic producers, cultivation of microalgae, and water testing experiments, each student submitted their results along with the answers for two types of questions, review and thought questions. The review questions involved recalling information based on the experiment conducted and the results obtained. Examples of these questions are: (i) why is it necessary to provide a light source to culture algae, (ii) explain how pure culture techniques were used in the experiment on isolation of antibiotic producers and why they are important to this experiment, and (iii) state one advantage of using the Colilert water testing method rather than multiple tube fermentation and membrane filtration. The thought questions involved applying knowledge gained from carrying out the experiments to broader situations. These questions usually involved further investigation in other sources, such as the textbook or websites, to develop an answer. Examples of these questions are:

- Why does an oil spill off the coast of Alaska affect the availability of seafood in major cities, such as New York, London, and Hong Kong?
- Why are antibiotics so expensive? What reasons do pharmaceutical companies give for the cost of drugs?
- Coney Island, a New York City public bathing area, provides recreation for up to one million people. Various sewage treatment plants have periodically overflowed raw sewage into the area forcing beaches to be closed. What are

the implications of this activity? What alternatives are there to avoid the mandatory closing of beaches due to high coliform counts?

The laboratory portion of this course constituted 50% of a student's grade. The cited projects and other lab reports assigned were valued at 20%. Announced quizzes were 20%. Five percent of the grade was based on the student's laboratory technique. This included setting up experiments properly, grading slides they prepared, and reporting their data to the entire class. An oral presentation based on the student reading an article on a microbiological topic was 5% of their grade. The remaining 50% of the total grade calculation was allocated to the lecture-discussion portion of the course.

Student evaluations, carried out in all courses towards the end of the semester, provided a means for students to anonymously comment on the learning activities and their experiences in this course. Since only the instructor viewed these comments after the course was completed, the students were usually uninhibited in writing any comments they perceived could affect their grade if seen earlier. Examples of such comments included: "The instructor inspired me. Before this course, I did not have much of an appreciation for the field of microbiology." "This class created a comfortable environment for me to work. Even when I made mistakes, I didn't feel bad." "One of the best classes I ever took. The labs weren't boring and we did so much work." "This class was too much work. I learned a lot although my grade may not reflect that." These statements, as well as others, supported the students' appreciation for the instructor's enthusiasm and their awareness that carrying out varied learning activities in this revised course format fostered their interest and further educational and personal development.

Previous reports have demonstrated active-learning approaches integrated into microbiology courses improved student learning (3, 8). Future assessment of student learning will involve a pre- and post-test consisting of questions such as: (i) name the five groups of organisms studied in microbiology, (ii) how long have microbes existed, (iii) name five harmful activities carried out by microbes, (iv) name five beneficial activities carried out by microbes, (v) name three current news media events concerning microbes, and (vi) do you believe microbes have a greater positive or negative effect on the world. The survey in Fig. 1 will be used to assess the student's perceptions of which lab activities they felt improved their learning and will help determine if activities designed to foster student involvement improved their learning.

As we concluded the section of laboratory activities on environmental microbiology, information regarding various career options in the field of microbiology was presented. Many of our students expressed an interest in pursuing careers in the sciences, but usually referred to the professions of medicine, dentistry, and related health science fields. Prior to these experiences and subsequent discussions, they did not realize the number of career possibilities available in microbiology. There are occasions when the instructor would lend the students books about microbiological topics and

Rate each laboratory activity on a scale from 1 to 4 based on how you believe it assisted you with your learning.
4 = most helpful; 3 = somewhat helpful; 2 = minimally helpful; 1 = least helpful.

	Water and sediment sampling	Isolation of antibiotic producers	Winogradsky column	Culturing microalgae	Water testing (Colilert)
Showed the relationship of microbiology to other fields					
Assisted in demonstrating the lecture concepts					
Developed a greater understanding of course content					
Showed the various functions of microbes					
Increased your direct involvement in your learning about microbes					
Increased your ability to carry out and record experimental data over a series of weeks					
Provided you with a better understanding of scientific research					
Increased your ability to work with a partner					
Improved your writing skills					

FIG. 1. Student assessment tool for laboratory activities. Students will be asked to complete this table towards the end of the semester in order to assess how these lab activities assisted their learning.

provide further information about microbiology research opportunities and careers available through library and computer searches. Two pamphlets prepared by and obtained from the ASM, "A Million and One: Careers, Discoveries, and Opportunities" and "Fellowships: Fellowship Opportunities for Students, Young Investigators, and Early-Career Scientists in the Microbiological Sciences," were distributed to the class. The students were also encouraged to visit the ASM website for further information about careers and related microbiological topics.

In conclusion, well thought out, challenging labs provided an excellent format to increase student participation in their learning without sacrificing content (13). By performing experiments demonstrating the many facets of the field of microbiology, students were actively engaged and more interested in and committed to their work. Possibly this increased sense of commitment was partially due to the fact that the students were using the samples they collected for most of the semester's work. They were self-directed and working in a team approach. Creating an active-learning environment helped students develop thinking and problem-solving skills and encouraged them to integrate information into a conceptual framework (9).

Effectively completing laboratory experiments involved a wide variety of skills. By paying attention to the written instructions, students learned to follow procedures. By using equipment in these experiments, they developed increased manual dexterity. Working with a partner promoted cooperative learning, and students began to appreciate the importance of positive group interactions. Studies on cooperative learning have demonstrated it produces cognitive, affective, and interpersonal benefits (5, 10). As students recorded their results and made observations throughout the 12 weeks of lab, they sharpened skills needed to reach sound experimental conclusions. The group process led students to discuss

the accuracy of their work, explain any contrasting results, and make correlations based on the exchange of ideas. Such group activity is rather typical of most life situations. Through the examination of their results, students developed a capacity to think critically, ask more questions, and make an important discovery about experiments—that is, research processes and resulting data often lead to just more questions requiring further experimentation rather than any definitive conclusions. The overall effect of these laboratory activities was that students, by carrying out scientific practices, experienced the nature and joy of science in the best traditions of investigative work. They were being scientists.

ACKNOWLEDGMENTS

A special thanks to the faculty and staff of The Office of Marine Education, Joseph N. Muzio, Gail Saccoccio, Captain Robert Brennan, and the crew of the RV Kingsborough, for providing the boat, equipment, and personnel to take the students on boat trips and assist them in collecting samples. The three named individuals have contributed greatly to providing an invaluable educational experience for my students and me.

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