INQUIRY & INVESTIGATION

Student Scientists: Transforming the Undergraduate Biology Lab into a Research Experience

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Abstract

This article features an authentic research-lab experience developed for use in a freshman-level general biology course for nonmajors at a two-year college. Students work in groups to select and investigate factors affecting microalgal cell growth and relate their findings to a real-life application of social significance. This lab experience was designed using a four-step pedagogical framework originally developed at a four-year university in a sophomore-level molecular and cell biology course. The creators of the pedagogical framework at the four-year university mentored the instructor at the two-year college through the process of using the pedagogical framework to design and implement the authentic research lab experience described in this article. This example shows that adaptation of successful pedagogical models, particularly within mentoring partnerships, can greatly increase the implementation of authentic research experiences in biology lab courses at varying levels of study.

Key Words: *Algal cell culture; inquiry-based lab; educative mentorship; authentic research; undergraduate research.*

○ Introduction

Advancements in technology, the increasing ability to acquire and process vast amounts of data, and novel ways to communicate and collaborate with others have changed the way that we approach and practice science. Not surprisingly, these advancements have also changed the way that we view science education, particularly in laboratory courses. Overall, there has been a shift in focus from cookbook-style lab experiences, in which students participate in scientific demonstrations or follow a set of instructions to complete a lab experiment with a known outcome, to more authentic research lab experiences in which students more fully participate in the scientific process by asking and answering their own novel questions while using modern scientific practices. Authentic research lab experiences not only allow students to learn science while *doing* science, but also offer students the opportunity to develop other important life and career skills, such as critical thinking, creative problem solving, collaboration, written and oral communication, information literacy, flexibility, leadership, and responsibility. In fact, over the past couple decades, many respected science education associations have issued a collective call to include more authentic research experiences in all biology courses (Boyer Commission on Educating Undergraduates in the Research University, 1998; Project Kaleidoscope, 2002; National Research Council, 2003, 2005; AAAS, 2011, 2015). However, authentic research experiences remain rare at the undergraduate level, largely due to two main obstacles: uncertainty surrounding the definition of authentic research, and the time required to develop these types of laboratory experiences (Spell et al., 2014).

In this article, we present an authentic research lab experience intended for a freshman-level biology nonmajors laboratory course at a two-year college.

One way to overcome both of these barriers is to utilize a pedagogical framework that incorporates all essential elements of authentic research and effectively guides the instructor through the creation of these types of lab experiences. Using such a framework ensures that the lab experience indeed contains the essential components of authentic research, including the reading of scientific literature, novel and student-generated questions, formulation of hypotheses, experimental design, data collection and analysis, working toward significant findings, and oral and written presentation of results (Seago, 1992; Lopatto, 2003; Spell et al., 2014). Additionally, the flexible structure built into a pedagogical framework reduces the amount of time required to develop a lab experience that can be effectively implemented in the unique environment of one's own institution and course. We have found that this process can be particularly fruitful within a professional

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mentoring partnership (Goedhart & McLaughlin, 2015) and can ultimately function to increase the amount of authentic research experiences within a variety of biology classrooms.

In this article, we present an authentic research lab experience intended for a freshman-level biology nonmajors laboratory course at a two-year college. This lab experience was designed using a pedagogical framework developed by McLaughlin and Coyle (described in the preceding article in this issue of *ABT*) and is the result of a mentoring partnership between the instructors at the four-year university who created the pedagogical framework and an instructor at the two-year college (Goedhart & McLaughlin, 2015). Because of infrastructure and equipment limitations at the two-year college, it was not possible to replicate the original research project utilized at the four-year university, which involved mammalian Vero cells. Instead, we designed a research project involving the growth of microalgal cells. We chose microalgae for two main reasons: (1) algae are largely nontoxic and can be easily and efficiently grown in a regular laboratory classroom setting with common and inexpensive lab equipment; and (2) algae can provide many socially important products (e.g., energy-rich biofuel, nutrient-rich food and fertilizer, and biochemicals used in pharmaceuticals) and services (e.g., wastewater treatment and carbon sequestration). However, large-scale use of algae for these products and services is possible only if we are able to grow algae quickly. Thus, by investigating factors that affect microalgal growth, students at a two-year college with limited access to research infrastructure and equipment can engage in an authentic research experience and could utilize scientific practices while working to solve a relevant, real-life issue. For a short video documenting this lab experience from both instructor and student perspectives, please visit the following link: https://vimeo.com/118326855.

The Pedagogical Framework as Implemented in a Freshman-Level General Biology Lab Course at a Two-Year College

This particular lab experience took place over the course of a 16-week semester. The first couple weeks of the semester were spent learning about microalgae and the potential of these organisms to provide important products and services needed to combat current and future food, energy, water, climate, and human health challenges of the 21st century. Students were then introduced to relevant scientific practices associated with studying algae by participating in a class experiment in which they all worked together to investigate the effect of varying student-selected pH environments on algal growth. During this preliminary experiment, students learned how to set up different treatments using algal culture and nutrient media, transfer liquid volumes using variously sized pipettes, measure pH, and use a microscope and hemocytometer to count cells. They also experienced the process of science: they made observations, formed hypotheses based on their own research utilizing scientific literature, tested their hypotheses in a controlled experiment, collected and analyzed data, formed conclusions, and presented results in written and oral forms. Following this preliminary experiment, groups of three or four students were challenged to manipulate a variable of their choosing to increase the growth of *Chlorella vulgaris*, a microalgal species used in the algal biofuel industry. The variables chosen by the groups included the following: added nitrogen, added glucose, added glycerol, agitation, and changes to the light:dark hour regime. Student groups chose their variable and subsequent parameters based on their research in the scientific literature. All members of each group were tasked to complete an experimental protocol prior to setting up the actual experiment. While a control treatment was set up and maintained by the class, each group also set up an experimental treatment with all conditions identical to the control treatment (light availability, temperature, nutrient media, pH, etc.) except the one variable they had chosen to alter. All members of each group worked together to measure cell counts at the beginning of the experiment and again after one week. Following data collection, group members worked together to analyze the data, draw conclusions, and present the results in a scientific poster in front of an audience of their peers and members of the campus faculty and administration. Each group member also communicated the results of their study in a formal scientific paper that served as an expansion of the protocol they had completed prior to the experiment. Members of several groups went on to present their posters at a student conference in the following semester.

In the process of investigating the effects of different variables on the growth of algae, students were also exposed to other core scientific topics typically included in a nonmajors biology lab, including biochemistry, cellular structure and function, energy dynamics, evolution, genetics, and ecological interactions, among others. We utilized videos and current news items to relate these concepts to the real-world example of growing algae for the purpose of providing various products and services. In this way, students were able to engage with all of the broader biological topics usually included in a general biology lab, but within the context of a relevant and real-world issue.

○ Lab Protocol

The Future of Food, Fuel, Fertilizer, Pharmaceuticals & Phytoremediation: An Undergraduate Lab in Microalgal Growth

1. **Objective**

   In this lab you will improve your ability to utilize the scientific process and relevant scientific practices while investigating the factors involved in microalgal growth.

2. **Background Information**

   Microalgae is a broad term that includes ~100,000 microscopic algal species found in a variety of terrestrial, freshwater, and marine habitats. These algae can exist as single cells or in colonies. Their ability to perform photosynthesis and their rapid growth rates make microalgae an important part of many ecosystems. Collectively, they make up a large component of the base of aquatic food chains and are a major source of aquatic and atmospheric O_2. They are also responsible for taking up great quantities of aquatic and atmospheric CO_2, helping to partially mitigate human-caused atmospheric CO_2 accumulation due to the burning of fossil fuels.

   Like plants, these cells generally obtain energy via photosynthesis; however, unlike plants, they do not produce roots, stems, or leaves – enabling them to invest energy and resources into very rapid cellular reproduction. Excess energy is primarily stored in the form of oils, which can be extracted and refined to supply biofuel that is compatible within our current energy infrastructure. In addition to supplying a usable fuel, algae can also serve as a reliable and nutritious food source

for humans, livestock, and aquaculture. Many algal species contain important carbohydrate, lipid, and protein compounds, including simple sugars, fiber, omega-3 fatty acids, and essential amino acids. They also contain a variety of important vitamins and minerals. Additionally, algae can supply other products and services, such as high-quality fertilizer, pharmaceutical biochemicals, and phytoremediation (wastewater treatment).

However, before microalgae can be utilized on a large scale to provide these important products and services, we must first determine how to maximize their growth. In this lab you will investigate how a variable of your choosing affects the growth of the microalgal species *Chlorella vulgaris*.

Video, "Algae Biofuels and Biotech" – Stephen Mayfield UC San Diego: https://www.youtube.com/watch?v=jPfYjOMNGX0

Video, "Algae-to-Fuels": https://www.youtube.com/watch?v=IxyvVkeW7Nk

Video, "Algae Fuel": http://www.pbs.org/wgbh/nova/tech/algae-fuel.html

Video, "Algae in Agriculture": https://vimeo.com/54406876#at=0

Video, "Feeding Farmed Fish": https://www.youtube.com/watch?v=PC4eTMA4smU#t=17

***Algae Industry Magazine* (up-to-date information on the algal products and services industry):** http://www.algaeindustrymagazine.com/

3. Laboratory Assignment

1. Learn the following experimental techniques: microscopy, pipetting, dilution, and cell counting using a hemocytometer.
2. Develop a hypothesis that explains how the alteration of one variable of your choosing might affect the cellular reproduction of algae over a seven-day period.
3. Describe a research strategy based on the above hypothesis that includes these components: Purpose (which includes background information, hypothesis under investigation, and scientific reasoning), Materials, Procedure, Data Interpretation, and References (using a standard "protocol" format). Algal cells will be counted initially and again after seven days.
4. Conduct the experiment as per research design, collect and interpret data, and present research findings in a professional scientific manner.

Website, "Scientific Writing": http://www2.lv.psu.edu/jxm57/irp/sciwrit.html

4. Techniques

I. **Algal Cell Treatment Setup**

Materials & Equipment:

- *Chlorella vulgaris* algal culture (Carolina, no. 152075)
- Sterile Proteose medium (recipe at http://utex.org/products/proteose-medium)
- Sterile 250 mL Erlenmeyer flasks
- Sterile autoclavable tubing
- Sterile air stones (using bleach or autoclave)
- Sterile cotton balls or silicone stoppers
- Aluminum foil
- Pipettor and sterile serological 5 mL pipettes

Procedure:

1. Set up growing station as described in video below and prepare nutrient media.
2. Fill 250 mL flask with 100 mL sterile nutrient media.
3. Add 5 mL of algal culture solution and shake gently to homogenize.
4. Insert sterile tubing with air stone into algal solution and cover flask with sterile cotton and aluminum foil or sterile silicone stopper.
5. Allow for slight bubbling (a bubble each second).
6. In addition to a class control treatment, each group of students will also set up a treatment group in which they investigate the modification of a single variable of their choosing, based on their own background research of the study system (e.g., light availability, nutrient availability, agitation).
7. Algal cells in all treatments will be counted at the beginning of the experiment and again after one week to determine growth rate.

Video, "Growing algae for fuel – Getting started": https://www.youtube.com/watch?v=NAYnZbsIhY8

Video, "Growing algae for fuel – Supplies": https://www.youtube.com/watch?v=iWyZlo1FWBg

II. **Algal Cell Counting Using a Hemocytometer**

Materials & Equipment:

- Hemocytometer
- Distilled water and compressed air (to clean hemocytometer if disposable)
- Lens wipes
- Microscope with 400× capability
- Microcentrifuge tubes
- 1000 μL pipette and tips
- 10 μL pipette and tips
- Vortex

Procedure:

1. Sufficiently shake the flask to homogenize the sample of algae.
2. Take a 1 mL sample from each flask using a fresh micropipette tip and put into a microcentrifuge tube.
3. Vortex the 1 mL sample in the microcentrifuge tube and remove a 10 μL sample using a fresh micropipette tip.
4. Carefully load the 10 μL to one side of the hemocytometer (counting chamber). Take care not to flood the slide.
5. Repeat step 4 to load the other side of the hemocytometer.

6. Count and record the total number of cells in the four large outer squares. Repeat the process for the cells loaded on the other side of the hemocytometer (see Appendix 1).
7. If using a disposable hemocytometer, clean the two chambers by blowing out fluid with compressed air and rinsing with deionized water. Repeat this process at least three times.
8. Allow the hemocytometer to dry on a lens paper.

Calculation:

$$\text{Number of cells/mL} = \frac{(\text{Total number of cells counted})(\text{dilution factor})(10^4)}{\text{Total number of squares counted}}$$

Video, "Counting Cells with a Disposable Hemocytometer": https://www.youtube.com/watch?v=69T755-J33A

○ Conclusion

Collaboration and building upon the work of others is a hallmark of science and has allowed for rapid scientific progress and discovery over time. The same can be true in science education. Adapting and building upon successful pedagogical frameworks developed by others can serve to provide structure and to greatly decrease the time involved in developing authentic research experiences. Here, we have presented an authentic research-lab experience designed for use in a freshman-level nonmajors biology course at a two-year college where students use the experimental system of algae to investigate an issue of real-world importance.

This research experience was developed through a mentoring partnership, using a four-step pedagogical framework originally utilized in the design of a sophomore biology majors course at a four-year university. Available equipment, infrastructure, and student populations often differ between institutions – particularly between four-year universities and two-year colleges. The flexibility involved in this four-step pedagogical framework allows it to serve as a model that can be utilized to increase authentic research experiences in a variety of biology courses that serve various student populations. The project study system and timeline can be modified according to the needs and resources of the institution, instructor, and students. For example, more support and resources are required to maintain mammalian cells, whereas microalgae can be effectively grown in an institution with less support and fewer resources.

There is no need to reinvent the wheel. It is much easier and faster to utilize an already successful pedagogical framework – and modify it according to the needs and resource constraints of one's own institution, course, and student population – than to create an authentic research experience from scratch. In our experience, this adaptation process worked best within a mentoring partnership, in which the creators of the successful pedagogical framework guided and supported the community college instructor through the process of modifying the framework to fit within the specific environment of a freshman-level nonmajors biology course at a two-year college. By helping one another to purposefully adapt and modify successful pedagogical frameworks, we can effectively and efficiently meet the call to provide students with authentic research experiences in *all* biology courses.

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APPENDIX 1: Counting Cells Using a Hemocytometer

Hemocytometers contain a grid system of known dimensions and are used to measure the density of cells in a given sample. We will be using hemocytometers to calculate the amount of algal cells in our samples during our experiments this semester.

Loading the cells onto the hemocytometer

Thoroughly shake your flask so that you have a homogeneous sample of your algal population. Then, using a micropipette, extract 10 μL of sample and load the entire sample into one of the loading chambers of the hemocytometer.

Counting the cells

We will be utilizing the four large outer squares (1 mm^2) to calculate the number of cells in the sample. Count all cells that fall within this area. *Note:* Only count cells that are completely within the area or are touching the left and top sides of these squares. Cells that touch the bottom and right lines of a square *should not* be counted (see Figure 1).

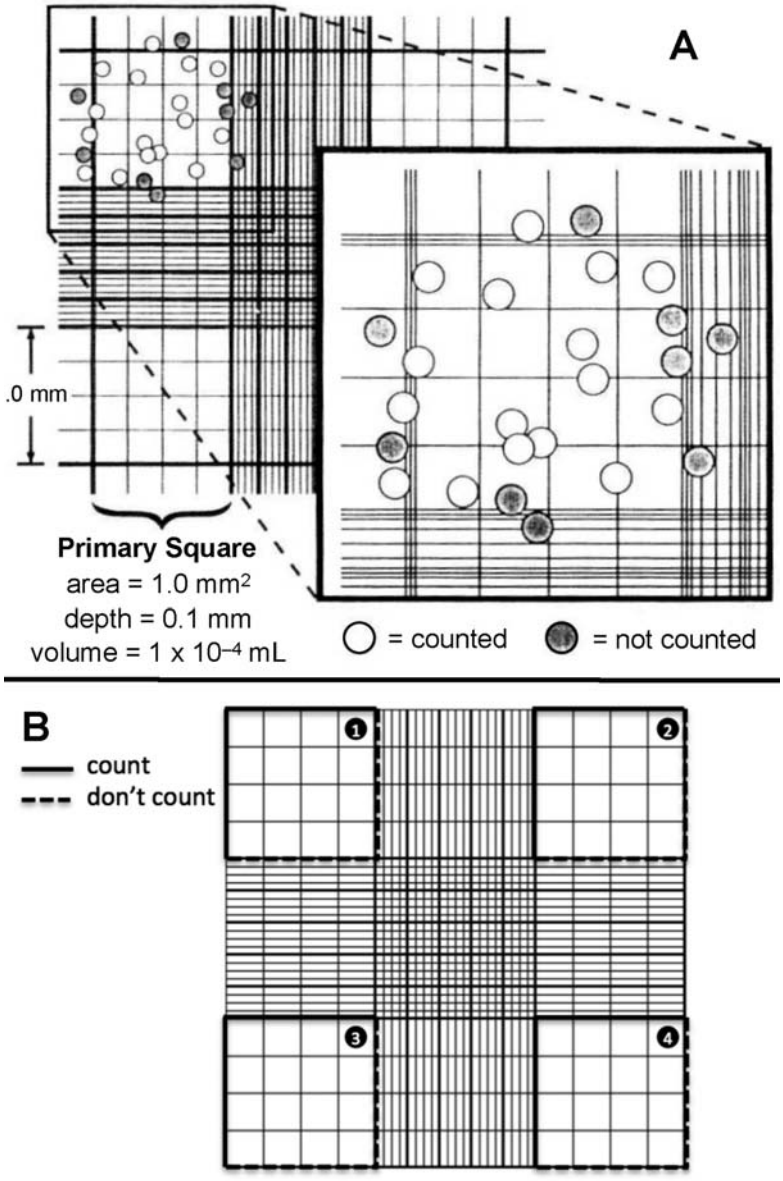

Figure 1. Counting cells using the grid of a hemocytometer. (**A**) From http://web.mnstate.edu/provost/CountingCellsHemocytometer.pdf. (**B**) From http://www.hemocytometer.org/2013/04/04/hemocytometer-protocol/.

Calculating the cell density

The calculation to determine the amount of cells in 1 mL is as follows:

$$\text{Number of cells/mL} = \frac{(\text{Total number of cells counted})(\text{dilution factor})(10^4)}{\text{Total number of squares counted}}$$

Practice calculating the cell density

Let's say that you count 56 cells in large square 1; 71 cells in large square 2; 65 cells in large square 3; and 68 cells in large square 4. What is the number of cells per milliliter in your sample?

APPENDIX 2: Laboratory Protocols

A lab protocol is the outline that you make prior to conducting an experiment to ensure that you are correctly following the scientific method and that you are designing an experiment that will best test your hypothesis. An effective protocol provides the purpose, expectations, and instructions for your experiment and enables you and others to assess your experimental design so that all inconsistencies and problems can be addressed before you actually begin your experiment. Lab protocols are usually about one page in length and generally consist of the following sections.

Purpose

This is where you provide any and all background information necessary to understand the system, the purpose of your study, and the premise for your study question. This section should flow from more general information about the "big picture" topic to more specific information about the particular problem you will address in your study.

- What is already known about this topic and organism? (Cite references from the scientific literature)
- What is the specific problem that you will address in your study? (How are you going to build upon what is already known?)

Question

This is where you directly articulate your study question.

- What do you want to know?

Hypothesis

This is where you give your best guess at the answer for your study question. This statement should be based on information gathered from relevant and reliable sources and should align with your review of the scientific literature in the Purpose section.

- What do you think that you will find?

Materials

This is where you list all materials that you will need to be able to answer your study question.

- What materials do you need to fully complete this study?

Procedure

This is where you list the procedural steps that you will take to answer your study question. This section should contain enough detail so that someone else would be able to duplicate your experiment on the basis of your stated procedure.

- How will you set up your experiment?
- What is/are your experimental group(s)?
- What is/are your control group(s)?
- What are some constant variables in your experiment?
- What will you measure? How will you complete your measurements?
- How long will your experiment last?

Data interpretation

This is where you state how you plan to analyze your data after the experiment.

- What type of data analysis do you expect to use to analyze your results?
- How do you expect your data figures to look?

References

This is where you list any and all relevant and reliable references (books, journals, websites) you used to design or conduct this study.

- Where did you get your information?

The process involved in producing a protocol can be tedious, but it will prove to be beneficial in several ways: (1) it will allow you to address any problems before you begin your experiment, (2) it will give you a roadmap to successfully answer your study questions, and (3) it will serve as a framework for your lab report. The more effort and care that you put into the up-front creation of your protocol, the less work and headaches you will have while conducting your experiment, analyzing the results, and writing your report.