Supplemental Material

CBE—Life Sciences Education

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Supplemental Material. Sample CURE laboratory exercise (scientific inquiry).

Lab 1: Scientific Inquiry

Objectives:
At the end of this experiment, students will be able to:

1. Identify components of the experimental design process
2. Critique experiments to determine their validity
3. Design high-quality scientific experiments that include all the necessary components
4. Draw conclusions from data and present alternate hypotheses to explain those data

Introduction

For the laboratory portion of this course, you will be designing and carrying out a series of experiments to discover new information about our model organism, *Tigriopus californicus*. Because you will be spending most of the semester involved in the specifics of experimental design and data analysis, it is crucial that these experiments are well designed and carried out successfully. If the experiment is flawed, the results, too, will be flawed. In today's lab, we will review the basics of the scientific method, specifically elements related to the experimental design process. The first step in the scientific method is to make observations. Interestingly, many people believe that the first step is to make a hypothesis, but no hypothesis can be made without first observing something. In some cases, this can be a physical observation, such as noticing that one type of plant only grows in sunny areas, or that the cells growing in the lab don't grow as well at colder temperatures. Another type of observation that is crucial in how science is actually done is to make an observation based on data published in the scientific literature. Most scientists start by reading journal articles on their topic of interest and collating the information available. From this, they then see where there are areas where more information is needed or questions need to be asked. From this, a scientist can then start the next step in the scientific method, making a hypothesis.

When developing your hypothesis, it is important to consider if it is a valid hypothesis. To be valid, it must be both testable and falsifiable. A testable hypothesis is one that can be tested and measured using the equipment we have today. For example, a hypothesis that states that there are living beings in another solar system is not yet testable. The other important component of a hypothesis is that it must be falsifiable, meaning that one could gather data to prove or disprove the hypothesis. Some things cannot be determined by an experiment, such as any statement that is a judgment or opinion. For example, the hypothesis that pit bulls are evil dogs is not testable or falsifiable. There is no way for us to define evil and, therefore, the hypothesis is not valid.

Once you have formed your hypothesis, the next step in the scientific method is to design and conduct the experiment. The idea behind designing an experiment is to test your hypothesis in a manner that is objective and that will either support or falsify your hypothesis. In order to understand experimental design, let's test the following hypothesis: if roses require sunlight to grow, then they will grow more slowly if placed in the shade. So, I want to put some roses in the shade and measure how fast they grow. However, there is more to experimental design than just that. First of all, we need a **control group**. This is a group of plants that receive the same conditions as the **experimental group,** except for the experimental treatment. To test my hypothesis, I have taken 40 rose plants, each in separate pots. I have placed 20 in the sun in the greenhouse and 20 in the shade in the greenhouse. Each pot gets the same amount of water and fertilizer, and all are kept at the same temperature. Those in the sun are the control group and those in the shade are the experimental group. If my initial hypothesis were correct, we would expect those roses in the shade to grow slowly while those in the sun to grow more quickly. If this happens, we can conclude that the sun is likely to be the important factor in plant survival since the only difference between the two groups was the amount of sun.

As we consider experimental design, there are a number of terms that need to be clearly defined. The first is the **independent variable**. This is the variable that is being tested or manipulated in the experiment. In our simple experiment, we are testing the effect of the sun on plant growth and we are manipulating the amount of sun available to the plants, so the amount of sunlight is the independent variable. It is critical to have only one independent variable being tested in each experiment. That way, if changes are observed, you can be sure that the one independent variable is the reason for the change. For example, suppose I had some plants that had more water and more sunlight and other plants that had less water and less sunlight. The plants with less water and less sunlight did not grow as well. Was this because they lacked water or light? I cannot answer this question because I tested two independent variables at the same time.

Another variable to consider is the **dependent variable**. This is what is being measured in the experiment. For our study, we are measuring the growth of the plants in response to sunlight, so growth is the dependent variable. One quick way to remember these is to put them into the following sentence – I am testing the effect of the independent variable on the dependent variable. So, in our experiment "I am testing the effect of the amount of sunlight on plant growth". If you reverse them, you can immediately see the problem. "I am testing the effect of plant growth on the amount of sunlight" makes no sense and therefore cannot be correct. In many experiments, researchers will measure more than one dependent variable. For example, I could measure the height of the plants, the number of leaves, the number of flowers and the length of the roots. All of these could be affected by light and would be considered dependent variables. Basically, the dependent variables are the data that you collect in your experiment as your results.

Another important term in experimental design is the **standardized variable.** There are many of these, and these are all the conditions of the experiment that must remain the same for both the control and experimental groups. In our plant growth experiment, all plants must receive the same amount of water, plant food, soil, soil type, humidity and carbon dioxide so that the only thing that differs between the control and experimental groups is the amount of light. All of these other variables are called the standardized variables.

Before we move away from this simple experiment, there is one more thing to consider. Why did I use 20 plants in both the experimental and control groups? Why wasn't one enough? This is something we call **sample size**, which is simply the number of individuals in each test group. If we had only one plant in the sun and one in the shade, we could have inadvertently chosen plants with very different genetics that would skew our results. By choosing more than one, we reduce the chance that random factors will affect the data. Instead, we will get what is referred to as a representative sample, meaning that the individuals in the experiment represent all of the roses in the world. Determining the sample size for an experiment typically depends on how difficult it is to make the measurements and get the test subjects. For roses, larger sample sizes are not difficult, but if you were working with elephants, the sample sizes would necessarily be much lower.

Now that we have designed and carried out our experiment, the next step in the scientific method is to analyze the data. Using the rose example, we will have growth rates for plants in the sun and plants in the shade. We can calculate an average growth rate for each group and determine if there is a difference. This determination is typically made by using statistical techniques. We will look at this process in more detail in a few weeks in the lab. Once we have our data, we can use this to draw our preliminary conclusions. These are the initial statements to determine if the data support or refute our hypothesis. Finally, we are at the last step in the scientific method, making a new hypothesis based on our results. In some cases, this new hypothesis is an extension of our original one. From our example, we may want to know what level of sun is best for growth. Another type of new hypothesis is what is called an alternate hypothesis. This can be a hypothesis that is based on your data, but provides a different explanation for the results. You will find that in many cases, there is more than one explanation for the data you collect. Additionally, if your hypothesis was not supported, you may want to develop new hypotheses to attempt to explain the phenomenon of interest. What you will discover this semester is that as soon as you collect some data, you will immediately discover new questions that you want to answer. **In this way, science is a continuing process with no definite end.**

Additional Considerations:

Before we move on with the procedures for today, it is important to consider a few more aspects of how science is done. The first consideration is the idea of a **model organism**. If you read the primary literature in biology, you will notice that there are a significant number of studies done on the same organisms (rats, a worm known as *C. elegans,* a plant called Arabidopsis, yeast and *E. coli*, to name a few). These are examples of model organisms, and they have a number of features that make them amenable to research in the laboratory. They often are small in size, have a short life span, a high reproductive rate, an ability to grow well under laboratory conditions, and similar features to other organisms in the wild. Rats are often used as an approximation for mammals, Arabidopsis are used in genetics and other experiments as a model for plants, and *E. coli* is used as a proxy for other species of bacteria. What is learned from these model organisms can then be extrapolated and used as a basis for understanding related species. For the laboratory portion of this course, we will also be using a model organism, the copepod species *Tigriopus californicus*. You will learn a great deal about this organism later in the lab manual and through your research, but we will be using it as a proxy for other planktonic species as well as other marine organisms that live in the intertidal area. The information that you gather can then be applied to a wide range of species in the oceans and will add to our base of knowledge.

Procedures

EXERCISE 1: Making Observations and Developing Hypotheses
One critical step in the scientific method is to gather information from the literature and use is to develop new and testable hypotheses. This reading of the scientific literature is one means of making observations. Rather than making the observations directly in the lab or field, you are observing what others have done and then building on it. In this exercise, you will need to read the following and create three valid hypotheses based on the information presented. I have started with an example for you to help you understand the process.

1. Almost two thirds of traded goods worldwide are transported by ship (Kumar and Hoffmann, 2002). To ensure ship buoyancy, stability and maneuverability, oceangoing ships need ballast water. Based on an estimation that the world seaborne trade in 2013 amounted to 9.35 billion tons of cargo, the global ballast water discharges in 2013 are estimated to about 3.1 billion tonnes (David, in prep.). There is significant transfer of ballast water between different continents and oceans, and it has been known for decades that ballast water also transports organisms into new ecosystems, where, under favorable conditions, they can become invasive (Carlton, 1985 and Williams et al., 1988). The introduction of invasive aquatic species into new environments has been identified as one of the four greatest threats to the world's oceans. When including terrestrial species, invasive species were identified as key factor in 54% of all known species extinctions as documented in the Red List database maintained by the International Union for Conservation of Nature (Clavero and Garcia-Berthou, 2005). Aquatic invasions are virtually irreversible and, once the newcomers are established, their impacts may also increase in severity over time. The transfer of invasive species does not occur only over larger distances, between continents, but also as a secondary spread in regional seas (David et al., 2013). (This information copied directly from Werschkun et al., 2014).

    Hypothesis 1:
    Ballast water of ships arriving to North America from the Mediterranean will have live species of plankton native to the Mediterranean.

    Hypothesis 2:
    Planktonic organisms can survive transoceanic trips in ballast water.

    Hypothesis 3:
    One aquatic invasive species from seas around Europe has been found in the ballast water of ships entering the Great Lakes.

2. The mating behavior of male organisms is strongly influenced by the reproductive biology of conspecific females. Of particular importance to the male are the timing of the female's receptivity to mating, her capacity to store sperm (keep sperm viable in her body for prolonged periods of time), and the probability of successful sperm displacement (removing sperm from the female's body) by future matings of the female with other males. Among crustaceans, male mating behavior frequently involves a period of time during which a male clasps a female without actively attempting copulation (the pre-copulatory "passive" phase); during this period, which may last a week or more, a male cannot inseminate a female other than the one he is holding. (Modified from Burton, 1985).

    Hypothesis 1:

    Hypothesis 2:

Hypothesis 3:

3. *Tigriopus californicus* is a harpacticoid copepod found in small upper tidepools along rocky areas of the west coast of North America. Occupying this habitat, the copepod is exposed to relatively large and sometimes sudden changes in seawater concentration due to evaporation or dilution of the media (Kontogiannis, 1973). In response to this stress, *Tigriopus* has evolved adaptive mechanisms allowing it to survive over a wide range of salinities. Survival and reproduction have been observed at salinities between 21.2 and 75.3 parts per thousand under laboratory conditions (as described in Burton and Feldman, 1982).

Hypothesis 1:

Hypothesis 2:

Hypothesis 3:

EXERCISE 2: Determining the Validity of Hypotheses

Read over the following hypotheses to determine if they are testable. If not, explain why not.

1. Glucosamine supplements reduce joint pain in elderly human patients.

2. Retinal cream A reduces the depth of wrinkles around the eyes.

3. Dogs with long hair prefer cooler climates.

4. The higher the intelligence of the horse, the easier it is to train it to jump over obstacles.

5. High-fat, high-sodium diets for humans increase the risk of atherosclerosis (hardening of the arteries).

6. The *Tyrannosaurus rex* could not digest the citric acid in lemons.

EXERCISE 3: Understanding Experimental Design
For each experiment, answer the questions about the experimental design.

1. A researcher is studying the effect of the pesticide Cypermethrin on the survival of juvenile salmon in the laboratory. He has 100 salmon in a solution of 10mg/L of cypermethrin for 96 hours and measures their survival, which was 87%. He then places another 100 salmon in 100mg/L of cypermethrin for 96 hours and measures their survival, which was 52%. He concludes that Cypermethrin levels should be regulated to be less than 10mg/L in waterways where salmon are found.

   Independent variable:

   Dependent variable(s):

   What other variables should he standardize?

   Control group:

   Experimental group(s):

   What are the strengths of his experimental design?

   What are the weaknesses of his experimental design?

2. A marine biologist wants to examine the effect of salinity on the plankton *Tigriopus californicus*. He takes 100 individuals and places them into a beaker where the salinity is 35 parts per thousand (normal seawater). He then takes another 100 individuals and places them into a beaker where the salinity is 45 parts per thousand. Finally, he takes another 100 individuals and places them into a beaker where the salinity is 25 parts per thousand. After 48 hours, he counts the overall mortality (i.e., death) in each beaker.

   Independent variable:

   Dependent variable(s):

   What variables should he standardize?

Control group:

Experimental group(s):

What are the strengths of his experimental design?

What are the weaknesses of his experimental design?

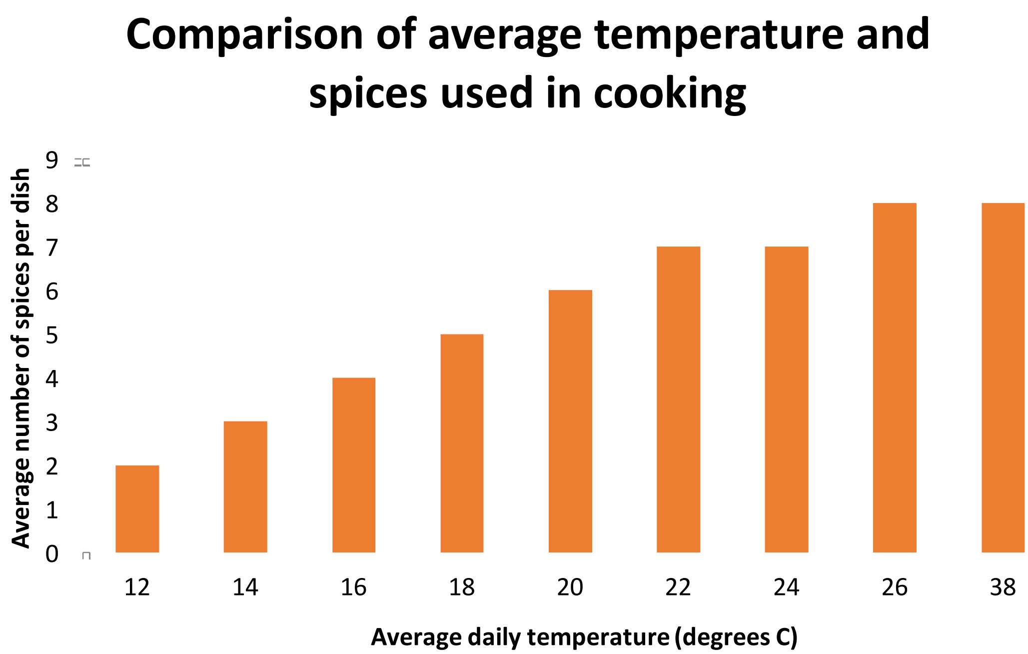
EXERCISE 4: Collecting Data

Create a table to collect the data, and draw a graph of how the data would look if the hypothesis was supported.

1. A researcher hypothesizes that survival rates will decrease when *T. californicus* is exposed to increased salinity. He sets up 3 96-well plates, each of which has 96 copepods in individual wells. These are filled with water at 35 parts per thousand. He then sets up 3 more plates the same way but with water at 45 parts per thousand. At the end of 96 hours, he measures the percent survival in each plate. Create a table for him to collect his data as well as a table for him to summarize his data. Then, create a hypothetical graph with summary data that would support his hypothesis.

EXERCISE 5: Interpreting the Data

1. The data shown below were collected to test the following hypothesis: foods in warmer countries have more spices to prevent bacterial growth (many spices have mild antibiotic properties). Examine the data, and answer the questions below:

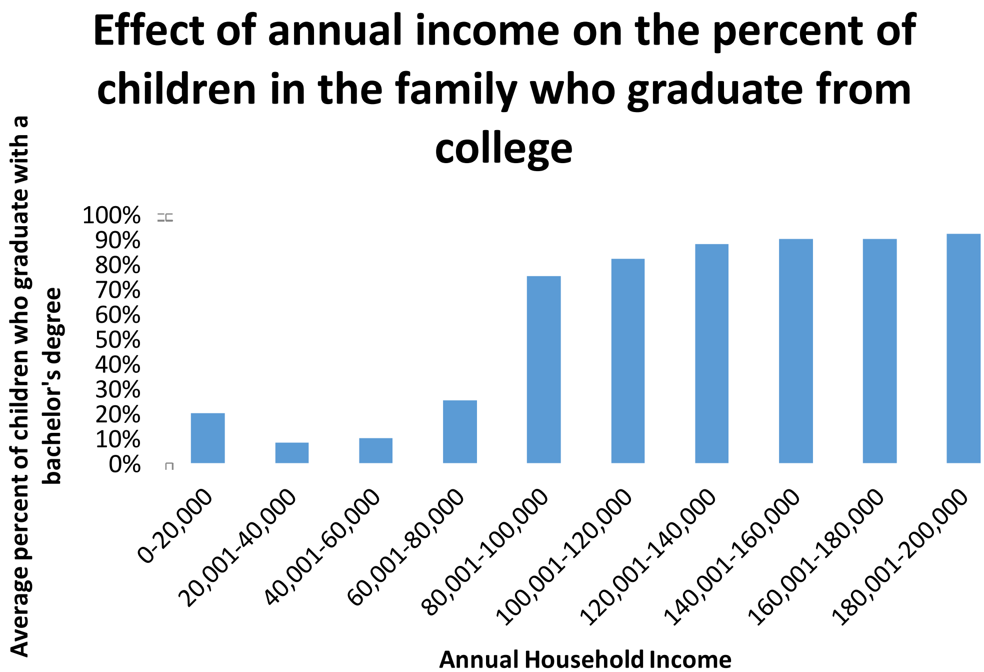


Do the data support the hypothesis? Why, or why not?

List two other hypotheses that you could develop based on the data shown.

What other conclusions can you draw from these data?

2. The data shown below were collected to test the following hypothesis: families with higher annual income will have more children who complete an undergraduate education. Examine the data, and answer the questions below:



Do the data support the hypothesis? Why, or why not?

List two other hypotheses that you could develop based on the data shown.

What other conclusions can you draw from these data?

Exercise 6: Conduct an Experiment

Conduct an experiment to test the following hypothesis: If the length of the femur is correlated to height, then we can use femur length to predict overall height in college students.

This type of inference is used widely in paleontology, when it is rare to have an entire intact skeleton. Instead, individual bones may be recovered and used to infer information about the individual. In this experiment, you will determine if femur length is a good proxy for overall height of an individual.

General Instructions

1. In your groups, assign each member to one of the roles for the semester (these roles will only be for today). For more information on the roles, refer to the section entitled "Format of the CURE Lab". All students in the group should be involved in each role.
2. The PI will read over the provided background information and summarize it.
3. The Protocol Expert will write up the methods/protocol that you will follow. Be sure to pay attention to detail, and be specific.
4. The Data Expert will then design the tables for data collection and record the results.
5. The entire group will carry out the experiment that you have designed.
6. The Analyst will summarize the data and relate it to the original hypothesis.

Protocol:

Tables for Data Collection:

Summary and Analysis of the Data:

Questions for Exercise 6:

1. Did your results support your hypothesis? Why, or why not?

2. Some paleontologists use the following equation to determine height: Femur length (in cm) x 2.6 + 65 = height (cm). Complete this equation for three of the individuals in your group, and write the results below.

3. Based on your data from question #2, does this equation “work”? If not, why do you think it was not successful for you?

4. In your experiment, what was the independent variable?

5. In your experiment, what was the dependent variable?

6. What were the factors of your experimental design that were problematic or needed refinement?

Homework

1. Explain the advantages and disadvantages of using a model organism (include at least 3 of each).

2. A researcher was testing the hypothesis that students in graduate school have higher GPAs than students working on undergraduate degrees. He looked at the transcripts of 10,000 undergraduate students and 10,000 graduate students at universities all across the country. He included students from a wide range of university types (state schools, liberal arts schools, Ivy League colleges, etc.) and was careful to balance the genders of the students so that he had nearly equal numbers of males and females. He found that the average GPA of an undergraduate student was 2.9 and the average GPA of a graduate student was 3.6. He concluded that his data support his hypothesis, and he argues that the reason for this difference is grade inflation (teachers giving higher grades) in grad school. Explain the strengths and weaknesses of his experiment and his conclusions (give at least 2 of each).

3. From question #2, develop an alternate reason that we see this discrepancy between undergraduate and graduate student GPAs.

4. You are designing an experiment to test the following hypothesis: Temperature affects the ability of students to think clearly, and, therefore, students in a hotter room will have lower exam scores than students in a cooler room. For your experiment, list the following:

   Sample size

   Independent variable

   Dependent variable

   Control group

Experimental group

Standardized variables

How will you ensure that you have a random sample?

5. Now, describe the methodology you will use to test the hypothesis from question #4.

NOTE: For comparative purposes, a copy of the "Scientific Inquiry" exercise implemented within the traditional laboratory course can be located in Dickey, 2003, pp. 1 – 17.

Supplemental Material. Demographic questionnaire and data collection methods.

1. **What is your race/ethnicity (select the one with which you most identify)?**
    a. Caucasian (white)
    b. Black/African American
    c. Hispanic
    d. Asian
    e. Multiracial/Multiethnic or Other

2. **Are you a first generation college student? (i.e., are you the first individual from your family to attend college?)**
    a. Yes
    b. No

Data Collected through Institutional Reporting:

- Index score (a pre-collegiate predictor of college readiness/success combining high school grade point average and standardized exam scores)
- Program and major area of study
- Gender
- Laboratory Graduate Teaching Assistant

Supplemental Material. End-of-Term Student Perceptions of Learning Gains (SPLG) Survey.

1. **What elements or characteristics of this semester's laboratory experience did you find most enjoyable, and why?**

2. **What do you believe you learned from taking part in this experience?**

3. **Participation in this semester's laboratory experience increased my confidence in developing my own scientific questions/hypotheses.**
   - **a.** Strongly Agree
   - **b.** Agree
   - **c.** Neutral
   - **d.** Disagree
   - **e.** Strongly Disagree

4. **Participation in this semester's laboratory experience increased my confidence in designing an experimental protocol to examine a research question of interest.**
   - **a.** Strongly Agree
   - **b.** Agree
   - **c.** Neutral
   - **d.** Disagree
   - **e.** Strongly Disagree

5. **Participation in this semester's laboratory experience increased my confidence in interpreting and presenting my results.**
   - **a.** Strongly Agree
   - **b.** Agree
   - **c.** Neutral
   - **d.** Disagree
   - **e.** Strongly Disagree

Table S1. Distribution of STEM and Non-STEM Majors Enrolled in the *Tigriopus* CURE.

STEM[a] (Major; % of Population)		Non-STEM (Major; % of Population)	
Biological Sciences	9.6%	Audiology and Speech	4.0%
Pre-Nursing	14.4%	Psychology	10.4%
Chemistry	8.8%	Human Services	18.4%
Environmental Sciences	4.0%	Criminal Justice	5.6%
Mathematics	0.8%	Art	3.2%
Nutrition	2.4%	General Education	4.8%
		Special Education	1.6%
		Music (Performance)	1.6%
		Sports/Exercise Science	8.0%
		Journalism	0.8%
		Philosophy	0.8%
		Sociology	0.8%

[a]Each participant's major was determined utilizing the following published guidelines: Louis Stokes Alliance for Minority Participation (LSAMP) Annual Survey (2015). https://www.lsamp.org/help/help_stem_cip_2010.cfm (accessed 10 September 2015).

Table S2. Percentage of Summative Assessment Items Categorized According to Bloom's Taxonomic Level Stratified by Semester/Type of Laboratory Experience.

	Fall 2014 (Traditional)				Spring 2015 (CURE)			
	Exam 1	Exam 2	Exam 3	Final	Exam 1	Exam 2	Exam 3	Final
Level 1: Knowledge	62%	58%	58%	65%	62%	58%	58%	65%
Level 2: Comprehension	36%	40%	40%	32%	36%	40%	40%	32%
Level 3: Application	2%	2%	2%	3%	2%	2%	2%	3%
Level 4: Analysis	0%	0%	0%	0%	0%	0%	0%	0%
Level 5: Synthesis	0%	0%	0%	0%	0%	0%	0%	0%
Level 6: Evaluation	0%	0%	0%	0%	0%	0%	0%	0%
% LOCs[a]	98%	98%	98%	97%	98%	98%	98%	97%
% HOCs[a]	2%	2%	2%	3%	2%	2%	2%	3%

[a]LOCs = Lower-Order Cognitive Questions; HOCs = Higher-Order Cognitive Questions (Crowe *et al.*, 2008). Note: The following topics were covered on each assessment: a) Exam #1 – introduction to biology, chemistry fundamentals, and macromolecules; b) Exam #2 – cells and energy; c) Exam #3 – cellular respiration, photosynthesis, replication, transcription, and translation; and d) Final Exam – mitosis/meiosis, genetics, and cumulative review of previous material.

Table S3. Descriptive Statistics and Multivariate Analysis of Variance (MANOVA) Output for Average Pre-/Post-Semester Percent Favorable Shifts on CLASS-Bio Attitudinal Factors, Stratified by Type of Laboratory Experience.

	CURE (*M; SD*)	Non-CURE (*M; SD*)	F-statistic	*p*-value	Cohen's *d**
Real-World Connections	-0.69 (27.08)	-13.83 (27.27)	14.62	< 0.001	0.48
Problem-Solving Difficulty	-1.71 (26.08)	-4.80 (21.39)	1.05	0.307	0.13
Enjoyment	1.73 (24.94)	-9.73 (27.12)	12.11	0.001	0.44
Problem-Solving Effort	-2.06 (24.92)	-16.57 (30.54)	16.95	< 0.001	0.52
Conceptual Connections	-3.00 (26.25)	-12.00 (24.82)	7.76	0.006	0.35
Problem-Solving Strategies	3.80 (33.91)	-16.60 (36.33)	21.06	< 0.001	0.58
Reasoning	-6.88 (28.63)	-20.64 (35.19)	11.50	0.001	0.43

* Cohen's *d* values were tabulated based on individual, planned comparison analyses (data not shown) of between-group differences in mean percent favorable shifts on factors presented in the CLASS-Bio diagnostic.

Table S4. Descriptive Statistics and Multivariate Analysis of Variance (MANOVA) Output for Average Pre-/Post-Semester Shifts on BMQ Motivation Factors, Stratified by Type of Laboratory Experience.

	CURE (*M; SD*)	Non-CURE (*M; SD*)	F-statistic	*p*-value	Cohen's *d**
Intrinsic Motivation	-0.58 (3.43)	-2.20 (3.51)	13.56	< 0.001	0.47
Career Motivation	-1.17 (4.25)	-3.10 (4.41)	12.48	< 0.001	0.45
Self-Determination	-0.62 (3.88)	-2.84 (3.95)	20.05	< 0.001	0.57
Self-Efficacy	-1.50 (4.09)	-3.52 (4.35)	14.26	< 0.001	0.48
Grade Motivation	-1.34 (3.53)	-2.21 (3.79)	3.48	0.063	0.24

* Cohen's *d* values were tabulated based on individual, planned comparison analyses (data not shown) of between-group differences in mean shifts in motivation on factors presented in the BMQ diagnostic.

Table S5. Criteria for classification of student responses to open-ended prompts on the Student Perceptions of Learning Gains survey.

Theme	Classification Criteria
1. Autonomy	Selected quote indicates that students possessed ownership over one or more aspects of the experimental design process (i.e., that they designed their *own* experiment) and/or that they were self-sufficient in the laboratory
2. Techniques/Skills (General)	Selected quote focuses on one or more experimental techniques or skills discussed during the course of the semester (e.g., microscopy; statistical analysis) and/or the acquisition of problem-solving skills in a general sense
3. Collaboration and Science Communication	Selected quote highlights the opportunities for interaction among peers and/or the instructor; selected quote focuses on the process of scientific communication (e.g., presenting research findings; writing a laboratory report)
4. Appreciation for Process of "Doing" Science	Selected quote reflects an appreciation for the effort needed to conduct rigorous scientific research and the multi-faceted, complex nature of the scientific process (e.g., troubleshooting; application of science to real life; intricacies of setting up an experiment)