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Lesson

Student-Driven Design-and-Improve Modules to Explore the Effect of Plant Bioactive Compounds in Three Model Organisms

Iglika V. Pavlova

Biology Department, University of North Carolina Greensboro

Abstract

Engaging and supporting introductory level students in authentic research experiences during required coursework is challenging. Plant bioactive compounds attract students' natural curiosity as they are found in many familiar items such as tea, coffee, spices, herbs, vegetables, essential oils, medicines, cleaning supplies, and pesticides. Over the course of one semester, students work in teams to design experiments in three experimental modules to test whether bioactive compounds have effects on *Daphnia* heart rate, antibacterial activity, or caterpillar behavior. In a fourth module, they research solutions to an environmental problem. Students are involved in multiple scientific practices as they make their own experimental decisions, analyze data including using statistics to carefully justify their preliminary conclusions, and have the opportunity to improve their experiment and repeat it. Iteration is also emphasized by the fact that students go through the whole process from design to presentation repeatedly for three experiments. In the process, students experience for themselves the real complexity of scientific investigations and what it takes to rigorously show cause-and-effect relationships. The pedagogical focus is on providing introductory students with a supportive structure in a way that empowers them to make informed experimental decisions and be successful. At the end of the semester, the majority of students displayed a strong sense of personal involvement and an appreciation of the difficulties of scientific experimentation in open-ended written reflections. Students reported that statistics was one of the most difficult yet valuable experiences in these labs and demonstrated significant gains on a statistical test.

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*__Correspondence to:__ Sullivan Science Building Rm 307, Biology Department, 321 McIver Street, Greensboro, NC 27403. Email: ivpavlov@uncg.edu

Learning Goals

The overarching learning goal is for students to understand more accurately and deeply the scientific process of making inferences from data to answer biological questions and make sense of the world around us. To this end, students will experience for themselves the realities of authentic scientific investigation in three experiments. Specifically, they will learn to conduct scientific investigations employing a wide range of scientific practices by making their own experimental choices, using methods for rigorous analysis and careful interpretation of data, and presenting their experiments in oral and written form. Students will also be able to describe the advantages and disadvantages of three different experimental systems to answer the same kind of question. Students will develop a plan for an ecologically-sound habitat to address a current ecological problem and gain an appreciation for the web of complex and mutually dependent interactions that connect living organisms on Earth.

Learning Objectives

Students will be able to:

- Perform background research of peer-reviewed literature to make informed hypotheses.
- Design a controlled experiment and write a protocol.
- Conduct laboratory investigations from a written protocol.
- Perform laboratory tasks such as pipetting and dissecting microscope work.
- Record data in a table in Excel.
- Calculate means and standard deviations.
- Perform & correctly interpret statistical significance testing (chi-square and t-test) in Excel.
- Graph data in Excel to include error bars.
- Evaluate experimental results to suggest improvements to the experimental design or to answer further questions stemming from the results.
- Perform further experiments based on the evaluation of a previous experiment.

Learning Objectives continued

Students will be able to:

- Work in groups to design and perform experiments.
- Write a guided lab report, a modified traditional lab report that is broken down into questions with embedded specific instructions.
- Present with their research group to the whole class using PowerPoint (or equivalent).
- Compare the advantages and limitations of different model organisms to answer scientific questions.
- Propose a plan for an ecologically-sound habitat to address a current environmental problem.

INTRODUCTION

The national trend in biology education the last two decades has been to transform traditional cookbook labs to use not only a student-driven inquiry approach, but inquiry that uses scientific practices that are closer to authentic research as it is practiced in research laboratories (1). Scientific practices include choosing questions, performing background research, designing experiments, analyzing data, repeating the process as needed, and presenting results. Scientific inquiry in research laboratories is a complex process with significant variation in scientific practices in different sub-fields and research teams. Scientific practices vary as well in how they are taught in the biological laboratory, with a continuum of student agency ranging from highly structured to inquiry-based experiences (2).

Curricula that incorporate more authentic scientific practices are often referred to as course-based undergraduate research experiences, or CUREs (1). CURE laboratory modules have been described previously in this journal (3-9). CUREs are expected to better prepare students for research careers (10). Early engagement in CUREs as part of introductory courses has been demonstrated to increase graduation rates and may thus be an important method to reduce unequal access to research careers in STEM (11). Based on current research, my goal for this Lesson was to develop a semester-long experience for introductory biology students that places emphasis on student agency across the range of scientific practices across three different experiments.

Auchincloss and colleagues (1) conceptualize a framework to define research-rich CURE approaches. The Lesson presented here uses the five dimensions from the framework: Use of Scientific Practices, Discovery, Iteration, Collaboration, and Broader Relevance. Students engage in multiple scientific practices from design to analysis similar to that in authentic scientific investigations (Use of Scientific Practices). Significantly, the outcomes of student experiments are unknown to students or the instructor (Discovery). The experiments are student-driven as students choose the test compound and develop the details of the experimental protocol rather than working with a known compound using a protocol perfected by the instructor. In this way, they get close to the real complexity of research that generates “messy” data. Students are given the opportunity to evaluate the data and to perform further experiments (Iteration). In fact, this Lesson expands on this aspect by having iteration not only for each experiment, but also a meta-iteration as students go through the process over three different experiments. Students also collaborate with each other and with instructors to plan and conduct the experiments, with instructors serving as research mentors (Collaboration). Because this Lesson allows significant student choice, the extent of the Broader Relevance dimension depends on the choices the student teams make. Often, teams are testing compounds that have never been tested in a certain experimental organism, and their results have implications for discovery of new biological effects or for the use of, for example, *Daphnia* as a bio-indicator of environmental pollution for a particular substance.

The course is distinguished from other research-rich curricula through its use of meta-iteration, a unifying theme, and bioactive compounds as the research focus. First, the meta-iteration of the scientific process over different experiments distinguishes it from most published CUREs that have one research project as one module or over the course of the semester (3-9,12). An advantage of the meta-iteration in this Lesson is that students practice repeatedly the range of scientific practices, growing their skills and understanding over time. There are additional advantages of repetition when it is placed in different experimental contexts. One, to allow students to identify the important shared features of the scientific process in rigorously inferring cause-and-effect. Two, the differences in experiments provide them with firsthand knowledge of the strengths and limitations of different experimental systems. Comparison of the pros and cons of model organisms is an explicit learning goal in this Lesson that distinguishes it from many undergraduate curricula. Second, the modules in this Lesson are united under a theme of bioactive compounds. An advantage of the thematic approach is that students experience a sense of curricular cohesion and are able to explore different aspects within the shared theme. Third, the unifying theme naturally engages student interest as bioactive compounds are common in the household in tea, coffee, spices, herbs, vegetables, essential oils, medicines, cleaning supplies, and pesticides. The theme has the benefit of both linking to central concepts in biology such as energy movement in ecosystems and linking to practical applications such as drug discovery. As a result, this Lesson can be used in a variety of courses or presented in different ways as part of either standard or innovative curricula. Possible themes include biotechnology, pharmacology, microbiology, physiology, or ecology.

The dynamic inquiry modules by Jo Handelsman and colleagues (13) were the initial inspiration for this Lesson. In this teaching approach, students are presented with an interesting issue and their experimental “challenge” for the day and work in groups to design, get feedback on their design from the class, and execute their experiment. They are only provided with sufficient background and a demonstration of required techniques. Students perform multiple modules of this nature throughout the semester. Through such repeated practice, students build skills and confidence in designing experiments to answer scientific questions. This Lesson preserves the iterative inquiry backbone of “challenge design by team – feedback in class – train on technique – perform experiment” to give a sense of dynamic experimentation to find answers to a question. I added to this framework a more complete set of scientific practices to more accurately represent authentic research practices. Specifically, students work to build scientific rationale through background research, perform statistical analysis, integrate multiple pieces of information to make carefully justified conclusions, and present in both written and oral form.

Plant bioactive compounds are the running theme that unites the modules in this semester-long Lesson. To protect against herbivores and pathogens as well as a multitude of other needs, plants produce bioactive compounds such as caffeine, quinine, aspirin, nicotine, and morphine. "Bioactive" refers to the fact that these compounds can have a multitude of powerful effects in relatively small doses in living organisms - with some of the effects on the human species familiar to all of us, be it in the daily cup of coffee or through news reports about the opioid crisis. However, many have evolved as protection against herbivores such as insects, who can get intoxicated at lower doses than the much larger humans. The bioactive compounds can also disrupt insect development or imitate alarm signals in insects, scaring them away before they can even start eating at the plant. Still others inhibit digestion; legumes such as beans have a number of such compounds. It is the latter that likely contributed to the death of Chris McCandless, the adventurer of "Into the Wild" fame (https://www.npr.org/sections/thesalt/2015/05/01/403535274/into-the-wild-author-tries-science-to-solve-toxic-seed-mystery). Bioactive compounds can also have a variety of medically-relevant properties, such as anti-bacterial, anti-fungal, anti-viral, anti-inflammatory, and anti-cancer effects (14). John King's "Reaching for the Sun" and Judith Sumner's "Natural History of Medicinal Plants" are excellent popular science books suitable for all audiences with chapters on bioactive compounds (15,16).

The theme of plant bioactive compounds combines several important educational benefits. First, the theme connects to students' daily lives. Students can, and do, bring materials or substances from their home to test, such as spices or herbs. Second, students can make connections to current research needs and diverse career options. With the constant need for new medicines, drug discovery from natural products such as plants is a field of active research (17-19). Significantly, the uniting theme prompts students to link plant science (bioactive compounds) to other organisms (their test subjects). Promoting the understanding of and excitement for plants can help counter "plant blindness" (20,21), opening students to the possibility of exciting and important careers related to plant science. Together these two benefits lead to a third benefit, to allow students to extend beyond the required curriculum to learn the biology behind something from their daily life – and this time they are the researchers in the exciting field of discovery of new effects and new sources for bioactive compounds.

A fourth benefit of having a unifying theme is that it supports the standard curriculum. The fact that plants protect themselves from herbivores with bioactive compounds connects to a foundational concept in biology, the transformation of energy in ecosystems (*Vision and Change*, https://visionandchange.org/finalreport/). Plants are one of the major primary producers on Earth today that "fix" the energy from the only external source of energy to the Earth – the Sun – to ultimately build from carbon dioxide all of the different organic compounds that they need. Through the herbivores that eat them, plants provide food for consumers throughout the food (trophic) chain. Students study energy transformation most directly in the module that uses caterpillars as an experimental system where they measure caterpillar biomass at the start and end of their experiment. The Lesson also links to evolution and environmental issues. Due to the presence of defensive bioactive compounds, caterpillar populations have to evolve the ability to eat a particular plant species. Caterpillars typically cannot survive on imported non-native species which are widespread as decorative or invasive species. In this way, non-native species disrupt the food chain with multiple downstream consequences (22).

Within the unifying theme, students explore the effect of compounds of their choice to learn whether they have antibacterial effects and whether they impact the physiology, growth, and behavior of animals. Students study the effects in three types of experimental organisms: *Daphnia*, bacteria (*Escherichia coli* and *Bacillus subtilis*), and caterpillars. Working with live organisms is an important and exciting aspect of the experiments. The organisms are widely used in both research and teaching labs and available with instructions for care from Carolina or Ward (5,23,24). *Daphnia*, specifically, is a well-developed model organism to study the effect of different compounds on heart rate or viability, such as potentially toxic compounds in the environment (5,25). The Lesson Plan describes the specific use of these organisms to test bioactive compounds in a way that purposefully supports students to actively engage in collaboration with peers and the instructor to grow their skills and confidence.

Intended Audience

This Lesson was taught in an introductory biology laboratory consisting of mostly biology majors in a large public research level 2 institution. It was taught in two sections of a 15-section laboratory course, with one instructor and about 20 students per section present in the room. A technician prepared the needed reagents, cultures, and animals. By consideration of the background knowledge and laboratory skills of students, modifications can be made for use in high school or mid- to upper-level college biology courses.

Required Learning Time

The complete lesson with the four modules of three-four weeks each was taught in 12, three-hour sessions as part of a semester-long course. The four modules consisted of three experimental modules (Module 1, *Daphnia* physiology experiment; Module 2, Antimicrobial activity experiment; and Module 3, Caterpillar behavior experiment) and one non-experimental module (Module 4, Design project). Each module is a stand-alone unit that can be used individually.

Prerequisite Student Knowledge

Students should be able to read and perform basic arithmetic. Any knowledge of background research, scientific experiments, graphing data, statistical analysis, web browser searches, Excel or PowerPoint (or similar software), or the fundamentals of biology and chemistry can support student learning. However, modules are designed without assuming that students have more than minimal exposure to these components.

Prerequisite Teacher Knowledge

Instructors should be familiar with the scientific practices used in research and listed in the Learning Objectives, to include:

- library research
- common laboratory tasks with pipettes, microscopes, dilutions of substances, bacterial cultures (including the Kirby-Bauer diffusion test), and raising live animals
- data analysis (including statistical analysis)
- use of Excel for statistical analysis and graphing (or

alternatives, if desired)

- writing of lab reports and oral presentation (using PowerPoint or alternatives, if desired).

Content-wise, instructors should be familiar with the fundamentals of movement of energy and matter in ecosystems. Sufficient teacher background on bioactive compounds is provided in popular science books on the topic (15,16), with additional consultation as needed with compendiums of plant bioactive compounds (14) or through internet or literature searches. A list of common compounds that was shared with students in this Lesson can be found in Supporting File S9. Bioactive compounds – Module 1. Lab 3. Handout. Lab challenge #2. An overall understanding of common bioactive compounds and their potential effects can help to better excite students about the topic and guide them in their experimental choices.

SCIENTIFIC TEACHING THEMES

Active Learning

Incorporating active learning was an explicit goal for the Lesson in each class session, with a large emphasis on student choice combined with team work driving the experiments. Small-team discussions and reporting out to the rest of class were used repeatedly in every class. For each of the four modules, students were presented with an experimental design "challenge" and then worked in their team to discuss and decide on different options in response to the challenge, to include both the choice of substance to test and the experimental procedure. They perform the first experiment and analyze the results. Students then consider the results from the first experiment and reflect on any technical issues that were encountered to make their own decisions on what changes to make for the second iteration of the experiment. When individual students had performed literature research on a topic, they would have to advocate for their choices based on the literature to their classmates. Students worked individually to write up assignments, to create graphs, perform statistical analysis, and complete a guided lab report for each of the experiments. Each student participated in two team presentations delivered to the rest of the class.

Assessment

Each lab period included products of student work that each individual student was responsible for, be it a pre-lab assignment (written or multiple-choice format), to prepare data in the form of a table, to graph data, or to perform statistical analysis. Students individually prepared a lab report (for the three experimental modules) and, in teams, a PowerPoint presentation (for two of the modules). Students were assessed for their understanding of experimental design and statistics at the start and end of the semester through a Pre/Post Survey as well as an end-of-semester quiz (Supporting File S2. Bioactive Compounds – Surveys). Students reflected on the process of science and their science attitudes at the start and at the end of the semester through a Pre/Post Survey. At the end of the semester, students additionally reflected on how the labs were interesting, useful, or difficult for them, and how their understanding of science and undergraduate science labs changed.

Inclusive Teaching

The Lesson was developed with a strong emphasis on engagement and support of all students, regardless of level of preparation. The topic links easily to daily life, and students were encouraged to pursue ideas that were interesting to them and to bring materials from their home to test. Structure with clear goals and support has been shown to particularly improve learning in students belonging to groups underrepresented in the sciences (26-28). To this end, the goals of achieving mastery in the different science processes in order to prepare them for future classes and careers were explicitly stated to students at the start of the semester (including in the syllabus, Supporting File S1. Bioactive Compounds – Syllabus) and when transitioning between modules. That learning is a process and that skills build over time was also explicitly stated to the students in every lab session. The emphasis on growth was enacted in every class where students were provided with support and sufficient in-class time to plan their experiment, to learn to use Excel, to make a graph, to perform statistical analysis, and to write up their work as a lab report and to prepare their presentation. The lab report was guided with built-in instructions for the individual elements (e.g., Supporting File S12. Bioactive compounds – Module 1. Lab 4. Guided lab report). Students had time to work on different parts of the lab report during class over several weeks. Information needed to complete a task was provided before class as part of homework assignments. Sessions started with a whole class discussion of daily tasks from the homework, followed by demonstration of any laboratory techniques. All assignments were considered formative: students were given feedback on how they might improve their work and then given the opportunity to make said improvements. Since they used the same or similar skills over the course of the four modules, students had the opportunity to grow their skills and improve throughout the semester – and explicitly recognize that in self-reflections.

Group work was also structured to support the success of all students centering around the principles of positive interdependence, individual accountability, and equal and simultaneous interaction (29). Students were individually accountable by completing their own individual assignments that prepared them for a class session or as they learned new skills during class. Being individually accountable at every stage of the research process prepared students to participate equally and productively during group interactions. Group work relied on positive interdependence, as students needed each other both for the overall success of the project as well as for tasks that were complicated enough to elicit mutual need (29-31). Specifically, students worked in groups of four for cognitively difficult tasks that benefit from multiple inputs such as planning the experiment or discussing experimental results. For performing the experiment or recording the results, students worked in smaller groups of two to allow both students to be fully engaged. For example, often both students worked on the experiment together, while in other cases the two students had specific, complementary tasks of either completing or recording results. Successful group interactions in this Lesson were also supported by clear goals, tasks being highly relevant to the goal, and outcomes in the form of lab reports or presentations with multiple parts that clearly linked to future value in their science careers (31). Active learning in general has been shown to support inclusive learning by all students (32). CUREs implemented early in the curriculum have been shown to improve scientific self-efficacy, identity, and student persistence in STEM degrees among diverse students (11,33) and hold particular promise for underrepresented minority students (33-35).

LESSON PLAN

Over the course of one semester, students design experiments in three experimental modules to test whether bioactive compounds from plants have biological effects on *Daphnia* heart rate, antibacterial activity, or caterpillar growth and behavior (Figure 1). Students design an ecologically-sound habitat of their choice in a fourth, non-experimental, module. The three experimental modules have a similar structure, where students are prompted with an experimental design "challenge" and then work in groups of either two or four to design and perform the experiment. Each lab session was about three hours long. Students analyze their experimental results and decide on an iteration of the experiment based on their analysis, either to improve their experimental design or collect more replicates. In the first two iterations of the experiment in Module 1, students work in groups of four to design, test, and finalize an experimental procedure with a known compound (caffeine or ethanol). Teams decide on their own compound to test for the third experiment in Module 1 using the protocol they developed. Students agency increases in subsequent experiments. They work in teams of two and choose the test compound (Modules 2 and 3) and the habitat problem to solve (Module 4). The focus is on students' making their own choices, while the instructor serves as a guide and mentor to the inquiry process. Each student completes a guided lab report for all three labs. The guided lab report is a modified traditional lab report that is broken down into questions with embedded specific instructions (e.g., Supporting File S12. Bioactive compounds – Module 1. Lab 4. Guided lab report). Student teams prepare a presentation for the experimental module of their choice as well as for the design module.

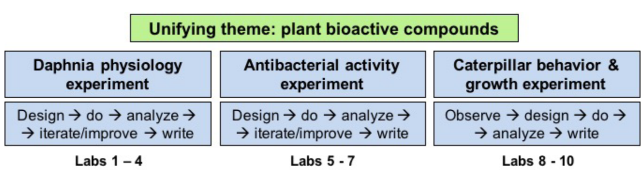

Figure 1. Lesson design emphasizes student choice and iteration. An overview of the three experimental modules united by the theme of understanding the effect of plant bioactive compounds. Labs 10-12 include the fourth, non-experimental, module to design an ecologically sound habitat.

I designed the course to allow students to experience growth over the semester, developing their thinking and building a range of skills in scientific experimentation. Students are empowered to take responsibility for their own experiments and individual assignments for each class session while also collaborating closely with their teammates and the instructor. To accomplish this, establish, early on, a pattern of providing support together with a clear expectation of individual responsibility and collaboration. This pattern should be re-enforced as often as possible. Make sure that every aspect of class – the syllabus, the first class session, the first activities during each session, and all subsequent activities – creates conditions for students to both individually engage in the process and to be supported by the instructor and their peers. I address students directly using second person pronouns in the Syllabus (Supporting File S1. Bioactive Compounds – Syllabus). Starting with the Syllabus, tell students the larger significance of the labs, the specific learning goals, and what they will do in the individual modules. I recommend describing each experiment as a process - one that involves thinking, planning, getting feedback, experimenting, and evaluating. To set those expectations, emphasize the growth in skills and independence from one experiment to the next in the Syllabus.

The start of the semester is particularly important for setting the tone for the rest of the semester. I start this process before the first class meeting, with an enthusiastic email and presenting a mystery organism (a hard-to-identify caterpillar such as that of the black-waved flannel moth). The introductory email includes a link to the Syllabus and asks students to post their guess on the mystery organism in a discussion forum. Students also introduce themselves in the forum and share what they are excited about for the upcoming lab from their review of the Syllabus. In this way, students know even before they enter the classroom the expectation of independence and growth.

Module 1: Laying the Foundations & Daphnia Physiology Experiment

Daphnia magna, the larger *Daphnia* species, can be obtained from Ward or Carolina 1–3 days ahead of the labs with instructions for care (Carolina # 142330). Additional details on working with *Daphnia* to test the effect of compounds on heart rate in teaching laboratories are available (5,24). The emphasis in the description below is how to use it as part of an inquiry process with students actively deciding and improving on the experimental protocol (Labs 1-3), testing a compound of their own choice in Lab 4, and then continuing to grow these skills in the subsequent modules.

Lab session 1

Class begins with introductions, first in groups of four at a lab bench and then the whole class. The Syllabus is projected to the class and used to introduce the lab philosophy of giving students agency to design experiments while guiding students to build expertise over time toward fluency for future coursework and careers. Fairly quickly, we move into an introduction to the first experiment so that students can experience for themselves how we will be working together throughout the semester. In the first experiment of Module 1, students are given a choice of compound (caffeine or ethanol) and come up with their own procedure. The compounds are familiar to students, so engage them to contribute. What are the effects of caffeine and ethanol on humans? On heart rate? Where does caffeine come from? Why do plants produce caffeine? Now, it is time for the first experimental design "challenge": how would you test what effect caffeine or ethanol have on *Daphnia* (Supporting File S3. Bioactive compounds – Module 1. Lab 1. Handout. Experimental Challenge #1)? Before designing their first experiment, students individually complete Pre-Survey (part 1) to reflect on their current lab skills and perceptions of science as well as an assessment to design an experiment (Supporting File S2. Bioactive compounds – Surveys). The Pre-Survey serves as a warm-up with every student trying to activate their prior knowledge and becoming primed to participate in their group.

The next step is to briefly introduce available lab supplies and "Key questions to consider" from Supporting File S3. Bioactive compounds – Module 1. Lab 1. Handout. Experimental Challenge #1. Let students discuss in their group how they want to complete the experiment and work on the experimental design worksheet (Supporting File S4. Bioactive compounds – Module 1. Lab 1. Worksheet. Write-up I). Then, have teams report out to the class. Foster critical thinking by asking students to explain their reasoning, and have different class members contribute possible pros and cons of different experimental choices. Avoid the temptation to provide answers by asking guiding questions: What are benefits and issues in using the same *Daphnia* versus different individuals for control versus drug readings? Emphasize the importance of considering confounding factors: How do you know whether it is caffeine, and not something else, that is affecting the heart rate? What will you do to make sure that caffeine is the only difference between control and treatment groups? Discuss with students the handling of *Daphnia* to avoid the confounding influence of stress on heart rate as it presents a major technical difficulty for the novice researcher. Compassionate and ethical work with live organisms should be mentioned here and how it is important in good experimental design: What do you think aquatic organisms such as *Daphnia* are experiencing as you are replacing the water between treatments? What are other things that are stressing the *Daphnia*? Do they have an effect on what you are trying to measure? I keep the conversation focused on the idea of accounting for confounds without emphasizing formal terminology until the second session. This method keeps students engaged in thinking authentically about the experiment, rather than stressing about what they "should" know in a science class.

Before students begin experimentation, introduce basic lab safety and demonstrate the use of the dissecting microscope with students in groups of two, finding the parts of the microscope as you speak. Show a video of *Daphnia* to point to the hard-to-see transparent heart on the back of the organism (https://www.youtube.com/watch?v=2g-04Uk0ut0&t=2s). Students can then practice using the hand counters to measure the heart rate from the video. Students perform Experiment 1 working in groups of two and help each other with tasks, including working with the microscope. The instructor circulates and helps those groups that are having difficulties. Emphasize the importance of taking notes on changes in the protocol or observations on possible confounds that can be used to improve the experiment in the next class. Students individually graph their results on graphing paper and complete the write up for the first lab (S4. Bioactive compounds – Module 1. Lab 1. Worksheet. Write-up I). Complete the first session with an enthusiastic note on how, in the next session, they will have the opportunity to improve their experiment. The homework assignment is a graphing tutorial (https://mathbench.umd.edu/modules/visualization_graph/page01.htm) and an associated online quiz (Supporting File S5. Bioactive compounds – Module 1. Lab 2. Homework. MathBench graph activity).

Lab session 2

Before Lab 2 begins, students record their height in centimeters using a ruler that is placed on the door of the lab for use in the statistics activity later in lab. Start lab by providing quick feedback on the Lab 1 write-up, mostly regarding the clarity of the writing. Students warm-up by discussing, in groups, the value of writing for their learning and careers. Move to feedback on graphing, answering student questions from the homework and providing correction for Lab 1 graphs. Most notably, without instruction, students usually plot individual *Daphnia* and not the averages from the experiment. The graphing tutorial is purposefully mostly a refresher to support student confidence while setting up an expectation of quality work. I give a brief chalkboard presentation focusing largely on scatter plots and line graphs (more familiar to students) and bar graphs (common in biology but may be new to students). Then, students apply their knowledge in teams to decide what graph would be best to use for their *Daphnia* experiment. In this way, Lab 2 also starts with a demonstration in action of how the instructor is there to support students while also allowing them to link to the larger purpose and take responsibility for their learning in a non-threatening manner.

Students complete Pre-Survey (part 2) on statistics (Supporting File S2. Bioactive compounds – Surveys) before instruction moves into an interactive unit on experimental design. Students share their difficulties from Experiment 1, and their classmates chime in with possible solutions. The central limitation in this experiment is that the heart rate in *Daphnia* ranges from 91 to 521 beats per minute (24). It is difficult to count accurately over a hundred clicks per minute. In fact, a college professor has demonstrated that caffeine does not increase the heart rate in *Daphnia*, and the purported increase observed in teaching labs is because of this inaccuracy and experimenter bias (24). What can be done? We can record and count the heart rate from a slowed video (we did not have this capacity in our lab). Students can also decide to slow down the heart rate by cooling down the *Daphnia* in ice water, and groups can help each other to blind the experiment to limit bias. This experimental difficulty serves as an important lesson on the limitations of experiments, which we may not always be able to overcome even if they significantly affect the interpretation of the results.

Next, an interactive unit on statistics serves as the formal introduction into the role of descriptive and inferential statistics. A helpful manual on statistical analysis for biology is provided by HHMI Biointeractive, https://www.biointeractive.org/sites/default/files/media/file/2019-05/Statistics-Teacher-Guide.pdf. This Lesson uses the interactive statistics instructional module by Marsan and colleagues (36). Students form a "live" bar graph in the classroom by lining up according to their height in class, prompting a discussion of normal distributions and sampling. After this interactive introduction to statistical concepts, show students how to calculate averages and standard deviation (SD) and to make bar graphs with error bars in Excel. It is best if students are following along with you and getting help from their classmates or instructor as they are experiencing difficulties. Provide a summary of statistical functions as a handout (Supporting File S6. Bioactive compounds – Module 1. Lab 2. Handout. Statistics in Excel). We find that our students have very limited skills in Excel, and so have purposefully added significant in-lab time for students to practice and get help. By doing this work in class, we have learned that students experience a range of challenges including ones as simple as selecting a set of data with the mouse. If they are alone at home at this initial stage, they can spend a lot of time in frustration, resulting in a negative emotional experience and poor performance. I highly recommend having students work with Excel in class to enter data, make graphs, and perform statistical analyses, if your timing allows it. The result is increased student confidence from a more positive experience and completing the task satisfactorily.

Teams are now ready to use all the feedback and new information from the first part of class to perform an improved Experiment 2, recording the data in Excel. Students individually calculate averages and SD from the data, construct graphs with error bars, and complete a revised experimental write-up (Supporting File S7. Bioactive compounds – Module 1. Lab

2. Worksheet. Write-up II). Circulate continuously to help as students move at their own pace. Encourage help from peers, though this should be limited to providing information. Remind students that each person needs to be able to perform any analysis and create any graphs on their own. Conclude Lab 2 with words on work accomplished and looking forward to choosing their own test substance next time. For homework, students read and summarize the "Chemical warfare" reading (16) (Supporting File S8. Bioactive compounds – Module 1. Lab 3. Homework. Chemical warfare reading). The reading provides ideas for compounds to test and explains the different possible roles that bioactive compounds, such as caffeine, play for plants.

Lab session 3

Before Lab 3 begins, teams record the results of Experiment 2 in a shared file for statistical analysis later on in lab. At the start of class, students discuss the most interesting and important points from the reading and report out to class. Encourage them to share what made the most impression on them: excitement is a big part of the experience here. Guide discussion toward aspects that most influence the experimental design. For example, the dose, the size of an organism, and how quickly an organism is exposed to the compounds all determine how much of an effect there will be. Different compounds also have different effects, be it on the nervous system, the heart, digestion, development – or by alerting insects to stay away. Students need to pay attention to these aspects when doing library research. Mention here the upcoming two other experiments – testing for antibacterial activity and effects on caterpillars – so students have the larger context for their decisions. Note that if there is a known effect of a compound, it is still worth testing to see whether it has other currently unknown effects – that is how many new medicines have been discovered!

The discussion of the reading prepares students for the literature search that comes next. Students use the *Daphnia* Challenge #2 handout to help them make their decisions for Experiment 3 and guide them through the library search for scholarly articles (Supporting File S9. Bioactive compounds – Module 1. Lab 3. Handout. Lab challenge #2). There are different ways to organize group work (see Discussion). In this version, I gave teams of four ample time in lab to follow their interests as long as, by the end of lab, they completed the plant compound choice worksheet (Supporting File S10. Bioactive compounds – Module 1. Lab 3. Worksheet. Compound choice). Besides the rationale for the compound, students also have to think about where to obtain the compound from and how to prepare it. The instructor needs the worksheet by the end of lab if the experiment is to be performed in the next class in order to have time to obtain anything that is not available on hand. Students can also bring materials from home, such as spices or herbal extracts. Most of the compounds or plant extracts can be obtained in health food stores or online pharmacies. The solvent most often is water, glycerin, or alcohol. *Daphnia* is an aquatic organism, so, in this experiment, we avoid testing oil-soluble compounds. Assure students who get excited from the reading about essential oils that they can test them in the upcoming two experiments. Such difficult choices are an important lesson in the benefits and limitations of different model organisms. It is one of the stated Learning Objectives (#13) for this Lesson.

In the last part of Lab 3, we continue the interactive statistics module from the previous session by delving deeper into inferential statistics (36). Introduce hypothesis testing and the logic behind t-tests. Students apply what they have learned to two datasets, the class height data and *Daphnia* Experiment 2. For the rest of class, students individually complete a worksheet on statistics (Supporting File S11. Bioactive compounds – Module 1. Lab 3. Worksheet. Statistics activities). Each student also creates bar graphs with 95% confidence interval (CI) error bars and uses them to infer statistical significance for the two experiments. Students move at different speeds here, so allow them to submit a mostly completed worksheet and complete the rest at home. The last question about the t-test is left for the next lab session.

Lab session 4

Have teams discuss what conclusions can be drawn from the statistical analysis of class height and Experiment 2 from the previous session and then report out to class. Use the opportunity to bring back the issue of which confounds were controlled well and which ones were more difficult to control as well as the imprecise measurement of heart rate. Emphasize the point that statistical analysis cannot compensate for a poorly-designed or executed experiment – it cannot "fix" your experiment. Now that students have been deeply involved with their experiment, it is a good time to introduce more formally some of the terms used in experimental design. The effects measured are the dependent variable, while the causal factor under investigation is the independent variable. The other possible causal factors that can impact the effect you are interested in are the possible confounding variables that we attempt to minimize the effect of so that we can determine the effect of the causal variable of interest. Controlled variables are those that are kept similar between the control and experimental groups; if they are similar between the two comparison groups, they are not influencing (and therefore confounding) the effect being measured.

Before students start Experiment 3 with their own compound, remind them about lab safety again. This time, it is more important, as they may be using compounds that are irritants. Students should wear gloves and avoid touching their skin or orifices. Most compounds come in concentrated form and need to be diluted. We watch a tutorial (http://biology.kenyon.edu/courses/biol09/tetrahymena/serialdilution1.htm) on making dilutions in class and discuss it. Students can also reference https://www.wikihow.com/Do-Serial-Dilutions as they work. *Daphnia* are much smaller than humans and so are expected to be generally affected by smaller dosages. There is good reason to test a more concentrated form first in order to get a noticeable effect. However, this is often not possible, since many compounds are dissolved in high concentrations of ethanol or glycerin. Ethanol has a strong effect in slowing the heart rate and toxicity in much lower doses, and glycerin is very viscous (24). I individually work with groups to answer questions and verify their dilution calculations – it is a new process for them, and they appreciate the check.

Students finally get to test their experimental compound in Experiment 3! By now, they are at ease with the experiment and have confidence in the experimental protocol they themselves developed and improved. Students are comfortable in taking on a new challenge, of preparing their extract from raw materials and making the dilutions. The level of engagement and excitement in this lab is palpably high! For homework, students will individually write up the rationale, procedure, results, and references for the *Daphnia* lab report (Supporting File S12. Bioactive compounds – Module 1. Lab 4. Guided lab report). Students already have experience writing some parts, and they will later have time to revise this draft. Remind students that they will be able to test for antibacterial activity in the next session, so that they can start thinking about the results from Experiment 3 in that light. In this lab, also show students how to perform a t-test in Excel and have them practice using Experiment 2 data (thus completing Supporting File S11. Bioactive compounds – Module 1. Lab 3. Worksheet. Statistics activities). Discuss the interpretations of the results with the class. Encourage them to think about how trustworthy the conclusions may be given what they know about how well they controlled the confounds and any technical difficulties they experienced.

At the end of class, remind students that, in the upcoming week, each student can test up to two compounds of their own personal choice for antibacterial activity (including any compounds from home that they would like to test). To stimulate student agency and excitement, I emphasize individual student's personal preference and curiosity in their choice for Module 2 compounds over having solid rationale backed by the literature. Most of the work on deciding Module 2 compounds is done in Module 1, and there is no separate time allotted for compound choice. Discussions on compound choice for Module 2 happened as students were planning and collecting data from the third *Daphnia* experiment with a compound of the group's choice in Labs 2 and 3. Specifically, students fell into one of three categories with regard to their choice for Module 2 compound: (a) students who became excited about essential oils from the Module 1, Lab 3 reading, but were not able to test non-water-soluble compounds in Module 1 and chose to test such a compound in Module 2; (b) students who tested the same compounds in both Modules 1 and 2; or (c) students who wanted to test spices or other household plant products that they brought from home (or we easily provided from our own homes). Available on hand were the compounds previously presented to the students in Supporting File S9. Bioactive compounds – Module 1. Lab 3. Handout. Lab challenge #2.

Module 2: Antibacterial Activity Experiment

Many bioactive compounds from plants have anti-bacterial, anti-fungal, or antiviral activity (14). The original inspiration for Module 2 came from the "Spice up your life" experiment in the Microbes in Action curriculum (https://www.umsl.edu/microbes/Classroom%20Activities/index.html). Adding the Kirby-Bauer disc diffusion test allows for quantification of results (23). Paper discs are soaked with the substance to test and placed equidistant on an agar plate inoculated evenly with the bacterial species of choice. We used *Escherichia coli* and *Bacillus subtilis*, a Gram-negative and Gram-positive bacterial species, respectively. It is better to have more than one species, because a certain compound may have activity against one, but not both, compounds. For example, some antibiotics are effective against Gram-positive, but not Gram-negative, bacteria. In the discs diffusion test, when a compound has bactericidal or bacteriostatic activity (killing or inhibiting growth, respectively) at the dose delivered, the bacteria do not grow there and form a "zone of inhibition" around the disc (23).

For this experiment, each student has materials to test two substances on each bacterial species. Students swab each plate with bacteria from an overnight liquid culture then place the disc soaked with the compound of their choice. The discs need to be sterile, either pre-purchased or hole-punched and sterilized in an autoclave. They are incubated at 37ºC overnight and refrigerated until the next class. Students take measurements on the zone of inhibition (in mm) using a ruler (23).

<u>Lab session 1</u>

Teams use the handout for this module's lab challenge (Supporting File S13. Bioactive compounds – Module 2. Lab 1. Handout. Experimental challenge) to begin a discussion of how they would perform a test for anti-bacterial activity. The experimental method for this lab is harder for students, so bring the class back together after five minutes or so to discuss initial ideas. Show them the materials and logic of the disc diffusion test. Teams can now go back to discussion with a clearer idea. This time, ask them to address the second and third questions under "Some questions to consider" in the handout. These questions prompt students to think about issues that lead to the formulation of positive and negative controls. Bring the class back together a second time after ten minutes, again because this issue can be quite difficult for some. It helps to draw on the board possible experimental results. Draw a plate with a disc with no zone of inhibition (no effect of test compound, a negative result). What would you do to figure out whether there is really no effect or something went wrong with your test? This leads to the idea that the experiment needs a positive control. A compound with a known effect can be used as a positive control. I recommend one with a moderate effect, such as oregano or thyme oils, as a large inhibition zone can interfere with other tests nearby on the plate. What if you do have an inhibition zone (a positive result)? Can you be sure that it is your substance and not something else in the mixture that caused the effect? This discussion leads to the idea that the experiment also needs a negative control. To help students generalize the purpose behind controls, ask them whether their *Daphnia* experiment had negative or positive controls and how those controls helped them understand their results.

Next, demonstrate in more detail the techniques for the disk diffusion test. A blackboard introduction to the metric system is followed by a demonstration and pipetting practice. For microbial work, explain clearly to students that they should label on the agar side of the plate and in small handwriting around the edge so as to be able to see the results. Have them put their initials and date as you speak. Sterile work is another important aspect; it is not necessary to use Bunsen burners if you use sterile swabs to spread the bacteria on the plate. Sterile swabs are also softer and have lower chances of disrupting the surface of the agar. Show students how to swab at an angle and to systematically cover the whole surface. This will create a lawn of bacteria throughout the plate. Students can do a dry run along with you. Since bacteria are microscopic, it helps to discuss what will happen next. What does it mean when we say that bacteria "grow"? They become visible as they divide and there is more of them, but the individual cells do not become bigger. Draw it on the board to help students visualize what is unseen by the eye.

Give teams time to discuss the experimental procedure, and have students work individually to complete the experimental design worksheet (Supporting File S14. Bioactive compounds – Module 2. Lab 1. Worksheet. Write-up I). They can then proceed at their own pace to complete the experiment. Students spend most of the time preparing their substances. In this lab, many students become excited to test compounds from household products such as spices, so there is a lot of activity grinding the spices and making extracts. Other students get excited about testing essential oils that they could not test in the *Daphnia* experiment. Students also have to make sure to label everything well and to prepare the controls. There is still ample time in this session for students to work on their *Daphnia* lab report in class and to ask questions and get feedback. The complete draft of the *Daphnia* lab report is due by the next lab.

Lab session 2

The lab session begins with students analyzing their experimental results. Remember that each student was able to test up to two substances of their own individual interest. Students excitedly share and talk about their results. They record their data and discuss what to do in the second iteration. Should you repeat an experiment with a negative result? Should you repeat one with a positive result or test something else? I recommend leaving it up to them to make those decisions as much as possible. Guide them through the process of looking at and thinking about their positive and negative controls. This experiment also presents a variety of challenges to solve. For example, they may not have a good lawn of bacteria or have put the disc too close to the edge, requiring repetition. The zone of inhibition for a compound with a strong effect may be so large that a disc may need its own plate in the repeat. Based on these results, students decide what to do and perform Experiment 2. They should record the experimental protocol, noting any changes compared to Experiment 1 on the worksheet (Supporting File S15. Bioactive compounds – Module 2. Lab 2. Worksheet. Write-up II). Students are given time to write up the experimental question, procedure, and graphs for the Antibacterial lab report (Supporting File S16. Bioactive compounds – Module 2. Lab 3. Guided lab report).

In this lab session, provide feedback on the *Daphnia* lab report. A fairly quick perusal of the lab reports before the class session can help you identify major issues. You can provide additional feedback on individual lab reports after lab, though this should happen fairly quickly if students are to have time to make their final edits before the next lab session. Model good writing behavior by allowing some time in class for students to edit their drafts. Emphasize to the class that editing is absolutely essential to any writing process. The final version of the *Daphnia* lab report is due before next session.

Lab session 3

Lab starts with students checking and recording the results of Experiment 2. They then move to literature research and writing the experimental rationale and references in the Antibacterial lab report. These, together with the discussion section of the lab report, are the most difficult parts of the experimental process for them. It is very valuable for students to be able to go through the process in consultation with peers and their instructor during lab.

Now, it is time to provide an overview of the components of a good presentation (Supporting File 17. Bioactive compounds – How to present slides). Students work in teams to decide which of the two experiments to present. This choice is yet another point in which they have agency to make their own decision. Make sure teams have time to work on the presentation during lab so that they can have interesting and important conversations about their experiment and how to present it. The goal is to have the presentation mostly completed, as, in the next session, they will need to make sure to have time to practice. Students will not have much time to work on the presentation outside of class, as the complete draft of the Antibacterial lab report is due before next class. This should not be very onerous, as they have completed most of it in class. What I have learned from allowing students to do work in class that is typically completed out of sight of the instructor is that the lab report is a big and complex undertaking. Writing a lab report requires significant time and skill. Allowing students to work during class promotes inclusive and equitable education. It also demonstrates to students the social nature of investigations.

Module 3: Caterpillar Behavior Experiment

In this module, we come full circle to the initial reading, since many bioactive compounds specifically deter or affect herbivores such as caterpillars. By testing plant-derived compounds on caterpillars, students truly “get” the idea of why plants produce bioactive compounds. Students enjoy observing the behavior of a live animal, a great strength of Module 3. The caterpillar experiment is the most complex, as it is not easy to design an experiment that conclusively shows whether an animal “prefers” or is “averse” to a compound. It challenges students to think about their experimental design and stimulates thinking with the rich data that is generated (described in more detail below). It is beneficial to do Module 3 last in the sequence of three modules for that reason, as students come to the experiment with more knowledge from the previous experiments.

The caterpillar of the Painted lady butterfly (*Vanessa cardui*) can be obtained from Carolina or Ward with materials and instructions for care (Carolina # 144026). I recommend having the caterpillars delivered about a week before the lab, as sometimes they come very small. If you have not worked with them before, it is best to order a test batch well ahead of time to become familiar with their growth and behavior over about three weeks as they complete their lifecycle. The provided prepared food is a paste that can be mixed in with various test substances. Live plant material can also be used, as the painted lady caterpillar eats the leaves of a wide variety of host plants (https://www.butterfliesandmoths.org/species/Vanessa-cardui). However, do note that caterpillars reared on the prepared food may not eat any other food. Use petri dishes for shorter term behavior experiments to test whether the caterpillar shows a preference or aversion to a particular test substance. To test for the effect of the test substances on growth over the week, make sure the caterpillars have enough humidity by using the cups that Carolina provides or a similar set-up. If students are using live material, they have to think about how to keep it alive (some come up with ingenious designs here!) or modify their experiment to use the prepared food which is known to provide both nutrition and moisture. The butterfly is a North American species and is considered to be relatively safe for release in the environment. However, make sure to consult with your university, as regulations may differ. For example, in our department, we are concerned that the purchased organism may not be genetically identical to local populations, and we do not release the butterflies.

Lab session 1

Introduce the lab challenge at the start of lab (Supporting File S18. Bioactive compounds – Module 3. Lab 1. Handout. Experimental challenge). Lead a brief discussion on caterpillar food and life cycle. It is important to discuss how they sense the food, as this impacts experimental design. For example, caterpillars may have taste receptors on their legs (http://unclemilton.com/manuals/Butterfly_Jungle_Manual.pdf). They may therefore be able to sense some substances better if they are directly on the food mound. Students have an opportunity at the start of this module to observe caterpillar behavior and do some informal experimentation. This initial interaction leads them to ask questions that are important for their experimental design. For example, caterpillars move, so they may not stay where you put them. At the same time, they move fairly slowly, so they may not get to the food mound in the time frame that you would like them to if they are too far away.

Central again is the question of designing the experiment in a way that helps you figure out whether the caterpillar actually prefers/avoids a certain substance or it is something else that led them to be on/away from the test mound. Students know by now how helpful controls are in making such decisions. If the test compound is diluted in a solvent, teams often decide to make two negative control mounds – one with nothing added and one with the solvent only. Testing animal behavior has additional challenges for controlled experiments. Many teams put the caterpillar in the center between the different mounds to prevent placement bias. Indeed, students have demonstrated a tendency of caterpillars to stay where they are. Others are rightly concerned about volatile compounds from the mounds interfering with each other, so choose to perform control and test experiments in different petri dishes. Some teams decide to put caterpillars directly on the mounds to take advantage of possible touch tasting. How to measure preference presents a challenge – do you count when caterpillar's front end is on the mound? What if it is its back end? Ultimately, teams make a decision how to score, and many take detailed notes of what the caterpillar was doing before the timed measurement. For example, was it on its way on or off a mound? After much lively discussion, teams complete their experimental design worksheet (Supporting File S19. Bioactive compounds – Module 3. Lab 1. Worksheet. Experimental design).

The rest of lab, students have time to edit and practice their presentations. Those that feel more prepared sign up for presentations in the following week, the rest will go in the week after. There is time as well for students to work on editing their Antibacterial lab report, including to get feedback and ask questions. The final version of this lab report is due by next lab session.

Lab session 2

Provide feedback on students' caterpillar behavior experimental design, and give an opportunity for suggestions and questions by all students. Teams then re-evaluate and finalize their experimental design and perform the caterpillar experiment for short term behavioral outcomes. They record the data and make graphs from the results. The graphs for this experiment are not trivial, as most teams have three mounds and have taken multiple measurements per caterpillar. Students in the same team may decide to present the same data in different ways. Given how complicated this experiment is, expect that many will want to run over their logic with you and seek confirmation that their graphs are correct. It is beneficial for students to work on these complex graphs in class. They are very engaged in the process, but some may be overwhelmed by the amount of information and choices.

Students also set up the caterpillars for a longer-term experiment on the effect of the test compounds on growth over a week. Make sure that students measure the starting mass of caterpillars and their food in this lab. They will compare these measurements to the resulting mass in a week. The caterpillars of *V. cardui* are very small at this stage, so we pool them together, and estimate the size of individual caterpillars. There are some difficult decisions to be made here, since if caterpillars avoid or are poisoned by the test compound, they will starve to death. Students bring up this issue on their own; just make sure to discuss it with the whole class. It is another example of how ethical considerations inform experimental design. Students typically decide to provide the prepared food source and note any changes in their consumption compared to controls. Indeed, in some cases, caterpillars will eat less of the food but are not starved to death. Give time for students to work on the background research, references, and procedure for the behavior experiment for the Caterpillar lab report (S20. Bioactive compounds – Module 3. Lab 1. Guided lab report).

The last part of class is dedicated to the team presentations. During the presentations, the instructor completes a grading rubric, while students complete a peer evaluation (Supporting Files S21. Bioactive compounds – Presentations grading rubric & S22. Bioactive compounds – Presentations student worksheet). There is time for comments and questions after each presentation. Presentations in our class were about 10 minutes per team.

Lab session 3

Lab starts with students taking measurements on the mass of caterpillars, their frass (excreted pellets), and the remaining food. Students perform calculations to figure out how much of the energy from the food was transferred to the growing caterpillar. If a caterpillar has not eaten as much of the food that contains a test compound because it avoids it or is poisoned by it, it would have gained less mass. A comparison with control caterpillars that have the prepared food can show the effect of the test compound. The "energy dynamics" Investigation 10 from the AP Biology lab manual provides a teacher guide (https://apcentral.collegeboard.org/pdf/ap-biology-teacher-lab-manual-effective-fall-2019.pdf), which is summarized for this Lesson as part of the Caterpillar lab report. Students individually perform the calculations in class and can get help as needed.

The second set of teams presents their chosen experiment with the same format as in the previous session. Students begin work on the Habitat design project that is part of Module 4 (see below). The complete Caterpillar lab report is due before next class.

Module 4: Habitat Design Project

Lab sessions 1 & 2

In the third lab session of the caterpillar experiment, introduce students to the Design project challenge (Supporting File S23. Bioactive compounds – Module 4. Lab 1. Handout. Design project challenge). Teams discuss possible ideas to pursue from several options offered in the handout. Students can pursue any topic of interest as long as it broadly relates to designing a habitat that solves an existing problem. They perform preliminary background research. Some decide to switch topics when they find something else that is more interesting and do not find sufficient information on solutions. In the following session, students have most of the time in lab to perform the research and prepare their presentation in the following session. Give students the freedom to choose a format for their presentation. Most of my students chose to make pamphlets that many shared with friends and family. Alternative formats allow for a creative outlet and are a nice break from the rigor of their investigations. They also support inclusive education.

Use the second-to-last class meeting to interactively review the major concepts in experimental design and statistics following the Lab quiz study guide (Supporting File S25. Bioactive compounds – Lab quiz study guide). Students complete the Post-Survey part 1 (Supporting File S2. Bioactive compounds – Surveys). Here, students reflect on what lab skills improved, how their thinking about science changed, and what aspects of labs were most interesting, difficult, or useful. The homework for next class is to prepare for the lab quiz and to be ready for the Design project presentations.

Lab session 3

Students complete an end-of-class lab quiz together with the Post-Survey part 2 (Supporting File S26. Bioactive compounds – Lab quiz). Each of these is a small part of their grade, similar to what a regular homework assignment would be. I find that this is enough for students to take it seriously. My goal is to de-emphasize the formal assessment flavor of the assignment. Instead, I emphasize the learning over the course of the semester and the quiz as representing their take-home lessons to truly learn to be able to use in the next classes. The Post-Survey part 2 is identical to the Pre-Survey at the start of the semester. The Pre-Post Survey is used to measure attitudes and learning as a more formal assessment of this Lesson.

In the last class meeting, teams present their design project research by rotating to other teams. I tend to sandwich it between the lab quiz and post-survey; it can also work as the very last thing we do as a class together at the end. Students alternate listening to each other and discussing each other's projects. Rotate and listen to each team's presentation. Both students and instructor use a template to take notes during the presentation (Supporting File S27. Bioactive compound – Design Project Presentation Notes). Teams improve their presentations as they present to each next team – they get ideas and feedback from other teams. The rotating format is low stress and very energizing to students. The presentations on the Habitat design projects are a great way to finish off the semester, with solutions to important environmental issues caused by humans!

TEACHING DISCUSSION

Effectiveness of the Lesson

The Lesson was implemented in the Spring 2017 semester in two experimental (CURE curriculum) sections in a 15-section introductory biology laboratory. The remaining sections followed the traditional curriculum with different lab topics each week. All sections have one instructor and are capped at 24 students. The assessment approach is summarized in Table 2. The curriculum was assessed under IRB exempt protocol 18-0182. I assessed the effectiveness of the Lesson to understand both student attitudes and confidence in performing research (Metric 1) as well as student learning of statistics and experimental design (Metric 2). In one approach, I evaluated students in the CURE sections at the end of the semester for their science attitudes in written reflections (Metric 1) and for learning on an end-of-semester lab quiz and in lab reports (Metric 2; Supporting File S26. Bioactive compounds – Lab quiz). In a second approach, I compared students in the two CURE sections to those in two traditional sections using a Pre/Post Survey (Supporting File S. Bioactive compound – Surveys). The Survey tests for confidence in research skills (Metric 1) and for learning of statistics and experimental design (Metric 2). The study has a quasi-experimental design, as students did not know which type of section they had signed up for ahead of time. The two groups performed similarly in the Pre-Survey (e.g., see Figure 2, average SRBCI scores, 4.0 in Traditional, 3.7 in CURE; Figure 3, average E-EDAT scores, 6.7 in both groups) indicating a similar level of preparation and disposition at the start to allow for meaningful comparison of any post-differences between the groups.

Metric 1: Student attitudes and confidence in performing research

Students in the CURE sections had a significant increase in their confidence in performing laboratory research tasks compared to students in the traditional sections (Table 3). The traditional sections also ask students to generate hypotheses and to analyze experimental results. However, the framework is that of predetermined "cookbook" labs where opportunity for student choice is low. In contrast, the CURE curriculum was intentionally designed to center on student choice in multiple ways during the research process and repeatedly over four modules. This emphasis on student choice may explain the notable sense of strong personal involvement and agency that CURE section students displayed in the end-of-semester written reflections (Table 4). Student answers were scored blindly using open-themed scoring, noting themes in student answers (and not a pre-determined rubric, 37). The most common code on the prompt on what was the most interesting part of lab was "design my own experiments" (62% of students, prompt 1 in Table 4). Further, 85% of students formulated answers on how they now understand the scientific process better through their personal experience in response to prompt 4 (Table 4; Box 1 representative quotes).

Students further specified that they have a better grasp of how complicated the scientific process is (36%), the need for conclusions to be based on evidence (21%), or specifically mentioned the importance of accounting for confounding factors (18%). When asked to describe how the lab experience changed their attitudes toward science labs in prompt 5, 46.2% of student responses communicated a positive effect toward the CURE labs, 33% identified specific benefits to other labs, and 28% described appreciating a more genuine research experience that is like "actual" science (Box 2, representative quotes). Similar to the other prompts, students described experiencing a sense of autonomy in prompt 5 as well (28% of students).

Importantly, the aspects that students in the CURE sections identified to be the most difficult – lab reports, statistics, and designing their own experiments – were also the ones most commonly identified as the most valuable for their future careers (Table 3, prompts 2 and 3). Thus, the lab experience seems to have provided a challenge that students persisted through and felt positive about at the end of the semester (see also next paragraphs regarding their learning gains). The responses to the reflections together with the Pre-Post lab skills survey indicate that the curriculum increased student self-efficacy. Self-efficacy is defined as the belief that one can accomplish the tasks at hand (38). Students' self-efficacy has been linked to STEM performance and interest (39) that also contributes to research interest (40). These effects may be particularly important for students who belong to groups typically under-served in STEM (41).The strong emphasis in this Lesson on accomplishing challenging, yet achievable, tasks combined with promoting high standards and learning supports may have contributed to increasing self-efficacy (39).

"I started to think outside the box more in this lab since we had to worry about confounding factors and other variables where with other experiments being prefixed, we didn't have to think about much."

"I learned that scientific experiments contain many confounding factors that must be accounted for. Designing a good experiment can be difficult and obtaining clear results is even more so. It made me have a greater appreciation for the science field and made me want to take more science course."

"I realized that even the best experimental design can be improved upon. Even though the experimental question that is being investigated is important, it is equally important to examine your design and look for ways to improve the experiment."

"I still do not like the intensiveness that I need to do in order to get the results that are needed, but now I understand the importance of not cutting corners even if I do not like the results."

Box 1. Students describe how their personal experience has led them to realize important aspects of the scientific process. Representative quotes from student responses to the prompt # 4 open-ended question, "Overall, what do you think changed the most about how you think and feel about the process of science - experiments, scientific research."

"Our undergraduate science labs are flexible and want students to learn and perform experiments in the best way possible."

"It was really engaging. I got to not worry about grades in general and just do the experiments and think deeper about the issues at hand. "

"I found it more useful than the previous labs I took where we just wrote lab reports and never really learned anything. I actually learned something in the class that I can apply to my future lab experiments in other labs or my career."

"I feel that it actually has a purpose. Compared to my other labs that seem pointless I feel that this lab taught me more about how science works in the real world."

"I really enjoyed this version of undergraduate labs. In the past labs have been typically long, boring, and caused me to be very anxious. I had more control, and felt like I learned more as I was more comfortable."

Box 2. Student reflections convey a positive attitude toward the lab experience, with a sense of autonomy and a more genuine scientific process being the top reasons given. Representative quotes from student responses to the prompt # 5 open-ended question, "Overall, what do you think changed the most about how you think and feel about undergraduate science labs?"

Metric 2: Student statistical reasoning and experimental design ability

I also incorporated assessments of student learning of statistics and experimental design (Metric 2) to complement the above-mentioned measures of students' subjective appraisal. Students were evaluated both using familiar examples from lab (on the lab quiz or in lab reports) and new examples (using published instruments). The latter assess whether students can transfer their learning beyond familiar contexts. Most notable is the statistically-significant Pre-to-Post improvement on the biology-based SRBCI instrument (42) for students in the CURE, but not traditional, sections (Figure 2, $p<0.001$). Analysis of individual Pre-to-Post changes on the SRBCI showed that 68% of participants experienced positive learning gains with +6 points being the highest (out of 12 points total possible), compared to 47%/+3 points, respectively, for the traditional sections. The end-of-class lab quiz provided an alternative evaluation of statistical learning pointedly targeting major misconceptions about statistics using hypothetical scenarios. In written answers, many students were able to correctly identify that average values do not account for sample variation (57% of students, question 15), that standard deviation is only meaningful in relation to the average value (57%, question 16), as well as to correctly interpret error bars (69%, question 17) and statistical significance (57%, question 18). This measure is fairly strict as it requires students to demonstrate understanding and not just memorization by explaining in writing. In contrast, on the *Daphnia* lab report, 83% were able to complete statistical analysis correctly, 74% to interpret statistical significance correctly, and 83% to interpret error bars correctly. The lab report analysis was performed on a smaller subset (n=23) of lab reports that does not include undergraduate and post-baccalaureate students that have since graduated. The students not included in the analysis are generally higher-performing. Therefore, I expect that the above percentages for the lab reports underestimate the actual class performance. To summarize, the statistics assessments demonstrate that students value learning statistics despite the difficulties and can improve their understanding of statistics. These findings support the use of statistics education as part of experiments that students are actively involved in and care about deeply as they did in this Lesson. Early engagement in personally relevant statistical analysis in introductory biology can help begin the difficult process of transfer of statistical knowledge to new situations and, ultimately, to achieving mastery.

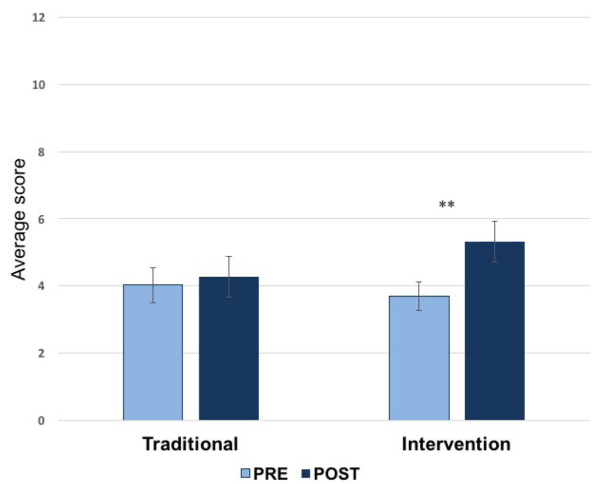

Figure 2. Statistical reasoning ability on the standardized SRBCI instrument. Statistically-significant Pre-to-Post improvements were observed in the CURE sections, but not in the traditional ones. CURE sections, n=39 students; Traditional sections, n=36 students.

With regard to experimental design, most students were able to identify on the end-of-class lab quiz the independent and dependent variables for the experiments they performed. Student understanding of cause-and-effect was strongest in the experiments with live *Daphnia* and caterpillars, where they could easily observe changes in heart rate and behavior (93-95% correct). Students were more unclear about the antibacterial assay, with 81% being able to correctly identify the dependent variable. A large proportion of students were also able to describe correctly on the quiz two confounding variables for the *Daphnia* experiment (88%). That students understood well the confounding variables in their experiments and how that impacted their conclusions was confirmed in their individual lab reports. In the *Daphnia* lab reports, all students were able to identify several confounding variables for the experiment, with 87% clearly understanding that confounds affect the heart rate (the dependent variable) and 70% explaining how the confounds affected their conclusion. Again, this analysis does not include more senior students that have since graduated. A senior faculty member also performed an informal evaluation of the lab reports. In his experience, it is rare to find such clear and specific treatment of confounds and qualified conclusions even in the mid- or upper-level courses that he teaches. An independent test of experimental design ability was administered using the E-EDAT instrument (43), with students improving in the CURE, but not traditional, sections, though the results were not statistically significant in this semester (Figure 3, $p>0.05$). Since the E-EDAT requires students to write out, in detail, an experimental plan for a hypothetical experiment, the Pre-to-Post drop in the traditional group and the increase in the CURE sections may be more reflective of their motivation for completing the test (and perhaps buy-in) rather than their knowledge.

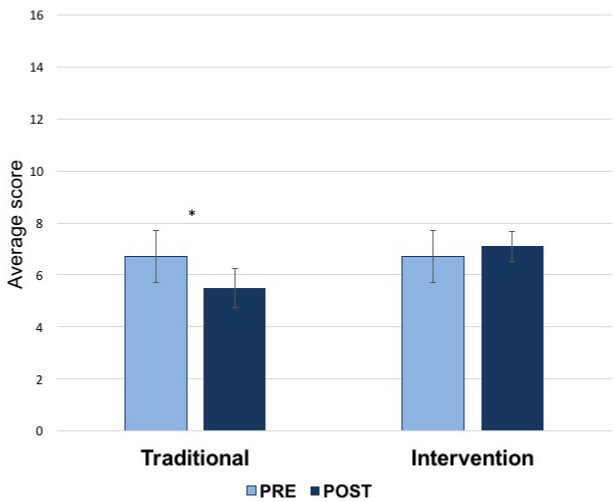

Figure 3. Experimental design ability on the standardized E-EDAT instrument. The Pre-to-Post improvements in the CURE sections, but not in the traditional ones, were not statistically significant. CURE sections, n=39 students; Traditional sections, n=36 students.

Possible Modifications

Student choice and teamwork are central features to this Lesson that promote inclusive learning, and there are different ways the decision-making process can be organized to achieve a good balance between individual student's independence, the team's cohesiveness, and scientific rigor. For example, in some semesters, each of my students performs background research for a compound of their own interest (individual choice), then defends the rationale and feasibility of their choice to their team (individual's scientific rigor), and the team, together, reaches consensus on which compound has the best scientific rationale (team cohesiveness and scientific rigor). I have also used a variation where the team first decides on a compound based on their individual preferences and knowledge (individual choice and team cohesiveness), and then each team member finds two scholarly articles on that compound. In this way, the team has a larger pool of scientific resources than they would have been able to have otherwise (scientific rigor). This second variation is more subject to team imbalances at the initial choice junction by students who know more or dominate socially. There is also risk that the compound that the team settled on before library research has feasibility problems, so guidance from the instructor at the first step is very important in this variation. A modification to further support effective student group work is to have students reflect on their and their classmates' attitudes and behaviors during group work one or more times during the semester. For example, the instructor could ask students to reflect on group work after the first weeks to encourage students to make productive adjustments in their attitude and behavior for the rest of the semester. Student peer evaluations can be influential in improving group interactions and can help set positive group dynamics from the start when students know they will be evaluated by their peers (44).

Students also have a strong role in this Lesson in deciding on the experimental protocol. I have used a variation where the whole class participates together in making decisions after teams have had a chance to discuss their plan. For each experimental decision, teams are invited to give reasons for different alternatives (scientific rigor), and then everyone votes their choice (individual choice and rigor expressed via democratic voting). For example, one could control for individual variation by choosing to use the same *Daphnia* for both control and experimental treatment. However, this experimental choice introduces confounds, since individual *Daphnia* are manipulated more. On the other hand, one could choose to use different *Daphnia* for control versus experimental treatments to minimize the handling of individual organisms though this would not control for individual variation. There are no easy answers here! Democratic voting after scientific discussion can be a very energizing approach that supports introductory students in developing a deeper understanding of the real complexities of scientific decisions (and any real-life decisions). I especially recommend this approach when the instructor would like for the whole class to follow the same protocol, for example to allow for data to be compared between teams. The decision of whether the whole class is to follow the same procedure can also be discussed and voted on by the class! Some classes vote for a common protocol when the scientific rigor argument has really sunk in. Other teams vote against it when there are enough students that are extremely vested in their experiment. I encourage instructors to enjoy the class decision, regardless of their own preferences!

An important caution for instructors is that there can be a strong pull for more clarity and structure. There is danger here in moving the Lesson away from the benefits of deeper student intellectual and emotional engagement. The Lesson Plan describes how to provide a supportive structure for growth while also allowing students to experience for themselves difficult choices and messy results. For example, the instructor should not expect to be fully knowledgeable about the vast number of possible bioactive compounds. Instead, the instructor can bring value to the Lesson by learning together with the students as a collaborator in those instances when students are pursuing a compound outside of the common list. The success of this Lesson, with its strong emphasis on student agency, can be supported by having additional instructional support during lab, for example an undergraduate or graduate assistant. Undergraduate assistants who are former students can make some of the most committed and knowledgeable assistants.

CURE approaches hold the promise of bringing access to authentic research experiences in large introductory college classes to diverse students, including students who do not have the time or resources to pursue research experiences beyond required coursework. To this end, the Lesson can be modified to allow teaching in multiple laboratory sections with different instructors. The overarching goal for adoption in large classes continues to be to maintain the fine balance between the clarity that increased structure by the instructor brings and students' deeper engagement when they are allowed to make their own decisions (as described above). It is best to make decisions that improve student experiences and also help instructors. For example, grading rubrics help communicate to both students and instructors the goals of each assignment while also simplify grading. Similarly, team lab reports improve team cohesion, provide an excellent introduction to the challenge of lab reports, and help with grading. Peer evaluations of team work twice during the semester set expectations for fair team participation, with the first one administered in the first half of the semester. I also require that team lab reports include an acknowledgements section where the work by each member is described. Team work should be organized to include individual responsibility and agency from start to end. Specifically, there needs to be a clear mechanism for individual students to participate in the decision-making process during group work (as described in Lesson Plan for smaller classes). Students also need to have personal responsibility for any skills development such as background research, data recording, statistical analysis, and graphing. Instructor workload can be alleviated if they can check off from their roster as each student completes a skills milestone during lab, especially when it is the student's first time working on a skill. Overall, in larger classes, fewer assignments are graded formally. Instead, the first formative assignments focus on rubrics for the most important elements (e.g., of a graph). Template files with embedded instructions for graphing and analysis serve a similar role to grading rubrics for clear communication to both students and multiple instructors. The number of assignments can also be limited by having students prepare presentations or lab reports, not both. The first labs can use presentations, as they are somewhat simpler. As presentations can be intimidating at first, one approach that is readily accepted by students is to have one team present to another rather than to the whole class (as described in the Lesson Plan for Module 4 presentations). Team presentations with a random draw of which team member presents which part (introduction, methods, etc.) right before the presentation encourages all team members to make sure they understand all aspects of the experiment (individual scientific rigor).

Modifications for the *Daphnia* experiment target the significant issues of inaccurate heart rate measurements and experimenter bias. Students can do preliminary tests before even starting the first experiment to understand these issues. Using the video recording, all students can individually measure the heart rate of the same *Daphnia* and compare the range of variation. What are the differences due to? What is the actual heart rate of this individual in the recording? Encourage students to propose solutions. Two additional tests drive the point home: (a) everyone measuring the heart rate using a slowed speed in the video; and (b) everyone clicking as fast as they can on the clickers. The results are memorable – the heart rate is undoubtedly faster when counted with the slow-speed video, and the measurements without slowing correspond to the fastest clicking speed! These activities motivate students to solve the issue. Icing of the *Daphnia* slows down the heart rate, so be prepared to have ice and trays available in the first lab. The ideal solution is video recording (24), if each student group can have access to the equipment. If not, then the lab becomes a class demonstration and not a student-driven experiment.

For the experiment testing for antibacterial activity, we did not perform statistical analysis. I highly recommend adding a t-test analysis comparing their test substance and the negative control. Students are already familiar with t-testing from the *Daphnia* lab, and this will serve to re-enforce their skills. The major issue for us was that students needed to repeat the first experiment to solve technical challenges. Rigorous training in microbiological technique and a practice run with known positive and negative controls will help build students skills. However, since they are testing novel compounds with unknown effects, many will still need to repeat the experiment a second time. Make sure to have enough resources on hand so that they can set up replicate experiments. A third iteration that has 2–3 replicates to allow for statistical comparison is advisable, because students are still optimizing the technique even in the second iteration.

The number of modules and the length of each module presented in this Lesson has some flexibility. A unique aspect of this Lesson is that students experience the research process for themselves in four modules, and for two of the modules, they were able to improve and repeat their experiments. The number of modules can be reduced to three or even two, allowing more time for the different lab tasks and for repetition within their experiments. Similarly to the three experiments in the *Daphnia* module in this Lesson, this modification would allow more iterations within each module. This can also ease the instructional and technical burden of having a larger number of modules. Many published CUREs have only one research project over the whole semester, and that is certainly a valid option. The value of having several experiments as in this Lesson is for students to be able to experience more than one research context. I believe this gives them a broader and more realistic understanding of the possibilities of biological research while also solidifying for them what makes rigorous science. Additionally, experiments with different organisms allow students to have "favorites" and to engage the emotional domain that can be so important for their continued interest and persistence in science.

SUPPORTING FILES

- S1. Bioactive compounds – Syllabus.
- S2. Bioactive compounds – Surveys.
- S3. Bioactive compounds – Module 1. Lab 1. Handout. Experimental Challenge #1.
- S4. Bioactive compounds – Module 1. Lab 1. Worksheet. Write-up I.
- S5. Bioactive compounds – Module 1. Lab 2. Homework. MathBench graph activity.
- S6. Bioactive compounds – Module 1. Lab 2. Handout. Statistics in Excel.
- S7. Bioactive compounds – Module 1. Lab 2. Worksheet. Write-up II.
- S8. Bioactive compounds – Module 1. Lab 3. Homework. Chemical warfare reading.
- S9. Bioactive compounds – Module 1. Lab 3. Handout. Lab challenge #2.
- S10. Bioactive compounds – Module 1. Lab 3. Worksheet. Compound choice.
- S11. Bioactive compounds – Module 1. Lab 3. Worksheet. Statistics activities.
- S12. Bioactive compounds – Module 1. Lab 4. Guided lab report.
- S13. Bioactive compounds – Module 2. Lab 1. Handout. Experimental challenge.
- S14. Bioactive compounds – Module 2. Lab 1. Worksheet. Write-up I.
- S15. Bioactive compounds – Module 2. Lab 2. Worksheet. Write-up II.
- S16. Bioactive compounds – Module 2. Lab 3. Guided lab report.
- S17. Bioactive compounds – How to present slides.
- S18. Bioactive compounds – Module 3. Lab 1. Handout. Experimental challenge.
- S19. Bioactive compounds – Module 3. Lab 1. Worksheet. Experimental design.
- S20. Bioactive compounds – Module 3. Lab 1. Guided lab report.
- S21. Bioactive compounds – Presentations grading rubric.

- S22. Bioactive compounds – Presentations student worksheet.
- S23. Bioactive compounds – Module 4. Lab 1. Handout. Design project challenge.
- S24. Bioactive compounds –Module 4. Lab 1. Worksheet.
- S25. Bioactive compounds – Lab quiz study guide.
- S26. Bioactive compounds – Lab quiz.
- S27. Bioactive compounds – Design Project presentation notes.

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Table 1A. Bioactive Compounds teaching timeline for Module 1: *Daphnia* physiology experiment.

Activity	Description	Time	Notes
Module 1: Daphnia physiology experiment.			
Module 1: Lab 1			
Introduction & Challenge 1	Welcome to class, mutual introductions, and introduction to the Daphnia experiment Challenge 1 (caffeine/ethanol tests)	20 min	S3. Bioactive compounds – Module 1. Lab 1. Handout. Experimental Challenge #1.
Pre-Survey, Part 1	Students complete Pre-Survey, Part 1 (all but statistics).	20 min	S2. Bioactive compounds – Surveys.
Design experiment	Teams discuss their experimental design and report out to class with feedback from peers and instructor.	30 min	S4. Bioactive compounds – Module 1. Lab 1. Worksheet. Write-up I.
Lab techniques instruction	Instructor introduces lab safety and microscope work. Students practice measuring heart rate from video.	20 min	
Perform experiment	Teams collaborate to perform the experiment and record data.	45 min	
Data graphing & writing	Students individually graph their results and complete the lab write-up.	30 min	S4. Bioactive compounds – Module 1. Lab 1. Worksheet. Write-up I (complete).
Homework	MathBench graphing tutorial and online quiz.	5 min	S5. Bioactive compounds – Module 1. Lab 2. Homework. MathBench graph activity.
Module 1: Lab 2			
Class height activity	Students record their height in a shared class Excel file as they enter class or in between activities.		
Introduction & Lab 1 feedback	Instructor provides feedback on Lab 1 write-ups. Teams discuss the role of writing for their future careers.	10 min	S4. Bioactive compounds – Module 1. Lab 1. Worksheet. Write-up I (feedback).
Homework feedback & activity	Instructor provides feedback on graphing homework. Students decide on graph type for their experiment.	15 min	S5. Bioactive compounds – Module 1. Lab 2. Homework. MathBench graph activity (feedback).
Pre-Survey, Part 2	Students complete Pre-Survey, Part 2 (statistics).	15 min	S2. Bioactive compounds – Surveys.
Experiments & statistics instruction	1. Instructor leads interactive discussion of experimental design (based on students' experiences in Lab 1). 2. Instructor leads interactive mini-lecture on descriptive and inferential statistics (using class height data). 3. Instructor demonstrates how to perform mean and standard deviation (SD) calculations and add error bars to graphs in Excel.	40 min	S4. Bioactive compounds – Module 1. Lab 1. Worksheet. Write-up I (discussion). S6. Bioactive compounds – Module 1. Lab 2. Handout. Statistics in Excel.
Re-design & perform experiment	Teams collaborate to finalize the re-design as well as to perform Experiment 2 and record data.	45 min	S7. Bioactive compounds – Module 1. Lab 2. Worksheet. Write-up II (complete).
Data graphing, analysis, & writing	Students individually calculate SD and graph their results (with error bars) as well as complete the lab write-up.	45 min	
Homework	"Chemical warfare" reading and worksheet.	5 min	S8. Bioactive compounds – Module 1. Lab 3. Homework. Chemical warfare reading.
Module 1: Lab 3			
Record class data	Teams record Experiment 2 data in a shared class Excel file as they enter class or in between activities.		
Homework reading discussion & Challenge #2	Teams discuss the most interesting and important points from the homework reading and report out to the whole class. The reading provides a foundation for students' choice for bioactive compounds for Challenge #2.	20 min	S8. Bioactive compounds – Module 1. Lab 3. Homework. Chemical warfare reading (discuss).

Activity	Description	Time	Notes
Background research & design team experiment	Students perform background research on their individual ideas and decide as a group which bioactive compound to test and how to perform Experiment 3.	90 min	S9. Bioactive compounds – Module 1. Lab 3. Handout. Lab challenge #2. S10. Bioactive compounds – Module 1. Lab 3. Worksheet. Compound choice.
Statistics instruction & activity	Instructor leads interactive mini-lecture on inferential statistics and hypothesis testing. Students individually calculate standard error for class data on human height and from Daphnia Experiment 2.	60 min	S6. Bioactive compounds – Module 1. Lab 2. Handout. Statistics in Excel. S11. Bioactive compounds – Module 1. Lab 3. Worksheet. Statistics activities.
Homework	Complete S11, except t-test (electronic upload).		S11. Bioactive compounds – Module 1. Lab 3. Worksheet. Statistics activities.
Module 1: Lab 4			
Lab 3 discussion & feedback	1. Teams discuss what conclusions to draw from the statistical analysis of class height and Daphnia Experiment 2 data. Instructor provides feedback. 2. Instructor leads interactive discussion on experimental challenges with Daphnia experiments so far.	20 min	S11. Bioactive compounds – Module 1. Lab 3. Worksheet. Statistics activities (discuss).
Experiments instruction	Instructor leads interactive mini-lecture on preparing chosen compounds (issues of safety, dosage, dilutions) and control preparations.	20 min	
Perform experiment	Teams prepare their test compounds and then perform Daphnia experiment.	90 min	
Statistics instruction & activity	Instructor demonstrates how to perform t-test in Excel. Students individually perform t-test for Daphnia Experiment 2 data.	40 min	S11. Bioactive compounds – Module 1. Lab 3. Worksheet. Statistics activities (complete).
Homework	1. Daphnia lab report - part I (complete 1-6 & 13; experimental rationale, procedure, results, and references). 2. Students declare their choice of compounds to test for Module 2. They can bring compounds from home to test.	10 min	S12. Bioactive compounds – Module 1. Lab 4. Guided lab report.

Table 1B. Bioactive Compounds teaching timeline for Module 2: Antimicrobial activity experiment.

Activity	Description	Time	Notes
Module 2: Antimicrobial activity experiment.			
Module 2: Lab 1			
Challenge & experiments instruction	1) Instructor leads interactive discussion on the Challenge for the antibacterial activity experiment, including possible hypotheses and controls. 2) Instructor demonstrates laboratory techniques (Kirby-Bauer disc diffusion test, microbial work, metric system, and pipetting).	50 min	S13. Bioactive compounds – Module 2. Lab 1. Handout. Experimental challenge. S14. Bioactive compounds – Module 2. Lab 1. Worksheet. Write-up I.
Design experiment	Teams discuss their experimental procedure for Experiment 1 and students individually complete S4:2.	20 min	S14. Bioactive compounds – Module 2. Lab 1. Worksheet. Write-up I (complete).
Perform experiment	Students perform the experiment and record what they did.	60 min	
Time to write lab report	Students have time to work on the Daphnia lab report, ask questions, and get feedback.	60 min	S12. Bioactive compounds – Module 1. Lab 4. Guided lab report.
Homework	Daphnia lab report - part II (complete questions 7-12).		S12. Bioactive compounds – Module 1. Lab 4. Guided lab report.
Module 2: Lab 2			
Collect and record data	Students collect and record data from Antibacterial Experiment 1.	30 min	
Experiment evaluation & design	Teams collaborate to evaluate the Experiment 1 data and design Experiment 2.	30 min	
Perform experiment	Students perform Experiment 2 and record what they did.	30 min	S15. Bioactive compounds – Module 2. Lab 2. Worksheet. Write-up II.
Time to write lab report	Students complete parts of the Antibacterial lab report (experimental question, procedure, and graphs).	60 min	S16. Bioactive compounds – Module 2. Lab 3. Guided lab report.
Lab report feedback & time to write	Instructor provides detailed feedback on each section of the Daphnia lab report. Students have time to edit.	40 min	S12. Bioactive compounds – Module 1. Lab 4. Guided lab report.
Homework	Daphnia lab report - final edits & submission.		S12. Bioactive compounds – Module 1. Lab 4. Guided lab report.
Module 2: Lab 3			
Collect and record data	Students collect and record data from Experiment 2.	30 min	
Background research & time to write lab report	Students work on parts of the Antibacterial lab report (experimental rationale, references, discussion).	60 min	S16. Bioactive compounds – Module 2. Lab 3. Guided lab report.
Presentations instruction	Instructor mini-lecture on how to prepare a presentation	15 min	S17. Bioactive compounds – How to present slides.
Time to prepare presentations	Teams collaborate to prepare their presentation slides.	65 min	
Homework	Antibacterial lab report – draft		S16. Bioactive compounds – Module 2. Lab 3. Guided lab report.

Table 1C. Bioactive Compounds teaching timeline for Module 3: Caterpillar behavior experiment.

Activity	Description	Time	Notes
Module 3: Caterpillar behavior experiment.			
Module 3: Lab 1			
Introduction & Challenge	Instructor leads interactive discussion on the Challenge for the caterpillar behavior experiment.	10 min	S18. Bioactive compounds – Module 3. Lab 1. Handout. Experimental challenge.
Design experiment	Students have an opportunity to interact with caterpillars. 1) Teams discuss their experimental design and report out to class with feedback from peers and instructor. 2) Teams complete their experimental design using S6:2.	60 min	S18. Bioactive compounds – Module 3. Lab 1. Handout. Experimental challenge. S19. Bioactive compounds – Module 3. Lab 1. Worksheet. Experimental design.
Time to practice presentations	Instructor reminds students about the features of quality presentations. Teams practice & edit presentations.	60 min	
Time to write lab report	Students have time to edit the Antibacterial lab report, ask questions, and get feedback.	60 min	
Homework	Antibacterial lab report – edits & final submission		S16. Bioactive compounds – Module 2. Lab 3. Guided lab report.
Module 3: Lab 2			
Feedback on experimental design	Instructor provides feedback on the caterpillar behavior experimental design with opportunity for questions.	10 min	S19. Bioactive compounds – Module 3. Lab 1. Worksheet. Experimental design (feedback).
Evaluate & perform experiment	Teams re-evaluate and finalize the experimental design as well as perform the caterpillar experiment (short-term behavioral outcomes).	60 min	
Background research & time to write lab report	Students work on parts of the Caterpillar lab report (experimental rationale, procedure, and references).	60 min	S20. Bioactive compounds – Module 3. Lab 1. Guided lab report.
Presentations	Presentations – round 1: 1. Instructor completes Presentations grading rubric. 2. Students complete Presentations peer evaluation.	60 min	S21. Bioactive compounds – Presentations grading rubric. S22. Bioactive compounds – Presentations student worksheet.
Homework	Caterpillar lab report – complete experimental rationale, procedure, and references.		S20. Bioactive compounds – Module 3. Lab 1. Guided lab report.
Module 3: Lab 3 *(Module 4: Lab 1 starts this session)*			
Collect and record data	Students collect and record data for long-term outcomes (effect on growth).	30 min	
Data analysis	Students perform calculations on energy dynamics of caterpillar growth.	30 min	S20. Bioactive compounds – Module 3. Lab 1. Guided lab report.
Presentations	Presentations – round 2: 1. Instructor completes Presentations grading rubric. 2. Students complete Presentations peer evaluation.	60 min	S21. Bioactive compounds – Presentations grading rubric. S22. Bioactive compounds – Presentations student worksheet.
Design project Challenge & background research	Teams discuss possibilities for their Design project and perform preliminary background research.	50 min	S23. Bioactive compounds – Module 4. Lab 1. Handout. Design project challenge. S24. Bioactive compounds –Module 4. Lab 1. Worksheet.
Homework	Caterpillar lab report – complete draft.		

Table 1D. Bioactive Compounds teaching timeline for Module 4: Design project.

Activity	Description	Time	Notes
Module 4: Design project.			
Module 4: Lab 2 *(Module 4 starts in the previous lab session)*			
Review	Instructor leads the class in an interactive review of major concepts in experimental design and statistics.	30 min	S25. Bioactive compounds – Lab quiz study guide.
Post-Survey, part 1	Students complete an end-of-semester reflection on lab skills improvement and overall learning in lab.	20 min	S2. Bioactive compounds – Surveys.
Design project	Teams perform research and prepare presentation in the form of a pamphlet or other media of their choice.	120 min	Teams choose format.
Homework	Prepare for Lab quiz. Prepare Design project presentation.		
Module 4: Lab 3			
Lab Quiz	Students complete Lab quiz.	30 min	S26. Bioactive compounds – Lab quiz.
Presentations	Teams present their project by rotating through other teams. Instructor takes notes on each presentation.	60 min	S27. Bioactive compounds – Design Project presentation notes.
Post-Survey, part 2	Students complete surveys on lab skills and science attitudes and assessments on experimental design and statistics.	80 min	S2. Bioactive compounds – Surveys.

Table 2. Overview of assessment strategy and data collected. Students were assessed on both their attitudes and confidence in performing research (Metric 1) and learning of statistical analysis and experimental design (Metric 2). For each metric, CURE section students were assessed at the end of the semester in written reflections, a lab quiz and lab reports (Approach A) and also compared in a Pre/Post-Survey to non-CURE section students (Approach B).

Metric 1: Attitudes and confidence in performing research	**Metric 2: Learning of statistical analysis and experimental design**
Approach A: Assess CURE section students at the end of the semester	
Written reflections	Lab quiz and lab reports
Approach B: Compare CURE and traditional section students at the start (Pre) and end (Post) of the semester	
Pre/Post Survey: Confidence in research skills	Pre/Post Survey: Learning of statistics (SRBCI) and experimental design (E-EDAT)

Table 3. Students in the CURE, but not traditional sections, report increased confidence in performing research tasks. Student responses to the closed-choice question, "How confident do you feel in your ability to perform the following lab-based tasks?" Five answer options were provided, ranging from "not confident" to "extremely confident." Statistically significant Pre-to-Post differences were observed in seven dimensions in the CURE sections (in red) compared to one dimension in the Traditional groups (in blue) using 2-tailed Pearson correlations. CURE sections, n=39 students; Traditional sections, n=36 students. * <0.05, **<0.01, ***≤ 0.001.

"How confident do you feel in your ability to perform the following lab-based tasks?"	
1. Work collaboratively and productively in a team.	8. Use Excel to make graphs. ***
2. Perform background research of the scientific literature on a topic. **	9. Present lab results to my lab members.
3. Critically read the scientific literature on a topic.	10. Communicate the rationale for doing an experiment to others.
4. Develop my own scientific question for an experiment. ***	11. Discuss a scientific issue by using evidence and developing logical arguments. *
5. Design my own experimental lab protocol. ***	12. Write a lab report (with Intro, Methods, Results, Discussion). ***
6. Interpret experimental data (such as finding trends or patterns in data). *	13. Write scientifically, but in my own words and avoiding plagiarism.
7. Perform statistical analyses. *	14. Work as an undergraduate research lab assistant in a biology lab.

Table 4. Student reflections speak to an appreciation for having experimental ownership and of the value of learning difficult statistics concepts. Student responses to four open-ended questions, on what they found 1) most interesting, 2) most valuable, 3) most difficult in the course, as well as 4) how their thinking about the process of science changed during the semester. Open coding for themes was performed blinded to student identity by an independent graduate research assistant; a single response could score for more than one theme. CURE sections, n=39 students.

Emerging themes in end-of-semester reflections *% responses to open-ended prompts 1-4, by frequency*	
1. Most interesting	
Designing my own experiments	61.5
Working with live organisms	48.7
Specific labs	35.9
2. Most valuable	
Statistics	30.8
Designing experiments	28.2
Lab reports	28.2
Excel	20.5
Presentations	15.4
Background research	7.7
Critical thinking	7.7
3. Most difficult	
Lab reports	38.5
Statistics	30.8
Designing experiments	20.5
Excel	10.3
Not having lab manual	10.3
Not having enough time	10.3
Data interpretation	5.1
4. How thinking changed about the process of science	
Understanding the scientific process through personal experience	84.6
Understanding the complexity of the scientific process	35.9
Basing conclusions on evidence	20.5
Understanding the importance of confounding factors in experiments	18.0