Three Research-Based Quantitative Reasoning Modules for Introductory Organismal Biology Laboratories

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Abstract

We have designed three laboratory modules for an introductory organismal biology course with an emphasis on quantitative reasoning and data analysis skills. Module 1 tests for dimorphism in crayfish chelae using a paired statistical design. Module 2 tests for allometric growth of tapeworm hook structures using a regression model. Module 3 tests for differences in stomatal densities between two groups of plants using a two-sample statistical approach. For all three modules, we emphasize the use of confidence intervals to draw statistical conclusions about hypotheses. Knowledge about the basic biology of animals and plants is required, including arthropods, platyhelminths, and vascular plants. Background reading on dimorphism, allometry, and transpiration provides the necessary foundation to develop questions and hypotheses. Some familiarity with R is necessary for both students and instructors, although the activities can be modified for analysis with Excel or another statistical package. These modules can be taught independently or together as a unit within a course. As stated in the AAAS document, Vision and Change: A Call to Action, the ability to use quantitative reasoning is a core competency that must be developed by all biology students. These modules address the call for instruction in quantitative reasoning and provide a hands-on active introduction to key tools that will be required to build students’ statistical repertoire in more advanced courses.

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Learning Goals

Students will:
◊ gain an appreciation for common biological phenomena including dimorphism, allometry, and transpiration.
◊ learn about the process of science, including asking questions, formulating hypotheses, collecting data, analyzing data, presenting data, interpreting results, and drawing conclusions.
◊ understand how confidence intervals are used to draw conclusions about hypotheses.

Learning Objectives

Students will be able to:
◊ clearly state testable hypotheses for three research projects.
◊ collect data using calipers, microscopes, and software.
◊ use R scripts to calculate basic statistics and make graphs.
◊ interpret results to draw conclusions about hypotheses using confidence intervals.

INTRODUCTION

Introductory organismal biology courses geared towards first-year science majors generally include a survey of organismal diversity, including organismal evolution, ecology, structure, and function (1–3). These courses typically include both lecture and laboratory components, where the latter typically consists of hands-on modules that often involve dissections and observations of whole organisms and microscope slides. These labs traditionally did not include much in the way of inquiry-based learning or student-centered research questions calling for exploration and hypothesis testing. However, there has been a push in recent years to include more quantitative analysis in undergraduate biology programs and to incorporate inquiry-based laboratory projects (4).

The importance of integrating quantitative reasoning into biology courses is increasingly recognized (5). The AAAS Vision and Change in Undergraduate Biology Education report notes that quantitative analysis should be integral to the Core Concepts for Biological Literacy (6). The ability to use “quantitative reasoning,” including statistics, was identified as one of the Core Competencies that all biology students need to develop (6). Traditional biology labs that focus on observation rather than inquiry leave little opportunity for students to hone their quantitative skills. The use of statistics is often relegated to statistics courses taught by mathematics faculty and is thus often not integrated into biological themes and courses. A recent survey of biology and environmental science instructors across institution types indicated that while many instructors teach data
management, analysis, and visualization and view these skills as important, a lack of space in the curriculum was identified as a barrier to integration of more of these skill-building activities (7). Furthermore, this survey revealed that learning coding was less valued by instructors as a skill for undergraduate students, although instructors were themselves interested in receiving training on how to teach coding skills. Technical skills such as writing scripts and computer programming are integral components of data science acumen (8, 9).

When one of us (EC) was first tasked with developing introductory organismal biology laboratory activities in 2012, very few resources for student-centered inquiry-based laboratory activities existed for this field. Although many excellent activities have since been published, none fill the specific niche for a course on the diversity of organisms and their forms and functions, while also including quantitative literacy and data analysis components. For example, laboratory course modules involving DNA barcoding focus on organismal diversity but include molecular lab work in place of quantitative analysis (e.g., 10, 11). Some activities focus less on organismal form and function, and instead on ecology (e.g., 12, 13) or biochemistry (e.g., 14). Others are limited to a single taxonomic group, such as arthropods (e.g., 10, 15, 16) or plants (e.g., 17); and/or require expensive specialized equipment (e.g., 16, 18). We thus designed a set of three inquiry-based laboratory project modules, where each module focused on a different taxon but contained the same three essential components: (A) examination of variation in structure and function at the intra-individual, intraspecific, and/or interspecific levels, (B) data collection, data analysis, and hypothesis testing, and (C) minimal equipment and supplies (S0.1 Organismal Biology Quantitative Reasoning – Preparatory Materials).

The introductory organismal biology lab modules presented here each require 1–2 lab sessions of 2–4 hours each. With additional reflection sessions, including student oral or poster presentations, the three modules combined can comprise half of a semester-long course. The remaining half of the course is composed of activities on evolution (natural selection, genetic drift, phylogeny, homology). The activities were created using backward design, with the objectives and assessments in mind (19). The ultimate goal was for students to have the ability to test hypotheses via the use of confidence intervals from data they collected (as in ref. 20). Intervening steps to achieving this goal included the students gaining hands-on experience with organismal diversity (both macro- and microscopic), explaining biological phenomena, and making predictions based on acquired knowledge. During this entire process the students also gain experience and competence with various scientific tools for data collection, including calipers, microscopes, and computer software. The course we teach consists of multiple sections with several instructors in an urban setting, making coordination of field work challenging. We therefore designed activities that could be completed in a laboratory setting with minimal equipment and supplies. Versions of these modules have been taught by one or both of us nine times since 2012, with edits and improvements made each successive semester, including a progression from the use of Excel to the use of R scripts for data analysis to provide students with a technical skills toolkit.

**Intended Audience**

We designed the modules for first-year science majors (biology, chemistry, biochemistry, forensic science, environmental science, behavioral neuroscience) at a large, four-year, private college. The course is a large lecture course (maximum enrollment of 118 since 2012) divided into classes of ~20 students for laboratory sessions. All the modules are completed in the laboratory sessions, with background material provided in the lectures.

**Required Learning Time**

The inclusion of all three modules ideally requires four lab sessions, each 2–3 hours long (Table 1). Each module can be taught individually, requiring 1–2 lab periods of 2–4 hours each. The second module is the longest and would require 2 lab sessions of 2–3 hours each, or 1 lab session of 4 hours. The first and third module can be comfortably completed in 1 lab session of 3 hours each. An optional fifth lab session can be added during which students present their research findings from one of the modules. The time spent outside of lab sessions is expected to be 3–6 hours per week.

**Prerequisite Student Knowledge**

The lecture component of the course should include components of animal diversity (Modules 1 and 2) and plant transpiration (Module 3). Students should be instructed to read sections of their introductory biology textbook on arthropods (Module 1), platyhelminths (Module 2), and plant transpiration (Module 3). Nature Education provides online information for students on dimorphism (21), allometry (22), and water transport in vascular plants (23). Additional content-specific papers that students should read include one on chela dimorphism (Module 1; 24) and one on parasite allometry (Module 2; 25). Finally, students are required to read a paper on error bars, with specific emphasis on the use of confidence intervals to test hypotheses, as well as appropriate graph-making techniques (20).

**Prerequisite Teacher Knowledge**

In addition to competence in introductory biodiversity science and basic statistical principles, instructors are required to have some familiarity with R and RStudio to assist students with troubleshooting (S0.2 Organismal Biology Quantitative Reasoning – Getting Started with R and RStudio). Alternatively, instructors can modify the modules for data analysis in Excel or a different statistics package (S0.3 Organismal Biology Quantitative Reasoning – Excel Data Analysis Option).

**Scientific Teaching Themes**

**Active Learning**

Students complete the work independently or in small groups, including all the data collection and analyses, and work through written worksheets to draw conclusions.

**Assessment**

Formative assessments consist of weekly worksheets that students complete while performing the lab activities. Students have the option of working independently or in groups of 2–4 to complete the worksheets, but each student submits their own worksheet on which they receive feedback that they can
use for future assignment preparation and studying for exams. Students can ask instructors for assistance. We assign a small grade (1% of final course grade) to each worksheet to ensure that students put in adequate effort. Traditionally, we have used a final written exam as a summative assessment. Student oral or poster presentations based on their research projects can be used as a summative assessment as well as or instead of written exams.

**Inclusive Teaching**

The modules fundamentally explore the theme of biodiversity. The central discussions revolve around why diversity is important at the intra-individual (Module 1), intraspecific (Modules 2 and 3), and interspecific (Modules 2 and 3) levels. Examples used in class should generalize the material to other problems and systems, specifically those related to human health because many science majors are interested in careers in health care. For example, the paired study design used in Module 1 should be related to clinical trials in which data are collected before and after a treatment is administered to a patient. Examples of allometry, the focus of Module 2, can refer to the hypo-allometric growth of the brain and the heart in humans (22). Finally, Module 3 requires students to bring their own leaves to the lab for analysis, thus providing students the opportunity to explore nature in their surrounding environments, such as their homes or neighborhoods.

Students work in groups of 2–4 students so that individuals with restricted motor skills or with vision impairment can delegate microscope and caliper work to students without these limitations. Formative assessments include low-stakes quizzes (to assess preparation for class) and worksheets that can be completed collaboratively during class period. Students are required to bring a laptop computer to laboratories. At Pace University, laptop computers can be borrowed from the campus library, which might also be an option at other institutions. When access to laptop computers is limited, students can work in groups of 2 to 4 students for data entry and analysis.

**LESSON PLAN**

**Overview**

This lesson consists of three modules that build upon one another in a way that results in a deep understanding of how confidence intervals are used to test hypotheses. All three modules involve students in every step of the scientific process, from asking a question, generating testable hypotheses, collecting data from specimens, analyzing and graphing the data using RStudio, calculating confidence intervals, and using the confidence intervals to draw conclusions about their hypotheses. A peer-reviewed article about error bars is required reading for the lab sessions and is discussed during brief pre-lab discussions before the first and third modules (20).

The first module involves a one-sample paired design, the second module covers slopes, and the third module introduces a two-sample design. Each successive module gives students more autonomy than the preceding one. The first module asks whether chela (claw) dimorphism exists in crayfish as it does in crabs, a close relative of crayfish. Students find the difference in chela area in each crayfish and test whether the mean difference is significantly different from zero. Data collection is collaborative in that data collected by the entire class is collated for analysis. The second module asks whether allometry exists in tapeworms as it does in trematodes, close relatives of tapeworms. Students estimate the slope of the relationship between the rostellum (hook structure) and body size (both variables log transformed) and test whether this slope differs significantly from one (a slope of 1 indicates isometry). Data collection is done in groups of four instead of class-wide, thus giving students more autonomy, because each group will have different results. The third module asks whether mean stomata densities differ between two groups of plants. Students individually collect specimens, and the data collection process is completely autonomous. Before each lab session, students should read the entire lab handout for that session. Instructors can use low-stakes lab quizzes as an incentive to encourage students to come prepared. The quizzes can be administered either at the beginning of the lab session or after a short pre-lab discussion to allow for students to ask questions. Worksheets are completed and handed in during each lab session.

**Module 1**

The goal of the first module is to test for dimorphism in crayfish chelae. This module can be completed in one 2–3 hour laboratory session. Background information and instructions are provided in a lab handout (S1.1 Organismal Biology Quantitative Reasoning – Module 1 Lab Handout). Begin with a short presentation on dimorphism in general, including that it can occur between individuals (e.g., sexual dimorphism) or even within a single individual (S1.2 Organismal Biology Quantitative Reasoning – Module 1 Discussion Slides). A good example of the latter is dimorphism in crab chelae. In green crabs Carcinus maenas, the right chela is usually the crusher and the left chela is the cutter in crabs that have not lost chelae due to predation or combat (24). Crab chelae often show sexual dimorphism, with males typically displaying more extreme differences in chelae sizes (26). It is not known, however, whether dimorphism also exists in crayfish. The class discussion should include the difference between a question and a hypothesis. An appropriate question would be: “Is there dimorphism in crayfish?” Appropriate testable hypotheses would be: “The left chela will be larger than the right chela, on average, in crayfish” (one-sided) or “One of the chelae will be larger, on average, than the other” (two-sided).

At this point, instructors can continue the discussion to include the use of confidence intervals to test hypotheses. We prefer to move on to the data collection and return to the pre-lab discussion slides before beginning a discussion of how the data will be analyzed. Divide ~100 crayfish among pairs of students, in a class of 20 students (i.e., ~10 crayfish per pair). We buy uninjected crayfish by the pail from Carolina Biological Supply. If storing crayfish for reuse, it is important to check to ensure that the chelae have not become detached from the chelipeds. Instruct students to wear disposable gloves when handling the crayfish. Large forceps are needed to remove the crayfish from the pails, and dissection trays should be available to hold the crayfish. Give each pair of students
two dissection trays—one for holding unmeasured crayfish, and another for holding crayfish that have already been measured. Ask the students to label the side of their dissection trays with labeling tape so they do not get confused. Provide each student with Vernier calipers.

After students are seated with their crayfish, instructors can optionally discuss the external morphology of the crayfish. At this step, explain how to use the calipers so that students can examine the calipers and follow along. Show students how to measure both the depth and the width of each chela as well as the sex (as shown in S1.1 Organismal Biology Quantitative Reasoning – Module 1 Lab Handout). We have found that metal calipers tend to rust and get stuck over time, and that cheaper plastic calipers work fine. Both types of Vernier calipers (metal and plastic) are available from Carolina Biological Supply. Students then work to measure chelae and enter data into a spreadsheet (S1.3 Organismal Biology Quantitative Reasoning – Module 1 Data Template). After the data collection is complete, the data are collated and shared among the class. This can be done by creating a Google Sheets spreadsheet where students enter their data. The instructor then shares this spreadsheet with the class, which can be done by clicking the Share icon within Google Sheets and copying the link, ensuring that “Anyone on the internet with this link can view” is selected. Students must then download the file as a .csv file (from the File dropdown menu).

The next step involves analyzing the data using RStudio. Instructors will probably need to spend time helping students troubleshoot installation problems, although students are told to attempt installation before the class (Table 1). For instructors who prefer to use Excel, we provide alternative instructions (S0.3 Organismal Biology Quantitative Reasoning – Excel Data Analysis Option). If not already discussed, return to the pre-lab discussion slides to complete the discussion on how confidence intervals are used to test hypotheses. Discuss Table 1 and Figures 1, 2, and 4 in Cumming et al. (20) and the importance of sample size in drawing conclusions using data. Both instructors and students should have some familiarity with R and RStudio before beginning this module, but we assume students have not been introduced to confidence intervals prior to this module. For students requiring guidance on R and RStudio, a short introductory document is available (S0.2 Organismal Biology Quantitative Reasoning – Getting Started with R and RStudio). Have students open the R script in RStudio and work through the problems (S1.4 Organismal Biology Quantitative Reasoning – Module 1 R Script). Detailed instructions for working through the R script are available in the lab handout (S1.1 Organismal Biology Quantitative Reasoning – Module 1 Lab Handout). Specifically, the script contains missing information that will need to be completed by the students. Students will calculate the area (length*width) of each chela measured and subtract the area of the left chela from the area of the right chela within each specimen. They will do this for the entire data set and then by each sex separately. Then, they will calculate the mean difference in chela area overall, for males only, and for females only and construct 95% confidence intervals for each mean difference, as well as display the data and confidence intervals in graphs.

While analyzing their data, students will need to simultaneously complete a written worksheet (available at the end of S1.1 Organismal Biology Quantitative Reasoning – Module 1 Lab Handout). The purpose of the worksheet is to keep students focused on the course objectives and to ensure that the goals and objectives have been achieved. Answers to the worksheet questions, as well as a completed R script, are available from one of us (EC) upon request. In summary, ensure that the students understand that as our sample size increases, so does the chance that we capture more of the variation in the data within our sample, and thus we become more confident in our results. For this reason, standard error, which becomes smaller as sample size increases, is used to make inferences, whereas standard deviation is used only to describe variation in the data. Also, students must be able to note that this “paired” design, with left and right chelae compared within each specimen, is important due to variation in size among individual specimens. Students must be able to clearly state a testable null and alternative hypothesis, such that the null hypothesis states that the alternative is not true (e.g., the alternative may be that the right chela are larger than the left, on average; then, the null hypothesis must state that the right chela are not larger than the left, on average).

Finally, students must be able to indicate that their confidence intervals are the range of values we compare to the value of our null hypothesis. If our null hypothesis indicates that there is no difference in size between left and right chelae, on average, we are determining whether zero falls within our confidence interval. If it does, we fail to reject the null hypothesis. If it does not, we reject the null hypothesis.

Module 2

The goal of the second module is to test for allometry in tapeworm hook structures; tapeworms use these structures to attach to a host's intestinal lining. This module takes longer than the first module and can be completed in two 2–3 hour lab sessions. Lab handouts with background information and instructions are available for each of two lab sessions (Supporting Files S2.1a, S2.1b). Students should install the required software for microscope cameras and ImageJ before coming to class, which is indicated (with links to download the installers) on the lab handouts. Be sure that students also install the latest versions of R and RStudio if they have not already done so. Instructors will probably need to spend time at the beginning of class helping students troubleshoot any installation difficulties. A supplemental activity on microscopes is available to use as a preliminary lab session if students do not already have a background in microscope use (S0.4 Organismal Biology Quantitative Reasoning – Using Microscopes).

Begin the lab session with a short discussion of what allometry is, including isometry versus hyper-allometry and hypo-allometry (S2.2 Organismal Biology Quantitative Reasoning – Module 2 Discussion Slides). Good examples are available from the Nature Education Knowledge Project (22). A study conducted on endoparasitic trematode worms made predictions about the scaling coefficient for the attachment structures (suckers) of parasites of ectothermic versus endothermic hosts, and also made predictions about the scaling coefficients of attachment suckers versus digestive and copulatory structures (25). The author predicted that the scaling coefficients would
be higher for attachment structures than for other structures and would be higher in parasites of endotherms, given the increased chance of becoming dislodged with increasing body size and in the digestive tracts of endothermic hosts. The results indicated, however, that scaling coefficients were largely consistent with isometric growth across 13 trematode families (25). Note that the body size measurement used in that study was surface area, which is two dimensional, whereas body structure measurements were one-dimensional (length). Thus, the relationship between logged variables was expected to be 1:2 (i.e., slope=0.5) under the null hypothesis of isometry. An interesting question extending from this research is whether similar relationships between attachment structures and body size also exist for tapeworms.

Tapeworms, or cestodes, are macroscopic parasites, the adults of which live in the digestive tract of vertebrate hosts. These long ribbon-like worms consist of numerous segments called proglottids that contain eggs and are released in the feces of the host. Some species have larval stages that are transmitted via intermediate hosts. The head of the adult worms is called the scolex and contains attachment structures including a crown of hooks (rostellum) and/or suckers. The scolex and segmented proglottids are connected by a neck. Depending on the genus of tapeworm, infections can be acquired via direct contact with proglottids or by consumption of an intermediate host such as a flea.

Pace University has microscope slides for four tapeworm genera, including Dipyldium (dog and cat tapeworm, transmitted by fleas), Echinococcus (causes hydatid disease, infects dogs, transmitted by cattle and pigs), Moniezia (infests mammals, transmitted by mites), and Taenia (transmitted to humans when eating undercooked beef or pork). We provide suggestions for where tapeworm slides can be purchased (S0.1 Organismal Biology Quantitative Reasoning – Preparatory Materials). Students work in groups of four for this research study. Ideally, each group of four students should receive 32 slides, with roughly equal numbers of 2–4 different genera of tapeworms. Each student requires a compound microscope and camera that attaches to the eyepiece. We use Motic Moticam2 microscope cameras and the current version of the compatible software, Motic Images Plus 3.0. In departments with tight budgets for equipment, pairs of students can share microscopes and cameras. Students also require calibration slides such as those that come with the Motic Moticam2 microscope cameras.

Detailed instructions for taking tapeworm photos and measurements from the photos are available in the first lab handout, including details on how to perform calibrations (S2.1a Organismal Biology Quantitative Reasoning – Module 2 Lab Handout A) and detailed instructions for data analysis in RStudio are available in the second lab handout (S2.1b Organismal Biology Quantitative Reasoning – Module 2 Lab Handout B). Alternative instructions for using Excel instead of RStudio are also provided (S0.3 Organismal Biology Quantitative Reasoning – Excel Data Analysis Option). If you have four-hour long lab sessions, the entire module can be completed in one lab session. Students divide up the work of taking photos and measurements in their groups of four. All students are required to perform data analysis independently. Provide students with a data collection template (S2.3 Organismal Biology Quantitative Reasoning – Module 2 Data Template) and an R script that they work through and edit (S2.4 Organismal Biology Quantitative Reasoning – Module 2 R Script). If the activity occurs over two lab sessions, the students should complete separate worksheets during each session. The first worksheet assesses the students’ ability to distinguish among isometry, hypo-allometry, and hyper-allometry (see end of S2.1a Organismal Biology Quantitative Reasoning – Module 2 Lab Handout A). Details on the second worksheet are below.

We take one-dimensional measurements including the rostellum diameter and neck width. Thus, when data are log transformed, we expect a slope of 1 for the relationship between neck width (a measure of body size) and rostellum diameter under the null hypothesis of isometry. The allometric scaling equation for two measurements in the one dimension is $Y = aX^b$ which becomes $\log Y = \log b + \log X$ after log transformation, where $b$ represents slope, $a$ represents intercept, and $X$ and $Y$ are the measurements for body size (neck width) and body structure size (rostellum diameter) respectively. The R script contains code for calculating the 95% confidence interval for the slope for the entire data set and for one of the tapeworm genera. Students are tasked with using this template code to calculate slopes and confidence intervals for each genus separately and to create a scatterplot of the data. The results are entered into a worksheet to be handed in at the end of the second lab session for Module 2 (see end of S2.1b Organismal Biology Quantitative Reasoning – Module 2 Lab Handout B). Students must be able to clearly state a testable hypothesis (S2.4.1 Organismal Biology Quantitative Reasoning – Module 2 Discussion Slides). Briefly, water vapor exits the plant leaf through pores called stomata (singular: stoma) when the air...
outside of the leaf is drier than the airspace within the leaf. The negative pressure potential that is created via the exiting of water through the stomata draws water up through the xylem and into the airspace in the leaf. The cohesion of water molecules due to their hydrogen bonds ultimately helps water move all the way from the roots to the shoots and leaves of the plant.

Which plants are expected to have higher and lower densities of leaf stomata? This question is addressed by the students for Module 3. At least one week prior to the laboratory session, ask students to independently collect leaves from two different plants or (ideally) groups of plants. The two groups could be two different species raised under similar environmental conditions, or the same species of plant grown under two different conditions (such as different levels of light, humidity, or heat, for example). For students who have limited access to plants, a student could use a single plant and collect leaves from the top and the bottom of the plant or take leaves from one of each of two different species. Discuss pseudoreplication in class so that students are aware of any sampling design limitations and how these limitations might impact their ability to generalize from their conclusions. Nice examples include testing the effect of gene deletion in mice using a single wild-type and a single knockout, and pipetting volumes of cells from the same control and experimental cultures, found on page 9 (Rule 2) of Cumming et al. (20). Each student should collect at least three leaves per plant group (i.e., species or environmental condition) and the leaves should be collected no later than the night before the class for morning classes or the morning before class for afternoon or evening classes. It will be difficult to take stomata impressions from wilted leaves and even if impressions are possible, the stomata will be closed and more difficult to observe from the impressions. It is difficult to take adequate impressions from leaves with trichomes or thick, waxy cuticles, so students should be advised not to collect leaves with these features. It is useful to have “back-up” leaves available in case a student is unable to collect leaves or if the leaves they brought are unsuitable for obtaining stomata impressions. Often other students will have extra leaves and are willing to share with a classmate in need.

During the lab session, provide each student with a compound microscope, blank glass microscope slides (one per leaf), clear nail polish, transparent tape (glossy, not matte or frosted, and not packing tape, which will tear the leaves), and a marker and marking tape to label the glass slides. Detailed instructions for taking impressions of the undersides of leaves are provided in the lab handout (S3.1 Organismal Biology Quantitative Reasoning – Module 3 Lab Handout). Students should work independently on this activity but any students with disabilities who are unable to perform the necessary motor or visual skills have the option of working in pairs. Students are also given a template for data collection, with each variable entered in a separate column (S3.3 Organismal Biology Quantitative Reasoning – Module 3 Data Template) so that the data can be analyzed using a draft R script also provided to them (S3.4 Organismal Biology Quantitative Reasoning – Module 3 R Script). As for modules 1 and 2, students edit the R script to complete the analysis independently and graph their data. Students must count the number of stomata in the field of view under the compound microscope and perform appropriate calculations to convert this count to stomata density (stomata/mm²).

Module 3 poses a new challenge in that students are comparing two groups to each other. An obvious way to compare two groups is to conduct a two-sample t-test, and this test can be conducted in introductory biology labs, which we have done in the past for this exercise. Our preference has been to use confidence intervals because they are demonstrative of the spread of data points within and between groups. Students should be directed to Figure 6 in Cumming et al. (20) to assist them in drawing statistical conclusions about their data. For a valid comparison of two groups using parametric methods, the standard deviations should be similar between groups, with data points roughly normally distributed within groups. If these conditions are met, with a sample size of 3 in each group, the confidence interval of one group must not overlap the mean of the other group to attain statistical significance at the α=0.05 level. In our experience, the standard deviations are often unequal between groups. The use of confidence intervals to test hypotheses is a nice way to demonstrate the importance of considering homoscedasticity in data analytical studies involving comparison of group means.

For this activity, students work through their worksheets during the lab session to draw conclusions about their study (see end of S3.1 Organismal Biology Quantitative Reasoning – Module 3 Lab Handout). As for modules 1 and 2, students must clearly state null and alternative hypotheses that together encompass all possible outcomes for the study. For example, the alternative hypothesis could be that there is a significant difference in mean stomata density between the plant groups. A corresponding null hypothesis is that there is no significant difference in mean stomata density. Another example alternative hypothesis is that the tropical plant species has, on average, a higher mean stomata density than the desert plant species. A corresponding null hypothesis would be that the tropical plant species does not have a higher mean stomata density than the desert plant species (which would be the case if the densities were identical or if the desert plant had a higher stomata density, on average). Students must justify their choice of hypotheses with a biological explanation, such as the tropical plant evolving via natural selection in a humid environment would have selected for plants that allowed for increased water exchange. Students must appropriately display their data using a strip chart with error bars depicting the 95% confidence interval and must use appropriate statistical reasoning to draw conclusions about their hypotheses.

TEACHING DISCUSSION

During the Spring semester in 2022, one of us (EC) conducted research on the effectiveness of our three modules on achieving the stated learning goals. A validated and published “Statistical Reasoning in Biology Concept Inventory” (SRBCI) was used in a pre-test/post-test format (27). This concept inventory consists of 12 questions, involving three biological scenarios, and categorized according to four different “core conceptual groupings”: repeatability of results, variation in data, hypotheses and predictions, and sample size. This concept inventory assessment was chosen because it assessed the use of confidence intervals for statistical reasoning (which was our focus) as well as assessing concepts that we highlighted in our modules (variation in data, the importance of sample size, and hypothesis testing using confidence intervals). We did not specifically address repeatability of
results in our modules, although students may learn about this concept via informal comparisons among student groups’ results. All of the necessary materials for administering the SRBCI and analyzing the data are available at the Questions for Biology (Q4B) website.

The human-subject research was approved by Pace University’s Internal Review Board (IRB # 1809843-1) and received exempt determination under the category of Educational Research. In each laboratory section, either the laboratory instructor or one of us (EC) administered the concept inventory test at the start of the laboratory period before the start of Module 1 (pre-test) and again after completion of Module 3 (and after students presented their research projects via oral PowerPoint presentations; post-test). The test was administered following the guidelines set forth by the concept inventory’s authors. The only adjustment that was made to the protocol was that the “Demographic Information” questions were altered to be relevant to our student body at Pace University. A Research Information Sheet was provided to students before each administration of the test and only data from students who selected “I agree to participate in this research study” both times were included in the analyses.

A total of 92 students completed both the pre-test and the post-test and had agreed to having their data used for research purposes. For 11 of the 12 questions, the percentage of students who scored correctly was higher on the post-test than on the pre-test (Figure 1). On one question, assessing the concept of variation in the data, students did equally well on the pre-test and post-test. This question assessed student’s ability to generalize across trials that had similar statistical results but for which the means within treatments differed among trials (which is not something our modules address). Some questions were inherently more challenging than others, with average pre- and post-test scores per question more similar to each other than to the scores for other questions (Figure 1). The largest learning gains were made for questions pertaining to “hypothesis testing and predictions” with an average 9.8% increase on the post-test relative to the pre-test across three questions in that category. Modest learning gains were also made in “sample size” and “variation in data”, with average increases of 8.5% and 4.7%, respectively. The lowest gains were made in “repeatability of results”, with an average 2.3% increase in on the post-test relative to the pre-test. The question with the single largest learning gain tested whether students understood that the null hypothesis could either be rejected or not rejected (as opposed to a hypothesis being proved). Taken together, these results suggest that our modules were effective at teaching the students the concepts that we aimed for them to learn.

Students’ evaluations of the course indicated that at least some students appreciated the laboratory format involving handouts with clear guidelines and worksheets that are completed with guidance of instructors and classmates during the laboratory session. Some characteristic student comments included the following:

“Labs and worksheets were completed in class with the help of lab partners and additional help from the professor. This allowed us to have a better understanding and complete knowledge of the questions and their answers, as opposed to guessing and potentially being wrong.” (Fall 2013 semester)

“The handouts and worksheets Dr Crispo provided was not only helpful in class, but also proved as a great study source.” (Spring 2017 semester)

“The fact that the worksheets were due during lab and not due the next lab also made it easier because we could just answer the labs during labtime.” (Spring 2018 semester)

Only two students made specific comments about the benefits of using R programming to analyze the data:

“The R program really helped me understand the material.” (Spring 2017 semester)

“I liked . . . being given the opportunity to learn R–Studio.” (Spring 2020 semester)

No students commented specifically on the importance of, or their interest in, learning quantitative reasoning skills or using confidence intervals to test hypotheses.

For courses without a laboratory component, it is possible to perform the data analysis and complete the worksheets without including the data collection component, or to perform the data collection from photos only. During Spring 2020 of the coronavirus pandemic, one of us (EC) shifted the course to remote learning prior to the second two modules. Data from previous semesters were shared with the students and class sessions met using Zoom. Students who worked together in class were put into breakout rooms to work through the R scripts and worksheets together. Students were able to call the instructor into their breakout rooms and share their screens to troubleshoot problems in RStudio. For Module 2, photos of tapeworm slides that had previously been taken were shared with the students, so that students were still able to take measurements using ImageJ on their own. For Module 3, students were given the data in a spreadsheet because images of stomata impressions were not available to send them. For courses that will be taught online or without a laboratory component, we recommend that instructors with access to a

![Figure 1. The percentage of students who correctly answered each question on the pre-test (red bars) and post-test (yellow bars) for each of 12 questions on the Statistical Reasoning in Biology Concept Inventory (SRBCI). The questions were divided into four core concepts that each question assessed: (A) repeatability of results, (B) variation in data, (C) hypotheses and predictions, and (D) sample size.](image-url)
microscope and cameras take photos of stomata impressions from different plants (either the same species grown under different environmental conditions, or different species of plants) and have students count the stomatal densities on their own. For Module 1, images of crayfish chelae (including a ruler for scale) could also be used for online courses and other courses without a laboratory component, and data collection could be performed using computer software such as ImageJ. A recent study found that students who collected their own data showed higher gains in “scientific identity” and “emotional ownership” of the research compared to students who were given data to analyze (29). Instructors who simply want to include a data analysis component without any data collection component can email one of us (EC) for example data sets that can be shared with students.

In the past, we have included statistical interpretation of the data using P values from one-sample t-tests (Module 1), regression analyses (Module 2), and two-sample t-tests (Module 3), as well as including three groups of plants and analysis of variance (Module 3). However, we have found that the confidence intervals are more intuitive than P values given the former’s graphical representation as error bars (Module 1 and 3). To avoid cognitive load, our preference is to include only the confidence intervals in introductory courses rather than to introduce both confidence intervals and P values. In a more advanced course, for statistics courses, or for courses that are taken after the completion of a statistics course, modifications can be made to the R script to include t-tests and regression analysis yielding P values, and the worksheets can be modified accordingly for interpretations based on P values.

Instructors might feel ill-prepared or intimidated to use R with undergraduate students in an introductory course. This lack of preparation might be a bigger barrier to integration in large classes with multiple instructors teaching different laboratory sections. One of us (EC) is part of a group called the Biological and Environmental Data Education (BEDE) Network whose mandate is to provide resources and support to educators who want to teach data science in their classrooms. BEDE Network hosts a group on QUBESHub and has a discussion board available for instructors who want to communicate with other instructors and network members for assistance with their courses. This nascent group has already led workshops in conjunction with meetings of the Ecological Society of America, and we encourage educators to communicate with us via the QUBESHub platform. One of us (EC) will personally be available to answer your questions or direct them to an appropriate person who can assist.

**SUPPORTING MATERIALS**

- S0.1 Organismal Biology Quantitative Reasoning – Preparatory Materials
- S0.2 Organismal Biology Quantitative Reasoning – Getting Started with R and R Studio
- S0.3 Organismal Biology Quantitative Reasoning – Excel Data Analysis Option
- S0.4 Organismal Biology Quantitative Reasoning – Using Microscopes
- S1.1 Organismal Biology Quantitative Reasoning – Module 1 Lab Handout
- S1.2 Organismal Biology Quantitative Reasoning – Module 1 Discussion Slides
- S1.3 Organismal Biology Quantitative Reasoning – Module 1 Data Template
- S1.4 Organismal Biology Quantitative Reasoning – Module 1 R Script
- S2.1a Organismal Biology Quantitative Reasoning – Module 2 Lab Handout A
- S2.1b Organismal Biology Quantitative Reasoning – Module 2 Lab Handout B
- S2.2 Organismal Biology Quantitative Reasoning – Module 2 Discussion Slides
- S2.3 Organismal Biology Quantitative Reasoning – Module 2 Data Template
- S2.4 Organismal Biology Quantitative Reasoning – Module 2 R Script
- S3.1 Organismal Biology Quantitative Reasoning – Module 3 Lab Handout
- S3.2 Organismal Biology Quantitative Reasoning – Module 3 Lab Handout
- S3.3 Organismal Biology Quantitative Reasoning – Module 3 Discussion Slides
- S3.4 Organismal Biology Quantitative Reasoning – Module 3 Data Template
- S3.4 Organismal Biology Quantitative Reasoning – Module 3 R Script

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Table 1. A proposed timeline of activities for students for each module, including time for preparatory work.

| Activity | Description | Estimated Time | Notes |
|----------|-------------|----------------|-------|
| **Student Preparation for Class** | | | |
| Download and install R and RStudio | Recent versions of R and RStudio will be used to graph and analyze the data. | 10 minutes | R must be installed before RStudio. [R for Windows link](#), [R for Mac link](#), [RStudio Desktop (free version) link](#). See S0.2 Organismal Biology Quantitative Reasoning – Getting Started with R and RStudio. |
| Download and install software for microscope cameras | Software is required to take images on laptops using microscope cameras. | 10 minutes | The type of software will be dependent on the type of cameras purchased. For Motic brand cameras, contact customer support for download links. The contact information will vary among geographical regions. |
| Download and install ImageJ software bundled with Java | Software required to take measurements from photos of tapeworm slides. | 10 minutes | Laptop security settings might need to be changed to permit opening a downloaded application. Download for ImageJ is available at this website. |
| **Class Session 1 - Module 1** | | | |
| Introductory lecture | Crab chelae dimorphism, generating a testable hypothesis, using confidence intervals. | 20 minutes | Lecture slides are in S1.2 Organismal Biology Quantitative Reasoning – Module 1 Discussion Slides. |
| Collect data | 100 crayfish are divided among students. Calipers are used to take measurements. Data are recorded in a data template file. | 60 minutes | Instructor demonstrates proper caliper use and should walk around the room to make sure that students are taking measurements correctly and address any student questions. |
| Data entry | Students record data on a master spreadsheet. | 10 minutes | Instructor collates student data and disseminates the master spreadsheet to the class. |
| Data analysis lecture | Data analysis using R. | 10 minutes | Instructor reiterates how confidence intervals will be used to test hypotheses and the use of R. |
| Data analysis | Data analysis using R. | 60 minutes | Students independently work through R scripts to analyze data. They can work in groups to complete their worksheets. Instructors should walk around the room to assist students with any difficulties. |
| Wrap-up and debrief | Ensure that students mastered the objectives. | 20 minutes | Students share their answers with the class at large and instructors address any lingering questions. |
| **Class Session 2 - Module 2** | | | |
| Introductory lecture | Tapeworms, allometry, generating a testable hypothesis about a slope. | 20 minutes | Lecture slides are in S2.2 Organismal Biology Quantitative Reasoning – Module 2 Discussion Slides. |
| Take photos | Each group of 4 students receives 32 tapeworm slides, roughly equal proportions of each tapeworm genera. | 90 minutes | Instructor demonstrates proper microscope use if necessary and should walk around the room to make sure that students are taking photos correctly and address any student questions. |
| Data entry | Students begin taking measurements from their photos. | 30 minutes | Time permitting, students will begin the measurement steps. Instructor should walk around the room to help students get started. |
| Wrap-up and debrief | Ensure that students mastered the objectives. | 10 minutes | Students share worksheet answers with the class at large and instructors address any lingering questions. |
| Activity                    | Description                                                                 | Estimated Time | Notes                                                                                                                                 |
|-----------------------------|-----------------------------------------------------------------------------|----------------|-------------------------------------------------------------------------------------------------------------------------------------|
| **Class Session 3 - Module 2** |                                                                             |                |                                                                                                                                 |
| Introductory lecture        | Using confidence intervals to test hypothesis about a slope.                | 20 minutes     | Lecture slides are in S2.2 Organismal Biology Quantitative Reasoning – Module 2 Discussion Slides.                                |
| Data entry                  | Students complete taking measurements from their photos.                     | 60 minutes     | Instructor walks around the room to ensure measurements are being made correctly and address any student questions.              |
| Data analysis               | Data analysis using R.                                                       | 90 minutes     | Students independently work through R scripts to analyze data. They can work in groups to complete their worksheets. Instructors should walk around the room to assist students with any difficulties. |
| Wrap-up and debrief         | Ensure that students mastered the objectives.                                | 10 minutes     | Students share their answers with the class at large and instructors address any lingering questions.                             |
| **Class Session 4 - Module 3** |                                                                             |                |                                                                                                                                 |
| Introductory lecture        | Transpiration, stomata, hypotheses about two group means.                    | 20 minutes     | Lecture slides are in S3.2 Organismal Biology Quantitative Reasoning – Module 3 Discussion Slides.                                |
| Make stomata impressions    | Each student collects their own leaves, uses nail polish and clear tape to make impressions of the underside of each leaf. | 20 minutes     | Students can work in pairs, but it is best to have each student collect their own in case some students’ leaves are not useable (e.g., because of desiccation or because the leaves have trichomes). |
| Collect data                | Students use microscopes to count stomata from leaf impressions.             | 60 minutes     | Instructor walks around the room to address any student questions.                                                               |
| Data analysis lecture       | Data analysis using R.                                                       | 10 minutes     | Instructor reiterates how confidence intervals will be used to test hypotheses and the use of R.                                 |
| Data analysis               | Data analysis using R.                                                       | 60 minutes     | Students independently work through R scripts to analyze data. They can work in groups to complete their worksheets. Instructors should walk around the room to assist students with any difficulties. |
| Wrap-up and debrief         | Ensure that students mastered the objectives.                                | 10 minutes     | Students share their answers with the class at large and instructors address any lingering questions.                             |