**Attack of the Killer Fungus: A Hypothesis-Driven Lab Module**

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Discovery-driven experiments in undergraduate laboratory courses have been shown to increase student learning and critical thinking abilities. To this end, a lab module involving worm capture by a nematophagous fungus was developed. The goals of this module are to enhance scientific understanding of the regulation of worm capture by soil-dwelling fungi and for students to attain a set of established learning goals, including the ability to develop a testable hypothesis and search for primary literature for data analysis, among others. Students in a ten-week majors lab course completed the lab module and generated novel data as well as data that agrees with the published literature. In addition, learning gains were achieved as seen through a pre-module and post-module test, student self-assessment, class exam, and lab report. Overall, this lab module enables students to become active participants in the scientific method while contributing to the understanding of an ecologically relevant model organism.

**INTRODUCTION**

The purpose of an undergraduate lab course should be to provide a true lab experience that allows students to be active participants in the scientific method. This can be accomplished through a variety of means, including the introduction of primary literature into the curriculum, increased student interaction, and discovery-driven experiments (5, 7, 14). It is equally important to demonstrate that these activities are enhancing student learning and fostering critical thinking skills through appropriate assessment techniques (4, 9).

Typically, lab courses are filled with “cookbook” experiments, in which all students perform the protocol with the end goal being to achieve the same outcome or a set of known outcomes. Students have little to no input in what occurs and thus are not participating in the scientific method. The lack of involvement also means there is no personal stake in the project. While logistically more difficult to establish, student learning is increased with research-oriented activities that can generate original results (3, 6).

To this end, a novel hypothesis-driven experiment was created regarding nematophagous fungi. Nematophagous fungi are soil dwelling organisms capable of trapping nematodes (13). Worm capture is a means of sustenance for the fungus and is important for the ecological balance in the environment. Many of the nematodes targeted by these fungi are parasitic and can infect crops or livestock. It is estimated that nematodes produce global crop losses of $125 billion annually (15). Modes of protection from these organisms include antibiotics or pesticides, both of which have negative side effects; so harnessing the abilities of a nematode predator may provide an alternative means to combat this problem.

This lab module focuses on the nematophagous fungus *Arthrobotrys oligospora* and its ability to trap the worm *Caenorhabditis elegans*. While non-parasitic, *C. elegans* is a well-established model organism that is easily manipulated in the lab. Students learn how to measure the rate of *C. elegans* capture by the fungus and use this protocol to design their own experiment. Working in groups of four, students conduct background research using primary literature, generate a testable hypothesis, perform the experiment, and analyze the data. This module requires students to take an active approach to their learning rather than only following a set of directions.

The goals of this lab module are two-fold. First, students can advance our understanding of nematophagous fungi. Despite the fact that fungal-dependent worm capture was first observed nearly a century ago, regulation of the process is poorly understood (10). Thus, students have the opportunity to contribute to the scientific literature. Second, this exercise will help students achieve the following learning objectives. Following completion of the module, students will be able to:

1. Perform dilution calculations.  
2. Use micropipettors with confidence.  
3. List the benefits of fungi in nature.
4. Describe how fungi and *C. elegans* are maintained in the lab.
5. Develop a testable hypothesis.
6. Search primary literature for hypothesis generation and data analysis.

Based on a number of measures of assessment (Table 1), students performing the lab module were able to achieve both goals. The class-generated experimental data agrees with the published literature and contributes novel information, and the students attained the established learning outcomes.

In addition, the module satisfies the following aims in the American Association for the Advancement of Science (AAAS) Vision and Change report (1). Aim 1, the integration of core ideas and competencies throughout the curriculum, involves the stimulation of student curiosity regarding the natural world. Aim 2, the emphasis on student-centered learning, is accomplished by making students active participants in their education by introducing research experiences in the classroom. And Aim 4 of Vision and Change is to engage the biology community in these changes by providing faculty with resources to develop novel courses. This module supports all of these goals and, in so doing, encourages the development of a student-centered classroom.

**Intended audience/Prerequisite student knowledge**

The lab module was designed for a microbiology lab course, Biological Sciences M118L, at the University of California, Irvine (UCI), with an enrollment of 160 students per ten-week quarter. This is an upper division biology majors course or at minimum have a basic understanding of the central dogma, cellular metabolism, and the relationship between genes, mutations, and phenotypes. This comprehension will aid in their ability to construct a hypothesis and analyze the module data. Prior lab experience or microbiology knowledge is not necessary, assuming the instructor provides sufficient background information. It is helpful if students are familiar with the use of micropipettors, but the module is designed to increase competence. In addition, students should be familiar with primary literature, including the basic components of a scientific paper and how to search for specific information within the literature. A brief primer is included in the supplemental material for students who have not encountered primary literature in previous courses or who are unfamiliar with hypothesis construction (Appendix 1).

**Learning time**

The UCI microbiology lab consists of a two-hour lecture which students in all lab sections attend before splitting into labs that meet for three hours one day and one hour an additional day per week. The course instructor leads the lecture, and a graduate student teaching assistant supervises each lab section (20 students per section). Three weeks of the quarter were spent on the nematophagous fungus module (Fig. 1(A)). In the week 1 lecture, students were introduced to the experimental background. Groups of four students conducted literature searches to choose a variable to test its effect on worm capture. In addition, each group developed a hypothesis concerning this variable and provided evidence to support this hypothesis. In week 2, the instructor assessed the feasibility of testing each group’s variable in the classroom and chose eight variables to examine in the week 3 experiment. During the week 2 lab, students performed a trial run of the protocol to identify possible problems. In week 3, each group tested an assigned variable and its effect on worm survival. The week 2 and 3 lab schedules are discussed in more detail below. At the end of the module, each student wrote a lab report in the format of a scientific paper based on their results.

**PROCEDURE**

**Materials**

1. *Arthrobotrys oligospora* can be obtained from the American Type Culture Collection (ATCC #24927). *C. elegans* (strain N2) and the *E. coli* feeding strain OP50 can be obtained from Cold Spring Harbor (http://www.silencinggenomes.org). *C. elegans* can also be obtained from the *Caenorhabditis* Genetics Center at the University of Minnesota (http://www.cbs.umn.edu/cgc).

2. Cornmeal agar (CMA) plates for *A. oligospora* consist of 1.7% cornmeal agar (Becton Dickinson

| Learning Objectives | Assessment Methods |
|---------------------|--------------------|
| Perform dilution calculations | Pre-/Posttest, Exam |
| Use micropipettors with confidence | Pre-/Posttest, Exam, Student Self-Assessment |
| List benefits of fungi in nature | Pre-/Posttest, Exam |
| Describe how fungi and *C. elegans* are maintained in the lab | Pre-/Posttest, Exam |
| Develop a testable hypothesis | Pre-/Posttest, Exam, Lab Report |
| Search primary literature for hypothesis generation and data analysis | Student Self-Assessment, Student Evaluation of Module |
A. oligospora is streaked on CMA plates one week prior to the experiment and plates are incubated at room temperature to allow growth.

3. Nematode growth media (NGM) plates for C. elegans consist of 2.3% NGM (Bioworld #30620040), 1 mM MgSO₄, 1 mM CaCl₂ and 25 mM phosphate buffer pH 6. 50 μl of an overnight OP50 E. coli culture grown in Luria Broth at 37°C is added to an NGM plate. C. elegans is added to this NGM plate by cutting a small block of NGM agar already inoculated with worms with a sterile tool and transferring the block to the new NGM plate. The worms crawl off the agar block onto the new plate, consume the E. coli and reproduce. A new NGM plate is seeded with C. elegans roughly four days prior to the experiment and is incubated at room temperature.

4. Necessary equipment includes micropipettors, corresponding tips, 1.5 ml microcentrifuge tubes, a microcentrifuge, sterile water, microscope slides, and compound microscopes (dissection microscopes can be used as well).

5. A detailed list of recipes and reagents, including the reagents per student, and faculty instructions is included in the supplemental materials (Appendix 2).

Faculty instructions

In the first lecture of the module, the instructor introduces the nematophagous fungus, the experimental protocol and how to search primary literature and design a hypothesis (Appendices 1–4). In the week 2 lecture, an hour is spent on a more detailed walk-through of the protocol and practice with the dilution calculations performed during the experiment. The trial experiment in week 2 requires one hour in the lab to rinse, count, and add the worms to the CMA plates. It also requires one hour in the second lab period of the week to remove and count the worms on each plate and determine the percent survival. The experiment in week 3 involves three time periods, the first to add worms to the CMA plates, and the second and third roughly 6 and 24 hours later to remove and count surviving worms. This requires students to come to lab twice outside of the normal period. Possible suggestions to accommodate this schedule are discussed later.

Experiment preparation will vary depending on the class size. With 160 students and 8 separate lab sections, it is manageable, but requires careful planning. Over the course of the two experiments (practice run and actual experiment), roughly 250 NGM plates and 700 CMA plates are used. A. oligospora requires one week of growth, which fits well with labs meeting weekly. Students can streak the fungus on a CMA plate, and it will be ready for use the following week. On the other hand, C. elegans requires roughly four days of growth once transferred to a new NGM plate for the worms to achieve the proper density and developmental stage, which does not fit well with most lab course schedules. Because of this, it may be necessary for the lab preparatory staff to prepare the NGM plates for each experiment.

Experimental variable suggestions are collected from the groups at the beginning of the second week. Unfortunately, time and resources are limiting factors, so all student suggestions cannot be accommodated. Instead, eight variables were chosen for study, based on novelty as well as feasibility. Because each of the eight lab sections had five groups, each variable was tested five times, making it possible to statistically analyze the class data.

Suggestions for determining student learning

Student learning was assessed through a premodule test and postmodule test that involved content and student self-assessment questions (Appendix 5). The pre- and posttest were identical, and students were not aware that either test would be administered. The test consisted of 11 knowledge and critical thinking questions and 4 questions which allowed students to self-assess their abilities based on a 7-point Likert scale (1). The pretest was given during lecture the week before the module began. The posttest was distributed in class the week after the lab report was completed. In addition, questions on a class exam given after the module were used for assessment. These
A. Lab Module Schedule

| Week 1 | Introduce fungus to the class  
|        | Students work in groups to research topic  
|        | - Choose 1 variable to test  
|        | - Develop hypothesis  
| Week 2 | Walk through experimental protocol and discuss calculations  
|        | Students perform practice run through protocol  
|        | - Add worms to CMA plates  
|        | - Rinse worms off CMA plates to calculate percent survival (48 hr later)  
| Week 3 | Students test the effect of variable on worm capture  
|        | - Add worms to CMA plates  
|        | - Rinse worms off CMA plates to calculate percent survival (6 hr later)  
|        | - Rinse worms off CMA plates to calculate percent survival (24 hr later)  

B. Sample data

Class data were collected from the week 3 experiment. Of the 40 participating groups, 39 were able to obtain results. One group did not clearly read the protocol and made an error during worm collection steps, and thus had no usable data (but they did analyze class data for the lab report). Data were collected in the form of percentage of surviving worms 6 and 24 hours after the addition of *C. elegans* to the CMA plates. Percent survival values were compared between the control and variable conditions. The class data are presented as a ratio of percent survival of the variable condition versus the control (Fig. 2). The eight tested variables include incubation of the CMA plates at 16°C and 27°C (compared to room temperature), use of 50% CMA plates, addition of 2% mannose to the fungus, addition of a *P. aeruginosa* culture or *P. aeruginosa* media.
(cells removed) to the fungus, incubation of CMA plates in the dark, and addition of worms prior to the experiment to induce fungal trap formation early. Some of these conditions, such as temperature variation, have been previously published, and our results agree with the literature (12). Others such as the worm pre-incubation and addition of bacteria to the CMA plates are variations on what has been published, providing the undergraduates an opportunity to add to the literature. For each variable, the average and standard error of the mean (SEM) were determined for the class data. As can be seen from Figure 2, the SEM is large for some variables, and thus it is important to continue to collect data to improve the reliability of the conclusions generated from these experiments.

Safety issues

Students work with C. elegans, the E. coli feeding strain OP50, and the fungus Arthrobotrys oligospora. These are all classified as biosafety level 1 organisms. In lab, students are required to wear lab coats and gloves and to follow proper sterile technique. No other potential safety issues are associated with this module. For this manuscript, student assessment data were collected following submission and acceptance of an Institutional Review Board (IRB) application.

FIGURE 2. Class data examining the effects of different variables on A. oligospora-dependent capture of C. elegans. A. oligospora was struck on CMA plates one week prior to the experiment and incubated at room temperature. The experiment was performed as outlined in Figure 1(B) and Appendix 4. At t = 6 h and t = 24 h following worm addition, students rinsed the worms off the CMA plates and calculated percent survival. Data are presented as a ratio of percent survival in the variable condition compared to the control. Data from at least 5 groups for each variable were averaged and the standard error of the mean (SEM) is noted. The pre-treatment worm variable involved the addition of C. elegans to CMA plates 24 hours prior to the start of the experiment. These worms were rinsed off immediately before the experiment began. For the mannose, P. aeruginosa and P. aeruginosa culture media variables, each was added to the CMA plates two hours before the experiment began.

DISCUSSION

Field testing

The nematophagous fungus lab module has been administered to an upper division microbiology lab at UCI, a ten-week class that consists of a weekly schedule of a two-hour lecture and four hours of laboratory spread over two days. The class has 160 students per quarter split into 8 sections. The lab focuses on medical microbiology topics and molecular biology experiments involving microbes.

After completion of the module, students achieved both of the established goals. First, they were able to obtain results that agreed with the published literature as well as produce novel data (Fig. 2). And secondly, the students achieved the learning objectives, as detailed below. In addition to the described methods of assessing student learning, the class was presented with a survey at the end of the quarter, a portion of which asked for their opinions regarding the module. The comments were overwhelmingly positive, with the most common remarks being that they enjoyed performing novel research and gained valuable experience in experimental design. Students responded to two statements on a 7-point Likert scale (7 = strongly agree, 4 = neutral, 1 = strongly disagree). The first was “the experiment allowed me to experience the scientific method” to which the average response was a 5.6 ± 0.10 (5 = slightly agree, 6 = agree) and the second was “the experiment improved my abilities to find information in the primary literature” which received an average response of 5.4 ± 0.14. While the final learning objective focused only on searching primary literature, the lab report rubric would allow one to measure whether the students appropriately utilized the literature for hypothesis construction and data analysis as well (Appendix 7).

Evidence of student learning

The expected learning outcomes and the specific means of assessment for each are listed in Table 1. Assessment measures included a pre-/posttest, class exam, and lab report. Students were allowed ten minutes of lecture time to complete the pre-/posttest. Class results for the knowledge and critical thinking questions along with the topic covered by each are displayed in Figure 3(A). Students made statistically significant gains for each question after the module. This was even the case for questions higher on Bloom’s Taxonomy, a classification system for the types of learning students can demonstrate (2, 8). On questions involving experimental design and hypothesis construction, which are more challenging than those dependent only on the ability to recall information, students still demonstrated significant improvements on the posttest.

Attainment of learning objectives was also demonstrated by a class exam that covered five weeks of course material. During the nematophagous fungus module, students were conducting other experiments related to bacteria isolation...
and identification. Class aggregate scores on six questions relevant to the fungus module are highlighted in Figure 3(B) and compared to the class average on the remaining exam questions (Appendix 6). For five of the six questions, students scored higher than on the non-fungus portions of the exam. This was even true regarding the dilution problems, which a number of students appeared to struggle with during the earlier lectures. The question regarding hypothesis construction based on information from an unfamiliar topic was the most challenging on the exam based on Bloom's classification, yet student scores were equal to scores earned for the non-fungus questions.

The ability of students to develop a testable hypothesis was also measured in the lab report. The assignment was for students to report their data in the form of a scientific paper, including a title, introduction, results section and discussion. Based on a 2-point rubric, students were assessed on the quality of the hypothesis written for the lab report (Appendix 7). The majority of students were capable of writing a clear, concise and testable hypothesis, with over 90% earning at least 1 out of 2 on the rubric (Fig. 3(C)). In the author's opinion, the learning gains observed regarding hypothesis construction were the most valuable for the students. From the pretest and class discussions, it appeared that students found it very challenging to develop a novel hypothesis. Although hypotheses are mentioned in biology courses from high school through college, it may be the scarcity of discovery-driven lab modules or independent research opportunities that limit the opportunities a student has to practice hypothesis construction. Thus, it is key that students be presented with activities, whether in lab or lecture courses, that involve the creation of a hypothesis.

Finally, the students self-assessed their abilities on the pre-/posttest. This included statements regarding proficiency with micropipettors, searching primary literature, and designing an experiment. In addition, their agreement...
with a statement discussing the connection between class activities and research laboratories was measured. From the data in Figure 4, it is clear that prior to the lab module, the students believed strongly in their abilities. This may have to do with the fact that most students taking the course were graduating seniors. Still, following the fungus experiment, there was a statistically significant increase in all measured abilities.

From the assessment data, the students achieved all of the established learning objectives. In addition, the class was able to conduct novel scientific experiments that add to the published literature. This highlights the value of discovery-driven experiments compared to traditional “cookbook” modules. The goal is to continue this module, both at the author’s university as well as collaborating institutions, and publish the collective data in a scientific journal. For a variety of reasons, including a lack of experience and logistical considerations, undergraduates in lab courses are not seen as a manageable workforce for scientific discovery. Yet with proper instruction and the right project, students can be significant contributors.

Possible modifications

Potential difficulties with the module include the abilities of the target audience and the chosen time points for measuring worm survival. This experiment can be adapted for non-majors and lower division biology majors, but would require an increase in the background material presented in the lab manual and class lectures (Appendix 3). While the student-driven experimental design is a benefit of the module, it can also be removed for students unfamiliar with searching primary literature, and instead the instructor can select the variables to be tested. Examples of potential variables to test include altering the incubation temperature, decreasing the percentage of the cornmeal present in the CMA plates (to create a nutrient poor environment), and adding various sugars (glucose, mannose, sorbitol, etc.) or microbes (E. coli, P. aeruginosa, etc.) to the CMA plates.

For the issue of student attendance outside of the normal lab period, this was a necessity based on the data collection time points. We have experimented with counting worm survival from 2 to 48 hours following worm addition to the CMA plates, with no worm death observed at 2 hours and complete worm death after roughly 24 hours. Time points at 6 and 24 hours allow for the identification of smaller effects that may be lost by 48 hours. Two students collected data at each time point; so the four members in each group were only required to come to lab once outside of the normal lab period. If this is not feasible, a possible modification is to have students in different sections working together to complete the experiment. For example, if a lab course has two sections, one in the morning and one in the afternoon, students in the morning class can begin the experiment while students in the afternoon can perform the first worm collection. In this scenario, it is recommended to form groups across sections so that a single group is carrying through the entire experiment and students are invested in the experiment’s completion and success.

SUPPLEMENTAL MATERIALS

Appendix 1: Primer for primary literature and hypothesis construction
Appendix 2: Instructor information regarding necessary equipment, reagents and recipes for the module
Appendix 3: Nematophagous fungi background information
Appendix 4: Protocols for Week 2 and Week 3 experiments
Appendix 5: Pre-/posttest taken by students in lecture to assess achievement of learning objectives
Appendix 6: Exam questions pertinent to the nematophagous fungi experiment.
Appendix 7: Rubric for nematophagous fungus lab report

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REFERENCES

1. American Association for the Advancement of Science. 2011, posting date. Vision and change in undergraduate biology education: a call to action. [Online.] http://visionandchange.org/finalreport/
2. Anderson, L. W., and D. R. Krathwohl. 2001. A taxonomy for learning, teaching and assessing: A revision of Bloom’s Taxonomy of educational objectives. Longman, New York.
3. Ault, J. F., B. M. Renfro, and A. K. White. 2011. Using a molecular-genetic approach to investigate bacterial physiology in a continuous, research-based, semester-long laboratory for undergraduates. J. Microbiol. Biol. Educ. 12:185–193.
4. Bissell, A. N., and P. P. Lemons. 2006. A new method for assessing critical thinking in the classroom. BioScience 56:66–72.
5. Call, G., et al. 2007. Genomewide clonal analysis of lethal mutations in the drosophila melanogaster eye: comparison of the x chromosome and autosomes. Genetics 177:689–697.
6. Casotti, G., L. Rieser-Danner, and M. T. Knabb. 2008. Successful implementation of inquiry-based physiology laboratories in undergraduate major and nonmajor courses. Adv. Physi. Educ. 32:286–296.
7. Colabroy, K. L. 2011. A writing-intensive, methods-based laboratory course for undergraduates. Biochem. Mol. Biol. Educ. 39:196–203.
8. **Crowe, A., C. Dirks, and M. P. Wenderoth.** 2008. Biology in Bloom: implementing Bloom's Taxonomy to enhance student learning in biology. CBE Life Sci. Educ. 7:368–381.

9. **DebBurman, S. K.** 2002. Learning how scientists work: experiential research projects to promote cell biology learning and scientific process skills. Cell Biol. Educ. 1:154–172.

10. **Drechsler, C.** 1937. Some hyphomycetes that prey on free-living terricolous nematodes. Mycologia 29:447–552.

11. **Likert, R.** 1932. A technique for the measurement of attitudes. Archives of Psychology 22:1–55.

12. **Morgan, M., J. M. Behnke, J. A. Lucas, and J. F. Peberdy.** 1997. In vitro assessment of the influence of nutrition, temperature and larval density on trapping of the infective larvae of *Heligmosomoides polygyrus* by *Arthrobotrys oligospora, Duddingtonia flagrans* and *Monacrosporium megalosporum*. Parasitology 115:303–310.

13. **Nordbring-Hertz, B., H.-B. Jansson, and A. Tunlid.** 2001. Nematophagous fungi, eLS. John Wiley & Sons, Ltd., Somerset, NJ.

14. **Quimby, B. B., K. S. McIver, G. Marbach-Ad, and A. C. Smith.** 2011. Investigating how streptococcus responds to their environment: bringing together current research, a case study and laboratory investigation. J. Microbiol. Biol. Educ. 12:176–184.

15. **Sasser, J. N., and D. W. Freckman.** 1987. A world perspective on Nematology: the role of the society. Society of Nematologists, Hyatsville, MD.