

# Bioscene

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# Bioscene: Journal of College Biology Teaching

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The objectives of ACUBE are:

1. To further the teaching of the biological sciences at the college and other levels of educational experience;
2. To bring to light common problems involving biological curricula at the college level and by the free interchange of ideas; endeavor to resolve these problems;
3. To encourage active participation in biological research by teachers and students in the belief that such participation is an invaluable adjunct to effective teaching; and
4. To create a voice which will be effective in bringing the collective views of college and university teachers in the biological sciences to the attention of college and civil government administrations.

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# Clarity in Teaching and Active Learning in Undergraduate Microbiology Course for Non-Majors

Gili Marbach-Ad, J. Randy McGinnis, Rebecca Pease, Amy H. Dai, Kelly A Schalk,  
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**Abstract:** We investigated a pedagogical innovation in an undergraduate microbiology course (Microbes and Society) for non-majors and education majors. The goals of the curriculum and pedagogical transformation were to promote active learning and concentrate on clarity in teaching. This course was part of a longitudinal project (Project Nexus) which prepares, supports and sustains upper elementary and middle level specialist science teachers. We sought to understand the impact of the course in terms of students' content understanding and in terms of students' feedback to the course. To determine students' content understanding we used a pre-post content survey. To document the use of teaching innovative approaches in the class we interviewed all participants, including the lecture and the lab instructors and their students, to get their perspectives. Also, all lecture classes and laboratory sessions were videotaped and observed by three university science educators. Our findings suggested that the instructor taught in the way that he planned. Positively, while the course placed an emphasis on modeling good pedagogy and promoting teaching science as a career option, significant gain in science content was achieved by the students.

**Key words:** Non-major science course, active learning, clarity in teaching, microbiology.

## INTRODUCTION

The primary objectives of non-majors science courses are to introduce students to the process of scientific thinking, to help students gain an appreciation for how science is conducted, understand how science impacts daily lives and to provide a knowledge base in a particular scientific field that students can use as a foundation for life-long learning in the sciences. In this study we investigated pedagogical innovation in an undergraduate microbiology course (Microbes and Society) for non-majors and education majors. For over five years the course, which weekly consisted of two lecture sessions and two laboratory sessions, had been under transformation by the same biology professor. The goals of the curriculum and pedagogical transformation were to promote active learning and concentrate on clarity in teaching. Studies have shown that student learning is enhanced when they are actively involved in the teaching-learning process (Allen, & Tanner, 2006, Ebert-May et. al. 1997, Johnson et. al. 1998, Udovic et. al. 2002). There are many different types of active learning, all of which involve the students being engaged with the material, rather than passively listening to lectures. Many active learning techniques involve some form of cooperative learning in which students work together in groups toward some goal (Slis, 2005). The Microbes and Society course active learning aspects included: encouragement of student questions, the use of multimedia (i.e., videotape films) and instructional technology, small

group activities, whole class discussions, student projects and the use of alternative assessments. All are best practice approaches for high-quality science teaching recommended by latest national standard reports (AAAS, 1993; NRC, 1996, 2007) and could be implemented in any science course for non-majors.

Clarity in teaching means teaching in a way that enables students to better understand the content, the processes, and their own learning. Research shows clarity to be a valid, distinct, and stable construct, unaffected by extraneous student or teacher variables (Hativa, 2000). Hativa (2000) reported that several dozen teacher behaviors were identified as components of clear teaching at the college level. Some of the main behaviors were: using examples and illustrations, presenting a material in a simple and logical manner, using questioning in class to gauge student understanding, repeating, stressing and summarizing important points, breaking down the material into small steps, and adapting the teaching to students' background knowledge and everyday life experience.

The Microbes and Society course is part of a longitudinal project (Project Nexus). Project Nexus [PN] is funded project by the National Science Foundation's Teacher Professional Continuum program (TPC). Project Nexus is designed to develop and test a science teacher professional development model that prepares, supports and sustains upper elementary and middle level specialist science teachers. Since the Microbes and Society course is a

facet of Project Nexus, an innovation by the instructor is to highlight the connection between science and teaching. Interested readers are invited to learn more about Project Nexus by visiting [www.projectnexus.umd.edu](http://www.projectnexus.umd.edu).

In this study we focused on the innovative teaching approaches (active learning, clarity in teaching and connection between science and teaching) that were used in the transformative lecture section. We assessed the success of achieving our goals using observers' evaluations, the instructor's perspectives and students' achievements and feedback.

## METHODS

### Course Description

The course *Microbes and Society* was a 4-credit course, with 75 minutes twice a week lecture sessions and twice a week one-hour laboratory sessions. *Microbes and Society* looked at the fascinating roles that microbes play in the world around us. The course helped students develop an understanding of basic concepts in biology: the unity of life, evolution, disease, antibiotic resistance, the roles microbes play in providing food and recycling waste, and the roles that cultural and societal influences play in the spread control of microbial diseases.

The course was designed to enable students to develop lifelong learning skills, an appreciation and understanding of science, and the ability to explain science to others. The course used a variety of teaching strategies applicable to both science and non-science courses from the elementary through college level. It was based on a 12-part video series *Unseen Life on Earth*

(<http://www.learner.org/resources/series121.html>).

The videos were used in the class in an interactive manner by incorporating small group and whole class discussions after each section of the video. The instructor asked questions and encouraged student questions. An important goal of the course was to model teaching for all (for different students with different background and different learning styles) with the hope that students who pursue teaching as a career would learn how to teach all learners.

### Learners in the Course

Twenty seven students were enrolled in the course. They were recruited by proactive techniques such as the posting of an engaging flyer for the course on bulletin boards throughout the campus, particularly in the College of Education and in the various non-science buildings. In addition, undergraduate advisors across campus were notified of the course and were encouraged to recommend it to students who needed a science course or wanted to experience learning science for diverse population in an active learning environment.

The recruited students represented diversity across several dimensions. Figure 1 shows students' demographic distribution (data obtained from 24

<b>Gender:</b>	15 females 9 males
<b>Number of Credit (earned up to entering the course):</b>	13 between 0 to 30 8 between 31 to 60 3 between 61 to 90.
<b>Ethnicity:</b>	1 African-American 3 Asian 2 Hispanic 13 Caucasian 5 others.
<b>Year at school:</b>	15 Freshmen 7 Sophomores 1 Junior 1 Senior.
<b>Major:</b>	12 in education programs with other major 12 are not in education program and are coming from different major such as Journalism, Computer Sciences, Sociology, Music Theatre, Mathematics, and Government & Politics.

**Fig. 1.** Student background information (self reported – 24 students).

students who responded to a survey that included questions regarding their self-reported demographic identification).

### Instrumentation

In this study we sought to understand the impact of the course in terms of students' content understanding and in terms of students' feedback to the course. To determine students' content understanding we used a pre-post content survey (the survey may be obtained by request from the authors). The course's instructor formulated the content questions based on information from the National Science Education Standards. The survey included 20 multiple-choice questions: Four chemistry questions (i.e. The smallest units of matter are: a. atoms; b. molecules; c. atomic particles; d. microbes; e. cells), 8 molecular biology questions (i.e., Genetic information is stored in: a. proteins; b. lipids; c. enzymes; d. DNA; e. RNA), and 8 biology questions (i.e., The smallest units of living matter are: a. organisms; b. cells, c. nuclei; d. organelles; e. viruses). The topics discussed in the course were primarily from the biology and molecular biology domains. Therefore the four chemistry questions served as a baseline to compare students' improvement on the topics that were taught in the class. Students' feedback for the course was obtained via an extensive end-of-semester survey. The questions had an opening sentence and five different

hierarchical categories. Students were asked to choose one answer (see examples in the results section).

To document the use of innovative teaching approaches in the class, one member of the research team (McGinnis) interviewed a representative sample of the class (N=6) on two occasions, once near the beginning of the semester, and once near the end of the semester. We used a structured interview protocol that consisted of four questions. As a research team we also interviewed the lecture and the lab instructors to gain their perspectives.

During class sessions it is difficult for science instructors to determine what impact their teaching has on students or even whether their teaching approach in actual practice matches their personal perception of practice. Discouragingly, a relatively large body of research suggests even those teachers who describe themselves as holding a constructivist perspective on teaching and learning, are seen by their students and by experienced observers to be teaching more conventionally than they thought they were (Salish I, 1997).

Therefore, in our study all lecture classes and laboratory sessions were videotaped and observed by three university science educators (our research team). One observer was a female professor who serves as the director of the University College of Chemical and Life Science Teaching and Learning Center, one was a male science education professor in the Curriculum and Instruction department, and the third was a female science education graduate student with a biology background. As a team they attended every class session, and took extensive notes that focused on documenting and interpreting the nature of the classes and the degree to which the instructional objectives were achieved.

## FINDINGS

Three observers regularly visited the class sessions to take notes and document how and to what extent the instructor included strategies that promoted “active learning,” “teaching in clarity,” and “connection between science and teaching.” All sessions were videotaped. Table 1 shows representative examples from the pedagogical strategies that were documented in the classroom. Below we elaborate on each of the innovative strategies reporting the instructor’s perspective that he gave in formal interviews.

### Active Learning Approaches

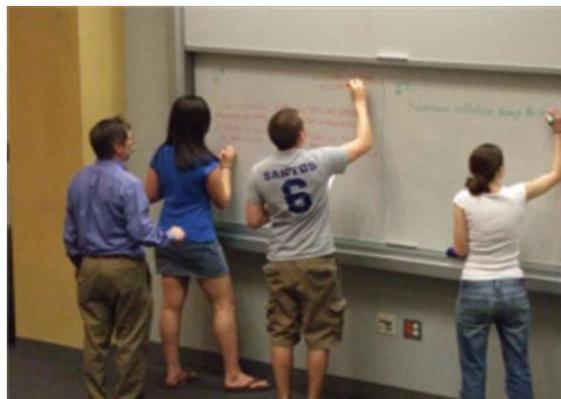
#### *Students’ and instructor’s questions*

The instructor encouraged students’ questions in-class and out-of-class, modeling for students that science is a constantly expanding field which is based on progressive research. The instructor also explained during the interview some of the strategies he used to encourage students’ questions and improve the quality of students’ questions. For example, instead

of answering a question directly, he might ask the group to respond to the question to encourage peer participation. As he reflected on the experience, he described his aspirations and observations, “And so what we’re looking for, is to move as many of the students as we can forward in terms of asking questions that go beyond will this be on the test or is this content directed, and trying to put it into a bigger context. With some students we were successful and with others we were not.” Students were also asked on ten occasions over the course of the semester to write as a homework assignment one question that they want to ask the instructor.

#### *Multiple channels of communication*

The instructor used multiple channels of communication with the students: Power-point presentations, white board, and videotapes. In an interview early in the course regarding his plans and



**Fig 2.** Small groups’ representatives presenting their responses on the white board.

goals for the course, the instructor described the lecture portion of the course, which was based on a 12 part video series, as a sequence of guided viewings of the videos followed by discussions. He indicated that he planned to make the class time interactive, spending only a small amount of time lecturing to the students. His hope was that the interactions would lead the students to the key concepts presented in the videos. In an interview towards the end of the course, the instructor discussed the use of the videos and how they were used to promote active learning. He described how the video presentation format was changed early in the semester, first to compensate for an unanticipated change in schedule, but then continued in attempt to improve the discussion portion of the lesson. During the interview in the end of the semester the instructor said, “At the beginning of the class for the first four to six weeks we would have them watch the whole video on Tuesday and then we would spend all of Thursday discussing it. About halfway through we changed that protocol for a number of reasons [mainly, as an adjustment to students’ reactions], and

**Table 1.** Examples from the observers' notes describing the class sessions.

Teaching Methods	Observers' documentation
<b>Active Learning</b>	
Instructor's questions	Type of questions: a) Do you have any questions? (the instructor stopped the lecture for questions); b) "These are [The instructor pointed on the board] the kind of questions that you will see on the test, would you like a little bit of help on any one of them?" c) The instructor asked content knowledge questions while lecturing, allowing student a minute (wait time) to think about their answers.
Students' questions	The instructor encouraged students to write in-class and out of class questions. If there was not enough time for the instructor to answer all the questions immediately, he asked the student to hold the question and answer later. The instructor modeled "just-in-time" teaching (i.e., collecting students' written questions from the previous class and answered some of them in the next class session).
Multiple channels of communication	Power-point presentations, white board, video-tapes.
Group study and whole class discussions	Students were asked to answer questions within their small group, each group (4-8 students) presented their answer on the board, followed by a whole class discussion, while the instructor added information to the discussion.
Assessment	Assessments included 6 mini exams with opportunities to retake each test, modeling that science should be taught as small units with assessments following each unit, not as a summative assessment. The exams also included alternative assessment elements, such as, analysis and evaluation of scientific articles.
<b>Clarity in Teaching</b>	
Features of clear teaching	The instructor a) simplified explanations (using drawings and videos); b) emphasized main ideas (using repeated explanations, vocal emphasis); c) presented flexibility in teaching - adjusting teaching to students' reaction – the instructor asked the students to vote on the way that they liked to watch the films (small segments vs. large segments).
Connection to students' interest	The video tapes used examples and explanations from the students' world like, football game or pizza preparation to demonstrate terms in microbiology.
Features of interesting teaching	The instructor a) walked up and down the stairs, circulating among the students; b) used historical view (genetic diagnostics) – showing the process of scientific research; c) used humor.
Connection to everyday life and prior background knowledge	The instructor opened most of class sessions with questions such as, "Did you read about this topic in the newspaper?" and discussed the past week's "microbiology in news" with students. He also raised questions such as, "Do you know of any new diseases emerged in the past few years?" ("...Maybe you remember this from school, biology classes at 4 <sup>th</sup> grade or 8 <sup>th</sup> grade..."). The instructor built interconnections between previous and current sessions.
<b>Linkages to teaching</b>	The instructor explained the pedagogy he used and why he was using it (i.e., Why he put students into groups, why he allowed taking a test retake.)

began showing the videos on both days, stopping after each segment to discuss it."

#### *Group study*

In almost all class sessions students were asked to work on in-class assignments in groups. The small group assignments included discussions about questions provided by the instructor and then each group shared the information with the whole class. Figure 2 is representative of the small groups presenting their responses on the white board.

#### *Assessment*

When asked about assessment, the instructor shared his thoughts on his experience from a variety of perspectives. While he believed that the course was "over-assessed," he was confident that the assessments were justified and modeled good teaching practices. He felt that the assessments were

designed to encourage the students to complete the reading assignments. He also expressed his feeling that assessment was another way to model good teaching practices. When queried about making the course learning-centered rather than content-centered, the instructor described his intentions and how he assessed the outcome, "What we are interested in is having them acquire the skills, and practice the skills of learning about science; in this case, science within microbiology. So that's what I mean by learning-centered not content-centered."

#### **Clarity in Teaching**

##### *Features of clear and interesting teaching*

The instructor stated that he made his teaching clear and interesting in the following manner, "...I strive to teach clearly by using examples and illustrations, in the class we make extensive use of simple illustrative

diagrams, presenting a material in a simple and logical manner, repeating, stressing and summarizing important points.”

#### *Connections to everyday life*

The instructor explained his intention to guide the students to an understanding of microbiology that would allow them to appreciate how it might apply to their lives and how they may keep informed through the use of popular literature. He commented, “What I am interested in is their understanding of science process and microbiology from an application standpoint. Meaning that they have an appreciation for how it fits within their world. And they have the skill and ability to read what I will refer to as lay literature; Washington Post, Discover, Science News, and be able to read it from a semi-critical fashion.”

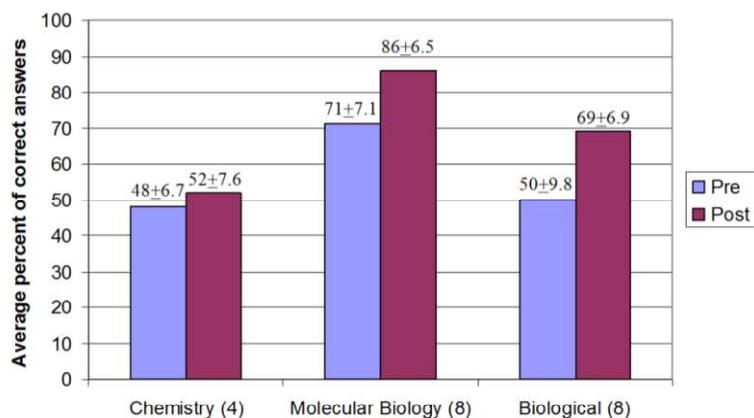
#### **Linkages to Teaching**

The course instructor expressed his intentions to make the course relevant to prospective teachers, especially those interns who may be considering teaching science to elementary or middle school aged learners, and to model teaching practices that could be used in those settings. He explained, “I have a particular interest in how those pre-service teachers, those students in the College of Education, the kind of science they get exposed to during their time here at the University. So within this course we model and refer back to science teaching perhaps more than you would expect to find in other comparable non-majors general education courses within the university outside of the College of Education.” The instructor strived to make the course explicitly connect with teacher education. He planned to explain the pedagogy behind his teaching methods and assignments, so that the students would always be aware of the learning goals, “I make, whenever possible, explicit and transparent, the pedagogy I’m using and why I’m using it. Why we put students into groups, why we do discussions, why is it that I allow them after taking a test to retake the test in a window of time for an average of the two grades.”

#### **Students’ Perspective**

##### *Students’ content knowledge*

The instructor collected pre and post content surveys which included identical twenty questions in three subtopics: Chemistry – 4 questions, Molecular Biology - 8 questions, and Biology - 8 questions. Twenty-one students responded to both the pre and post surveys. Encouragingly, a t-test analysis showed that students significantly ( $p < 0.001$ ) improved their scores on the post survey in the content areas that were taught, Molecular Biology and Biology. The average score on the pre-test was 58.5% (11.7 out of 20) while the average score for the post survey was 73% (14.6 out of 20). There was no significant change in scores on the Chemistry content, which served as the control variable. Figure 3 summarizes students’ scores on the three subtopics of the survey. At the end of the semester, 25 students provided responses to a final course evaluation. The questions had an opening sentence and five different hierarchical categories. Students were asked to choose one category. To the question, “the weekly learning guide was...” 32% of the students answered “very beneficial”, 36% “useful” and 32% responded “marginally useful”. None of the students chose “a waste of time” or “have no opinion”. One question asked about the unusual use of assessment in the course. To the statement, “The ability to retake the first two in-class test was...” 52% of the students answered “very useful in helping to understand the course material”, 20% useful in helping to understand the course material and 28% responded “No opinion”. None of the students chose “not useful” or “not appropriate for college course”. Another question asked students to refer to the small group discussion. To the statement, “The in-class group discussion activities were...” 16% of the students answered “very useful in helping to understand the course material”, 44% useful in helping to understand the course material, 36% responded “not useful” and 4% “No opinion”. None of the students chose “too simplistic”. One question referred to the video tapes that were used in the class, 8% of the students answered that the videos were



**Fig. 3.** Students’ average percent of correct answers ( $\pm$  Standard Error) in the three content domains: Chemistry, Molecular Biology and Biology (n=21).

“the best part of the course”, 52% answered that “they were interesting and enriching”, 32% chose “of limited value” 8% chose detracted from the course. None of the students chose “they added nothing to the course”. To the question, “How worthwhile was this course in helping you to understand the roles of microbes in the world?” 36% of the students answered “very”, 52% “somewhat”, 8% chose “a little” and 4% responded “have no opinion”.

#### *Undergraduate students' feedback and gain of content knowledge*

One open-ended question in the end-of-semester course evaluation instrument applied to the connection between science and teaching. We asked only students who considering teaching science to answer the following question: “*What teaching practices/techniques did you see modeled in this course that you think would benefit your science teaching? Explain.*” Seven students answered this question. Responses included: “Peer-to-peer learning, working in groups”, “I would provide feedback to my students as well as facilitate discussions in lecture”; “I liked how the videos illustrated the information. It made it easier to understand certain material”; “The best way for your students to learn is if they are invested and see it done hands on”; “Relating science with everyday life”.

One of the homework assignments asked students to report if they thought that the course has stimulated them to think at all about a career in teaching and if not if they feel that it helped them at all to educate other populations (like their future kids). Twenty-one students responded to this question. Twelve students reported that the course stimulated them to think about teaching career. Most of them (10) already thought about a teaching career before, but they reported that the course contributed to their teaching skills. One student commented, “...One thing this class has showed is how important it is to engage all of the senses when really trying to educate. In this class we do physical things with our hands, we listen, we watch, we read, this class really touches all bases. Even though I am going to teach English, I can still incorporate physical things into it. Also, this class has shown how a mixture of independent and group work can enhance the learning environment. Overall, I feel this class has taught me to keep things diverse in the classroom when I am teaching.”

Nine students reported that the course didn't stimulate them to think of teaching career, but most of them (7) wrote that the reason for it is that they came to the course with the notion that they want to do something else in their lives. They did stress that the course helped them in different ways, “I have never wanted to be a teacher and have no desire now to be a teacher.... [But] the class did teach me how to explain things to people who are new to a subject. In this sense perhaps it will help me in my career when I am briefing interns or entry level jobs.”

Two students said that they don't feel that the course contributed to their teaching abilities at all, “I don't feel that this class has necessarily made me think about my teaching. I think that a large part of this is that this course does not directly target teaching procedures. I am not overly interested in science and am planning to teach general education for grades 2 and 3 and therefore have a hard time relating microbes to my teaching. I did not learn about microbes until I was much older, at least in high school.”

## **DISCUSSION**

In this study we investigated a curricular and pedagogical innovation in an undergraduate microbiology course (“Microbes and Society”) for non-majors. Generally, most science courses are content driven and re-enforce students' negative view that science is a collection of facts unrelated to their world. For non-majors who must take and pass science content courses to satisfy degree requirements, this undesired outcome is especially true. An established set of studies (e.g., Vance-Chalcraft, et al., 2007) reveal that most of these kinds of science courses that emphasize facts use a reproductive model where information is presented in didactic lectures, memorized and reproduced on assessments. Therefore, the aim of the Microbes and Society course was to improve students' understanding of science, using active learning approaches, concentrating on clarity in teaching and connecting science to teaching.

In this study we documented the instructor's perspective, and we provided his rationale and explanation for the teaching and learning approaches that he used. We reported on the notes of observations that were taken by the three science education observers. The observations supported the pedagogical claims of the instructor. He did indeed teach in the way that he planned. We reported the students' feedback to the course. A key finding was that while the course placed an emphasis on modeling good pedagogy and promoting teaching science as a career option, significant gain in science content was achieved by the students.

Our findings also suggested areas that the course could be improved. Both the researchers and the instructor thought that the course included too many assessments and the use of videotapes as an instructional strategy were too extensive. The students also shared these thoughts and recommended future changes in the course.

Specifically, they recommended having more class discussions. We observed that the instructor was very flexible and attentive to the students' requests throughout the semester, and he changed his teaching accordingly. For example, mid-semester after the students made the suggestion, he began to show the videotapes in short chunks, followed by explanation

and some class discussion instead of in large chunks that filled up a class session. Our assumption was that by appreciating the unique needs and characteristics of students it will set an educational environment that would enhance enduring learning for each student. Since this course was part of a science teacher preparation project (Project Nexus), it was important to demonstrate to the education majors and others what clear teaching is and how to teach for understanding. Positively, most of the students in the course expressed that the innovative activities in the course (i.e., group discussion, the videotapes, the test retake) were useful in helping to understand the course material, something they believed would assist them in becoming better parents even if they did not decide to become teachers.

We believe that our findings showed that we implemented a non-major's science course that enhanced students' science understanding and increased their motivation and satisfaction. This course could serve as a model for other non-major courses.

As a postscript, for the next semester's offering of Microbes and Society, we have recruited 32 diverse students, with 2 on the wait list. We look forward to building on what we have learned to further improve the course.

#### ACKNOWLEDGMENT

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# Exploring Evolutionary Patterns in Genetic Sequence: A Computer Exercise

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**Abstract:** The increase in publications presenting molecular evolutionary analyses and the availability of comparative sequence data through resources such as NCBI's GenBank underscore the necessity of providing undergraduates with hands-on sequence analysis skills in an evolutionary context. This need is particularly acute given that students have been shown to bring misconceptions about evolution to the classroom, and these misconceptions can hinder their learning about genetic sequences, mutation, and evolutionary processes. However, undergraduate institutions sometimes lack sophisticated analytical software in student computer laboratories. Here we present a computer laboratory exercise utilizing freely available analysis software, and which is designed to analyze sequences that can be obtained from GenBank or other online sources. The exercise is flexible in its complexity, allowing instructors to modify the lab to suit the needs and skills of their classes, and was significantly helpful to introductory biology students in understanding the basics of sequence variation and analysis.

**Keywords:** genetic data, DNA sequence, evolution, computer exercise, sequence alignment, phylogeny, population genetics

## INTRODUCTION

The integration of exploratory labs in undergraduate science courses is based upon the ideas that students learn science best by doing, and that difficult concepts are best internalized by thinking, discussing, and working with them actively, instead of simply hearing about and/or memorizing them (von Glaserfeld, 1995; D'Avanzo, 2003). The challenge in designing laboratory activities is to foster active exploratory discussion and debate from which all students benefit.

Collaborative learning and discussion can be particularly important when students are working with difficult concepts, or those about which they have some misconceptions. It is common for students to hold onto misconceptions in the face of data that fail to support them (D'Avanzo, 2003), and also for the retention of misconceptions to hinder development of a correct understanding of such concepts (Driver, 1995). This has been a particular problem for the field of evolutionary biology, to which students bring a wide array of misconceptions (Anderson, Fisher, and Norman, 2002; Garvin-Doxas and Klymkowsky, 2008). In particular, analysis of evolutionary patterns in genetic sequence is problematic, as it relies heavily on a central understanding of mutation as a random process, while students want to assign a driving force of positive change to evolution (Anderson, Fisher, and Norman, 2002; D'Avanzo, 2003; Garvin-Doxas and Klymkowsky, 2008). We tend to discuss these ideas theoretically in class, without always giving our students active exercises working with genetic

sequence data, in order to force them to challenge, and move past, their misconceptions.

Population genetic analyses of nucleotide sequence data rely on the fact that the genetic code is degenerate, meaning that there are multiple nucleic acid substitutions that do not change the amino acid specified by a codon. Such silent mutations are called synonymous substitutions, and under most conditions are expected to occur at a random, baseline, rate that represents the rate of mutation, and thereafter be unaffected by selection pressure. On the other hand, substitutions that code for a different amino acid (nonsynonymous substitutions) could be subject to selection pressure. If no selection is taking place at a locus, variations are selectively neutral, and the ratio of nonsynonymous to synonymous substitutions (the dN/dS ratio) should represent the rate at which these mutation types arise and persist in a population by chance alone (Freeman and Herron, 2007). A population with excess nonsynonymous substitutions suggests that positive selection has occurred as part of an adaptive shift at the locus in question. Likewise, excess synonymous substitutions suggest that balancing selection has been maintaining the level of amino acid polymorphism in the population as it is, for example in highly conserved genes such as those central in development (Freeman and Herron, 2007). While the analysis of dN/dS is taught as part of a standard treatment of evolutionary genetics, students often have a difficult time internalizing the neutral model as a baseline, and understanding how sequences look under these different selection scenarios. A more complicated

analysis involves a comparison of coding and non-coding regions, such as introns, as selection pressures shouldn't affect the dN/dS ratio as they do in coding regions.

Due to its complexity, evolutionary analysis of genetic sequence is an ideal topic for an active learning exercise. Such an exercise can foster a deeper understanding of patterns of molecular evolution through hands-on work with sequences, and discussion of possible selective outcomes with peers. A well-designed exercise would provide students with multiple datasets, leading to different outcomes through which students would be able to observe and compare the different signatures of past selective events. With the vast array of freely available sequences, it is possible to obtain sequences for the same or related species, but with different signatures of past selection, so that multiple datasets may be used within the same class. Student pairs may each be given their own unique set of sequences, some of which will yield different outcomes, fostering student discussion about different types of selection and the conditions that lead to each.

The teaching exercise we've designed, which utilizes freely available computer software to analyze the genetic sequence data, is composed of four parts. As a result, it can be geared for students at different levels, from introductory biology to those taking a course in evolutionary biology or population genetics. In the laboratory exercise, students receive a unique set of sequences that includes coding sequence for some number of alleles (individuals) of the species of interest, plus one outgroup. Part I allows students to first examine the sequences, and get a feel for moving back and forth between nucleotide and amino acid sequence. In Part II, they perform a multiple sequence alignment, examine the aligned sequence for substitutions, and explore the difference between synonymous and nonsynonymous substitutions in the sequences. Part III uses the aligned sequences to generate a phylogenetic tree. Finally, in Part IV advanced students can perform a population genetic analysis for signatures of past selection, the McDonald-Kreitman test. This is one of the most widely-used tests that employs the nonsynonymous/synonymous substitution ratio in comparing aligned sequences from two closely-related species (McDonald and Kreitman, 1991).

Our laboratory exercise is a case study of allelic diversity based on the *Rps2* gene of the annual plant *Arabidopsis thaliana*. *Rps2* is a resistance gene that has a function in recognizing an infecting pathogen. Due to its role in resistance, there is a fair amount of variation maintained in the gene in natural populations (Caicedo, Schaal, and Kunkel, 1999; Mauricio et al., 2003). In the laboratory exercise,

students are challenged to characterize the substitutions in the *Rps2* gene from a set of samples: do the sequences from wild plants all look similar, or do some diverge? What is the pattern of relatedness among the samples? Do they find roughly equal proportions of synonymous and nonsynonymous substitutions, or does one type predominate? And from this information, can they determine whether selection has occurred in the past, and if so what type? Throughout, they can compare their results with those of classmates who have different datasets that provide different answers. While we have had good success with our exercise that focuses on *Rps2*, this general laboratory framework can be easily adapted to any number of species or genes of interest.

The computer lab exercise can be used as is, or modified for either more basic, or more advanced, students. All of the software necessary for this teaching exercise are freeware, and can be easily downloaded and installed on any PC. A basic version of the exercise, comprised of Parts I and II, has been tested in an Introductory Biology course during 2009, and a more advanced version that includes Parts III and IV has been tested in several smaller upper-level courses.

## MATERIALS AND METHODS

### Sequence for analysis

The laboratory exercise uses nucleotide sequence, and in its simplest version it uses only continuous coding sequence (no introns or untranslated regions). For more advanced classes, instructors may choose to use sequence that includes introns or untranslated regions, and allow students to compare them. Sequence in the standard FASTA format is usually available for your favorite gene from NCBI, EMBL or species specific data resources (for example, WormBase for *Caenorhabditis elegans* sequence). In order to obtain continuous coding sequence, it is best to use assembled CDSs, cDNAs or mRNA sequences; such processed sequence is usually available for common focal species for a variety of genes of interest in the common genetic databases. A great deal of sequence that includes introns or untranslated regions is also available through these resources. If instructors wish to have advanced students compare coding and non-coding regions as part of the exercise, they should choose to use sequence for which the gene of interest has been annotated and in which coding and non-coding regions are clearly identified; this is available for many important genes through these online resources.

### Software for student lab

The prepared student exercise uses three freely available programs which can be easily installed and run on a standard PC. BioLign is a user-friendly

program for sequence analysis and alignment, written by Tom Hall in consultation with the lab of Ed Buckler, and available at (<http://en.bio-soft.net/dna/BioLign.html>). Molecular Evolutionary Genetics Analysis, or MEGA, is a phylogenetic tool for biologists (Kumar et al., 2008), and is used in this exercise to build a phylogenetic tree with the alleles (<http://www.megasoftware.net/>). Finally, DNA Sequence Polymorphism, or DnaSP, is a software package for evolutionary analysis of polymorphism patterns in nucleotide sequence data (Rozas and Rozas, 1999), and is used in the teaching exercise to perform a McDonald-Kreitman test (available at <http://www.ub.es/dnasp/>).

### Student lab exercise

Objectives of the laboratory exercise:

At the completion of the (complete) lab, students will be able to:

1. Explain the difference between synonymous and nonsynonymous substitutions.
2. Discuss expectations for synonymous and nonsynonymous substitutions under different evolutionary scenarios.
3. Manipulate and align sequence, construct phylogenetic trees based on these sequences, and perform analyses in MEGA and DnaSP.
4. Describe the importance of outgroups in evolutionary genetics and molecular phylogenetics.
5. Complete a McDonald-Kreitman test for selection, and explain whether a coding region is likely to have evolved neutrally, or to have been undergoing positive or balancing selection.

The student exercise is best completed in a computer lab with the instructor present, to answer questions and pose further ones that generate meaningful discussion. Students may work individually or in pairs, however, we have found that working in pairs is very productive, in giving students an immediate opportunity to talk through each step of their results. Students need only the alleles file provided by the instructor (obtained through databases), and a computer with the above three freeware programs installed. A printer may be helpful to print the phylogeny and McDonald-Kreitman test output, but is not necessary.

Parts I and II of the exercise were used as a laboratory exercise in an introductory Biology course in spring 2009, and a follow-up assessment tested student understanding of the conversion between nucleotide and amino acid sequence; synonymous and nonsynonymous substitutions; and the technique of multiple sequence alignment. Students completing the computer laboratory exercise were compared with a group that completed a traditional module covering

the same information in a wet lab and by examining the identical sequences on paper and answering the same questions. Both groups were given a quiz after completion of the exercise, and their results were compared using a two-tailed T-test to detect a difference in group means. Additionally, students completing the computer lab were asked to rate the usefulness of the exercise in helping them to understand three different topics: the difference between synonymous and nonsynonymous substitutions; the difference, and connection, between nucleotide and amino acid sequence; and the process and usefulness of a multiple sequence alignment. In each category, they could rate the helpfulness of the exercise in four categories: A) Not at all helpful, I still do not understand; B) Somewhat helpful; C) Very helpful; and D) Does not apply to me—I already understood.

### RESULTS AND DISCUSSION

The computer lab exercise allows instructors to provide students with hands-on sequence analysis experience without purchasing costly programs for processing genetic data. The exercise can be tailored to an instructor's course goals and topic, in that the same multi-step exercise can be utilized for genetic sequence from any species or gene or interest. Additionally, since sequence data is freely and easily obtainable through NCBI's GenBank or other sources, this exercise can be used even if the instructor is a novice at such analyses or does not have access to his or her own sequence for analysis.

The modular design of the accompanying computer lab exercise affords instructors a great deal of flexibility to tailor the exercise to the level of a particular course. Parts I and II of the lab, the exploration and alignment of the sequences, have been adapted to serve as an introductory DNA sequence lab in a first-year Biology course; the entire lab has been used as designed in an upper-level course.

Parts I and II of the lab exercise were very successful in helping introductory-level students understand basic sequence analysis. The exercise was used during two laboratory sections in a first-year Biology course, replacing a wet lab and sequence examination exercise that had been used in the past and was still utilized in two additional laboratory sections. Students completing the computer sequence analysis lab scored significantly higher on a follow-up quiz testing understanding of codons, mutations, synonymous vs. nonsynonymous mutations, and sequence alignment than did their counterparts who completed the traditional lab (Table 1;  $t=3.622$ ,  $df=52$ ,  $p=0.0007$ ). Of 28 students completing the computer exercise and a survey, 78.6% found it helpful (either "somewhat" or "very

helpful”) in understanding the difference between synonymous and nonsynonymous substitutions, 89.2% found it helpful in understanding the connection between nucleic and amino acid sequence, and 82.1% found it helpful in understanding the process and usefulness of a multiple sequence alignment. Students completing the computer lab additionally reported that they appreciated seeing how “real geneticists” analyze sequences, and that the lab exercise felt “more advanced” and “like real science” than an exercise looking at sequences on paper.

The approach of this lab exercise provides a near infinite range of possibilities to the instructor who wants to adapt it for a unique set of goals, while still providing an off-the-shelf possibility for the instructor with less experience with population genetic analyses. It is easy to use, requires no investment in computer programs or supplies, and gives students realistic hands-on experience working with sequence data. Further, it preserves the exciting mode of scientific discovery, as each group can be given a different set of alleles, adding a notable dimension to the exercise in which each student or

**Table 1.** A comparison of quiz scores for students completing the computer sequence analysis exercise with those who completed the traditional laboratory exercise covering the same material. The sample size, average score and standard deviation, and range of the number of points (out of 70 points total) on a quiz covering codons, mutations, conversion between nucleotide and amino acid sequence, synonymous and nonsynonymous substitutions, and the technique of multiple sequence alignment.

	Sample size	Average $\pm$ 1 SD	Range
Traditional exercise	26	49.3 $\pm$ 8.2	30 - 61
Computer exercise	28	56.6 $\pm$ 6.6	40 - 70

The full population genetics laboratory, including parts III and IV, was also used in a small upper-level course in which students reported that it was helpful in understanding phylogenies, and in evaluating how the  $d_N/d_S$  ratio yields evidence of past selection. Due to the small class size, however, a comparative assessment was not possible, as all students completed the computer exercise.

The advanced version of the laboratory exercise, including population genetic analysis, is particularly well-suited to a jigsaw-type active learning exercise. A jigsaw activity is one in which students begin working in topic groups, each with its own unique challenge or topic, to solve a particular problem and become experts on it (Aronson et al., 1978; Perkins and Saris, 2001). Once these topic groups have worked together to each form a complete understanding of their topic—or in this case, dataset and mode of selection—they reorganize into groups composed of a single individual representing each of the topic groups. In these reorganized jigsaw groups, students each explain their own group’s unique dataset and conclusions, taking turns being the expert (Aronson et al., 1978; Perkins and Saris, 2001). This type of collaborative approach in which different students possess unique pieces of the puzzle has recently been used in systems biology and was shown to be beneficial (Kumar, 2005). Our experience suggests that this exercise works well as a jigsaw, and in the future we would like to test its efficacy in advanced courses of the appropriate size for collection of outcomes data.

group has to reconcile his or her own results with the differing results of others. Lastly, it is important in providing an opportunity for students to delve deeply into a topic that many find confusing and laden with misperceptions. Through studying their sequences and performing these analyses, students can develop a more intuitive understanding of the meaning of neutral variation and the effects of selection on genetic sequence data.

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# Emphasizing the “Literacy” in “Scientific Literacy”: A Concise Blueprint for Integrating Writing into Biology Classes

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**Abstract:** Effectively integrating writing into biology classes gives students the opportunity to develop a better understanding of and engagement with course content. Yet many instructors remain reluctant to emphasize writing. Some are concerned about the time commitment writing assessment requires. Others shy away from emphasizing writing in their classes because they have limited experience doing so and no training in responding to student writing. Here, drawing on both research and direct experience, we address those concerns, providing a justification and concise blueprint for integrating writing into biology classes in such a way as to maximize the benefits to students and minimize faculty effort. As a starting point, we suggest that faculty approach student writing as attempts at communication rather than as texts in need of proofreading. Building on that starting point, we introduce the main classroom practices, assignments, and pedagogical considerations that have yielded benefits, including drafting and revision techniques, rubrics, sample questions, a prioritization scheme for responding to student writing, and time-management tips for faculty. Instructors can use these strategies singly or in combination to add a more significant writing component to their classes.

**Key Words:** Course Development, Writing Assessment, Critical Thinking

## INTRODUCTION

In our experience, students frequently misunderstand the nature of disciplinary boundaries—believing, for instance, that writing is only important in English classes. Instructors, however, typically recognize the importance of students’ core-area abilities to their performance in biology courses. Students require considerable fluency, e.g., to discern the shades of meaning needed to correctly answer well-crafted multiple choice questions or write effective essays. Consequently, we observe that various student assignments succeed or fail on the basis of both biology competence and linguistic fluency. Routinely, however, some biology students still imagine that only English classes require “good writing,” however they might define that phrase. At one public university, 74% of biology faculty surveyed rated scientific writing as an important component of undergraduate education. Although 63% of students surveyed agreed, of the eight factors assessed, students rated only one, a historical viewpoint on biology, lower than they did scientific writing (Marbach-Ad and Arviv-Elyashiv, 2005). These data indicate that a significant minority of students markedly de-emphasize writing’s importance.

We biology educators—and educators in general—rather than our students bear most of the blame for that misconception. More than half of the

biology students surveyed placed an emphasis on scientific writing (Marbach-Ad and Arviv-Elyashiv, 2005); by not correcting the minority’s misconception, biology faculty passively encourage its spread. Research also suggests both that a majority of students at more selective universities want to strengthen their writing and that they consider writing instruction most effective when it is organized around a “substantive discipline” (Light, 2001: 54) such as biology. Yet in our experience due in part to time limitations, faculty who teach large introductory classes find it difficult to rely on writing-intensive assessment. As a consequence, our students’ foundational experiences usually center on easily scored short answer questions. Since students need only develop the minimum language skills necessary to pass such introductory classes, their faulty assumptions about the necessity of core-area abilities appear justified. Because short answer questions tend to emphasize rapid response over reflection, they also discourage the more in-depth understanding of course content that writing assignments foster (Paxton, 2000).

Biology faculty routinely note the poor quality of student writing (e.g., Moore, 1992), often while declining to take action to improve the situation. Some assume that English departments bear responsibility for developing underprepared students’ writing competence. Others would like to help but feel unqualified (Marbach-Ad and Arviv-Elyashiv,

2005). In our experience, some colleagues see so many problems in their students' writing that they don't know where to begin, despite accepting that they should try to help.

As a first step we suggest that faculty concerns—and complaints—about student writing are also concerns about students' sometimes uncertain grasp of biology. By adopting a more writing-intensive assessment strategy, faculty can address these overlapping concerns (Curto and Bayer, 2005). After exploring the connection between these issues, we address writing assessment: the area about which we have found faculty express the most uncertainty. Subsequently, we address a broader range of classroom practices, assignment design issues, and pedagogical considerations in order to provide a broad perspective on how biology faculty can best integrate writing into their teaching. We conclude with suggested strategies suitable for a variety of classrooms.

Although we review the relevant research in light of our own experiences in seven biology departments, eight language and literature programs, and two writing centers and provide advice for its application, we do not offer an exhaustive list of references. Our purpose is to provide a concise, user-friendly blueprint for colleagues addressing the concerns that have attracted our professional attention. We do not expect practicing biologists to table their research programs while they develop expertise in writing instruction. But we provide readily accessible avenues for further exploration, referencing representative sources that reflect the standard practices of process-oriented writing instruction and, wherever possible, open-access online articles and books commonly available in

academic libraries.

### **Bloom's Taxonomy and the Gap between Short Answer and Written Response**

Bloom's Taxonomy (or the Taxonomy of Educational Objectives) hierarchically classifies educators' objectives in three domains (i.e., affective, psychomotor, cognitive) (Anderson and Krathwohl, 2001; Bloom, 1956), the last of which is relevant here. Cognitive domain objectives range from recollection to three progressively higher order tiers: understanding/comprehension; application; and analysis/evaluation/creativity. Although there is disagreement about the categorization of these domains as higher- or lower-order cognitive activities (e.g., Paul, 1990), many faculty recognize similar hierarchies. Remembering facts, for example—which short answer questions typically emphasize—typically merits less credit than analyzing or applying course content to novel problems in essay responses. Instructional practices can reflect a comparable pedagogical stance, as is demonstrated by an excerpt from an upper-level ecology syllabus (Figure 1).

In our experience, undergraduates frequently have limited preparation for biology classes integrating a significant writing component. Such a situation can become particularly problematic when writing-intensive assessment requires critical thinking (e.g., the application of foundational knowledge to a novel problem). In such cases, the mnemonic training of introductory class multiple choice exams provides virtually no preparation. Thus, some students struggle in transitioning from multiple choice questions (which provide correct answers and, typically, a 25% chance of a correct guess) to situations in which they must formulate responses

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Exam questions will fall into three categories or levels:

**Level 1.** These direct, straightforward questions will test your **knowledge** of key ecological principles and concepts. Approximately 70% of the exam questions will fall into this category. In order to receive a grade of "C" you will be expected to correctly answer all of these questions.

Example: *How are populations of "wild" animals regulated?*

**Level 2.** These questions will test your ability to **apply** ecological principles in a variety of different situations, some of which will not have been discussed in class or in your textbook. Approximately 15% of exam questions will fall into this category. Students who receive a grade of "B" or better will be able to answer Level 1 and Level 2 questions.

Example: *How might density influence population regulation mechanisms?*

**Level 3.** These more complex questions will test your ability to understand the **interrelationships** between different ecological principles and their various applications. Approximately 15% of the exam questions will fall into this category. Students wishing to receive an "A" will be expected to answer Level 1, 2, and 3 questions.

Example: *How might population density and habitat quality be related?*

**Fig 1.** Excerpt from the syllabus for a 300-level ecology class. At the university in question, the course is required for biology and environmental science majors. The "levels" of questions the syllabus describes generally parallel Bloom's Taxonomy in identifying a hierarchy of learning objectives. In addition, the explanation clarifies to students that higher course grades can only be earned if students demonstrate competence in activities that correspond to the "higher" learning domains. The course in question requires writing on all exams and in lab, including a final lab paper analyzing and interpreting data collected by the students.

independently.

The potential gap students face is summed up by a note one student left on an exam in a required upper-level cellular biology class: “Why can't you at least have some multiple choice? Sometimes I forget the exact words your [*sic*] looking for but would recognize it if I saw it.” Yet the exam required responses no more than 2-3 sentences long. The student's note emphasizes the gulf between quick-to-score short answer assessment and questions that require more input, from factual recall to critical thinking. The student's difficulty was with content, a problem that written responses are far more likely to reveal than multiple choice questions. Yet given the level of discomfort and anxiety with which so many approach writing and its assessment, faculty can be tempted to reduce or entirely eliminate writing in their classes. But sound strategies for assigning and assessing writing yield so many benefits that we are far better off resisting that temptation.

### **A First Priority in Assessing Student Writing: Response Not Proofreading**

We begin this section with a worst-case scenario. A writing instructor at a community college where one of us was a writing center tutor graded papers solely on the basis of grammar and punctuation. Each error resulted in a set deduction (e.g., 1 point for a missing comma, 5 points for a sentence fragment). Content was irrelevant. A thoughtful essay that repeated a few types of comma mistakes could earn a lower score than an unfocused, poorly reasoned essay that relied exclusively on simple five-word declarative sentences to avoid errors. The professor mentioned several times (and with evident delight) that his record was a grade of -163 on a 100-point scale.

While grammar and punctuation are important, the scenario described above represents an extreme end of the response spectrum that biology faculty should avoid. In addition to representing a worst-case scenario pedagogically, this example demonstrates the value of following a Hippocratic-oath inspired maxim: whatever else your feedback does, start by taking care that it does no harm. Fear of error can lead inexperienced writers to performance anxiety so severe that they never manage to answer our questions (Bartholomae, 1985; Shaughnessy, 1977). Overemphasizing error also sends the same message as the instructor in the example above, that “good writing” is “correct writing”—an impression that has long been prevalent among students because it reflects the feedback they typically receive (Shaughnessy, 1977; Sommers 1982). Composition theory recognizes that an excessive emphasis on error is unreasonable for inexperienced writers—both because they are still learning writing's rules and

because few readers share a proofreader's concern with error (e.g., Huot, 2002; Williams, 1981).

When integrating writing into your classes we suggest a two-part approach, focusing first on content and subsequently on grammar, punctuation, and format. Begin by recognizing that your primary goal is to respond rather than proofread. Doing so allows students to write without worrying unduly about grammatical error, frees you from developing grammatical expertise, and allows you to focus on writing's main purpose—communicating ideas (Williams, 1981). Responding primarily to content encourages student investment in both writing and course content by taking student writing seriously as an expression of ideas. The prioritization of content is pedagogically sound, benefiting both us and our students (Curto and Bayer, 2005; Huot, 2002; Straub, 2000).

Focus on addressing errors that inhibit communication. Although writers will self-correct errors as they gain experience (e.g. Shaughnessy, 1977), inexperienced writers have not yet attained the best writing of which they will be capable. Instructors can best facilitate the self-correction process by giving feedback that sends the message, “I want to understand your ideas, but the writing here made that hard for me” rather than leaving the impression, “Your writing here breaks the rules of grammar, so what you have to say may not be worth reading.” The latter emphasis can easily discourage and exclude students who most need effective invitations to participate.

Emphasizing content does not require that we ignore presentation. It simply encourages us to address presentation within a broader context, understanding that for less experienced writers grammar or punctuation feedback is most useful in moderation. Since so many writers are at least mildly anxious about errors, it can be useful to bypass a focus on error in favor of strategies that allow students to strengthen their writing by making it clearer and more concise (e.g., “Best Practices” below).

In addressing error with inexperienced writers, prioritize fundamental mistakes centering on the sentence as a unit—i.e., sentence fragments (partial sentences punctuated as complete sentences), run-on sentences (two or more conjoined sentences punctuated as a single sentence), and comma splices (two sentences incorrectly linked by a comma) (Moore, 1992). Shifting verb tenses and errors in subject-verb agreement are generally seen as less serious. Failing to maintain parallel structure within individual sentences represents a still less serious problem. Our prioritization of error will vary, reflecting our particular concerns (Moore, 1992). But

one way to assess the seriousness of errors is to read student writing with a more trusting eye; errors that impede our ability to understand the writer's ideas merit more attention than those we would typically overlook in a peer-reviewed article (Williams, 1981).

Whatever one's preferences, focusing on patterns of error typically yields greater benefits than attempting to identify all errors (Bartholomae, 1980; Kroll and Schafer, 1978). Writers tend to repeat errors due to misunderstandings about grammar or punctuation rules. Identifying the most serious and frequently occurring error types allows students to prioritize their efforts, yielding maximal improvement by clarifying the underlying rules being violated while keeping the writer's task from becoming overwhelming (Straub, 2000). It is similarly helpful in making assessment more manageable; by focusing on content and addressing only the most serious one or two recurring punctuation/grammar errors, instructors need not provide detailed proofreading and editing.

### **Writing: Formal vs. Informal**

By distinguishing between different sorts of writing we can encourage our students to better understand biology and help ourselves to provide appropriate feedback. One relevant distinction is between formal and informal writing, analogous to Elbow's (2000) distinction between high stakes and low stakes writing. Classes that rely on a balance of formal and informal writing encourage a reasonable perspective on error. Formal writing requires adherence both to grammar/punctuation and assignment-specific format requirements (e.g., citation methodologies). Formal writing (e.g., research papers, lab reports) emphasizes both content and presentation. Informal writing (e.g., a journal response to assigned readings) de-emphasizes presentation (e.g., grammar, format) while emphasizing content.

The distinction between these writing modes has important pedagogical consequences. Informal writing can give students valuable experience with writing in contexts that de-emphasize mechanical correctness and formatting, perhaps reducing their anxieties about writing. Informal writing also provides opportunities—particularly when students can write without strict time limits—to engage with, synthesize, and analyze course content. Informal writing (e.g., journal responses to readings) can also alert instructors in advance of formal assessment situations about the content with which students are having difficulty.

In order to obtain maximal advantage, we should provide appropriate responses to these different sorts of writing. On an exam, for example, requiring students to define terms using complete sentences

rather than independent clauses would unproductively emphasize grammar/punctuation over content comprehension. It is neither realistic nor productive to expect students writing under the stress and time constraints of exams to demonstrate the performance typical of less time constrained conditions. In some respects, then, while exams are among the most formal writing contexts, they can be productively assessed as informal writing. Thus, if a student's exam essay demonstrates an exceptionally high degree of biology competence, it would merit an "A" despite errors in spelling and grammar. Conversely, however polished the writing, a response failing to demonstrate minimal biology competence cannot merit a "C."

### **Drafts and Drafting**

A gulf exists between the theory of writing instruction, which emphasizes drafting and revision, and grading, which emphasizes assessment of final products. This divide can be apparent even in English departments, where professors may teach graduate student composition instructors the necessity of acclimating undergraduates to revision. Yet literature professors in the same departments might never mention revision or respond to student drafts. Biology faculty can profitably avoid this oversight.

Some might object that since they already lack the time necessary to assess writing, they certainly cannot assess it more than once during its production. But a method for avoiding this largely theoretical hurdle is an established feature of process-oriented writing instruction: dedicate the majority of time allocated to providing feedback on formal writing assignments to drafts. The time allotted to assessment remains unchanged but is reallocated. While feedback on graded writing arrives too late for many students to see it as anything other than an unfortunate post mortem, they can use feedback on drafts to produce stronger finished work. This general strategy can be tailored to fit a variety of situations. For instance, Chuck and Young (2004) conclude that formal assessment and text-specific feedback on draft reports led to improved student writing when students were given time to reflect on their work. Chuck and Young (2004) also report that while the average assignment took longer to complete, since they achieved target goals for writing improvement using fewer assignments, responding to drafts resulted in a net time savings.

Teaching writing as a process- rather than product-oriented activity involves a variety of potential strategies, most beyond the scope of the current discussion. (For brief introductions to teaching revision see Heard (2002) and Willis (1993).) One additional point is central, however: we have a better chance of successfully integrating

writing into our classes if we recall a general truth about undergraduates. Whether students consider themselves “good” or “bad” writers, they typically do so without recognizing that their first drafts rarely represent their best work (Emig, 1971; Willis, 1993). Our students’ most common misconception about writing is that “good” writers produce flawless texts quickly and effortlessly. Emphasizing the drafting process demonstrates that effective writing is achieved via regular effort extended over days or weeks rather than a single stressful evening.

### **Best Practices: Starting Points**

Numerous strategies increase the likelihood that our students will benefit from writing activities as they develop subject-area competence. Above and beyond particular options for use in biology classes that have been reported in detail (e.g., Chuck and Young, 2004), we conclude with several suggestions. These options—organized by the type(s) of assignment for which they are most appropriate—can be integrated into existing classes singly or in combination.

### **For Papers or Lab Reports:**

*Grading Rubric:* To make grading less mysterious and the writing process more productive for students, provide a rubric that includes the specific point breakdown you will use during grading. On a 100-point assignment, for instance, grammar/punctuation might count for 5-10 points, while clear articulations of the null and alternate hypotheses might count for 10-20 points. By quantifying our emphasis on various aspects of assignments, students can more effectively work to the standards we will apply (Mansilla et al., 2009).

Grading rubrics also simplify and speed the responding process while facilitating consistency, as was demonstrated for one of us who used a rubric in an upper-level Darwinian medicine class. One of the 90 students submitted identical copies of the 3-5 page research paper. Although the double submission went unnoticed until grades were being recorded, the two copies—graded a week apart—scored within 1.5 points of one another.

*Benefits:* reduced student anxiety; clarification of grading standards; faster, more consistent evaluation

### **For Papers:**

*Short Research Paper Revision Exercise Using Strunk and White:* Strunk and White’s *Elements of Style* (1918) is a public domain resource (<http://www.bartleby.com/141/>). This strategy uses chapters 9-13, an introduction to improving short writing assignments that covers organization, conciseness, and active voice. These short chapters consist mainly of brief examples that can be included in handouts or online posts. Although this strategy is described in terms of short papers, it is suitable for

other writing assignments. Guiding writers through the process of shortening a document by 30%-50% while making the writing more effective provides an exceptional learning opportunity.

Once students receive preliminary approval for their research topics, they consult Strunk and White (1918) chapters 9-10 in preparation for submitting 1-2 page synopses and references. After final instructor approval, students develop 4-5-page drafts. Students consult Strunk and White (1918) chapters 11-13 before and during the drafting process, making independent progress on conciseness. Instructors can quickly highlight areas requiring student attention (e.g., by writing the number of the relevant Strunk and White chapter(s) in the margins), allowing students to take responsibility for revisions and effectively produce 2-3-page final drafts.

Many instructors find Strunk and White’s prose especially clear, as do many students who use it to address the significant hurdles of identifying an appropriate voice and tone for their writing (Bartholomae, 1985). Nonetheless, some students might have difficulties—e.g., reconciling their expectations of what constitutes specific, concrete language with Strunk and White’s preferences (Ohmann, 1979). It is advisable to address the material in advance of and/or during the drafting process. Key points and examples can be covered in one 30-40-minute PowerPoint presentation/discussion or several shorter sessions. If time permits, use sample revisions from current students to demonstrate the strategy’s viability.

*Benefits:* students develop revision strategies without the need for grammatical expertise; instructors can quickly provide text-specific response to student writing

### **For Exams, Papers, Journal Entries, or Homeworks:**

*Sample Responses:* Identify an essay question you like but are willing to retire from use on exams. (It will be used to good educational effect, so you won’t be losing it.) After covering the relevant material, allow students to formulate practice essay responses individually and/or in small groups. Once students finish, provide sample answers (e.g., as handouts or PowerPoint slides) that range from A-level to F-level work. Allow students to read and discuss all sample responses before identifying/explaining the grade each response received.

*Benefits:* reduced test anxiety; reflective thinking/communication about course content; grade norming/clarification of grading standards

### **For Exams:**

*Sample Question Assignment:* As part of exam preparation, students write several multiple choice

and essay questions appropriate for an upcoming exam. In class discussion, explore the questions' strengths and weaknesses, possible revisions, and reasons why proposed questions are particularly appropriate/inappropriate given the emphases of the class and your assessment approach. Submitted questions can be graded as homework assignments, often with minimal written feedback because many questions will have been discussed in class.

*Benefits:* students receive clarification about requirements and develop more familiarity with course content; faculty obtain ideas for exam questions

## CONCLUSION

Integrating more writing into a class does not require that we abandon existing plans and assignments. Rather than moving suddenly into unfamiliar instructional territory, transition gradually. As you do, integrate assignments/activities using instructional strategies that will increase your students' likelihood of succeeding. Remember: students will require assistance and guidance engaging with course content outside of a short-answer-only paradigm. To assist with this process, we include a list of reminders (Table 1).

Understand in advance that regardless of your committed effort and pedagogically sound approach, some writing will merit failing grades. In one crucial respect, grading writing is similar to grading short answer questions: failing grades need not prove damaging for students. Quite the contrary: students need to know when their work does not meet minimum expectations. If we refrain from assigning

the low grades that students' work merits because we are unduly concerned with potential negative impacts, our good intentions risk doing students harm rather than good.

Inflated grades may rise to whatever level students consider acceptable. Such a situation is better avoided. It will give some students no compelling reason to improve, since their current performance has already proven sufficient. Grades that accurately assess students' work make clear that additional effort is not optional but necessary. Elbow (1983) addresses the sometimes conflicting demands of teaching—on one hand to the students whose intellectual growth we seek to foster and on the other hand to the disciplines whose standards our grading is meant to reflect.

Elbow's (1983) conclusion—that only by having high expectations of our students can we truly serve them—remains a useful touchstone as faculty integrate writing into their classes. Our emphasis, however, is not that we can help students improve their writing—that laudable goal remains an ancillary benefit. The best reason for integrating writing into our classes is that doing so gives students the opportunity to better understand biology. Creating a learning environment within which students write about, read about, and discuss course content will make them more literate, but it will also help them learn biology (Curto and Bayer, 2005; Krajcik and Sutherland, 2010; Saunders and Sievert, 2002).

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**Table 1.** Final Reminders

1. Clarify your expectations for what constitutes exceptional, acceptable, and failing work and articulate the criteria you will use in assessing student writing. Present examples of sample graded writing to students and discuss that writing with them. Develop grading rubrics and share them with students.
2. Spend at least 50-75% of the total amount of time you will allocate to providing feedback on writing assignments to response you provide before final grading. Require and respond to drafts of complete assignments or draft sections of longer assignments.
3. In responding to student writing, focus first on content—and remind yourself regularly that content is your main interest. When you respond to drafts, take particular care in this regard: while writers are still in the early stages of exploring, organizing, and clarifying their thinking about a subject, their work is subject to major revision. There is little to be gained by providing in-depth revisions of passages that will end up being deleted.
4. Consider incorporating a question development component to your classes in which students propose possible questions for exams and papers. Discuss the strengths and weaknesses of the questions students propose so that they can develop a sense of what topics are most important in your classes and what types of questions they can expect.
5. Recognize that some assignments merit failing grades, and don't be hesitant to assign them when appropriate.

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# Independent Gene Discovery and Testing

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**Abstract:** A clear understanding of basic gene structure is critical when teaching molecular genetics, the central dogma and the biological sciences. We sought to create a gene-based teaching project to improve students' understanding of gene structure and to integrate this into a research project that can be implemented by instructors at the secondary level or higher. We modified a previous project performed within a genetics course in which students designed and tested polymerase chain reaction (PCR) primers based on expressed gene products from specific biochemical pathways. Students used these skills to investigate a gene that they hypothesized would be related to the multiple sclerosis phenotype in humans. They were each required to identify the gene structure (exons and introns) of their selected gene and to design primers around these elements. A total of seven genes hypothesized to interact with the Multiple Sclerosis (MS) phenotype were used to design 154 primers (77 forward and 77 reverse). Based on the structure of each gene, these primers were combined as 83 unique pairs and tested by seven students. A total of 25 pairs were visibly polymorphic and 58 pairs were monomorphic. All materials, including primer design protocols, modifiable gene graphics files, screening preparation documents and assessment information are presented to allow replication of this project.

**Keywords:** Undergraduate research, Gene structure, PCR, Primer design, Independent project

## INTRODUCTION

### Goals, Expected Outcomes and Background

The central dogma was first proposed by Francis Crick in 1958 (Crick, 1970; Crick, 1958). It proposed a relationship between DNA, RNA and polypeptides. The transfer of information between these molecules is vital to living organisms and can be used to understand sources of biological data and how they are obtained. It is also very useful when describing how the polymerase chain reaction relates to the replication of DNA *in-vivo*.

An understanding of gene structure is critical when describing the central dogma, transcription, post-transcriptional modification of mRNA and translation. In order to fully illustrate this concept, we sought to introduce students to several key research resources, including online biochemical pathways at the Kyoto Encyclopedia of Genes and Genomes website, expressed sequences attributed to the genes in these pathways, bioinformatics in the form of BLAST at the National Center for Biotechnology Information (NCBI) and designing primers for the polymerase chain reaction (PCR) using the Primer3 program (Rozen and Skaletsky, 2000).

Expressed sequences are used extensively to create libraries of genes for different organisms. Of primary importance is the fact that these genes have been through the transcription process within the organism. Comparing these transcribed and edited sequences to known gene sequences (even those from

another organism) allows their identification as an enzyme in a particular pathway.

A drawback of these sequences is that they represent mature mRNA product, from which introns have been removed. This makes an understanding of the actual chromosomal structure difficult to discern without further analysis.

A comparison between the mature mRNA product and chromosomal DNA using the BLAST utility at NCBI can be used to determine the chromosomal location (in base pairs) of the gene and its introns and exons (NCBI, 2009). This information can be used to generate an ideogram of the gene and to illustrate how a gene can be post-transcriptionally modified.

The polymerase chain reaction exponentially replicates DNA with specificity derived from short oligonucleotides (primers) designed from the target DNA. In order to illustrate this procedure, students can design and test primers based on information obtained from a comparison of expressed vs genomic DNA.

### Objectives

We sought to develop a procedure that integrates several aspects of scientific research within an overall educational goal of understanding how genomic sequence, pre-mRNA, introns and exons relate to each other. The introduction of students to as complete a research experience as possible within the confines of an academic year was an equal goal. In order to accomplish these tasks, we executed the

project in four steps, which included planning, grant writing, wet-lab research and reporting results.

Planning included the identification a gene of interest within a biochemical pathway, followed by the creation of an ideogram delineating exons and introns within the genomic region. After a gene was selected and described, students were expected to perform limited grant writing using a template document, indicating reasons for selecting their gene and incorporating their gene ideograms and primers designed into the grant proposal. If approved for funding, these primers were to be ordered and tested for polymorphism. Successful project completion was recognized after presentation of their research results at a college research symposium.

## **MATERIALS AND METHODS**

### **Equipment and Consumables Required**

The following equipment is required to perform this project: A computer with Internet access, thermocycler, horizontal electrophoresis gel rig and power supply, mini-centrifuge, heat block/water bath/incubator, pipettors and documentation capability. Consumables include: PCR tubes, Pipette tips, agarose, TBE Buffer, Taq Master Mix, TE buffer and micro-centrifuge tubes. Students taped their gel pictures into a combined lab notebook as they were generated.

### **Source of Human DNA**

The DNA used for this study was provided by students participating in undergraduate genetics labs at Louisiana Tech University. All DNA was extracted using the included protocol and information such as height, weight, eye and hair color, birthplace and family history of MS or Cystic Fibrosis was obtained. As described in Supplemental File 1, students performed an oral saline rinse, followed by extraction using the BioRad Instagene Matrix (cat #732-6030). This project was approved by the Human Use Committee of Louisiana Tech University. All samples were re-labeled to maintain anonymity and all participants signed a Human Subjects Consent Form.

### **Gene Selection**

Students were presented with basic information about the human multiple sclerosis phenotype (Supplemental File 2) which included hyperlinks to proposed pathways. After reading this brief synopsis, each student was encouraged to investigate a gene in a pathway that they felt might be a contributing factor to the MS phenotype. Once a gene was identified, students used the KEGG (2009) database to obtain expressed sequences attributed to that gene. Sequences were compared with the human genome using the BLAST utility on NCBI (2009), comparing against the Human genomic plus transcript (Human

G+T) sequences, allowing the creation of gene-specific figures that show exon and intron boundaries (the template for gene figures is also included in Supplemental File 2). The genomic sequence related to each gene was saved and used to design PCR primers.

### **Primer Design**

Primers were designed using the genomic sequences for each gene. All primers were created with the Primer 3 program (Rozen and Skaletsky, 2000) using the default settings. A detailed primer design and rehydration protocol is provided as Supplemental File #3. All primers were ordered from Integrated DNA Technology (IDT; Coralville, IA) at the 25 nmole scale.

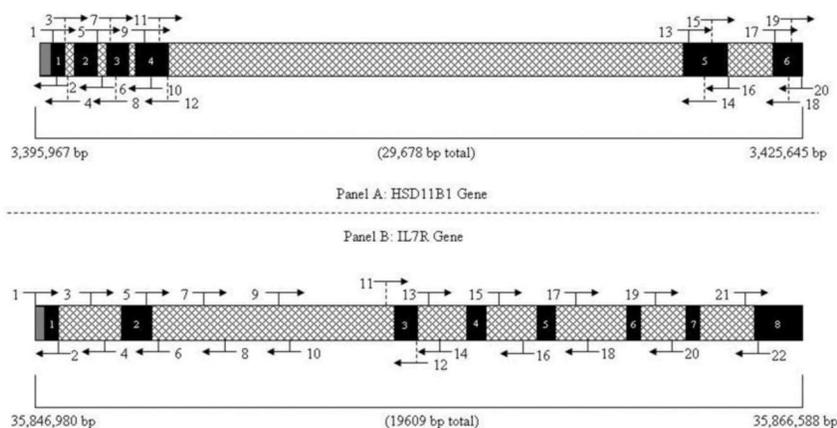
### **PCR Conditions**

Components in each reaction included 10  $\mu$ l 2X PCR mix (cat # M7122, Promega, Madison, WI), 3  $\mu$ l H<sub>2</sub>O, 5  $\mu$ l DNA (5-10 ng/ $\mu$ l) and 1  $\mu$ l each of 10 mM forward and reverse primers. PCR conditions varied, based on procedure (testing for function or optimization) with lower annealing Tms used for non-functioning primer pairs and higher annealing Tms used for products that produced multiple or unclear banding patterns. The standard conditions were an initial 94° C denaturation for 5 min, followed by 36 cycles of denaturing (94° C for 60 s), annealing (50° C for 75 s) and extension (72° C for 45 s). After a final extension of 5 minutes at 72° C, PCR product was loaded into a 1.5% agarose (cat # BP160, Fisher Scientific, Waltham, MA) prepared with ethidium bromide (0.1  $\mu$ l:20ml gel matrix). Electrophoresis was performed @ 4v/cm for 3-4 hours in 0.5X TBE (TRIS:Boric Acid:EDTA) buffer and documented with a UVP Gel documentation system (Upland, CA).

## **RESULTS AND DISCUSSION**

A total of seven students each investigated a single gene that they selected from a pathway, identifying introns and exons within the IL-7, CD28, HSDB3B1, HSD11B1, IL7R, NMUR1, PKA and TSHR genes using genomic sequence and the BLAST program. Examples of two student-designed gene ideograms (HSD11B1 and IL7R) are shown in Figure 1. Students designed PCR primers based on data shown in these figures (example primers from the two genes in Figure 1 are shown in Table 1).

A total of 154 gene-based primers were designed and tested by seven students. Of the 83 tested combinations, 25 were visibly polymorphic and 58 were monomorphic (Figure 2). Although designing primers based on mature mRNA sequence is effective (Shultz, 2008), the likelihood of PCR not working because a genomic primer spans exon boundaries (panel A, Figure 3) or amplifies unknown lengths of



**Fig. 1.** Graphic representation of 29,678 bp of the human HSD11B1 gene located on chromosome 1 (panel A) and 19,609 bp of the human IL7R gene located on chromosome 5 (panel B). Exonic sequence is numbered and represented by solid black fill, intronic sequence is “hashed” and the promoter region (-100 bp upstream) is grey.

intronic DNA (panel B, Figure 3) can be eliminated by determining the structure of the gene prior to design.

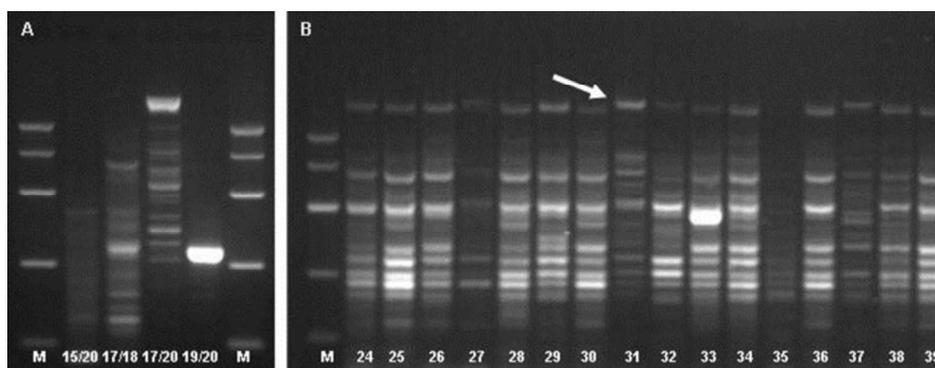
There are two ways to look at the data generated from this research. Multiple products from each sample can be useful because they increase the chance of identifying polymorphism. Multiple products, however, are difficult to replicate using agarose detection methods and must be considered transient in nature. If a polymorphism is observed within multiple bands, it should be excised from the gel and stored. By storing the detected band you make it possible to sequence the product and design primers that amplify that fragment only. Single amplification products are easy to replicate, but generate fewer polymorphisms. If polymorphic between samples, these single products require no further processing to be useful as molecular markers. If a single amplification product is monomorphic, it is still useful in SNP genotyping as a pre-detection step (Lee et al., 2004; Shultz et al., 2008).

This procedure can be performed as several steps, each of which entails increasing educational benefit and cost. The initial identification of exon and intron boundaries is a free process requiring computers with access to the Internet. An additional step of identifying alternatively spliced mRNA can also be performed without cost. Although the design of primers is free, there would be limited instructional usefulness to this step without ordering the oligonucleotides. Initial testing of the primers can be performed at a limited cost (approx \$0.20 per reaction), but requires the hardware resources listed in the materials and methods. The primers themselves represent a cost of \$0.20 – 0.30 per base (we typically estimate \$5 each). Additional testing can be

performed on multiple DNA samples, creating a population genetics study but also increasing the cost as primers are used on an expanded DNA set. Additional work can be based on primers found to be polymorphic, such as sequencing of products and/or detection of single nucleotide polymorphisms (SNPs), but this expansion of the protocol requires significant equipment expenditures and/or vastly increased consumables cost, and would not typically be appropriate for course-based undergraduate research.

In this study, the instructor decides what phenotype will be the focus of the investigation. This is supported by specific information in Supplemental File #2 for multiple sclerosis. A strength of this procedure is that by modifying only this file, an instructor can focus on their own phenotype of interest. The difficulty level of this project can be increased substantially by requiring students to create their own summary file, then act on their own collected information to select a gene of interest.

Because this research involves consumables such as agarose, pipette tips, buffers and other miscellaneous supplies, a faculty mini-grant equivalent to four student mini-grants (~\$500) was also applied for and received. These funds were available for use within the project and included additional funds for primers in case fine mapping of a polymorphism was necessary. In this study, one of the student mini-grants was declined due to a technicality. A portion of the faculty mini-grant was used to cover this particular student’s costs.



**Fig. 2.** Panel A) Initial test of primer function for 4 primer pairs within the HSD11B1 gene. Primer pairs tested based on proximity to each other predicted by the configuration shown in Figure 1. Panel B) Test of primer pairs from the HSD11B1 gene on members of a population of 46 samples. A polymorphic product is indicated by a white arrow for primers 17 and 20 (subjects 24-39). The “M” annotation indicates a size standard of 100, 250, 500, 750 and 1000 bp.

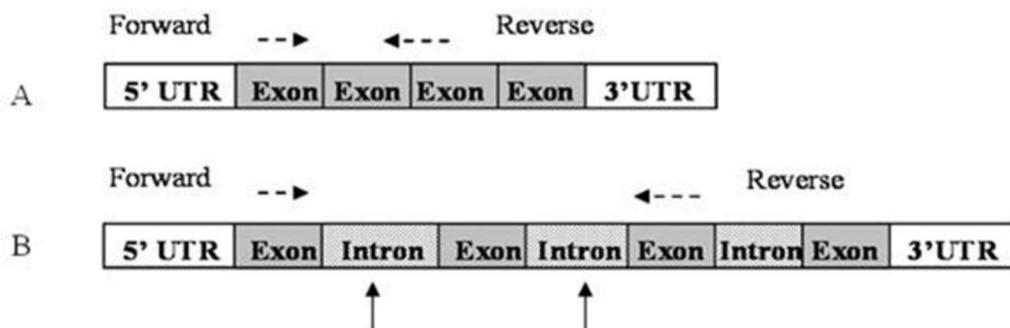
### Student Background and Assessment

The students involved in this project had two, 10 week terms of exposure to the required lab procedures (DeCaire et al., 2008; Shultz, 2008). Each student initially enrolled in an upper-class Genetics course. Students were selected from this course based on class participation and interest in the subject. These students then enrolled in one quarter of lab

assisting for the same Genetics course. This process ensured that all students had a good working knowledge of pipetting, knew the location of materials within the lab and were familiar with the general procedure. This level of student preparedness is not necessary, but an instructor could expect an inverse relationship between the time required for training and the background of each student.

**Table 1.** Primers designed from Human HSD11B1 and IL7R genes. Primer numbers correspond to indicator arrows within the gene ideograms shown in Figure 1.

Primer Number	Sequence	Distance from 5' end	Primer Number	Sequence	Distance from 5' end
HSD11B1_1	AGGAGTCTTCAGGCCAGCTC	100	IL7R_1	CCTCCCTCCCTTCTCTTAC	1
HSD11B1_2	TCCTCAGGAACACTCAAGCA	187	IL7R_2	TCACCATTTTGAGCATAGCC	186
HSD11B1_3	AAACCTCCCACTTCAGAGCA	800	IL7R_3	TCAGGTCCCAGTGGGTGTAT	3029
HSD11B1_4	GCCCCTGTGACAATCACTTT	964	IL7R_4	AGCAGGATGCTGGAAGAAAG	3236
HSD11B1_5	TGGGTATGGTCTCACTTCC	964	IL7R_5	CCAGGAGACTTGGAAGATGC	3971
HSD11B1_6	TGCGATATGCTAGCTTCTGTG	1114	IL7R_6	AACCACCATCCCTCACCATA	4129
HSD11B1_7	CCCCAAAAATCTGCAGCTAA	1864	IL7R_7	TTGGGGCCTGTAATTGGATA	5889
HSD11B1_8	CCTCTGCAAGAGATGGCTA	2014	IL7R_8	GGACAAACATCAGGTGGAAG	6102
HSD11B1_9	TCCTCTGAACCTTGCCCTTG	2099	IL7R_9	TGTAGTGCTTTTGTATCCTTGC	7972
HSD11B1_10	ACTTCTCTGCATGGAAGCTGA	2344	IL7R_10	TGACGAGTTAATCGGTGCAG	8194
HSD11B1_11	TCAGTTCCATGCAGAGAAGTA	2400	IL7R_11	TCAGGTTCAATTGCATCTAATTCT	10197
HSD11B1_12	TCATGGAGGAAGCAAAGTCC	2900	IL7R_12	GTGGAGGCATGTCAGGAAT	10303
HSD11B1_13	CCACCCTATGCCTTCGATAC	27450	IL7R_13	TGGTTGTCACCTTTGGGCTA	10610
HSD11B1_14	CATTGACCCTGGACACTGAA	27650	IL7R_14	CCAGCTCCAGTTAGCCACTT	10850
HSD11B1_15	TTGCTTTGGATGGGTCTTC	27700	IL7R_15	CTTGGCTGCCCTTAGACAG	14135
HSD11B1_16	CCACAATGGTAAGGCAGACA	28000	IL7R_16	CCGTCCATTTGTTTCATCC	14337
HSD11B1_17	GGCTGAGTCTCACCACATTC	28250	IL7R_17	TGCATGGCTACTGAATGCTC	17527
HSD11B1_18	TGGGTCTCGGTCTCTCTCTC	28800	IL7R_18	TGACCAACAGAGCGACAGAG	17637
HSD11B1_19	CCCTACTCTCCCTTGTCAATC	29459	IL7R_19	CTGTGGTCTCTGGTCCAACC	18426
HSD11B1_20	AACAGTCCAAAAATCCCTCA	29678	IL7R_20	GGAGACTGGGCCATACGATA	18663
			IL7R_21	ACATGCTGGCAATTCTGTGA	18984
			IL7R_22	TCTGGCAGTCCAGGAAACTT	19151



**Fig. 3.** Segment A shows primer design based on mature mRNA sequence only, with the reverse primer spanning 2 exons. This PCR reaction will fail because the reverse primer will not be able to anneal (bind) to the target (genomic) DNA. Segment B shows primers designed in exons, but with two introns of unknown length in between. If the total distance between the forward and reverse primers is too long, the reaction will likely fail because the target genomic DNA is too long for standard PCR.

An effective implementation of this course may also include the creation of a multi-instructor course, including technical communication for grant and poster preparation and a separate, hands-on laboratory section. In this form, the course would provide excellent preparation for graduate or professional school.

This procedure could also be performed in a standard laboratory course (i.e. not as an independent-study course). Factors that would increase the chance of successful execution include the utilization of students from the previous term as lab assistants, (which decreases the student/teacher ratio) and the use of available instructional materials (see supplemental files). We have found that most students can easily perform this procedure working independently with minimal instructor input. Obvious modifications for a standard course would be the removal of the grant writing component and replacement with a research report or lab notebook for assessment.

Assessment of students was conducted at three points during this independent study project. Completion of the schematic figure and the creation of a primer list were required in order to complete College mini-grant requests during the first quarter. Primer screening, optimization and completion of record-keeping were required for completion of the second quarter. Finally, the preparation and presentation of a poster at a University symposium during the third quarter completed the project.

#### Technical Discussion

Although we used gradient PCR to optimize the primers, in a large class setting it is entirely appropriate to set up a single thermal cycler at an annealing temperature of 55° C. If the product is still non-specific, the temperature can be raised to 60° C. If the products are faint, the temperature can be decreased to 50° C. The complexity of obtaining the

genomic DNA sequence can also be circumvented by simply identifying the exonic boundaries, then designing primers that only amplify within a known exon.

An important concept to remember is that the expressed genes identified on the KEGG database may exist in several forms due to alternative splicing of pre-mRNA. These alternative splices may cause the misidentification of exons as introns. At this time, there are no plans for accounting for this possibility within this procedure, but it could be pursued by comparing multiple copies of the cDNA attributed to the same gene.

#### CONCLUSION

We present a procedure in which students independently identify a gene's structure, design primers based on this information, test these primers and present their findings. This project demonstrates how an integrated approach to teaching and research can be beneficial to both students and instructors. All materials used in this study are presented within the text or as supplemental material.

## ABBREVIATIONS

BLAST Basic local alignment search tool  
cDNA Complementary DNA  
KEGG Kyoto encyclopedia of genes and genomes  
mRNA Mature RNA  
MS Multiple sclerosis  
NCBI National center for biotechnology information  
PCR polymerase chain reaction  
SNP Single nucleotide polymorphism  
TBET Tris, Boric acid and EDTA buffer  
TET Tris and EDTA buffer  
Tm Melting temperature

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## AUTHOR CONTRIBUTIONS

VP acted as a resource to DC-AW and wrote portions of the manuscript. DC-AW performed the in-lab portions of the study and are listed in alphabetic order. JS conceived of the work, created the support documents, wrote portions of the manuscript and supervised the study.

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# An Investigative Alternative to Single-Species Dissection in the Introductory Biology Laboratory

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**Abstract:** Dissections of single species (e.g., fetal pig) are a common student learning activity in introductory biology courses. Such dissections demonstrate location of anatomical parts and provide dissection practice but provide less opportunity for student critical thinking, numeracy and demonstration of the scientific method. A comparative anatomy lab was implemented over two years at a small, rural, private, liberal arts college. Students dissected their choice of five vertebrate species, mastered the location, function and gross evolution of the digestive and cardiovascular systems, and analyzed intestinal length and heart mass among sets of species. Instructors and students reported significantly more collaboration and reflective learning than in other exercises. The comparative approach highlights several evolutionary trends and created testable data at an equivalent cost to previous fetal pig dissections.

**Keywords:** vertebrate evolution, comparative anatomy, quantitative data, hypothesis testing

## INTRODUCTION

Undergraduate introductory laboratories often use dissection as an educational tool. Gross anatomical dissection is a useful skill for many biologists and reinforces the links between structure and function during the teaching of physiological and evolutionary concepts. Many published laboratory exercises regarding vertebrate dissection are simple explorations of terminology, fulfilling only the first cognitive domain of learning in Bloom's (1956) taxonomy. In addition, the model species of frog and fetal pig dissections are so common that students can claim college-level dissections are only review. Although dissections are new to some first year undergraduates, the value of repeating a single species dissection is lost to others. Some students surreptitiously avoid the dissection and instead memorize diagrams or photographs while others enjoy the dissection procedure but give little or no attention to mastering the biological concepts. Less common, but also disturbing, is when high-achieving students appear bored with re-memorizing from the same animal that they had seen previously. A need exists to improve students' dissection skills and mastery of anatomical terminology while engaging student creativity, hypothesis testing and critical thinking.

Vertebrate dissection exercises usually are limited to a few commercially available species that together provide a spectrum for anatomical comparison. Meuler (2008) described a multiple species approach that she tested with 15 students. She reported increased engagement after students answered qualitative questions and wrote a research

paper based on two dissections per student pair over a four-week period. Our organismal biology course does not allow that much time for vertebrate anatomy or for the grading of that number of research papers. Instead we desired a more cost-effective alternative that stresses quantitative data analysis (a theme of the course) as well as evolutionary trends.

Here I introduce a comparative approach to dissection that requires critical thinking skills. Students choose which hypotheses to evaluate, and how best to combine and display their data. While students are required to learn all the same structures, they also choose which species to dissect. Some species (e.g., pigeon) have a highly specialized anatomy that might distract an introductory student from basic morphological trends. Some species are too small (anole), taken from overharvested populations (dogfish shark), or are too time-consuming (bowfin, turtle) or expensive (cat, rabbit) for large undergraduate laboratories. Therefore I chose perch, mudpuppy, frog, pig and mink as study specimens.

Experience in the first year showed that two class periods (three hours each) provided only enough time for students to learn two organ systems. Although students are exposed to the reproductive and skeletal systems, grades depend upon recall only of the digestive and circulatory systems. Previous laboratories all emphasize learning about hypothesis testing, data type and organization, and graphing and error, and this exercise shares these learning outcomes. Anatomical parts and functions are memorized outside of class without their instructor,

with course time devoted towards dissection and concept application.

I designed a two week laboratory activity with two distinct experiences. In week one, students choose their specimen, and then (singly or in pairs) they dissect a species different from the one used by most of their peers, using customized dissection instructions. In the first week, all those students who have chosen to work on the same species work together and the instructor provides technical expertise in dissection skill only. The second week creates new student groups. Each new group contains at least one dissector of each vertebrate class examined. Each member of these new groups gives a brief oral presentation on anatomical function to the whole class, earning half of the dissection grade. Presentations are followed by the same groups exploring evolutionary trends across Vertebrata and then writing and evaluating hypotheses.

Students evaluate hypotheses from the digestive system as a class, and then choose from a variety of hypotheses about the cardiovascular system. The whole class first evaluates relative intestinal length by using student generated data and information on feeding ecology. Vertebrate intestine length varies greatly according to diet type, ranging from of 3-6 times the body length in carnivores to over ten times the body length in herbivores (Kardong, 2008). Students and the instructor use graphing to evaluate differences among mean relative intestinal lengths. Hypothesis writing and evaluation is usually helped by the few students already familiar with the relationship between diet and gut length. Less familiar to students is any trend in relative heart mass. Students are supplied with several ideas about cardiovascular evolution from which they can generate and test a hypothesis. I expected student groups to discover the large difference between fishes (whose relative heart masses range from 0.06-0.20% of body mass) and tetrapods (amphibian's relative heart mass = 0.46%, reptile's 0.51%, mammal's 0.59%; Farrell, 1991).

These comparative anatomy exercises were implemented over two years at a small, rural, private, liberal arts college. The exercise spans the last two labs (each three hours long) in an organismal biology class that covers physiology and evolution across kingdoms. Lectures concurrent with these laboratory exercises consist of basic vertebrate and invertebrate physiology on at least the digestive and cardiovascular systems. Here I report the exercise procedures, student data and outcomes as perceived by instructors and students. The complete laboratory, including preparatory guidelines, recommended sources for specimens, dissection instructions, natural

history handouts, worksheets and data templates is available for use.

## PROCEDURES

### List of Materials (to supply 20 students)

#### *Supplies required for weeks 1 and 2*

- 2 American minks, 2 fetal pigs, and 4 each of mudpuppy, leopard frog and yellow perch. Except for the perch, all specimens' circulatory systems are double-injected.
- Dissecting supplies and equipment
- The class shares dissection atlases for each species
- Every student is provided with species-specific dissection instructions and a Natural History Handout detailing the classification, evolution and diet of each species.

#### *Supplies for Week 1 (Dissection) only*

- Microscopes showing digestive and cardiovascular system tissues and cells
- 2 pairs of blunt-nose pliers (required for fully opening mink oral cavity)
- Balances (0.01-2.00 g and 0.1-2.0 kg) and a 10 m tape measure

#### *Supplies for Week 2 (Comparative Anatomy) only*

- Articulated skeletons (3-4 each) and live representatives of bony fishes, amphibians, and mammals
- Multiple computers with compiled Week 1 data
- Statistical test results and graphs comparing relative intestinal lengths among trophic lifestyle, prepared in advance by the instructor

### Pre-Lab Preparation

The students' pre-lab readings include introductions to dissection technique and to the available species. Instructors can survey student preferences for specimen type the week prior to dissection, and then combine the need for class time efficiency with respect for student opinion when choosing which student receives which species. Students working with fish and amphibians will finish dissections sooner than those working with mammals. Yet there usually are several students that are quite interested in dissecting mammals despite the extra time commitment. Prior to week 2, instructors compile data from all groups and laboratory sections into a single spreadsheet and email this to the students, along with a reminder of their oral presentation. In week 2, students read the Natural History Handout for each species. This handout (available from the author) summarizes the life history traits, ecological roles, diet (with per cent composition of each food type), interactions with humans and the geologic age of the specimen's class and family. Completion of readings is not evaluated,

but the benefits of reading in advance are clearly demonstrated by the end of week 1.

**Activity 1. Specimen Dissection (Week 1, 3 h)**

An initial 15-20 m lecture by the instructor covers dissection ethics, specimen storage, dissection technique and anatomical orientation terminology. By the end of the lab period, the students need to report the sex, snout-vent length (total length for the perch), total mass, small intestine length and heart mass of their specimen. Students with ethical and/or religious objection to dissection can work with an online fetal pig dissection (Fleck *et al.*, 2010). Instructors explain the oral presentation due at the beginning of Week 2. Because fish and amphibian dissections are completed earlier than mammal dissections, the instructor asks anatomy questions of these students.

**Activity 2. You are the Expert (Week 2, 10 min preparation + 25 min X 2 groups = 1 h 10 min)**

At the beginning of the second week, the class is split into two groups, each with an equal number of mammal, amphibian and fish dissectors. Groups are assigned randomly to present either the cardiovascular system or the digestive system, and are given 10 min to prepare. Each student is allowed 2 min to speak, and the students in each group must give their presentations in evolutionary order. Students are not only required to point out major anatomical features but are also responsible for

naming differences between their specimens and whether these differences are typical of that class or are specializations (these are addressed in the specimen natural history handouts). Group presentations are graded for content, preparation, accuracy and usage of actual specimens.

**Activity 3. Terrestrial Adaptations (Week 2, 30-45 min)**

The class is divided into small groups (3-4 students), each with at least one dissector of fish, amphibian and mammal. First, each group member answers questions about the vertebrate class they dissected using articulated skeletons and live animals (which do not have to be the same species as those dissected). The seven questions are nearly identical but their answers may vary with the type of specimen. After answering the questions individually, students compare answers and, as a group, identify anatomical trends in vertebrate evolution (Table 1).

**Activity 4. Comparative Anatomy and Heart Evolution (Week 2, 30-60 min)**

The instructor demonstrates to the class how graphing can evaluate (but not statistically test) hypotheses. The class is asked to consider that perhaps relative intestinal length is constant, changes among vertebrate classes, or changes according to diet. After discussion the class is shown previously prepared histograms with error bars and statistical test results. The small groups from Activity 3 then

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**Table 1.** Sample instructions and questions (Q) asked of each student. Answers (A) highlight evolutionary constraint or change across three vertebrate classes. Specimen-specific answers are separated by a backslash (/). Questions 1 and 5 demonstrate recall while questions 4 and 6 require application and analysis.

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Observe an articulated specimen. Ask your instructor for help distinguishing true bone from cartilage and (soft rays / claws).

Q1.Examine the placement of the (pectoral fin/forelimb) A: (laterally in fish / in between in amphibians / ventrally in mammals) where it joins the pectoral girdle. Is the (fin/forelimb) emerging from the body laterally, ventrally, or in-between?

Q2.(tetrapods only): How many bones are in the middle toe? A: Four

Observe a live specimen as it moves.

Q3.Is the head capable of freely moving from side to side at the neck? A: (no/some/yes)

Q4.From what part(s) of the body is most of the forward motion generated? A: (slow movement from swaying of pectoral and caudal fins and fast movement from spine and caudal fin / hindlegs in frogs pushing up and forward / tail in mudpuppy / legs only in mouse)

Compare your answers to those found by other members of your group. Consider the differences and similarities across the vertebrate classes and answer the following questions as a group:

Q5.Are the perch's fin rays homologous or analogous to tetrapod footbones? A: analogous

Q6.Consider the vertebral column and how its muscles must be attached. When you compare aquatic to semiaquatic to fully terrestrial animals, what trends do you see in how forward motion was generated? How does this relate to flexibility and power? A: Initially, the spine is the source of leverage for muscular power, then later is relegated to a balancing force for limbs.

**Table 2.** Student-generated anatomical data including relative intestinal length (small intestine length divided by snout-vent length) and heart mass (heart mass divided by rinsed specimen mass x 100%). Data are compiled from eight introductory biology laboratory sections taught in spring 2010.

Species	N	Relative Intestinal Length			N	Relative Heart Mass (%)		
		mean	range	SD		mean	range	SD
Yellow perch	17	0.85	0.33-1.41	0.35	17	0.21	0.13-0.67	0.13
Mudpuppy	18	1.23	0.55-1.93	0.39	18	0.47	0.11-0.83	0.20
Leopard frog	12	1.27	0.63-2.35	0.51	14	0.48	0.14-0.88	0.22
Domestic pig	14	7.38	3.05-11.85	2.50	13	0.84	0.55-1.37	0.22
American mink	12	3.31	2.29-4.67	0.65	12	1.12	0.64-1.57	0.28
All specimens	73	2.67	0.33-11.85	2.70	74	0.58	0.11-1.57	0.37

examine three ideas of vertebrate heart evolution. Heart size is presented as being influenced by the presence of limbs, a terrestrial lifestyle or endothermic metabolism. Each small group writes hypotheses for each idea as well as a null hypothesis, and then examines one hypothesis graphically. Each small group is graded on their hypotheses, figure and interpretive figure caption.

#### Assessment

I performed Kruskal-Wallis (K-W) tests for significant difference ( $\alpha=0.05$ ) in means among species groups using IBM SPSS Statistics 18 (SPSS Inc., Chicago IL). The K-W non-parametric test is less powerful than other statistical approaches but also has fewer assumptions (Sokal and Rohlf, 1981). Heart mass data were evaluated in a number of comparisons (e.g., by class or by aquatic lifestyle) and thus K-W *P* values were adjusted by Bonferroni correction (Sokal and Rohlf, 1981). Student learning and exercise logistics were evaluated by instructor opinion (gathered in weekly meetings) and then by anonymous student evaluations. At the end of the 2009 semester, students answered the same eight questions about each lab activity. Scores were adjusted to reflect a consistent scale (i.e., 5 = very positive, 1 = very negative) and a Mann-Whitney *U* test (Sokal and Rohlf, 1981) was used to detect a difference in mean student response between the comparative anatomy and the pooled responses for all other exercises pooled together.

#### RESULTS AND DISCUSSION

Comparative anatomy data on heart mass and intestinal length were generated successfully by students in 2009 and 2010. In the first year of implementation, data were problematic due to numerous student data entry errors. By 2010, data collation was supervised by the instructor and was restricted to sex, snout-vent length (mm), total mass (g), small intestine length (mm) and heart mass (g). A summary of data from 2010 only is in Table 2. Kruskal-Wallis testing revealed significant differences in mean relative intestinal length among dietary type (Figure 1a,  $P < 0.01$ ), supporting the well-documented adaptation of increased intestinal

length with increased plant ingestion (Kardong, 2008). After students were shown these results, they examined data regarding relative heart mass (RHM). Although the greatest differences in RHM occur between fishes and other vertebrates, student data showed significant differences ( $P < 0.01$ ) among each species, class, aquatic lifestyle and thermoregulatory group (Figure 1b). Heart mass varies within each vertebrate class by activity level (Farrell, 1991), and it could be interesting to have more advanced students regress each species RHM by a metric of physiological activity.

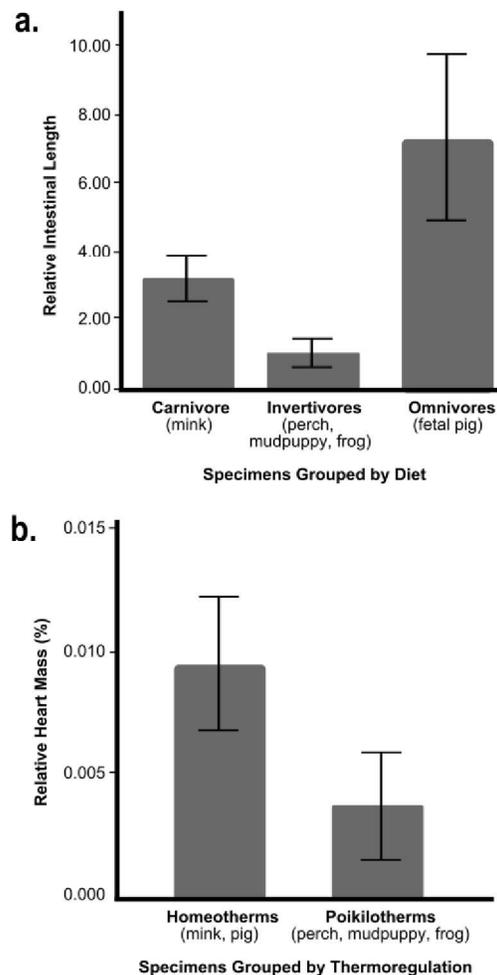
Laboratory sections held a mean of 19.1 students (345 total students over two semesters, nine lab sections each semester), of which the majority were first year students. Each lab section was taught by one instructor and one (sometimes two) undergraduate teaching assistants. An anonymous survey administered after the first year of implementation revealed that a significant difference in student response between comparative anatomy ( $n = 156$  responses among 8 questions) and all other lab procedures ( $n = 1872$  responses). The mean score for all responses was  $3.79 \pm 0.89$  for this exercise and  $3.59 \pm 0.00$  for all other activities. The greatest differences were more positive responses to the questions “Collaborative work is a valuable part of this lab” (dissection =  $4.20 \pm 0.07$ , all other labs =  $3.82 \pm 0.02$ ;  $P < 0.01$ ) and “The lab provided opportunities for reflective learning” (dissection  $4.14 \pm 0.06$ , all other labs  $3.93 \pm 0.20$ ;  $P < 0.01$ ). The lowest scoring question was “I felt rushed in this lab,” which was not significantly different from the other activities (dissection =  $2.83 \pm 0.10$ , all other labs =  $2.77 \pm 0.03$ ;  $P = 0.68$ ). Instructors reported that the laboratory’s primary weaknesses were the time required and student difficulty with graphing software, although these varied greatly among course sections. When compared to fetal pig dissection alone, instructors judged that the strengths of this revised laboratory were an increased critical examination of specimens, student investment and emphasis on evolutionary concepts.

These laboratory activities address all five cognitive domains of Bloom’s (1956) taxonomy.

The oral presentation activity requires students to master two organ systems as students cannot know in advance which system, and which portion of that system, that they will speak on to their peers and their instructor. The only materials allowed as visual aids are the specimens, meaning that students must interpret an actual specimen instead of recognizing and listing parts from a photo atlas. The graded portion of Week 1 therefore involves both knowledge and comprehension. Application of knowledge and analysis of new data are performed in the comparative anatomy portion (Table 1). The students infer evolutionary trends and are then presented with a novel example of comparative anatomy hypothesis evaluation. The students must work in groups to manipulate a raw dataset by making numbers comparable (calculating the relative heart mass) and separating it into appropriate categories (e.g., homeotherms vs. poikilotherms). These higher-order modes of learning are evaluated by examining student-generated hypotheses and graphs. Grading of the figure caption involves assessing students on their ability to evaluate, the most complex cognitive domain in Bloom (1956). Instructors noticed more evaluation than this, however, as students conversed and even argued about trends in anatomy and evolution.

The fetal pig is a common and appropriate choice for learning anatomy, but a single species dissection may not allow students to visualize evolutionary trends or practice meaningful data collection and interpretation. The strength of a comparative anatomy approach is the emphasis of differences resulting from evolutionary adaptation. Such differences may lead to students having evaluative experiences, such as determining the sex of non-mammals, or defining the beginning and endpoints of the perch heart or the small intestine. In the latter case, the ancestral aquatic habitat of fishes and amphibians meant little need for water reclamation or fecal storage and thus these classes lack a true large intestine. Similarly, the boundary between large and small intestine in the carnivorous American mink is indistinct at best. Students asking for a definitive answer are directed to consider the adaptations involved and then trusted to make their best evaluation. Evaluation was extended to numerical literacy as well, as students were surprised that trends could still be evident despite the presence of some investigator error. The subsequent questioning of when and how to trust data led to better interpretive sentences. Such learning outcomes are made possible by the simultaneous dissection of multiple species.

As with many laboratory activities, the major limitations of dissection are money and time. The



**Fig. 1.** Comparative vertebrate anatomy histograms (error bars represent a single standard deviation) on intestinal length (instructor-generated, top) and heart mass (student-generated, bottom). The instructor uses intestinal length to demonstrate how to graphically evaluate a hypothesis by combining specimens into categories before students generate their own heart mass graphs.

cost of using multiple species is equivalent to or actually less expensive than providing a fetal pig to students working in pairs. A disadvantage is in taking up two laboratory sessions, but this is not a problem for our course considering the lecture time spent on vertebrate evolution and physiology. The loss of attention to other systems results in less recall practice for the students, but instructors have agreed that they prefer the greater depth of knowledge provided by the comparative approach.

If more than two laboratory sessions can be made available, then other systems with other investigatory activities can be created. Additional exercises written, but not continued into the second year due to time constraints, included examinations

of the reproductive, urinary and nervous systems with graphical evaluations of brain mass and kidney volume. The exercises could be expanded to include a basal vertebrate (lamprey) or a true herbivore (although rabbits are currently prohibitively expensive). Another obvious expansion of this activity is including invertebrates, but this would necessitate less emphasis on readily visible homologies. A third direction for improvement is in teaching numeracy. If time allows, the data generated can be explored by statistical testing.

Even without such modifications, course instructors and students appreciated the small group work, peer-to-peer learning and independent investigation. The exercise sacrificed evaluating student mastery of some systems, but did require in-depth knowledge of the digestive and cardiovascular system as well as the scientific method. The class dissected five species at nearly the same cost as our previous fetal pig activity, and many students appreciated choosing their species. The comparative approach to dissection demands greater self-reliance for recall, allows more room for student creativity and can facilitate a more critical understanding of anatomy, evolution and the scientific method.

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# Incorporating a Literature-Based Learning Approach into a Lab Course to Increase Student Understanding

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**Abstract:** Scientific literature was used to give a research oriented context to our immunology lab course. Immunology lab, a senior level course (60 students/year) was formerly taught in a traditional mode, with exercises aimed at learning lab protocols. To engage students in understanding we connected the protocols to their use as reported in research articles, employing an interrupted case study method called Literature Based Learning (LBL). Our goals were to give a research context to learning basic protocols, engage students in reading scientific literature, and increase students' understanding of how standard techniques are employed to address a research question. An end of semester experimental design project using mock research scenarios culminated the research oriented learning. Pre-course surveys revealed that nearly 25% of students had never been asked in the course setting to read a primary research article. Post-course surveys revealed that 94% of students thought that after the course they knew more about research and over half of the students agreed that the literature-based assignments had raised their confidence in their ability to understand scientific articles. We found that the LBL approach was an effective mechanism to engage our students in research oriented learning. While easy to implement, it had a dramatic outcome.

**Key words:** Literature-based learning, biology laboratory, group work, experimental design project, undergraduate course.

## INTRODUCTION

When baking a cake for a party there is generally little concern over why eggs are added as long as the cake is delicious at the dinner party. Similarly students who are asked to generate a prescribed outcome from completing a protocol generally don't much care why they are doing each step as long as they get the "right" answer. Students begin to believe that science is about the answer and not about the process. When aiming only for the end result students are less likely to be engaged and as such miss the opportunity for understanding of both the scientific process and underlying scientific concepts (Schamel and Ayres, 1992).

The goals of the National Research Council's *National Science Education Standards* (1996) are for students to gain a long-term understanding of science concepts, an insight into the nature of science, and an appreciation of the skills that are necessary for scientific research. Laboratory courses are an ideal environment to meet these aims. Unfortunately, the way many labs are structured works against these goals. As Bransford, Brown, and Cocking (2000) state, there are important differences between tasks and projects that encourage hands-on doing and those that encourage doing with understanding. In traditional "cookbook" labs, many if not all of the questions and decisions that are a part of the scientific inquiry process are provided for students (Bybee, 2002).

The National Research Council's BIO2010 report (2003) states that lab courses should allow students to think independently, introduce them to authentic scientific questions and incorporate cooperative learning. The report recommends that project-based laboratories with discovery components replace traditional scripted "cookbook" laboratories to develop the capacity of students to tackle increasingly challenging projects with greater independence. These changes should increase student interest and participation and improve student written and oral presentation of scientific information. A review by Lawson (1992) indicated that changes in lab design towards investigative labs increases students' reasoning skills, concept understanding and, overall how much students "like" lab.

A specific skill that is often overlooked in the national discussions of teaching the process of science is the use of scientific literature. As an undeniably important source of information for scientists, the research literature should be a critical training ground for young science professionals. Not only does teaching students to work with the scientific literature model the activities of scientists, but having students read, interpret, analyze, and report on the research literature provides opportunities to develop skills of critical and analytical thinking and written and/or oral communication (Mulnix, 2003). Even when aware of the literature, students often find scientific papers

difficult to understand and digest. In part this is because the style of scientific papers is very different from that of most other reading, including textbooks (Porter, 2005).

With this in mind, we set out to modify our existing immunology lab course to increase student understanding of standard immunological techniques using a “Literature-Based Learning” (LBL) approach. To actively involve all students in the LBL approach we used the “Interrupted Case Study Method” (Herreid, 2005) in which information is given to the students in a series of steps (“progressive disclosure” of information) allowing students to think about and discuss each step before receiving information that reveals the authors’ thought process. For example Herreid (2005) suggests that a research article could be used as an interrupted case study: students receive and read the introduction section; from this alone they propose methods to address the research question. Then, students receive and read the remainder of the research article and discuss how their proposed research approach compared with that described by the authors.

This course re-design was part of the Host Pathogen Interactions Teaching group project to introduce research-oriented learning into our microbiology courses. In this paper, we will report on our teaching approach and the assessment of student engagement, and understanding of methodologies in context of immunological techniques and research design.

## METHODS

Our course re-design had three specific aims: 1. learning of standard immunological techniques/protocols, 2. understanding the theory and the use of techniques/protocols, and 3. applying the techniques/protocols to authentic research problems. Aim 1 was achieved by teaching the techniques and protocols as is typically done in a lab course. Aims 2 and 3 were achieved by placing the use of the techniques/protocols in the context of research design – either from published articles or as developed by the students.

Our design involved a two credit 400 level Immunology lab course that met for two hours twice a week (separate from the 400 level Immunology lecture course which is a co-requisite). Students were divided into three lab sections of 20 or fewer students taught by graduate teaching assistants. Course enrollment consisted of 56 upper-class students in three sections. Lab sections were each taught by one graduate teaching assistant per section, and the lab was coordinated by one lab coordinator. Assessment was achieved through a post-course survey that 52 of the 56 students completed.

## Immunology lab design prior to innovation

The course focused on teaching standard immunological techniques/protocols which were implemented by the students as they were instructed (see Table 1, not bold). Prior to each lab period students were given background information on the protocol of the day. During the lab period the teaching assistant gave a brief lecture and students performed the protocols. After the lab period students were expected to write a brief lab report highlighting the method and results obtained. In addition to lab reports students were assessed by exams (two during the semester and a final exam) and a final project on immune-related diseases. Each student was assigned a disease and was expected to prepare and present a poster on the symptoms and other characteristics of the disease.

## Revised course design: Overlay of Literature-Based Learning

In the revised course, learning immunology techniques/protocols was put into an authentic research context by requiring students to read and discuss relevant primary literature (Table 1 – Bold). To orient the students to reading primary literature, a primer article was assigned. This article (Parent [Appendix 1], 2010) was carefully chosen to be short, (7 pages and 5 figures) straightforward, at the level of the beginning immunology student and following a standard research article design with sections: Introduction, Materials and Methods, Results and Discussion.

Beginning in lab period six, research articles (Parent [Appendix 1], 2010) were distributed to engage students in understanding the context of immunology protocols. As with the primer article, we chose articles that had clear introduction, methods, results and discussion sections. We avoided articles from journals such as *Science* or *Nature* as these do not have these clearly defined sections. They also have very strict page limits which means much of the jargon, techniques, etc. are not explained in any detail. The articles we chose needed to be well written, with an introduction that had good background and a clear question/hypothesis and results that were understandable to undergraduate students. The articles were chosen because of their relevance to topics of immunology (the course topic) and pathogenesis (the focus of the Host Pathogen Interactions Teaching Team). We chose articles that used *E. coli* or *Streptococcus pneumoniae* as these were selected by the Host Pathogen Interactions Teaching Team as the “anchor organisms” for teaching concepts of Host Pathogen Interactions (Authors, 2007). Finally, the articles were selected for their use of the immunological methods that students would learn over the course of the semester.

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**Table 1:** The Literature-Based Learning overlaid on a traditional laboratory

Lab 1: Organs of the Immune System

**Paper 1: PRIMER Students read complete research paper—Bring all students to a comfort level in reading a research paper**

Lab 2: Peripheral Blood Smear

Lab 3: Blood Cell Separation

Lab 4: Precipitation

Lab 5: Agglutination

Lab 6: Antibody Conjugation

**Paper 2: Part 1: Students receive introduction of research paper, propose research question and techniques –Labs 1-6 are put into context. Part 2: One week later students receive complete paper and discuss—Their understanding is compared to the experts.**

Lab 7: Immunofluorescence microscopy

Lab 8: ELISA

Exam I

Lab 9: Immunoprecipitation

**Paper 3: Part 1: Students receive introduction of research paper, propose research question and techniques –Labs 7-8 are put into context. Part 2: One week later students receive complete paper and discuss—Their understanding is compared to the experts.**

Lab 10: SDS-PAGE and Western blot

**Paper 4: Part 1: Students receive introduction of research paper, propose research question and techniques –Labs 9-10 are put into context. Part 2: One week later students receive complete paper and discuss—Their understanding is compared to the experts.**

Lab 11: Flow cytometry

Exam II

**Experimental Design Project Lecture and group work: Students receive a research scenario, determine a research question and design experiments, using immunological techniques—Requires that all course learning is applied in a research context.**

Lab 12: Antibody mediated cytolysis and cell count

Lab 13: Lymphocyte proliferation

Lab 14: Microbial killing by macrophages

**Students carry out experiments for three lab periods**

**Students present all information in poster format with an oral presentation—Requires students to demonstrate understanding of techniques and present in mode of a research scientist.**

**Paper 5: Students read complete paper—culminating activity: Was there an increase in appreciation for information presented?**

Final Exam

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We found that some older papers were the best choices to give context to the more standard/classic immunologic methods taught in our course.

Articles were distributed to students in sections according to a modified interrupted case study approach (Herreid, 2005). First, the students received the introduction section: they were asked to determine the specific research question, and to choose a technique (from the techniques previously learned in lab) that would be most appropriate to address the proposed research question (Parent [Appendix 2], 2010).

In lab students would discuss their responses and then receive the complete research article. At this point the authors' research question was revealed along with the experimental design and the

techniques used (Parent [Appendix 1], 2010). The interrupted case study approach was used to engage students in understanding scientific research by engaging them in the same process that was first followed by the authors. Student discussion was meant to help students learn that there may be more than one way to state a research question, and a variety of experimental approaches or techniques may be appropriate to address the question. Throughout the semester students had the opportunity to see the protocols they learned in lab used in authentic context and they were engaged in the research process. Like the authors of the research articles, the students developed research questions and chose from protocols or techniques that they had

learned to address open ended research questions (cf. Table 1 and Parent [Appendix 2], 2010).

To cement student understanding of how standard techniques/protocols are applied to address research questions students completed an end of semester Experimental Design Project (EDP). Students worked in teams to address a research scenario (Parent [Appendix 3], 2010). Because of the complicated nature and potential high cost of immunology research, we chose to use mock scenarios. Students received authentic research stories, but most of the samples with which they worked were mock. For instance, bovine serum albumin (BSA) was used to simulate various proteins and B cells were used to simulate other types of cells. This way we only had to purchase a limited number of antibodies that were against common antigens (BSA, B cell receptor). This helped keep the cost of the projects within the course budget. Teams of 3-4 students were established at the start of the semester based upon student self-selection. Students were expected to discuss the problem, define a testable research question and apply any of the immunology techniques/ protocols learned over the semester. Following completion of the lab work (3 lab periods) students presented their research methods/design, results and conclusions in an oral poster presentation to their peers.

#### Profile of students

Students that responded to the pre- and post-course survey (52 students) were asked to provide details about their background. We respect students' sensitivity towards exposing background information; therefore we present the distribution of data that we gathered from the survey and not from their transcripts. Figure 1 shows the percentages for those who provided the information.

#### Research instruments

##### *Pre-course survey*

To assess the students' competency and comfort in reading scientific literature and their knowledge of immunological techniques prior to this class a pre-course survey was administered through WebCT (Web Course Tool). Students were asked to complete open ended questions about their:

- familiarity with immunological techniques in general and with respect to the exact techniques and protocols that would be covered during the course.
- background (year in school, gender, race, GPA etc.), their expectations for the class and their plans after graduation.

Since these questions were open-ended, similar responses were grouped into categories. This approach was validated by the lab coordinator of the

**Gender:** (47 students)  
55% females  
45% males

**Age:** (47 students)  
15% between 19 to 20  
72% between 21 to 25  
6.5% between 26 to 30  
6.5% above 30

**Ethnicity:** (43 students)  
2% African –American  
26% Asian  
67% Caucasian  
5% Hispanic

**GPA:** (40 students)  
10% GPA 2.0-2.5  
26% GPA 2.6-3.0  
44% GPA 3.1-3.5  
20% GPA 3.6-4.0

**Final grade in the prerequisite General Microbiology class:** (40 students)  
55% A; 30% B; 15% C.

**96% of the students were Biology majors**

**Fig. 1.** Student background information

course, another science faculty member and a science education expert.

##### *Post-course survey*

To receive student feedback regarding the incorporation of the Literature-Based Learning and the Experimental Design Project, a post-course survey was administered through WebCT (for the full survey cf. Parent [Appendix 4], 2010). Analysis of the open-ended questions was performed as stated for the pre-course survey. To assess students' level of competency in reading and understanding primary literature (Table 1 and Parent [Appendix 2], 2010), we assigned the reading of one last research article to students.

## RESULTS

The pre-course survey was given to the class in the beginning of the course. Fifty-two students responded to the pre-course survey. Fifty-one students responded to the pre-course question "What are your expectations from this course?" The majority of the students (26) that responded to this question indicated that they expected to learn more about immunology in general. Fourteen students indicated that they wanted to learn about procedures and hands-on techniques that are used in labs. Nine students referred to their expectation to be prepared for medical school, research lab, or other career choice. Six students referred to the applicability of the laboratory to everyday life. Three students reported that they expected the lab to apply to or reinforce concepts learned in the Immunology lecture course. Three students mentioned their expectation to

learn about current research/articles and understand them. Three students wished they would pass the course with a good/decent grade. Only one student brought up the expectation that the course would be interesting.

Fifty students answered the pre-course question “What are your future plans?” The majority (43 students) reported that they intend to pursue an advanced degree (medical school, graduate school, dental school, physician assistant program, veterinary school, pharmacy school, optometry school and law school). The others (7 students) responded that they intend to obtain jobs that do not require further education (military, government, high school teacher and pharmaceutical sales).

As mentioned in the methods section, the post-course survey included twenty-five questions (Parent [Appendix 4], 2010). The questions targeted the evaluation of the three major innovative initiatives in this lab: reading research papers, implementing the Experimental Design Project (EDP), and working in groups.

### **Reading Research Papers**

The main goal of the primer paper (research article 1) assignment was to share with the students how scientific work is presented to other scientists through a formal research paper. Therefore, in the post course survey we asked students to reflect how much they learned about how scientific work is formally presented (Question 11, Parent [Appendix 4], 2010). Fifty-eight of the students (30) reported that by completing the paper 1 assignment they learned more about how science is presented. Interestingly, most of these students (17) reported that they already understood how scientific work is presented prior to the course, but the assignment helped to increase their understanding. From the 42% of the students (22) that reported that they did not feel that the assignment increased their learning on this topic, thirteen students reported that they already understood how scientific work is presented prior to the course instruction.

The primary rationale for assigning research articles 2-4 was to expose to students the process scientists use to formulate a research question (Parent [Appendix 4], 2010, Question 12) and to select appropriate techniques/protocols to address a given research question (Parent [Appendix 4], 2010, Question 13). The majority of the students (81%) reported that they learned more about how to formulate a research question from the assignments for papers 2, 3 and 4 (Question 12). Most of these students reported that they had some understanding (33%) or that they understood (31%) how to formulate a research question prior to the course, but gained more understanding from the assignments.

Only 19% of the students did not report any increase in learning on this topic. The response pattern for question 13 was similar to that of question 12. Most of the students (88%) reported that they learned more about how to choose appropriate research techniques from the assignments for papers 2, 3 and 4.

Overall, students were very positive regarding the use of the papers during the course. In response to the question “Five research papers were used in this course. What did you like about the research papers and the way that they were used?” (Question 4), the majority of the students (92%) responded with positive comments. Students reported that reading the papers gave them an understanding of the research process and how research is presented and refined their ability to read research papers (“I appreciated how the research paper assignments taught me how to be able to pick up a scientific paper without being overwhelmed... I liked that the assignments for each paper were primarily the same each time. The repetition of assignments for the papers really helped me to practice on how to read and analyze these papers”).

Students also commented that the papers allowed them to see how techniques learned in the lab are applied (“...It was nice to see how the exact assays we performed in class were applied in actual scientific studies,” “...It really helped me reinforce the knowledge of immunology techniques that we learned over the course of the semester”). Other students mentioned that the papers were interesting and made them think (“...It also made us critical thinkers in regards to other possible techniques that could have been done”). The four negative comments indicated that these students found that the reading was not challenging and did not extend their learning.

### **Implementing Experimental Design Project (EDP)**

All students responding to the survey submitted a positive comment regarding the completion of the EDP. Table 2 shows categories of students’ responses to the question “What did you like about the Experimental Design Project (EDP)?” (Question 15). Students reported that they liked that the EDP allowed them to be creative and work independently on their own project. One student stated,

“I liked the idea of being left to figure out the research question on my own. This broke away the repetitive four year lab process where I would normally be given protocols and forced to carry them out. When you know what the end result is supposed to be, you feel less concerned about answering your question and the goal basically becomes ‘getting through the lab’. However, the research project forced me to use some creativity to try and figure out what I need to do. I mean our group almost figures out how to design our experiment by ourselves...”

Students also liked that the EDP required them to work in groups (“... I liked the fact that we actually carried out the experiment in a group like labs in the work place”).

### Working in Groups

Students were asked to describe the one thing that they found most rewarding about working in a group for the Experimental Design Project (Question 17). Students reported that the group work allowed them to share ideas (16 students), learn more from others (11 students) and share responsibilities (10 students). One student wrote, “The people in the group complemented one another’s strengths and weaknesses. It seemed like, while one person might be good at coming up with the methods, another would be good at interpreting results... I felt like I learned from other people”. Students also mentioned that the group made the work more fun, allowed them to make friends and learn how to think (8 students).

Overall most of the students thought that after the course they knew more about research than before the course (94%). Eighty-one percent reported that the course met their expectations and 12% reported that the course exceeded their expectations. Ninety-six percent of the students indicated that they would recommend this course to their friends. Students found the course interesting and challenging. They enjoyed learning immunological techniques and found that the design of the course gave them an exposure to and appreciation for the research setting.

undergraduates have little sense of how scientific knowledge is generated, how research projects progress over time, or how scientists think about and actually do research (Pukkila, 2004).

The Host Pathogen Interactions teaching group (website will be given after review process) is concerned with meeting the goals of the NRC, AAAS and BIO2010 and creating experiences for research oriented learning. The Literature Based Learning model was designed to engage students in learning basic immunology techniques in the context of current literature of Host Pathogen Interactions, specifically targeting *E. coli* and *Streptococcus pneumoniae* (Author, 2007). In the model reported here students learn and apply basic immunology techniques/protocols in the context of authentic research. Students completed readings relevant to protocols and later through the Experimental Design Project students worked co-operatively to apply these protocols to authentic research problems.

The majority of the students responded with positive comments regarding the research papers, and all students submitted positive comments regarding the completion of the EDP. Students liked the exposure to the scientific literature which made them think, showed them how techniques are applied and helped them understand how science information is presented. Students mainly reported that they liked the independence of working on a project of their own design. They appreciated the opportunity to apply techniques that they had learned in a manner

**Table 2:** Frequency of students’ responses to the question about the use of the Experimental Design Project (EDP) in the course.

15. What did you like about the Experimental Design Project (EDP)? (Categories)	No. of responses*
The EDP allowed an opportunity for me to design my own project/be creative/work independently.	27
The EDP allowed me to apply what I learned in lab this semester.	18
The EDP was like a true research experience (an authentic learning opportunity).	14
The EDP required me to think/understand.	8
The EDP allowed me to work with my peers.	8

\* The sum of a single column can exceed 100% because there are students whose responses fell into more than one category.

## DISCUSSION AND IMPLICATIONS

The teaching standards set forth by the National Research Council (1996), the AAAS (1993), and BIO2010 (2003) call for the science curriculum to emphasize research processes such as understanding, reasoning, and problem solving. Students need to acquire skills that are necessary for scientific research and understand how scientists work (Handlesman, 2004). However, the sense of discovery felt by scientists involved in generating new information is unfortunately rarely communicated to undergraduates. Instead, instructors often feel compelled to teach their students an ever growing body of facts. As a consequence, many

that was designed to mimic the authentic research setting. Most of the students also reported that the research papers and the course assignments were useful in preparing them for the Experimental Design Project. Students liked and seemed to benefit from the group work where they had the opportunity to share ideas, work in a collaborative and collegial setting on a project that was their shared responsibility.

The Literature-Based Learning approach could be overlaid upon any technique-oriented lab course. The most significant increase in time required for implementation comes from the time needed to select appropriate readings and develop the scenarios for

the Experimental Design Project which are to be based on published research studies and must utilize the techniques learned in the course. Our impression of the revised course is that it is dramatically improved. The students are more engaged and enthusiastic about their work. From student reactions and the level of knowledge expressed by students during poster sessions it is clear that student understanding of scientific research and immunological methods are increased from the level in the previous course design. Using the interrupted case study method to parse out the reading of primary literature in connection with learning standard protocols provided the basis of a research-oriented approach to lab science instruction. This Literature-Based Learning approach was first implemented in spring 2005 and continues to be used with similar success.

#### ACKNOWLEDGMENTS

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# An Open-Ended Investigative Microbial Ecology Laboratory for Introductory Biology

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**Abstract:** In this article we describe a multi-week investigative laboratory in microbial ecology/diversity and nitrogen cycling that we have used in our introductory biology course. This module encourages active student involvement in experimental design, using the scientific literature and quantitative analysis of large data sets. Students analyze soil microbial community diversity using EcoPlates (sole source carbon utilization patterns). Also, functional analysis of these communities includes assays for nitrate, nitrite and ammonium to determine experimental effects on the nitrogen cycle. Students are continually assessed by various strategies throughout the module.

**Keywords:** Investigative, introductory biology, quantitative analysis, microbial diversity, soil microcosms, nitrogen cycle

## INTRODUCTION

Numerous reports continue to emphasize the need for reform in science curricula (Handelsman *et al.*, 2004; DeHaan, 2005). These reform efforts encourage active learning, investigative approaches and collaboration. Laboratory experiences are extremely important in learning the process of science. Here, we describe a multi-week investigative laboratory module based on microbial community ecology for an introductory college biology laboratory course (Jones-Held, *et al.*, 2004). Typically, we use this module with first year students from various science disciplines. The scheduled laboratory sessions are three hours in length.

We focused on microbial community ecology because this theme illustrates that organisms do not exist in isolation from each other and from their environment. Furthermore, the community ecology approach introduces the concept of biodiversity and its relationship to microbial communities that cannot be directly observed. Additionally, the method used for evaluating microbial diversity generates large data sets that students analyze and this helps improve and reinforce their quantitative and computer skills. We coupled the microbial community analysis to the microbial cycling of nitrogen as it illustrates the role of living organisms on chemical transformations, reinforcing the link between biology and chemistry that is so important for students at the beginning stages of their science studies. The specific learning objectives of this module are to design and initiate an experiment that will examine environmental effects on soil microbe community diversity; to understand the importance of soil microbes and their diversity in nutrient cycling within ecosystems.

Ecologists often characterize communities in terms of species diversity. Species diversity is usually determined by using diversity indices that take into account both richness, reflecting the number of species present, and evenness, indicating the relative abundance of species in a community (Molles, 2008). These indices rely on taxonomic identification that is fairly straightforward for plants and animals. Microbial diversity in the environment is much more difficult to address or quantify because taxonomic identification of naturally occurring microbes is usually inconclusive and difficult (Kirk *et al.*, 2004). However, characterizing naturally occurring microbial communities is extremely important because of their essential role in ecosystem functions like nutrient cycling and in assessing the effects of any environmental perturbations on the community. An important component to terrestrial nutrient cycling is the soil environment and its diverse microbial communities. There are existing methods for characterizing soil microbial diversity (Kirk *et al.*, 2004). One of these methods uses a commercially available 96 well plate (EcoPlate) to characterize the functional diversity of the microbial community using sole source carbon utilization patterns (Figure 1). The basis for the analysis is metabolic profiling using 31 different carbon sources and a blank. In the presence of a carbon source that bacteria can use for growth, their respiratory activity will reduce the tetrazolium dye present in each well of the plate producing a visible color change. Using these plates, microbial diversity can be quantitated using existing diversity and evenness indices that have been modified to reflect plate color development (Zak *et al.*, 1994; Staddon *et al.*, 1997; Staddon *et al.*, 1998;

Derry *et al.*, 1998). Plate color development reflects functional diversity not taxonomic diversity (Staddon *et al.*, 1998). From an instructional standpoint, these plates are straightforward to use and each EcoPlate generates a large data set that students can organize and quantitatively analyze (Jones-Held *et al.*, 2004).

The nature of the individual components of the module and their integration for successful completion provide a framework for applying tools and strategies designed to provide both formative and summative assessment of essential student skills at this level such as: (a) understanding and appreciating the biodiversity and roles within ecosystems; (b) application of the elements of the scientific method within a specific context; (c) appropriate execution of laboratory practices and use of laboratory equipment; (d) collection, analysis, interpretation and evaluation of data; (e) search, retrieve and utilize published and electronic forms of scientific information. A timetable and list of activities for this module is presented in Table 1. Each activity is subsequently described in detail.

## MATERIALS AND METHODS

### Pre-laboratory Preparation

**Materials:** Plastic Containers (27.9 cm X 44.5 cm X 17.8 cm); Commercial Potting Mix or Soil; Seeds (i.e. peas, beans, corn, grass)

Typically, this module of our introductory course is offered during the winter months when it is difficult to obtain field-collected samples. Using field-collected samples would shorten the scheduling of the module and provide the advantage of having students collect their own field samples. Instead we set up soil microcosms 4 to 6 weeks prior to the start of semester so that the plants have time to establish and grow for several weeks before the students begin their treatments. Microcosms are small self-contained ecosystems that have been used in many ecological research studies. Plants are all started at the same time by randomly sowing seeds across each microcosm. Even though all microcosms have the same potting mix we try to add some diversity to each microcosm by using different plants or combinations of plants. We maintain the microcosms under greenhouse conditions until student use. Within

the 4 to 6 week growth period under greenhouse conditions all plants are of sufficient size for students to initiate their experiments.

### Laboratory Sessions 1 – 3

**Materials:** Microcosms; 7.6 cm diameter pots; Balances, Spatulas, Distilled water; pH Meters; Other standard laboratory equipment; glassware and chemicals as needed

The first week of lab involves an overview of the module providing the framework for students to search the literature and to develop an experimental design. Some designs have included the effects of salinity, temperature or metals such as copper on the soil microbial community. Laboratory instructors discuss with the students what they need to consider in setting up an experiment. Within this one week period, student groups (3 to 4 students/group) must search the literature and develop a written experimental design. During the previous semester our students have had two information literacy modules that provide a proper context for conducting literature searches. Alternatively, the fundamentals of literature searches could be integrated into the first laboratory session. Instructors meet with each of the groups during the second week session to discuss the scientific rationale and feasibility of the proposed experiment. Students need to be aware that they must coordinate with other group members in order to be consistent in their experimental treatments. This component introduces students to the need for collaboration in experimental research studies.

In laboratory session 3, after student groups have formalized their experimental plans, we provide each group with a microcosm of their choice. The groups are provided with all the appropriate materials according to their individual experimental protocols. Instructors are present to assist if questions or problems arise. Some of the questions that are typically encountered relate to chemical solubility and making stock solutions. Also, students are required to maintain a proper lab notebook so they know their experimental set-up and how to consistently prepare solutions over their two-week treatment periods. Students are permitted to come into the lab whenever it is open and available.

Using the microcosms as their soil source, students subdivide the microcosms into 7.6 cm

**Table 1.** A timetable and sequence of laboratory activities for the module.

Time	Activity
Four to six weeks prior to start of module	Set-up soil microcosms
Session 1	Literature search and developing an experimental design
Session 2	Review and discussion of experimental design
Session 3	Setting up experiments - Initiating experimental treatments
Session 4	Continued treatments of microcosms
Session 5	Prepare soil extracts, inoculate EcoPlates and cultures for nitrogen analysis
Session 6	Read EcoPlates. Complete ammonium, nitrate and nitrite analyses
Session 7	Data Analysis

diameter pots by randomly removing soil plugs. The soil plugs are removed using bulb planters and spatulas. Students have the option to include the plants with the soil plug or not. The number of pots depends on the experimental design and number of replicates. Once students establish their samples they are maintained on growth stands on a 16-hour light and 8-hour dark cycle at room temperature in the laboratory. During the treatment periods groups are responsible for maintaining their samples. Optionally students can make plant growth measurements (i.e. height) or record digital images of their samples that can be used in their presentations.

### Laboratory Session 5 – Initiate EcoPlates and Samples for Nitrification

**Materials:** EcoPlates (Biolog Inc. 3938 Trust Way, Hayward CA 94545, USA - \$10.51/plate); Multichannel pipettor; Disposable reservoirs (Biolog Inc. 3938 Trust Way, Hayward CA 94545, USA); Microplate Reader (Tecan U. S. Inc., Research Triangle Park, N.C. 27709); Buchner funnels; Whatman #1 filter paper

#### Preparation of soil microbial extracts

In the preparation of the soil microbial extracts all essential glassware is autoclaved and sterile technique is emphasized as much as possible throughout the preparation of the microbial extracts and subsequent inoculations. Soil (10 g) from the experimental treatment(s) or control sample are each transferred to a separate flask containing 90 mL of sterile 0.85% NaCl (flasks are labeled appropriately). The samples are placed on a shaker at room temperature for one hour in order to release the bacteria from soil particles. After shaking, the soil in each sample is allowed to settle out before collecting the supernatant. Alternatively, if particulate soil

matter remains in suspension, the sample is filtered through a Buchner funnel with Whatman #1 filter paper or centrifuged to obtain a particulate-free filtrate or supernatant. Adjustments in the inocula densities can be made at this point if necessary. In our experiences, preparing the soil microbial extracts as described always provides sufficient inocula densities without adjustment to obtain results with the EcoPlates. As a guideline, typically, a soil microbial extract with an absorbance value of at least 0.100 at 592 nm is used.

#### Inoculation of EcoPlates

Soil filtrate (20 mL) from the experimental extract or control sample is transferred to a pipette reservoir. Using a multi-channel repeating pipettor, with tips, 150 µL of soil filtrate is transferred to each well of the EcoPlate. The EcoPlates are covered by lids and each plate is appropriately labeled. The plates are incubated at 28°C for 3 to 7 days (temperature and length of incubation are variables that can be part of the experiment).

#### Nitrogen cycle

The production of soil ammonia/ammonium from organic matter decomposition involves a variety of bacterial species as well as fungi. Depending on the environmental conditions, this released ammonium may be oxidized to nitrite that is further oxidized to nitrate. The oxidation of ammonia in soils is primarily accomplished by members of the genus *Nitrosomonas*. Nitrite oxidizers are in the genus *Nitrobacter*. The biological transformations of ammonia and nitrite comprise the process of nitrification, part of the global nitrogen cycle. In order to isolate and analyze the different microbial genera involved with ammonia production and its

A1 Water	A2 β-Methyl-D-Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine
B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D-Galacturonic Acid	B4 L-Asparagine
C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L-Phenylalanine
D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine
E1 α-Cyclodextrin	E2 N-Acetyl-D-Glucosamine	E3 γ-Hydroxybutyric Acid	E4 L-Threonine
F1 Glycogen	F2 D-Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L-Glutamic Acid
G1 D-Cellulose	G2 Glucose-1-Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethylamine
H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine

**Fig. 1** Ecoplate Carbon Sources. Each carbon source is repeated three times across the microplate. Only the first four columns of the plate are shown in this figure.

subsequent oxidation, various enrichment media for the different nitrogen transformations are used and inoculated with soil from the microcosms.

**Materials:** Sterile Nitrate Producing Medium (Nitrite oxidizers) in tubes (10 mL per tube; Paerl, 1998); Sterile Nitrite Producing Medium (NH<sub>4</sub> oxidizers) in tubes (10 mL per tube; Paerl, 1998); Sterile Peptone Broth (4% peptone, w/v) in tubes (10 mL per tube)

#### Culture set-up

Tubes of each type of media are obtained in replicate. To each tube 0.1 g of soil from the experimental treatment is added. This step is repeated using the control sample and another set of replicate tubes of each type of media. All tubes are appropriately labeled. The tubes are incubated at 28°C for one week (temperature and length of incubation are variables that can be part of the experiment).

### Laboratory Session 6 - Complete Experimental Analyses

**Materials:** Test tubes (13 X 100 mm); Cuvettes; Distilled water; Balances; Weigh boats; Spatulas; Spectrophotometer or Spec 20; Pipettors; Pipette tips; Vortexer; Concentrated HCl; Incubator; Ammonium Ion-Selective Electrode (Vernier Software & Technology, 13979 SW, Millikan Way, Beaverton, OR 97005-2886 - \$179); High and Low Standard Ammonium solutions (Vernier Software & Technology); Vernier LabPro – Computer Interface; Computer; Low Range Test Kit (The Nitrate Elimination Co., Inc. 334 Hecla St. Lake, Linden, MI 49945 - \$40.00 for 25 assays)

#### Analyses

**EcoPlate:** After one week, sample absorbances are read at 592 nm using a microplate reader. Results are printed out.

**Ammonium Determination:** Students measure ammonium using an ammonium electrode interfaced with a computer. Direct measurements for ammonium can be made using the calibrated electrode according to the manufacturer's instructions. There are ammonium electrodes commercially available that connect to a pH meter providing an alternative for ammonium determination.

**Nitrite and Nitrate Assays:** Nitrite and nitrate assays are performed according to the described protocol by the supplier. The assay is based on the enzyme nitrate reductase, catalyzing the reduction of nitrate to nitrite using the electron donor NADH. The nitrite reacts with color reagents under acidic conditions to produce a visible color. For both assays,

25 µL from each of the sample tubes are routinely used. Assay buffer is added to each sample and standard. Then a supplied NADH source that is appropriately diluted is added to each tube and vortexed. Nitrate reductase is added to each tube (samples and standards). The tubes are incubated at room temperature for 20 minutes. Subsequently, the supplied color reagents are added and mixed with the samples and standards and incubated at room temperature for 10 minutes. The absorbances of all the standards and samples are read at 540 and recorded after appropriately blanking the spectrophotometer. These values are used for nitrate determination in the samples. The assay for nitrite follows the same steps as described for nitrate except nitrate reductase and NADH are omitted. After completion of the assay, a standard curve (using the supplied and appropriately diluted nitrate standard) is constructed and the concentration of nitrite and nitrate in each of the samples are determined. For the nitrate determinations it is essential to include samples that only contain the nitrate producing medium since this medium contains nitrite which is the end product determined in both assays.

### RESULTS AND DISCUSSION

As this is an introductory biology course, we developed specific guideline questions for students because they are at the early stages of learning how to analyze and interpret data. The following questions and experimental analyses are addressed and included in student laboratory papers and presentations. We encourage student groups to think of alternative ways to analyze data or other questions that could be addressed.

Initially, the students do find the task of transforming the raw data ultimately into summary diversity and evenness indices daunting. Additionally, students need the basic background information on diversity indices and evenness and what these parameters indicate about a community. Usually we devote an entire laboratory period to explain how to organize the raw data for subsequent analysis. This process involves using a sample set of data and going through the different steps of data transformation as described in Tables 2 and 3. Also, at this time we further explain the concept of diversity indices. We have included examples of actual EcoPlate data from a student group experiment and the subsequent data transformations (Figure 2;

**Table 2.** Calculations for microbial community analysis (from Derry *et al.*, 1998).

Parameter	Equation	Variable
Shannon Diversity Index	$H' = -\sum p_i \ln p_i$	$p_i$ = proportional color development of the $i^{\text{th}}$ well over total color development of all wells of a plate
Shannon Evenness	$E = H'/\ln S$	$S$ = number of wells with color development

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.477	2.787	2.106	2.057	0.330	2.791	2.263	2.230	0.307	2.716	2.306	2.365
B	2.248	2.953	2.790	2.510	2.115	3.047	2.522	2.711	2.097	2.799	2.596	2.395
C	1.956	1.640	0.801	1.365	2.108	1.812	0.698	1.345	2.267	1.708	0.445	1.472
D	1.951	3.165	1.461	2.475	1.832	3.048	0.943	2.453	2.063	2.969	1.526	2.376
E	2.489	2.438	1.941	1.356	2.805	2.830	1.771	1.487	2.506	2.841	1.918	0.988
F	2.535	1.237	1.712	1.876	2.762	1.783	1.500	1.467	2.512	1.726	1.335	1.395
G	2.949	1.809	1.522	2.435	2.808	1.889	0.921	2.623	2.936	1.765	1.524	2.478
H	2.337	0.755	2.084	1.937	2.400	0.728	2.161	1.460	2.325	0.576	0.502	1.471

**Fig. 2.** A sample result of a microplate scan of an EcoPlate. The absorbances were derived from a student experiment examining the effects of salinity on the soil microbial community. The soil extract used to inoculate this plate originated from a soil microcosm treated with 50 mM NaCl for two weeks. The EcoPlate was incubated at 28°C for one week. Each absorbance value reflects microbial growth on a specific carbon source and a blank (n=3).

Tables 2 and 3).

### Questions posed to the students

#### Microbial Community Analysis.

- For the analysis of your plates refer to Table 2. Complete the calculations for microbial diversity and evenness as described in Table 2. Since the EcoPlate wells are in triplicate, calculate the Mean Diversity Index and standard deviation. Calculate the mean Evenness value and standard deviation.
- Explain the results of EcoPlate analyses.
- What other possible effects may have contributed to the results you found with the EcoPlates?
- What other experimental variables may affect the interpretation of the data generated by EcoPlates?
- What may be possible limitations to using EcoPlates in the analysis of microbial communities?
- Was there any correlation between nitrification and microbial diversity?

#### Nitrogen Cycle

- Was the extent of ammonification the same in all the samples? If not, what factors could account for the differences between samples? Explain.
- Was the extent of nitrification the same in all samples? If not, what factors could account for the differences between samples? Explain.
- Summarize and present your nitrification data in an effective manner.
- Using the appropriate scientific literature resources, find information on whether your experimental treatments (i.e. salt, temperature, pH) have been found to have an effect on elements of nitrification or the Nitrogen Cycle.

#### Student assessment

Student assessment consists of the following components:

**Lab Conduct and Demeanor:** The first semester of our laboratory schedule is largely devoted to learning techniques and proper lab protocols for

**Table 3.** Sample calculations for diversity and evenness based on one set of replicates from Figure 2. These calculations are based on the absorbance values in cells A1 through H4 of Figure 2. A spreadsheet (i.e. Excel) or an appropriate statistical application (i.e. Minitab) is used for data calculations and reduction.

Sequence	Description of Calculations
Step 1	Subtract blank (Cell A1 – Figure 2) from all absorbance values (A2 through H4).
Step 2	After subtracting blank, divide each absorbance value by the sum of all the absorbance values of a set of replicates. In this example, sum all the absorbance values from A2 through H4 and divide each absorbance value by this sum. This calculation will give the ( $p_i$ ) for each well or cell.
Step 3	Take the ( $\ln$ ) of each ( $p_i$ ) and multiply by the ( $p_i$ ). Sum all of these values and take the negative of this sum. In this example, that value is 3.353 and is the calculated $H'$ (diversity index) for that set of replicates.
Step 4	Repeat Steps 1-3 for the other two replicate sets (i.e. A5 to H8 and A9 to H12).
Step 5	Determine the Mean $\pm$ S.D of the replicates (Table 4). Repeat calculations using other plates in experiment. Use an appropriate statistical test for the analysis of sample data (i.e. unpaired t-test).
Step 6	The evenness is calculated by taking each $H'$ and dividing by natural log of the number of wells showing color development. For example, with the first set of replicates (Figure 2) $S = 31$ . The $\ln$ of 31 = 3.43. $E = 3.353/3.43 = 0.98$ . Repeat calculations for the other sets of replicates and plates. As with the diversity index, determine the Mean $\pm$ S.D of the replicates (Table 4). Use an appropriate statistical test for the analysis of sample data (i.e. unpaired t-test).

**Table 4.** Means and standard deviations (n = 3) for Shannon Diversity Indices and Evenness. This transformed data is based on raw data (**Figure 2**) generated by a student group examining the effects of salinity on microbial community diversity. Experimental Group A – soil samples were treated with 50 mM NaCl for two weeks. Experimental Group B – soil samples were incrementally subjected to increasing NaCl to a final concentration of 140 mM NaCl over two weeks. The student group used soil microcosms and protocols described in this paper.

Treatment	Shannon Diversity Index	Shannon Evenness
Control (distilled water)	3.236 ± 0.041	0.95 ± 0.010
Experimental Group A (50 mM NaCl)	3.341 ± 0.017**	0.977 ± 0.006**
Experimental Group B (Increasing to 140 mM NaCl)	3.311 ± 0.007**	0.967 ± 0.006

\*\*significantly different from the Control at  $p \leq 0.05$  using an unpaired t-test

gathering data, determining statistical relevance and presenting data. Lab exercises such as micropipetting, spectrophotometry, preparation and use of standard curves and Minitab applications, etc. provide a sound basis for further laboratory exercises and experiments. An important part of the laboratory is the emphasis on proper attitude and behaviors required for effective and efficient operation of a working scientific laboratory. Students are made aware that they will be evaluated on these characteristics as well as appropriate “results” of laboratory work. The projects conducted by the student groups described here allow the instructors to assess the ability of the students to apply what should have been learned during the first semester to the specific applications of their project. Observations made by instructors during the laboratory projects, specifically, errors of omission or commission relative to procedures, techniques, use of equipment, etc. are communicated to students as negative evaluations to be included in grading. These evaluations are conveyed in individual meetings with students and represent 10% of their laboratory grade.

**Lab Notebooks:** At the beginning of the semester, each laboratory instructor provides the students in each lab section with a handout, **Guidelines for Laboratory Notebooks**, which provides detailed description of the types of notebooks allowed for use, the manner in which entries of different types can be made, and examples from lab notebooks from past semesters. These descriptions provide the students with the assessment criteria by which their notebooks will periodically be evaluated. Routine evaluations are made at mid-semester and at the conclusion of the course; some instructors choose to evaluate the notebooks more frequently.

**Written Reports:** As part of the requirement for the course, a lab report, documenting the procedures and results of the experiment(s) is completed by each student group. Again, each instructor provides the students in their section(s) with a handout(s) describing the elements that will be required for a

written report. Typically, these descriptions provide explanations and examples of the usual components of a scientific lab report in the biological sciences including Introduction, Materials and Methods, Results (including construction of Tables and Figures) and Discussion. The descriptions (and examples) again provide the student with the assessment criteria that will be used for evaluation of their written reports. Most instructors provide anonymous past reports for student scrutinies which are separated into “Poor”, “Acceptable” and “Good” categories to provide the current students with examples of various applications of the assessment criteria. Additionally, most of the students have taken or are taking concurrently courses in Critical Thinking and Effective Writing. These Core Courses are part of their liberal arts education requirement. Instructors make a conscious effort to relate the scientific method components that are the core of the biology courses the students are taking to the critical thinking skills they are accumulating in the liberal arts course. Instructors also try to relate specific instructions given to students in the Effective Writing course which apply to “descriptive writing”, “argumentation”, etc. to the instructions given related to writing scientific reports in the lab sections as a way to demonstrate transferability of learning and application of assessment criteria to similar situations.

**Oral Reports:** Each student group completing a project is asked to present an oral report, of 15-20 minutes, using Power Point presentation techniques, to the other students in the lab section and instructor (other students and instructors are also invited to attend). Each instructor conducts a “mini-workshop” on Power Point presentations for those students who might not be familiar enough with the process. Each instructor provides specific guidelines for all aspects of the presentation which serve as the assessment criteria for this component of the lab requirement. Prior to the oral presentation, each student group must submit a hard copy of the proposed Power Point presentation for instructor evaluation; the instructor

may then suggest changes to be made before final presentation. Some lab instructors utilize peer evaluation of the Power Point presentations, using a score sheet that reflects the guidelines given by the instructor to the students which serve as the assessment criteria. Students are encouraged to “rehearse” their Power Point presentations within their group and/or other groups; instructors are also available to provide evaluations of the “rehearsals”.

For many of our students this is the first time they have been involved in a multi-week investigative laboratory activity. They find the experience stimulating and challenging. We have found this module to be very effective in actively engaging students in the process of science as well as refining their analytical and quantitative skills.

The lab is open ended with the intention of encouraging revisions, modifications and new additions to the labs. For example, after the first time using this module, we decided to include a component on serial dilutions, a common microbiological technique but one where students easily become confused. The serial dilutions were easily incorporated into the sequence at the time the students prepared their soil microbial extracts. Alternatively, this sequence of investigative analysis could be extended to include an analysis of 16S rDNA by isolating and culturing a single bacterial isolate from the community (Ros *et al.*, 2008).

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## Editorial

When I volunteered to replace Steve Daggett as editor of *Bioscene* last October, it was with a mix of excitement and trepidation. I could see that he had had enough and, having put in five long years as editor, deserved a rest. Steve had improved the Journal immensely, making a number of changes that served ACUBE well. He also reorganized the editorial board and made the editorial process much more efficient with the help of Janice Bonner.

I was fairly sure I would be up to the task, but I knew that there would be a learning curve and that there would be an assortment of obstacles that would have to be overcome. Of course, Steve's remarks in his final editorial were hardly encouraging: "I felt a great weight had been placed upon me when I took this over." Now that I have gone through the first round of reviews and editing of the journal, including 23 revisions of this issue, I think I truly understand what he was talking about. I now know how many (correct & incorrect) ways there are to format a sentence/paragraph/page footer in Microsoft Word. I can also say that I know almost everything one should avoid when using and formatting text boxes and paragraphs. I now have a much more comprehensive understanding of the subtleties of wrapping text and creating tables of contents, the most daunting of which looks something like this: { **TOC** \o "1-3" \n 1-1 \h \z \u }. The human genome project seems to pale by comparison. Nevertheless, I think I'm getting the hang of it.

Steve graciously left me six suggestions to help me through the process. I have kept them in mind and I have used them as guidelines in trying to get up to speed. I have included some of the progress I have made relative to each below:

**1. Maintain and work closely with the chair of the editorial board.**

I have changed the name of Janice Bonner's position to Associate Editor and I have added a second Associate Editor, Karen Sirum, to assist with what is probably the hardest part of the editorial process - serving as a liaison between author and reviewer (i.e., prodding the reviewers to provide their reviews in a timely fashion).

**2. Keep authors informed of manuscripts' progress from submission to publication.**

I am trying a couple of web-based workflow options to make sure that we always know where a manuscript is in the pipeline so that authors are always aware of the status of their manuscripts. This is in its formative stages.

**3. Solicit opinions about the direction of *Bioscene* from our membership.**

We had healthy discussions about the future of *Bioscene* in the editorial board meeting and in the final steering committee meeting at our last meeting. I have changed a few things in *Bioscene* and I'd love to hear from our members about the changes.

**4. Fine tune our submission guidelines.**

I'll get to this one - I promise!

**5. Do not be afraid to experiment.**

No problem here. You will see some changes in the format of the journal. In most cases it is due to changes discussed previously. For example, I have changed some of the sections for submissions. We are now soliciting three different types of submissions - "Articles" (same as previously), "Innovations" (name and scope changed from "Laboratory Exercises;" this would now include techniques related to other aspects of instruction, such as lectures, recitations and online), and "Viewpoints" or "Perspectives" (This sort of replaces "News and Views" and is designed to include Op-Ed type pieces, synopses of meeting round table discussions, and any other opinion based submission. Which term do you like better?). Other changes have to do more with the eccentricities of the editing software.

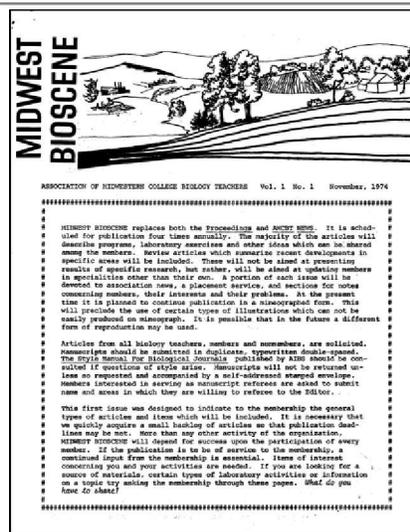
**6. Do not forget that this is a volunteer position.**

Hmmm... As one prone to obsessive behavior (aren't we all?), I'll probably have trouble with this one.

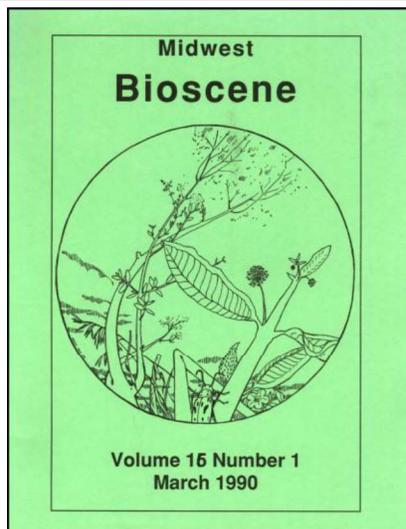
In summary, I am starting to understand what Steve learned during his tenure. I am not yet very far up on the learning curve, but I believe that I am getting there.

Finally, almost every worthwhile endeavor is somewhat painful and progress is always too slow. I think it is healthy every so often to sit back and reflect on how much progress has been made. Although you can't judge a book by its cover, I have included samples of *Bioscene* covers from the past so that we can remind ourselves how far this journal has come under the guidance of Steve and all of the editors who served before him.

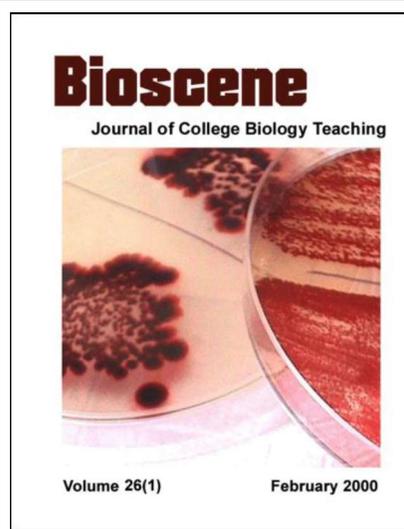
There is some controversy as to whether the changes in the covers represent evolution or intelligent design (☺). The cover changes may simply reflect changes in technology, they may reflect the growth of the organization, or perhaps a little of both. Regardless, *Bioscene* (and ACUBE for that matter) obviously has a rich history, one that we can all be proud of.



**Midwest Bioscene**, Vol. 1(1), 1974.  
Editor: John R. Carlock



**Midwest Bioscene**, Vol. 16(1), 1990.  
Editor: John R. Jungck



**Bioscene**, Vol. 26(1), 2000.  
Editors: Timothy Mulkey & Ethel Stanley

I wish you all a happy holiday break and a productive, rewarding New Year.

James W. Clack, Ph.D  
Department of Biology  
Division of Science  
Indiana University - Purdue University

# Bioscene: Journal of College Biology Teaching

## Submission Guidelines

### I. Submissions to *Bioscene*

*Bioscene: Journal of College Biology Teaching* is a refereed quarterly publication of the Association of College and University Biology Educators (ACUBE). Submissions should reflect the interests of the membership of ACUBE. Appropriate submissions include:

- Articles: Laboratory and field studies that work, course and curriculum development, innovative and workable teaching strategies that include some type of evaluation of the approaches, and approaches to teaching some of the ethical, cultural, and historical impacts of biology.
- Reviews: Web site, software, and book reviews
- Information: Technological advice, professional school advice, and funding sources
- Letters to the Editor: Letters should deal with pedagogical issues facing college and university biology educators

### II. Preparation of Articles

Submissions can vary in length, but articles should be between 1500 and 4000 words in length. This includes references, but excludes figures. Authors must number all pages and lines of the document in sequence. This includes the abstract, but not figure or table legends. Concision, clarity, and originality are desirable. Topics designated as acceptable as articles are described above. The formats for articles are as follows:

- A. Abstract: The first page of the manuscript should contain the title of the manuscript, the names of the authors and institutional addresses, a brief abstract (200 words or less) or important points in the manuscript, and keywords in that order.
- B. Manuscript Text: The introduction to the manuscript begins on the second page. No subheading is needed for this section. This supply sufficient background for readers to appreciate the work without referring to previously published references dealing with the subject. Citations should be reports of credible scientific or pedagogical research.

The body follows the introduction. Articles describing some type of research should be broken into sections with appropriate subheadings including Materials and Methods, Results, and Discussion. Some flexibility is permitted here depending upon the type of article being submitted. Articles describing a laboratory or class exercise that works should be broken into sections following the introduction as procedure, assessment, and discussion.

Acknowledgment of any financial support or personal contributions should be made at the end of the body in an Acknowledgement section, with financial acknowledgements preceding personal acknowledgements. Disclaimers and endorsements (government, corporate, etc.) will be deleted by the editor.

A variety of writing styles can be used depending upon the type of article. Active voice is encouraged whenever possible. Past tense is recommended for descriptions of events that occurred in the past such as methods, observations, and data collection. Present tense can be used for your conclusions and accepted facts. Because *Bioscene* has readers from a variety of biological specialties, authors should avoid extremely technical language and define all specialized terms. Also, gimmicks such as capitalization, underlining, italics, or boldface are discouraged. All weights and measures should be recorded in the SI (metric) system.

In- text citations should be done in the following manner:

Single Author:

"... when fruit flies were reared on media of sugar, tomatoes, and grapes" (Jaenike, 1986).

Two Authors:

"... assay was performed as described previously (Roffner & Danzig, 2004).

Multiple Authors:

"... similar results have been reported previously (Baehr *et al*, 1999).

- C. References: References cited within the text should be included alphabetically by the author's last name at the end of the manuscript text with an appropriate subheading. All listed references must be cited in the text and come from published materials in the literature or the Internet. The following examples indicate *Bioscene's* style format for articles, books, book chapters, and web sites:

(1) Articles-

(a) Single author:

DEBURH, L.E. 1991. Using *Lemna* to study geometric population growth. *American Biology Teacher* 53(4): 229-32.

(b) Multi-authored:

GREEN, H., GOLDBERG, B., SHWARTZ, M., AND D. BROWN. 1968. The synthesis of collagen during the development of *Xenopus laevis*. *Dev. Biol.* 18: 391-400.

(2) Books-

BOSSEL, H. 1994. *Modeling and Simulation*. A.K. Peters, London. 504p.

(3) Book chapters-

GLASE, J.C. AND M. ZIMMERMAN. 1991. Population ecology: experiments with Protistans. In Beiwenger, J.M. 1993. *Experiments to Teach Ecology*. Ecological Society of America, Washington, D.C. 170p.

(4) Web sites-

MCKELVEY, S. 1995. Malthusian Growth Model. Accessed from <http://www.stolaf.edu/people/mckelvey/envision.dir/malthus.html> on 25 Nov 2005.

Note that for references with more than five authors, note the first five authors followed by *et al.*

D. Tables

Tables should be submitted as individual electronic files. Placement of tables should be indicated within the body of the manuscript. All tables should be accompanied by a descriptive legend using the following format:

**Table 1.** A comparison of student pre-test and post-test scores in a non-majors' biology class.

E. Figures

Figures should be submitted as individual electronic files, either TIFF or BMP. Placement of figures should be indicated within the body of the manuscript. Figures include both graphs and images. All figures should be accompanied by a descriptive legend using the following format:

**Fig. 1.** Polytene chromosomes of *Drosophila melanogaster*.

III. Letters to the Editor

Letters should be brief (400 words or less) and direct. Letters may be edited for length, clarity, and style. Authors must include institution address, contact phone number, and a signature.

IV. Other Submissions

Reviews and informational submissions may be edited for clarity, length, general interest, and timeliness. Guidelines for citations and references are the same for articles described above.

V. Manuscript Submissions

All manuscripts are to be sent to the editor electronically. Emails should include information such as the title of the article, the number of words in the manuscript, the corresponding author's name, and all co-authors. Each author's name should be accompanied by complete postal and email addresses, as well as telephone and FAX numbers. Email will be the primary method of communication with the editors of *Bioscene*.

Communicating authors will receive confirmation of the submission within three days. Manuscripts should be submitted either as a Microsoft Word or RTF (Rich Text File) to facilitate distribution of the manuscript to reviewers and for revisions. A single-email is required to submit electronically, as the review process is not blind unless requested by an author. If the article has a number of high resolution graphics, separate emails to the editor may be required.

## VI. Editorial Review and Acceptance

For manuscripts to be sent out for review, at least one author has either joined ACUBE or agreed to page charges. Charges will be the membership fee at the time of submission per page. Once the authors' membership or page charge status has been cleared, the manuscripts will be sent to two anonymous reviewers as coordinated through the Editorial Board. Authors' names will be withheld from the reviewers. The chair of the editorial board will examine the article for compliance with the guidelines stated above. If the manuscript is not in compliance or the authors have not agreed to the page cost provisions stated above, manuscripts will be returned to authors until compliance is met or the page cost conditions have been met.

Reviewers will examine the submission for:

- **Suitability:** The manuscript relates to teaching biology at the college and university level.
- **Coherence:** The manuscript is well-written with a minimum of typographical errors, spelling and grammatical errors, with the information presented in an organized and thoughtful manner.
- **Novelty:** The manuscript presents new information of interest for college and university biology educators or examines well-known aspects of biology and biology education, such as model organisms or experimental protocols, in a new way.

Once the article has been reviewed, the corresponding author will receive a notification of whether the article has been accepted for publication in *Bioscene*. All notices will be accompanied by suggestions and comments from the reviewers. Acknowledgement of the reviewers' comments and suggestions must be made for resubmission and acceptance. Further revisions should be made within six months if called for. Manuscripts requiring revision that are submitted after six months will be treated as a new submission. Should manuscripts requiring revision be resubmitted without corrections, the chair of the editorial board will return the article until the requested revisions have been made. Upon acceptance, the article will appear in *Bioscene* and will be posted on the ACUBE website. Time from acceptance to publication may take between twelve and eighteen months.

## VII. Revision Checklist

Manuscripts will be returned to authors for not following through on the following:

- A. Send a copy of the revised article back to the chair of editorial board, along with an email stating how reviewers' concerns were addressed.
- B. Make sure that references are formatted appropriately.
- C. Make sure that recommended changes have been made.
- D. Figures and legends sent separately, but placement in manuscript should be clearly delimited.

## VIII. Editorial Policy and Copyright

It is the policy of *Bioscene* that authors retain copyright of their published material.