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Bioscene is published in May, (online) and December. Please submit manuscripts according to the guidelines for consideration

Cover image: Cover image was provided by Neval Erturk of Converse College showing a flower of a member of the genus *Kalanchoe*.

Laboratory Exercises

Articles

News & Views

Bioscene: Journal of College Biology Teaching
Volume 35 (2) · December 2009
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ACUBE Mission Statement

The Association of College and University Biology Educators (ACUBE) focuses on undergraduate and graduate biology education. Members of ACUBE share their ideas, concerns, and course innovations; present their work at the annual meeting; publish their work in Bioscene, our peer reviewed journal; and participate in the friendly collegiality of the organization.

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 the college and university teachers of the biological sciences to the attention of college and civil government administrations.

Bioscene: Journal of College Biology Teaching

Guidelines for Submissions

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:

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

or

" Ulack (1978) presents alternative conceptual schemes for observations made…"

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-

(2)

(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.

(3) Books-

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

(4) 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.

(5) 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:

Figure. 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.

BIOPS Interactive
An e-Learning Platform Focused on Protein Structure and DNA

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Abstract: One of the difficulties in teaching basic molecular biology concepts to the students with little biological background is the lack of hands-on exercises that combines the challenges of the concepts with visualization and immediate feedback. BIOPS Interactive is a web-based interactive learning environment for molecular biology that complements traditional lecture and text coursework. It includes a set of exercises of basic concepts about molecules through the use of interactive graphics and scientific visualization. Students can gain immediate feedback of their exercises, and the instructor can get the real-time feedback about students' performance. In addition to the existing exercises, BIOPS provides a simple platform for instructors to create exercises of their own, or to modify existing ones. The preliminary feedback from BIOPS users has been positive.

Keywords: molecular biology, web-based education, interactive animation.

Introduction

The need for interdisciplinary education with respect to the field of biology has grown significantly during the past decade, fueled by the growing popularity of biologically motivated courses in disciplines like mathematical and computer science, and the prominent role that bioinformatics and computational methods have gained in biological investigations (e.g., (Johnson, 2001)).

The introduction of basic concepts from molecular biology to students with limited biology background (e.g., beginning biology undergraduate majors and students from other disciplines) is a challenging problem. Molecular biology concepts such as protein and DNA are often blended with the basic chemistry, 3-dimensional geometry, and biological relevance. Because of the underlying complexity of these concepts, it is vital to go beyond the text and pictures that can be found in a textbook.

The literature has also highlighted the importance of interaction and constructivism in the teaching of traditional sciences (Driver *et al.*, 1994; Yeany, 1991). In particular, several studies have underlined the need to introduce components of creative enquiry and collaborative learning in introductory biology courses (Allen and Tanner, 2005; Lord, 1997; Millen, 2003). In presenting fundamental biology concepts to a novice student audience, it is desirable to have a set of well-designed exercises to challenge the students using an *interactive* and *graphical* environment. The literature has highlighted the importance of providing continuous feedback on the progress in the learning process, and the great impact that interactive environments have on retaining students' attention (Windelspecht, 2001). A natural consequence of this is the need for the instructor to be able to easily modify the exercises to address the needs of the class.

Computational tools offer solutions to enable viewing and investigating molecular biological entities (Honts, 2003; Musante, 2004). These tools are interesting, as they combine 3-dimensional visual representations with interactive behavior – e.g., selecting and deselecting parts of a molecule, zooming and rotation. Nevertheless, many viewers are often awkward to use and not designed to serve the educational mission – being designed as independent tools to be used by expert scientists.

This paper presents a novel tool to address these problems, called *BIOPS Interactive* (or, simply, *BIOPS*). The tool proposes to narrow the gap between theory and practice, students and instructors

. BIOPS is designed to enhance the learning of fundamental concepts from molecular biology, and it is meant to be used in introductory courses for biology majors as well as students for students that are approaching the field of biology from other disciplines (e.g., Computer Science students).

BIOPS Interactive is a web-based learning environment that enables students to practices with concepts in molecular biology – with particular focus on DNA and protein structure. BIOPS complements traditional classroom learning, by offering opportunities for both self-paced and synchronous lab activities. Through the use of scientific visualizations the students are engaged in the discovery of biology concepts, by *interacting* with the problems. BIOPS is also designed to provide in-class interaction functionalities, allowing instructors to post problems to the class and interactively observe the class progress in the problem resolution. Instructors can create their own material and exercises, or modify existing BIOPS modules. BIOPS is a web-based (Figure 1) learning environment, where students can go online to exercise learned theory in a new interactive way.

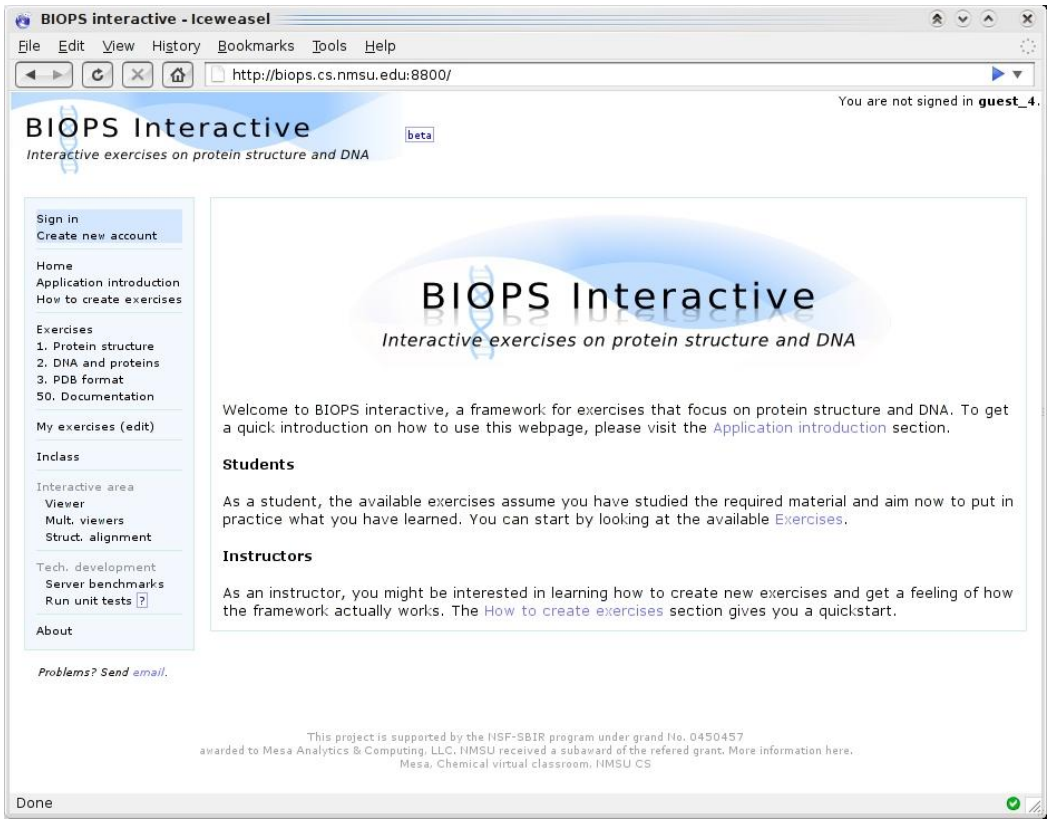

Figure 1: BIOPS website front page.

In BIOPS, the interactivity is achieved by proposing to the students exercises and online tools with direct visualization and interaction, and enabling hands-on practice of the content acquired in traditional textbook-based lectures. Software tools and a dedicated e-learning platform of exercises, with molecular biology content, have been developed, and these are discussed in the rest of this manuscript.

Materials and Methods

Basic concepts in molecular biology, like amino acid side-chain properties and protein secondary and tertiary structure, can be more easily acquired by biology and non-biology students if such concepts can be related to a visual presentation. Traditional book-based learning relies on the use of static images to illustrate key concepts. Static images have the drawback of offering a single and flat presentation of concepts that are inherently three-dimensional.

The domain of computational biology offers a variety of resources, such as data repositories, search tools, and visualization tools; these resources have the potential to be useful in the educational setting, offering dynamic, mechanical, and operational views of biological entities. Nevertheless, the majority of such resources have not been designed for educational purposes, and their use in a classroom setting requires integration and refactoring.

A first step in addressing this problem is represented by the adoption of software tools that generate a three-dimensional presentation of molecules, with the ability to dynamically interact with the presentation, e.g., by performing rotation, translation, and zooming. In addition, the software tools are expected to link the graphical representation to other representations of the same concepts (e.g., nucleotide and amino acid sequences, secondary structure components) and place them in the context of a learning process.

Sequence and protein data (including information about secondary structures) are readily available in several repositories (e.g., the Protein Data Bank (PDB) (Berman *et al.*, 2000)). The data in these repositories has a fairly complex representation, it has a purely textual representation, and it is not readily usable as an educational instrument. A popular tool that can be used to provide graphical representation is *Jmol* (Herraez, 2007), a Java application that allows a user to interact with molecular models through three-dimensional motion graphics.

In BIOPS, Jmol is used as the visualization component, but its capabilities to provide interaction have been enhanced by integrating it in a coherent framework, and enhancing its capabilities to enable interaction with other tools and resources (such as PDB) – via control modules, programmed using a web scripting language. The interactivity helps the user, for instance, to select parts of a protein, highlight a chain, or visualize the amino acids that compose a helix.

Figure 2 shows an example of the integrated display of sequence and structure information for the student. This design can link the symbolic notation of amino acids or nucleic acids on the sequence to their 3-dimensional location and physical properties shown in the molecular viewer. This type of connection is very helpful for the students with little biology background to quickly realize the concepts behind the symbols. In this DNA-protein complex – named 1SKN in the PDB and representing a DNA-binding domain in eukaryotic transcription factors that specifies mesoderm in *C. elegans* (Rupert *et al.*, 1998) – two nucleotides of chain A are highlighted in the viewer (in yellow) by a user, as a result of making the selection of the "CC" symbol in the sequence module (in red, bottom). Both the sequence and the viewer display identical colors on the secondary structure for easy identification. Shortcut buttons for movement actions are immediately below the viewer to aid the user.

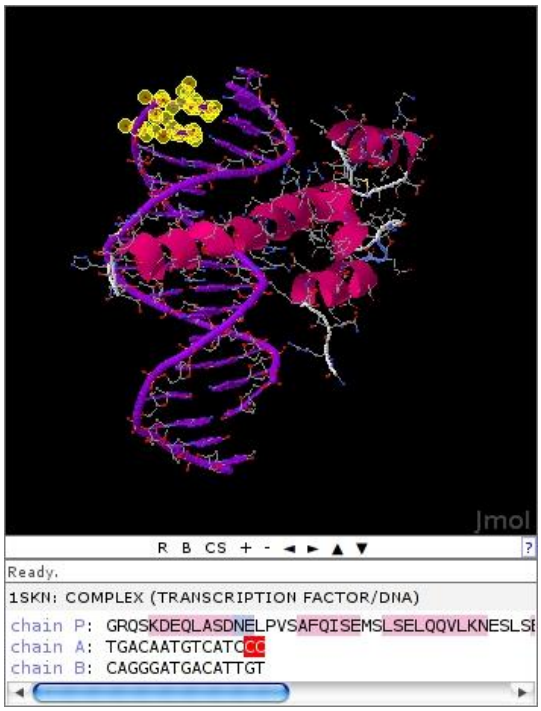

Figure 2: Sequence display module, showing the structure sequence displayed in Jmol

The tool is directly linked to the PDB repository, from which the data can be directly downloaded and used. In order to facilitate the interaction with PDB, BIOPS includes a module with which students can become familiar with the PDB format (Figure 3). The module is connected to the sequence and the viewer modules.

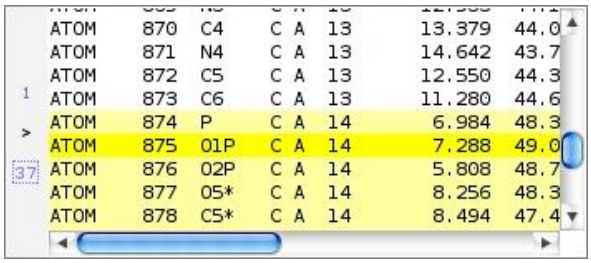

Figure 3: PDB file format module, highlighting the selected atoms in Jmol

Results

BIOPS for Students and Instructors

From a student perspective, BIOPS aims at presenting exercises with a rich interactive interface that motivates the student to further understand and explore the topic. The available exercises are presented to the students in a simple menu, organized in separate sections based on topics.

The BIOPS platform supports different degrees of interaction between the student and the instructor (i.e., the creator of the exercise). The students can provide solutions to each exercise as well as provide feedback about the content of the exercise – in the form of textual comments and a simple rating of 1 to 5 – to help instructors improve the content. In turn, the instructor can keep track of progress made by the class and by the individual students in solving the exercises.

Instructors can use BIOPS to create exercises with interactive content. These exercises can be deployed on the web, for self-paced student practice, or used directly in the classroom. An instructor can receive students answers in real-time during the class, in order to monitor progress and to help to decide if the class understood the material, or if a review should be made.

BIOPS is available online. It includes a direct viewer section where proteins and DNA can be accessed through their PDB identifier, and a multi structural alignment tool accessible through the web. In the successive sections we will illustrate the features of BIOPS through examples of its use.

An Exercise Performed by the Student

Figure 4 shows an exercise that introduces amino acids. This exercise is designed to test if a student can distinguish the 20 different amino acids when their chemical structures are given. Through this exercise, a student is challenged with the concepts of side chains and their different properties. The student is asked to look at the viewer displaying a dipeptide in 3-D. One of the amino acids, arginine, is given, while selecting the other one is the student's task. There is a table of amino acids on the right, which allows the student to view a two-dimensional representation of one or more specific amino acids. By comparing the 3-D representation with the 2-D representations, the student can analyze different amino acids and select the one that matches the best. The ability to manipulate the 3-D representation, via rotation and zooming, allows the student to determine a view of the dipeptide that facilitates the comparison. This process engages the student in finding the answer using his/her own intuitive analytical skills.

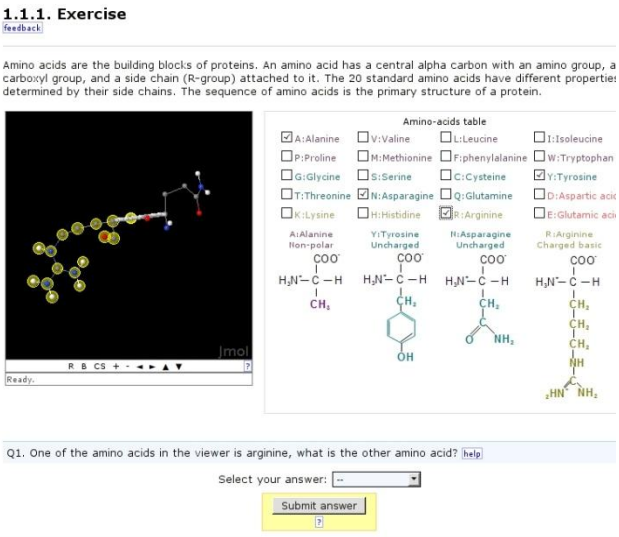

Figure 4: An exercise, with the amino acids table presented in 2-D to aid the identification of the 3-D representation of a molecule

After identifying the amino acid, the student is asked to select the answer from a list of possible answers (Figure 5).

Figure 5: Feedback returned after submitting the answer to an exercise

An Exercise Used by the Instructor In-Class

The web-based nature of BIOPS allows us to take advantage of internet-enabled classrooms, and it enables students to participate in guided in-class sessions. With this scenario in mind, the instructor initiates a new in-class session and chooses the exercise to be solved. Students connect to the instructor session and try to solve the problems. The instructor session automatically presents group and individual statistics regarding the answers submitted by the students connected to the session. If the instructor decides to change the session exercise, the session on each student's computer will automatically change as well, providing a synchronized focus on the lecture.

Figure 6 shows a table where students tried to access a session and tried to solve three sections of the exercise

section	correct	wrong	unanswered
section_1	2	0	0
section_2	0	1	1
section_3	1	0	1
total: 2 users	50%	17%	33%

user name	correct	wrong	unanswered	submissions
guest_31	2	1	0	3
guest_27	1	0	2	1

Figure 6. Statistics for an in-class exercise.

After the results have been submitted, the instructor can decide, based on the feedback returned, what material to cover next. The immediate feedback gives the instructor a way of dynamically and objectively adjusting the next step in the class (e.g., revise materials, propose new exercises on the same topic, move to a new topic).

The ideal use of this feature is in a computer lab setting, where students practice the material learned in class. Furthermore, BIOPS enable interactive remote participation of students to lab sessions, instead of the passive model promoted by traditional online management systems (e.g., Moodle).

Bringing the Community into the Loop: Exercise Creation

In recent years, the web has gained a prominent role as a framework the "grassroots" development of repositories of community-provided knowledge – this trend has been referred to with different terms, such as Web 2.0 (Shuen, 2008). Web 2.0 technologies offer the ability to share knowledge and provide community-based evaluation of data and hypotheses (e.g., CBioC (Baral, 2006)).

The potential for involvement of a community of educators in the development of a shared educational infrastructure has been recognized by several authors (Alexander, 2006; Vonderwell, 2008). BIOPS provides the ability for a community to contribute exercises, which can be used and shared with other instructors. Exercises are stored in BIOPS in a XML format, making it easy for educators to use existing exercises as templates and modify them as suited. The feedback mechanism enables continuous improvement of exercises and the creation of a community-based repository of user-ranked exercises.

To support this approach, BIOPS provides a repository of exercises, along with an online section where new exercises can be created. Any registered user can create exercises and save them for later

reference and share them with other BIOPS users. A relatively limited amount of computing knowledge is required to develop exercises, and online documentation is provided on how to create them in a simple manner, from simple questions to more advanced features that require scripting.

BIOPS provides a graphical user interface for the interactive development of new exercises. Each exercise is described using a specialized XML format specifically developed within BIOPS.

An exercise is composed of two parts. The first part describes the question to be proposed to the student. The question can be formatted using standard HTML; in addition, the problem description can include a script section, used to perform processing of the answers and to invoke Jmol. The second component of an exercise includes information concerning the correct answer to the problem. A new exercise can be created by modifying one of several templates provided by BIOPS.

We noticed that teaching the basic molecule structures often involves teaching the data that represents the structure. BIOPS encourages students to first understand what a molecule is and have a hands-on experience with the molecule through the 3-dimensional viewer, and only later learn about the underlying data. One of the exercises was designed to enable loading a protein structure, visualizing it, and manipulating it.

The interactive area allows also access to an online structural alignment tool, enabling the display of the alignment of up to four chains. As seen in Figure , after the alignment is performed, the user can perform the desired selection and visualize it in two viewers. One of the viewers (left) shows the overall alignment, the second (right) shows only the selection made.

challenge the understanding of the structural data provided in PDB format. Custom-made PDB files may be uploaded by instructors who want to create an exercise that shows molecules, ranging from simple molecules up to multiple proteins within the same viewer. More advanced displays, with two or more viewers for the same PDB file are also possible (Figure).

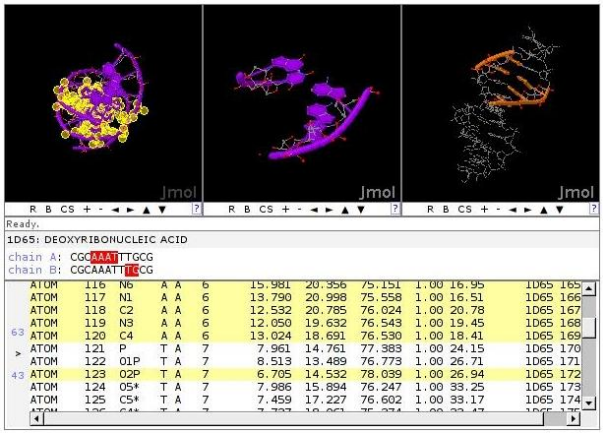

Figure 7: Multiple viewers showing the same molecule differently

Interactive Areas

BIOPS provides users with the ability to open interactive areas on the web site. A common use of the interactive area is to connect to PDB and to

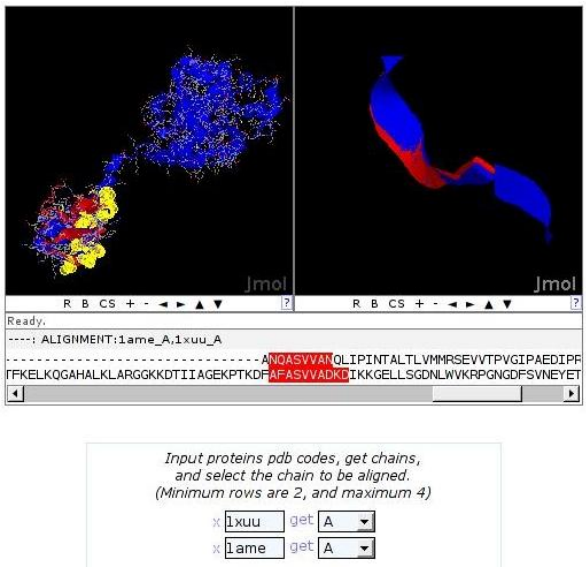

Figure 8: Interactive use of the alignment tool

System Organization and Implementation

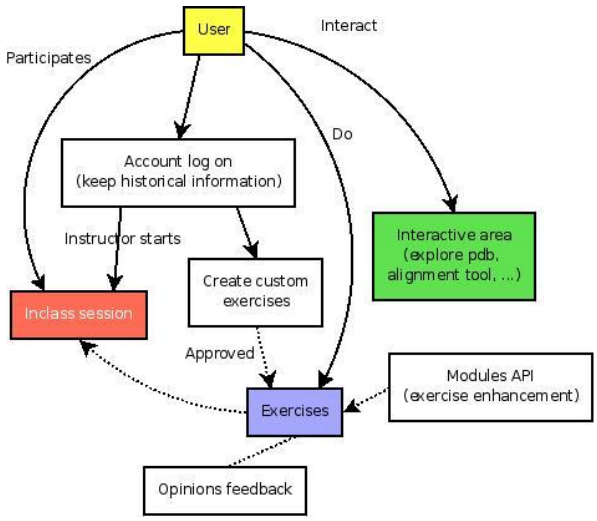

Figure 9: System design overview

The key components and interactions of the BIOPS framework are depicted in Figure 9. The role of the user in the system and the progress in problem solving and class participation are managed through a centralized accounting mechanism. An account can be freely created, and it allows BIOPS to maintain user profiles and user-specific historical information, such as answers submitted. The accounting mechanism for the instructor includes the ability of tracking exercises developed and historical data concerning success and failure rates per problem.

The core of the system is represented by the collections of modules used to manage exercises. Any user of the system can create exercises. Exercises are stored in a central repository, where they can be retrieved, reviewed, and included in organized collections for in-class sessions.

The exercises management component provides a graphical user interface and an XML language for the development of new exercises. The specialized language allows access to a collection of enhanced modules Application Programming Interface (API), which supplies the improved interactive resources mentioned earlier.

Figure 10 shows a more technical system overview of BIOPS. The web pages requested by users from the Internet are enhanced with Javascript and AJAX, making use of Java Applets, like Jmol, to show 3-D representation of molecules.

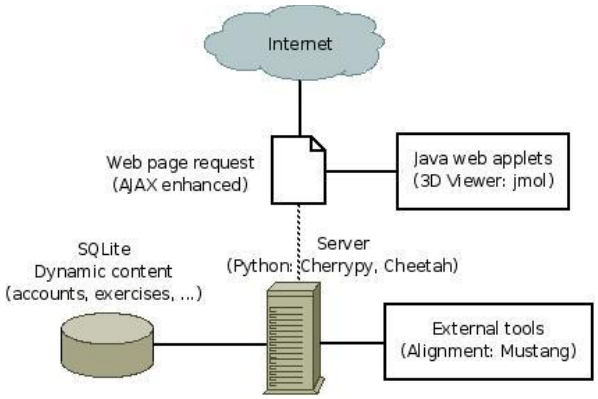

Figure 10: Technical system overview

The main BIOPS server has been developed in Python, using Cherrypy as web server and Cheetah (Hellegouarch, 2007) as the template engine. The repositories of exercises and user accounts have been implemented using a lightweight SQL-based relational database. External tools are used in real time by the server to provide interactive functionalities.

Topic	Question number	Question	Score	Spring 08	Spring 09
Environment Used:	1	What type of computer did you use to access BIOPS?			
	2	What operating system is installed on your computer?			
	3	Which web browser did you use to access BIOPS?			
BIOPS Interface Design	4	Was the interface easy to use?	|Very Easy|Easy|So-So|Not Easy|Difficult|	4.8/5	4.5/5
	5	Did you know what you needed to do?	|Very Obvious|Obvious|So-So|Confusing|Not Obvious|	4.2/5	4.1/5
	6	Were you able to figure out how to manipulate structures in Jmol?	|Very Easy|Easy|So-So|Not Easy|Difficult|	4.1/5	4.0/5
	7	Were the Jmol widgets (e.g., amino acid structures) useful?	|Very Useful|Useful|So-So|Somewhat Useful|Not Useful|	4.9/5	4.6/5
BIOPS Content	8	Was the content appropriate to learn about protein structure?	|Very Appropriate|Appropriate|So-So|Inappropriate|Very Inappropriate|	4.4/5	4.6/5
	9	Did you gain better understanding of protein structure after working through the exercise?	|Much Better|Better|About the Same|	4.6/5	4.6/5
Clarity of Content Presentation	10	Were you able to answer the questions in the tutorial correctly and understand why you were correct on your first attempt?	|Most of them|Many|Some|A Few|None|	4.6/5	4.6/5
	11	Were you able to answer the questions in the tutorial correctly and understand why you were correct after multiple attempts?	|Most of them|Many|Some|A Few|None|	4.2/5	3.9/5
General Experience Evaluation	12	What did you learn by using BIOPS Interactive?			
	13	What parts of BIOPS Interactive did you like best?			
	14	What parts of BIOPS Interactive did you like least?			
	15	Do you have suggestions on how to improve the educational value of BIOPS Interactive?			

Table 1. Evaluation questionnaire and summary results.

Preliminary Evaluation

The development of a first release of BIOPS has been recently completed and informal evaluations have been performed.

In July of 2007, BIOPS was presented at the 21st Symposium of The Protein Society in a workshop for educators. A total of 35 educators participated in the session. After the presentation, the workshop allowed hands-on activities, where participants interacted with exercises in an in-class session. The experiment was successful both technically and as a learning event, where the participants engaged in interacting with the exercises and followed the session. The feedback was positive with several expressions of interest for classroom use of BIOPS.

BIOPS was deployed at Virginia Tech, as part of two graduate level classes in Computational Biochemistry (Spring 2008 and 2009). The background of the students was primarily Computer Science. We distributed to the students an evaluation form with 15 questions. The questionnaire is summarized in Table 1. The students had never seen BIOPS before. The evaluation included sections requesting an evaluation of the interface design, including usability of the modules. The evaluation also covered the perceived strengths and weaknesses of BIOPS, especially concerning the effectiveness as a learning instrument.

The evaluation indicated a high level of satisfaction in the use of the system, including praise for the easy to use interface and the easy way to solve and develop exercises. References were made, in particular, to the BIOPS modules where sequences can be selected and highlighted. Regarding the strengths of BIOPS, the ability to interact with the molecules and with the three-dimensional representation was often cited. The evaluation overwhelmingly confirmed that BIOPS helped the students understanding the material – students stressed the impact of being able to associate theoretical concepts to the graphical structures (e.g., representations of amino acid structures or by highlighting secondary structures). The main weakness reported was the need to expand the exercise repository – something that will naturally occur as the BIOPS user base expands. Figure 12 summarizes the scores given to questions 4-11 for the Spring 2009 edition of the class (10 students). Analogous results were collected in Spring 2008 (15 students). The scores of each column corresponds to one of the categories (1=worst, 5=best).

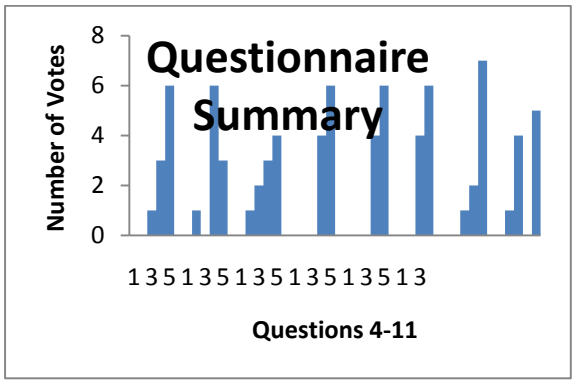

Figure 12: Summary of answers to Questions 4-11 (5=highest score, 1=lowest score)

We performed another evaluation in the Computer Science Department at NMSU, with a group of students who are in a local mailing list. Seven people participated in the evaluation (6 graduate students and 1 undergraduate student). The participants are students who have little biology background but interest in bioinformatics. The participants performed a series of exercises (protein structure, PDB, and DNA); these exercises were not conducted in the context of a classroom, so the students were asked to learn the material as they proceeded through the exercises. In spite of this, on average 75.4% of the questions were correctly answered. 52% of the participants rated the exercises "very good". The overall satisfaction is mostly rated as "helpful" and "very helpful".

Conclusions

The BIOPS project has resulted in the successful development of a web-based interactive system for instruction in molecular biology. The system is accessible at http://biops.cs.nmsu.edu:8800. BIOPS has already received more than 2,500 unique visitors (as July 2009) since its first public release was made available (July 2007).

BIOPS was developed as an independent but complementary system to the *ChemInformatics Virtual Classroom* implemented by Mesa Analytics to help students understand the relationship between drug design and protein structure. In the near future, the two systems will be integrated, to enable the introduction of exercises about protein structure within interactive sessions dedicated to drug design (e.g., illustrating the concept of protein-protein docking).

Acknowledgments

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Group Projects as a Method of Promoting Student Scientific Communication and Collaboration in a Public Health Microbiology Course

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Abstract: Communication of scientific and medical information and collaborative work are important skills for students pursuing careers in health professions and other biomedical sciences. In addition, group work and active learning can increase student engagement and analytical skills. Students in our public health microbiology class were required to work in instructor-assigned groups to research a human pathogen and associated disease, and to create a presentation appropriate for their classmates. Objectives of the project included building students' abilities to research and critically assess relevant scientific and medical information, increasing their scientific communication skills, and improving group collaboration skills. Another goal was for students to be the class "expert" on their chosen pathogen. Group projects were presented orally to the class, and in written formats as either posters or pamphlets. A peer evaluation was utilized to allow students the opportunity to evaluate their group's effectiveness. Students were surveyed after the projects for self-evaluation of content knowledge and confidence in scientific communication and research skills. Many students expressed enthusiasm for the project, and 96% and 65% of students reported increases in content knowledge and communication skills, respectively. We conclude that group projects are an effective means of delivering content while increasing students' confidence in science communication skills.

Keywords: group work, microbiology, science communication

Introduction

Undergraduate students majoring in fields within the allied health professions need to gain appropriate content knowledge in areas of biology including microbiology. In our curriculum, pre-nursing students and majors in other allied health programs typically take a one-semester course in medical and public health microbiology. This course focuses on basic microbiology with an emphasis on human pathogens and infectious disease, including both lecture and laboratory components. Although increasing students' content knowledge and exposure to laboratory methods in microbiology are certainly primary goals of our course, the development of other skills is also important for students planning careers in nursing and other allied health fields. For example, the ability to critically assess medical and science literature, to communicate technical information to different audiences, and to work effectively in groups are all valuable for students in these majors, and indeed are useful skills for students in many, if not all, fields in science, medicine, and technology. To address the development of these skills, we have implemented a new group project into this class. Students were assigned by the instructors to groups of three to five and were required to research and present information on a human pathogen and the associated disease. The objectives of this project included: 1) building students' abilities to research and critically assess relevant scientific and medical information, 2) increasing their scientific communication skills, and 3) improving group collaboration skills. In addition, we expected students to demonstrate in-depth knowledge about the pathogen chosen by their group.

Over the past two decades, increasing emphasis has been placed on teaching the process of science as well as content, and on exposing undergraduates to scientific research through primary literature and/or independent projects (National Research Council, 2003). Primary literature relevant to the pathogen would be the optimal source for the most current scientifically accurate information. However, students in this course had generally not been exposed to reading primary literature in the prerequisite introductory biology course, and were not expected to be familiar with

methods for searching for or reading current primary literature in microbiology and infectious disease. Our primary goal was for students to find and assess the credibility of accurate information from secondary sources and present it at a level appropriate for their classmates and the public. The internet serves as a primary source of information of science health care information for college students (Escoffery, 2005), and indeed for patients and the public as well (Wilson, 2002). Interestingly, a study suggested that students with stronger internet searching skills learned more and were more critical of information accessed through internet searching than students who were less familiar with internet searching (Tsai, 2003). We therefore allowed students to use reliable but non-primary literature sources such as textbooks and internet-accessible information, and in our view, this served as an important exercise for students in evaluating the reliability of science and health-related information on the internet.

The development of skills in communication of scientific and medical information is also important for students pursuing careers in health professions as well as in other areas of biomedical science. Students who successfully enter the nursing profession, for example, will be expected to accurately and clearly discuss science and medical information with their supervisors, colleagues, and patients, and must be able to communicate effectively with each of these groups. Evidence from several studies indicates that ineffective communication skills in nurses and other medical professionals can negatively impact patient satisfaction and compliance with recommended treatments (Chant, 2002; Fallowfield, 1999). We therefore gave students the opportunity to improve their skills in communicating technical scientific and medical information to their peers through an oral presentation and through an informational pamphlet or poster.

Substantial evidence suggests that collaborative group work and activities are effective means of learning for students (Michael, 2006; Tanner, 2003). The ability to work well as part of a team is also an important skill in many careers in current society, including the nursing and other allied health fields that students in our course are planning to enter. This project included both collaborative work and active learning elements, since students were required to work within a group to research their topic and present their findings. Because this project included several objectives that could not be directly tested with a content- and application-based quiz or exam, we used a survey to assess students' perceptions of their gains in skills from this project. We describe here our results after three semesters of using this group project assignment.

Materials and Methods

This project was first implemented in the Fall 2006 semester, and has subsequently been utilized in the Fall 2007 and Spring 2008 semesters as well. The Fall 2006 and Fall 2007 courses were taught by one of the authors (Walton) and the Spring 2008 course was taught by the other author (Baker). Some relatively minor changes in project format were implemented in the Fall 2007 and Spring 2008 semesters; these will be highlighted below. However, the general format of the project, grading rubric, and assessment survey were consistent among the three semesters described in this manuscript.

Students were assigned by the instructors to groups of three to five students. In the Fall 2006 semester, group assignments were completely random. In the Fall 2007 and Spring 2008 semesters, the groups were not assigned until at least one major exam grade had been recorded, and we used these exam scores to distribute students so that each group contained a mix of stronger and weaker students. Group size was largely determined by the number of students per section and the amount of time available for presentations. Each group was required to select a human pathogen from a list that we provided. The list typically contained 9-15 pathogens that were not otherwise covered extensively in the course, but were generally of high interest to students. The list of pathogens included species from four major groups of microbes (viruses, bacteria, fungi, and protozoa). Examples of popular choices included rotavirus, *Bacillus anthracis*, and *Trichomonas vaginalis*. Students were provided with a handout that contained guidelines for the project, including project objectives; requirements for content depth, format, and references; and a copy of the grading rubric. A sample grading rubric is shown in Table 1.

Groups were required to use a minimum of three reputable, peer-reviewed secondary sources for their project. Suggested sources were given in the guidelines, including the course textbook, reputable online sources such as Medline Plus (http://www.medlineplus.gov) and the Centers for Disease Control and Prevention (http://www.cdc.gov), and optionally current primary literature. On the day that the project was due, each group gave an oral presentation of no more than 10 minutes with information about their selected pathogen and the associated human disease. In

addition, groups prepared either a poster (Fall 2006 and Fall 2007 semesters) or an informational pamphlet (Spring 2008) to accompany their presentations. The target audience for the oral presentation and the poster/pamphlet were emphasized to students as an important consideration. In the Fall 2007 and Spring 2008 semesters, the posters or pamphlets were to be designed as public awareness materials that would be appropriate for any general audience, while the oral presentation was expected to include more technical information at an appropriate level for the rest of the class.

We graded each group and individual student on the day of the presentation, using the rubric shown in Table 1. As shown in the rubric, a student's grade for the project included a group component, which was the same for all members of the group, and an individual component based on how well that student presented his or her part of the group presentation and answered questions posed by the instructor and students in the audience. On the day of the presentation, all students were also required to turn in a peer evaluation that asked students to evaluate the performance of their group members. The average peer evaluation score for each student was factored into their overall grade for the project. Students' learning of the material from all of the presentations from their section was assessed by instructor-constructed quizzes or exams one to two weeks following the presentations.

Table 1. Group presentation grading rubric (Fall 2007)

	Poorly done, missing, many mistakes	**Average, some mistakes or omissions**	**Excellent, thorough. few to no mistakes**
Oral presentation			
Content (20 pts):			
Appropriate level of information	0 1	2 3	4 5
Description of microbe	0 1 2	3 4 5	6 7 7.5
Description of disease (case study, treatments)	0 1 2	3 4 5	6 7 7.5
Format (10 pts):			
Contains all required information	0	2	4
List of sources and proper citations	0	2	4
Style (well organized, easy to follow)	0	1	2
Presentation (5 pts):			
Individual able to describe presentation or poster content; answered questions	0 1	2 3	4 5
Poster			
Content (5 pts)			
Appropriate and accurate level of information	0 1	2 3	4 5
Design (5 pts)			
Poster is readable, clear, attractive	0 1	2 3	4 5
Mean peer evaluation score for group participation (0-5 pts)			_____

Total: _____ out of 50 possible points

Notes and suggestions:

Results and Discussion

Upon completion of the group infectious disease project, students were asked to provide anonymous input regarding their perception on the project's effectiveness at achieving instructor-established goals. Students were asked to rate their confidence level before and after the project in three

areas: ability to research information, ability to communicate, and depth of knowledge for their selected infectious disease. Table 2 provides the mean (+/- SD) student responses by semester. All means in a pair-wise t-test comparing before and after project responses for a given survey item showed significance at $p<0.05$. Our results support the general impression that many students lack confidence in communicating with their peers prior to this project, reporting an average which ranged from 3.2 to 3.6 on a 5-point scale among the three semesters. Survey numbers indicate the project significantly enhances their confidence in this area, increasing student average confidence by 0.8 to 1.3 beyond their initial response, with 65% of students reporting an increase when data from the three semesters were combined. Likewise, we do not expect students taking this course to come into the class with a high level of pathogen-specific knowledge. This is supported by the average response to this survey question, which ranged from 2.0 to 2.6 across the three semesters. Students clearly took responsibility for their topic as evidenced by the dramatic increase in their opinions of how well they understand the pathogen and disease following the project (a 1.9 to 2.4 increase), with 96% of total students surveyed reporting an increase. Additional support of this subject-specific knowledge increase comes from scores on quizzes over the material researched and presented. For example, students scored an average of 13.7 out of 15 pts (91.3%) on an open-note quiz over all pathogens presented, not just their own, in the Spring 2008 semester and averaged 8.6 out of 10 pts on a closed-note quiz in Fall 2007. Regardless of semester, the quiz was written by the instructor and consisted of multiple-choice questions. These scores show that, in addition to learning from their own group's research, students also learned effectively from their peers' presentations.

Interestingly, though still a significant increase, the least change in student response scores occurred in their opinions of their own confidence in researching and evaluating relevant data. We have found that most students feel capable of researching information in general, though not necessarily scientific information, prior to this course. This is likely due to the required Research and Writing course all Missouri Western State University students take as part of general studies education. Students generally take this course in their first academic year, almost always before advancing in their science curriculum to this course. Our data show an initial, confident response of 4.0 to 4.2 with only a 0.3 to 0.6 average confidence increase in adding to this skill. We feel that this relatively minimal increase in students' perceptions of their research skills may be due to our project parameters, which allowed students to use everyday resources they were already comfortable with, such as textbooks and the internet.

In the second two semesters of our project, students were also asked to evaluate their overall impression of this project as an effective way to learn about their chosen pathogen. Their high response averages of 4.4 and 4.8 indicate our project design is effective in stimulating student learning. This is an important piece of evidence showing that an active learning process is effective and well accepted by our students.

Table 2. Student response survey data. Numbers listed are response means (+/- standard deviation). Response scale used is 1=strongly disagree, 2=disagree, 3=neutral, 4=agree, 5=strongly agree. All data comparing before and after responses for each survey statement in a given semester show $p<0.05$ in a paired-samples t-test.

	Fall 2006 (N=43)		Fall 2007 (N=50)		Spr. 2008 (N=47)	
	before	after	before	after	before	after
I am confident in my ability to research and find accurate microbiology and health information.	4.2 (0.9)	4.7 (0.5)	4.1 (1.0)	4.4 (0.7)	4.0 (1.0)	4.6 (0.5)
I am confident in my ability to communicate infectious disease-related information to my classmates and other peers.	3.6 (1.0)	4.6 (0.5)	3.5 (1.1)	4.3 (0.8)	3.2 (1.1)	4.5 (0.6)
I have an extensive understanding of the biology of the pathogen and related disease that my team selected.	2.6 (1.0)	4.5 (0.6)	2.1 (0.8)	4.3 (0.7)	2.0 (1.0)	4.4 (0.7)
Overall, this project was an effective way for me to learn about pathogens.	----	N/A	----	4.4 (0.6)	----	4.8 (0.4)

As a part of the grading rubric for assessment (table 1), students were given the opportunity to grade their group members and comment on any aspects of group interaction. Of the 140 students over three semesters who completed the project and survey only 14 received a score of less than 5, the top score, from their peers. For the peer evaluation, students were given a scale from 0 to 5 with explanations of what each score represented. A score of 5 indicated the group member "was a full participant in all aspects of the group project, and contributed his or her full share of the work, and was generally a positive influence on the group's work." Student peer evaluation numbers indicate that students are working well together despite some initial reservations in doing a group project, as seen in Table 3 which lists select student comments. In all three semesters of this evaluation students were assigned to groups of three to five students. In the Fall 2006 class, our first use of this project format, students were required to meet and coordinate efforts but were given only two 30 minute portions of lecture periods dedicated to group work time. All other interaction had to occur outside regular class times. This semester precipitated the greatest number of comments about dislike for working in groups and difficulty finding time to get everyone together. This is in agreement with other published studies using collaborative groups within the sciences, which note that negative student comments frequently relate to difficulty scheduling group meetings (Hume, 2006; Mulnix, 2003). As a result of student comments, and knowing we have many non-traditional students with extensive non-academic obligations, the next two semesters we placed students into groups that would contain stronger and weaker students based on exam scores, we provided contact information for group members, and we designated group meeting/work time within the laboratory portion of the course during a time period while students were also working independently on a bacterial unknown project. Although we cannot rule out the possibility that students were just being nice to their group members, after these changes, fewer students commented on problems with getting the group together and fewer students received a score of less than 5 from their peers. We also noted that groups worked independently, requiring very little instructor oversight, unless group dynamics were an issue or source validity was in question.

Table 3. Select student comments from the student survey. Comments presented here are representative of the types of comments for each semester of this project.

Select student comments	
Fall 2006	It was alright, overall I think we worked together well as a group and we all did our part of the project & it was actually pretty informative.
	The project would not have taken as long to complete if all of the members in the group had participated instead of just two of us.
	I think a presentation (power point) might be better. Very crowded looking at the posters. Pamphlets I think is a good assignment.
	I enjoyed the poster assignment, it gave me a chance to get to know my classmates and it was a nice break from every day class routine.
Fall 2007	I thought the project was a good assignment. It helped me get to know some of my classmates better, and we actually had fun working together. The biggest thing that worried me was presenting the pathogen because I am scared of public speaking! All in all, I thought it was good.
	Enjoyed it, enjoyed getting to know & work with new classmates in research.
	The only thing I could suggest for you to change is to hand out an information sheet for the team members to contact each other. I found my teammates helpful & willing but the first few days nobody thought to trade emails & phone numbers.
	I really enjoyed the research with the group but the project and the unknown at the same time was time consuming for me.
Spr. 2008	I think this was a good idea, to make people work together as a group. I know this was one of the most challenging parts of the entire project for some groups
	It was a good way to learn about other important pathogens that are not as popular as like the AIDS or the Ebola virus. I really liked that each of these pathogens is very important to the health related fields.
	I thought the project was a great way to cover certain pathogens in more detail. The Power Point and pamphlet made the presentation more interesting and educational. A good end-of-the-year project!
	Group projects can be fun. It was hard to get together as a whole group.

This project was designed to engage students in active learning through researching a pathogen, improve group collaboration skills, and increase both written and oral communication skills even though the specific delivery of this project was slightly different each semester. In Fall 2006 the project involved the preparation of a technical poster on the chosen infectious disease and the presentation of that poster to peers during a designated lecture period. Some groups chose of their own initiative to also prepare a pamphlet to provide to all classmates, which proved to be very popular with the other students. Each team submitted exam questions based on their material and the specific information researched and presented by each group was tested on the course's final exam. In Fall 2007 the project was adjusted to include the presentation of the material in an oral PowerPoint during laboratory sessions and the preparation of a poster for use as a public awareness tool. Groups therefore had to prepare materials appropriate for two different audiences, and were graded on the appropriateness of the oral presentation for their classmates and of the poster or pamphlet for the general public. We considered the general public a particularly appropriate audience to focus on because the student population in this course is primarily interested in allied health careers. A recent study in a nonmajors chemistry course also described the value of requiring students to communicate scientific information to nonspecialist or lay audiences, and students in this course generally agreed that communicating scientific information to this audience was a valuable learning experience (Shane, 2008). Students were allowed access to the posters for several days followed by a closed-note quiz. In Spring 2008 the project included an oral PowerPoint presentation in laboratory sessions and the preparation of a single sheet public awareness pamphlet provided to each classmate that could be used during a quiz. Regardless of the format used to fulfill our objectives we saw similar student survey response increases as shown in table 2, and similar class quiz scores ranging from 86.0% to 91.3%. In addition, the overall quality of work accomplished by the students across the three semesters of our project was very high and met our expectations with students averaging 93.4%, 92.6%, and 91.9% on instructor-determined grading. These data show that minor alterations to this project to suit the logistical needs of a class or the preference of an instructor still result in high student achievement of the stated objectives.

In conclusion, our project requiring students in a public health microbiology course to work in groups to research and present an infectious disease and demonstrate content knowledge was very successful. Our data support that, regardless of minor adjustments in project format, the students self-assessed an increase in comfort with researching relevant information, communicating with others, and understanding their pathogen. In addition, we observed that students worked well together in their groups and required very little instructor guidance. Our data demonstrate that allied health students can actively learn science, communicate science information, and learn science from their peers. This type of project has given us the opportunity to play a role in helping students to develop essential skills needed by future health care providers.

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A Simulation of DNA Sequencing Utilizing 3M Post-it® Notes.

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Abstract: An inexpensive and equipment free approach to teaching the technical aspects of DNA sequencing. The activity described requires an instructor with a familiarity of DNA sequencing technology but provides a straight forward method of teaching the technical aspects of sequencing in the absence of expensive sequencing equipment. The final sequence analysis can be used as a springboard to a number of activities including literature reviews and writing projects.

Keywords: DNA sequencing, low technology

Introduction

As a teaching professional in the area of molecular biology, I have seen a substantial increase of student interest in learning how bands on X-ray film or within a gel can be interpreted as irrefutable evidence. Many small colleges and universities have little or no molecular equipment which they can utilize to foster the interest their students show in learning more about the topic.

This article provides informed college professors with a simulation of DNA sequencing that can be easily depicted with four colors of 3M Post-it® notes. The article will describe how DNA sequencing is carried out in a wet lab and will provide a dry lab simulation of the critical steps of the process. Upon completing the "dry lab", students will be able to determine the nucleotide sequence of genes, confirm the names of those genes and, if desired, use this as a starting point for generating a literature review based paper on the role these genes play in diverse organisms. This novel technique was presented to secondary science teachers (during a 2 hour session) from around the state of Nebraska in a workshop for Rural Academic Secondary School Science Partnership (RASSSP). It was found very useful by all participants and comments by these teachers are included at the end of the article.

Materials

(listed per student or group)

- Approximately 200 sheets (73 mm x 73 mm) each of four Post-it® note colors. In this example yellow, pink, orange and green sheets were utilized.

INSTRUCTOR PREPARATION (Preparation time per student or group is approximately 20 minutes).

STEP 1 – Simulation of an "unknown" single-stranded gene sequence.

Obtain a sequence from The National Center for Biotechnology Information (NCBI) website (see instructions under IDENTIFICATION OF YOUR GENE FRAGMENT) or utilize one or more of the gene sequence fragments provided (see appendix A). It is recommended that if choosing a gene sequence from NCBI on your own, that you utilize bacterial or viral genes to eliminate confusion that may result from introns present within eukaryotic genes.

Representing the sequence with Post-it® notes is carried out by assigning a colored Post-it® note to each of the four types of nucleotides. The following code is suggested:

Yellow sheets represent the base adenine.
Pink sheets represent the base guanine.
Green sheets represent the base cytosine.
Orange sheets represent the base thymine.

For example, a gene fragment with a known sequence of GATCGTCAC would be represented with Post-it® notes that were stuck together in the following sequence: pink-yellow-orange-green-pink-orange-green-yellow-green. The sequence of Post-it® notes will serve as "template DNA" in our sequencing reaction (described below) and should be about 25 sheets (nucleotides) in length. Shorter sequences may result in less specificity when gene identification is carried out.

STEP 2: Generation of primers.

For each gene sequence generated in step 1 (above) you will need to generate approximately ten "primers" that are each the same four nucleotides in length. A primer is a short segment of nucleic acid

that will hybridize (form hydrogen bonds between bases of nucleotides) to complementary DNA at a designated location and provide a chemical structure (3'-OH) needed for further extension of a growing DNA strand from that specific location on the DNA template strand. Using our sample sequence above of GATCGTCAC this primer set of Post-it® notes would complement the first four bases of GATC. A single strand of DNA can only hybridize to a second strand of DNA in a guanine/cytosine or adenine/thymine manner (Weaver, 2008). So the complementary strand of GATC would be CTAG and would be represented with approximately ten Post-it® note sets of a green-orange-yellow-pink sequence, all to be given a black border (Figure 1) to designate these short sequences as primers that provide the necessary starting point for extending DNA from that specific "primed" location.

Figure 1. Sequence elongation. Shown are both the template DNA stand (bottom) and a newly generated DNA strand (top) that could be produced in reaction Tube A. Note that the primer (the first four nucleotides of the top strand) are outlined in black and a newly incorporated deoxynucleotide (fifth sheet shown in green) and terminal dideoxynucleotide (yellow crossed-out sheet) are added in a fashion that complements the template strand in a green-pink (cytosine-guanine) and orange-yellow (thymine-adenine) manner.

STEP 3: Simulation of components needed for Sanger Sequencing.

Wet lab sequencing requires a polymerase enzyme, primers, template DNA and both deoxynucleotides and dideoxynucleotides. A description of the role of each of these components can be obtained from various sources (Sanger 1977, Dolan). Each of these major components of the "wet" sequencing reaction needs to be represented in the "dry" Post-it® note reaction. The role of the polymerase will be carried out by the students. Template DNA and primers were prepared in steps 1 and 2 above. Individual deoxynucleotides will be represented by Post-it® notes of various colors and dideoxynucleotides should also be represented with Post-it® note sheets. However, the sheets representing dideoxynucleotides should have an X drawn on them. About $1/5^{th}$ of your Post-it® notes should be crossed-out to represent dideoxynucleotides. Sequencing in a "wet" lab also requires a buffer which can be represented by the air in the room. The level of detail describing each of these components will, of course, be dictated by the individuals you are teaching.

Assemble four table tops for each sequencing reaction. Each table will represent one of four "reaction tubes" for DNA sequencing which we will call Tube A, Tube C, Tube G and Tube T. Each "reaction tube" (table top) includes all the following reagents:

1. A simulated Post-it® sequence (DNA template approximately 25 nucleotides in length) derived from appendix A or other source. Note that this sequence must be the exact same sequence on each of the four tables.

2. Approximately ten complementary Post-it® note primer sequences (four nucleotides in length). Again, these should be exactly the same primer sequence on each of the four tables.

3. Deoxynucleotides represented by all four Post-it® note colors. Since each of the four deoxynucleotides will be utilized multiple times, approximately 200 sheets of each color of Post-it® note should be made available on all four tables.

4. Dideoxynucleotides represented by crossed-out Post-it® notes for one of the four dideoxynucleotides, EITHER A-adenine (yellow), G-guanine (pink), C-cytosine (green) or T-thymine (orange). Each of the four tables will have a different color of crossed-out Post-it® notes.

A student or group will work with one of the four tables or "reaction tubes" to identify an unknown sequence. If more sequences are to be deciphered, any number of groups can be set up as long as each group utilizes a set of four table tops.

Instructor preparation should have resulted in a simulation of sequencing reaction tubes. Each of the four tables will represent 1 of 4 possible reactions in the sequencing process. The primer (with the help of a student playing the role of polymerase) will start matching the DNA template with appropriate deoxynucleotides and 1 of the 4 dideoxynucleotides. The first table represents the reaction in Tube A which includes the following "dry" reagents:

1. Template DNA (Post-it® note sequence prepared in step 1 of Instructor Preparation).

2. Deoxynucleotides of all four bases (Approximately 200 sheets of each of the four colored Post-it® notes).
3. Dideoxynucleotides of adenine only (Approximately 40 yellow Post-it® notes with an X drawn on them).
4. Primers (approximately ten).
5. Polymerase (A student will play the role of this enzyme).

A second table will act as "Tube C" and should contain the exact same components as "Tube A" with one exception: dideoxynucleotides of cytosine (green crossed-out Post-it® notes) should replace the adenine sheets. "Tube G" and "Tube T" should also be represented at individual table tops, in each case replacing Post-it® notes that represent the dideoxynucleotide adenine with guanine and thymine, respectively.

STUDENT ACTIVITY

Simulation of the sequencing reaction (using Tube A as an example):

DNA sequencing relies on the generation of many single-stranded DNA products that are the result of copying a single-stranded DNA template. Each step of sequencing will be described below, followed by a simulation procedure to be used in our dry lab. For a more detailed account of sequencing, see the Dolan animation provided in the reference section. In each reaction tube, the entire sequencing process is actually a series of reactions described below:

Reaction 1 (Denaturation). Wet lab sequencing is initiated by heating the components in each of the four reaction tubes (A, C, G and T) to 94°C. This will denature our separate double-stranded DNA into single-stranded template. In our "dry" reaction, the DNA is already "denatured" and represented as a single-stranded template. The dry lab could be easily modified to include a double-stranded DNA molecule by attaching complement Post-it® notes to the single-stranded template assembled by the instructor during simulation preparation. Simulation of heating is achieved by breaking the hydrogen bonds between guanine/cytosine and adenine/thymine bases. This would be simulated by separating the linear Post-it® note sequences from one another (separating pink from green and yellow from orange). The end result is a single-stranded DNA template.

Reaction 2 (Annealing). In a wet lab, the reaction tubes are cooled (approximately 55 °C) to facilitate the binding of primers to the single-stranded template DNA. The role of a primer (normally a 10 to 20 nucleotide single-stranded sequence) is to anneal (form complementary double stranded DNA by hydrogen bonding between guanine/cytosine and adenine/thymine bases) to a specific complementary region of DNA you wish to sequence. The primer provides a necessary chemical structure (known as a 3'-OH group) needed for the addition of nucleotides to a growing DNA strand (Stefan, 1998).

The "dry" lab simulation of this step is demonstrated with primers that are merely 4 nucleotides in length. The students should anneal a primer to the region of DNA that is complementary at all four bases. This should, if set up properly in the instructor preparation portion of the lab; result in priming at a terminal end of your single-stranded DNA template. This primer will provide a necessary starting point for the addition of more nucleotides that complement the template DNA strand during the elongation phase (immediately below).

Reaction 3 (Elongation). Wet lab elongation steps generally occur during a slight elevation of temperature (approximately 60-72°C). This allows a polymerase enzyme to attach an additional nucleotide to the primer that is complementary to the template strand. Hydrogen bonds are formed only between guanine and cytosine or adenine and thymine. As a result, if a template strand contains a thymine, only a nucleotide containing the base adenine can form the second strand of this DNA molecule. If the template strand consists of a guanine at the terminal end of the primer, a cytosine will be added to the primer. This will continue until a dideoxynucleotide is incorporated into the growing DNA strand. Since dideoxynucleotides do not contain a necessary 3'-OH group, no new nucleotides can be added to this chain and elongation stops.

In the dry lab simulation of the extension step, students play the role of the polymerase enzyme and Post-it® notes (standard and crossed-out, respectively) play the role of deoxynucleotides and dideoxynucleotides. The student, reading the primed template strand, progresses by attaching randomly chosen Post-it® notes in only a yellow to orange and green to pink fashion (see figure 1) from the priming site. When the student randomly places a crossed-out Post-it® note (representing a dideoxynucleotide) on the extending strand, that particular fragment of DNA is terminal and cannot be elongated further as dideoxynucleotides lack the necessary 3'-OH chemical structure needed for continued addition of DNA nucleotides.

Repeat reactions 1-3 (Repeat of Denaturation, Annealing and Elongation). At this point, the wet lab would repeat starting with the denaturation step that would separate the newly formed DNA strand from the template strand. Primers would again anneal to the template and a second elongation step would take place. This cycle

would repeat several times, generating a large population of newly formed DNA strands which terminate at various locations due to the random addition of a dideoxynucleotide. Simulation of this process for the dry lab consists of removing the newly generated Post-it® DNA strand, priming again and carrying out elongation as described above. This cycle should continue until all possible termination sites are represented in that particular tube.

Because the only terminators available in "Tube" A are adenine dideoxynucleotides, all termination sites in this particular tube will contain a terminal nucleotide of adenine. By choosing adenine (yellow Post-it® notes) randomly, it should become obvious that many of the sequences you are generating will be repeats, just as they would be in a "wet" sequencing reaction. However, eventually all possible sizes of fragments ending in adenine will be generated.

The same process is to be carried out in "Tubes" C, G and T. Note that each of these tubes, due to the presence of their respective dideoxynucleotides, will terminate with a cytosine, guanine or thymine, respectively. Again, all possible lengths of DNA with the proper termination nucleotide in each tube will eventually be generated. The number of nucleotides in each sequence of Post-it® notes should be counted and written on the terminal Post-it® note for use in the gel electrophoresis portion of the activity.

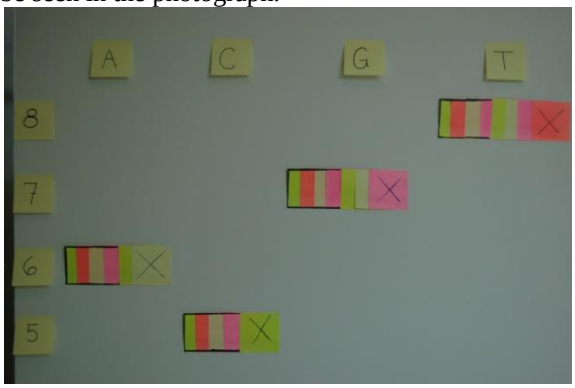
The result of the reactions in Tubes A, C, G and T are a population of newly synthesized DNA strands in the form of Post-it® notes that represent every possible length and terminal nucleotide of the original template strand. However, all the products of tubes A, C, G and T are still in separate reactions and need to be resolved through a simulation of acrylamide gel electrophoresis.

SIMULATION OF ACRAYLAMIDE ELECTROPHORESIS:

In a wet lab, the newly synthesized DNA fragments generated in Tube A, C, G and T are each loaded into a separate lane of an acrylamide gel. Once a current is applied, the newly formed DNA fragments migrate through the gel and are separated based on size, with the smallest fragments traveling through faster then larger fragments (Dolan).

To simulate electrophoresis, utilize a section of floor or wall space. Linear tile patterns on a floor work particularly well. Label a column of space numerically 25 (at the top) to 1 (see figure 2). Next to the numerically labeled column, label a new column as Tube A. Add columns for Tubes C, G and T as well. These will act as your acrylamide gel lanes. The samples need to be loaded in the gel and separated based on size. This is simulated simply by a student holding generated Post-it® note DNA fragments from Tube A. As the student walks down the gel (s)he looks for matching sizes of fragments with the numbers labeled on the gel. As the student in Column A moves down the gel from 25 nucleotides to 1 nucleotide, (s)he should lay corresponding length fragments at the appropriate location in the gel. For example, if (s)he does not posses any Post-it® notes fragments of 25 nucleotides in length, (s)he steps forward to position length 24. If (s)he does possess Post-it® note DNA fragments at this length, (s)he lays them on the floor at this site and continues moving down column A through length 23, 22, 21……..1. This same thing should be done in column C, utilizing only Tube C products. Additionally columns G and T should follow with Tube G and T products, respectively.

Figure 2: Simulation of Gel Electrophoresis. The numbers on the left represent the number of nucleotides (including primers) in the newly generated Post-it® note DNA fragments. Columns from reaction Tubes A, C, G and T are labeled at the top of the photo. Post-it® note fragments are pooled from Tubes A, C, G and T reactions and run in a single gel (albeit separate lanes) to generate the pattern revealed. By reading nucleotide lanes from the bottom to the top, a sequence can be obtained. In this case the sequence is C-A-G-T. Note: Only four nucleotide fragments are shown to ensure detail can be seen in the photograph.



READING THE SEQUENCE

In a wet sequencing lab, DNA strands separated on the acrylamide gel are not visible unless they are tagged with either radioactive or fluorescent markers. For more details on methods of detection please see the following reference (Dolan). In our example, the fragments are visible. Students should have generated a band of DNA five nucleotides long (four nucleotides representing the primer, plus an additional dideoxynuclotide) in only a single reaction tube (A, C, G or T). Similarly, each reaction tube

should have only specific fragment lengths because of the deoxynucleotide randomly complemented by the specific dideoxy variant of that reaction tube. As you look at the Post-it® notes on the floor/wall (in your gel), you will simply be able to see which lane (column A, C, G or T) contains the smallest newly generated DNA fragments (five Post-it® notes in size). If this happens to be in column G for your particular sample, then your first base in the unknown sequence is marked as a G. Look for the next largest and identify it (A, C, G or T) based on the column in which it is present (See figure 2). Continue the process until you reach the top of your gel. You have just generated a sequence of your unknown gene. The next step will be to figure out what protein results from the DNA sequence you just deciphered.

IDENTIFICATION OF YOUR GENE FRAGMENT.

This part of the procedure is the same whether you determined your DNA sequence in a "wet" or "dry" lab. The sequence you have deciphered from your "dry" lab can be identified utilizing the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/). On the home page click on BLAST and then nucleotide blast. Type your sequence (nucleotides 1-25) into the prompt area and submit your query with your "search set" marked as "other" (as opposed to either the human or mouse genome). The best matches for your sequence will be displayed. By clicking on link sites, the name of the gene and organism in which it is housed can be readily determined. The instructor can then ask for detailed reports based on researching the role this gene plays within the organism in which they are housed. The depth and quality of these reports can be modified to fit your particular audience.

RASSSP Review Comments:

"The hands on activities were great. I can take most of the activities and implement them directly into my curriculum."

"The lab Dr. Christensen did with us was great. I wish we could have that lab written down for us because I'd like to do it but I'm not sure I could repeat it right from memory."

"The activity that Dr. Christensen provided was great!!"

"Great stuff! Just what I wanted, a great, hands-on low technology lab that I can do w/ students. We want more of this type of low technology."

"Doug did a very good job of using a do-able activity to teach a difficult concept!"

"I can tell my students about the technology and answer "How do they do that" questions."

"Excellent prep and decent activities."

"Wonderful presentations by Christensen. I appreciated how we were able to do the activities. … provided good background information for the content/activities they delivered."

"Very good for biology teachers. Nice activity! Extremely well done."

"Well presented and usable at our level."

"This gave me a much better understanding of DNA sequencing."

"This was one of my favorite sessions. It was new and very usable information."

Acknowledgement

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DOLAN DNA LEARNING CENTER. ©Copyright, Cold Spring Harbor Laboratory. Available online at: http://www.dnalc.org/ddnalc/resources/sangerseq.html

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APPENDIX A:

Bacterial/Viral Sequences

1. gcgaaagacg atgcggcagg tcaggcgatt (fliC Esherichia)
2. tatatagtga gaaaaaacga tatgtatggt (Internalin A Listeria)
3. aaagtgagaa atacgaacaa agcagaccta (actA Listeria)
4. caatgggaac tctgccggga ttcccactgc (invA Salmonella)
5. agtgccggag tttgacatcg acgatgaggt (rpoS Pseudomonas)
6. aatagcatgt aagcaaaatg ttagcagcct (OspA Borrelia)
7. aaaaaagcag aagaaaacaa acaaaaaggc (mip Legionella)
8. gttggacccg aagaccaggt ccacgcggct (katG Mycobacterium)
9. aaagcaggtc agaaaacgga tgatatgctt (slo Streptococcus)
10. tcacatcaat gacagtaatt tctgcatctg (yop Yersinia)
11. gagcgggagg tgcgacatat acatatagaa (gag HIV)

Thermoregulatory Behavior in Diurnal Lizards as a Vehicle for Teaching Scientific Process.

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Abstract: Field experiments offer the opportunity for hands on experience with the scientific process. While this is true of a wide variety of activities, many have pitfalls both experimental and logistical that reduce the overall rate of success, in turn, influencing student learning outcomes. Relying on small, territorial, diurnal lizards and an array of inexpensive data loggers and lizard models provides a reliable method of testing a hypothesis in the field. This approach virtually guarantees that every student obtains a useable data set within a short period of time. Behavioral responses in lizards are dependent on becoming active only after external, sun driven, heat sources are adequate to permit daily activity on the one hand and avoiding excessive heat uptake above a critical maximum. Activity is dependent upon three primary modes of heat transfer (gain or loss): solar radiation, conductive exchange and convection, concepts that all students seem to understand. Over 11 of the past 15 years all students (more than 80) who undertook this project obtained useable data sets (data log records matched to focal observations). We relied on two different subspecies of *Holbrookia maculata*. Numerous other lizards are amenable to this approach.

Keywords: scientific process, hypothesis testing, behavior, thermoregulation

Introduction

The majority of small iguanine lizards native to the United States are territorial and many have densities high enough in good habitat to expect to find several adults within a grid of 30 m x 70 m and often in a much smaller area. Lizards are best described as ectothermic (Pough et al., 2006) and many are also homeothermic during diurnal activities, often maintaining a body temperature of approximately 35-39 C. Obtaining body temperatures above ambient conditions is generally accomplished by some form of basking behavior. To achieve this, lizards typically take advantage of direct and indirect sources of solar radiation. Herein I outline the major steps in executing a project that carries each student through the complete process of testing and extending a generally accepted paradigm of thermoregulation in small diurnal lizards.

This exercise was conceived and refined over the past 15 years in part to insure that each student would obtain a solid, acceptable data set that would virtually assure that everyone would be able to move through all the phases of the scientific process and present a term paper written in the format that one might use for submitting a manuscript. In our case it is an extension of what is already know in general terms about thermoregulation in small lizards. Each student is expected to achieve a fundamental understanding of what constitutes a valid scientific question; how to carry out a project that tests the validity of a general paradigm; how to analyze and present data graphically and then write a paper based on a set of format criteria. In this case students ask whether they see evidence of behavioral responses to extensive thermal conditions

The process begins when students read a prepared set of published papers. We read papers that lead students from the general to the specific. The first paper considers the physics of heat transfer, then we move to classical (pioneering work) of a general nature, more specific papers that relate to lizard performance and then papers that report results of conspecific subspecies from other locations. Students then write the introduction to their term papers which contains two crucial elements: 1) a distillation of what we know now and 2) a statement of what their papers are going to test. There are no published reports on thermal responses in *Hobrookia maculata thermophila.* Student/faculty discussions have established that we are attempting to test whether this subspecies does in fact show behavior patterns indicative of thermoregulation. data loggers to quantify microclimate, how to explore a variety of ways to present data and use spread sheets as well computer programs that rely on object oriented graphics and how to apply basic statistical methods. These skills are all parts of a coordinated effort culminating in a manuscript that adheres to all of the guidelines required for submission to a particular scientific journal.

Materials and Methods

In our studies in both Nebraska and Sonora, Mexico students laid out grids in areas where lizards were abundant. The long axis ran from south to north. In Nebraska we chose to use wooden stakes 0.5 m x 13 mm x 25 mm to mark the grid; in Mexico we used inexpensive bathroom tiles approximately 100 x 100 mm. Each was labeled with black permanent felt marker pens with letters and numbers large enough to be read at a distance of seven m, the dimensions of each grid quadrat. Thus numbering began at the southwest corner of the grid with A column (A0, A1…A20). The B column was parallel to the A column and again extended from south to north as B0 through B20. Columns C through F were laid out in turn and parallel to the first two.

To capture lizards for focal observations, each student used white No. 50 carpet thread in the form of a small noose attached to one end of a 1 m x 8 mm diameter wooden dowel purchased at a hardware store. Most lizards were readily noosed during the early part of their daily activity period. We then measured the snout vent length (SVL) and tail length to the nearest mm to obtain body size. Each lizard was weighed and sexed at the point of capture. To make it easier to identify focal lizards on successive days, each was identified with a small dab of fingernail polish applied to the neck, mid body, a shoulder, or a thigh. When additional codes were needed, we substituted fingernail polish for a small dot of airplane dope obtained at a hobby store. This permitted the use of the same codes but in different colors. If a lizard paint code began to slough off during the study, the individual was noosed and repainted. After data were obtained from a lizard, we recorded where the lizard was first seen and returned it to that location.

Students spent about two hours in preliminary observations cataloguing what lizards did during the day in terms of changes in posture and exposure to the sun. This resulted in identifying five postures in which less and less contact was made with the substrate (Figures 1 and 3). In addition to this, students quickly noticed that lizards spent more time facing west in the cool of the morning so we incorporated not only posture, but also compass orientation to the sun. During mid day when the sun bore down almost vertically on the study area, lizards shuttled in and out of the sun. At times *Holbrookia* also moved off of solid substrates (in the sun at times and shade as well) so we noted substrate in addition to posture and body orientation to the sun. Students also grasped the heat transfer implications of lizards that chose a posture when it was quite warm in which the body was more or less parallel to the light, body inclined, so that only the head was receiving direct incident light.

In discussion of the three primary modes of heat transfer (direct solar radiation, conduction and convection) we explored the solar consequences and decided that east and west orientation (especially if the lizard adopted an elevated body attitude parallel to the sun's rays) permitted scoring compass orientation as head to sun (east) or tail toward sun (west). Lizards facing north or south received the same incident solar radiation at a given moment, so we had effectively three categories for orientation, even though students noted north and south in their focal observations records.

To determine the heat transfer conditions of lizards, we deployed an array of small battery operated data loggers from Onset Computers (PO Box 3450, Pocasset, MA 02559-3450). To estimate the body temperature a lizard would experience in full sun or full shade, we constructed two lizard models (Figure 2). Each consisted of copper pipe 10 mm in dia. and 52 mm in length; available in most hardware stores as 3/8 inch dia. water pipe. To provide a realistic level of absorbance of natural light, we did several tests of actual lizards in full sun. Adult lizards (55 mm SVL) were temporarily tethered to a block of Styrofoam and recordings of body temperature were made at 5 second intervals until the lizard showed signs of discomfort; no tests were conducted above 39C which is near the upper thermal limit for most lizards. We did this in a container that eliminated wind. All lizards were maintained at a temperature of 30 C before the tests began. The air temperature for tests was 34C. This produced a heat up, cool down curve that we incorporated into a spreadsheet (See Appendix, Table 1). Tests of various lengths of pipe and paint color resulted in a close approximation to real lizard responses to solar uptake when we used flat gray, car primer. A cork was placed in each end of the pipe. One cork accepted the temperature sensor probe and the other accommodated the wire stand used to hold the pipe about 1 cm above the substrate (Figure 2). By placing one model lizard in open sun and the other in full shade, provided students with realistic estimates of body temperatures under those two extremes. At both locations and over multiple years we also used the noosing technique to sample lizards using a fast reading cloacal thermometer (Model T-6000, Miller and Weber, 1637 George St., Ridgewood NY, 11385) to the nearest 0.2 C in both open sun and in full shade. Body temperatures agreed well with estimates from the model lizards.

To further enhance the student's ability to comprehend the thermal regime of lizards, we placed a bare probe in open sand 3 mm below the surface and another in the sand in full shade. The latter we found useful when it became apparent that part of the thermoregulatory repertoire of *Holbrookia* occasionally involved burying in the sand in the shade for substantial intervals. Another data logger recorded burrow temperature 10 cm into the den. Onset computers also make data loggers that record direct solar radiation, relative humidity and barometric pressure. Of those, sunlight was deemed a valuable addition. Days when clouds come and go were among the most interesting. Having sunlight variation records at five min intervals synchronized to focal observations allowed students to look for changes in posture and orientation that were either consistent with or at odds with the hypothesis.

Results

With the advent of compact digital projectors we now routinely review lizard postures with students prior to making focal observations. We practiced scoring lizard posture based on photos (Figures 3 A-C). Data sheets contained a small series of stick figures (Figure 1) labeled A-D depicting posture on the basis of the amount of substrate contact. Posture A (Figure 3A) represented full contact with the substrate and posture D (Figure 3B) indicated a lizard with only toes and heels touching the substrate. Postures in between A and D are fairly stereotypic and involved intermediate substrate contact while posture E entails a strategy off of hard, high thermal heat solid substrates (Figure 1, Legend). Data sheets contained columns to the right of the defined postures that permited students to record grid location, posture, compass orientation and substrate as well as activities such as foraging, territorial defense or courtship**.** Each row constituted a record that was synchronous with the data logger array records. Before students began focal observations, we synchronize our watches with each other and with the data loggers. All loggers were armed to record on the hour and at 5 min intervals thereafter. On a typical day in the field after the grid has been laid out and marked and the data logger array had been deployed, students searched, located, noosed and painted lizards. After a student had marked his or her lizard, focal observations began. If students entered the field shortly before lizards became active in the morning, the data logger array provided information useful in determining the initial thermal conditions (sand temperature minima) required before lizard activity commenced. A single data sheet contained 12 focal observations (one hour). Four hours of observations thus provided a long enough period to see lizards initiate day time activities, and if the air and substrate temperatures were high enough by noon, too see a shift from uptake of heat to reach operating temperatures to conditions in open sun that exceeded a critical thermal maximum for activity. When sand temperatures exceeded voluntary operating temperatures, lizards either shuttled in and out of the sun, adopted postures and compass orientation that minimized radiant and conductive energy uptake, or adopted perches above the sand (Figure 3C) or moved into partial or full shade.

Figure 1. Depicts all the postures that *Holbrookia maculata* adopted. In posture A the venter was in contact with the substrate; in posture B the front limbs were fully extended raising the anterior part of the body off of the substrate; in posture C all four limbs were extended, minimizing body contact with a hard substrate. Posture D differed from C because the lizard is on an incline. Posture E depicted a lizard that had adopted an elevated stance off of the sand substrate. Figure 2F indicated that a lizard had either entered a burrow or (most often) buried in loose sand in the shade at the base of a plant.

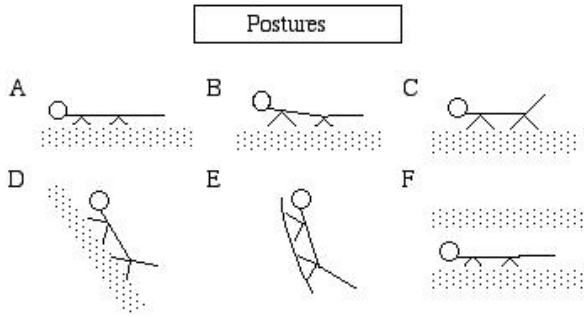


Figure 2. Represents a Hobo, four channel temperature data logger connected to a model lizard made from copper pipe.

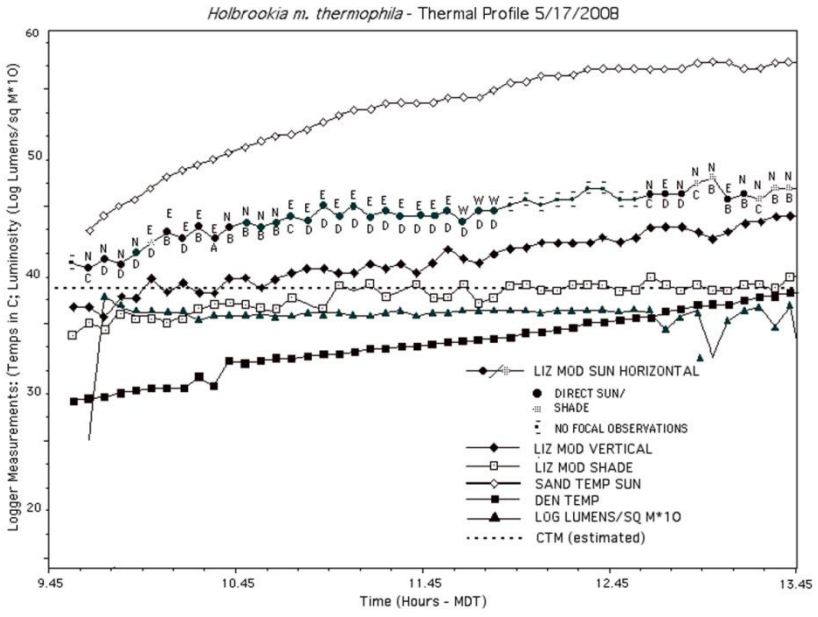
Figure 3. Depicts three of the postures *Holbrookia maculata* adopted to modulate conductive heat exchange. Figures 3 A and B were on hard, high thermal substrates such as rock or sand. In 3A the lizard was in posture A with full body contact with the substrate; In 3B all four limbs were extended as in posture D. Figure 3C depicts a lizard that was in contact with a low thermal mass substrate in an inclined position and without contact with the surrounding sand**. 3A**

3B

3C

Once out of the field, all data logger records were downloaded as tab delimited data matrices and imported into a spreadsheet. Students then began the process of building graphs that permitted viewing all of the logger records for the day. Together we used a digital projector to examine graphs of microclimate conditions. Figure 4 contains a typical, minimal array of data logger records. At this point I requested that students each write up a general summary of the changes in microclimate for the logger records for that day. The next process involved examining what a given lizard did under a particular set of climate conditions. The goal at this point was to test the following hypothesis: Was there evidence to support or refute the proposed hypothesis that *H. maculata thermophila* used behavior to regulate body temperature?

Figure 4. Represents a typical composite graph of environmental conditions recorded by the data logger array with posture, compass orientation and sun conditions experienced by a single focal lizard.



We typically made focal observations over a span of three days but two are sufficient. Three increased the likelihood of different weather conditions that could result in behaviorally driven thermoregulatory differences between days. Once students had two or three days of observations, we spent a considerable amount of time graphing the results. I encouraged each to experiment with presentation methods. This worked better as a one-on-one activity than as a group using projected graphs. Once the data were graphed, each student was required to examine the behavior of his or her focal lizard. Four hours of observations synchronized to the data logger records for a day provided 48 records of orientation, posture and shade choices. We operated under the assumption that body temperatures close to 39 C or higher (Figure 4) represented an approximate upper limit for activity. As the process of presenting data matured, students used an object oriented drawing program to place a small circle on the graph line depicting the model lizard in full sun (Figure 4). We used symbol color to designate whether the lizard was in full sun (yellow), partial sun (gray) or full shade (black). For publication purposes we made sure that the symbols we chose worked in black and white. Just above each circle students placed a small capital letter to designate compass orientation at that moment. Just below each circle, an upper case letter (A-F) indicated posture at each focal observation. I lead students in a discussion as to how best to present the data in such a way that they could identify all of the conditions on a single figure. We found that this facilitated the discovery of patterns in behavior. These could then be used to provide evidence that lizards are indeed using posture and orientation (behavior) to avoid temperatures that exceed the maximum or in fact dropped below a body temperature that permitted lizards to function efficiently, or in fact did not support our hypothesis. From the required readings (Huey and Kingsolver, 1989) students are familiar with the concept of optimal performance including sprint speed (for predator avoidance) as well as digestion rates, hearing and reproductive activities. In warm climates most lizards in full sun do not tolerate solid substrates above 40 C for more than about 5 min if they are already at or near the upper end of thermal tolerance. Warm lizards on substrates in the 40 to 60 C range were expected to be there for less than 5 min (Table 1). Lizards generally responded in less than 2 min. This time worked well because we took focal observation at 5 min intervals. When air and sand temperatures exceeded 40 C and the sun was out, lizards on solid substrates retreated to partial or full shade and buried in shady sand at times. Focal lizards tolerated full sun at slightly lower air temperatures (less than 40 C) for prolonged periods if they are off of a solid, high thermal substrate (Figure 3, Posture E). Students soon realized that by using this simple color-coding, depicting orientation and posture, all in one part of the graph, allowed them to look for sun-shade shuttling and long stretches of time during which a lizard stayed in one posture and oriented in the same direction. We plotted colored circles on graphs (Figure 4) on the logger curve for model lizards in open sun for two reasons. One is practical. The graph was less crowded there. The second reason was to remind students that occupying a solid substrate in open sand is not a viable option for lizards unless they behave in such a way that they remove themselves from the substrate. Lizards that move into vegatation off of the sand virtually eliminated conductive heat uptake. This was almost always coupled with posture oriented parallel to solar input reducing direct solar heating to a minimum.

Discussion

Before fieldwork commenced, I spent some time in discussion with the class to be sure that they understand the limits of science. To be scientific, a hypothesis was restricted to the real, measurable world and hypotheses had to be vulnerable to falsification. We explored the utility of using hypotheses in studying biological systems. Because we are often in remote settings, I provided a list of papers to read and copies of key papers that introduce students to life as ectotherms; first with a classic paper (Cowles and Bogert, 1944) and then with a more modern treatment of heat sources available to lizards, both direct and indirect. Pough et al. (2006) is a fine source of first principals. For instructors and advanced students, Pough et al. (2004) is exceptional and much more detailed. The next required reading (Huey and Kingsolver, 1989) provided students with a nice treatment of the concept of thermal optima. From this point readings contained information that more specific for the species we studied (Stebbins, 2003) and finally published field studies germane to the subspecies we chose for our thermal studies (Hager, 2006). For those in the eastern half of the U S, Conant and Collins (1998) is a useful general reference for students and instructors. After the students had read the assigned papers we engaged in a group discussion before the introduction was written. Once focal observations were completed we spent time examining the best ways to present the results. Students generally converged on a best suitable way to render the results in graphical form. Students had the most difficulty with a tendency to

combine results and an explanation fo them in that section rather than waiting until the discussion section. In the discussion section, the greatest challenge was to fully utilize the results to support a general conclusion and to actually come to grips with the heat transfer modes that were operating at a given point in time. Students were encouraged to look for long bouts of the same posture and orientation and then consider what the lizard body temperature would be, given the external heat conditions. The short period of time required for a lizard to over heat (Table 1) was a powerful tool for students as they began to present results in favor of their argument. Identifying long bouts of stereotypic behavior could be treated with simple statistics. We spent a good deal of time with the logic of long bouts in one posture considering whether it was random or not. Because the student had a full set of graphs on computer, students can also do oral presentations as reinforcement to the written work. The sequence, oral presentation of data first has the advantage of refining or at times revising explanations that would go into the discussion section. There is also merit in having them do the oral presentation once the term paper is complete.

Alternative Enrichment Exercises:

For students who become curious during the execution of this exercise, there are a number of questions that can be addressed with the existing data and these include: (1) What is the drop in temperature that a lizard (or model) undergoes if oriented parallel to the sun? (This is a simple experiment and we now put a third model lizard in the field next to the horizontal one in open sun and plotted both on graphs (See Figure 4.) (2) Is there a difference in distances traveled on days that differ in weather? (3) Is there a difference in the size of the area (territory) that females vs. males cover in a day or as a composite over several days? (4) Do students see evidence of differences in behavior at a given time of day between males and females? (5) What is the sex ratio and density of adults on the study area? (6) Where do lizards spend the night?

Data sheets, a complete checklist of guidelines we use for the construction of a paper, a more extensive list of suitable papers relevant to thermoregulation in reptiles, heating curves and sample graphs are available in PDF format and free to download at: http://biology.creighton.edu/faculty/platz/

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Appendix

Table 1. Provides a heat/cooling curve for a 55 mm SVL, male *Holbrookia maculata*, from an initial body temperature of 30 C exposed at a 90 degree angle to full sun. Ambient air temperature was 34 C. Heating beyond 39 C was extrapolated from lower temperature rates of heating.

FUTURE Tb (C)		30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
DELTA Tb (C)		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
BODY T. (C)																
30	0	28	55	83	110	138	165	193	220	248	275	303	330	358	385	
31		0	28	55	83	110	138	165	193	220	248	275	303	330	358	
32			0	28	55	83	110	138	165	193	220	248	275	303	330	
33				0	28	55	83	110	138	165	193	220	248	275	303	
34					0	28	55	83	110	138	165	193	220	248	275	
35						0	28	55	83	110	138	165	193	220	248	
36							0	28	55	83	110	138	165	193	220	
37								0	28	55	83	110	138	165	193	
38									0	28	55	83	110	138	165	
39										0	28	55	83	110	138	
40											0	28	55	83	110	
41												0	28	55	83	
42													0	28	55	
43														0	28	
44															0	

A Comparison of Heat Versus Methanol Fixation for Gram Staining Bacteria

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Abstract: Gram staining bacteria is a fundamental technique introduced in general biology and microbiology laboratory courses. Two common problems students encounter when Gram staining bacteria are (1) having a difficult time locating bacterial cells on the microscope slide and (2) over-decolorizing bacterial cells during the staining procedure such that gram-positive bacteria, which should appear purple in color, are pink instead. In this study, we examined whether the method of fixation (heat versus methanol) that is used to adhere bacteria to the slide prior to staining might influence the staining results. We found that significantly greater numbers of *Staphylococcus aureus* (gram-positive) and *Escherichia coli* (gram-negative) cells adhered to slides following methanol fixation compared to slides that were heat-fixed. Additionally, methanol-fixed cells of *Staphylococcus aureus* were consistently stained the correct color (a dark purple) while the staining of heat-fixed cells was more variable with cells ranging in color from purple to pink. Overall, our results indicate that students are more likely to successfully visualize and Gram stain bacteria if the cells are fixed with methanol rather than heat.

Keywords: Gram stain, gram-positive, gram-negative, heat fixation, methanol fixation

Introduction

A fundamental laboratory technique that is introduced in general biology and microbiology courses is staining of bacterial cells on glass slides for visualization and characterization purposes. A common procedure, the Gram stain, differentiates between bacterial species based on the chemical composition of their cell walls. The staining procedure involves applying a primary stain, crystal violet, followed by Gram's iodine, which acts as a mordant, decolorizing with an organic solvent such as ethanol, and counterstaining with safranin. Following the procedure, gram-positive bacteria, which are more resistant to decolorization, appear purple in color while gram-negative bacteria, which are more sensitive to decolorization, appear pink.

Students encounter a number of problems when learning how to Gram stain and view bacterial cells. During the staining procedure, bacterial cells tend to be washed off the slide. Students then have difficulty locating bacterial cells on the slide, particularly the lightly colored (pink) gram-negative cells. Additionally, students often over-decolorize the cells, such that gram-positive cells, which should appear purple, are stained pink instead. This is particularly an issue when older cultures of bacteria are used for the staining procedure (Magee *et al.*, 1975).

Some evidence suggests that the means by which bacterial cells are "fixed" to the glass slide prior to staining may influence the results of the Gram stain (Magee *et al.*, 1975; Mangels *et al.*, 1984). Fixation increases the adherence of bacterial cells, and the most common method employed is heat fixation (Ederer and Lund, 1981). This is completed by passing a slide of bacterial cells through a flame until the underside of the slide is warm to the touch. Chemical methods of fixation have also been described. One is the use of methanol as a fixative agent. A number of studies have shown that methanol fixation gives more reliable Gram staining results than heat fixation (Magee *et al.*, 1975; Mangels *et al.*, 1984). That is, gram-positive bacteria are more likely to be stained purple, and gram-negative bacteria are more likely to be stained pink when cells are fixed with methanol compared to heat. Additionally, gram-positive bacteria fixed with methanol are more resistant to decolorization than cells fixed with heat (Magee *et al.*, 1975; Mangels *et al.*, 1984).

We were surprised, therefore, to find that of six general biology laboratory manuals we examined, five recommended heat fixation of bacteria (Hummer *et al.*, 1983; Dickerman, 2000; Scott and Wachtmeister, 2006; Dolphin, 2008; Vodopich and Moore, 2008), and only one recommended the use of methanol as a fixative agent (Singh and Gunn-Scissum, 2003). The same held true for the microbiology manuals we reviewed. All 15 manuals recommended heat as the preferred method of fixation (Norrell and Messley, 1997; Stukus, 1997; Alexander and Strete, 2001; Bey, 2001; Johnson and Case, 2001; Benson, 2002; Kelley and Post, 2002; Wistreich, 2003; Alexander *et al.*, 2004; Cappuccino and Sherman, 2005; Pollack *et al.*, 2005; Pommerville, 2005; Leboffe and Pierce, 2006; Harley, 2008; Morello *et al.*, 2008), and only two of the 15 (Johnson and Case, 2001; Morello *et al.*, 2008) even mentioned methanol as a possible fixative agent.

Our objective was to examine Gram staining results following fixation of both gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacteria using heat versus methanol as means of fixation. In particular, we were interested in evaluating the number of cells adhering to the slides following the Gram staining procedure. We also assessed the color of the Gram stained bacteria that were heat-fixed versus methanol-fixed. This research was completed exclusively by undergraduate students who were majoring in biology (Roland, Rossi, Weishalla, and Wolf), only one of whom had prior laboratory experience staining bacterial cells. Our overall goal was to determine whether students with little or no experience in completing microbiology laboratory exercises might achieve greater success in Gram staining and viewing bacteria using one method of fixation compared to the other.

Methods

Bacterial cultures. Stock cultures of *S. aureus* and *E. coli* (Presque Isle Cultures, Presque Isle, PA) were maintained on tryptic soy agar (Fisher Scientific, Pittsburgh, PA). Prior to each experiment, a test tube containing 10 mL sterile tryptic soy broth (TSB; Fisher Scientific) was inoculated with *S. aureus* or *E. coli*, placed in a Lab-Line incubator-shaker (Barnstead International, Dubuque, IA), and incubated at 100 rpm and 37^{o}C for 14 hours.

Fixation and Staining of Bacteria. For each experiment, 20 glass slides were cleaned with 95% ethanol, and a circle with a diameter of 2 cm was made on the surface of each slide using a wax pencil. The 14-hour culture of *S. aureus* was diluted 1/100 in TSB, and 20 □L of the diluted culture was spread within the circle on each slide. Alternatively, 20 □L of the 14-hour *E. coli* culture was spread within the circle on each slide. The slides were then allowed to air dry. One set of ten slides was heat-fixed by passing the bottom of each slide through the flame of a Bunsen burner until the slide was warm to the touch. The remaining ten slides were flooded with absolute methanol (200 □L per slide) for 2 minutes (Mangels *et al*, 1984; Singh and Gunn-Scissum, 2003). Excess methanol was decanted off the slides into a waste disposal container, and the slides were allowed to air dry. All 20 slides were randomly numbered 1-20 so the individuals who stained and viewed the slides did not know which slides were heat-fixed versus methanol-fixed. The slides were then stained using Hucker's modified Gram-stain technique (Harley, 2008) and viewed independently by three different individuals using a bright-field light microscope (Carl Zeiss MicroImaging, Inc., Thornwood, NY). The experiment was completed three and four times for *E. coli* and *S. aureus*, respectively.

Cell Counts and Statistics. For each slide, bacterial cells were counted in three random fields of view, and the mean number of cells in a field of view was calculated. The Student-t test was completed on the data to determine whether there was a statistically significant difference in the mean number of cells that adhered to heat-fixed slides compared to slides fixed with methanol.

Photographs of Staphylococcus aureus. Twenty microliters of a 14-hour culture of *S. aureus* was spread on each of 10 slides, and the slides were allowed to air dry as described above. Five of the slides were heat-fixed, and five were fixed with methanol. The slides were randomly numbered 1-10, Gram stained, and viewed under oil immersion on a Zeiss bright-field light microscope. Photographs of random fields of view were taken with a Canon Powershot G6 digital camera using identical camera settings for photographs of both heat-fixed and methanol-fixed slides. Slides and/or photographs were viewed and independently evaluated by three different individuals.

Laboratory Safety Guidelines and Microbiological Laboratory Techniques. Students were instructed in standard laboratory safety guidelines and proper microbiological laboratory techniques (Norrell and Messley, 1997; Stukus, 1997; Alexander and Strete, 2001; Bey, 2001; Johnson and

Case, 2001; Benson, 2002; Kelley and Post, 2002; Wistreich, 2003; Alexander *et al.*, 2004; Cappuccino and Sherman, 2005; Pollack *et al.*, 2005; Pommerville, 2005; Leboffe and Pierce, 2006; Harley, 2008; Morello *et al.*, 2008) prior to completing this study. Topics discussed included but were not limited to the following: proper handling of bacterial cultures (aseptic technique); proper disposal of microbiological waste; procedures for decontaminating accidental spills; and proper handling and disposal of toxic and flammable chemicals used in this study (e.g., ethanol and methanol).

Results

In all three experiments completed on *E. coli*, slides fixed with methanol had a significantly greater mean number of cells per field of view compared to slides that were heat-fixed ($p < 0.001$, 0.005, and 0.0001, for Experiments 1, 2, and 3, respectively). Representative results from Experiment 3 are shown in Figure 1. In this case, methanol-fixed slides of *E. coli* had more than ten times as many cells per field of view than did slides of *E. coli* that were fixed using heat. All three researchers who independently viewed the slides indicated it was much easier to locate and identify *E. coli* cells on the slides fixed with methanol than on slides fixed with heat.

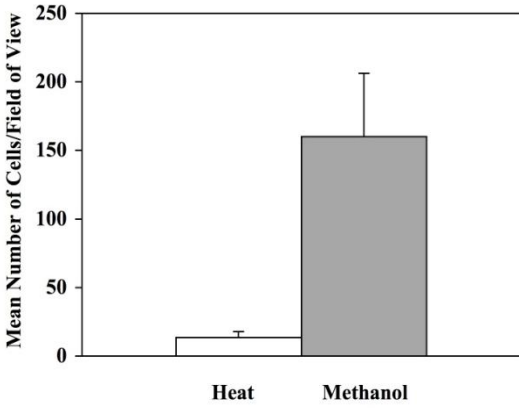


Figure. 1. Mean number of *Escherichia coli* cells per field of view on slides that were heat-fixed (n=10) versus methanol-fixed (n=10). Twenty microliters of a 14-hour culture of *E. coli* was applied to the surface of each of 20 slides and allowed to air dry. Ten of the slides were heat-fixed and the other 10 were fixed with absolute methanol. The slides were Gram stained and viewed under oil immersion with a bright-field light microscope. The number of bacterial cells in three random fields of view was counted for each slide and averaged. Methanol-fixed slides of *E. coli* had a significantly greater mean number of cells per field of view than did heat-fixed slides ($p < 0.0001$).

Similar results were obtained in the four experiments completed on *S. aureus*. In all cases, methanol-fixed slides had a significantly greater mean number of cells per field of view than did slides that were heat-fixed ($p < 0.0005$, 0.0001, 0.0001, and 0.0001 for Experiments 1, 2, 3, and 4, respectively). Representative results from Experiment 4 are shown in Figure 2. In this case, there were 2.5-fold more cells per field of view on slides of *S. aureus* that were methanol-fixed than those slides fixed with heat.

Figure. 2. Mean number of *Staphylococcus aureus* cells per field of view on slides that were heat-fixed (n=10) versus methanol-fixed (n=10). A 14-hour culture of *S. aureus* was diluted 1/100 in TSB, 20 μL of the diluted culture was applied to the surface of each of 20 slides, and the slides were allowed to air dry. Ten of the slides were heat-fixed and the other 10 were fixed with absolute methanol. The slides were Gram stained and viewed under oil immersion with a bright-field light microscope. The number of bacterial cells in three random fields of view was counted for each slide and averaged. Methanol-fixed slides of *S. aureus* had a significantly greater mean number of cells per field of view than did heat-fixed slides ($p < 0.0001$).

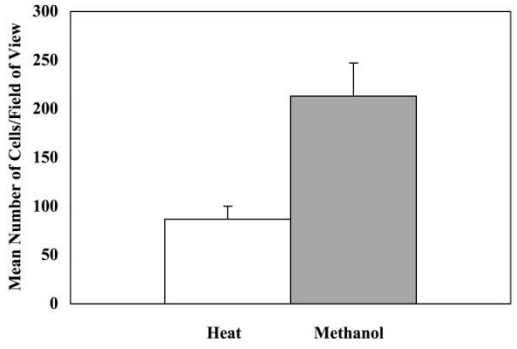
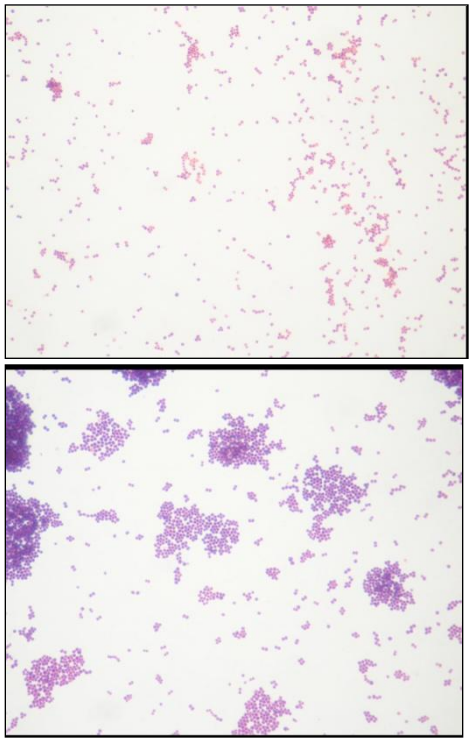


Figure. 3. Photographs of slides of *Staphylococcus aureus* cells that were heat-fixed (A) versus methanol-fixed (B). Twenty microliters of a 14-hour culture of *S. aureus* was applied to the surface of each of 10 slides and allowed to air dry. Five of the slides were heat-fixed and the other five were fixed with absolute methanol. The slides were Gram stained and viewed under oil immersion with a bright-field light microscope. Photographs of random fields of view were taken with a digital camera using identical camera settings for photographs of heat-fixed and methanol-fixed slides.

3A



3B

Methanol-fixed gram-positive bacterial cells were less sensitive to decolorization during the Gram staining procedure than were heat-fixed cells. Shown in Figure 3 are representative photographs of slides of *S. aureus* that were prepared identically except that one slide was fixed with heat (Figure 3A) and the other with methanol (Figure 3B). Clearly, a greater number of *S. aureus* cells adhered to the slide that was fixed with methanol compared to the heat-fixed slide. All three individuals who independently examined the slides agreed that bacterial cells fixed with methanol (Figure 3B) retained the crystal violet (purple) stain more readily than did the cells that were heat-fixed (Figure 3A). Virtually all cells viewed on methanol-fixed slides of *S. aureus* were purple in color. Staining results were variable on the heat-fixed slides where both purple and pink cells were observed. No difference in color was observed in *E. coli* cells fixed with methanol versus heat (data not shown), and therefore the method of fixation did not influence the Gram staining results of this gram-negative bacterium.

Discussion

We found that both gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria that were fixed to slides using methanol as the fixative agent adhered more effectively than did cells that were heat-fixed to slides. Slides of *S. aureus* fixed with methanol had two to four times as many cells present than did slides fixed with heat. In the case of *E. coli*, the difference was even more dramatic with five to ten times as many cells present on methanol-fixed slides compared to slides that were heat-fixed. Methanol fixation prior to Gram staining would clearly allow students to locate and view bacterial cells more easily than heat fixation.

Consistent with previous studies (Magee *et al.*, 1975; Mangels *et al.*, 1984), we also found that gram-positive bacteria were less likely to decolorize when fixed to slides with methanol rather than heat. In our experience, *S. aureus* cells that were fixed with methanol consistently were stained a dark purple color. Identically stained cells that were heat-fixed varied in color, ranging from dark purple to pink. The decolorization step is the most critical of the Gram-stain procedure. Students tend either to under-decolorize, leading to gram-negative cells falsely appearing gram-positive (purple), or, more commonly, to over-decolorize, in which case gram-positive bacteria falsely appear gram-negative (pink). Using methanol as a fixative agent would help to eliminate this problem. Gram-positive bacteria fixed with methanol would likely still appear purple in color even if excessive amounts of decolorizing agent were used.

We were curious as to why virtually all of the laboratory manuals we examined recommended heat fixation rather than methanol fixation when staining bacteria. In order to gain insight into this, we contacted a number of the authors of these laboratory manuals and asked them why methanol fixation was not included in their laboratory exercises on bacterial cell staining. Some indicated that they were not aware that methanol fixation was an alternative to heat fixation. Others indicated that since methanol is a toxic and flammable chemical, they were concerned for safety reasons, particularly since Bunsen burners are used when staining bacterial cells. While safety issues are a valid concern, we believe the dangers associated with the

use of methanol (and the ethanol decolorizing agent, for that matter) can be minimized by following certain laboratory safety guidelines. For instance, the experiments completed in this study were carried out in a well-ventilated research laboratory to minimize student exposure to fumes released from the chemicals. Alternatively, one could complete the methanol fixation step under a chemical fume hood to reduce exposure to the vapors. Additionally, the Bunsen burners used to aseptically transfer the bacterial cultures to the slides and to heat-fix the bacteria to some of the slides were turned off prior to methanol fixation and the staining procedure in which ethanol was used. It should also be noted that limited quantities of methanol (and ethanol) were used in this laboratory procedure. For instance, only 200 □L methanol was required to fix bacteria to each slide. If a classroom of 24 students were to complete this exercise, and each student was to Gram stain one slide of bacterial cells, this would result in less than 5 mL methanol being used for the entire class.

The research discussed in this paper was completed by four undergraduate biology students, (Roland, Rossi, Weishalla, and Wolf), who had little to no experience handling bacterial cultures or staining bacterial cells. Results from our study indicate that students are more likely to visualize successfully and Gram stain bacteria properly if the cells are fixed with methanol rather than heat. In light of our findings, it might be useful to reevaluate the method of bacterial fixation used in introductory biology and microbiology laboratory courses.

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Editor's Note: This article was published in last December's issue. Compatibility in word processing software resulted in a nonsensical document that made its way into our journal. The editor would like like to apologize to readers and to the author and the following reprint represents a complete version of the article.

Environmental Studies and Utilitarian Ethics

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Abstract: Environmental ethicists have focused much attention on the limits of utilitarianism and have generally defined "environmental ethics" in a manner that treats utilitarian environmental ethics as an oxymoron. This is unfortunate because utilitarian ethics can support strong environmental policies, and environmental ethicists have not yet produced a contemporary environmental ethic with such broad appeal. I believe educators should define environmental ethics more broadly and teach utilitarian ethics in a non-pejorative fashion so that graduates of environmental studies and policy programs understand the merits of utilitarian arguments and can comfortably participate in the policymaking arena, where utilitarian ethics continue to play a dominant role.

Keywords: Environmental Education, Environmental Studies, Environmental Ethics, Utilitarianism, Utilitarian Ethics

Introduction

The current generation of college students is expected to witness a dramatic decline in biodiversity, the continued depletion of marine fisheries, water shortages, extensive eutrophication of freshwater and marine ecosystems, a dramatic decline in tropical forest cover, and significant climatic warming (Jenkins 2003, Pauly et al. 2002, Jackson et al. 2001, Tilman et al. 2001, Adedire 2002, Karl & Trenberth 2003). The ethical implications of these anthropogenic ecological changes are clearly evident and have generated a tremendous interest in environmental ethics - a subject that has justifiably entered the environmental biology classroom.

The teaching of environmental ethics in environmental science courses has been heavily influenced by recent philosophical debates and many educators have followed environmental ethicists in rejecting the ethics of utilitarianism. Environmental science textbooks commonly exemplify this trend by associating utilitarianism with discredited "worldviews."

Despite the deprecatory treatment by environmental ethicists, utilitarianism continues to be widely accepted by professionals in other fields and utilitarian ethics still dominate the public policy arena. The derisive treatment of utilitarian ethics in environmental science courses may, consequently, have unfortunate consequences. Many graduates of environmental science courses are likely to be called upon to implement and defend policies they are ill prepared to understand or fully accept without a basic appreciation for the merits of utilitarian ethics. Environmental science graduates may also find themselves isolated from economists and other professionals if they fail to develop an appreciation for the limitations of competing theories and develop an antipathy for utilitarian ethics.

To prepare graduates of environmental science courses for participation in the policy process, it is important that environmental biologists teach the strengths, as well as the weaknesses, of utilitarian ethics in a non-pejorative fashion, and the limitations, as well as the strengths, of competing theories.

It must be appreciated that the training given most biologists seldom includes rigorous courses in philosophy. Consequently, environmental science instructors are likely to lack knowledge of, or an appreciation for, the relative merits of competing theories. I hope my treatment of this subject serves, in part, to address this issue by exposing biology instructors to several important philosophical debates, and by raising awareness of the unsettled nature of environmental ethics.

The Changing Status of Utilitarianism in Environmental Ethics

Utilitarianism, in its most traditional form, is both a theory of the good and a theory of the right. It holds that the greatest good is happiness and freedom from pain and suffering. Acts that promote the greatest good (i.e., have the greatest utility) are morally right. Acts that reduce overall happiness and/or promote pain are morally wrong.

Some advocates of utilitarianism have redefined the greatest good to be the satisfaction of personal desires or preferences. Preference utilitarianism is, of course, integrally associated with a host of contemporary economic theories, which commonly hold or assume that individuals are best served when they are able to pursue and satisfy their preferences within a free market.

No one familiar with the environmental movement in the United States can doubt or deny the important role utilitarianism has played as a justification for protecting wilderness, ecosystems, and species. Modern environmental ethicists have, however, criticized utilitarianism on various grounds and have distanced themselves and the field of environmental ethics from traditional theories of morality, including utilitarian ethics, by rejecting anthropocentrism, denying the importance of sentience, embracing intrinsic value theories, and affirming holistic ethics.

In the 1970s, several environmental ethicists and animal rights proponents challenged the inferior moral standing of other species and anthropocentrism (i.e., "speciesism" and "human chauvinism"). They persuasively argued that value and morality cannot be reduced to matters of interest or concern to human beings alone, and that there are no justifiable reasons for excluding the interests of other species

from moral consideration (Singer 1975, Fox 1978, Regan 1979, Routley & Routley 1979). Anthropocentrism was also attacked and rejected for failing to recognize the intrinsic value of non-human life forms and for justifying many of the environmentally destructive practices environmentalists oppose (e.g., Naess 1973, Devall & Sessions 1985).

The rejection of anthropocentrism did not necessitate a refutation of utilitarian ethics. However, a non-anthropocentric utilitarian approach to environmental ethics only broadens the set of morally relevant organisms to include, in addition to humans, elephants, cetaceans, great apes, and a handful of other sentient organisms. Utilitarianism has, therefore, been roundly criticized by those ethicists that reject sentientism and believe a legitimate environmental ethic must go further and assign moral standing to such insentient entities as plants, species and/or ecosystems. (e.g., Goodpaster 1978, Callicott 1980, Sagoff 1984).

Intrinsic value or inherent worth is what makes trees, species, and ecosystems the subjects of direct moral concern in the minds of many environmental ethicists, so its importance to the field can hardly be overstated. Because utilitarians recognize only the intrinsic value of pleasure or desire satisfaction, the commitment to intrinsic value in environmental ethics has also driven a rather deep wedge between environmental ethics and the ethics of utilitarianism.

In addition to rejecting anthropocentrism, sentientism, and utilitarian limits on intrinsic value, a number of environmental ethicists argue that an adequate environmental ethic must be holistic, as opposed to individualistic, and make ecosystems and species the subjects of direct moral concern. Such "holists" do not deny that we have duties to individuals, but they contend that our duty to preserve wild places, species, biotic communities, and ecosystems can trump the interests or rights of individuals. Following in the footsteps of Aldo Leopold, Callicott (1980) claims, in particular, that the *summum bonum* (i.e., greatest good) is the "land" and that an environmental ethic must provide environmentalists and conservationists with grounds for managing exotic, over-abundant, and problematic species - even when this involves killing, and otherwise harming, individuals.

While one can imagine a non-anthropocentric utilitarian environmental ethic, there can be no such thing as a holistic utilitarian environmental ethic. Utilitarianism is necessarily individualistic because only individuals can experience pleasure and pain or satisfy their interests. Environmental and utilitarian ethics have, therefore, become antithetical in proportion to the degree to which environmental ethics has embraced holism.

In Defense of a Utilitarian Environmental Ethic

Human beings and other sentient organisms depend on the ecological services natural environments and wild organisms provide. Natural systems and wild organisms regulate climate and biogeochemical cycles, are an important source of food, produce and protect fertile soils, pollinate crops, produce pharmacologically active compounds, control pests, and increasingly serve as a source of unique genetic material. The estimated economic value of all these and other ecological services easily exceeds the world's economic output (Myers 1996, Costanza et al. 1997) and, because many natural services and products are non-substitutable, the instrumental value of wild organisms and natural areas is, for all practical purposes, infinite.

Given the dependence of all sentient life on the ecological services natural environments and wild organisms provide, an ecologically-informed utilitarian ethic must, in some sense, be an environmental ethic. To be taken seriously, however, proponents of utilitarianism must respond to a handful of claims environmental ethicists have made regarding the nature of utilitarian ethics. In particular, proponents of utilitarianism must address claims that utilitarian ethics:

- Are inherently anthropocentric and/or sentientist,
- Ignore the rights and/or intrinsic value of other species and biological entities, and
- Justify environmentally destructive policies by making sentient individuals, rather than species and ecosystems, the locus of moral concern.

The claim that utilitarian ethics are anthropocentric constitutes a valid criticism of the way utilitarian ethics have generally been applied, but a utilitarian ethic that recognizes the pain and suffering of *all* sentient organisms does not arbitrarily favor humankind. Utilitarians were, in fact, ahead of their time in recognizing the moral standing of other animals (Bentham 1823), and have denounced anthropocentrism (i.e., "speciesism") (Singer 1974, 1975).

It is certainly true that utilitarian ethics ignore the rights and intrinsic value some ethicists believe insentient life forms possess, but this might well be considered a virtue of utilitarianism rather than a liability. Utilitarians can, of course, recognize legal rights and value species, ecosystems, etc., intrinsically - in the sense of valuing these entities for what they are and "as is." Ethicists that wish to go further and appeal to "natural rights" or "intrinsic value" in order to establish the moral standing of insentient entities have the burden of proving that such rights and/or values actually exist, are identifiable, and are of a very special kind. Insentient entities must be shown, that is, to have the same kind of rights and/or value that other entities with moral standing have (e.g., human beings). Demonstrating the existence of such rights and/or value has proven to be a difficult problem for environmental ethicists and they have largely failed to convince policymakers that trees, microorganisms, and communities have rights, or the kind of value that makes them legitimate objects of direct moral concern. Furthermore, no proof of such rights and/or value seems possible.

The assertion that utilitarianism can justify policies that environmentalists disapprove of has been made by ethicists claiming, in particular, that a utilitarian interest in individual welfare conflicts with an environmental interest in species and ecosystems. Callicott (1980), for example, argues that the holistic ethic he endorses is superior to the sentientist ethics of utilitarianism because the practitioners of the latter ethic would be prohibited from culling deer to protect sensitive ecosystems. A utilitarian environmental ethic would not, however, prohibit culling when the intended purpose is to promote the aggregate welfare of the population in question and/or to protect the ecosystem upon which the welfare ofsentient beings depends. Wildlife managers would only be required to minimize suffering by employing the most humane methods at their disposal. The land ethic Callicott favors places no such demands on wildlife managers, but it is difficult to see how this difference might be construed as commendable.

The above-mentioned claim takes many other forms and it is also argued, for example, that those interested in the pain and suffering of individuals would have to abstain from hunting, condemn "merciless" predators, guard the lives of wild animals, and liberate domesticated animals (Callicott 1980, Sagoff 1984). Such claims ignore the instrumental value of healthy environments, however, and can only be derived from a superficial characterization of utilitarian ethics (This point is convincingly made by Varner, 1995).

Critics of utilitarian ethics are not confined to the ranks of environmental ethicists and some educators may object to teaching utilitarianism on the grounds that it is flawed in ways that have little or nothing to do with environmental issues. A thoroughgoing defense of utilitarian ethics is beyond the scope of this paper, but it should be pointed out to the critics of utilitarianism that utilitarian ethics continue to be applied to a diverse array of 21st Century problems, including ethical problems encountered in public education, medicine, bioengineering, law, and economics. In all of these fields, utilitarianism has its proponents and utilitarian arguments are common.

Contemporary Environmental Ethics as a Problematic Alternative to Utilitarianism

Environmental ethicists have encouraged a vigorous and healthy debate regarding the attributes of a satisfactory environmental ethic, but no consensus has been reached concerning the specific nature of such an ethic and no single theory is widely accepted, even within the discipline.

Educators should recognize that environmental ethicists encounter both practical and philosophical problems when they attempt to make insentient beings the subjects of direct moral concern. As a practical matter, it is difficult to demonstrate that the moral standing of trees, insects, and bacteria can be established in time to prevent a significant worsening of the current environmental crises, given that the vast majority of Americans hold views that have been shaped by Christian theology and the anthropocentric ethics of Locke, Mill, Kant, and Descartes. As a philosophical matter, it is hard to argue that the interests of humans are no more important or of no greater moral concern than the similar interests of a tree or bacterium, but when moral standing comes in different colors or degrees, its meaning becomes vacuous and problematic. Does it mean anything to say, for example, that a tree has moral standing if it can justifiably be cut down to eliminate a threat to human life or to provide a family with firewood?

The only way to prevent a hierarchy of moral standing from developing and trivializing what it means to have standing is to treat the interests of all organisms, including human pathogens, equally. No ethicist is prepared to treat the "interests" all organisms have in living, etc., equally, and environmental ethicists have been forced to acknowledge that certain human interests must outweigh the interests of other life forms, including their interest in survival (e.g., Callicott 2003, Eckersley 1998). It might be argued that utilitarianism allows for dissimilar treatment and is subject to the same criticisms. However, utilitarians can weigh the interests of all individuals equally and still treat individuals differently because organisms differ with respect to their ability to appreciate pleasure and/or pain, and the concept of "interests" is typically limited to the interest sentient beings have in pleasure and the avoidance of pain.

The commitment to holistic entities in environmental ethics (e.g., species and ecosystems) also introduces what appear to be intractable practical and philosophical problems. Although holists acknowledge that we have duties to humans that can trump our duties to species and communities, the implications of a holistic approach to ethics cannot be escaped. All holistic ethics place the good of the whole (i.e., community, state, etc.) ahead of the welfare of individuals. In this respect, they resemble classically fascist doctrines that emerged in the mid-20th Century. Not surprisingly, environmental holism has in fact been dubbed "environmental fascism" (Regan, 1983).

Holistic ethics represent a radical departure from the normative ethics of human rights and concern for the welfare of individuals, and convincing the public that such a radical departure is ethically mandated presents enormous practical difficulties. There are also no holistic principles or rules for establishing the relative worth of different species or ecosystems, but to argue that a one-acre pond on "the back 40" is as morally important as a similarly-sized hot spring in Yellowstone would strike most Americans as absurd. To argue otherwise reintroduces a host of problems that are encountered when moral standing comes in differing degrees or is only recognized under certain conditions.

Any ethic that emphasizes the "interests" of species, communities and ecosystems may also rest on a shaky foundation because these are incorporeal entities (i.e., they are scientific abstractions). Such entities have no natural or clearly defined boundaries in time or space, and terms like *species*, *community*, and *ecosystem* are difficult, if not impossible, to precisely define.

Even if it is agreed that species, communities and ecosystems exist in some real sense, it is entirely unclear what "interests," if any, they might possibly have. It is also unclear how the extinction of a species can be regarded as unethical when the killing of individuals is not, without appealing to human values and utility. The loss of a species represents the loss of a unique assemblage of genes, but this is also what is lost when individuals and populations are destroyed. The difference is one of scale.

The value of species to communities and ecosystems is certainly greater than the value of individuals, but appealing to the ecological importance of individual species is problematic. Not all species are likely to play a crucial role in the functioning of ecosystems and some species may be ecologically interchangeable. Even when a particular species plays a vital role in a community or ecosystem, it is impossible to say that its removal is good or bad without appealing to human values and/or ascribing to questionable beliefs concerning the nature of biological communities and ecosystems.

The recognition of intrinsic value in environmental ethics creates further difficulties. An environmental ethic based on the intrinsic value of insentient organisms, species, communities and/or ecosystems is committed to an ethical position the validity of which cannot be objectively demonstrated. Unless all parties are willing to accept that such value exists, as a matter of faith or intuition, staunch advocates of intrinsic value theories can only presume to hold a superior moral position. Furthermore, even if it is agreed that species, etc. possess some form of intrinsic value, it must be demonstrated that such value is morally relevant or should

be preserved. As noted previously, this has proven to be difficult.

Assuming insentient organisms, species, etc. are intrinsically valuable, there is still no logical way to define the nature of intrinsic value so that the concept is not eviscerated, at least as a practical matter, by the development of a hierarchical value system. Assuming all organisms have intrinsic value, the eradication of pathogenic organisms can only be condoned if certain human interests and values are placed ahead of the "interests" and intrinsic value of other species. As Regan (1992) has pointed out, such a hierarchical concept of intrinsic value is indistinguishable from the concept of instrumental value. Any hierarchical value system is also necessarily anthropocentric because humans must, by default, construct the hierarchy of intrinsic value or the rules allowing for dissimilar treatment.

Not all environmental ethicists believe that a valid environmental ethic must be non-anthropocentric, holistic, or embrace the concept of intrinsic value. These are dominant themes in environmental ethics, however, and the lack of consensus only highlights the fact that there is no widely-accepted alternative to a utilitarian environmental ethic.

Conclusions

The environmental challenges today's students will face are truly daunting, and a strong environmental ethic, capable of discouraging destructive environmental policies, is desperately needed. Unfortunately, environmental ethicists have not yet produced a widely-accepted "environmental ethic" policymakers can fruitfully apply to the variety of "real world" problems they face, and it is still unclear what the attributes of such an ethic should be.

The majority of environmental ethicists appear to believe that a *true* environmental ethic is one that makes other organisms and/or holistic entities, like species and ecosystems, subjects of direct moral concern. This definition has helped to establish and define the scope of environmental ethics as an academic discipline, but it is too narrow to serve the present and future needs of environmental advocates and policymakers. It is also alienating, and environmental biology programs that are dominated by such a view not only risk producing graduates that are ill-prepared to participate in public policy debates, they risk losing potential students and collaborators with an interest in law, economics, civil engineering, etc. As Soule and Press (1998) have pointed out, mainstream neoclassical economists, for example, are rare in environmental studies programs, and this is probably because they find their views and those of their peers and professors ideologically incompatible.

Environmental ethics should not be shaped by practical concerns alone, but arguments that appeal to the moral standing of trees, species and ecosystems have not proven themselves to be logically superior to their more traditional alternatives, and should not be taught as such.

Many environmental ethicists and educators unjustly equate anthropocentric ethics and utilitarianism, in particular, with destructive environmental policies and methods of valuation that lead to environmental degradation. This is extremely unfortunate because traditional utilitarian and rights-based ethics can be used to reject the very practices they are often blamed for endorsing, and resonate with most Americans. When anthropocentric arguments are used to defend destructive and unsustainable environmental policies, the benefits to humans are nearly always exaggerated and/or the costs of environmental degradation to present and future human beings are underestimated. This being the case, such policies can usually be shown to be unethical from a utilitarian perspective.

In many environmental studies and policy classrooms, utilitarian ethics are unquestionably discussed in a fair and unbiased manner, but the tendency to associate utilitarianism with environmental problems and "environmental ethics" with their solutions is too often readily apparent. In one otherwise well-written environmental studies textbook, for example, the "western worldview" is described as "human-centered and utilitarian. It mirrors the beliefs inherent in the 18th Century frontier attitude" and is associated with "a desire to conquer and exploit nature as quickly as possible." The same textbook goes on to describe the principles of deep ecology in panegyric terms. "Deep ecology stresses harmony with nature," and a "respect for life" (Raven & Berg 2004). Another popular text claims that the "ecocentric environmental worldview is the environmental wisdom worldview" and differs from the "planetary management worldview" in holding that some forms of economic growth are environmentally harmful and should not be encouraged; inaccurately implying that ecologically enlightened homocentric views fail to recognize this fact (Miller, 2003).

The field of environmental ethics is fecund, exciting, and unquestionably important, but it is also nascent, fluid, experimental, and apparently incapable of providing near-term solutions to the ethical dilemmas attendant to modern environmental problems. Its failure, as a practical discipline, is an admitted source of concern to many environmental ethicists and the direction the field has taken over the last 30 years is now being extensively reevaluated from within. Our academic institutions need to recognize that this process will take time and that a genuine environmental ethic should and must be defined, for now, in broad enough terms to include utilitarianism.

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Book Review

Well-chosen non-fiction material can effectively compliment course content. It can introduce or culminate a lecture topic, provide elements for class discussion, and encourage students to read compositions besides textbooks and online features. Moreover, non-fiction books can offer an engaging and thought-provoking way of exposing students to recent advancements in biology and/or stories about the scientists themselves.

This article briefly reviews ten new non-fiction books that I thoroughly enjoyed, both for their intellectual merit and entertaining and accessible language. In addition, I offer suggestions on where to incorporate some of their best chapters into organismal courses. These books were specifically chosen because 1) the tone and level of technical writing were appropriate for undergraduates, 2) they are relatively new and based on primary literature, and 3) the chapters in each of them can stand-alone (e.g., although I think all of these books are worth reading cover-to-cover, I recognize that dedicating enough class time for an entire book may be difficult). For brevity, my chapter summaries do not encompass all of their content, but instead highlight one or more of the key points.

In alphabetical order by title:

"Darwin's Origin of Species" by Janet Browne, 2006
This book is part of a larger series ("Books that Changed the World"), and is a swift yet incredible summary of both Charles Darwin and his influential texts. I was particularly impressed in the latter chapters where she connected his life and times to other people and/ or events. Her writing is witty, relevant, and void of extraneous details. A nice follow up is Carl Safina's 2/9/2009 New York Times article,

"Darwinism must die so that evolution may live".

Chapter 1: Darwin's childhood, college, and voyage on The Beagle. 2: his experiences from the 1830's until the early 1850's. 3: receiving the letter from Wallace until publication of "The Origin of Species". *4: an excellent discussion of why his text and the controversy surrounding it are so unique. *5: the legacy of both Darwin and "The Origin of Species".

"Endless Forms Most Beautiful" by Sean Carroll, 2005
What is the one piece missing from the Modern Synthesis? Embryology. This fairly dense book is about the importance of some recent advancement in evolutionary developmental biology, and thus almost all of his material is extremely fresh. Carroll effectively makes the case that we need to focus on evo-devo instead of "evolution as changes in allele frequencies," because what is inspiring about that? I really appreciate this perspective as well as his implied suggestions in chapter eleven on how to alter my approach to teaching evolution. I recommend it for upper-division (rather than lower-division) students, as freshman may not understand its premise; how can they read about what is missing from the Modern Synthesis if they don't understand the Modern Synthesis?

Chapter 1: homologs, Williston's Rule (a recurring theme), and the task of development. 2: some creepy tidbits, ZPA, and "The Hopeful Monster". 3: gene switches and Hox genes. *4: the geography of an embryo. *5: an in-depth chapter on gene switches. 6: looking to DNA and development for clues about the Cambrian Explosion. *7: the evolution of novel traits such as wings. *8: summaries of work done at cutting-edge evo-devo labs such as Fred Nijhout's at Duke University. *9: the evolution of melanism, which is a really new idea for a chapter in a book like this. *10: brain, jaw, and speech differences between humans and chimpanzees (and this chapter brilliantly addresses how so many questions will be left unanswered if we can't study human embryos). *11: evo-devo as the new premise of evolutionary biology, and really the center of the book.

"The Future of Life" by Edward O. Wilson, 2002
There are a plethora of books and essays about the problems and potential demise of this planet, but Wilson's contribution is not just another addition to the pile. It is acute, full of statistics, and his (mostly) modern examples are appropriate for both freshman and seniors. He knows so much about the opposing arguments he is almost defensive about them, but his logic works, and works well. With little jargon and lots of soul, it is the most provocative book included in this review.

Chapter 1: an easy and fun descriptive survey of life. *2: the economist's and the environmentalist's view of how humans are defiling the planet, including some truly outrageous examples. *3: invasive species, endangered species, and vanishing ecosystems. 4: humans as the planetary killer. *5: addresses the question of "who cares?" 6: the abridged version of his 1984 book "Biophilia". 7: the solution presented mostly as a "to-do" list.

"Life Ascending" by Nick Lane, 2009
This book has ten chapters that cover the ten greatest inventions of evolution, and it is appropriate for almost any biology course. He includes powerful, current, and novel examples, and he regularly references the primary literature (e.g., the relevant peer-reviewed manuscripts can be easily retrieved by students). He also proposes several hypotheses for each major invention he tackles, which is a great reinforcement of the scientific method.

Chapter 1: the origin of life (primordial soup and LUCA). 2: DNA (history of its discovery, transcription, translation, and the "RNA world" hypothesis). *3: photosynthesis (this chapter is where the title of this book really shines). *4: cells (prokaryotes and eukaryotes, eukaryotic signature genes, mtDNA). *5: sex (meiosis, Muller's ratchet, and the Red Queen hypothesis). *6: motility (the discovery and evolution of muscle tissue). *7: sight (addresses the anti-evolution/eye argument, the Cambrian explosion, Pax6, and opsins). *8: hot blood (endothermy and stamina, comparative anatomy, and the Permian mass extinction). 9: consciousness (some interesting ideas about the evolutionary and physical roots of basic emotions). *10: death (e.g., death as the concept that makes

multi-cellular life possible and some discussion about selection on aging processes).

"The Making of the Fittest" by Sean Carroll, 2006
If DNA is used to prove crime, why not use it to prove evolution? Thus, each chapter of this book uses molecular biology to offer another bit of "proof" for evolution. Like the other three Sean Carroll books reviewed in this manuscript, it has nice illustrations, is captivating and easy to understand, and has some especially new and interesting examples (e.g., he doesn't use the traditional cases like Kettlewell's moths and the vestigial legs of snakes).

Chapter 1: introduction to how evolution works through tinkering. *2: math and evolution. 3: conserved genes (this chapter reminded me of "The Selfish Gene" by Richard Dawkins). *4: opsins and alcoholism. *5: fossil genes. 6: convergent evolution. *7: human resistance to disease. *8: the evolution of complex structures (this chapter is particularly packed with new examples). *9: dissecting the major arguments against evolution in the context of human vaccinations. *10: importance of fossils, introduced species, and human impact changing natural processes and habitats (e.g., Cape Cod-less).

"Only a Theory" by Kenneth Miller, 2008
This book is not a tribute to his experiences with the Dover trial, but rather a convincing address to the dangers of intelligent design and the importance of evolution to society. He has many easy-to-understand analogies that set up his rationale and/or position, and I found myself affirmatively nodding over the flow his logic accumulated in each chapter.

Chapter *1: setting the stage for the evolution debate *via* the viewpoint of "thinking like an American". 2: a general introduction to the debate, but easily covered with a lecture instead. *3: understanding intelligent design; lets take it seriously and then evaluate its merits. 4: the genome project and human evolution as evidence for evolution and not ID. 5: the success of ID, history of life on Earth, and evo-devo as more evidence for evolution. 6: "the meaning of life" from an evolutionary perspective. *7: how the ID movement and the evolution debate, for perhaps the first time in the sciences, are not free from politics and culture. 8: the larger implications of accepting ID as a science.

"Remarkable Creatures" / "Into the Jungle" by Sean Carroll, 2009
Students find science more interesting when they see the scientists behind it as real people, with real experiences, and Carroll does just that. These books are brilliantly written, captivating, have outstanding illustrations and photographs, and have nice modular organization (e.g., they have sub-sections within chapters that make them easy to put down and pick up). "Into the Jungle" is an edition issued by Pearson Education, and while a significant number of the chapters appear in both books, there are a few chapters present in "Remarkable Creatures" that are not in "Into the Jungle" and vice versa (see below; titles are abbreviated as RC and ITJ). As a bonus in "Into the Jungle", there are five questions at the end of each chapter that can serve as assignments.

Chapter topics in both books: *Charles Darwin (Chapter 2 in RC, 1 in ITJ), *Alfred Wallace (Chapter 3 in RC, 2 in ITJ), *Henry Bates (Chapter 4 in RC, 3 in ITJ), Eugene Dubois, Roy Chapman Andrews, and Luis Alvarez. Unique chapter topics in "Remarkable Creatures": *Alexander von Humboldt (Chapter 1), *Charles Walcott (Chapter 6), *John Ostrum (Chapter 9), *Neil Shubin (Chapter 10), Louis and Mary Leaky, *Linus Pauling (Chapter 12), and Allan Wilson's work on Neanderthals. Unique chapter topics in "Into the Jungle": Marjororie Latimer's discovery of the coelocanth, *Tony Allison's research on sickle-cell anemia (Chapter 8), and Johan Ruud's tale of the icefish.

"The Trouble with Testosterone" by Robert Sapolsky, 1998
This book explores ideas about animal behavior in relation to humans. It is very charming and he writes in a tone that 18-22 year olds will relate to and appreciate. The author is notably well rounded; in the lab he studies neurophysiology and in the field he follows a troop of baboons in East Africa. The chapters are shorter than some of the other books reviewed in this article, so they are especially easy to incorporate into class. If used in an upper-division course, he includes recommended primary literature for further reading at the end of each chapter.

Chapters 1 and 2: general introduction. 3: the fitness of the timing of puberty in mammals. *4: perceptions into how science is done. *5: adolescent mammals transferring to a new home. 6: stability of animal morphology and behavior. 7: a confusing and random chapter about ideas in science. *8: the history of poverty in medical research. *9: the "me generation" and the consequences of gluttony. 10: the implications of stress (a great read for that high-maintenance student). *11: the chapter that gives the book its name, but it is also about the philosophy of science. *12: sexual selection, male emigration, and aging. 13: zoopharmacognosy (the study of self-medication in non-human animals). 14: Western vs. Third world diets and the role of stress in health. 15: personalities and split brains. 16: the physiology of being sick. 17: science and religion. This final chapter is so brash at times he puts a disclaimer at the beginning of it; only pursue if you want to dedicate time to class discussion or have a serious opinion paper on it.

"The World Without Us" by Alan Weisman, 2007
Imagine if all of the sudden there were no humans on Earth; this is pages 1-3. For the rest of the book, Weisman describes what would happen to nearly twenty ecosystems without human inhabitants. Although not as entertaining as some of the other books in this review, it is both bright and toxic and provides some invaluable chapters for capstone discussions.

Chapters 1 and 2: general introduction. *3: New York City. 4: chimpanzees, ice ages and changes in CO_2. 5: a history of some extinct North American megafauna. *6: mammals of the African plains. 7: Cyprus. 8: underground cities in Turkey. *9: the plastic chapter. *10: Texas (enough said). 11: farmlands. 12: Panama Canal and other modern wonders. 13: Korea/DMZ. *14: the decline of bird populations. 15: Chernobyl and leftover nuclear waste. 16: mined areas. 17: the future of those that depend on us such as bacteria and viruses. 18: the leftovers of our art and digital media. *19: the ocean.

"Your Inner Fish" by Neil Shubin, 2008
This book explores the evolution of chordates, beginning with chondrichthyes. It is very approachable, quite funny, and like "The Trouble with Testosterone" it has many analogies 18-22 year olds will find humorous (e.g., "show me the body" and "an inconvenient tooth"). Since it ultimately revolves around the evolutionary history of the human body, its chapters are particularly relevant and interesting for health professions students.

Chapter 1: descriptive chapter about discovering *Tiktaalik* in the Artic Circle. *2: homologies and his personal experiences with a cadaver wrist. 3: the deep molecular similarities in animals (sonic hedgehog, Hox genes, and ZPA). 4: the importance of teeth in the transition from reptiles to mammals (great for pre-dental students). *5: the formation of the head with an amazing segment about his experiences and understanding of the cranial nerves. 6: general embryology, including some nice history. *7: evolution of a body from no body. 8: evolution of smell. 9: evolution of vision and opsin proteins. 10: the evolution of ears. 11: puts the last ten chapters together, and only appropriate if students have read the entire book.

In conclusion, these ten books are, in my opinion, some of the best non-fiction biology literatures to be published in the last decade. They contain over fifty stand-alone chapters that can be incorporated into a suite of organismal courses, and many of them summarize cutting-edge research. Quality, accessible books on evolutionary biology are especially critical in the current educational climate; getting students (particularly freshman and non-majors) engaged in evolutionary theory is a relatively recent but essential component of teaching biology. Thus, I think the books in this review not only offer instructors a substantial foundation for introduction or capstone discussions, but will also get students excited about scientific advancements in the minds of the scientists who accomplished them.
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Letter to the Editor

Soy milk is an excellent and economical blocking agent for immunoblots.
Immunoblotting is a technique that is easily

mastered by high school and college students and several laboratory exercises make use of this are available for blocking that work well; however, they can represent a substantial cost of each immunoblot and may represent a significant barrier to use in laboratory exercises that involve a large number of students. Popular, inexpensive agents include non-fat dried milk, gelatin, and bovine serum albumin. However, since commercial reagents (such as Pierce Superblock) remain very popular, it appears these self-made blocking solutions either do not work as well as the commercial reagents or are not as convenient to use (or both).

Since antibodies are made in animals, we thought that a non-animal protein source might provide an alternative blocking agent that would be less likely to have peptide epitopes that might interact with the primary or secondary antibodies. Initially, we tried textured vegetable protein, but found it difficult to dissolve. We then found that under our conditions soy milk, available at many local grocery stores, blocks as well as Superblock (Pierce) and was as convenient to use. We compared soy milk (purchased from Hy Vee (Columbia, MO) or Meijer (Normal, IL) grocery stores) to SuperBlock (Pierce 37545) as blocking agents. After the proteins from a crude kidney homogenate were separated via SDS-PAGE and electrotransferred onto PDVF, half of the blot was blocked with soy milk and half with Pierce Superblock. Then both halves were probed with anti-Na pump alpha-subunit antibody (Affinity Bioreagents, MA3-929) and secondary antibody-HRP conjugate ((Pierce, 41430). The signal to background ratio for both blocking agents were very similar.

One problem with non-fat dried bovine milk is that it contains a compound that inhibits the avidin or strepavidin interaction with biotin (Hoffman and Jump, 1989) Thus we also tested the ability of soymilk to adequately block membranes that were probed with HRP-conjugated avidin (Pierce, 29994) by examining biotinylated albumin was separated by SDS-PAGE. Once again, similar signal to noise ratios were observed for the soymilk blocked blot and the SuperBlocked blot.

Soymilk has been used for blocking Elisa plates (Santa-Marta et al., 2005), but we are unaware of any other studies using soy milk to block immunoblots, or studies using it for avidin-HRP detection. The use of soymilk can technique. A number of commercial products

substantially decrease the total cost of an immunoblot procedure-and unlike most other self-made approaches, there is no increase in labor.

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Web review: Discover Life at http://www.discoverlife.org

Many young persons express an interest in science and technology, but it seems that too few continue to choose it as a major in later high school years or college. Students have been found to be particularly "turned off" by the perceived extensive technical content in classes and lack of the ability for hands-on involvement. Many young persons have limited access to exciting and thought-provoking science activities to encourage and sustain their interest**.** The question becomes, "why has science become un-cool?"

As scientists and professors we are particularly interested in attracting students into our programs. To heighten student interest in the

sciences and help to involve students [and the community**]** may I suggest a look at the **Discover Life Web Site** that can be found at http://www.discoverlife.org. Discover Life is a website under the auspices of the Polistes Foundation. It is primarily served from the University of Georgia. The site is promoted and maintained through the untiring dedication of John Pickering PhD., Odum School of Ecology, University of Georgia, Athens, GA .

Promoted as an online encyclopedia, or digital resource, Discover Life is so much more! It is a link to providing educators and community action groups a means to enrich and share knowledge of the life sciences. The Discover Life mission "is to assemble and share knowledge in order to improve education, health, agriculture, economic development, and conservation throughout the world." The site is true to its word, providing interested persons with a wealth of online tools and information. The free on-line tools can assist groups in species identification; providing ways for educators [of all grades] to share teaching strategies and ideas and study nature's wonders; a hands-on online interactive means to report findings, build maps, and process images; it then also becomes a tool to- contribute to- and learn from a growing, interactive encyclopedia of life that now has 1,293,810 species pages.

More than just providing information Discover seeks to involve students, educators and researchers in their ongoing projects, welcoming those groups to contribute their findings to their growing list of contributors. A current major project includes organizing and working with community based groups around the U.S. and North America to study the impact of invasive species, weather, fire, pollution, and other environmental changes on biological systems.

The easy to navigate site includes links to Nature guides and Identification manuals/IDtrees, excellent nature photos, educational lesson plans and outreach activities, research opportunities and a vast wealth of additional links and information. There is even a life sciences labels data bank to assist you with mounted specimens, microscope slides, DNA samples, photographs, virtual images, and sound recordings, etc! And the "global mapper" is fun at any stage of scientific investigation! **Discover Life is an excellent tool to increase excitement and involvement and keep the FUN in science!**

Christine Bezotte

ACUBE is online at ACUBE.org. The site includes archived and upcoming editions of *Bioscene: Journal of College Biology Teaching*.

54th Annual ACUBE Meeting
October 8-9, 2010
Lourdes College
See ACUBE.org for further information.