Journal of College Biology Teaching
Bioscene

Volume 39(1) May 2013

Bioscene
Journal of College Biology Teaching

Volume 39(1) **May 2013**

ISSN 1539-2422

A Peer-Reviewed Journal of the

Association of College and University Biology Educators

Editor-in-Chief:
James W. Clack
Indiana University – Purdue University

An archive of all publications of the Association of College and University Biology Educators (ACUBE) can be found at http://acube.org/bioscene

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

Cover image: Herbarium sheet of *Darlingtonia californica Torr.* created by Charles Wilkes, 1842. Reproduced with permission from the United States National Herbarium of the Smithsonian Institution

CONTENTS

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

ARTICLES

Plant Collections Online: Using Digital Herbaria in Biology Teaching

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Abstract: Herbaria are collections of preserved plants specimens, some of which date back to the 16th century. They are essential to botanical research, especially in systematics. They can also be important historical documents. The collections of Lewis and Clark, Carolus Linnaeus, and Charles Darwin, to name a few, are primary sources for the study of these individuals' work. Now many of these herbarium specimens are being scanned and the images are freely available on the Web. This article deals with how these online collections may be used in teaching about biology and its history. It will highlight the JSTOR Plant Science project which is making available electronically about two million plant specimens, many historically significant, as well as the entire runs of important plant journals. In addition, it will discuss other valuable online resources including how links to social media can bring the history of botany to 21st-century students.

Key words: botany, herbarium, history of biology, online resources, systematics

INTRODUCTION

Collecting specimens is a major part of what biologists have done in the past and what they do today. Amassing plant and animal specimens is key to taxonomic and anatomical work, and collecting gene sequences obtained from specimens is central to present-day biological inquiry. Though in many ways these two approaches are different ways of doing biology, it's becoming more common for both sequence data and organism specimen data to be stored electronically and accessed via the Internet which allows for interesting comparative work that would have been difficult, if not impossible, in the past. Having data accessible online means that it's available not only to researchers but to students as well. And just as museum curators are necessary so collections can be presented to nonspecialists in an intelligible form, teachers need to be curators of such data collections so students can understand the valuable information available in them (Siemens, 2008). Many resources are available for dealing with gene sequence data; however, the focus here is on herbaria, collections of preserved plant specimens, which were first created in the 16th century, often became neglected in the 20th century, and are experiencing a resurgence, in part because of efforts to digitize these collections. After describing what herbaria are and how they are used, this paper will explore how herbaria, both real and virtual, can play an important role in teaching about biology and its history.

What Is a Herbarium?

It's not uncommon for someone to take a flower and press it between the pages of a book in order to preserve it. The paper absorbs the water in the flower and pressing prevents the petals from curling up as they dry. Once dried, the flower will last indefinitely because the lack of water inhibits bacteria and fungi from causing deterioration. This practice is similar to that used in preparing herbarium specimens which are pressed and then mounted on acid-free paper, most often with glue or linen tape, utilizing more sophisticated equipment. Some specimens cannot be preserved in this way. Fruits, for example, may have to be stored in alcohol and large nuts in boxes; they simply cannot be flattened between two pieces of paper. However, all specimens must be carefully labeled as to species, date and place of collection, and name of collector.

Looking at a herbarium specimen of, for example, the pitcher plant, *Darlingtonia californica* Torr. (Figure 1), can be disappointing for a nonbotanist. What was a stately plant in shades of green, yellow, and red (Figure 2), is now a mass of brownish material. The vitality has left the plant, but the important taxonomic information is still there in terms of what a botanist needs to know in order to identify the species. As natural history developed in the 18th century, descriptions of plants and animals became focused on a few key properties to the exclusion of others. The great taxonomist Carolus Linnaeus saw four qualities as particularly significant: the form of the elements of the organism, the quantity of the elements, the manner in which they are distributed in space relative to each other, and the relative magnitude of the elements. For a plant, all these are usually present on a herbarium sheet. It might be argued that a clear photograph of a plant would be an excellent substitute for a herbarium sheet and actually provide more information, such as that on position of elements in space and color,

Fig. 1. Herbarium sheet of *Darlingtonia californica* Torr.; the specimen was collected on the United States Exploring Expedition (1838-1842) in the United States National Herbarium.

particularly for the flower which is usually the part of a plant that varies the most in color. Indeed, photographs are very useful in plant identification guides. However, photographs often misrepresent scale, may not display all of a plant's identifying features, and obviously aren't sources for chemical or microscopic analysis.

Types and History

Type specimens are the most important herbarium sheets in any collection. A type specimen is the particular plant upon which the botanist who names the species and publishes its description bases that description (Bridson & Forman, 1998). Often several specimens are collected from the same plant, or several plants are collected from the same area at the same time as the type is collected. These are mounted on separate sheets, designated as isotypes, and often sent to other institutions as insurance that if the type is destroyed, there will be a similar specimen available to represent the species. The oldest known herbarium specimens are from the mid-16th century. Some herbaria were part of the cabinets of curiosity that were popular among moneyed Europeans during the Renaissance and morphed into massive collections during the Enlightenment. One of the most notable compilations was that of the British physician, Hans Sloane; it became the foundation of the British Museum's collection (MacGregor, 1994).

As with many collectors, Sloane eventually became overwhelmed by specimens and when Carolus Linnaeus consulted the herbarium on a trip to London in 1732, he considered it in "complete disorder" (Dandy, 1958). Just the storage method used presented a problem. Bound in 265 volumes, the specimens could not be rearranged as species were reclassified. Linnaeus saw this system's flaws and stored his specimens on separate sheets kept in folders with other specimens of the same genus and laid on shelves in cabinets he had specially made for the purpose where all the genera of a family were stored together. In this way he could easily move sheets around if he decided a particular specimen

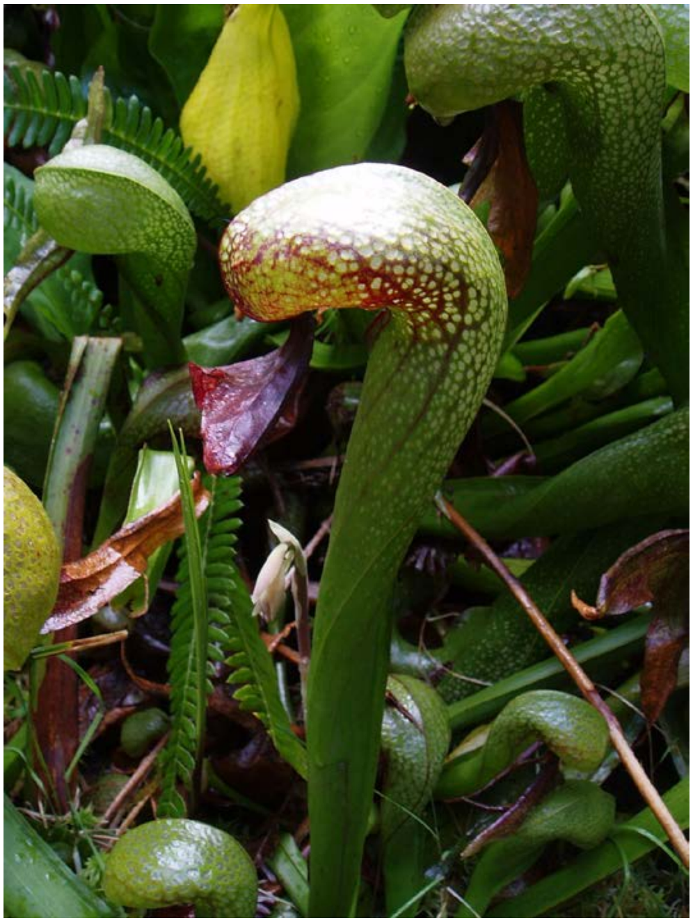

Fig. 2. *Darlingtonia californica* Torr. The image published into the public domain by Adam Harris on Flickr: EOL Images.

belonged to a different family. It is essentially this system that is still used today.

Linnaeus's classification enterprise was driven by the need to organize information about the ever-increasing number of plants arriving in Europe during the Age of Exploration. These collecting efforts are reflected in herbaria, especially in Europe. The specimens of one collector are usually spread over many herbaria. In the past, there were professional plant collectors who sold sets of specimens to interested botanists. Even today most collectors gather more than one specimen of a particular species; these are the capital of the plant collector and of the botanist. One student likened herbarium sheets to baseball cards. Indeed, there is a similarity in that the most valuable sheets are often those of rare species, or are very old, or were collected by someone like Charles Darwin, obviously the Babe Ruth of biology. While a single sheet isn't often sold as a baseball card may be, collections are sold and have been for centuries; that's how Sloane acquired many of his specimens.

Changing Perspectives

By the end of the nineteenth century, the great age of exploration was coming to an end. While there were still many plant species yet to be discovered and many collectors still at work, biology began to move away from taxonomic work as its center to experimental research in cell biology and physiology. Daniel Crawford (2001) argues that with the eventual shift to DNA work, there was less fieldwork and population sampling, and therefore fewer herbarium specimens were created. However, there is now a renewed interest in herbarium collections for a variety of reasons. First, ecologists are coming to appreciate herbaria as essential archives for documenting biodiversity; the only way to know how many species there are and where they can be found is with reliable records such as those in herbaria and other natural history collections (Joppa et al., 2011). Secondly, herbaria are vital for documenting environmental change. For example, flowering plants are usually collected when they are in bloom because flowers are key to identification. If a specimen in flower is collected from a locale in April, and a herbarium specimen of the same species, also in flower and from the same area, was collected 100 years ago in May, then this could represent one more piece of evidence for climate change (Primack et al., 2004). Thirdly, entomologists have used herbarium specimens to discover when a particular beetle species first invaded an area: specimens collected before a certain date contain none of the anti-beetle chemicals present in later ones (Zangerl &

Berenbaum, 2005). In addition, sheets created during plant surveys provide valuable information on how plant communities change over time (Kohler, 2006). These examples indicate that collections become more valuable with time; they are not just libraries of species but time capsules providing historical evidence for what was growing where at a particular moment. Each specimen is unique in this respect; each is irreplaceable.

Recently, herbaria are also receiving attention from molecular biologists. Many plant specimens harbor intact DNA that can be used in genetic studies. Even 200-year-old sheets have yielded DNA which could be sequenced (Andreasen et al., 2009). In addition, in herbaria today, researchers are systematically preserving plant samples for use in sequencing: fresh material is dried in silica gel and then stored at low temperature. This is one more kind of plant specimen found in herbarium collections along with boxes of pinecones and jars of alcohol with fruits or flowers floating in them. There may also be a seed bank where seeds are stored for future germination as a way to preserve the genetic diversity of species. If possible, all these specimen types should remain together in part because the different kinds of collections mentioned at the start of the article—specimens and sequencing data—are like reference libraries where researchers come to consult plant material instead of books. And, like libraries, there is a long tradition of borrowing and lending among herbaria, as researchers in different parts of the world work on plant genera or families representatives of which may be housed in dozens of herbaria.

Digitization

Just as libraries have been at the forefront of digitizing information about their books and the books themselves, the same thing is true of herbaria; both types of institution are dealing principally with two-dimensional material, which makes creating digital images relatively easy. This is a massive undertaking and data on labels are more often digitized than images of specimens. There have been a number of United States grant programs, often through the National Science Foundation (NSF), to support digitization efforts so all the major herbaria have some of their collections online (iDigBio, 2013). On an international scale, the Global Biodiversity Information Facility (GBIF) has created a portal where almost 400 million records about species of all kinds are available electronically (GBIF, 2013). However, there is a threat to herbaria that underlies such massive digitization efforts. As early as 1990, it was suggested that once a herbarium sheet had been imaged and its information digitized, the sheet was no longer needed; it would only be necessary to retain type specimens (Clifford et al., 1990). Needless to say, there were prompt rebuttals to this proposal (Harley, 1990).

Databases are amazing resources not just for botanists, but for teachers and students as well. They can serve as virtual museums of plants and as libraries of information about plants. Because of their fragility and value, herbarium collections are closely guarded, with access sometimes limited to researchers in the field. This is a major reason why collections are being digitized—so the sheets and the information on them can be accessed without damage to the originals. There are also other accessibility issues. Most plants collected by former colonial powers reside in these nations, while their former colonies have only a fraction of the documentation of their botanical wealth. It's ironic that many of the nations with the most diverse flora have the greatest difficulty in maintaining collections of this richness.

To address this problem in the most needy area, the African Plants Initiative was established in 2003 with the aim of digitizing type specimens of African plants and making these images available on the web (Patmore, 2010). Four years later the Latin American Plants Initiative grew out of this effort and has since become the Global Plants Initiative, now focusing on Asian plants. The Andrew W. Mellon Foundation funds this project, providing scanning and photography equipment to institutions. This initiative led to the digitization not only of herbarium specimens but of the botanical literature needed to support research in systematics. All these resources are presented through JSTOR Plant Science (JPS) which is much more than a database linking to specimens and journal articles. It provides a suite of search and social media tools that greatly enhance the value of the database itself. However, this doesn't deal with access to the technology needed to use these resources which often remains a challenge.

Within JPS, the specimen images are of high quality with a zoom feature for examining details. When a species name is entered in the search box, what appears is not only a thumbnail image of the specimen (or specimens) but also links to articles and citations related to that species, including JPS and other JSTOR resources, as well as the Biodiversity Heritage Library (BHL), the Tropicos database, and GBIF. In addition, on the opening page of JPS, there are links to comments from users concerning specimens in the collection. This is one way mistaken species determinations are corrected. JPS is also active on Twitter and Facebook, has a number of videos on Vimeo, and ran a blog as well (JSTOR Plant Science, 2012). With tools like these, JPS is creating a virtual and global taxonomic community for scientists and for biology teachers and students. One page with all these resources available for a particular species is a goldmine and shows students how reference materials can be organized to make them more useable. It also gives them a sense of the literature available, ranging from articles aimed at experts to ones easily read and understood by nonprofessionals. Other resources in JPS include manuscripts and letters by such botanists as Asa Gray, Joseph Hooker, and Carolus Linnaeus; in addition, there are thousands of drawings and paintings of plants.

The JPS blog can be useful in teaching many topics, from evolution and genetics to biodiversity and economic botany. Some posts describe resources available through JPS. Others deal with the project's accomplishments and its outreach to the global plant community; posts included videos made at partner institutions around the world. They are reminders that our instant access to information is hardly the case globally. The majority of posts concern items in the specimen collection and often focus on history: everything from William Dampier, a pirate-botanist, to figs and how their leaves came to have a strategic place in art.

JSTOR is a widely-available database, but even without this access, the blog is open to all web users. One or more blog posts could be used as assigned readings in themselves, and many of the links are to freely available resources outside of JSTOR, so these too, can be explored by students. The posts are essentially curated guides to a topic. If students can access JSTOR, then the posts become more than just interesting reading and can develop into lessons in information literacy and the use of social media in research. This might be the best way to use the JPS blog: as a model for students to use in creating their own. Also, students can carry the social media connection further; groups involved in creating posts can share ideas, information, and resources via Twitter or Facebook, and they can embed the videos in their blogs. Such assignments combine writing, visual presentation, and evaluation of information in a way that standard PowerPoint presentations that students often create cannot.

Teaching with Digital Herbaria

There are many different ways to incorporate digital herbaria into biology classes. I'll describe three approaches here to suggest how these resources can encourage active learning about plants. For those who stress history, there are sites like the one presenting plants Charles Darwin gathered on his *Beagle* voyage and sent to his mentor, John Stevens Henslow, professor of botany at Cambridge University (Darwin's Plants from the Beagle Voyage, 2012). The site links to a video describing the importance of the collection to Darwin's work (Parker, 2009). The American plant systematist and historian of botany, James Reveal, has created a rich website on Lewis and Clark's botanical collection (Reveal, 2008). On the Linnean Society's website are scans not only of Linnaeus's plant specimens, but of insects, fish, and shells (The Linnean Collections, 2012).

Depending on what's being taught, students could explore one of these sites. On the Darwin site, there's a page on reading a herbarium sheet that might be a good place to begin. Then students could look out for specimens labeled "Type" and investigate what this means. They could also compare historical specimens with recently collected ones of the same species. This might lead to issues of name changes and synonyms; the Plant List (2010) is a comprehensive entry point for this research. Also, students could examine how labels have become more information-rich, especially in terms of location data with GIS coordinates. As an exercise in biological information literacy, they could compare the sites for two or more of these historical herbaria to see how they differ in terms of information, accessibility, etc.

For ecology courses, digital herbaria are useful in investigating biodiversity, environmental change, and phenology. LifeMapper (2013) is a website which provides georeferencing data for many species worldwide. As with most of these global sites, some of the information is sketchy, but that in itself is a good lesson for students that ecology, biodiversity studies, and biodiversity informatics are all developing enterprises. As an exercise, students can collect plants, identify them, create specimen sheets, and geo-reference them using Google Earth (2013). They can then compare their specimens with online examples of the same species. There are also two national digital projects, examples of Citizen Science, in which they can participate. Part of the National Environmental Observatory Network (NEON), Project Budburst (2013) involves plant budding, flowering, and fruiting times. The data input will document climate change in the future and can also be compared to historical data. An activity like this doesn't just fulfill the requirement for a grade but also makes a valuable contribution to environmental studies. The same is true of another national program, Nature's Notebook (2013), one element in the National Phenology Network. Participants sign up to take notes on a particular area, looking for specific organisms. This is a great way for students to begin to appreciate the nature that surrounds them. It also helps them to realize that even though they may live in a very human-altered environment, there are still habitats and organisms to study. They could, for example, take notes on the spread of an invasive species just moving into an area or be on the lookout for rare plants. Searching for these species in digital herbaria might be the next step. Students can also learn to use one of the many online plant checklists or local floras. Learning about these resources may help to make them lifelong students of nature.

Because biology is the most visual of the sciences, I stress visual literacy when I teach. Observation is frequently taken for granted as an obvious skill, but it needs to be nurtured. Collecting plants in the field and identifying them are ways of developing this skill, as are exercises in comparing specimens of the same species collected at different times or in different locations. Large digitized plant collections available to students include those at the Smithsonian Institution (2013), New York Botanical Garden (2003), Royal Botanic Gardens, Kew (2013), and the Muséum National d'Histoire Naturelle (2013). Most digitized specimens are of high quality and can be magnified, so students can inspect texture and fine structures. It is also valuable for them to compare specimens with photographs of the same plant, and with illustrations. Each approach has advantages and disadvantages in communicating information about a plant; it's useful for students to investigate these differences and describe them. And as an ultimate exercise in visual literacy, they could draw plants from life and from herbarium specimens.

While this paper has focused on plants, there are superb zoological and fossil collections online as well. NSF is heavily funding digitization of all types of natural history collections, so more and more of these resources will become available on the web. The related literature is also being digitized through the BHL (2013), and field notebooks for American naturalists are coming online through a Smithsonian Institution project (Field Book Project, 2012). The material available is so rich that it's open to a variety of approaches that can attract different types of learners and also renew a sense of discovery in faculty as well.

ACKNOWLEDGEMENTS

I would like to thank the herbarium curators who have been so helpful to me. This article was written with support from the St. John's University Faculty Writing Initiative.

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INNOVATIONS

Focusing on the Hard parts: A Biomechanics Laboratory Exercise

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Abstract: As part of a biomechanics course aimed at both upper-division Biology and Physics majors, this laboratory exercise introduces students to the ingenious ways in which organisms vary the composition and form of support and defensive structures such as bone and shell to maximize their strength while minimizing the energetic cost needed to produce them. Students design and build physical analogues that take advantage of strategies found in nature such as the use of composites and variations in form and internal structure. These are then tested in a competition to determine whose design can withstand the greatest force with the lowest mass per unit length (a proxy for the energetics of production). From this exercise students gain a better understanding of how these structures can be optimized, as well as providing an opportunity to discuss basic biological concepts such as fitness, variation and evolution.

Key words: Biomechanics, bone, physics, biological materials

INTRODUCTION

The study of biomechanics provides biology students with an opportunity to apply their education in physics to a biological context and bring together biological concepts normally spread across a wide range of coursework (e.g. evolution, physiology, behavior). At Saint Joseph's University the course in biomechanics covers a broad range of topics including fluid dynamics, biomaterials and locomotion. Lectures taught by members of the Physics and Biology departments are broken into weekly units that cover the physical concepts and theories as well as their biological applications. This manuscript describes a three-week laboratory exercise designed to help students understand the effects of material composition and form on the strength of solid biological structures.

The module on “hard parts” starts with an overview of the relevant material mechanics including stress (the force per area deforming a solid), strain (a measure of the degree of deformation), the elastic modulus (the ratio of the former to the latter), strength (the force which a material can withstand before fracture) and elasticity (the ability of a material to return to its original shape after deformation). Descriptions, examples and supporting information on these topics can be found in any college physics textbook, and many students may already have been exposed to them during their high school or undergraduate courses.

In contrast to the physics involved, the biological implications of those properties are usually only understood at a very basic level: e.g. that structures such as skeletons, snail shells, and carapaces need to be strong and light and energetically efficient in their production and use. To help the students develop a deeper understanding of how material properties can affect an organism’s fitness we use examples that illustrate two dichotomies: offense vs. defense and composite materials vs. blended materials. Examples of offense are teeth and claws, both of which provide examples of the latter dichotomy. Teeth use the combination of two materials: enamel and dentin. The outer layer of enamel is made of a tough but brittle crystalline form of calcium phosphate called hydroxyapatite. Enamel resists fracture under sudden impact but is prone to fracture with little to no deformation. To balance the strengths and weaknesses of the enamel, it is bonded to the more energy absorbing dentin. Dentin, like enamel, is also a calcified tissue, but mixed with collagen to change its properties (Vogel, 2003). Claws, such as those used by scorpions or crabs, use a different strategy, namely changing the composition of a single material rather than layering two separate materials. By doping the tips of their claws with metals such as zinc they produce material that is much more chip-resistant (Schofeld, 2005). This is particularly important for those that use their claws as forceps to pick up and manipulate food items, since a fracture at the tip may limit their effectiveness till the next molt, thus reducing fitness.

The development of any offensive capability on the part of a predator is usually countered by the evolution of a corresponding defensive adaptation in the prey. Here we focused on two examples, the snail shell and mammalian bone, to illustrate how different biomaterials can act in isolation and in combination. Snail shells provide an excellent example as their strength arises from three levels of organization: the materials used, the arrangement of those materials, and the overall shape of the shell. Shells of most gastropods are composed primarily of calcium carbonate bricks that are arranged in an offset pattern and held together along their long axis by proteinaceous glue. This arrangement allows the strength of the bricks to be augmented by the energy absorbing qualities of the protein, similar to the arrangement in teeth. Absorption of impact energy occurs by allowing any cracks to travel over a tortuous pathway through the shell, thus dissipating more energy than would otherwise take place if the crack traveled directly through a monolithic piece of calcite (Menig et al., 2000). The strength of the shell is increased by its arched shape, which helps to distribute and redirect forces placed on it over a larger area, just as an arch helps distribute the weight of a roof. A final “trick” that has recently been found in a deep water gastropod is to cover the outside of the shell with a layer of hard metal crystals which are thought to dull the claws of would be predators, increasing the area over which their crushing force is transmitted and thus lowering the force per unit area they can impart to the shell (Yaoa et al., 2010).

Mammalian bones, in spite of their very different evolutionary lineage, arrangement, and location, use similar strategies for dealing with impact and fracture. Long bones such as the ulna or femur use properties of their constituent materials and overall shape to provide the greatest strength with the least weight. This is particularly important for land-based organisms that cannot take advantage of water’s buoyant assistance to support their bodies. Similar to the shell’s brick and mortar approach, mammalian bones use concentric layers of mineralized material arranged in osteons which are in turn connected to other osteons via a proteinaceous glue (Fung, 1993). These osteons can absorb the energy of impact through both delamination and “pulling out” whereby entire osteons break their connection along their entire length, thus dissipating energy. The presence of “spongy” trabecular bone in the ends and core of the bone provides additional energy absorption. This webbing of bone forms a matrix that shatters and dissipates energy while the overall integrity of the bone is maintained by the outer layer of compact cortical bone. Additionally, bones are often non-circular, reflecting the anisotropic (unequal along different planes) forces they encounter either from the weight of the organism or stress placed on them by muscular action. By increasing the size or thickness of the bone in the direction of the greatest stress, and by maximizing the mass of material at the outer rim, the flexural stiffness of the bone is increased, allowing it to resist bending when under load (Fung, 1993).

By presenting these examples, we aim to impress upon the students that a few basic strategies to increase the strength of structures, however complicated their implementation may be biologically, can be found across a wide range of taxa. Lamination, tortuous crack propagation, combination of strong but brittle with soft but energy absorbing materials, changing material composition and the specific shape they form, can all produce strong structures while minimizing the materials, and therefore the energy, needed to produce and maintain them.

This exercise was developed to allow students to experiment with the biological strategies that organisms have developed to resist impact forces. The students are asked to design and construct analogues of biological structures using their knowledge of biomaterials and the ways in which organisms use them to resist fracture. To increase student interest we ran the exercise as a competition with two-person teams. Each team was allowed to produce and test as many prototype bones as they wished, but could only enter one design in the final competition. These constructs were then tested for their ability to withstand both static loading and impact. To emphasize the idea that most biological systems are limited in the energy they can put into building and maintaining elements of their body, designs were scored based on the force they withstood divided by their mass per unit length. The mass, in this case, represented the energy necessary to produce the structure.

Students were given free rein to develop their own designs, many of which were rather complicated. In this manuscript, however, we present data for a series of simple designs to illustrate specific comparisons: 1) the effect of hollow vs. solid bones of similar size, 2) the effect of similar masses being arranged as either solid or hollow bones, 3) the effect of a trabecular-like matrix and 4) strength of an ellipse along either of its axes.

METHODS

The competition was designed to challenge students to design and build a structural analogue to bone, shell or other hard biological structure (hereafter referred to as a “bone”) that would withstand the greatest force without failure. Failure was defined as a complete break, or sufficient fracture to leave the structure without the necessary rigidity to bear weight along its long axis. This included situations where the bone was only held together by flexible material or flopped over but did not separate into two pieces.

Bones were limited to a cross-sectional area of 5 cm^2 and a length of 15 cm to preclude students from building giant objects that would be impossible to break. In order to make the results more biologically relevant, the bones could not have any internal elements that were greater than 10% of their length or width. Bones were constructed out of plaster of Paris, available at any home improvement or hobby store.

Fig. 1 Cylindrical bone construction techniques. A. Sand bed with shower rod cover pieces being prepared for filling with plaster. B. Sand bed with shower rod cover pieces with straws added to produce hollow cylinders. The straws are removed after the plaster cures.

To limit students from simply coating a mass of foreign objects in plaster, we dictated that the bone must be greater than 50% plaster by volume. Aside from this and the size rules, we left it up to the students' imagination to apply the biological concepts and examples covered in class to their designs.

Depending on the desired shape, bones could be molded either in tubes or in sand molds. For the former, a plastic shower rod cover was cut to length and taped shut along its length. These tubes were then plugged at one end with modeling clay and held vertically in a bed of moist sand where they could be filled and allowed to cure (Figure 1A). As long as the inside of the tube was smooth no release agent was needed. To produce hollow cylinders, large greased straws were inserted into the molds before the plaster was added (Figure 1B). The straws were removed after the plaster cured to leave a hollow cavity running the length of the cylinder. To produce more complex shapes, plastic storage containers were filled with wetted sand into which depressions could be made. These molds were then lined with plastic wrap to keep the plaster in the mold and aid in the release of the bones after curing. This allowed layers of the bones to be poured at different times or with different "additives" in different places. For both methods, bones were allowed to cure overnight, after which

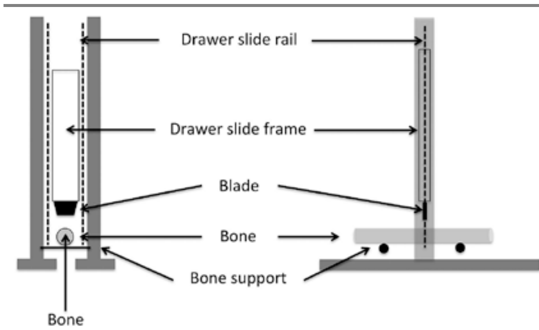

Fig. 2. Schematic of the guillotine used to load the bones.

they were removed from their molds and allowed to air dry for at least a day. The laboratory exercise took place over a three-week period, allowing students to build and test a number of prototypes before settling on their final design.

Bones were tested under both static and dynamic loading, recreating a crushing force from a claw or jaw and an impact from a strike, respectively. Static loading can be difficult as the bones are quite strong. The problem was solved by using a metal guillotine (Figure 2) onto which a container was hung and slowly filled with water from a second spigoted container (Figure 3). This allowed for large masses to

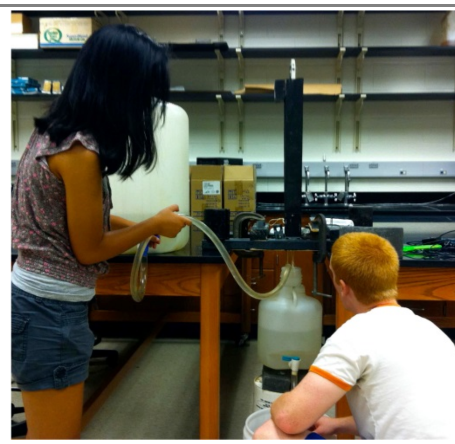

Fig. 3. Students using the guillotine with three reservoirs for filling, weighing and measuring the water mass.

be applied to the bones (students are often surprised how heavy water is) and easy measurement of the load. After the bone failed, the water was drained into a third receptacle and the mass determined. Using this method a bone could be tested and the load determined in about a minute. Dynamic loading was accomplished using the same guillotine, dropping its blade from increasing heights. Testing of the blade's impact in clay blocks showed that the depth of the indentation, and therefore the forces produced, were relatively consistent between replicate drops and at different heights (RMS values of 0.17, 0.13 and 0.17 for heights of 10, 20 and 30 cm respectively).

Bones were scored according to the following formulae: Static Score = maximum mass supported/mass per unit length of the bone, and Dynamic Score = maximum height from which the mass was dropped/mass per unit length of the bone. This made it possible to compare across designs regardless of size and shape. Larger, heavier bones might resist more force, but they would be penalized for their increased mass. Though the force exerted on the bones during static loading was easy to quantify using the formula $F=ma$ with m being the loading mass and a the acceleration due to gravity, determining the force exerted by dropping the mass onto the bone was less straightforward. The time it takes for the falling mass to come to rest determines the force exerted per unit time on the bone. This would vary from bone to bone, and within bones between heights. As we could not measure this parameter, we could not determine the forces exerted during the dynamic testing and therefore could not make direct comparisons between the two scores.

Students in the course came up with a range of designs that changed cross sectional shape, types of material mixed into the plaster, moisture levels of the plaster mix and even the curve of the bone along its length. Time constraints prevented us from testing more than three replicates of any given design, limiting statistical power in analyzing their results. We therefore present additional data for six designs which represent modification of both the shape and material composition: 1) round solid, 2) round hollow, 3) round small cylinder with the same mass as the round hollow, 4) round hollow filled with “spongy bone” (plaster mixed with 10mg of powdered bicarbonate for every 250 ml of water), 5) ellipse tested along its longer axis and 6) ellipse tested along its smaller axis. Ten replicate bones were tested under both static and dynamic loading.

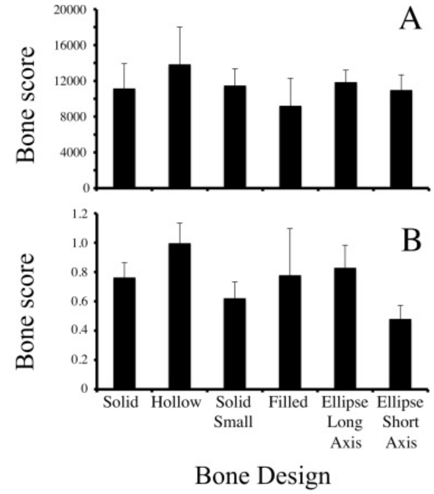

Fig. 4. Bone Scores (average ± standard deviation) for both static (A) and dynamic (B) loading of all six designs. Note that bone scores are not directly comparable between testing schemes as static loading used maximum mass before brekaing and dynamic loading used maximum height before breaking.

RESULTS

Though replicates were very consistent in their mass/unit length within treatments (RMS 4-13% average 8%) there was considerable variation in the force they could withstand (Table 1). Therefore, bone scores within each design under both testing schemes varied more (RMS 13-33% average 22%, Figure 4). Even with this variation, significant differences (one-way ANOVA) in three out of four comparisons of interest were seen under the dynamic loading, and in two out of the four under static loading, though hollow vs. filled results were the opposite of expectations (Table 1).

DISCUSSION

This lab was the most popular exercise of the semester. Without any pressure from the instructors, students spent a significant amount of time planning, building and testing their bones both in and out of scheduled laboratory times. Though it was a fun

Table 1. Results of testing for all treatments under both static and dynamic loading. Score was calculated as loading (the maximum mass before breakage under static testing or the maximum height under dynamic testing)/the mass per unit length of the bone. Averages and standard deviation for all parameters are provided in addition to the results of one-way ANOVA's for the four comparisons of interest.

Design	Average Score	S.D.	Average mass (g/cm)	S.D.	Maximum Loading (g or height in cm)	S.D.	ANOVA
Static							
Solid	11164	2754	4.5	0.4	37869	14122	df = 1.19 F = 11.9 p < 0.01
Hollow	13842	4166	2.4	0.3	34059	11521	
Hollow	13842	4166	2.4	0.3	34059	11521	df = 1.19 F = 6.7 p = 0.02
Filled	9206	3084	4.1	0.4	40489	11455	
Hollow	13842	4166	2.4	0.3	34059	11521	df = 1.19 F = 2.7 p = 0.12
Solid Small	11480	1876	3.2	0.1	36472	6502	
Ellipse Along Long Axis	11861	1345	3.8	0.2	44892	1345	df = 1.19 F = 1.69 p = 0.21
Ellipse Along Short Axis	10969	1709	3.6	0.2	10969	7022	
Dynamic							
Solid	0.76	0.10	4.6	0.4	3.4	0.5	df = 1.19 F = 20.4 p < 0.01
Hollow	1.00	0.14	2.8	0.4	2.8	0.6	
Hollow	1.00	0.14	2.8	0.4	2.8	0.6	df = 1.19 F = 4.1 p = 0.06
Filled	0.78	0.32	4.0	0.5	3.2	1.6	
Hollow	1.00	0.14	2.8	0.4	2.8	0.6	df = 1.19 F = 46.7 p < 0.01
Solid Small	0.62	0.11	2.9	0.2	1.8	0.3	
Ellipse Along Long Axis	0.83	0.15	3.8	0.2	3.1	0.5	df = 1.19 F = 36.1 p < 0.01
Ellipse Along Short Axis	0.50	0.09	3.6	0.2	1.7	0.3	

exercise, to keep the students centered on the concepts we wished to emphasize, each bone entered into the competition was accompanied by a one-page explanation of the design, construction and the biological examples it was based on. The students' writing showed that they had a firm grasp on the adaptations we discussed in class and that they understood their purpose as well as the mechanisms by which they worked.

Student designs tended to be quite imaginative adding various glues, reinforcing elements (e.g. mesh, fibers), and mass-saving additives (e.g. foam, pearlite). The limitations of the students' resources and experience lead to crude approximations of natural structures, and though most of those designs were unsuccessful, we do not consider that a weakness of the exercise. We encouraged students to try for relatively complex designs both to keep their interest and to illustrate a specific point, namely that though the concepts may be fairly straightforward, (e.g. a mix of different materials can make the structure stronger), the implementation of that concept is exceedingly difficult. Students came away from even the worst failures with a better appreciation for both the ability of biological systems to produce marvelously engineered structures and the remarkable evolutionary processes that have led to those abilities.

For this manuscript the authors chose designs that illustrate some of the basic characteristics of solid biological elements adapted to withstand large forces. The first of these strategies is placing much of an element's mass as far from the central axis as possible, thus increasing its area moment of inertia. The significantly higher scores for the hollow design illustrate the efficacy of this design. Though the solid rod held more mass under static loading than the hollow rod of equal diameter (Table 1), its extra mass did not add enough to its strength to make it energetically efficient and thus it produced a lower score. Similarly hollow bones performed much better than solid bones of similar mass. The failure of the filled bones, which were designed to mimic the pairing of dense cortical and spongy trabecular bone, was a surprise. This may have been due to the necessity of adding the spongy plaster mixture after the hollow cylinder had been produced and dried. The introduction of a considerable amount of moisture to the outer cylinder as well as the stress placed on its walls by the expanding spongy plaster may have weakened the final combined product even after allowing it to re-dry.

A second comparison that we set out to illustrate was differential growth (in terms of length of axes) in response to anisotropic forces. Though the ellipses did not perform differently under static loading, there was a large, statistically significant difference when ellipses were tested against impact along their long and short axis. As expected, larger forces could be withstood if delivered against the long axis of the ellipse. This strategy can be seen in the shape of long bones, such as the ulna or femur, which adapt over time to resist stresses along a specific axis. The difference in responses between the two loading schemes may reflect the different failure pathways initiated under each type of loading. Static loading causes failure through bending of the beam and an inability of the structure to withstand compressive forces along the top, tensile forces along the bottom, or shear forces along the cross-section. Dynamic loading, however, would most likely cause fracture (and therefore failure) through alternative scenarios, the specifics of which are beyond the scope of this manuscript, and likely most biomechanics courses aimed at biologists. The lack of a significant difference between the two ellipse orientations under static loading is probably due a combination of the differences in their second moment of inertia being small, the inherent between-bone variability, and the sensitivity of the testing apparatus.

Though trends did exist in many of the non-statistically significant comparisons, the presence of large variances themselves provided a teaching moment. Variation between the strength of replicate bones is a useful example of how small changes in construction techniques, materials, or moisture levels can make a large difference in the properties and success of the final product. This variability, which was more pronounced with the students' bones compared to ours, provides a perfect opportunity to discuss some of the basic concepts of evolution (e.g. variation, differential fitness) which can be particularly useful if non-biologists are part of the student body since they may not think about this important topic as much as biology majors.

Though this relatively simple exercise was very successful, more complicated variations could introduce further "trade-offs" that biological systems often face. In the current version the major trade-off was between weight and strength, a common biological theme. However, there are other examples, such as the need to maintain a certain amount of flexibility or resilience as well as strength, or the ability to withstand forces along different axes. Such a two-part testing scheme would provide an opportunity to introduce further discussion and appreciation for the challenges faced by organisms using solid biological elements and the ingenious methods by which they respond to those challenges.

Overall, we feel that this exercise provides a number of opportunities for student learning. First, it provides students a chance to apply physical and engineering principles to a biological issue. Such interdisciplinary opportunities are rather rare, in our experience. Second, as students struggle to successfully apply these principles, they develop a greater appreciation for how well organisms are able to do so. Lastly, students in this exercise have the opportunity to work hands on, prototype, test, revise and otherwise go through the process used by science, engineering and other real-world applications of their education. While there are rules, the students are allowed to work towards their goal on their own, instead of following a set recipe. We feel that this kind of exercise is very important for students to experience and one that is all too rare in many curricula.

ACKNOWLEDGEMENTS

The authors wish to thank the Howard Hughes Medical Institute for their support in developing this course and laboratory exercise and the Saint Joseph's University Summer Scholars program for funding the students' research.

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Using RNAi in *C. elegans* to Demonstrate Gene Knockdown Phenotypes in the Undergraduate Biology Lab Setting

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Abstract: RNA interference (RNAi) is a powerful technology used to knock down genes in basic research and medicine. In 2006 RNAi technology using *Caenorhabditis elegans* (*C. elegans*) was awarded the Nobel Prize in medicine and thus students graduating in the biological sciences should have experience with this technology. However, students struggle conceptually with the molecular biology behind the RNAi technology and find the technology difficult to grasp. To this end, we have provided a simple, streamlined and inexpensive RNAi procedure using *C. elegans* that can be adopted in upper level biology classes. By using an unknown RNAi-producing bacteria, students perform novel techniques, observe and determine which mystery gene was knocked down based on phenotype and experience a new research organism. By bringing this technology to the undergraduate lab bench, the gap between blackboard concept and proof of concept can be bridged.

Keywords: *C. elegans*, RNAi, gene knockdown

INTRODUCTION

During their tenure in the undergraduate science environment, science students should become familiar with experimental techniques and skills needed for their postgraduate careers. A frequent challenge for professors in the biological sciences is finding effective ways to ensure student engagement and provide students with the opportunity to experience original and valuable research techniques (Adams, 2009). The goal of any instructor should be to introduce new techniques and model organisms that relate to the real world science environment as well as enhance student engagement.

In 2006, the Nobel Prize in Medicine was awarded jointly to Andrew Z. Fire and Craig C. Mello for their discovery of RNA interference (RNAi), a gene silencing mechanism by double-stranded RNA. The number of scientific publications involving RNAi has jumped from zero in 1998 to over in 4500 in 2005. Currently there are over 13,200 publications referencing RNAi in the literature, reflecting the explosion of RNAi research and the importance of the technology. In 2002, *Science* Magazine named RNAi "technology of the year" and in 2003 *Forbes Magazine* called RNAi "Biotech's billion dollar breakthrough" (Adams, 2004).

RNAi technology spans both basic scientific research and medicine and is an important concept and technology that graduating students in the biological sciences should understand. For example, RNAi therapies have been used in the treatment of ocular diseases like macular degeneration, viral infections including hepatitis and HIV, cancers, inflammatory conditions and neurodegenerative diseases (Lares et al. 2010). However, the concept of how a small RNA molecule can elicit a radical gene knockdown phenotype is often difficult for students to conceptually understand.

Caenorhabditis elegans is a very popular model organism utilized in many fields of study. *C. elegans* is a simple organism amenable to studies in genetics and development, cell biology, neuroscience, evolution and ecology (Girard et al. 2007). The popularity of *C. elegans* rises from its genetic manipulability, fully described developmental life-cycle, fully sequenced genome, ease of maintenance, short and prolific life cycle and small body size (Leung et al., 2008)

This laboratory exercise provides a hands-on approach to demonstrating the Nobel Prize winning RNAi mechanism and introduces students to an important and popular research model, *C. elegans*. By bringing this technology to the undergraduate lab bench, we can bridge the gap between blackboard concept and proof of practice. Because RNAi is multi-disciplinary and crosses many scientific fields, this exercise is applicable to the undergraduate developmental biology, cell and molecular biology or biotechnology lab setting.

MATERIALS AND METHODS

Acquiring *C. elegans* strains

Wild-type *C. elegans* strains are available free of charge to educational institutions through the *Caenorhabditis* Genetics Center (CGC) at the University of Minnesota, St.Paul, by emailing a request describing the requested strain and a brief statement of intended use. Requests can be emailed to cgc@umn.edu. Strains of their choice are sent on NGM petri plates seeded with *E. coli* OP50 as feeding bacteria.

Preparing *E. coli* OP50 food source

C. elegans utilizes *E. coli* OP50 as a food source when the *E. coli* is spread as a lawn on culture plates. A starter *E. coli* OP50 culture can also be obtained through CGC. A starter culture is prepared by aseptically transferring a single colony from the streak plate into 250ml sterile Luria Broth (10g Bacto-tryptone, 5 g Bacto-yeast, 5g NaCl, H_20 to 1-liter, pH to 7.0 using 1M NaOH). The innoculated cultures grow overnight at 37°C after which the bacteria can be used to seed NGM plates. The liquid culture is stored at 4°C and is stable for several months (Stiernagle, 2006).

Preparing NGM petri plates

Standard Falcon 60mm petri plates are used to maintain *C. elegans*. Nematode Growth Medium (NGM) agar is prepared by mixing 3g NaCl, 17g agar, 2.5g peptone and 975ml H_20 in a 2-liter flask which is autoclaved for 50 minutes. After the flask cools to 55°C in a water bath, 1ml 1M $CaCl_2$, 1ml 5mg/ml cholesterol dissolved in ethanol, 1ml 1M $MgS0_4$ and 25ml 1M KPO_4 are added and mixed. Using sterile technique, warm NGM mixture is poured into the 60mm petri plates until they are 2/3 full. The plates sit for 2-3 days at room temperature before they are seeded to allow moisture to evaporate and to detect contamination (Stiernagle, 2006).

Seeding NGM Plates

A volume of 50 µl of OP50 bacterial culture is aseptically transferred to the center of an NMG plate on a flat surface. The bacterial lawn will grow overnight at room temperature. The seeded plates are stored on a countertop in a sealed air-tight container for 2-3 weeks (Stiernagle, 2006).

Maintaining Worm Cultures

The worms are maintained by transferring a chunk of NGM agar every three days to a new seeded bacterial plate. This is performed using a sterilized spatula to cut a 0.5cm x 0.5cm piece of agar with worms from an old plate and flipping the chunk over and placing it near the bacterial lawn of a fresh seeded plate. The worms will crawl out from under the chunk and feed on the new lawn (Stiernagle, 2006). Worms are best maintained at 20°C in a humidified incubator, but development can be accelerated at 25°C. Cross usage of the incubator with other lab species is acceptable. Old plates should be autoclaved to kill any biohazardous material prior to disposal.

Preparing RNAi bacteria

RNAi bacterial libraries can be purchased at Source BioScience LifeSciences ($15,500) or from Thermo Scientific **(***C. elegans* ORF-RNAi library Comprehensive coverage for RNAi screening). However, purchasing libraries can be costly for a small undergraduate lab budget. Although buying a whole library (19,762 clones) provides the ability to knock down any gene, this is not necessary for the confines of this undergraduate lab experiment which only requires five to ten strains. Contacting *C. elegans* labs and asking if they are willing to send a few RNAi bacterial strains is less costly. Most labs are willing to donate a few RNAi bacterial clones for undergraduate student use. The RNAi bacterial cultures are prepared in 5ml LB with 50µg/ml ampicillin or 10µg/ml tetracycline and 25µg/ml carbenicillin depending on the antibiotic resistance genes present in the plasmid (plasmid maps are provided by the donating lab). The cultures are grown overnight in a shaking incubator at 37°C.

Preparing RNAi/NGM plates

Standard NGM plates are prepared as described above with the addition of 25µg/ml carbenicillin and 1mM IPTG to the NGM agar mix prior to pouring plates. The plates must be poured 4 days before being seeding with RNAi bacteria to allow time for them to dry, but covers should remain on during this time. If the plates are wet, the RNAi phenotype will not be as strong (Stiernagle, 2006). Due to addition of IPTG, plates must be stored in the dark. The plates must be stored at 4°C. After the plates have dried, 50µl of overnight RNAi bacterial culture is spread and left to dry for 2-3 days in the dark at room temperature.

Preparing *C. elegans* transfer tool

The transfer tools are prepared using Fisher brand 5 ¾ inch glass pipets. Platinum wire (99.95%), 0.05% iridium, 0.01-inch diameter, 30G can be purchased at Tritech Research (PT-9901, www.TritechResearch.com). The transfer tools are prepared by cutting a 1-inch piece of platinum wire and flattening one end with standard 5-inch flat-nose pliers. The wire is bent into an S-shape and the non-flattened end is placed into the open end of the glass pipet. The wire is set into place by melting the glass under a flame.

Worms are transferred using bacteria as a sticky source. The bacteria stick to the flat surface of the transfer tool when it is touched to the seeded lawn. The worms are "lifted" as they stick to the OP50 bacteria and deposited to a new OP50 lawn for

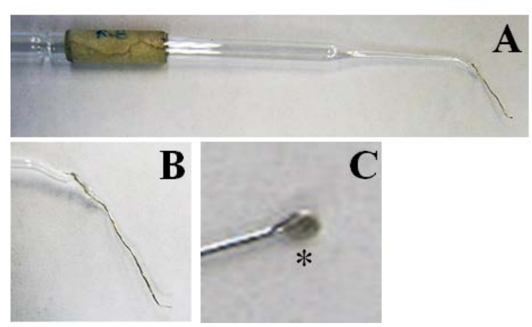

Fig.1. A. Full length glass picking tool. B. Close up view of platinum wire inserted and melted into glass pipet tip. C. Close up view of flattened end used for worm lifting. Asterisks show lifting surface in side or front views

continual culture. The wire tool should be flamed between transfers. Transfers are performed under a dissection microscope.

Microscopy

The specimens are observed under a Leica Zoom 2000 stereomicroscope with 10x ocular eye pieces and a zoom magnification range from 10.5x to 45x with both reflected and transmitted light illumination.

Transferring worms to RNAi plate

Using the transfer tool two or three L2 to L3 stage worms are moved to an unseeded NGM plate and given 30 minutes to allow the worms to wiggle off excess OP50 bacteria. *C. elegans* prefer to eat OP50 bacteria to RNAi bacteria and excess OP50 will cause a weaker RNAi phenotype. Using the transfer tool, the RNAi bacteria are scooped up from an RNAi seeded plate. Using the transfer tool with the RNAi bacteria stuck to it, the worms are picked up from the unseeded plate. The worms are transferred onto the RNAi seeded plate. The plates should be kept in the dark on a countertop. The phenotypes can be scored 3 days later. The worms are staged using a standard staging reference (http://www.wormatlas.org/).

Student Exercise

The exercise began with student groups growing a control and an unknown RNAi bacterial culture and treating the L2 to L3 staged *C. elegans* worms. The project was intended to supplement a lecture on the molecular mechanisms of RNAi, provide a hands-on activity using RNAi and *C. elegans* and demonstrate the technology. The goal of the lab was for student groups to determine what gene the unknown RNAi knocked down based on the phenotype of their progeny.

Students first observed wild-type worms using a stereomicroscope. These observations introduced the students to the basic worm morphology, life stages of the worms, use of the stereomicroscope and the transfer tool. After students became competent, they were able to determine which worms were suitable to pick for RNAi treatments (L2 to L3 as referenced in the standard staging series). Students fed L2 to L3 staged worms with the unknown RNAi-producing bacteria as well as with a control (empty vector) bacteria. After 3 days students scored the phenotype of the progeny.

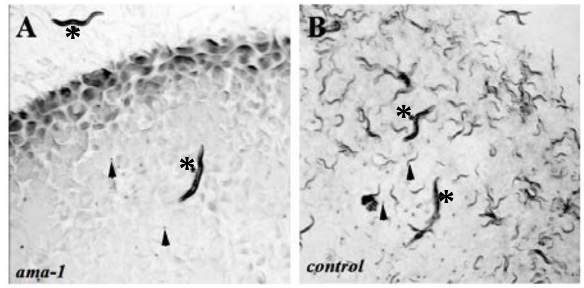

Fig.2. A. RNAi knockdown of *ama-1* gene resulting in dead eggs due to knockdown of RNA polymerase II. Dead eggs are noted with arrow. Asterisks denote original parent worms. B. RNAi knockdown using control empty vector. Live eggs produced by parental worms (shown by asterisks) grow and multiply. Arrows show live eggs and larva

Student Assignment: Lab Day 1

1. Draw and anatomically describe the wild-type worms on your petri dish. Note and describe the differences among all worm stages from gastrula, L1, L2, L3, L4, young adult and adult. Select three worms at the L2 or L3 stage and transfer them to an unseeded plate. What were the key anatomical structures that defined the L2 or L3 stage worm?
2. Transfer the worms from the unseeded plate to the RNAi plate and also to a control plate. Why must an unseeded plate be used? What are you trying to avoid in your transfer process and why? How would it affect your results?

Student Assignment: Lab Day 2 (3 days post-treatment)

1. Obtain your RNAi treated and control worms. Describe what you see. How many worms are now on the plates?
2. Describe and draw any phenotypic changes you see in the progeny. Count the number of progeny that display a phenotype and rank the phenotypes along a scale from 0 to 3; 3 demonstrating a strong phenotype and 0

Table 1. Genes knocked down using RNAi technology.

Gene	Normal Function	Knockdown Phenotype	Reference
bli-4	Post-embryonic cuticle development and stability	Fluid filled blisters	Page, 2007
unc-22	Regulates actomyosin contraction-relaxation cycles	Uncoordinated head muscle twitching	Edgley, 2006
dpy-5	Cuticle procollagen necessary for body length	Abnormal cuticle, shortened football shaped body	Page, 2007
lon-2	Negative regulator of growth factor signaling/regulates body length	Elongation of body length	Page, 2007
ama-1	Encodes large subunit of RNA polymerase II required for mrna transcription	The laid eggs never hatch	Blackwell, 2006
rol-6	Cuticle collagen necessary for cuticle morphology	Rolls around, horseshoe shaped	Page, 2007

demonstrating no phenotype (a wild type worm). You will define intermittent phenotypes graded 1 and 2 from your observations. From these numbers, calculate the total number of progeny that display a phenotype. Why do you think some worms do not demonstrate a knockdown phenotype and appear wild-type? Why do some show a stronger phenotype than others?

3. Monitor the worms' behavior, but do not touch them. Do you see any behavioral differences in your RNAi treated worms? Touch your worms gently with your tool. Describe their behavior. Are they different than control worms and, if so, how?

4. Based on your progeny phenotype, speculate on what gene/pathway you might have knocked down with your RNAi treatments and explain why.

5. Based on your progeny phenotype, log on to www.wormbase.org and determine which gene you knocked down. Were you surprised? Did this fit with your speculated pathway above?

RESULTS

Genes that were knocked down in this experiment included *bli-4, unc-22, rol-6, lon-2, dpy-5* and *ama-1*. Table 1 lists the gene wild-type product and description of the knockdown phenotype. As an example, Figure 2 demonstrates representative phenotypes for the RNAi knockdown of *ama-1* from student treatments. Adult worms shown by asterisk are approximately 1mm in length. Students were given a list of possible loci their RNAi might target. From the observed phenotypes, students had to determine which mystery gene they knocked down.

Laboratory Evaluation

A pre-exercise survey/quiz determined that only 20% of 15 students had heard of RNAi, and only 13% knew what RNAi could be used for. Sixty seven percent of students had heard of *C. elegans*, but only 27% knew why it was used as a research model (Figure 3).

Additionally, student enjoyment of the project was surveyed. Sixty seven percent of students reported they extremely enjoyed working on a technology that won the Nobel Prize and 33% reported they strongly enjoyed it.

DISCUSSION

For many students, the mechanism of RNAi may seem overwhelmingly complex and confusing initially. While explaining a methodology in a classroom is an essential first start, laboratory exercises provide a stronger and hands-on method for teaching methodology and concept. Many undergraduate biology majors will be pursuing careers in research, academia or medicine and thus introducing RNAi at the college level is essential. Furthermore, introducing a novel organism like *C. elegans* at the undergraduate level further engages students in scientific inquiry.

Our data demonstrate that junior and senior level biology students had very little familiarity with the *C. elegans* model or the RNAi technology at the beginning of the project, despite its relevance in the real world science environment. This demonstrates the need for such an experiment in the undergraduate setting. When students considered how well they liked working on this project, 67% of students reported that they extremely enjoyed working on this project and 33% reported they enjoyed this project a great deal. Students were very excited and even shocked to see the phenotypes that they created! Seeing how knocking down one gene could lead to such dramatic phenotypes was exciting and emphasized the process they learned about in lecture. Although explaining and drawing out the molecular mechanisms of RNAi in a lecture setting is important for students, performing the experiment and generating a mutant phenotype provides them hands-on proof. Having a tangible product of a molecular mechanism occurring is a helpful educational tool. Additionally, having student groups use different examples of RNAi enhanced the exercise as students were able to see other group's phenotypes. At the end of the project, each student group presented their data in oral presentation format to the class in a mock scientific conference format. Student enjoyment is an

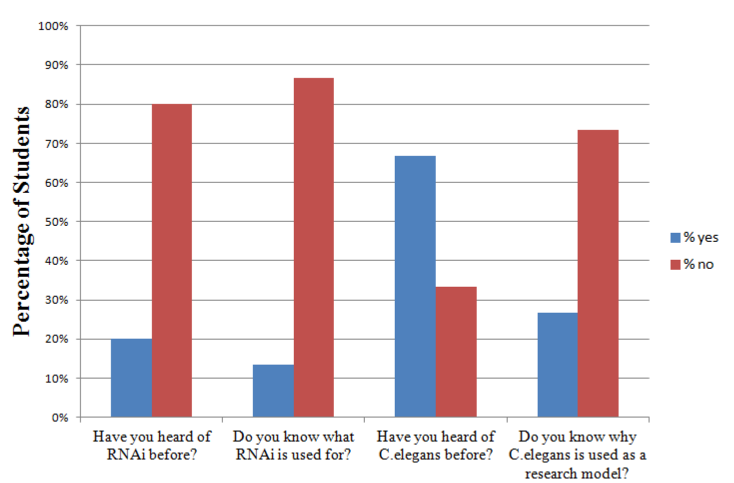

Fig. 3. Pre-exercise survey determining student familiarity with RNAi.

important factor when designing or planning lab exercises. If the exercise does not stimulate student interest or spark curiosity, then students are not invested in the project and will get less out of it (Adams, 2009).

Although the lab was successful, students did encounter problems with the laboratory exercise. Initially, transferring worms from one plate to another was a challenge. If the transfer tool does not have a well-flattened surface, the bacteria and worms tend not to stick well. Additionally, students had difficulty with microscope depth of field and struggled to either find the worms for lifting or place them gently on the transfer plate. Students tended to gouge the agar plate with their tool, creating holes where the worms would nest. Furthermore, the RNAi bacteria are not as "sticky" as OP50 bacteria. Thus, several practice rounds of worm transfers were necessary before performing the RNAi exercise and students were encouraged to make multiple transfer tools. Furthermore, the students' first round of phenotypes were rather weak due to residual OP50 bacteria that were transferred. The worms prefer the OP50 bacteria over the RNAi-producing bacteria and as such did not eat the RNAi bacteria resulting in a weaker phenotype. For the second round of treatments, an additional transfer to another unseeded plate was incorporated to allow the worms to wiggle off excess OP50.

Demonstrated here is a simple RNAi exercise using the *C. elegans* model organism amenable to the undergraduate lab setting. The experiment can be performed in one week or two, depending on how many replicates the instructor wishes to perform and can easily fit into a tightly scheduled undergraduate lab calendar. In this exercise, students learned about *C. elegans* as a research model organism, the life cycle of the worms and the practicality of using worms in research. Furthermore, the lab takes a complex molecular mechanism like RNAi and demonstrates proof of concept. Students enjoyed the hands-on approach of working with *C. elegans* and were excited about the mutant phenotypes they generated utilizing RNAi technology.

Lastly, the lab module could be followed up with a truly investigative open-ended research project. For example, students could select pairs of genes to knock down. Of particular interest could be selection of genes which are described to have no individual knock down phenotype, for example *sin-3* with *tbx-34* or *set-31* with *scrm-6*. Perhaps the dual gene knock down would yield an interesting result. These experiments would bridge the gap from introductory "skill building" lab activities to truly investigative scientific research.

ACKNOWLEDGEMENTS

I would like to thank the Hammarlund lab at Yale University for donating wild type worms and HT115 bacteria carrying the desired plasmids. Additionally, I would like to thank Rachid El Bejjani for all his assistance with this project.

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Promoting Science Literacy through an Interdisciplinary Approach

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Abstract: Recognition of the value of a scientifically literate citizenry has driven American science education reform since the 1950s. We have seen some improvement in the comprehension of science facts in the past 10-20 years, but far less improvement in Americans' understanding of the nature of science. College science courses are ideal venues for promoting science literacy. However, in an effort to condense a complicated subject into a single semester, the nature of science is often lost amidst the facts presented in a freshman survey course, often the entirety of a non-science major's experience in science. We argue that an interdisciplinary approach that integrates the sciences and the humanities can attract non-science majors, increasing these students' exposure to scientific concepts by relating them to students' existing interests and knowledge. This fosters science literacy by teaching students that science is a process of human inquiry with a distinct methodology, instead of simply a litany of facts. We recommend that a successful interdisciplinary course should present an engaging topic with which students can identify, incorporate opportunities for student research, and offer site visits to working laboratories.

Key words: science literacy, education reform, interdisciplinary teaching, chronobiology, history

INTRODUCTION

Recognition of the value of a scientifically literate citizenry has driven American science education reform and standards since the end of World War II. The relevance of science and technology as demonstrated in the Cold War was dramatically underscored in 1957 when the Soviet Union launched the world's first orbiting satellite, Sputnik 1, and it is no coincidence that the term "science literacy" first appeared in print the following year (DeBoer, 2000). However, the precise meaning of science literacy is not always clear. It is often loosely defined as a basic understanding of the nature of science. The ability to comprehend science journalism as represented in the *New York Times* is frequently cited as evidence of science literacy. Numerous authors have noted, however, that this definition is imprecise and elastic. Jon Miller (2004), Director of the International Center for the advancement of Science Literacy, argues that the *New York Times* standard is sufficient, while others have advocated for a spectrum of literacies that range from the average citizen to the scientist or policy expert (Bybee, 2010). In contrast, George DeBoer (2000), Deputy Director for Project 2061 of the American Association for the Advancement of Science, claims that the imprecision of the term is itself an asset. Because there are multiple paths to science literacy, argues DeBoer, the goal of educators should be to introduce students to the "world of science so they may pursue it throughout their lifetimes."

On the other hand, our inability to reach a consensus on the meaning of scientific literacy raises serious questions, for how do we measure and assess scientific literacy if we cannot readily define it? According to Miller (2004, 2010), approximately one in four of American adults currently possesses science literacy. This figure is based on surveys by University of Michigan researchers and the National Science Foundation (NSF) which assess factual knowledge (e.g., is an electron bigger or smaller than an atom) as well as a basic understanding of scientific inquiry represented by rudimentary probability questions and comparisons of experimental designs (NSF, 2010). American adults scored considerably higher on the fact-based questions than on questions intended to test their understanding of scientific inquiry. When asked to use their understanding of science to answer more conceptual questions, few Americans were able to do so. Since science and technology form the underpinning of our economy, medical system, communications, and entertainment, science literacy touches the lives of everyone. Society must be able to understand science in broad terms and provide constructive criticism and meaningful social oversight of the scientific and technical establishment.

For our purposes, we wanted our students to understand science as a process of inquiry which we defined to include the basic scientific method, how research questions are developed, the role of technology in scientific ideas and research, and an understanding of the implications of science in students' lives as citizens, consumers, and –hopefully–lifelong learners. We also sought to demystify how scientific knowledge is created or tested by exposing students to working laboratories and scientists.

Although college biology courses are an obvious venue for promoting science literacy, introductory courses are often taught as a deadening litany of facts that describe the natural world without significantly aiding students' understanding of science as a process of inquiry with a distinct methodology. Compounding this problem, most students complete their science courses as freshmen. Many math- and science-phobic students avoid additional coursework in biology or other sciences. This is especially unfortunate since the number of science courses taken in college is the strongest predictor of scientific literacy in American adults (Miller, 2004). On the other hand, the United States is fairly unique in requiring any science courses at all in college; nowhere else do colleges and universities require science courses for non-science majors (Miller, 2002). This may help to explain why the US ranks slightly above most Western European nations and Japan in science literacy (Scearce, 2007).

To attract a broader range of students, and to increase non-science majors' exposure to science, we recommend an interdisciplinary approach that integrates science and the humanities. To this end, we designed a sophomore-level seminar titled "Body Clocks: How Nature Tells Time" to investigate the biology, psychology, and history of chronobiology (e.g., biorhythms). In this paper we argue that an interdisciplinary approach that includes a humanities field is key to increasing non-science majors' involvement in science education and effectively enhancing students' understanding of the nature of science. We fostered science literacy by blending traditional lectures, class discussions, hands-on experiments, site visits to clinics and laboratories, and student research. Participatory learning, i.e., learning by doing, was an integral component of our course.

COURSE DESCRIPTION AND MECHANICS

Troy University offers an interdisciplinary course housed in the honors program in which two or three professors from different disciplines teach a seminar on a topic of their choosing. Our course, "Body Clocks," combined professors from biology, psychology, and history. Our central topic, chronobiology, was chosen as part of a National Science Foundation grant on the history of chronobiology. This history offers case studies which illuminate the nature of science, and by integrating history with biology and psychology, the interdisciplinary approach offered science and non-science students a way to investigate chronobiology as both a body of knowledge and as an intellectual endeavor–in other words, both the facts of chronobiology and the nature of science.

The semester was divided into four units: chronobiology as it relates to sleep, performance, health, and evolution. Throughout the semester we employed a variety of strategies to engage students with different learning styles, such as traditional lectures, class discussions, hands-on experiments, site visits to laboratories, demonstrations, and student research and presentations. Student assessment consisted of class participation, unit exams, and group research projects. As discussed below, the research projects were especially important because they involved students in all stages of scientific research. In the course of preparing for their projects, students completed the University's Institutional Review Board (IRB) certification and submitted their research proposals for formal IRB approval. Their experience with the IRB greatly enhanced their understanding of science as a process that often involves human experimentation, and it required students to consider the possible consequences of their research.

There are two approaches to teaching an interdisciplinary course involving two or more professors. In the first, the course is segmented by specialties–biology, psychology, and history in our case–to accommodate each professor's portion of the course with the individual fields covered serially. In the second, faculty integrate their material to create a cohesive course. We chose the second approach. Although initially we divided the responsibility for each class period into halves or thirds, we quickly learned that to accommodate spontaneous class discussions, it was better to have one professor lead on any given day. Each professor contributed several lectures for each unit, and we tried to provide bridges among our three fields as much as possible. Accordingly, each professor attended all lectures, and we frequently took advantage of questions or tangents that came up in class to interject our own expertise. We believe that a truly interdisciplinary approach requires that professors and students find the common ground in order to make the connections across disciplines. Our focus on science as a process held the topics together. Each unit considered fundamental questions about how scientists develop questions, test hypotheses, and draw conclusions. To do this effectively, good communication in the form of weekly faculty meetings was essential. Each week we discussed what had worked during the previous week, what did not, and how we would integrate our topics in the upcoming weeks.

Combining the biological and psychological approaches to chronobiology allowed us to provide students a way of understanding the topic as a body of knowledge that is highly relevant to their lives and interests. The history of chronobiology set this knowledge in a broader context. For example, in our first unit on the chronobiology of sleep, our biologist, Dr. Cohen, lectured on the biology of circadian rhythms and the neurological phases of sleep. Students learned the basic anatomy of the brain and the role of the pineal gland and regulatory hormones and chemicals in the sleep cycle. Then, psychology professor Dr. Hooten discussed the actions of common sleep aids and the physical and mental effects of sleep deprivation. Students were able to relate their personal experiences to this more technical information, leading to a lively class discussion of strategies for the "all-nighter" and how academic performance is affected under these conditions. Lastly, historian of science Dr. Ross discussed how scientists' understanding of sleep changed over the course of the 20th century. The dominant paradigm was that sleep was essentially a passive state: in the words of 19th century surgeon Robert Macnish, sleep was the "the intermediate state between wakefulness and death" (Macnish, 1834). This persisted more or less into the early 20th century. However, with the application of the electroencephalogram (EEG) on humans in the 1920s, researchers had the ability to peer into the brains of sleeping subjects for the first time. Scientists then challenged long held assumptions and asked new questions. With the discovery of the rapid eye movement (REM) phase of sleep in the 1950s, sleep was redefined as a dynamic process that included bursts of brain activity correlated with dreaming. By linking these diverse topics, students were able to understand the biology of sleep, apply what they had learned to their own lives, and form ideas about how technology can shape scientific research.

The hands-on learning activities in class and site visits to laboratories were valuable additions to the course. On the first day of class students estimated the passage of a minute under different conditions: eyes closed, eyes open, or one hand held in cold water. Working in small groups, students timed one another's estimates and then collated the data as a class. Such an activity not only helped break the ice, it also dramatically introduced the concepts of how we perceive time and rudimentary experimental design. As became our habit, after completing the time experiment, we immediately discussed ways to improve it and the statistical significance of the collected data. In addition to other in-class activities, students visited a sleep clinic, which demonstrated how data on sleep disorders were collected and evaluated. During the chronobiology of performance unit, we were able to visit the Army Aviation research center at Fort Rucker in Enterprise, Alabama which tests pilot performance under various conditions, including sleep deprivation.

These activities and excursions accomplished more than reinforcing the material covered in lectures and readings. For the non-science majors in particular, the site visits were invaluable introductions to working laboratories and real scientists. Students met researchers, asked questions, viewed equipment, saw real-world examples of how circadian rhythms are studied and why, and discussed how such research is funded. They were able to encounter science as an active process and laboratories as sites of knowledge production, rather than science as simply a body of knowledge disconnected from human actors and buried in textbooks. This aided in their understanding of the nature of science and, therefore, the acquisition of science literacy.

Student research comprised about 20% of their course work and further conveyed the concept of science as a dynamic process. Students were divided into three groups of six to seven students and assigned a general topic. With help from one of the professors, each group then developed its research questions, collected and analyzed their data, and presented their findings in-class and publicly at a student psychology conference held annually at Troy University.

Students were deeply involved at each stage of research. For example, one group focused on the factors that affect the quality of sleep. Students brainstormed to design a sleep journal that all the students and professors involved in the course would keep for three weeks. The class debated what data should be collected, how to maximize compliance, and what demographic information to collect. After completing IRB training and certification, the students also grappled with the ethical concerns of their experiments. How would they protect the privacy of participants while still gathering the information they felt was important to their study? For instance, is it appropriate to ask "do you normally sleep alone or with someone else?" Students thought this was important information to have, but considering that the participants might feel uncomfortable answering or might share a bed with a range of partners, they reworded the question to read "Do you normally sleep alone? (no pets, kids, bed partners, etc…)" After finalizing the questions and demographic data to be collected (and receiving IRB approval), students and professors kept track of their sleep for three weeks. The sleep experiment group worked with their professor to evaluate the data statistically and form their conclusions about which factors most affected quality of sleep.

Student research, such as the sleep experiment, involved students in all stages of scientific inquiry: formulating the research questions and hypotheses; developing questionnaires or other research tools; collecting and analyzing data; and presenting their results. This practical experience aided their understanding of the nature of science in ways that more typical science instruction does not. For the non-science majors, this experience was unique in their science education. Very few of the students had ever given a presentation at a conference, and most commented on the value of this experience in a survey at the end of the semester. We found that these research projects greatly enhanced students' understanding of the scientific method and the process by which scientific knowledge is generated, analyzed, and shared.

ASSESSMENT

We treated the interdisciplinary course as a pilot study. Twenty-one students enrolled in the course from five majors: biology, psychology, history, English, and American Sign Language interpreter training. Eight of the 21 were non-science majors, nine of 21 were women, and all but two of 21 were upper-classmen. Because this was a relatively small sample size, our analysis of the course was necessarily more qualitative than quantitative, but we believe the students fairly represented a range of majors and interests. As mentioned above, the interdisciplinary seminar is housed in the University's honors program, but all students are eligible to register for the course. Due to the novel interdisciplinary nature of the course, the students who registered tended to be more intellectually curious and engaged in the material than those in a required general education science course. This worked to our advantage in generating discussions and classroom participation, but it also demonstrated that by combining science with the humanities we were able to attract non-science majors to what was largely a science course. By this approach, science and non-science majors were exposed to disciplines they normally would not explore.

We assessed our success in increasing our students' science literacy through their unit exams, class discussions, research, and anonymous end-of-course surveys. The exams were written by all three professors and included multiple choice, fill in the blank, short answer, and essays. Student discussions were evaluated based on participation and the quality of student questions and comments. Shared experiences, such as the field trips, hands on class activities, or personal study habits, provided a spring board for class discussions that could lead to deeper conversations about the science of chronobiology. The research projects also provided shared experiences and a basis for student participation, as well as end products, papers and presentations, that the faculty assessed. Student surveys consisted of nine questions concerning the strongest/weakest features of the course, exams, and the value of the site visits, research projects, and presentations. Nineteen of the 21 students submitted these surveys. Using these various assessment tools, we found that we were most effective in the following three areas.

Demonstrating interdisciplinary connections

When asked what the strongest feature of the course was, about one third of the students cited the interdisciplinary nature of the course. Others commented on the "diversity" of information as the strongest feature or noted its "interdisciplinary nature" and "different perspectives." This was supported by the in-class discussions during which students were able to draw on information provided from different lectures and readings to examine chronobiology and by their essays on the unit exams. We used our separate fields to examine science as a process of inquiry, emphasizing *how* the science of chronobiology developed alongside the *facts* of chronobiology. That the students clearly recognized these connections is indicative of their development of science literacy.

Expanding students' knowledge of the scientific method

On the surveys several students self-identified as non-science majors and commented that they developed a new understanding of how the process of science worked or even a new interest in science. One student commented that the course explained "the scientific process" in a new way and another that he or she "learned how to conduct an experiment properly and follow through with it." Students also commented on the value of the research projects: "I appreciated that we were given simple ways to learn the experiment process," "it broadened my education and caused me to approach things in a different way, including many fields," "it allowed the class to experience what it is actually like to perform a research experiment and present it to our peers," and "invaluable." Based on the survey comments, class discussions, and the completed research projects, we believe that students successfully learned about the scientific method and the role of experiments in knowledge production.

This was supported by their exam and project grades. Of the top five grades in the course, three were biology majors, one was from the school of education, and the other was a history major. This was hardly a large enough pool from which to draw significant conclusions, but, as a pilot study, it indicated that our approach is worth pursuing.

Explaining the transmission of scientific knowledge

We required students to present their findings to the class and at the conference as described above. Developing students' presentation skills was a secondary goal of the course which fared better than we had anticipated. We expected this would help students understand how scientific knowledge is debated and shared. However, the conference proved to aid their professional development significantly. Each group planned their paper presentation together and selected two representatives to read their papers at the conference. We used the in-class presentations as rehearsals and the conference as the final product of their research. The quality of the final presentations was very high and far exceeded our expectations. The students also fielded questions at the conference, where they were required to explain their experimental designs, or to defend their conclusions.

On the end-of-semester survey we asked students about their reactions to the conference and they were nearly unanimous that it was very valuable, even exciting. Students remarked that "I enjoyed presenting, it gave me a sense of accomplishment" and "I am not a great public speaker so I was a little nervous; however I was very excited to have the opportunity to present our research in a professional environment." Some students noted that they believed the presentations would improve their résumés and that they were planning on attending future conferences. Again, this advanced our goal of promoting science literacy by demonstrating how scientific knowledge is created and then transmitted via public presentations and debates.

DISCUSSION

In order to attract both science and non-science majors to an interdisciplinary course, we believe it is essential to select a topic that will engage students broadly and on a personal level. Sleep deprivation (the all-nighter) or other personal experiences proved useful for generating discussion and engaging students from all backgrounds. This tended to short-circuit the science-phobia of non-majors by focusing on a topic with which they could identify. Inclusion of the humanities makes the material more accessible to non-science majors and broadens the education of science students as well. In our case, the history of science provided every student with an understanding of science as a human endeavor–a process of human inquiry.

Student research is invaluable if the goal is science literacy. Ideally, students should be involved in each step: developing research questions and methods, collecting and analyzing data, and presenting their conclusions. Through student research, non-science majors in particular gained a much better understanding of the nature of science. Science majors gained considerable experience formulating their own research questions (rather than the prescribed experiments typically found in class labs) and especially benefitted from presenting their findings in a more formal environment.

If possible, visits to sites where research is conducted are beneficial. Although field trips can be time-consuming, they offer most non-science majors their first experience with experimental science outside of the freshman biology lab. They were able to see the real life applications of the concepts they were learning in class and the relevance of chronobiology outside of their lectures and readings.

The benefits to students are worth the investment of time required for interdisciplinary courses and greatly outweigh the costs. In schools where this kind of labor-intensive team teaching may not be possible, science faculty may wish to consider adding lectures, readings, or field trips that demonstrate science as a process or that discuss science from the perspective of the humanities. History of science is an obvious choice, but science fiction, films, or art can also engage students and help them explore the nature of science and its implications for society. Classics such as *The Island of Dr. Moreau* or *Frankenstein* are two examples that come to mind and can lead to discussions about students' concerns in the 21st century: bird flu, genetically modified foods, or global warming. Interdisciplinary teaching serves as a powerful way to expand students' understanding of science and draw in those students who would otherwise avoid further science education.

After more than 50 years, the goal of increasing science literacy is still an important one, and one that has slowly been bearing fruit in America (Miller, 2004). In this interdisciplinary course, we were able to capitalize on the expertise and skills of three professors from different fields and effectively expose non-science majors to what was largely a science course. The inclusion of a humanities field was key to attracting these students and also benefitted science majors. Through lectures, active learning, research projects, and site visits, students learned about the nature of science, and developed a sense of how science is actually practiced. Students employed the basic scientific method, learned how scientific knowledge is generated and debated, and gained an understanding of the implications of science in their lives.

ACKNOWLEDGEMENTS

The development of this course was supported by a grant from the National Science Foundation and the University of Minnesota's Program in the History of Medicine. In addition, we would like to thank Dr. Hal Fulmer and the Associate Provost's office for support of interdisciplinary education on the Troy campus and Dr. Jennifer Gunn of the University of Minnesota.

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PERSPECTIVES

Challenges and Opportunities for Learning Biology in Distance-Based Settings

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Abstract: The history of learning biology through distance education is documented. A review of terminology and unique problems associated with biology instruction is presented. Using published research and their own teaching experience, the authors present recommendations and best practices for managing biology in distance-based formats. They offer ideas on resources for content, laboratory activities, safety and interaction among class participants. The need for research on the efficacy of virtual labs and simulations in adult biology education is noted.

Key words: online biology instruction, online science instruction, web based teaching, distance education

INTRODUCTION

Distance-education and internet-based learning are no longer novel concepts, as demonstrated by exponential growth of such courses (Dobbs et al., 2009). In science this trend is slower (Kennepohl & Shaw, 2010). We suspect that biology teachers fall into three camps with regard to distance education: (1) those to whom the process is so routine that they may give our paper only a passing glance (2) those who are new enough to the process that they are actively seeking information, and (3) those who have a deeply entrenched opposition to the whole idea. Our writing is aimed at all three. In this paper, we will provide a review/discussion of distance learning issues which are particularly relevant to biology.

Both authors, instructors in a small but nationally ranked community college in the rural southeast, have taught online for more than six years. In addition to utilizing various online strategies over the past 10-15 years, we both developed and teach a fully online non-majors biology course. One author also teaches online education courses. The other teaches a course for biology majors, combining face-to-face and online teaching. In addition to formal objectives for this paper, we will offer commentary on teaching biology in alternative formats as we draw examples from our experiences.

A Short History of Distance Learning

The exact origins of instruction delivered by the internet are foggy. Many writers place such instruction under the more inclusive moniker of *distance-learning* and claim roots as far back as the early 1800s in Europe. Correspondence courses, delivered by post, may represent the beginning of our modern practices (Casey, 2008; Dobbs et al., 2009; Schlosser & Simonsom, 2010). In biology the great Anna Botsford Comstock is of note. Her *Handbook of Nature Study* (1911) began as a series of lessons for an at-home study package for teachers. In early years, some colleges combined on-campus intensive summer study with correspondence courses (Schlosser & Simonsom, 2010).

As technology advanced beyond the press and post so did distance education. Audio conferencing, then visual media, became utilized (Anderson, 2008). In homes, television brought students to the screen for enrichment courses or certificate programs (Dobbs et al., 2009). Respected universities began to offer fully distance-based degree programs at least as early as the 1960s (Schlosser & Simonsom, 2010). Today, the process of offering courses online is fostered by commercially available *learning platforms* such as eCollege, Blackboard and webCT which allow for organization of course materials and communication among participants (Landry, et al., (2008). A trend among universities requires students to complete some minimum number of hours in online courses before they are awarded a degree (Dobbs, Waid & del Carmen, 2009). Online classes are generally more flexible in time and space than their traditional counterparts (Anderson, 2008). Students with extensive family or work commitments remain the target group (Schlosser & Simonsom, 2010). They have fueled the market for alternative options in education. There is little doubt that distance instruction will continue to develop with emerging technology.

Problematic Terminology

So far we have used the terms *distance* and *internet* almost interchangeably. We argue that internet-based learning is one form of distance-education. An idea developed further in our paper is that *internet-based learning* may not take place exclusively by computer. So, we refer to our work as *distance-based teaching*. The United States Department of Educational Research notes that distance education is characterized by the separation of the learner from the source of instruction. Technology allows learners to get information and to interact (Anderson, 2008; Casey, 2008). Interpreted loosely, that technology may be mail delivery. The internet is the predominant technology today. So terms such as *electronic learning (e-learning), internet-based learning* and *web-based instruction* have found their way into our lexicon (Rivera & Rice, 2002; Anderson, 2008). The phrases *face-to-face instruction* or *seat-based classes,* often refer to instruction taking place in a traditional classroom or lab. *Web enhanced* or *hybrid* refers to a mixture of formats in which students meet in a classroom for some required number of hours per term and participate in online instruction for the balance of time (Rivera & Rice, 2002; Shea et al., 2006). Our school uses the term *web enhanced* a bit differently; the enhancement is seen as purely supplemental. Table 1 summarizes many of these commonly used terms.

Anderson (2008) offered a set of terms to distinguish the timing of instruction. If a student may access the course material at any time the delivery is *asynchronous*, even when deadlines or timeframes for assignments are specified. If a student is required to complete two lessons per week for sixteen weeks, the format is asynchronous if he were free to access content on Wednesday as easily as Friday or at 3:00 am as easily as 2:00 pm. *Synchronous* delivery involves access in real time. For example, all distance students may be required to access the course each Friday at 5:00 pm and participate for three hours in a discussion. Alternatively students may interact by remote television or webcam at a specified time. In short, activity occurs live; absences are treated the same as for a seat-based class. Dobbs et al., (2009) reported that students favored asynchronous delivery while Bernard, et al. (2009) noted that synchrony produced higher student achievement. Classes may combine both formats.

An additional set of terms (*interaction treatments*) describes how course participants access content and communicate. The essentials (as listed in Bernard et al., 2009) follow. *Student/teacher interaction* involves communication by telephone, e-mail or discussion forums. *Student/student interaction* may utilize discussion boards, group web pages, chat rooms, or student-created slide presentations (such as PowerPoint™). Anderson (2008) stressed its importance in building in a sense of community to a course. The presence of a community of learners (as opposed to a group of people, each learning in isolation) appears to be essential to student success in online courses (Shea et al., 2006; Anderson, 2008), though student/student interaction may place unwelcome constraints on some students even if required in an asynchronous format (Anderson, 2008). *Student/content interaction* may take several forms, including reading textbooks, visiting web sites, listening to lectures on sound files or watching laboratory videos. Table 2 provides examples of course components with a checklist of which interaction treatments they may satisfy.

Why the Reluctance?

As noted, science is not taught as often in a distance-based setting (Kennepohl & Shaw, 2010) perhaps due to concerns specific to science teaching and general distress about online instruction. Casey (2008) reported that distance education is viewed

Table 1. Terminology related to distance learning with examples of class activities.

Generally Synonymous Terms	Definition	Examples
Traditional class Face to face class Seat based class	Students and teacher meet exclusively to almost exclusively in an archetypal classroom or classic laboratory setting	A typical freshman level biology class with lecture and lab. An environmental biology class with two field trips.
Hybrid class Web enhanced class	Students and teacher have required face to face meetings in addition to interacting by way of the internet to complete the mandatory number of class hours.	Physiology students go to campus once per week to complete a lab. Assignments and discussions are done by way of the internet. Graduate students work independently on botany field projects. They communicate in chat rooms but come to campus monthly for poster presentations and guest speakers.
Web based class Internet class Online class Networked class Electronic class (E-class)	Students complete all (or nearly all) course requirements at a distance, mostly using the internet for delivery and interaction.	Freshmen complete discussions and hand in assignments using a course delivery platform. They do lab at home using a kit they purchased. They visit campus (or an approved proctor) to take the required final exam. Genetics students view digitized lectures twice weekly. They use the class web site to post questions. The class takes three exams by way of a testing feature built into the course delivery platform.

Table 2. Possible elements for distance based biology classes and checklist of interaction treatments.

Possible Elements for Distance Based Biology Classes	Interaction Treatments Checklist		
	Student-Teacher	Student-Content	Student-Student
Course Cartridges or Access Codes from Publishers	√	√	
Discussion Boards and Chat: Live and Asynchronous	√	√	√
Electronic Mail	√	√	√
Interactive Television or Video Conferencing	√	√	√
Lab Demonstration Videos	√	√	
Lab Procedures Instructional Videos	√	√	
Lectures on Sound Files	√	√	
Library Resources: Supplemental Books, Journal Articles, etc.		√	
Listservs	√	√	√
Slide Presentations from Publisher, Teacher or Students	√	√	√
Student Group Pages	√	√	√
Telephone: Individual and Conference Calls	√		√
Tests & Quizzes: Online or Written, Proctored or Unproctored	√	√	
Textbooks: Paper or Electronic		√	
Web Sites Related to Course Content		√	

with more suspicion than other modes of instruction. Many faculty view online education as inferior and predict decreased learning (Kirtman, 2009). Ward (2008), in a survey of over 100 college science instructors from various disciplines, noted that a strong majority viewed distance science courses negatively and were resistant to accept them. Why? Some teachers may lack the technical knowledge or support to teach online (Kennepohl & Shaw, 2010). The practice requires a different mindset and teaching style, and prior training is recommended (Miller, 2008). One way to get a feel for online education is to enroll in online courses or seminars.

Science is a complicated discipline to learn and teach, and specialized equipment or complex models are often required (Downing & Holtz, 2011). It is not surprising that the greatest challenge for many distance instructors is implementing laboratory and field work effectively (Cancilla & Albon, 2008; Ward, 2008; Reuter, 2009; Downing & Holtz, 2011).

Research Findings, Best Practices & Advice

Important factors to consider in distance-based science learning environments include transmission of course material and inclusion of content-based student assignments and activities. We will now present some of our own practices and experiences with these factors, and review the current literature.

Transmission of Web-Based Learning Content

Distance instructors may ask, "How do I convey the content knowledge that would normally be presented during lecture?" Pursued alone or in combination, options such as pre-existing web sites, printed books, e-books, recorded lectures, and other resources provide flexibility. Most learning platforms allow teachers to include *Uniform Resource Locators* (URLs; i.e. web addresses) for use by students. Teachers simply need to select a few to meet their needs.

Traditional classes use textbooks as a primary content source, and distance classes are often similar. Fully accessible, free of charge, quality texts are available online. One example (Kimball's Biology Pages: home.comcast.net/~john.kimball1/BiologyPages/) is based on a classic text by John Kimball (1994). Publishers often include online content for their books, free or fee-based. Others offer content as CD ROMs or *cartridges* where an entire course is loaded into a learning platform. Cartridges may be electronic versions of textbooks or may include interactive quizzes and animations. Some contain grading packages and other features (Landry, Payne & Koger, 2008). As a rule, we are not enthusiastic about course cartridges due to challenges in finding balance between content and quality.

The challenge of transmitting content can actually become an opportunity for increased learning. One of the easiest transmission tools is slide-based lectures which can include text, graphics, animations, instructor narration, and even written scripts for the hearing-impaired. Students may move through material at their own pace, so challenges regarding absences and concentration lapses are eliminated. The convenience of online lectures may contribute to the overall satisfaction students attribute to distance learning (Walker & Kelly, 2007).

Web-based lectures do have disadvantages, including lack of visual cues and feedback which help teachers evaluate understanding (Miller, 2008); inability of students to ask real-time questions, and lack of in-depth conversations about content during lecture. A variety of options can meet these challenges. Telephone, instant messaging and electronic mail may be used to communicate with any student, distance or traditional. Most learning platforms include discussion boards allowing asynchronous exchanges among students. *Live chat*

Table 3. Sample teaching, learning and assessment sequence from a web-based freshman genetics unit.

Activity		Description
Step 1:	Student Preparation	Students read material from textbook, external links and instructor-made slide presentations.
Step 2:	Guided Practice	Students view animations, video clips, and they participate in interactive games and quizzes.
Step 3:	Formative Assessment	Each student completes a low-stakes quiz online. Depending on the result, he or she may move ahead or complete additional practice.
Step 4:	Short Writing Assignment	Students write an essay on genetics. The instructor evaluates these. Students may then revise the essay as needed and then expand the essay into a project.
Step 5:	Project	Each student completes a slide presentation and posts it to the class web site.
Step 6:	Peer & Instructor Evaluation	All slide presentations are evaluated by the instructor and by peers.
Step 7:	Final Product, Class Debate	On the discussion board, everyone participates in an online debate regarding genetic engineering and its implications.

options are available too. Further solutions include creating review activities and assignments that build on lectures or apply the material to real-world problems.

From Transmission to Application: Moving Beyond Memorization

Many distance activities reinforce content and allow students to apply knowledge. These include web-based review centers provided by text publishers, online content quizzes, narrated (and often interactive) animations, links to web content, and discussion board activities. These create opportunities that would be difficult to incorporate into traditional classes, but they must be carefully employed to ensure effectiveness. For instance, Muchovej (2009) found that optional online quizzes did not significantly improve scores when quiz questions were recycled on exams. A low number of students completed the optional quizzes, suggesting that how the learning strategies are employed is important.

Web-based technologies allow instructors to utilize sequentially built knowledge and skills. An example from one of our freshman biology courses is summarized in Table 3. Notice that the activities, in this case centered on learning genetics content, build upon one another to progress from knowledge to application.

Laboratory Activities

Published research concerning labs in distance-based science courses is spotty (Kennepohl & Shaw, 2010). Reuter (2009) reported no significant differences between online and traditional lab grades in various science courses. In a small study, Lunsford & Bolton (2006) reported similar success rates on biology content exams for nonmajors taught traditionally and online. By its nature, science involves frequent laboratory work, a fact reflected in most biology courses. Providing laboratory experiences is challenging in a web-based course (Kennepohl & Shaw, 2010). Specifically concerns about lab content, materials, and safety arise. The quality of a class may suffer if planning is not done carefully (Miller, 2008). We recognize these concerns while challenging our colleagues to honestly question the quality of seat-based lab practices, especially those offered to undergraduates and non-majors. As uncomfortable as it may be to admit, Cancilla & Albon (2008) reminded us that seat-based lab practices are often rushed, prescribed and lacking in authenticity. Lab activities in any format always require careful planning. Some of the choices for distance courses will be discussed below.

Hybrid, Power-Lab and Mentoring Options

An easy solution is to offer a traditional laboratory meeting via a hybrid class (Kennephol & Shaw, 2010). A variation is the *power lab.* These are required, extended lab meetings (Cancilla & Albon, 2008) which may be offered several times per semester, in the evenings, or on weekends. Students satisfy the laboratory component in a traditional way despite the unconventional scheduling. If only one or a few lab objectives require a traditional solution, a laboratory mentor working with individual students may be an option. For example, if a teacher places high value on microscope use, students may locate a site for completion of such work (e.g. high-school teachers or hospital lab technicians are eager to assist our students). Colleges and universities have been sending students to clinics, classrooms, labs, field stations and other locales to enhance their on-campus studies for decades. You may wish to invest time to formulate a preapproved list of off-campus mentors or lab sites. Safety and liability issues should be considered when having students work off-campus.

Instructor responsibilities for safety are the same whether lab is conducted in a traditional or a distance setting. Specific challenges related to liability and insurance are too complex for this article, but educators and institutions should examine their practices to ensure that students are not exposed to unreasonable risk. A good overview is provided by the National Science Teachers Association (NSTA) (nsta.org/about/positions/liability.aspx). Table 4 also provides some tips.

Virtual Labs, Simulations & Demonstrations

By definition, a virtual or simulated experience is approximated by a computer. Virtual labs are becoming commonplace in distance-learning (Anderson, 2008). In online and traditional classes, we recommend limited use of simulations. We prefer to utilize simulations as a supplement, as we have

Table 4. Tips for safety and liability concerns involving off campus lab or field work.

General Safety Tips	Working From Home	Potential Legal Issues	Safety at Home
Remember that all lab activities (even paper and pencil materials) could have some potential safety or legal risks. Strive to keep use of chemicals, sharp objects, heat, etc. to a minimum. If your labs require a lot of potentially dangerous items consider an on campus, a hybrid or a power lab option.	Enclose required safety materials such as goggles or gloves in lab kits. Supply detailed instructions for disposal of chemicals or specimens. Put Material Safety Data Sheets (MSDS) in lab kits or post them on the class web site. Supply demonstration videos to emphasize safety for specific lab procedures.	Require students to view a safety video or attend a safety training session, even at off campus facilities. Require students to sign a safety contract specifying liability and attesting that they understand safety procedures. Requires students to purchase and maintain accident insurance if appropriate. Ask off campus sites to treat students as they would treat employees concerning safety and liability.	Post contact information for the Poison Control Center on the class web site. Post a flow chart concerning first aid procedures and when it is necessary to call 911 for a safety emergency. Provide specific precautions in lab procedures such as "Do not mix with other chemicals" or "Avoid contact with eyes" or "See MSDS".

found virtual activities ranging from useful to terrible. One can access quality film images of dissections online at no cost (e.g., exploratorium.edu/learning_studio/cow_eye/step01.html). Also, teachers have the option of filming their own demonstrations (Kennepohl & Shaw, 2010). In other cases publishers offer flashy, expensive computer simulations that, in general, are heavy on production but ineffective at building scientific process skills. We are not aware of a recent, quality study of the efficacy of virtual labs and simulations in adult biology education. Clearly there is a need for research. Asbell-Clarke & Rowe (2007) noted a study comparing traditional and virtual labs for introductory chemistry classes. Students, by and large, learned no lab skills or techniques but did pick up additional content knowledge about chemistry. Reuter (2009) reported that virtual labs are coming to be viewed as secondary in value to authentic lab and field experiences. One advantage is that students are able to access simulations asynchronously and therefore at their own convenience (Cancilla & Albon 2008).

Working at Home: Kitchen Labs, Remote Instrumentation and Other Options

Though some courses do not lend themselves to web-based lab assignments due to need for equipment or concerns for safety (e.g., microbiology), many freshman-level labs can be easily and safely completed by students in their own homes. So called *kitchen science labs* tend to require little to no specialized equipment. They are particularly common in non-majors courses (Reuter, 2009). Asbell-Clarke & Rowe (2007) found that well planned kitchen activities can promote mastery of inquiry skills. From their homes our students have completed labs concerning scientific measurement, observation of metamorphosis, experiments involving diffusion, mark and recapture modeling, and use of homemade pH indicators. Be sure to supply students with a list of required materials in advance.

Another option involves offering materials (slides, reagents, dissection specimens, etc.) to students as *lab kits* (Kennepohl & Shaw, 2010). Teachers may mail kits to students, distribute them during face-to-face meetings or collaborate with college bookstores for dispensing. There is a growing industry involving the commercial packaging of laboratory kits. One source with a variety of options is Hands on Labs/LABPAQ (holscience.com). While all of these alternatives require a significant commitment from instructors, the outcomes allow students flexibility in scheduling. Also, labs can be sequenced into the flow of lectures, and lab results can be discussed online.

Instructors should provide safety instructions. Directives on organization of work areas, chemicals, clothing, equipment, and clean-up may be needed. Teachers may also consider a *safety contract* which must be completed by students to show they have understanding of safety considerations. Another approach is the use of a pre-lab *safety quiz* which could be easily integrated into many distance-learning platforms. An excellent example of safety considerations specifically focused on distance-learning labs is given by Hands-On Labs, mentioned above. Table 4 provides more suggestions.

Remote instrumentation is commonplace in biology and involves manipulation of scientific apparatus from distant locations. Control and data transmission are usually accomplished via the internet. Examples include sensors, cameras, chromatographs and machinery for collecting samples. (Educase Learning Initiative, 2006; Kennepohl & Shaw, 2010). Remote instrumentation fits well with distance-based learning. Equipment is usually accessible at any time and data are often easily shared. In fact they may be shared and contributed to not only within classes but among them, even among multiple colleges (Cancilla & Albon, 2008). This can save time and travel, and provide authenticity as students work with actual data (Educase Learning Initiative, 2006). Practical lessons on sample size may be cleverly built into large data sets. On the downside, remote instrumentation may be costly. Some researchers or sponsors may object to sharing raw data (Educase Learning Initiative, 2006).

Library and Internet-Based Review Projects

Teachers have long sent students to libraries to complete what could pass as lab work. Perhaps students study classification by writing a review of organisms from various taxa or they may read research articles for class discussion. These tasks are easily accomplished online. Respected journals are available online and most libraries maintain access to databases for retrieving articles. In addition, many internet search engines provide full articles for free. Examples ideal for academic research include Google Scholar (scholar.google.com) BioOne Journals (bioone.org) and Scirus: For Scientific Information Only (scirus.com). Scientists routinely use online sources for their research so it follows that students should as well (Cancilla & Albon, 2008).

Inquiry-Based Learning

Inquiry activities, those which mimic scientific practice (NRC, 2000) are possible in distance-based formats. One option we have found effective is long-term independent research projects providing experience with scientific inquiry. Our students complete a research project in which they formulate a question, hypothesis and design. They present findings via slide presentations or research papers posted on the class website. Lunsford (2008) reported a guided inquiry in which distance students monitored yeast respiration using a bottle and balloon apparatus. Other examples include a project in which a student investigated correlations between firefly lighting and temperature and humidity. Projects of this type require several conditions for success. Goals and guidelines must be clear. Detailed instructions, with examples of previous student work, help meet this requirement. Students can sequentially build the skills necessary to complete the project. Leading up to inquiry assignments, our labs are designed to give students practice with observation, measurement, sampling and statistics. Lectures and labs also cover design, variables, replication, randomization, etc.

DISCUSSION

Internet-based learning is no longer a novel concept, though science has been slower than other disciplines to embrace the genre. Reluctance comes from concerns about effectiveness of distance-learning and issues specific to science education. In this paper, we have addressed concerns involved with the transmission of biology material in distance-learning. Specifically, we have discussed the effectiveness and challenges of transmission strategies such as traditional and electronic texts and slide-based lectures. We have outlined student activities and assignments (like essays, presentations and discussions) which build knowledge sequentially. Challenges related to labs have been reviewed and solutions offered. We have called for teachers and researchers to explore virtual laboratory activities and simulations and what role, if any, they should have in our distance teaching practices. We have considered other learning approaches like research and review projects and inquiry-based learning. We believe that there are many obstacles related to effective biology teaching in distanced-based settings. Most of these challenges can be overcome with careful planning and proper application of technologies and educational theories. Distance-based biology teaching can offer many advantages over traditional settings for those willing to explore the possibilities.

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Bioscene: Journal of College Biology Teaching
Submission Guidelines

I. Submissions to *Bioscene*

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

- Articles: Course and curriculum development, innovative and workable teaching strategies that include **some type of assessment** of the impact of those strategies on student learning.
- Innovations: Laboratory and field studies that work, innovative and money-saving techniques for the lab or classroom. These do not ordinarily include assessment of the techniques' effectiveness on student learning.
- Perspectives: Reflections on general topics that include philosophical discussion of biology teaching and other topical aspects of pedagogy as it relates to 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, Innovations and Perspectives

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 all submissions are as follows:

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

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

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

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

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

Single Author:

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

Two Authors:

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

Multiple Authors:

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

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

(1) Articles-

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

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

(2) Books-
BOSSEL, H. 1994. *Modeling and Simulation*. A.K. Peters, London. 504p.

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

(4) Web sites-
MCKELVEY, S. 1995. Malthusian Growth Model. Accessed from http://www.stolaf.edu/people/mckelvey/envision.dir/malthus.html on 25 Nov 2005.

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 in Word (2003+) or RTF format. 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 high resolution (≥ 300dpi) individual electronic files, either TIFF or JPEG. Placement of figures should be indicated within the body of the manuscript. Figures include both graphs and images. All figures should be accompanied by a descriptive legend using the following format:

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

III. Letters to the Editor

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

IV. Other Submissions

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

V. Manuscript Submissions

All manuscripts are to be sent to the editor electronically. *Authors must clearly designate which type of article they are submitting (see Section I) or their manuscript will not be considered for publication.* 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 necessarily blind unless requested by an author. If the article has a number of high resolution graphics, separate emails to the editor may be required. The editors recommend that authors complete and remit the Bioscene Author Checklist with their submission in order to expedite the review process.

VI. Editorial Review and Acceptance

For manuscripts to be sent out for review, at least one author must be a member of ACUBE. Otherwise, by submitting the manuscript without membership, the corresponding author agrees 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 associate editors 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 associate editors 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 associate editor, 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.