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Avila University

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Cover image: Red onion (*Allium cepa*) cells in a sucrose solution. Note cells undergoing plasmolysis among other cells without plasmolysis activity. Again, several cells are void of any color and are not scored. Photograph supplied by Richard V. Kowles.

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Bioscene: Journal of College Biology Teaching

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ACUBE Mission Statement

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

The objectives of ACUBE are:

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

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Bioscene: Journal of College Biology Teaching
Guidelines for Submissions

I. Submissions to *Bioscene*

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

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

II. Preparation of Articles

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

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

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

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

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

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

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

or

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

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

(1) Articles-

(2)

(a) Single author:

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

(b) Multi-authored:

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

(3) Books-

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

(4) Book chapters-

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

(5) Web sites-

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

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

D. Tables

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

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

E. Figures

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

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

III. Letters to the Editor

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

IV. Other Submissions

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

V. Manuscript Submissions

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

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

VI. Editorial Review and Acceptance

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

Reviewers will examine the submission for:

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

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

VII. Revision Checklist

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

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

VIII. Editorial Policy and Copyright

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

Influence of Population Density on Offspring Number and Size in Burying Beetles

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Abstract: This laboratory exercise investigates the influence of population density on offspring number and size in burying beetles. Students test the theoretical predictions that brood size declines and offspring size increases when competition over resources becomes stronger with increasing population density. Students design the experiment, collect and analyze the data, and discuss the results in the context of adaptive phenotypic plasticity.

Keywords: Population density, contests, brood size, offspring size, burying beetles

Introduction

Some animals have only a few large offspring at a time, whereas others have many small offspring. This negative relationship between offspring number and size can be observed not only among species, but also within many species (Roff, 2002). Negative relationships between offspring number and size are the consequence of mechanical constraints or limited resource availability (Roff, 1992, 2002). For example, body size of a female limits the number and size of offspring she can produce. For a given body size, a female can either produce many small or a few large offspring. When resources are limited, a trade-off between offspring number and offspring size may occur. How females solve this dilemma when resources are limited, may be a function of the intensity of competition over these resources, especially when large individuals are more likely to prevail (Leips and Travis, 1999; Both, 2000; Creighton, 2005; Goubault et al., 2007). If high population density results in more intense competition over resources and large individuals are more successful in monopolizing resources, females should have fewer, yet larger offspring (Mesterton-Gibbons and Hardy, 2003). This would ensure that their offspring have a higher probability of gaining access to resources necessary for reproduction. Reciprocally, if population density is low and thus competition over resources is weak, females should have many small offspring (Mesterton-Gibbons and Hardy, 2003).

This laboratory exercise tests the theoretical predictions that offspring number declines and offspring size increases when competition over resources becomes stronger with increasing population density.

Burying Beetles

In the United States, there are 15 species of burying beetles (Coleoptera: Silphidae: *Nicrophorus* sp.; for distribution, natural history, and keys for identification of each of these species see: Anderson and Peck, 1985; Peck and Kaulbars, 1987; Ratcliffe, 1996). These species are widely distributed and occur in many habitat types (e.g. forests, prairie, mountains). The most widespread species is *N. marginatus* which can be found in open fields, prairie, open woods, or other open habitats. Most burying beetle species are active at night and reproduce in spring or early summer.

Burying beetles use carrion as a food resource for their offspring (for reviews on burying beetle biology see: Ratcliffe, 1996; Eggert and Müller, 1997; Scott, 1998). Carrion is a high quality, but rare, resource. Contests (i.e. fights) over carrion are common, especially when population density is high. The largest individual of either sex usually wins in a contest, thus gaining access to the carrion. Generally, a male and a female beetle bury the carrion. While burying the carrion, the beetles remove fur or feathers. As early as 12 hours after encountering the carrion, the female lays eggs in the surrounding soil. About two days later, the larvae hatch and crawl to the carrion. During the first two to three days on the carrion, the larvae are fed by the parents. Subsequently, the larvae feed by themselves from the carrion. The larvae of many species can survive and grow without being fed by the parents, although less well (e.g. *N. defodiens*, *N. marginatus*, *N. pustulatus*, and *N. vespilloides*). Larvae of *N. mexicanus*, *N. orbicollis*, and *N. sayi*, however, require parental feeding for survival. After the carrion has been consumed, typically 6 to 10 days

after the larvae have hatched, the larvae disperse and pupate in the surrounding soil, emerging as adult beetles four weeks hence. Beetles reach sexual maturity after an additional three more weeks. The majority of species overwinter as adults (e.g. *N. defodiens*, *N. marginatus*, *N. pustulatus*, and *N. vespilloides*). Some species overwinter in the third larval stage as prepupae (e.g. *N. tomentosus*, *N. vespillo*).

Materials and Methods

Preparation Before Class

In summer, two months before the students conduct the experiment, approximately 30 burying beetles are collected using pitfall traps baited with decaying ground beef. A wide-mouth jar (at least 200 ml.), plastic bucket, or similar container can be used as pitfall trap. The trap is filled with about 2 to 5 cm of moist soil or leaves which provide hiding places and humidity for the trapped beetles thus reducing mortality due to aggressive interactions among the beetles or heat stress, respectively. As bait any well-aged meat (e.g. ground beef, chicken liver, or fish exposed to room temperature for two to three days) will attract beetles. The bait is placed in a small container (e.g. 30 ml plastic cup) that can be closed tightly with a lid to keep beetles and flies off the bait. To release the smell of the bait, small holes are punched into the container or the lid. The container with the bait is placed onto the soil or leaves in the trap. The trap is buried in the soil with the rim of the trap level with the surface of the ground. A rain cover or sun shield should be placed over the trap. A plastic plate secured with three large nails can serve as a rain cover and sun shield. A one-foot square piece of one-inch mesh screen wires placed over the pitfall trap and staked down securely will keep scavengers out. Instead of burying the traps, they can be hung, at least 3 feet above ground, from tree branches or stakes. Trapping rates are improved by avoiding dense vegetation, with the placement of traps in prairies, open woods, or less dense parts of forests. Additional information on trapping of burying beetles can be found in Ratcliffe (1996), Trumbo (1996), and Bedick et al. (2004). Traps should be checked daily, especially on hot or rainy days. Beetles caught in the pitfall traps are transferred to containers filled with moist peat or soil and transported to the lab.

The beetles are sexed in the lab by comparing the tarsi of the front legs and the last segments of the abdomen (Figure 1). Ten to 15 pairs of beetles are placed individually into clear plastic containers of sufficient size to accommodate a mouse carrion (e.g. 17 × 12 × 6 cm). The containers are filled with about 2 cm of moist peat and supplied with a previously frozen mouse available from pet food supply sources. Mice weighing approximately 20 to 30 g work best. Burying beetles rear very small broods on lighter mice and they have difficulties keeping larger mice carrion clean of bacteria and mold. The containers with the beetles and the mouse carrion are kept in a darkroom. Containers are checked daily for eggs that are easily visible on the bottom of the containers. The day after the first eggs are visible, the beetles and the carrion are removed. The peat is carefully searched for eggs using forceps. Eggs are transferred onto a small piece of wet paper towel. The paper towel with the eggs is afterwards placed into a container that can hold a mouse (e.g. 17 × 12 × 6 cm), filled 1 cm deep with moist peat and containing a previously frozen mouse. To facilitate consumption of the carrion by the larvae, a small incision with scissors is made in the back of the mouse. The containers with the eggs are kept in a dark room at room temperature. About two days later, the larvae hatch. When the larvae have consumed all or most of the carrion and are crawling along the container walls, they are transferred to a new container of the same size filled to the top with moist peat. No more than 15 larvae are placed in a single container (17 × 12 × 6 cm). If smaller or larger containers are used, the number of larvae should be adjusted accordingly. The containers are kept in a darkroom at room temperature until the new beetles emerge about four weeks later. The rearing of larvae without the parents reduces transfer of mites and parasites carried by all field-caught beetles. Parasites and mites introduced by field-caught beetles can cause problems with laboratory exercises (e.g. brood failure). This procedure works well for burying beetles that overwinter as adults and the larvae can be reared without parents (e.g. *N. defodiens*, *N. marginatus*, *N. pustulatus*, and *N. vespilloides*; Peck and Kaulbars, 1987; Trumbo, 1992; Eggert and Müller 1997).

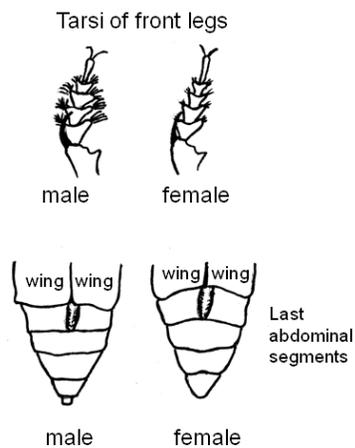


Figure 1. Dorsal view of tarsi of front legs and last segments of abdomen of adult female and male burying beetles, *Nicrophorus pustulatus*.

The newly emerged beetles are sexed (Figure 1) and can then either be used to establish a laboratory colony (see below) or for laboratory exercises. Beetles used for this laboratory exercise are randomly assigned to the low-population density treatment or the high-population density treatment. Low-population density beetles are placed individually in containers (about $17 \times 12 \times 6$ cm; much smaller containers do not work as well with the high-population density treatment) filled with 1 cm of moist peat. Beetles assigned to high-density population treatment are kept in groups of four beetles in containers of the same size. Beetles are kept in these containers for at least three weeks at a 15:9 h light:dark cycle at room temperature, and are fed wet cat food or decapitated mealworms twice weekly.

To establish a laboratory colony, the newly emerged beetles are maintained either individually or in single-sex groups. Up to six beetles can be kept in containers of about $17 \times 12 \times 6$ cm. For smaller or larger containers the number of beetles should be adjusted. The containers are filled with 2 to 3 cm of moist peat or soil. The beetles are kept at 15:9 h light:dark cycle at room temperature (i.e. summer conditions) and fed wet cat food twice weekly. Small pieces of liver, fly maggots, decapitated mealworms, or dead crickets are also suitable as food. Three to six weeks after emergence of the adult beetles, the next generation of beetles is produced by placing a male and female beetle in a container able to hold a mouse, filled about 1 cm with moist peat or soil, and

containing a previously frozen mouse. The parent beetles are removed when the larvae are about five days old, to prevent that the parent beetles eat the larvae after the carrion has been consumed. Dispersing larvae are transferred to new containers filled with moist peat or soil for pupation.

In Class (Week 1)

Introduced to the ecological concept of reproductive strategies including the trade-off between offspring number and size, students are asked to design an experiment that tests whether population density influences offspring number and size in burying beetles. While designing the experiment, the students are directed to consider: 1) the null hypothesis and alternative hypotheses, 2) the factor they have to manipulate (i.e. population density), 3) the measurements they should take (i.e. number and weight of larvae), 4) the factors they must control for or measure (e.g. carrion size, room temperature, light cycle, number of parents providing care), and 5) sample size. Students work in small groups to draft the experimental design that is subsequently subject to review and criticism by the whole class. Once the class has decided on an experimental design, all students establish together a protocol for the experiment and prepare data sheets. With the protocol set and the data sheets ready, the students start the experiment.

The experiment begins by filling 40 containers that are able to hold a mouse (e.g. $17 \times 12 \times 6$ cm) with about 2 cm of moist peat and by placing a previously frozen mouse on top of the peat. Female and male beetles are added to these prepared containers as follows: ten containers receive each one high-density female and one high-density male; into ten additional containers one high-density female and one low-density male are placed; to ten containers one low-density female and one high-density male are added and the last ten containers receive each one low-density female and one low-density male. The containers with the beetles are maintained in a darkroom at room temperature for the next two weeks. It takes approximately two weeks from the time the females encounter the carrion until the larvae have consumed the carrion and are dispersing, i.e. crawling along the container wall. To simplify the system, males are removed two days after the matings have been initiated. To avoid cannibalism of dispersing larvae by the females, the female beetle is removed when the larvae are five days old. If no dark room is available, a black cloth can be placed over the containers.

In Class (Week 3)

Two weeks after the experiment has been started, the larvae of each brood are counted and the weight of the whole brood is determined. Average larval weight for each brood is calculated by dividing the weight of the whole brood by the number of larvae in the brood. Several minutes are required to count the larvae of a brood and to weigh the whole brood. During a short break the instructor compiles the data that the students will use to calculate means and standard errors, graph the results, and conduct statistical analyses in class.

At the end of class, all the larvae are collected and used to start a new laboratory colony or added to a previously established laboratory colony of burying beetles. Burying beetles are also regularly used for additional behavioral laboratory exercises such as studies of mating systems or conflicts between individuals over resources (Trumbo, 1996; Scott, 2003).

Results and Discussion

This laboratory exercise allows students to design and conduct an experiment and to perform statistical data analyses. I use this laboratory exercise as the first laboratory exercise in my behavioral ecology course for junior and senior biology majors to reinforce the concept of trade-offs and to prepare the students for their lab research project in the second part of the course. The week between the first and second part of the experiment is used to introduce the students to statistical analyses, in particular to linear regression analyses as well as to one-tailed and two-tailed t-tests (e.g. Sokal and Rohlf, 1995). Using linear regression analysis, students can test for the trade-off between number and size of offspring, while the one-tailed t-test allows to test whether high-density females have fewer, but heavier offspring than low-density females.

Typical results from this exercise are shown in Figures 2, 3, and 4. Average larval weight decreases with increasing brood size (equation for linear regression line: $Y = 368.8 - 6.4 X$; $F_{1,69} = 47.51$; $P < 0.0001$; Figure 2). Brood size is significantly smaller for high-density females than for low-density females (one-tailed t-test: $t = 2.3$; d.f. = 67; $P = 0.01$; Figure 3), but the larvae of high-density females are heavier than those of low-density females (one-tailed t-test: $t = -1.8$; d.f. = 56; $P = 0.04$; Figure 4). These results illustrate the trade-off

between offspring size and number, corroborating the findings of Creighton (2005) in *N. orbicollis*. These results also support the prediction of the theoretical model by Mesterton-Gibbons and Hardy (2003) that brood size should decrease when contests over resources become more frequent.

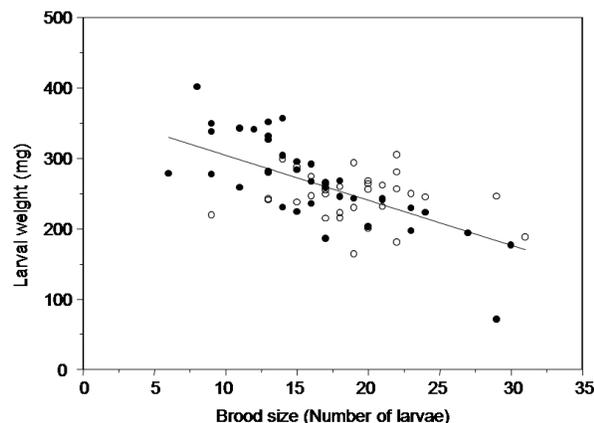


Figure 2. Average larval weight in relationship to brood size in *N. pustulatus*.

Open circles: low-population density beetles. Filled circles: high-population density beetles.

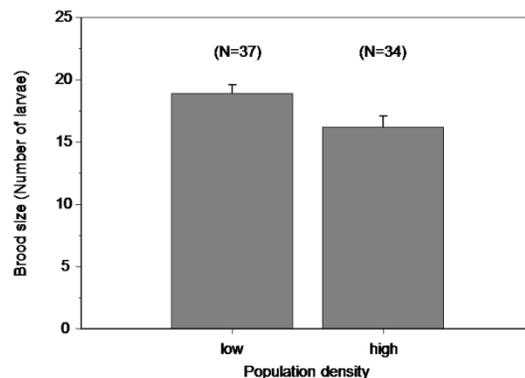


Figure 3. Brood size in relationship to population density in *N. pustulatus*. Means and standard errors are shown. Sample sizes are given in parentheses.

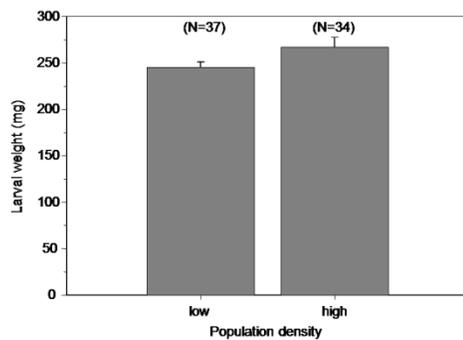


Figure 4. Average larval weight in relation to population density in *N. pustulatus*. Means and standard errors are shown. Sample sizes are given in parentheses.

Students discuss in class the importance of the ability of females to adjust offspring number and size in relation to environmental conditions so that they maximize their fitness. This discussion introduces the students to the concept of phenotypic plasticity (i.e. capacity of a genotype to produce different phenotypes depending on the environmental

condition the genotype experiences) and its adaptive value (i.e. the consequences for the animals' fitness).

This laboratory exercise can be simplified by increasing the guidance and providing the basic experimental design. On the other hand, the rigor of this exercise can be raised by increasing the complexity of the experimental design. In a separate offering of this exercise, the experimental design was made more complex by adding additional levels of population density and including the weight of the mouse as covariate in the experiment.

Acknowledgements

I thank the students of my Behavioral Ecology class of fall 2008 and the course Early Undergraduate Research in Behavioral Ecology of summer 2004 and 2007 at the University of Nebraska at Omaha for testing earlier versions of this laboratory exercise. Data collection in the course Early Undergraduate Research in Behavioral Ecology was financially supported by NSF grant IBN-0309532 and NSF STEP grant NSF-0336462. I thank Mark Schoenbeck for comments and suggestions on earlier versions of the manuscript.

References

- ANDERSON, R.S. AND S.B. PECK. 1985. The Carrion Beetles of Canada and Alaska. *The Insects and Arachnids of Canada*, Part 13: 1- 121.
- BEDICK, J.C., RATCLIFFE, B.C. AND L.G. HIGLEY. 2004. A new sampling protocol for the endangered American burying beetle, *Nicrophorus americanus* Olivier (Coleoptera: Silphidae). *Coleopterists Bulletin* 58: 57-70.
- BOTH, C. 2000. Density dependence of avian clutch size in resident and migrant species: is there a constraint on the predictability of competitor density? *Journal of Avian Biology* 31: 412-417.
- CREIGHTON, J.C. 2005. Population density, body size, and phenotypic plasticity of brood size in a burying beetle. *Behavioral Ecology* 16: 1031-1036.
- EGGERT, A.-K. AND J.K. MÜLLER. 1997. Biparental care and social evolution in burying beetles: lessons from the larder. In Choe, J.C. and B.J. Crespi. *The Evolution of Social Behavior in Insects and Arachnids*. Cambridge University Press, Cambridge. 541p.
- GOUBAULT, M., MACK, A.F.S., AND I.C.W. HARDY. 2007. Encountering competitors reduces clutch size and increases offspring size in a parasitoid with female-female fighting. *Proceedings of the Royal Society of London, B* 274: 2571-2577.
- LEIPS, J. AND J. TRAVIS. 1999. The comparative expression of life-history traits and its relationship to the numerical dynamics of four populations of the least killifish. *Journal of Animal Ecology* 68: 595-616.

MESTERTON-GIBBONS, M. AND I.C.W. HARDY. 2003. The influence of contests on optimal clutch size: a game-theoretic model. *Proceedings of the Royal Society of London, B* 271: 971-978.

PECK, S.B. AND M.M. KAULBARS. 1987. A synopsis of the distribution and bionomics of the carrion beetles (Coleoptera: Silphidae) of the conterminous United States. *Proceedings of the Entomological Society of Ontario* 118: 47-81.

RATCLIFFE, B.C. 1996. *The Carrion Beetles* (Coleoptera: Silphidae) of Nebraska. Bulletin of the University of Nebraska State Museum Volume 13. Lincoln. 100p.

ROFF, D.A. 1992. *The Evolution of Life Histories: Theory and Analysis*. Chapman & Hall, New York. 535p.

ROFF, D.A. 2002. *Life History Evolution*. Sinauer Associates, Sunderland. 527p.

SCOTT, M.P. 1998. The ecology and behavior of burying beetles. *Annual Review of Entomology* 43: 595-618.

SCOTT, M.P. 2003. Competition for breeding resources by burying beetles. In Ploger, B.J and K. Yasukawa. 2003. *Exploring Animal Behavior in Laboratory and Field*. Academic Press, San Diego. 472p.

SOKAL, R.R. AND F.J. ROHLF. 1995. *Biometry: The Principles and Practice of Statistics in Biological Research*. 3rd edition. Freeman, New York. 887p.

TRUMBO, S.T. 1992. Monogamy to communal breeding: exploitation of a broad resource base by burying beetles (*Nicrophorus*). *Ecological Entomology* 17: 289-298.

TRUMBO, S.T. 1996. The role of conflict in breeding systems: burying beetles as experimental organisms. *The American Biology Teacher* 58: 118-121.

Biomedical Research Experiences for Biology Majors at a Small College

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Abstract: A program-level assessment of the biology curriculum at a small liberal arts college validates a previous study demonstrating success in achieving learning outcomes related to content knowledge and communication skills. Furthermore, research opportunities have been provided to complement pedagogical strategies and give students a more complete science education.

Keywords: Program-level assessment, content knowledge, primary literature, communication skills, undergraduate research

Introduction

Davis & Elkins College (D&E) is a private, four-year, comprehensive liberal arts college that stresses small class sizes and strong faculty-student interaction. The Department of Biology and Environmental Science offers a curriculum designed to promote science as a process of inquiry and instill within students an appreciation of the underlying unity and diversity of life. To meet the needs of students with diverse career interests, three degree options are offered: the Bachelor of Science (B.S.) in Biology, the Bachelor of Science in Environmental Science, and the Bachelor of Arts (B.A.) in Biology and Environmental Science.

In 2004, we published a program-level assessment of the B.S. in Biology degree (Stover and Mabry, 2004). We focused on student competence in the five areas outlined by our 2001 departmental assessment plan: 1) content knowledge, 2) writing skills, 3) speaking skills, 4) ability to interpret primary literature, and 5) research skills (laboratory and/or field).

As we indicated in the previous study, the interpretation of biological data requires a specific knowledge base. To comprehend and integrate information from different areas of biological inquiry, students should be familiar with fundamental concepts associated with each area, including the molecular basis of heredity, cell structure and function, biological evolution, and the interdependence of organisms. Furthermore, communication skills are essential for biologists as they generate data, share their findings, and build on the work of others. Successful communication

depends on both the ability to effectively convey information (written and oral) and the ability to interpret and evaluate information presented by peers (Feldman *et al.*, 2001). Analyzing primary literature allows students to enhance their critical thinking skills as they participate in the dissemination of scientific information (Houde, 2000; Smith, 2001). Finally, to fully understand and appreciate the scientific process, biology students should be engaged in hands-on, investigative activities (Glasson and McKenzie, 1998; Lewis *et al.*, 2003).

In addition to math, physics, and chemistry requirements, biology majors at D&E must take five core content courses. Principles of Biology I (BIOL 101) introduces first-semester biology majors to cell structure and function, genetics, and developmental biology, while Principles of Biology II (BIOL 102) is taken in the second semester and deals primarily with the ecology and evolution of organisms. Genetics (BIOL 205) is a survey of prokaryotic and eukaryotic inheritance; Cell and Molecular Biology (BIOL 302) investigates metabolism, gene expression, and differentiation of eukaryotic cells; Evolution (BIOL 305) emphasizes the evidence, mechanisms, and genetics of organic evolution.

All D&E students are required to take the *College Basic Academic Subjects Examination (C-BASE)*; Assessment Resource Center, University of Missouri) prior to graduation. As indicated by the departmental assessment plan, biology majors are expected to score “medium” or “high” on the *Fundamental Concepts of Life Science* component of the C-BASE. This component of the exam assesses knowledge of basic biology, botany, zoology, and ecology. Scores are reported by the Assessment

Resource Center as low, medium, or high based on students' success in answering biology-related questions. Although the exam is used primarily to evaluate the college's general education curriculum, results of the life science section complement course grades as an external, standardized measure of biology majors' basic content knowledge.

With the exception of BIOL 305, all core courses require written laboratory reports (BIOL 305 requires a term paper). Biology majors are introduced to laboratory reports in BIOL 101, allowed to hone their skills in BIOL 102, and expected to demonstrate competence in scientific writing by scoring between 80 and 100% on all reports required for BIOL 205 and 302. Figure 1 provides a sample laboratory report format.

<p>BIOL 302 – Cell & Molecular Biology</p> <p><u>Abstract</u> (8 points) Briefly summarize the report. Include basic aspects of each section in the summary. Start with an introductory sentence or two to describe the underlying question. In a few sentences, describe the experiments you performed to address the question. What were your results and how do they compare with information you found in the literature?</p> <p><u>Introduction</u> (20 points) The introduction should consist of general background information. Basic concepts associated with the topic should be covered, but you should <u>not</u> discuss the actual experiments you performed. Reference all material you obtain from outside sources in the body of the text (author, date).</p> <p><u>Methods</u> (8 points) Write out, in complete sentences, the procedures that you followed to perform each experiment.</p> <p><u>Results</u> (12 points) Write out the result of each individual experiment. Include graphs and/or tables, as well as text, to present your data. Do not interpret data in this section; simply present your findings.</p> <p><u>Discussion</u> (40 points) This is the most important section. In the discussion, you should attempt to interpret the data you have collected. Analyze the result of each experiment individually. How do these results compare to information found in the literature? Does the literature support or contradict your results? Offer potential reasons for conflicting results. Suggest alternative or follow-up procedures to further analyze the question. Reference any information you find to help explain your results.</p> <p><u>Miscellaneous</u> (12 points) Lab reports should be written in paragraph form (double-spaced). Check spelling and grammar. Each section should have its own heading. You must reference <u>all</u> information you obtain from outside sources and include an alphabetical "literature cited" section at the end of the report. Items in the literature cited section should contain the following information: Author(s). Year of Publication. Title of Article. <i>Title of Journal</i> Volume: Page Numbers.</p>

Figure 1. Laboratory Report Format for BIOL 302.

To fulfill departmental requirements, biology majors must complete Current Topics in Biology (BIOL 335), a one-credit course that involves the analysis and discussion of current research articles. As described in the 2004 study, students in BIOL 335 carefully analyze recent journal articles, which are given to them one week prior to an in-class discussion. Students and a faculty facilitator

then discuss the paper and compare notes. The goal is for students to become comfortable with graphical representations of data and the interpretation of experimental results. The instructor usually selects articles with a common theme. Figure 2 contains instructions for analyzing journal articles, while Figure 3 provides citations of representative articles.

BIOL 335 – Current Topics in Biology

1. Citation

Include authors' names, year of publication, title of article, name of journal, volume of journal, first & last pages of article.

Example:

Cunningham, P., Geary, M., Harper, R., Pendleton, A., and Stover, S. 2005. High intensity sprint training reduces lipid peroxidation in fast-twitch skeletal muscle. *Journal of Exercise Physiology Online* 8(6): 18-25.

2. Research question

What biological question does this research address? What was known & unknown prior to this research? This information is generally found in the Introduction of the paper.

3. Hypothesis

A hypothesis is a statement of explanation to a question concerning some biological phenomenon. A hypothesis may not be clearly stated. You may have to infer what the hypothesis is, based on the procedures being used to address the research question.

4. Prediction

An "If... then..." statement. If the hypothesis stated in step 3 is correct, then we would expect certain things to be true. The experiments conducted should test these predictions, leading to data that either supports or refutes the overall hypothesis.

5. Experimental approach

What, specifically, was measured or determined? Summarize the approach in your own words.

6. Results

What new information was produced? Summarize the results in your own words.

7. Conclusion

What do the authors make of the data? Are their conclusions valid? Is there any other possible interpretation? Do the data support the hypotheses? Are there any alternative hypotheses?

8. Now what?

A good paper may generate more questions than it answers. After reading this paper, what is the next question these authors (or other researchers in this field) should address?

Figure 2. Instructions for Analyzing Journal Articles (based on an instrument created by Dr. Catherine Gardiner, University of Northern Colorado).

BIOL 335 – Current Topics in Biology

Diet and Exercise

Halberg, N. *et al.* 2005. Effect of intermittent fasting and refeeding on insulin action in healthy men. *Journal of Applied Physiology* 99: 2128-2136.

Williams, N.I. *et al.* 2001. Longitudinal changes in reproductive hormones and menstrual cyclicity in cynomolgus monkeys during strenuous exercise training: Abrupt transition to exercise-induced amenorrhea. *Endocrinology* 142: 2381-2389.

Alternative Medicine

Schwartz, E. *et al.* 2002. Oral administration of freshly expressed juice of *Echinacea purpurea* herbs fail to stimulate the nonspecific immune response in healthy young men: Results of a double-blind, placebo-controlled crossover study. *Journal of Immunotherapy* 25(5): 413-420.

Solomon, P.R. *et al.* 2002. Ginkgo for memory enhancement: A randomized controlled trial. *Journal of the American Medical Association* 288: 835-840.

Sexual Reproduction

Mabry, M. and P. Verrell. 2003. All are one and one is all: Sexual uniformity among widely separated populations of the North American seal salamander, *Desmognathus monticola*. *Biological Journal of the Linnean Society* 78: 1-10.

Mack, P.D. *et al.* 2002. Sperm competitive ability and genetic relatedness in *Drosophila melanogaster*: Similarity breeds contempt. *Evolution* 56(9): 1789-1795.

Figure 3. Representative Journal Articles for BIOL 335.

To demonstrate an ability to communicate information related to biological research, biology majors are also required to participate in Senior Seminar (BIOL 397). Satisfactory completion of BIOL 397 involves oral presentations of research data. Students select scientific journal articles of

interest to them, use the skills acquired in BIOL 335 to analyze the articles, and present the data (using Microsoft PowerPoint) to their peers and a faculty facilitator. Figure 4 shows the presentation analysis form utilized by the course instructor.

BIOL 397 - Senior Seminar					
Student _____					
		Poor	Acceptable	Good	Excellent
<u>Content</u>					
Research Question:	1		2	3	4
Overall Hypothesis:	1		2	3	4
Prediction:	1		2	3	4
Methods:	1		2	3	4
Results:		1		2	3
Conclusion:		1		2	3
Personal Interpretation:		1		2	3
Follow-up Studies:	1		2	3	4
<u>Presentation Style</u>					
Eye Contact:		1		2	3
Articulation/Volume:	1		2	3	4
Movement/Interaction:		1		2	3
Fielding Questions:	1		2	3	4
					Score _____

Figure 4. Presentation Analysis Form for BIOL 397.

Finally, biology majors are expected to demonstrate competence in basic biological research methods associated with laboratory components of

the five core content courses. See Figure 5 for an example of a laboratory skills checklist.

BIOL 205 - Genetics	
Student _____	
Biology majors must demonstrate competency in the following areas:	
1. <u>Basic statistics</u>	
• Probability	_____
• Chi-square test	_____
2. <u>Laboratory techniques</u>	
• Slide staining/light microscopy	_____
• Use of micropipette	_____
• Restriction enzyme digestion of DNA	_____
• Gel electrophoresis	_____
• Culture maintenance	_____
• Bacterial transformation	_____
3. <u>Experimental protocols</u>	
• Testing hypotheses	_____
• Interpreting results	_____

Figure 5. Laboratory Skills Checklist for BIOL 205.

The 2004 study tracked the progress of the five biology majors who completed each of the core content courses, the C-BASE, BIOL 335, and BIOL 397. All five students scored “medium” or “high” on the *Fundamental Concepts of Life Science* component of the C-BASE, scored 80% or higher on all laboratory reports in BIOL 205 and 302, successfully completed both Current Topics and Senior Seminar, and demonstrated competence in all core course laboratory skills.

We found that the greatest weakness of the assessment plan was the evaluation of research skills. Participating in laboratory exercises may reinforce biological concepts, and students may pick up some valuable skills in the process. Ultimately, however, students must be actively involved in scientific research to fully understand it. In the 2004 paper, we outlined a plan to implement and expand experimental systems developed as part of *Research Link 2000*, a project initiated by the National Science Foundation’s Council for Undergraduate Research to provide convenient model systems for undergraduate teaching and research. We hoped that the experimental systems would facilitate the introduction of research-based laboratory activities into the undergraduate curriculum.

Unfortunately, budgetary constraints and a lack of release time prevented implementation of the proposed plan. However, we were able to capitalize on another opportunity. D&E was recruited to participate in the West Virginia IDeA Network of

Biomedical Research Excellence (WV-INBRE). The WV-INBRE is a consortium of institutions of higher education in West Virginia, organized under the direction of the Marshall University School of Medicine (MUSOM) and the West Virginia University Health Sciences Center (WVUHSC). The consortium is funded through the National Institutes of Health (NIH) to develop biomedical research programs at the state’s predominantly undergraduate institutions (PUIs) and to stimulate student interest in graduate training in the biomedical sciences. WV-INBRE provides equipment grants and pilot research grants to investigators at PUIs to fund undergraduate research. Furthermore, students at PUIs have the opportunity to work in biomedical research laboratories during the summer, under the direction of mentors at MUSOM and WVUHSC.

In the current study, we reassess the content knowledge, communication skills, and research experiences of our B.S. in Biology graduates, five years after the original assessment.

Methods

We tracked the progress of the 10 students in the B.S. Biology program (from 2005 to 2009) who completed the five core content courses, the C-BASE, BIOL 335, and BIOL 397. Student competence was evaluated according to criteria outlined in the 2001 departmental assessment plan. Five of the students participated in WV-INBRE-sponsored undergraduate research on the D&E

campus or as a summer intern at MUSOM or WVUHSC (Figure 6). Two of the five participated in research both at D&E and as summer interns at WVUHSC. One of the five interned at WVUHSC for two consecutive summers. Research projects spanned various biomedical disciplines, including cell biology, immunology, neuroscience, physiology, and toxicology. Research on the D&E campus required students to spend approximately

nine hours per week in the laboratory, for a total of 24 weeks, during the school year. Summer interns worked, on average, about eight hours per day, five days per week, for a total of nine weeks. Students participating in research were given up to four credit hours of BIOL 390 (Undergraduate Research in Biology) and were required to orally present the results of their work in a public forum.



Figure 6. A D&E student at work in a toxicology lab at MUSOM.

Results

All 10 students scored “medium” or “high” on the *Fundamental Concepts of Life Science* component of the C-BASE (since 2005, just under 60% of non-science majors at D&E have scored “medium” or “high” on this component of the exam), scored 80% or higher on all laboratory reports in BIOL 205 and 302, successfully completed Current Topics and Senior Seminar, and demonstrated competence in all laboratory skills associated with core courses. All 10 students graduated with the B.S. degree in Biology. All five research participants put in the required workload and presented their data, first in poster format at a WV-INBRE research symposium (Figure 7), then as an oral presentation,

either as part of D&E’s annual Biology and Environmental Science Forum, or as part of the Chi Beta Phi (a national science honorary) National Conference. Of the five research participants, two went to medical school, and three entered graduate programs (in biotechnology, exercise physiology, and neuroscience). Of the five students who did not participate in undergraduate research, one is completing a graduate degree in sports medicine, one has graduated from optometry school, one is currently in pharmacy school, one has a hospital administrative position (she was a double major in biology and business), and one is working as a veterinary technician and is planning to apply to veterinary school.



Figure 7. A D&E student participating in a WV-INBRE research symposium.

Discussion

In many ways, a small liberal arts college can provide the ideal setting for learning science. Classes are generally small, and active learning is often encouraged. Previous studies have demonstrated that a small group dynamic (even in a large enrollment class) can improve student learning and performance in the science classroom (Tessier, 2007). Furthermore, class discussions can be utilized to stimulate critical thinking (Stover and Mabry, 2005), and case studies can be employed to address students' prior knowledge (Gallucci, 2006) and train them to develop experimental procedures (Dinan, 2005). Finally, it is sometimes possible to integrate the lecture and laboratory sections of a science course. Recent research has demonstrated that this "seamless" classroom experience can have a positive influence on both conceptual understanding and academic achievement (Burrowes and Nazario, 2008). The Department of Biology and Environmental Science at D&E has been able to incorporate all these pedagogical strategies into the curriculum.

Hands-on, independent research, as indicated by previous studies, allows students to hone the skills necessary to do science (in both laboratory and field), nurture a deeper appreciation of science, and sample scientific research as a career choice (Lopatto, 2004; Seymour *et al.*, 2004; Clerkin, 2007). However, while it is always encouraged, actual scientific research may not be emphasized (or even possible) at a small liberal arts college. WV-INBRE has made it possible for some biology students at D&E to experience the best of both worlds: the

informal, student-centered environment of the small liberal arts college and the high tech, hands-on environment of the biomedical research laboratory. While individual research experiences were certainly unique, each student who completed the 9-week internship and presented data was given a "pass" grade for BIOL 390. Presentations varied in terms of quality. Some students presented their "piece" of the research puzzle, while others attempted to explain the "big picture." However, they all had an experience that could not have been provided without the WV-INBRE grant.

It should be noted that the biology curriculum at D&E is quite rigorous (as it is at other institutions). Students who survive the gauntlet of biology, chemistry, and physics courses are, in general, very good students. Our placement of students in graduate and professional programs is consistently between 85 and 90%. However, personal communications from representatives at various graduate and professional programs suggest that research experiences can dramatically improve an applicant's chances of being accepted. Students interested in attending graduate programs in the sciences, in particular, can strengthen their applications significantly by participating in undergraduate research. Previous studies have suggested that students participating in undergraduate research have a higher likelihood of pursuing advanced study and, ultimately, a career in research (Nagda *et al.* 1998; Bauer and Bennett 2003).

We are still not completely satisfied with the B.S. in Biology program. Because the WV-INBRE internships are competitive, we cannot guarantee a

research experience for every biology student. Furthermore, not every biology student is interested in doing biomedical research. We recently obtained funding to support a few undergraduate research projects in the ecological and environmental sciences, and we will continue to provide our students with as many opportunities for experiential learning as possible. Although we still have a long way to go, our ultimate goal is to provide an

independent research experience for every student in the program to complement the active learning environment we provide in the classroom.

References

BAUER, K.W. AND BENNETT, J.S. 2003. Alumni perceptions used to assess undergraduate research experience. *Journal of Higher Education* 74 (2): 210-230.

BURROWES, P. AND NAZARIO, G. 2008. Promoting student learning through the integration of lab and lecture. *Journal of College Science Teaching* 37(4): 18-23.

CLERKIN, P.J. 2007. Research experience for undergraduates: A student's reflection on an authentic learning experience. *Bioscene* 33(3): 18-21.

DINAN, F.J. 2005. Laboratory based case studies: Closer to the real world. *Journal of College Science Teaching* 35(2): 27-29.

FELDMAN, S., ANDERSON, V., AND MANGURIAN, L. 2001. Teaching effective science writing. *Journal of College Science Teaching* 30(7): 446-449.

GALLUCCI, K. 2006. Learning concepts with cases. *Journal of College Science Teaching* 36(2): 16-20.

GLASSON, G.E. AND MCKENZIE, W.L. 1998. Investigative learning in undergraduate freshman biology laboratories. *Journal of College Science Teaching* 27: 189-193.

HOUDE, A. 2000. Student symposia on primary research articles. *Journal of College Science Teaching* 30(3): 184-187.

Acknowledgments

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LEWIS, S.E., CONLEY, L.K., AND HORST, C.J. 2003. Structuring research opportunities for all biology majors. *Bioscene* 29(2): 9-14.

LOPATTO, D. 2004. Survey of undergraduate research experiences (SURE): First findings. *Cell Biology Education* 3: 270-277.

NAGDA, B.A., GREGERMAN, S.R., JONIDES, J., VON HIPPEL, W., AND LERNER, J.S. 1998. Undergraduate student-faculty research partnerships affect student retention. *Review of Higher Education* 22 (1): 55-72.

SEYMOUR, E., HUNTER, A.B., LAURSEN, S.L., AND DEANTONI, T. 2004. Establishing the benefits of research experiences for undergraduates in the sciences: First findings from a three-year study. *Science Education* 88 (4): 493-534.

SMITH, G.R. 2001. Guided literature explorations: Introducing students to the primary literature. *Journal of College Science Teaching* 30(7): 465-469.

STOVER, S.K. AND MABRY, M.L. 2004. The five-tool biology major. *Bioscene* 30(2): 13-19.

STOVER, S.K. AND MABRY, M.L. 2005. Merging science and society: An issues-based approach to nonmajors biology. *Journal of College Science Teaching* 34(4): 40-43.

TESSIER, J. 2007. Small-group peer teaching in an introductory biology classroom. *Journal of College Science Teaching* 36(4): 64-69.

An Inexpensive Method to Simulate a Monohybrid Cross Using Wild-Type Zebrafish (*Danio rerio*)

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Abstract: Monohybrid crosses are taught in biology labs across the country using peas and corn. Students studying monohybrid crosses using zebrafish (*Danio rerio*) would encounter a new model organism, and those interested in human or veterinary medicine would have increased interest. To this end, our college has implemented a laboratory exercise at the introductory level using wild-type and non-pigmented zebrafish embryos to simulate a monohybrid cross. By using 1-phenyl-2-thiourea (PTU) to generate non-pigmented fish, the necessity of maintaining a separate albino line of fish is eliminated. Live embryos are preferable for generating student excitement; however, the option to use preserved embryos allows for re-use of fish over multiple years without relying on embryo production on a particular day.

Keywords: Mendel, zebrafish, monohybrid, genetics

Introduction

In the classroom, we are often challenged with presenting material in new and exciting ways. Monohybrid crosses in particular can be difficult to present in a novel way in an introductory college biology course for either majors or non-majors. Mendel famously used the pea, *Pisum sativum*, to discover the 3:1 phenotypic ratio of dominant:recessive traits (Mendel, 1866). Many high schools and colleges continue to use this model organism, as well as corn, to exemplify genetic crosses. In our introductory biology course serving a population of approximately 120 students per year, we have used corn to demonstrate both monohybrid and dihybrid crosses (Genetics of Corn Kit, Carolina Biological).

Students often have preconceived notions brought into the classroom and laboratory environment (Lazarowitz and Lieb, 2006). While not the intent of educators, some students may receive the impression that monohybrid genetic crosses are the domain of plants after exploring the concept exclusively in corn. Those students with prior exposure to Punnett squares and Mendelian genetics would benefit from new examples. In addition, critical thinking skills can be developed in laboratory exercises through their inquiry-based nature (Howard and Miskowski, 2005). Here I will describe a laboratory exercise involving a simulated monohybrid cross in zebrafish (*Danio rerio*) to complement common Mendelian laboratory exercises involving corn.

This laboratory exercise emphasizes that the phenomenon of 3:1 phenotypic ratios can be found in animals as well as plants. In addition, the exercise

introduces a common model organism to students. Finally, the exercise simulates albino zebrafish without the instructor having to maintain a line of albino fish. Therefore, the exercise is adapted for high schools and colleges having minimal zebrafish facilities and circumvents the need to maintain multiple genetic lines. While live embryos are the most advantageous for student interest, preserving the embryos ahead of the lab exercise allows for specimens to be used multiple years and without relying on embryo production on a particular day.

Materials and Methods

Wild-type zebrafish adults (*Danio rerio*) were purchased from Carolina Biological and housed in ten gallon tanks. Adults were kept on a 14h:10h day:night cycle at 28.5°C and fed a diet of Tetraamin flakes and freeze-dried brine shrimp (Omega One). Approximately 20 fish were bred over a collecting tray and embryos were collected 2 hours after simulated dawn. Embryos were transferred to 60 mm Petri dishes with 0.3x Danieau solution (Table 1) and kept at 28.5°C (Nasevicius and Ekker, 2000).

At 7 hours post fertilization (hpf), clutches of embryos were placed into either a new Petri dish of 0.3x Danieau or 0.3x Danieau containing 0.2 mM 1-phenyl-2-thiourea (PTU). PTU blocks melanin synthesis resulting in embryos that appear albino (Karlsson *et al.*, 2001). Over the course of 3 weeks, approximately 75% of embryos were placed into 0.3x Danieau and 25% of embryos were placed into the solution containing PTU (Figure 1). Mating of adult zebrafish was continued until approximately 200 embryos were obtained for each student group in a lab section. For our class of 120 students, no more

than four lab groups were in a session at

any one time, so 800 embryos were needed.

Table 1. Recipes used in zebrafish Mendelian genetics laboratory exercise

Reagent	Recipe
50x Danieau buffer stock	2.9 M NaCl 35 mM KCl 20 mM MgSO ₄ 30 mM Ca(NO ₃) ₂ 250 mM HEPES pH 7.6
100x 1-phenyl-2-thiourea (PTU) stock	120 mg PTU in 40 mls 0.3x Danieau buffer (20 mM) Freeze in 5 ml aliquots
0.3x Danieau buffer	3 mls 50x Danieau buffer 497 mls H ₂ O
0.3x Danieau buffer with 1x PTU	3 mls 50x Danieau buffer 5 mls 100x PTU stock 492 mls H ₂ O
4% Paraformaldehyde (PFA)	0.4 g PFA 1 ml 10x Phosphate Buffered Saline (PBS) 9 mls H ₂ O
PBT wash	5 mls 10x PBS 50 ul Tween 20 45 mls H ₂ O

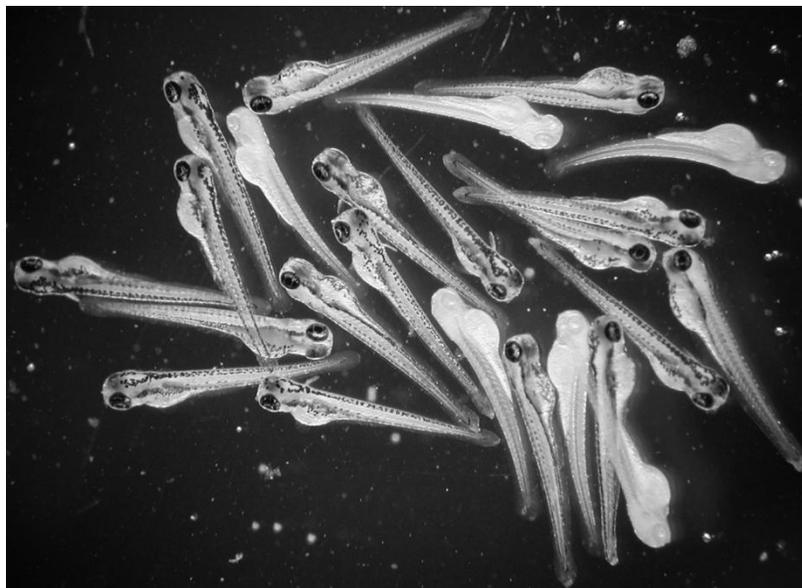


Figure 1. Clutch of 72 hpf embryos with approximately 25% treated with PTU. A ratio of approximately 3:1 wild-type:non-pigmented embryos is used to simulate a monohybrid cross between wild-type and albino zebrafish.

At 24 hpf, dead embryos were removed from Petri dishes to maintain a clean environment for surviving specimens. This removal of dead embryos is common in the zebrafish field. Mortality in zebrafish embryos is variable depending upon egg and sperm quality. PTU did not affect mortality. At 72 hpf, embryos were transferred to a glass vial and fixed in 4% paraformaldehyde in phosphate buffered saline (PBS) overnight at 4°C. Embryos were then

washed three times for 5 minutes each in PBT at room temperature (Table 1). Embryos were sorted into an approximate 3:1 phenotypic ratio of wild-type:non-pigmented and stored at 4°C in glass vials. To accurately simulate a natural mating, the ratio was not exactly 3:1. Immediately before the laboratory procedure began, embryos were transferred to covered 60 mm Petri dishes containing PBT

Laboratory Procedure

The first exercise in the laboratory (not shown here) introduced the terminology of dominant and recessive alleles using the Genetics of Corn kit from Carolina Biological mentioned above. In brief, students examined purple and yellow corn kernels and counted to find an approximately 3:1 phenotypic ratio of purple:yellow corn kernels. Students were asked if their observed values were near expected values, and introduced to the Chi-Square test for Goodness-of-Fit. Students then proceeded to the zebrafish exercise described below.

The zebrafish exercise began with students observing a wild-type adult fish. Then, using a stereomicroscope, students were asked to observe a preserved wild-type zebrafish embryo. Both of these observations allowed us to introduce students to the zebrafish model organism, use of a stereomicroscope, and basic embryo anatomy (head, eye, tail, yolk sac). After this familiarization was complete, students used the stereomicroscope to count approximately 200 embryos (in one Petri dish) for wild-type and non-pigmented phenotypes. Results were then analyzed by the Chi-Square test for Goodness-of-Fit. The laboratory exercise lasts 30-40 minutes. Students then proceeded to dihybrid crosses using the Genetics of Corn kit mentioned above.

For the purposes of the assignment, non-pigmented embryos were called albino. Instructors may or may not choose to communicate the melanin inhibition to students. If the instructor does not, he or she should make sure to indicate that these fish are phenotypically albino but not genetically albino. A Chi-Square table was provided with the exercise.

Student Assignment

1. You have tested one of Mendel’s Principles on a plant. Does his Principle of Segregation also apply to animals? You will test this question using pigmented and albino zebrafish. Obtain a live zebrafish adult, representing the F₁ generation of a cross between a true-breeding pigmented fish and a true-breeding albino fish. Which phenotype is dominant?

2. Draw a Punnett square showing the cross of a true-breeding pigmented fish (AA) with a true-breeding albino fish (aa). What genotype does the F₁ fish have?

3. Draw a Punnett square showing a cross between two F₁ fish. What are the expected genotypes (and ratios)? What are the expected phenotypes (and ratios)?

4. Obtain 3-day old preserved zebrafish embryos from your instructor. The Petri dish contains a clutch of embryos, all siblings. Observe the embryos. Can you see the eye? The heart? Record the embryo pigment phenotype counts in Table 2 under the “observed” column.

5. Calculate the expected number for each phenotype based on the ratio in part 3. For example if you expected a ratio of 8 pigmented:1 albino and counted 180 embryos, you would expect (8/9)*180 embryos to be pigmented (160 embryos) and (1/9)*180 embryos to be albino (20 embryos). Record the expected numbers in Table 2 and complete the Chi-Square calculation.

Table 2. Chi-Square calculation of F₂ zebrafish pigmentation phenotypes

	observed	expected	observed-expected	(observed-expected) ² / expected	(observed-expected) ² / expected calculated
Pigmented					
Albino					
Sum					

6. State your null hypothesis before checking your p-value.

7. Check the Chi-Square table for your p-value and interpret your results. Write a well developed paragraph that addresses the following: What are you testing (what was your expected phenotypic ratio and why)? What is the observed phenotype ratio? Is

it near your expected? Do you accept or reject your hypothesis? Why or why not? Explain your reasoning.

Laboratory Evaluation

Approximately 85% of students either thought the laboratory exercise was fine or liked it a great deal (Figure 2). Students felt that both

zebrafish and corn were equally effective in four areas assayed (Figure 3), but enjoyed the zebrafish portion of the exercise more than the corn portion (Figure 4). Only 11% of students had used a vertebrate in a high school genetics lab exercise. A free-form question asked what students liked best and least about the complete laboratory exercise, which included corn and zebrafish exercises. The highest percentage “liked best” answer was zebrafish (40.5%), while 14.4% liked corn the best. In contrast, 7.6% liked zebrafish least, while 8.7% liked corn least. The highest percentage of any “liked least” free-form answer was the Chi Square test at 29.3%.

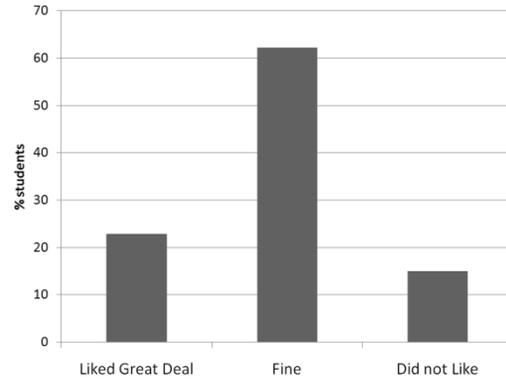


Figure 2. Assessment of student enjoyment of the complete laboratory exercise, including both zebrafish and corn portions. Most students liked the lab a great deal or thought the lab exercise were fine.

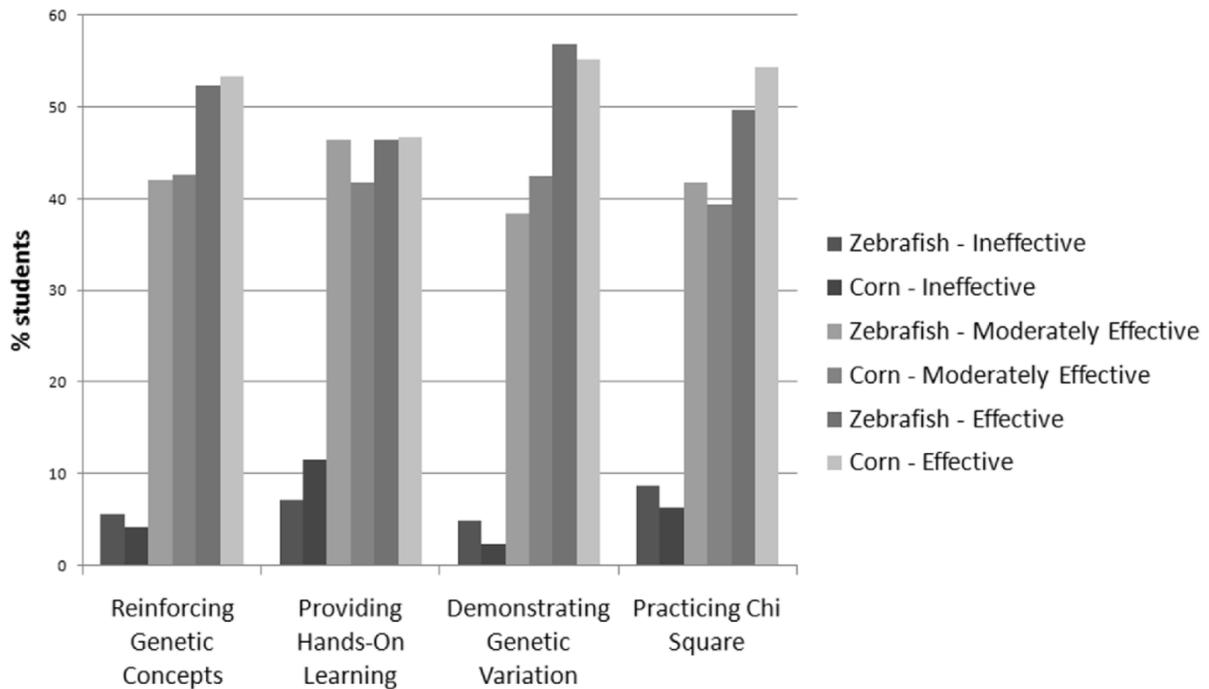


Figure 3. Assessment of laboratory exercise effectiveness, as reported by students. Students rated the corn and zebrafish laboratory exercises as equally effective.

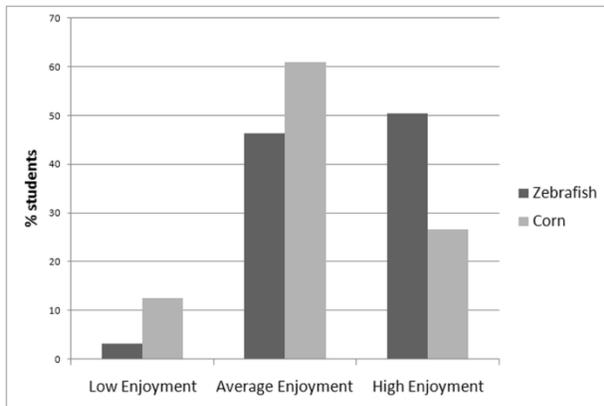


Figure 4. Assessment of student enjoyment of the zebrafish and corn portions of the laboratory exercise. Students rated the zebrafish exercise as “high enjoyment” nearly twice as much as the corn exercise.

Discussion

For many students, Mendelian genetic principles, when combined with Chi-Square calculations, are daunting. While the often-used corn laboratory exercise provides an invaluable method for introducing monohybrid and dihybrid crosses, our college wants to stimulate student interest in genetics by using an animal model. Many of our prospective undergraduate biology majors are interested in careers in human or veterinary medicine. Introducing zebrafish as a model system at this level engages those students. In addition, this encounter with embryos is often the first for many students. Reigniting the spark of curiosity in students is a primary concern for educators.

Students participating in the laboratory exercise were excited about using an animal model. We found that 89% of our students had not worked with a vertebrate animal model in a genetics laboratory setting during high school. In the qualitative portion of the survey, one student enjoyed learning about Mendelian concepts in animals rather than plants. Another indicated that zebrafish were the primary reason that the lab held his or her attention. Students did not rank the zebrafish as more effective than the corn examples provided during the laboratory exercise, but expressed “high enjoyment” with the zebrafish more than twice as much as the corn portion of the exercise. We provided live adult zebrafish, which the students found useful to visualize the adult form of the embryos used.

Students did encounter two problems with the laboratory exercise. Some students were hesitant when asked to identify embryo anatomy (such as the heart, yolk sac, or eye) before looking under the

microscope. Zebrafish embryos have readily identifiable organs, such as the eye and heart, which students were able to identify once they engage with the provided materials. The use of zebrafish embryos in introductory biology courses has become more widespread (D’Costa and Shepherd, 2009). Another common problem was unfamiliarity with the Chi Square Goodness of Fit test, the least liked portion on the qualitative portion of the survey. Students did perform the test once before (a monohybrid corn cross used immediately before the zebrafish exercise), and the exercise discussed here increases familiarity with the test by repetition. Students go on to perform the test on sex-linked *Drosophila* crosses to find other genotypic ratios and to discover parental genotypes the following week. By repetition and instruction, most students are able to grasp the Chi Square test by the end of the two weeks of genetic laboratory exercises.

Active learning exercises such as laboratories are important in introductory biology courses (Smith *et al.*, 2005). Laboratory exercises are almost always active learning experiences and are important in building critical thinking skills. These skills are desired in Bloom’s taxonomy of critical thinking and can help students perform better on exams (Chaplin, 2007). In addition, the exercise described here integrates mathematics and statistics into the biology classroom, which is often desirable (White and Carpenter, 2008). Asking questions and using statistical tests begins the important process of incorporating inquiry and research into the curriculum.

In conclusion, our college has implemented a zebrafish laboratory exercise to complement a well-established corn genetics laboratory. This laboratory exercise simulates a monohybrid cross between wild-type and albino zebrafish using PTU to inhibit melanin synthesis. Because the cross is simulated, this exercise can be done for low cost (only the cost of PTU) compared with maintaining an albino line of zebrafish. Students enjoyed the hands-on aspect of working with a vertebrate in a genetics laboratory. Finally, the preserved embryos can be used over multiple laboratory sections and for many years.

Acknowledgments

I would like to thank A. Udvardia for sharing her PTU treatment protocol. R. Collins and F. Bosch provided helpful feedback during construction of this laboratory exercise. K. Filer assisted in survey construction, while C. Lord, L. Roshetar, and S. Melanaphy assisted in data collection. J. Doughman, G. Schaperjahn, C. Waterstraut, S. Webb, and T. Underwood assisted in fish husbandry.

References

- CHAPLIN, S. 2007. A model of student success: Coaching students to develop critical thinking skills in introductory biology courses. *Int. J. Scholarsh. Teach. Learn.* 1(2). Accessed from http://academics.georgiasouthern.edu/ijstol/issue_v1n2.htm on 3 July 2009.
- D’COSTA, A. AND I.T. SHEPHERD. 2009. Zebrafish development and genetics: Introducing undergraduates to developmental biology and genetics in a large introductory laboratory class. *Zebrafish* 6(2): 169-77.
- HOWARD, D.R. AND J.A. MISKOWSKI. 2005. Using a module-based laboratory to incorporate inquiry into a large cell biology course. *Cell Biol. Educ.* 4(3): 249-60.
- KARLSSON, J., VON HOFSTEN, J., AND P.E. OLSSON. 2001. Generating transparent zebrafish: a refined method to improve detection of gene expression during embryonic development. *Mar. Biotechnol.* 3(6): 522-7.
- LAZAROWITZ, R. AND C. LIEB. 2006. Formative assessment pre-test to identify college students’ prior knowledge, misconceptions and learning difficulties in biology. *Int. J. Sci. Math. Educ.* 4(4): 741-62.
- MENDEL, G. 1866. Experiments in plant hybridization. *Proc. Nat. Hist. Soc. Brünn.* 4: 3-47.
- NASEVICIUS, A. AND S.C. EKKER. 2000. Effective targeted gene 'knockdown' in zebrafish. *Nat. Genet.* 26(2): 216-20.
- SMITH, A.C., STEWART, R., SHIELDS, P., HAYES-KLOSTERIDIS, J., ROBINSON, P., AND R. YUAN. 2005. Introductory biology courses: A framework to support active learning in large enrollment introductory science courses. *Cell Biol. Educ.* 4(2): 143-56.
- WHITE, J.D. AND J.P. CARPENTER. 2008. Integrating mathematics into the introductory biology laboratory course. *PRIMUS* 18(1): 22-38.

Analyzing Population Genetics Using the Mitochondrial Control Region and Bioinformatics

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Abstract: The 14-base pair hypervariable region in mitochondrial DNA (mtDNA) of Asian populations, specifically Japanese and Chinese students at Plattsburgh State University, was examined. Previous research on this 14-base pair region showed it to be susceptible to mutations and as a result indicated direct correlation with specific ethnic populations. Earlier studies provided the 14-base pair region sequence analysis for Asians in general. This inquiry-based project was generated in a junior/senior college general genetics course. The project produced sequences of the 14-base pair hypervariable region for Japanese and Chinese populations, specifically. The investigation examined 28 Japanese and 27 Chinese international students. A Control group, consisting of 20 random students was also sampled. The 14-base pair hypervariable region for each sample was analyzed through comparison using bioinformatics. Analyzing the samples showed that, overall, 46% of the Japanese samples, 59% of the Chinese samples, and 75% of the Control samples had the same 14-base pair sequence. In addition to this, it was observed that the Japanese, Chinese, and Control group all had their own specific mutations within the 14-base pair hypervariable region.

Keywords: DNA, mitochondria, hypervariable region, bioinformatics, data mining

Introduction

In the summer of 2001, the authors of this journal article were chosen to attend the Vector Bioinformatics Workshop held at the Trudeau Institute in Saranac Lake, N.Y (<http://www.dnalc.org/ddnalc/about/annreppdf/annrepp2001.pdf>, 2001). The workshop was held under the auspices of the Dolan DNA Learning Center (DNALC) located in Cold Spring Harbor, NY and was funded through a Howard Hughes Grant. The five day workshop focused on analyzing patterns in DNA sequences, online algorithms helpful in identifying gene features and using genome browsers to find genes in online databases. Participants were taught to recognize chromosome locations, identify homologs in other organisms, and explore their involvement in normal and disease processes. The workshop also centered on the use of the DNALC's custom *Bioservers*. Participants provided a sample of their own mitochondrial DNA, prepared the sample (<http://www.geneticorigins.org/mito/intro.html>), using Polymerase Chain Reaction (PCR), and submitted it to the DNALC for sequencing. This professional development opportunity subsequently opened the doors to a variety of learning experiences

that could be incorporated into an undergraduate genetics' curriculum. One such opportunity is the focus of this article.

The Nature of Science is such that as one slowly collects information, formulates hypotheses, and explores testable possibilities, the end result is always another fork in the road. Answering one question only creates many more questions. Two General Genetics students did not stop at the knowledge of their own mitochondrial control region sequence of DNA returned to them by the DNALC *Bioserver*. Once the Vector Bioinformatics Workshop was completed, participants were allowed to submit their students' DNA samples for the sequencing of the mtDNA control region. As a result, undergraduate genetics students were able to use their *own* DNA sequences to investigate some population genetics questions but the answers still did not satisfy the curiosity factor. The student team found that reading some related journal articles (Anderson et al., 1981; Horai and Hayakawa, 1990; Horai et al., 1993; Lewis et al., 2007) presented information about a 14 base pair hypervariable section (found between mitochondria bases 16180-16193) within the control region of the mtDNA

The team's subsequent research endeavor was designed to compare Japanese students, Chinese students, and a control group (no Asians were included), using the garnered 14-base pair hypervariable region within mitochondrial DNA (mtDNA). It was hypothesized that Chinese and Japanese students would have a 14-base pair region that was more similar than those of the control group. Additionally, the team wanted to know if testing specific Asian populations (Japanese and Chinese) would support the analyzed data published in the Horai et al. (1993) literature that investigated Asians as a whole entity. It should be noted that one of the student team members was from Japan.

Research was initiated after reading the previously mentioned journal articles that analyzed the 14-base pair region of different populations of people, including Asians, Africans, Native Americans and Europeans. For each of these groups, a common 14-base pair sequence was identified (Horai and Hayasaka, 1990; Horai et al., 1993; Lewis et al., 2007). In the Horai et al., (1993) literature, Asians were tested (along with Europeans, Africans and Native Americans). However, Asian groups were not specified (Japanese, Chinese, or Korean, etc). Noting this, the team chose to test DNA samples taken from two specific Asian groups, Japanese and Chinese, to compare the 14-base pair region. There is a high population of International students on the State University of New York at Plattsburgh campus (7-8% of total student population). A vested interest in the investigation outcome (Japanese team member) and availability of test subjects allowed for the study to be done.

Using the campus student population and under the auspices of the Committee on the Protection of Human Subjects (COPHS), DNA samples were obtained from 28 Japanese international students, 27 Chinese international students, and 20 students from within the genetics class, the latter being used as the Control group. The Chinese, Japanese, and Control group samples were randomly collected (again with proper, signed consent forms). Both the Chinese and Japanese students represented many regions across China and Japan (Figures 1 and 2).

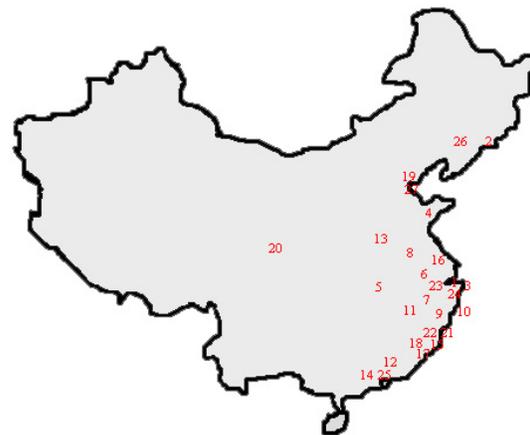


Figure 1. Map of China; the numbers indicate original residence of the International Chinese students from whom DNA was collected.



Figure 2. Map of Japan; the numbers indicate the original residence of the International Japanese students from whom the DNA samples were collected.

The focal point for the research was the 14-base pair hypervariable region found within the mitochondrial DNA control region (Figure 3). This 14-base pair sequence is supported as being

hypervariable, since it is more susceptible to mutations, including insertions, deletions, transversions, and transitions (Walker et al. 2003). These point mutations or single nucleotide polymorphisms (SNPs) accumulate at a rate 10 times that of nuclear DNA (Walker et al. 2003).

Employing protocols from both molecular genetics and bioinformatics, DNA sequence comparisons of the 14-base pair region were done.

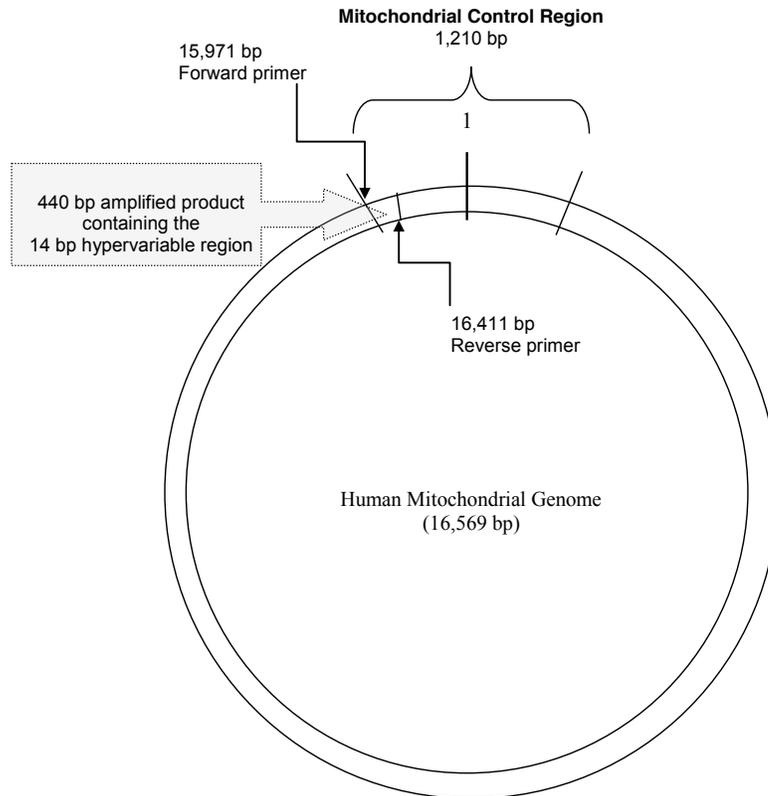


Figure 3. Human mitochondrial DNA map including the targeted hypervariable region. A 440 base pair (bp) region was amplified within the mitochondrial control region. The locations of the Forward (5'-TTAACTCCACCATAGCACC-3') and Reverse (5'-GAGGATGGTGGTCAAGGGAC-3') primers are indicated. The researched 14 bp hypervariable region is located within the 440 bp amplified region. Graphic produced and edited by Sharon Clarke.

Material and Methods:

DNA isolation and amplification were completed using the Cold Spring Harbor Dolan Learning Center protocol (<http://www.geneticorigins.org/mito/mitoframeset.htm>). Briefly, each participant swished 10 mL of saline (0.9% NaCl) for 30 seconds; this solution was expelled into a collection tube. Each tube was carefully numbered and labeled accordingly (for example: Japanese 1, Japanese 2, Chinese 1, Control

1, etc..). One mL of each sample was spun in a microcentrifuge for one minute to concentrate the cheek cells within the saline solution. Once concentrated, the excess saline solution was poured off and the pellet of cheek cells was suspended in 100 µl of a Chelex solution. This mixture was boiled for 10 minutes. After boiling, each sample was spun in a microcentrifuge for 1 minute. Thirty microliters were drawn from the top of each spun sample and placed into the labeled tubes. These served as the DNA source for all of the samples. Amplification

was done according to the above mentioned protocol with the following exceptions:

- 15.0µl dH₂O, 2.5µl of 20µM forward primer, 2.5µl of 20µM reverse primer and 5µl of human DNA were added to the Ready to Go™ PCR tube. This replaced the 22.5µl pre-mixed (water, primer mixture) and 2.5µl of DNA.
- The thermal cycler was programmed for 35 cycles instead of 30 cycles.
- The annealing temperature was set to 54°C rather than 58°C.

Following DNA amplification by Polymerase Chain Reaction (PCR), 10µl of 25µl (stock) sample were removed for gel electrophoresis. Then 2.5µl of a 5x loading dye were added to each sample. The samples were then loaded into a 2% agarose gel. Each gel also received 10µl of a 100 base pair standard. Once a 440 base pair band was visually confirmed in the agarose gel for each sample, the stock sample was used to retrieve another 10µl amount which was then sent to the Dolan DNA Learning Center (according to their instructions found at: <http://www.geneticorigins.org/mito/mitoframeset.htm>) at Cold Spring Harbor, NY to be sequenced.

The sequenced samples were accessed using the Dolan Learning Center database (<http://www.bioservers.org/html/sequences/sequences>). Analysis required locating the 14-base pair hypervariable region within the 440 sequenced bases. The 14-base pair hypervariable region immediately

follows the sequence CACATC, which is located approximately 182 bases within the 440 sequenced bases.

Sequence comparisons were determined using CLUSTAL W (<http://align.genome.jp/>). This program determined the number of nucleotide differences amongst the sequences.

The phylogenetic tree was created using on-line San Diego Supercomputer Center (SDSC) Biology Workbench program (<http://workbench.sdsc.edu/>).

Results:

From the 75 mtDNA samples analyzed, there were 23 different 14-base pair sequences (Table 1). The conserved 14-base pair hypervariable region was found in 46% of the Japanese population, 59% of the Chinese population, and 75% of the control group. This conserved region is represented by AAAACCCCTCCCC (Table 1). In addition to this, there were two other sequences (AAAACCCCCCCCC, AAAACCCTCCCCC) found in all three sample groups, although they occurred in far less frequency than the conserved region. It was also noted that each population had unique mutations within the 14-base pair sequence. The Japanese sample group had twelve other 14-base pair sequences in addition to the conserved one, the Chinese samples had six, and the Control group had two additional and distinctive 14-base pair sequences (Table 1).

Table 1. Observed 14 base pair hypervariable sequences found within the tested Japanese, Chinese, and Control samples

	14 bp mtDNA Sequences	Number of Subjects			Total
		Control	Japanese	Chinese	
1	AAAACCCCTCCCC*	15	13	16	44
2	AAAACCCCGTCCCC	1			1
3	AAAACCCCGCCCC	2	1	2	5
4	AACACCCCGCCCC	1			1
5	AAAACCTCCCCC	1	2	1	4
6	AACCCCGCCCC		1		1
7	AAAACCCCTCCCC		1		1
8	AAACCCCTCCCC		1		1
9	AAAACCCCTCGCC		1		1
10	AAACCCCTCCCG		1		1
11	AACCCCGCCCCC		1		1
12	ACAACCCCTCCCC		1		1
13	AAACCCCTCTC		1		1
14	AAAAACCCCGCCCC		1		1
15	AAATAACCCCGCTCC		1		1
16	AAATCCCGCTCC		1		1
17	AAACCTC		1		1
18	AAACCCCGCCCC			2	2
19	AAAACCCCTCCTC			2	2
20	AAAACCTCCCCC			1	1
21	AAACCCCGCCCC			1	1
22	AAAATCCCGCCCC			1	1
23	AACCCCGCCCC			1	1
	Total	20	28	27	75

* conserved sequence.

It was noted that the Japanese group had the greatest number (48) of total mutations (Table 2). In addition, they also had the greatest number of mutations in each of the categories (Table 2). This included insertions, deletions, transversion, and transition point mutations. All three groups tested

had a variety of point mutations. Finally, the Control group had no deletions and insertions, and just a small number of transversion and transition point mutations. The most common mutation for all three groups was a transition from a T to a C (Table 2).

Table 2. Observed number of nucleotide substitutions, deletions and insertions found with the three tested populations.

Mutation	Control	Japanese	Chinese
Transition			
T→C	4	11	7
C→T	1	6	4
Total	5	17	11
Transversion			
C→G	2	2	0
C→A	0	3	0
A→C	1	4	0
Total	3	9	0
Deletion			
A→_	0	6	4
C→_	0	4	
Total	0	10	4
Insertion			
_→A	0	1	0
_→T	0	1	0
_→C	0	10	7
Total	0	12	7

Discussion

The experimental results supported the data in the published literature. The experimental conserved 14-base pair region was very close to the published conserved 14-base pair region (Horai et al., 1993). There were two sequences, in addition to the conserved sequence, that were common in all three sampled groups (AAAACCCCCCCCCC and AAAACCCTCCCCC in Table 1).

The Japanese samples were seen to have a higher mutation rate than the Chinese or Control group sequences and as a result had a greater variety of 14-base pair sequences (Table 1). This may be due to the fact that most of the Japanese samples were from students from heavily populated regions of Japan (including Tokyo). It is a possibility that pollution, or other epigenetic factors associated with heavily populated areas could affect the DNA by causing mutations. Also, it should be noted that a number of the Japanese students are descendents of family members who lived close to where the atomic bombs were detonated during World War II. The radiation

could have induced additional mutations. However, neither one of these hypotheses were supported through this research.

The Chinese samples were also observed to have a higher mutation rate than the Control group. This could be due to the fact that a majority of the individuals sampled came from the eastern part of China where industrial development is heavy. Here again, pollution could be an epigenetic factor, and may have caused mutations in the DNA sequence.

As mentioned, part of the research findings were very close to those in the Horai et al. (1993) publication. Both studies had the same conserved 14-base pair sequence. In the Horai et al. (1993) report, 55.8% of the combined Asian and Control samples had the conserved sequence, while this investigation found the conserved 14-base pair sequence in 58.7% of the samples tested (Table 1). In the Horai report, investigators found 19 different 14-base pair sequences within the mitochondrial hypervariable region, while this study ended with 23 different

14-base pair sequences (Table 1). The additional four sequences were found within the Japanese test group. Out of the 23 different sequences found, 12 of these sequences were found in the Japanese population alone.

Educational Components:

This investigation indicated the usefulness of bioinformatics in order to show both relationships and difference in the sequences tested. This included the CLUSTAL program which showed conserved regions and another program that can be used (but was not included in this article) was a phylogenetic tree program which shows relationships of DNA sequences. Also, the experiment demonstrated how a research project in a college genetics course can be built on previous research and publications for the purpose of comparing experimental results to those already published (Horai et al, 1993). All of these mentioned comments address the Nature of Science:

- Ask a question
- Explore and discover
- Test ideas
- Analyze the outcomes
- Consider the next step

The 14 base pair hypervariable region is one of the easiest DNA sequences to acquire mutations. From the results, the sequences showed similarities of different ethnic groups at the genetic level, especially

References

ANDERSON, S., BANKIER, A.T., BARREL, B.G., DE BRUIJN, M. H., COULSON, A.R., SANGER, F., SCHREIER, P. H., SMITH, A.J.H., STADEN, R. AND YOUNG, G. (1981). Sequence and organization of the human mitochondrial genome. *Nature* 290:457-465.

CLUSTAL W. San Diego Supercomputer Center (SDSC) Biology Workbench, Accessed from <http://seqtool.sdsc.edu/CGI/BW.cgi> on September 2008. Used for creating Phylogenetic tree.

DOLAN DNA LEARNING CENTER 2001 Annual Report. Accessed from <http://www.dnalc.org/ddnals/about/annreppdf/annrep2001.pdf> on July 5th, 2009

DOLAN DNA LEARNING CENTER, *Sequence Server*. Accessed from <http://www.bioservers.org/html/sequences/sequences.html> on September 23rd, 2008. Used for obtaining sequences.

similarities between the Japanese and Chinese individuals. In addition to isolating and amplifying the DNA, it was also important for students to analyze and present their data which they did at both a state and national conference.

Not all biology curricula can provide students an opportunity to isolate and amplify DNA, but there is still an opportunity to engage students in data mining. This allows them to compare sequences already in databases using bioinformatics. For example, the 14-base pair hypervariable region studies in this inquiry based experience can be examined within other ethnic groups found listed in the Dolan DNA Learning Center database. Within this database alone are sequences from African, European and Native American populations for the mitochondrial control region. Comparisons can be made using the same approach as was done in this study. Even though one may not have the opportunity to conduct a research project, using literature and present databases for data mining can still provide a very meaningful learning experience.

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HORAI, S., K. HAYASAKA. 1990. Intraspecific Nucleotide Sequence Differences in the Major Noncoding Region of Human Mitochondrial DNA. *American Journal of Human Genetic* 46:828-842.

HORAI, S., KONDO, R., NAKAGAWA-HATTORI, Y., HAYASHI, S., SONODA, S., *et al.* 1993. Peopling of the Americas, Founded by Four Major Lineages of Mitochondrial DNA. *Molecular Biology and Evolution*. 10(1): 23-47.

Kyoto University Bioinformatics Center, Multiple Sequence Alignment. Accessed from <http://align.genome.jp/> on September 2008. Used for creating CLUSTAL sequences.

LEWIS *et al.* 2007. Mitochondrial DNA and the peopling of South America. *Human Biology*. April 2007 v 79(2): 159-178.

MULTIPLE SEQUENCE ALIGNMENT BY CLUSTAL W program. Accessed from <http://align.genome.jp/> on September 13, 2008. Used for creating Phylogenetic tree.

WALKER, JERILYN A., RANDALL K. GARBER, DALE
J. HEDGES, GALE E. KILROY, JINCHUAN XING, *et al.*

2003. Resolution of Mixed DNA Samples Using
Mitochondrial DNA Sequence Variants. *Analytical
Biochemistry* 325:171-173

Regulation of Water in Plant Cells

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Abstract: Cell water relationships are important topics to be included in cell biology courses. Differences exist in the control of water relationships in plant cells relative to control in animal cells. One important reason for these differences is that turgor pressure is a consideration in plant cells. Diffusion and osmosis are the underlying factors involved in the control of water in plant cells; however, additional attention must be given to osmotic pressure, osmotic potential, and water potential. This discussion shows how these parameters relate to each other, and how they are explained by thermodynamics and the universal gas laws. Detailed laboratory exercises are described that demonstrate these principles. The laboratory exercises include data collection, graphing of data, statistical analysis of data, and calculations of osmotic potential and diffusion pressure deficit from the data.

Keywords: Turgor, osmotic pressure, osmotic potential, water potential, diffusion pressure deficit

Introduction

Cell water relationships are deemed by most cell biologists to be an important topic for inclusion in cell biology courses. Still, some cell biology textbooks do not thoroughly cover the topic. In most textbooks, the topic is at least discussed with an explanation of osmosis; however, some of these discussions are without any reference to the thermodynamics involved. In other cases, there is a complete omission of the concept of turgidity. The control of water in plant cells is considerably different from the control in animal cells because of cell walls and turgor, a topic not always covered in detail in some cases.

Almost all of the textbooks reviewed have general explanations of osmosis. Pollard and Earnshaw (2002), however, did not include any discussion on the subject. In addition to osmosis, a number of textbooks also included some discussion on water relationships in plants by providing descriptions of plant cell walls, vacuoles, turgor, and plasmolysis. The concept of thermodynamics, however, was not related to water movement in these textbooks (Bolsover *et al.*, 2004; Lewin *et al.*, 2007; Karp, 2008; Alberts *et al.*, 2010). Cooper and Hausman (2007) included an excellent discussion of the contrast of water relationships between plant and animal cells. Becker *et al.* (2009) additionally discusses the 2nd Law of Thermodynamics, free energy, and entropy. The most quantitative descriptions of water movement were found in

outdated textbook editions that are probably out of print (Wolfe, 1993; Tobin and Morel, 1997).

Background Information

Osmosis, in a very technical sense, is the movement of water across a membrane whereby the net movement occurs from a lesser negative water potential to a more negative water potential. The following discussion explains the basis of that definition. Osmosis is an event of diffusion, and it occurs because of the random movement of molecules due to kinetic energy. Diffusion is the tendency of molecules to undergo a net movement in the direction of lesser concentration. The rate of diffusion is proportional to one over the square root of the molecular weight of the molecule involved. Temperature, the distance to travel, and the area of diffusion are other factors involved in the rate of diffusion. A diffusion gradient, therefore, will result in a substance undergoing a net movement from a region of greater concentration (C_1) towards a region of lesser concentration (C_2). The diffusion of solute particles is similar to the diffusion of gases, except that the particles are in a solvent. Hence, gas laws can be applied in order to analyze the movement of solutes.

Osmosis generally refers to the net movement of water across a membrane. Osmosis occurs, for example, when an aqueous solution is separated from pure water by a semi-permeable membrane. One way to demonstrate osmosis is a simple set-up using a thistle tube. For example,

consider the thistle tube containing a sucrose solution, covered with a semi-permeable membrane, and placed into a beaker of pure water (Figure 1). Such a device, called an osmometer, is a simple way to measure osmotic pressure in addition to demonstrating osmosis. The aqueous solution in the thistle tube will rise because of the entrance of water from the beaker due to osmosis. The amount of rise of water in the tube, designated h for height, is a crude but valid way to measure osmotic pressure. A more sophisticated osmometer is a device equipped with a moveable piston in the tube connected to a pressure gauge. Such a system would provide a more quantitative measure of the amount of pressure necessary to prevent water from rising in the system's tube due to osmosis.

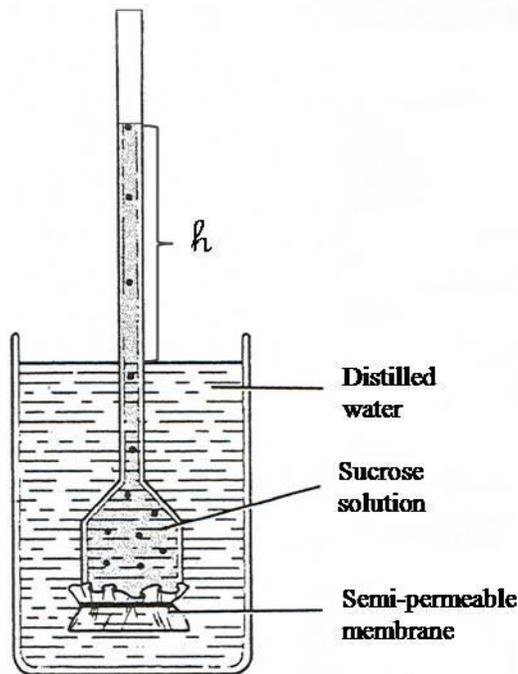


Figure 1. A typical demonstration of osmosis. The rise of water (h) represents osmotic pressure.

Unconfined aqueous solutions not in an osmometer also have an osmotic pressure. However, such solutions are described as having an osmotic potential (OP) rather than an osmotic pressure. All aqueous solutions have an osmotic potential. A beaker of tap water, a cup of coffee, a mud puddle, etc. all have an osmotic potential if solute particles are dissolved in the water forming the solvent of the solution. The magnitude of the osmotic potential is based on the colligative property of the solution; that is, OP is directly proportional to the solute

concentration. The colligative property is determined solely by the number of solute molecules per unit of the solvent volume and not by the size of the molecules. For example, no difference in the colligative property exists between soluble large protein molecules and an equal number of soluble miniscule ions. Osmotic potential is referred to as Ψ_s (psi solutes).

Additional terms and their acronyms are often applied to water relationships in both animal and plant cells. The concept of thermodynamics (second law) and free energy can be incorporated into the understanding of these relationships. Water potential given as Ψ_w (psi water) is a good starting point. Water potential is an expression of the free energy of water, and it is equal to the difference between the free energy of water in an aqueous solution at any point in a biological system and that of the free energy of pure water under standard conditions. Water potential reflects the ability of water to diffuse. Pure water has the highest Ψ_w , and it is given the arbitrary value of 0. Therefore, all water potentials of solutions are less than 0 meaning they have a negative value. Water potential is actually equal to osmotic potential when turgor is not involved.

The departure of water potential of a solution from the water potential of pure water (0 value) can be expressed using a thermodynamic equation:

$$\Psi_w = R T \ln (e/e^0)$$

where R = ideal gas constant (1.987), T = absolute temperature (degrees K), \ln = the natural log with base 2.718, e^0 = vapor pressure of pure water, and e = vapor pressure of water in the system. Calculations are usually made in bars or atmospheres. When e/e^0 is less than 1, Ψ_w will be calculated as a negative number, the usual situation for an aqueous solution. The more negative the water potential of a system, the greater the tendency for water to diffuse into it. In other words, the more solute particles, the more negative the value assigned to the water potential.

In animal cells, water potential (Ψ_w) is equal to osmotic potential (Ψ_s). Animal cells regulate the movement of water by controlling the movement of ions; that is, they cope with osmotic pressure by balancing ionic concentrations on the two sides of the cell membrane. Also, animal cells have membranes containing aquaporins or water channels. In this way, animal cells can, to some extent, prevent

severe swelling or shriveling. In this way animal cells have selective water transport mechanisms.

With plant cells, turgor (Ψ_{tp}) must be considered. Plant cells are highly vacuolated and thus they act like an osmometer. Vacuoles are selectively permeable and can make up 75% of the volume of a plant cell, accumulating high concentrations of soluble molecules. Turgor pressure occurs due to osmotic pressure that causes the cell to take in water and, in turn, causes the cell contents to push against the cell wall. An equal and opposite pressure to turgor pressure is wall pressure (Ψ_{wp}). The cell walls of plants are relatively inelastic, and this rigidity is sufficient to prevent plant cells from bursting under osmotic pressure. In plant cells, therefore, overall water potential is equal to osmotic potential plus turgor pressure,

$$\Psi_w = \Psi_s + \Psi_{tp}$$

Matrix (Ψ_m) forces, which are water-binding forces, can also be involved in water uptake by plant cells, but these forces are generally small and therefore ignored. As previously mentioned, Ψ_s is negative but Ψ_{tp} is a positive pressure, and the two pressures work in opposition to each other. Ψ_w is made less negative by turgor. When a cell is fully turgid, water flows equally in both directions across the membrane, and no net water flow occurs.

It is evident then, that water relationships of plant cells can extend beyond the usual definition of “water movement occurs from greater concentration to lesser concentration.” Nonetheless, solutions in the external environments of plant cells that have greater solute concentrations than the cellular contents will draw water out of plant cells, causing wilting. Plants residing along the edges of sidewalks are often killed because of salt application to the walkways. This same situation can become problematic for farmers who apply too much fertilizer on their crops (called burning). On the other hand, these water relationships also explain why vegetable sections of grocery stores are periodically sprayed with tap water. The maneuver keeps the plant tissues turgid and more appealing to consumers. Turgor pressure also contributes to the movement of water into roots, and to some extent to the movement of water through the cellular tissue in stems. Root cells contain protein molecules in solution to cause water to flow into the plant.

Laboratory Exercises

Maintenance of the physiologically active state in cells and whole organisms depends upon the relative constancy of a number of conditions, one of which is a favorable water balance. Plant cells are ideal for demonstrating this concept. The purpose of this exercise is to learn about the physical principles governing net water fluxes in osmotic systems and to become familiar with simple methods for measuring water parameters in plant cells. These exercises, however, require meticulous attention to measurement techniques. The data collected are conducive to statistical analyses.

PART 1. Determination of the osmotic potential (OP) of plant cells

Use clean watch glasses (or comparably small glass vessels) to set up the following series of sucrose solutions made with distilled water. Volumes of 10 mL can be made for each vessel by mixing 1.0 M sucrose with distilled water in appropriate proportions. For example, combining 5.5 mL of 1.0 M sucrose solution with 4.5 mL of distilled water makes the 0.55 M sucrose solution. Concentrations other than those listed sometimes have to be used, depending on the osmotic potential of the tissue.

0.55 M	0.35 M	0.15M
0.50 M	0.30 M	0.10 M
0.45 M	0.25 M	0.05 M
0.40 M	0.20 M	0.00 M

A red onion is one type of tissue that can be used in this exercise. Carefully obtain thin layers of the membrane-like layer of tissue from the outer purplish-red side of concave pieces of the bulb. This layer is partly made up of pigmented cells, making microscopic examination of the protoplasm (cellular contents) relatively easy. Use small strips of the tissue (3 to 10 mm long) to avoid folding of the tissue in the sucrose solutions. Tissue samples are obtained by bending a layered piece of the onion bulb towards the purple side until it breaks and then gently pulling it apart. Use a forceps to obtain small sections of the thin tissue, carefully choosing tissue that shows considerable color. This tissue is usually only one cell layer thick. Students who react to onion odor may want to wear goggles and gloves. Place a strip of tissue into each of the sucrose preparations at approximately 2-minute time intervals. Make sure that the tissue stays submerged. A small paper clip can be placed on the tissue to prevent it from floating. At about 45 minutes after submergence, mount the tissue on a blank slide with a small drop of the same solution in which it was submerged and apply a cover

glass. Examine the dark red cells for plasmolysis using the 10X objective lens of the microscope. Ignore those cells devoid of color, which in some cases could be a considerable number. Plasmolysis is the shrinkage of the protoplasm and thus its separation from the cell wall due to loss of water. Score 40 to 60 cells from each solution as plasmolyzed or not plasmolyzed. Any amount of protoplasm shrinkage should be scored as a plasmolyzed cell (Figures 2 and 3).

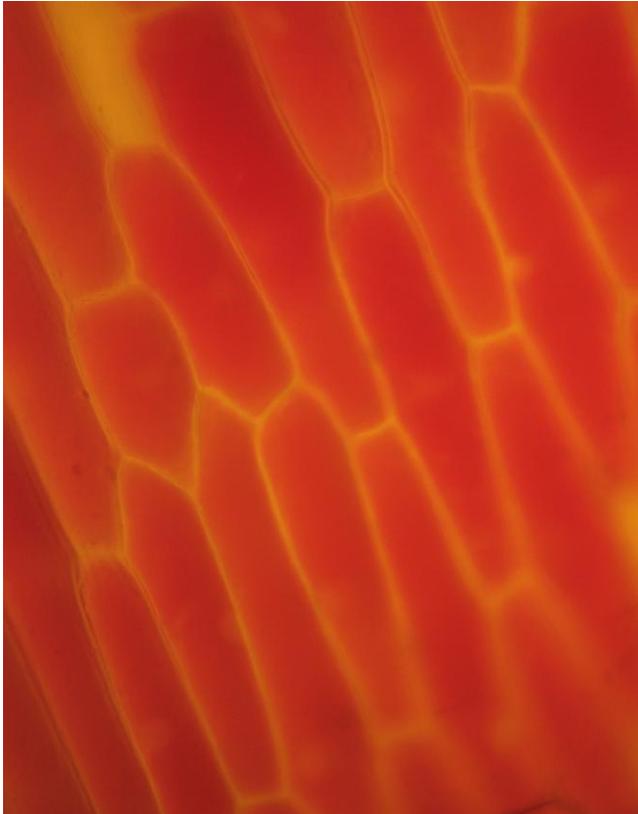


Figure 2. Red onion cells in distilled water showing all cells fully filled with protoplasm. Cells completely without color (upper left) are not scored.

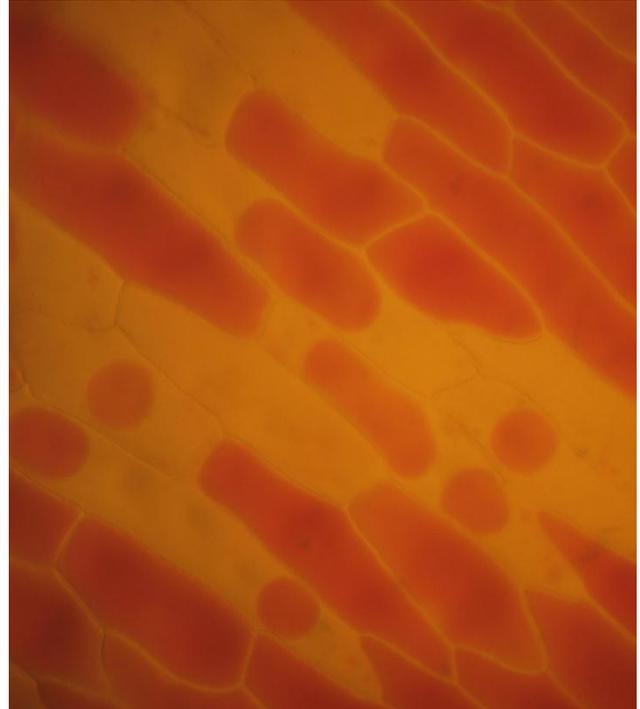


Figure 3. Red onion cells in a sucrose solution. Note cells undergoing plasmolysis among other cells without plasmolysis activity. Again, several cells are void of any color and are not scored.

Other tissues such as certain plant leaves can be used to determine OP, especially if they contain anthocyanin. The lower epidermis of leaves from *Rhoeo discolor* is a good source. However, an occasional student may be allergic to handling these leaves.

Record the number of cells plasmolyzed and not plasmolyzed from the series of solutions (See Table 1 for sample data). Then plot the percentage of cells plasmolyzed against the molarities using regression analysis (Figure 4). Vernier Graphical Analysis was used in this case. More reliable results are obtained if class data are combined. The concentration in which 50% of the cells are plasmolyzed is arbitrarily defined as incipient plasmolysis. Incipient plasmolysis is considered to be the same molarity as the osmotic potential of the cells. Always use the point of 50% plasmolysis obtained by the regression equation since that calculation is based on 10 to 12 different data points.

Table 1. Sample data plotting percent plasmolyzed cells of onion tissue over a range of different sucrose concentrations

Sucrose (M)	Plasmolyzed cells	Total cells	Percent Plasmolyzed cells
0.55	50	50	100
0.50	46	50	92.0
0.45	50	64	78.1
0.40	44	64	68.8
0.35	25	50	50.0
0.30	12	62	19.4
0.25	5	48	10.4
0.20	3	55	5.5
0.15	2	57	3.5
0.10	1	41	2.4
0.05	0	40	0.0

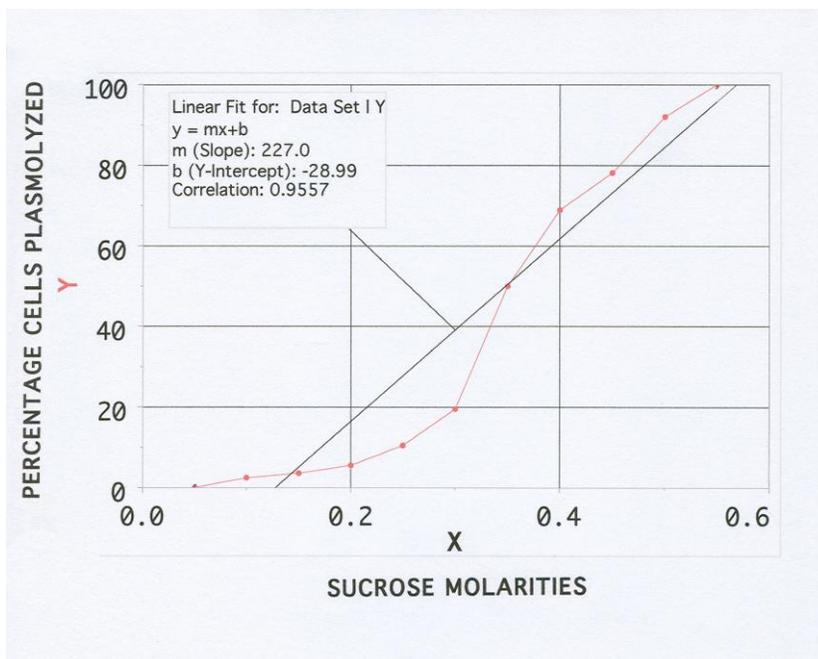


Figure 4. Regression analysis and linear fit of sample data generated by plotting percent cells undergoing plasmolysis on the Y-axis versus sucrose molarities on the X-axis. Incipient plasmolysis is arbitrarily assigned as 50% plasmolysis and used to determine OP.

The osmotic potential of the cells can be calculated with the following equation. The units are atmospheres, a familiar unit of measurement for pressure.

$$OP = (22.4) \times (M) \times (T)/273$$

Where: OP is osmotic potential
M is the molarity of the external solution at incipient plasmolysis
T is the absolute temperature calculated as room temperature in degrees Celcius plus 273

PART 2. Determination of the diffusion pressure deficit (DPD) of cells

Diffusion pressure deficit is older terminology, but still useful for analysis purposes. DPD is the difference in diffusion pressures between an aqueous solution and pure water. In the case of plant cells, $DPD = OP + TP$ where OP is negative and TP is positive. DPD is an actual deficit and therefore it too is regarded as an absolute quantity (positive).

Prepare the sucrose solutions of the following molarities by appropriately diluting a 1.0 M sucrose solution. Make volumes of 100 mL in clean beakers for this series. For example, the 0.55 M solution would be made by combining 55 mL of 1.0 M sucrose solution with 45 mL of distilled water. Again, other concentrations can be used depending on the DPD of the tissue.

0.55 M	0.35 M	0.15 M
0.50 M	0.30 M	0.10 M
0.45 M	0.25 M	0.05 M
0.40 M	0.20 M	0.00 M

The following steps should be carried out as quickly as possible. Use a standard cork borer with about an 8 mm diameter to push out a cylinder of tissue from a potato, beet, or similar plant tissue (beet is used for demonstration in this exercise). Discard the hard outer covering at the ends of the cylinder and cut about 5 cm of the cylinder into disks, each disk approximately 3 mm in thickness. If the tissue is hard and rigid, it may be necessary to remove the cork borer with the help of pliers. Also a metal rod may be necessary to force the cylinder of tissue out of the borer. If using beets, wear gloves. Weigh the complete group of tissue disks from one cylinder and immediately place the group of disks into one of the

solutions. Repeat this procedure for each of the sucrose solutions. Use a separate weigh paper each time.

After about 75 minutes, remove the tissue by pouring the solution with the tissue into an ordinary strainer. When dripping has stopped, very briefly blot the tissue in paper toweling to remove excess wetting due to the solution and weigh. Repeat this procedure for all samples, again using separate weigh papers each time. Keep the technique as uniform as possible from one sample to another. Using the equation below, calculate the percent change in weight in each case. These solutions can result in either positive or negative percent weight changes (Table 2).

$$\text{Percent change in weight} = \frac{\text{final weight} - \text{original weight}}{\text{original weight}} \times 100$$

Plot the percent changes in weight against the molarities using regression analysis (Figure 5). Again, results are more reliable when class data are combined. Use the equation below to calculate the diffusion pressure deficit (DPD) of the tissue. In this case, M is the sucrose concentration in which no change in weight occurred. The units are in atmospheres.

$$DPD = (22.4) \times (M) \times (T)/273$$

Analyses

Sample osmotic potential (OP) data are shown in Table 1, and these data when graphed are illustrated in Figure 4. The osmotic potential calculated from these sample data by regression analysis is shown below in which Y is set at 50 because osmotic potential is assumed to occur at the concentration in which 50% of the cells underwent plasmolysis:

$$y = (m)(x) + b$$

$$m \text{ (slope)} = 227.0 \text{ and } b \text{ (intercept)} = -28.99$$

$$50 = (227.0)(x) + (-28.99)$$

$$\text{Solving for } x \text{ at } y = 50 \text{ is } .348$$

The correlation of these data is 0.96

This molarity (.348) in which 50% of the cells were plasmolyzed at 23 degrees C (room temperature) is placed into the following equation:

$$OP = (22.4) (.348) (296/273) = 8.45 \text{ atmospheres}$$

Table 2. Sample data plotting percent change in weight of beet tissue over a range of different sucrose concentrations

Sucrose (M)	Original weight in g	Final weight in g	Change in weight in percent
0.55	2.865	2.460	- 14.14
0.50	2.732	2.407	- 11.90
0.45	2.807	2.666	-5.02
0.40	2.474	2.422	-2.10
0.35	3.101	3.152	+1.64
0.30	3.060	3.118	+1.90
0.25	2.549	2.642	+3.65
0.20	2.801	2.899	+3.50
0.15	2.357	2.428	+3.01
0.10	2.675	2.754	+2.95
0.05	2.413	2.528	+4.77
0.00	2.880	3.060	+6.25

Sample diffusion pressure deficit (DPD) data are shown in Table 2, and these data when graphed are illustrated in Figure 5. The diffusion pressure deficit calculated by regression analysis is shown below in which (x) is set at 0 because this is the point in which no loss or gain in weight occurred in the tissue:

$$y = (m) (x) + b$$

$$m \text{ (slope)} = -0.024 \text{ and } b \text{ (intercept)} = 0.264$$

$$y = (-0.024) (0) + 0.264$$

Solving for y at x = 0 is 0.264

The correlation of these data is - 0.88

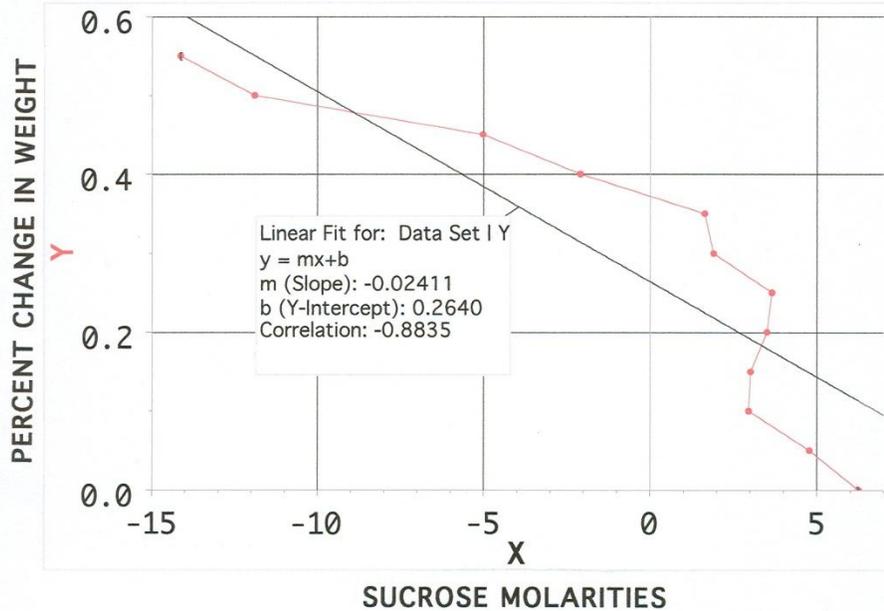


Figure 5. Regression analysis and linear fit of sample data generated by plotting percent change in weight on the X-axis versus sucrose molarities on the Y-axis. The percent change of weight equal to 0 is used to determine DPD.

This molarity (0.264) in which 0% change in weight occurred at 23 degrees C (room temperature) is placed into the following equation:
 $DPD = (22.4) (.264) (296/273) = 6.41$ atmospheres

Additional Explanations

Turgor pressure (TP) is equal to $-OP + DPD$, and this parameter could be calculated if the OP and DPD determinations were made from the same type of tissue. However, TP is always a positive quantity.

Osmotic relationships can be likened to the gas laws. The ideal gas law equation is given as follows:

$$P V = n R T$$

Where:

- P = pressure
- V = volume
- n = number of molecules
- R = universal gas constant
- T = temperature Kelvin

Therefore, $P = nRT/V$

Mathematically, this is the same as $n/V R T$
 And n/V is equal to M
 Hence, $P = M R T$

One mole of gas occupies a volume of 22.4 liters at 273 degrees K.

Thus, pressure = $(22.4) (M) (Temperature K/273)$, and the units of measurements are in atmospheres.

Osmotic potential is determined at the point of incipient plasmolysis. All turgor pressure has been removed at this point. Therefore, the osmotic potential in the cell is equal to the osmotic potential of the particular sucrose solution causing incipient plasmolysis without turgor pressure interfering with the measurement. Diffusion pressure deficit, on the other hand, is determined as the point in which no loss or gain in weight occurs in the tissue. Consequently, the measurement at this point takes into account both the osmotic potential and the turgor pressure of the cell, resulting in the diffusion pressure deficit.

References

- ALBERTS, B., BRAY, D., HOPKINS, K., JOHNSON, A., LEWIS, J., RAFF, M., ROBERTS, K., AND P. WALTER. 2010. *Essential Cell Biology*. Garland Science, New York, NY. 731p.
- BECKER, W.M., KLEINSMITH, L.J., HARDIN, J., AND G.P. BERTONI. 2009. *The World of the Cell*. Pearson Benjamin Cummings, San Francisco, CA. 789p.
- BOLSOVER, S.R., HYAMS, J.S., SHEPARD, E.A., WHITE, H.A., AND C.G. WIEDEMANN. 2004. *Cell Biology*. John Wiley & Sons, Inc., Hoboken, NJ. 531p.
- COOPER, G.M. AND R.E. HAUSMAN. 2007. *The Cell. A Molecular Approach*. The American Society for Microbiology Press, Washington, DC. 820p.
- KARP, G. 2008. *Cell and Molecular Biology*. John Wiley & Sons, Inc., Hoboken, NJ. 776p.
- LEWIN, B., CASSIMERIS, L., LINGAPPA, V.R., AND G. POPPER. 2007. *Cells*. Jones and Bartlett Publishers, Boston, MA. 863p.
- POLLARD, T.D. AND W.C. EARNSHAW. 2002. *Cell Biology*. Saunders, Philadelphia, PA. 805p.
- TOBIN, A.J. AND P.E. MOREL. 1997. *Asking About Cells*. Brooks/Cole, U.S.A. 698p.
- WOLFE, S.L. 1993. *Molecular and Cellular Biology*. Wadsworth Publishing Co., Belmont, CA. 1145p.

A Module-Based Environmental Science Course for Teaching Ecology to Non-Majors

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Abstract: Using module-based courses has been suggested to improve undergraduate science courses. A course based around a series of modules focused on major environmental issues might be an effective way to teach non-science majors about ecology and ecology's role in helping to solve environmental problems. I have used such a module-based environmental science course for non-majors. The course is divided into 5 modules, with each module addressing a key environmental issue, specifically global climate change, human population growth, sustainable use of natural resources, habitat loss, and the value of biodiversity. Each module follows the same basic structure: 1) an introduction to the question, 2) an investigation of the basic science underlying the question, and 3) an investigation and discussion of the human aspect of the question (i.e., what can we do?, what are the costs and benefits of addressing the question?, are there any social, economic, or cultural factors that affect our ability to address the issue?, etc.). Each module has associated laboratory exercises that culminate with field-based student-designed surveys of biodiversity on the Denison University Biological Reserve. The module-nature of the course allows for the integration of science and non-science disciplines around basic environmental questions, and appears to be an effective means of teaching environmental science and ecology to non-majors.

Keywords: ecology, environmental science, module-based, non-majors course

Introduction

Teaching ecology to non-majors, especially non-science majors, can often be challenging as students sometimes do not feel or see a need to learn what they see as material that is irrelevant to their real life or future career. Linking the teaching of ecology to discussions of real world environmental problems or controversies is one way to engage students in the study of ecological principles (Pallant, 1996; Gill and Burke, 1999; Battles et al., 2003). Different approaches to making these connections have been suggested (e.g., use of service-learning, Bixby et al., 2003; problem-based learning, Keller, 2002). Here I describe a course for first-year non-science majors designed around modules that use major environmental issues as a context in which students can learn and apply basic ecological principles to questions they may encounter as citizens once they leave college.

The Course.-- The course is a First-year Seminar, a writing intensive course that is part of the first-year experience at Denison University. The particular course, FYS102, is a multi-section course with each section taught by a different professor and focusing on a different theme related to the professor's

discipline - thus there are science, humanities, social science, and fine arts sections. Each section of FYS102 has 12 – 20 students, and represents a self-contained course lasting 14 weeks. My section was focused on the ecology of environmental problems and fulfilled a lab science general education requirement, as well as a quantitative reasoning competency requirement. My course met for three 1-hour long "lecture" periods and one 3-hour long lab period each week. I have taught this course twice (Spring 2005, Spring 2008). Both times I taught the course the majority of students were non-science majors, although in Spring 2008 there were a substantial minority of students who were science majors or environmental studies (ENVS) majors (in Spring 2008, this course could count as a core course for ENVS majors).

Basic Course Organization.-- The course was organized around 5 modules covering a variety of environmental issues and each module was focused on a guiding question. Each module started with an introductory exercise, explored the underlying science, and concluded with an exploration of broader issues (see Table 1 for details of the content covered in each module).

Table 1. Summary of content of the five modules used in the environmental science course for non-majors.

	Science Content	Other Considerations
Module 1: Is the Climate Changing?	<p>Determinants of climate – local determinants, Hadley cells, global determinants, ocean circulation</p> <p>History of Earth’s climate – ice age periodicity, what causes heating and cooling cycles</p> <p>Greenhouse effect – greenhouse gases, mechanisms</p> <p>Evidence of climate change – evidence of changes in CO₂, instrumental records, changes in glaciers, biological evidence,</p> <p>Predictions of future climate change – climate models</p> <p>Biotic consequences of climate change – changes in plant and animal distributions, phenological changes, health related issues</p>	<p>Economic and health-related consequences of climate change</p> <p>Political issues – Kyoto Protocol</p>
Module 2: Can the human population grow indefinitely?	<p>Life tables – m_x, calculating l_x, R_0, survivorship and fecundity curves</p> <p>Lotka-Volterra population equations – exponential and logistic forms</p> <p>Carrying capacity – definition, can it change?</p>	<p>Social, cultural, political, and religious aspects of addressing human population growth</p>
Module 3: Can exploitation of natural resources be sustainable?	<p>Food webs – trophic levels, food chains, terminology</p> <p>Trophic cascades – top-down control vs bottom-up control</p> <p>Keystone species – definition, how determine a keystone species, role in community structure</p> <p>Direct and indirect effects – definitions, how generated in food webs</p> <p>Ecological efficiencies – calculations of how much energy is lost in primary producers and in consumers, how ecological efficiencies impact how long food chains can be</p> <p>Maximum sustainable yield – definition, relationship to logistic growth curve</p>	<p>“Tragedy of the Commons”</p> <p>Government subsidies – how influence impact of fisheries on fish populations</p> <p>Economic and political issues related to natural resources and fisheries</p>
Module 4: How can the forests of Madagascar be saved?	<p>Edge and matrix effects – definitions, how impact abiotic conditions, how affect populations, how relate to habitat fragmentation and loss</p>	<p>Cultural, socioeconomic, and political aspects of habitat loss and fragmentation in Madagascar</p>

	Minimum viable population – definition	
	Succession – primary vs secondary succession	
Module 5: What is biodiversity worth?	Measures of diversity – species richness, relative abundance, evenness, dominance, Simpson’s Index	Traditional economics -- supply and demand curves
	Patterns and gradients of diversity – latitudinal gradients, altitudinal gradients, species-area curves, explanations for these patterns	Environmental economics – externalities, valuation of natural products and ecosystem services

The labs for each module supplemented and reinforced lecture material. In addition, some labs provided students the skills needed to become informed citizens (e.g., web page critiques in Module 1, biodiversity field survey projects in Modules 4 & 5).

Each module had an associated writing exercise. Such writing assignments can be effective in helping students understand ecological or environmental science concepts (e.g., Tessier, 2006). Writing assignments ranged in length and focus, as well as in style. In addition, each student wrote a media critique in which they compared popular media articles on an environmental problem or topic of their choosing to scientific papers on the same topic. As part of this assignment, I taught students how to access the scientific literature using Biological Abstracts On-Line as well as an electronic journal database provided by a consortium of Ohio College and University libraries. No special instruction was given on how to read a scientific paper beyond a general introduction to the structure of a scientific paper.

MODULE 1: Is the climate changing?

The introductory activity for this module was a screening of “An Inconvenient Truth” that takes place during the very first lab of the semester. Students were asked to note any questions on the science they had while watching the film, as well as any issues or topics (scientific, political, or ethical) that were brought up in the film that they would like to know more about. These observations were then used to guide the content of the rest of the module.

For this module I mostly lectured on the scientific underpinnings of global climate change, including lectures on the history of the Earth’s climate and determinants of climate on Earth, as well as details about the evidence for and potential causes of global climate change. In addition, I assigned topics related to the economic, social/ethical, and

political considerations of global climate change to small groups of students (usually 2 or 3). For example, a group assigned the social/ethical consideration was given the question “From a societal standpoint, what might be some acceptable solutions to global climate change? Are these solutions personal or collective?”, and a group assigned the political consideration was given the question “What are the arguments of each side of the ‘global warming debate’? What are the primary points of tension and disagreement? (focus on USA).” Each group then investigated the question(s) they were assigned and developed a presentation (5 to 10 minutes) that they gave to the rest of the class. I provided time in lab for them to work on gathering their information, primarily from web resources.

Laboratories in this module consisted of a critical evaluation of pro and con websites related to climate change. The initial lab had pairs of students find and evaluate websites from both sides of the climate change debate. I began the lab by discussing what characteristics can be used to determine the quality of a website (i.e., is it a credible source?). At the end of the lab students presented their critiques of the two websites, explaining to their peers how credible the websites are. One particular benefit of this exercise was that these websites could be used by students later in the module as they investigated the “Other Considerations” assignment (see above).

Students were given an assignment to write a brief report to the President outlining the evidence related to global climate change, and based upon this evidence, provide their perspective on what should be done, if anything, to address global climate change. Because FYS102 is supposed to help improve Denison students’ writing skills, this and all other writing assignments described below were graded for both content and composition (i.e., grammar, organization, writing style, etc.).

MODULE 2: Can the human population grow indefinitely?

During the first lecture period for this module, students completed an exercise on von Foerster et al. (1960) based on exercise created by Biology faculty at Earlham College that has students read excerpts and a summary of von Foerster et al.'s (1960) main assumptions, model, and conclusions. Based on population estimates available at the time the paper was written, von Foerster et al. (1960) predicted a "doomsday" in the near future when the human population would instantaneously double. In this exercise, students used up-to-date estimates of the human population (<http://www.census.gov/ipc/www/idb/worldpopinfo.php>) to compare observed human population growth to predicted growth, prompting discussions about what controls human population growth, as well as what can be done to slow human population growth.

I used lecture and worksheets to introduce students to basic population growth models, the concept of carrying capacity, and the use of life table calculations to determine population growth rates (see Appendix I for an example of a worksheet used in this class). Small groups of students (pairs or threes) also answered questions related to human population growth and carrying capacity using their textbooks. Questions included "What determines the carrying capacity for humans? Has this changed over human history?" and "What are some factors that might limit the increase of the human population? In other words how might we change/lower r or R_0 ?" These questions were designed to allow students to consider the ecological basis for human population growth and relate this to the social, cultural, political, and religious aspects of trying to control human population growth. Answers were discussed as a class.

The laboratories for this module included computer simulations using *Populus* (Alstad, 2001), and a cemetery demography exercise (Beiswenger, 1992). In lab, we used computer simulations in the program *Populus* to reinforce lecture material on population growth models (particularly the concepts of r and K). The cemetery demography lab used birth and death dates collected from a local cemetery dating back to the early 1800's. Students then used these birth and death dates to calculate survivorship curves for two periods in early Granville history. Comparisons of these two periods, as well as comparison with dates from a cemetery in South Carolina from the first half of the 19th Century (data provided in Beiswenger, 1992), made students think about how human demography and survivorship

trends have changed over time, and the factors that may be influencing such trends.

For the writing assignment for this module, students were tasked with using the information on population growth, and in particular human population growth, to write an update to von Foerster et al. (1960). They had to evaluate the arguments of the article in light of the new data, determine if human population growth should be controlled, and make recommendations on how to limit human population growth.

MODULE 3: Can exploitation of natural resources be sustainable?

To introduce this module, students read and discussed Zabel et al. (2003), a secondary article that examines how fisheries can influence marine communities. In particular the article considers the relative merits of maximum sustainable yield and ecologically sustainable yield. Students were asked to read the article before coming to class, and generate a list of questions about the article. During class, small groups of students (threes or fours) were asked to answer questions about Zabel et al. (2003), followed by a discussion of these questions as a class.

As with the other modules, I lectured on the ecological background for the module. I used a worksheet to reinforce the students' understanding of ecological efficiencies. I used small groups to explore questions about the broader considerations of fisheries and the exploitation of natural resources. For example, students were asked to consider the "Tragedy of the Commons", the consequences of subsidies and natural fluctuations in fish stocks for the sustainability of fisheries, and possible solutions to allow for more sustainable exploitation of the fisheries. In addition, small groups designed a sustainable fishery and presented their designs to the rest of the class.

To reinforce the concept of keystone species one of the labs used in this module was the keystone predator simulation in Ecobeaker (Meir, 1996) that recreates the classic removal experiments of Paine (1966) by manipulating the presence and absence of species in the rocky intertidal community and seeing how the manipulations affect the community. A second lab used the EDM simulation of fisheries from the collection of software found in BioQuest. This simulation allowed students to explore energy flow through a pond, and along the way students examined the limits to harvesting fish, reinforcing the

concepts of sustainability and the link to other ecosystem parameters, such as ecological efficiency.

For the writing assignment, students wrote short answers to questions arising from Zabel et al. (2003) that students brought to class after reading the paper. Students were required to answer two questions, each chosen from one of two sets of questions. One set of questions contained student-generated questions about the science in Zabel et al. (2003) (e.g., “If the ecosystem can already be altered at the turn of a dime by natural occurrences, is adding a fishery that bad?”). The other set of questions covered the conservation or sustainability issues raised by the article (e.g., “Compare and contrast ‘maximum sustainable yield’ and ‘ecologically sustainable yield’. What are some advantages and disadvantages of these approaches to fisheries?”).

MODULE 4: How can the forests of Madagascar be saved?

In both iterations of the course, the first class period of this module consisted of a screening of the video “Islands of Ghosts”, a video that explores the natural diversity of Madagascar and also considers the challenges this diversity faces, including issues of an economic, cultural, and spiritual nature that might affect the ability to conserve Madagascar’s biodiversity. Students were asked to note any questions or issues that the video raised. In the second iteration, I also invited Dr. Richard Lehtinen of the College of Wooster to give a guest lecture about Madagascar and his research on amphibians in Madagascar. His lecture provided background about habitat fragmentation, amphibians, as well as cultural factors that might affect the conservation of Madagascar’s forests.

Following the video and guest lecture, students answered questions, using their textbook, on the science issues raised by the video compiled from those students generated while watching the video. As part of this exercise, students created a reserve design for Madagascar. As with the basic science material, I used a series of student-generated questions that focused on the socioeconomic, cultural, and political aspects of conservation on Madagascar to prompt small-group discussion, followed by class-wide discussion.

For this module, as well as the final module, the lab periods were devoted to performing a study of the diversity of the Denison University Biological Reserve. Students worked in small groups (3 or 4 students) to design their study, collect and analyze

their data, and present their results in written and oral form. Each group designed their survey to compare the biodiversity of a taxonomic or ecological group (e.g., macroinvertebrates, plants, insects, fish, etc.) between two or more habitats (e.g., pools vs. riffles in a stream, upland vs. lowland woods, pine plantation vs. deciduous woods, etc.). Students designed their studies in consultation with me after getting a tour of the Biological Reserve and suggestions about possible habitats and organisms to study. As part of the design process, I discussed with the students the appropriate survey techniques to use, such as quadrat sampling, pitfall trapping, transect sampling. Based on this discussion, I provided students with the necessary field equipment. Students were given two lab periods to conduct their study, and one lab period to analyze their results statistically. During the field lab periods my TA and I circulated among the groups and helped them get started or trouble shoot any problems with their original design. Students then wrote a scientific paper and gave a 15 minute presentation of their study to the class.

For this module students wrote a plan to save Madagascar’s forest. Their plan had to be based upon the scientific material they learned about park design and habitat fragmentation. However, they also had to explicitly consider the many other considerations that would impact the ability to create such as system. For example, they had to incorporate Malagasy cultural and spiritual norms into their plan, as well as include economic factors (e.g., providing jobs and resources for the Malagasy who use the forests for subsistence).

MODULE 5: What is biodiversity worth?

The first lecture period revisited each of the previous modules and determined the potential economic considerations that each raised. This list formed the basis for thinking about environmental economics.

While most of this module was dedicated to traditional and environmental economics, I lectured on how to measure diversity and how diversity is distributed around the globe. The measures of diversity were also used in the group survey projects. Much of this module was devoted to understanding the basics of economics, starting with a small group exercise in which students answered questions about the principles of traditional and environmental economics. I then expanded on these topics in lecture. The module culminated with a discussion of sustainable economics.

Labs in this module were a continuation of the small group field project surveying biodiversity on the Denison University Biological Reserve outlined in Module 4. Students turned in the write-up of their survey project as the writing assignment for this module. I gave students guidelines on how to write a paper in the format of a scientific paper, and students had to use primary literature in the writing of their paper.

Throughout the semester, students were assessed using a variety of assignments and exams. I gave two 1-hour long exams (at the end of the 2nd and 4th modules) that were each worth 10% of the semester grade. A cumulative final exam accounted for 15% of the semester grade. Module specific writing assignments were weighted equally and together accounted for 25% of the semester grade.

The first draft of the media critique assignment was worth 5% of the semester grade, and the second draft 7.5%. The group project paper was worth 10% of the semester grade. Miscellaneous lab and lecture assignments (e.g., worksheets, computer simulation assignments, in-class presentations, etc.) accounted for 10% of the semester grade. Class participation in both lab and lecture was worth 7.5%.

Students generally responded positively to both iterations of the course, with high quantitative ratings. In addition, several students commented qualitatively about the course (see Table 2). Several noted that the course was applicable to their real lives. Other students commented on how they liked the structure of the class. Finally, some student comments clearly suggested that this module approach was effective at teaching ecological topics.

Table 2. Qualitative responses of students to the course.

General Category of Response	Student Responses
Applicability to real life	<p>“with the topics about global warming this class interested me because it seemed to have a topic which is hot now, about humans and their living in the environment.”</p> <p>“course material was relatable to current times, easy to understand, informative”</p> <p>“information relevant to everyday life”</p>
Structure of class	<p>“I enjoyed the research at the bio reserve. It got us out of the classroom and made us apply what we have learned.”</p> <p>“It was very interesting and something I would not normally take. I liked the structure of the class and the fact that we did a lot of group activities.”</p>
Effectiveness of course	<p>“I actually learned more about ecology in this class than in Biology 202 (Ecology and Evolution).”</p>

This module-based approach works well as it places abstract ecological principles in concrete and relevant contexts (see Swinehart and Mort, 1995, for a similar rationale for including environmental problems in chemistry curricula, and Swan and Spiro, 1995, for including environmental issues in teaching chemistry to non-science majors). The module approach can be tailored to address global or local environmental issues. For example, the next time I teach this course, I plan to replace some of the “global” modules with “local” modules (e.g., deer populations, invasive species). Such local modules will allow more hands-on data collection rather than computer simulations. In addition, such a module approach can be used to teach other biological subjects. For example, a course centered on bioethics or medical ethics could provide several modules focused on teaching cellular or molecular biology. One module could address the issue of genetically screening of embryos or individuals for genetic

References

- ALSTAD, D. 2001. *Basic Populus Models of Ecology*. Prentice Hall, Upper Saddle River, NJ.
- BATTLES, D.A., FRANKS, M.E., MORRISON-SHETLAR, A.I., ORVIS, J.N., RICH, F.J., AND T.J. DEAL. 2003. Environmental literacy for all students: Evaluation of environmental science courses developed for a new core curriculum. *J. Coll. Sci. Teach.* 32: 458-465.
- BEISWENGER, J.M., ed. 1992. *Experiments to Teach Ecology*. Ecological Society of America, Washington, D.C.
- BIXBY, J.A., CARPENTER, J.R., JERMAN, P.L., AND B.C. COULL. 2003. Ecology on campus: Service learning in introductory environmental courses. *J. Coll. Sci. Teach.* 32: 327-332.
- GILL, R.A. AND I.C. BURKE. 1999. Using an environmental science course to promote scientific literacy: Expanding critical thinking skills beyond the environmental sciences. *J. Coll. Sci. Teach.* 29: 105-111.
- KELLER, G.E. III. 2002. Using problem-based and active learning in an interdisciplinary science course for non-science majors. *J. Gen. Educ.* 51: 272-281.
- KRIST, A.C. AND S.A. SHOWSKI. 2007. Experimental evolution of antibiotic resistance in bacteria. *Am. Biol. Teach.* 69: 94-97.
- diseases. Lectures could consider basic molecular genetics, and labs could focus on techniques related to screening for genetic diseases. In addition, a “module” on natural selection could be used where students read a recent primary or popular article detailing antibiotic resistance in bacteria. Labs can experimentally demonstrate the evolution of antibiotic resistance (e.g., Krist and Showski, 2007).

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MEIR, E. 1996. *EcoBeaker 1.0: An Ecological Simulation Program*. Sinauer Associates Inc., Sunderland, MA.

PAINE, R.T. 1966. Food web complexity and species diversity. *Am. Nat.* 100: 65-75.

PALLANT, E. 1996. Assessment and evaluation of environmental problems: Teaching students to think for themselves. *J. Coll. Sci. Teach.* 26: 167-172.

SWAN, J.A. AND T.G. SPIRO. 1995. Context in chemistry: Integrating environmental chemistry with the chemistry curriculum. *J. Chem. Educ.* 72: 967-970.

SWINEHART, J.H. AND G. MORT. 1995. Bringing environmental problems into the science classroom. *J. Coll. Sci. Teach.* 25: 58-61.

TESSIER, J. 2006. Writing assignments in a nonmajor introductory ecology class. *J. Coll. Sci. Teach.* 35: 25-29.

VON FOERSTER, H., MORA, P.M, AND L.W. AMIOT. 1960. Doomsday: Friday, 13 November, A.D. 2026. *Science* 132:1291-1295.

ZABEL, R.W., HARVEY, C.J., KATZ, S.L., GOOD, T.P., AND P.S. LEVIN. 2003. Ecologically sustainable yield. *Am. Sci.* 91: 150-157.

APPENDIX I: Example of a worksheet used to help teach population equations and life table calculations. Additional worksheets used for teaching are available by contacting the author.

LIFE TABLES AND POPULATION GROWTH WORKSHEET

1. Complete the following life table (#1) by calculating the values of l_x , and $l_x m_x$.

x (yr)	n_x	l_x	m_x	$l_x m_x$
1	1000	_____	0	_____
2	500	_____	2	_____
3	250	_____	3	_____
4	125	_____	0	_____
5	0	_____	0	_____

- a. At what age (year) do females of this species start reproducing? _____
- b. What proportion of the females survive to age 3? _____
- c. At what age do females make their greatest contribution to the net reproductive rate? _____
- d. What is the value of R_0 for this population? _____
- e. Is this population increasing or decreasing? _____
- f. What will the population size be in two generations (assume $N_0 = 100$)? _____

2. Consider the following life table (#2). Calculate the values of n_x , and $l_x m_x$.

x (yr)	n_x	l_x	m_x	$l_x m_x$
1	1000	1.0	0	_____
2	_____	0.1	5	_____
3	_____	0.08	2	_____
4	_____	0.07	2	_____
5	_____	0.06	2	_____
6	_____	0	0	_____

- a. At what age do females make their greatest contribution to the net reproductive rate? _____
- b. At what ages do females reproduce? from age _____ to _____
- c. What is the value of R_0 for this population? _____
- d. Is this population increasing or decreasing? _____

3. Consider the following life table (#3). Calculate the values of l_x , and $l_x m_x$.

x (yr)	n_x	l_x	m_x	$l_x m_x$
1	1000	_____	0	_____
2	900	_____	0	_____
3	800	_____	0	_____
4	750	_____	2	_____
5	150	_____	2	_____
6	0	_____	0	_____

- a. At what age do females make their greatest contribution to the net reproductive rate? _____
- b. At what ages do females reproduce? from age _____ to age _____
- c. What is the value of R_0 for this population? _____
- d. Is this population increasing or decreasing? _____
4. Which life table shows a population with a survivorship curve closest to a Type I curve? _____. Which life table shows a population with a survivorship curve closest to a Type 3 curve? _____
5. Explain: How can it be that in Life Table 2 females breed for so many years and in one year each produced 5 young yet that population is decreasing compared to the one in Life Table 3 with females only breeding in two years and never having more than 2 young per year?
6. If you had two management options for the population in Life Table 2, but could only implement one of them because of the lack of funds, which would you choose and why? Assume you want to make the population increase as much as possible.
- One that doubles the number of individuals surviving to age 2 but doesn't change the number surviving to age 3.
 - One that increases the age-specific fecundity of 2 year olds to 6 from 5.

Editorial

This is my last issue as *Bioscene's* editor. I've been doing this since 2005. Like a lot of worthwhile things in life, it has been both simultaneously fun and challenging. The fun involved reading manuscripts and getting novel and exciting ideas about teaching biology. The challenging involved actually putting together the issues in a timely fashion so that they looked somewhat competent. This pressure was relieved a bit when budget issues forced us to trim the publication from quarterly to two times a year. But dealing with computer codes, formatting, and large numbers of authors without staff support made for some rotten weekends in front of the computer (altogether now: BOO-HOO!).

The new editor of *Bioscene* will be Jim Clack from the Department of Biology at Indiana University. Jim is a long-time reviewer for our journal and is extremely tech-savvy. It should be a smooth transition. Nevertheless, I feel it is incumbent upon me to share some of the wisdom I've accumulated these past five years. I've reduced this to the following six points:

- Maintain and work closely with the chair of the editorial board.** This position is crucial. When I took over as editor, I did both the editing and the editorial board management. It was tough because manuscripts are constantly streaming in and I felt it was my duty to get them out to reviewers and back in a timely fashion. I would like to take this opportunity to thank Janice Bonner for taking on this task. She has been a terrific asset and I hope when her term expires, an equally able member assumes that position.
- Keep authors informed of manuscripts' progress from submission to publication.** I'm aware that this has been a source of frustration to many. The turnaround time from submission to publication can be over a year. Many of our authors are pre-tenured and need publications for tenure packets. Some type of notice by the editorial staff, however brief, would be always be appreciated.

3. **Solicit opinions about the direction of *Bioscene* from our membership.** This journal is a reflection of ACUBE. So the opinion of our members matters tremendously. In addition, to the annual report at the fall meeting, I've encouraged (without much success) letters to the editor. I've also sought ideas during editorial board meetings and informal settings at meetings. What input I've received has been invaluable.
4. **Fine tune our submission guidelines.** Each year that I've been editor, I've tried to improve our submission guidelines. While this may be painful for authors, it should help make our review process more efficient and allow us to put forth a better product in a timely fashion. The instructions should be both in issues of *Bioscene* and easily accessed on the ACUBE website.
5. **Do not be afraid to experiment.** In my time as editor, I have standardized the "look" of a *Bioscene* article, but maintained the same cover format as it existed when I began. I've also added new sections (website reviews, book reviews, etc.). Moreover, the publishing process has changed. One of our two issues is published online. With more and more paper

publications moving in this direction, this is a terrific time to tweak how this journal is put together. There are some who loathe change. But change is inevitable and human constructs can always be improved.

6. **Do not forget that this is a volunteer position.** I felt a great weight had been placed upon me when I took this over. I felt that great weight leave last fall, when Jim said he would take over. It probably wouldn't have been so arduous if I had reminded myself from time to time, that I had volunteered to do this. Any deadlines that were imposed, were self-imposed. There should be opportunities to enjoy the experience. I hope my successor experiences many of those.

Well, that's it from me. The next editorial board meeting will be held during the October 8-9, 2010 meeting at Lourdes College in Toledo, Ohio. The next *Bioscene* issue will be the December one, most likely available in January 2011. As usually, I'm looking forward to receiving it. This one will be extra exciting, since for the first time in five years I will not have a clue what is in it.

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