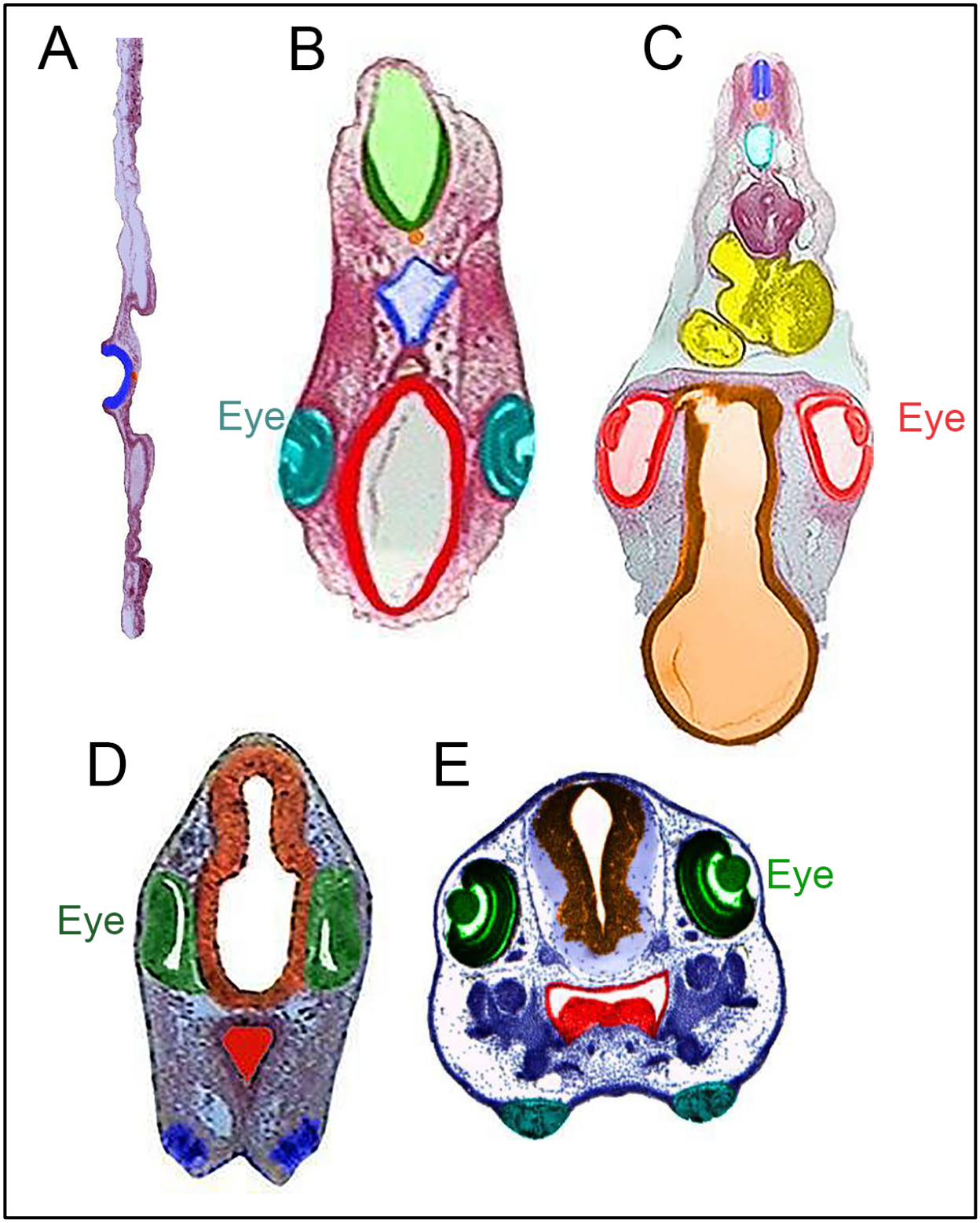
Bioscene
Journal of College Biology Teaching



Volume 41(2) December 2015

Bioscene Journal of College Biology Teaching

Volume 41(2) **December 2015**

ISSN 1539-2422

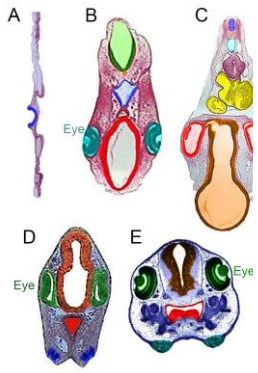
A Peer-Reviewed Journal of the

Association of College and University Biology Educators

Editor-in-Chief:
Debbie Meuler
Cardinal Stritch University

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

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



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Volume 41(2) ·December 2015
A Publication of the Association of College and University Biology Educators

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- Incubate new and innovative teaching ideas;
- Involve student research in the biology curriculum;
- Advise and mentor students in and out of the classroom;
- Enhance scholarship through our nationally, peer-reviewed journal, *Bioscene*.

ARTICLES

Investigation of Macrophage Differentiation and Cytokine Production in an Undergraduate Immunology Laboratory

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Abstract: We have developed a semester-long laboratory project for an undergraduate immunology course in which students study multiple aspects of macrophage biology including differentiation from progenitors in the bone marrow, activation upon stimulation with microbial ligands, expression of cell surface markers, and modulation of cytokine production. In the first part of the semester, students differentiate macrophages from mouse bone marrow stem cells and perform immunophenotyping on their macrophages using myeloid markers that are either constitutively expressed or expressed upon activation with microbial ligands. Students use a low-cost image cytometer to both visualize and quantify cellular expression of myeloid markers. Students then perform literature research, design, and execute a series of experiments aimed at investigating the role of natural anti-inflammatory compounds on TNF-α production in these macrophages. The soup-to-nuts investigative approach in which students generate "their own" macrophages and study their functions over the course of the semester fostered a sense of ownership and accomplishment

Keywords: Immunology, image cytometry, macrophage, ELISA

INTRODUCTION

Macrophages are myeloid lineage professional phagocytes that play a key role as a first line of defense against invading pathogens. In addition to their immediate role in phagocytosis of microbes, macrophages express toll-like receptors (TLRs) and other pattern recognition receptors that enable a powerful and exquisitely tailored cytokine response to infection. Macrophage cytokines coordinate an immune response that includes stimulation of vascular permeability and recruitment of neutrophils and other immune cells to the infection site. Along with dendritic cells, activated macrophages play an important role in antigen processing and presentation to T cells, placing them at a critical juncture between innate and adaptive immunity (Murray & Wynn, 2011).

Macrophage biology can be modeled using a variety of cell culture approaches ranging from macrophage-like cell lines such as RAW 264.7 to primary resident tissue macrophages. Macrophage-like cells lines such as RAW 264.7 are robust and fairly easy to culture with minimal expertise, and in this respect, offer an attractive model for studying macrophage function. However, exclusive use of cell lines presents two major drawbacks: 1) the immortalized nature of the cells and their associated genetic abnormalities may limit conclusions that can be drawn from their use, and 2) in an undergraduate teaching laboratory, a cell line-only approach makes it all too easy for students to overlook the fact that myeloid lineage cells such as macrophages are born, differentiate, and carry out their functions in the context of a complex network of immune cells and organs *in vivo*. However, for the undergraduate laboratory, isolation of primary macrophages such as thioglycollate-elicited peritoneal macrophages, splenic macrophages, and alveolar macrophages is often not realistic, especially in institutions that lack mouse facilities. Here, we propose that the generation and study of bone marrow-derived macrophages (BMDM) in the undergraduate laboratory represents a fertile middle ground between cell lines and primary cells that offers the ease of a cell line together with a higher degree of authenticity. BMDM are commonly used in research labs and are a widely accepted monocyte/macrophage model as they closely approximate the attributes of primary macrophages and do not display immortalized growth, but can be isolated in large quantities using well-established protocols and can withstand cryopreservation (Franke et al., 2011; Manzanero, 2012; Weischenfeldt & Porse, 2008; Zanzoni et al., 2009). The process of BMDM differentiation starting with isolation of mouse femurs allows students to observe and think about hematopoiesis from the starting point of a whole organism. The harvesting of bones from sacrificed mice and subsequent BMDM differentiation protocol involves a single visit to an animal facility. Instructors at small colleges lacking animal facilities can generally obtain extra mice no longer needed for research from investigators at nearby R1 universities.

Here, we describe a semester-long laboratory project for an undergraduate immunology laboratory course that reinforces multiple principles of cellular immunology, including hematopoiesis, TLR signaling, cytokine production, and co-activation. The course is designed for students with no prior experience in cell culture, but can be easily adapted depending on the level of the students. The goal of this paper is to present our course structure accompanied by detailed methods. We will also present a brief assessment of student engagement and discuss ways in which our methods can be adapted to other courses.

COURSE STRUCTURE

Immunology (BIO3012) at Merrimack College is an elective course typically taken by juniors and seniors who have previously completed Introductory Biology and Genetics. Cell Biology is not a prerequisite, therefore, no prior knowledge was assumed of the students regarding mechanisms of cellular differentiation and signal transduction. Like most science courses taught at Merrimack College, the course is four credits and includes a lecture and laboratory which are taught by the same instructor in order to maximize connections between the theoretical and practical aspects of the subject. This laboratory course is divided into three parts: 1) Introduction to Mammalian Cell Culture, 2) BMDM Differentiation and Analysis, and 3) Independent Investigation as described below and as shown in Table 1.

Introduction to Mammalian Cell Culture.

In the first week of lab, students were given a small flask of RAW 264.7 cells and were asked to seed several wells of a 24-well plate. The purpose of this exercise was to provide an opportunity to learn and practice aseptic technique, proper use of the Class II Biosafety cabinet, and use of the hemocytometer. Following cell seeding, students stimulated production of the pro-inflammatory cytokine TNF-α by treatment of select wells with whole microbes (*E. coli*) or purified lipopolysaccharide (LPS). LPS is a molecule that is present in the cell wall of all Gram-negative bacteria and is a strong stimulator of cytokine production via toll-like receptor 4 (TLR4)-mediated signaling (Poltorak et al., 1998). In the lecture portion of the course, the molecular aspects of innate immunity were discussed at length, including TLR signaling as well as signaling induced by other families of pattern recognition receptors. Culture supernatants from stimulated and unstimulated RAW 264.7 cells were collected and saved for use as positive controls for subsequent TNF-α ELISAs to be performed later in the course. (If time allows, the samples could be immediately analyzed via ELISA).

BMDM Differentiation and Analysis.

To introduce students to the techniques and concepts that will be used throughout the semester, we dedicated the second week of lab to the analysis of a current primary research article focusing on macrophage toll-like receptor signaling (Thanawastien et al., 2009). For this, students presented figures in a round-table format. In the third week of the lab the instructor travelled to the University of Massachusetts Medical School to sacrifice donated mice and harvest femurs under their Institutional Animal Care and Use Committees IACUC protocol. Although students did not directly participate in the handling or dissection of mice, we spent time in class discussing the importance and

Table 1. Semester laboratory schedule. The semester consisted of thirteen 2.5 hour laboratory periods; activities in each laboratory period are shown. In addition to the regularly scheduled lab times, students were occasionally asked to come in to the lab for short periods of time to treat or fix cells.

Week #	Activity
1	Introduction to Cell Culture: Students are given a 25 mm flask of RAW cells –students harvest cells, determine concentration via hemacytometer, seed in 24 well dish, treat wells +/- Lipopolysaccharide, harvest supernatants
2	Journal Club: Thanawastien et al., 2009.
3	Crush mouse femurs and stimulate myeloid differentiation via treatment with MCSF
4	BMDM harvest and creation of BMDM frozen stocks
5	Seed BMDMs and culture +/- LPS to stimulate activation, fix and save cells
6	Image cytometric immunophenotyping of BMDM with CD11b and CD80 fluorescent antibodies
7	Image cytometry data analysis
8	Grant revew panel
9	BMDM seeding and anti-inflammatory treatment, collect supernatant
10	TNFα ELISA
11	Cytotoxicity Assay
12	Extra time to repeat experiments, plan final presentations
13	Anti-Inflammatory "Lunch" and Student Presentations

function of IACUC in animal research, which is critical in the field of immunology. Additionally, a video was viewed in class to expose students to the entire procedure including femur harvest (Trouplin et al., 2013). Working in pairs, students liberated marrow cells from femurs and tibias, seeded cells, and cultured cells in the presence of macrophage colony stimulating factor (M-CSF) to induce monocyte/macrophage differentiation (Klappacher et al., 2002; Stanley et al., 1997).

Students were asked to perform their own literature searches to determine which cell surface markers will identify myeloid lineage cells, as well as markers of myeloid activation. Students came up with several potential markers, and reviewing the students' answers together as a class served as an excellent introduction to a general discussion of immunophenotyping. As part of the discussion, we stressed the fact that no single cell surface marker can positively identify the myeloid lineage, monocyotes, or macrophages. Through classroom discussions, we decided to analyze expression of CD11b and CD80. CD11b (complement receptor) is a marker commonly used to identify myeloid lineage cells, and CD80 (B7-1) is a macrophage co-stimulation signal for T cell activation whose expression is induced upon activation with microbial ligands (Linsley & Ledbetter, 1993).

Others have implemented creative teaching modules that incorporate flow cytometry in the setting of undergraduate teaching laboratories (Boothby et al., 2004; Fuller-Espie, 2010; Ott & Carson, 2014; Szeberenyl, 2007). As an alternative to flow cytometry, students used image cytometry to immunophenotype our BMDM with respect to CD11b (constitutive myeloid) and CD80 (activation-associated). Image cytometric analysis resulted in vivid images that correlated well with scatter plots representing cell population data.

Independent Investigation.

Following analysis of myeloid cell surface markers by image cytometry, students designed and executed a series of experiments aimed at investigating the anti-inflammatory properties of natural plant-based compounds. In lecture, the inflammatory response in health and disease was discussed at length. Students were introduced to literature on natural anti-inflammatory compounds. Students were asked to write a grant proposal detailing experiments they would conduct using their isolated BMDM in order to determine whether a chosen compound counteracts inflammation at the cellular level. For example, one student group proposed to test the hypothesis that epigallocatechin gallate (EGCG), a component of green tea, inhibits LPS-stimulated TNF-α production in BMDM. Compounds studied by all student groups are shown in Table 2.

To encourage students to deeply think about their hypothesis and the details of their experiments, they were asked to draft a miniature version of an NIH-style grant proposal; the instructor reviewed and commented on each student's draft, giving feedback for improvement in the final draft. Additionally, the class was divided into peer review groups in which each group (3-4 students) was given an equal number of draft proposals to review. By participating in the review process, students were able to see examples of others writing, and reported that the process was extremely helpful in helping them improve their own writing. We believe that the drafting process made a daunting assignment more approachable and allowed the students the chance to improve their writing skills.

The remainder of the semester was dedicated to performing experiments designed by the students to test their hypothesis that compound "X" modulated production of TNF-α by BMDM. We limited our analysis to TNF-α for financial reasons, however, a panel of two or more cytokines could be analyzed if desired. In addition to cytokine analysis, students examined cytotoxicity to ensure that variations in cytokine levels were not due to varying levels of toxicity in response to cell treatment.

Table 2: Compounds tested by students for anti-inflammatory effects in BMDM. The qualitative effect of each compound on inhibition of LPS-induced TNF-α production in BMDM is also indicated, with ++ indicating strong inhibition of TNF-α production, + indicating weak inhibition, and – indicating no inhibition or inconclusive data.

Compound	Source in Nature	Inhibition of TNFα Production observed?
Epigallocatechin-3-gallate	Green tea	++
Fucoidan	Brown algae, seaweed	+
Resveratrol	Grape skins	++
Neem	Neem leaf	+
Harpagophytum procumbens extract	Devil's Claw	-
Quercetin	Flavenoid with wide distribution	++
Parthenolide	Feverfew leaves	-
Ocimum basilicum (eugenol)	Eugenol	-
Curcumin	Turmeric	++
γ-aminobutyric acid (GABA)	Animals and a variety of plants	++

MATERIALS AND METHODS

The complete student lab manual and an instructor's preparation guide, including reagent ordering information, is available within the ACUBE Resources site.

RAW 264.7 Culture

RAW 264.7 cells were obtained from the American-Type Culture Collection and maintained in Dulbecco's Modified Eagle Medium (DMEM, Sigma) supplemented with 10% fetal bovine serum (Atlanta Biologicals) and penicillin/streptomycin (Life Technologies). For student experiments, RAW 264.7 cells were seeded in 24 well plates with 1.0 x 10^5 cells/well. Seeding was carried out during the regularly scheduled lab period, where students were also instructed on aseptic technique, proper use of the Class II Biosafety Cabinet, and enumeration of live/dead cells by trypan blue staining followed by hemocytometer counting. The following day, students came in briefly to treat cells with 10 μL overnight *E. coli* (DH5α) culture or 10 ng/mL LPS (Sigma). Cells were treated for 4 hours and supernatants collected and saved at -20°C.

Bone Harvest

Five six week-old male C57/BL6 mice were sacrificed by CO_2 euthanasia followed by cervical dislocation as approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Massachusetts Medical Center. C57/BL6 mice are commonly used in immunology studies, but many other strains would make a suitable substitute. Either male or female mice may be used. In C57/BL6 mice, the optimal age range for BMDM production is 6 weeks-3 months. Older mice (up to one year) may be used, although they typically produce lower BMDM yields. Femurs and tibias were separated from tissue as described previously (Zanzoni et al., 2009). Bones were harvested 20 hours prior to the scheduled laboratory period and were immersed in DMEM and stored at 4°C or on ice during transport.

BMDM Differentiation

BMDM media consisted of phenol red-free high glucose DMEM (Sigma) supplemented with 20% fetal calf serum (Atlanta Biologicals), 1 mM sodium pyruvate (Sigma), 2 mM glutamine (Sigma), 100 Units/mL penicillin/streptomycin (Life Technologies) and 10% L929 cell supernatant, prepared as described previously (Yamamoto et al., 1981). Phenol red-free DMEM was used throughout our experiments as the presence of phenol red in the media interferes with the colorimetric cytotoxicity assay used later in the semester.

Students worked in pairs to extract bone marrow from a single femur and tibia. Bones were crushed mechanically using an ethanol-sterilized mortar and pestle inside the Biosafety cabinet. The mechanical crushing method produces less pure cultures than flushing of bones with fine-gauge needles, however, we have found this method to be preferable in the hands of undergraduates as it avoids the unacceptable risk of needle-stick injuries. Crushed bones were submerged in 10 ml BMDM media and further dissociated by repeated pipetting. Samples were transferred to sterile 50-mL conical tubes and large debris (bone fragments and connective tissue) was allowed to settle by leaving tubes undisturbed for 2 minutes on ice. The supernatant was transferred to a new 50 mL conical tube, then centrifuged at 1200 rpm for 5 minutes to pellet cells. Cells were resuspended in 5 ml BMDM, counted and seeded at 1.0 x 10^7 cells per plate in 25 cm petri dishes (not cell culture-treated) in a total of 25 mL BMDM media. Typically, the femurs and tibias of a single mouse will produce enough material for 5-10 plates. Following three days of incubation at 37°C and 5% CO_2, cells were fed with 20 ml additional BMDM media and returned to the incubator for an additional four days.

Following seven days total incubation, media was removed and BMDM were washed three times in cold magnesium/calcium-free PBS and incubated in 10 ml cold PBS for 30 minutes on ice. The ice incubation causes BMDM to round up and loosen their attachment to the plates. To collect BMDM, cells were pipetted up and down several times in PBS. This process requires quite a bit of patience and "elbow grease", as BMDM are highly adherent. Students must be encouraged to keep pipetting up and down (typically at least 20 times on each plate), using the stream of fluid to force cells off the plates until no "cloudy" areas are observed. To check that most cells have been removed, plates may be checked in the microscope. Cells were transferred to a sterile 50 mL conical tube on ice and each plate was rinsed with 10 mL fresh PBS. Cells were then concentrated by centrifugation at 1200 rpm for 5 minutes and resuspended in the equivalent of 1 mL BMDM media per plate. Cells were counted and resuspended in freezing media (BMDM media supplemented with 10% DMSO) to a final concentration of 1.0 x 10^7 cells/mL and 1 mL aliquots stored in cryovials at -80°C (if available, liquid nitrogen is preferable, although cells will remain stable at -80°C for the remainder of the semester).

BMDM Culture and Activation

To recover frozen BMDMs, cryovials were thawed in a 37°C water bath only until just thawed, then placed on ice. The contents were diluted in 9 mL BMDM media in a 15 mL sterile conical tube. To remove DMSO, cells were centrifuged at 1200 rpm for 5 minutes, resuspended in 1 mL fresh BMDM media, and an aliquot was stained with trypan blue and counted in order to determine cell viability and concentration.

To generate samples of resting and activated BMDM samples for subsequent immunophenotyping, cells were seeded at 5 x 10^6 cells/plate in 10 cm

plates. The following day, two plates were treated with 10 ng/mL LPS (Sigma) in order to activate macrophages, and the other two were treated with the equivalent volume of media only (inactivated). Following 24 hours in LPS, cells were washed with 10 mL PBS and fixed in 4% formaldehyde/PBS, removed from wells by scraping, and stored at 4°C until the next laboratory period.

For investigation of natural compounds, students seeded BMDMs in 24 well plates at a density of 7.0 x 10^5 cells/well. Following seeding and overnight incubation, students treated their cells with a variety of concentrations of their test compound (typically, 1 μM, 10 μM, and 100 μM, although a variety of concentrations were used based on their literature research). Following 12-16 hour "pre-treatment" with putative anti-inflammatory compounds, a subset of wells was treated for 2 hours with 10 ng/mL LPS. It is important here to include controls that are exposed to putative anti-inflammatory but not LPS, as well as an LPS-only control and a no-treatment control.

Image cytometric immunophenotyping

Conical tubes containing fixed cells were centrifuged on high speed for one minute to pellet cells. Cells were then resuspended in PBS/10% Fetal calf serum to a final concentration of ~ 3 x 10^6 cells/ml. To reduce background staining caused by binding of the Fc region of fluorescent antibodies to macrophage Fc receptors, 5 μL Fc block reagent (BD Pharmingen) was added to each sample and incubated with agitation at 4°C for 5 minutes. 100 μL cells were transferred to new tubes and incubated in the presence of 1.25 μL phycoerythrin(PE)-anti-mouse-CD11b (Biolegend) AND 1.25 μL Alexa-fluor-488-anti-mouse-CD80 (Biolegend). Following 30 min incubation in the dark at 4°C to avoid photobleaching of the fluorophores, cells were washed 5X in PBS/10% Fetal calf serum, then resuspended in 100 μL PBS/10% Fetal calf serum.

The Cellometer Vision instrument (Nexcelom Biosciences) has been described in previous publications (Chan et al., 2011a, 2011b). The system utilizes bright-field (BR) and dual-fluorescent imaging modes to quantitatively analyze and measure the concentration and fluorescent intensity of target cells. The fluorescence optics modules (FOMs) utilize filters with Excitation/Emission of 470 nm/535 nm and 525 nm/595 nm for FITC and PE detection, respectively. The cell samples (20 μL) were pipetted into the Nexcelom disposable counting chambers, inserted into the instrument, and then focused using the BR imaging. Following image capture, data was exported to a proprietary .NXDAT file format, which was then analyzed in the FCS Express Software (De Novo, Los Angeles, CA).

Natural Compounds

Compounds were purchased from Sigma and resuspended in DMSO unless otherwise directed. Solutions were sterilized by filtration through 0.22 μm filters. Final concentrations used were typically 1, 10, and 100 mM.

ELISA

TNF-α ELISA (eBioscience) was carried out according to manufacturer's instructions using 100 μL cell supernatants. If desired, the RAW cell supernatants generated earlier in the course may also be tested here (RAW cells typically produce higher levels of TNF-α, requiring dilution of supernatants prior to adding to ELISA wells (1:10) to avoid saturation. Absorbance at 450 nm was quantified using a BioTek ELx800 plate reader.

Cytotoxicity Assay

To rule out the possibility that TNF-α levels are modulated due solely to cytotoxicity, we used the Cytotox Assay (Promega) to measure culture supernatants for the presence of lactate dehydrogenase, a cytosolic enzyme. Assays were carried out in 96-well plates and A_{680} measured using the BioTek ELx800 plate reader.

Institutional Review Board Approval

Student surveys were approved by the Merrimack College IRB.

RESULTS AND DISCUSSION

Representative student-generated data of image cytometric analysis of CD11b/CD80 surface expression is shown in Figure 1. As expected, scatterplot analysis of the non-activated BMDM shows that 93.3% of are $CD11b^+/CD80^-$ and only 4.9% are $CD11b^+/CD80^+$. We observed a shift in the subset of CD80 positive cells following LPS-mediated activation, with 36.6% of cells $CD11b^+/CD80^+$. Previously published values typically show higher levels of CD80 induction upon LPS treatment of BMDM (Franke et al., 2011), however, we suspect that the mechanical crushing method used to extract marrow from bones led to decreased purity in our sample. One extremely powerful aspect of image cytometry is that students can observe fluorescence in the captured images, which provides a visual framework to help them better understand abstract scatterplots and histograms.

A list of all compounds tested and their qualitative effects on TNF-α production in BMDM is shown in Table 2. One of the most potent inhibitors of LPS-inducted TNF-α production was the green tea compound epigallocatechin gallate (EGCG). TNF-α ELISA results show a dose-dependent decrease in TNF-α levels upon pre-treatment with EGCG (Figure 2). To determine whether variations in TNF-α levels were due to variations in cell viability, students also tested cellular supernatants for release

of lactate dehydrogenase (LDH), an abundant cytosolic enzyme released from cells upon death. LDH levels remained constant regardless of EGCG concentration (data not shown).

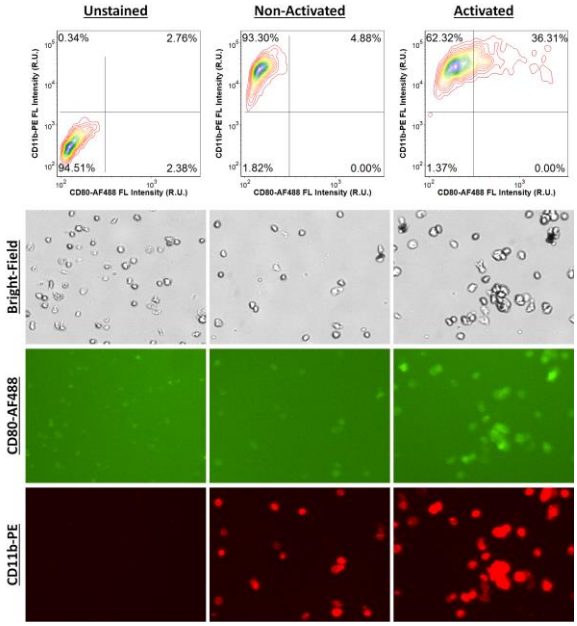

Figure 1. Image cytometric analysis of bone marrow-derived macrophages. BMDM were cultured in either DME/20% FBS/M-CSF (Non-activated) or DME/20% FBS/M-CSF + 10 ng/ml LPS for 24 hours (Activated). Cells were fixed with 4% formaldehyde/PBS and incubated phycoerythrin (PE)-anti-CD11b and Alexfluor-488-anti-CD80. Representative bright-field, CD80 positive (green pseudo color) and CD11b positive (red pseudo color) images resulting from Cellometer Vision analysis are shown. Scatterplots show that the majority (93.3%) of non-activated BMDM are $CD11b^{+}/CD80^{-}$. Activation of BMDM by treatment with LPS induces a shift in CD80 expression, with 36.61% of cells $CD11b^{+}/CD80^{+}$. Fluorescent images also show activation with LPS induces an increase in the intensity of CD11b staining. In addition, the cell morphology in bright-field shows increase in cell size that can be observed by the students. To assess background levels of autofluorescence, a sample of unstained BMDM is shown (left panel).

We have found that image cytometry is ideal for use in the undergraduate laboratory for both practical and pedagogical reasons. Observation of raw images shows a clear difference in the proportion of cells expressing CD11b and CD80 when comparing activated and resting BMDM. Also, direct observation of bright-field and fluorescent images shows that LPS treatment induces an increase cell size and in the *intensity* of CD11b staining. Direct observation of these images enabled students to quickly understand and explain the population-based scatter plots generated based on multiple images. Exam questions showed that all students were able to apply their understanding of cytometric analysis to other situations (sample questions are available within the ACUBE member resources site).

Practically speaking, image cytometry instrumentation has a smaller footprint, lower price, and minimal maintenance, making it an ideal tool for teaching and research at smaller colleges and departments. All of the kits and reagents needed for this laboratory can be purchased for approximately $1500. In addition to its use in immunophenotyping, image cytometry can be used to visually and quantitatively assess multiple cellular phenotypes, including viability, apoptosis, necrosis, autophagy, cell cycle progression, and mitochondrial potential (Chan et al., 2011a, 2011b; Robey et al., 2011).

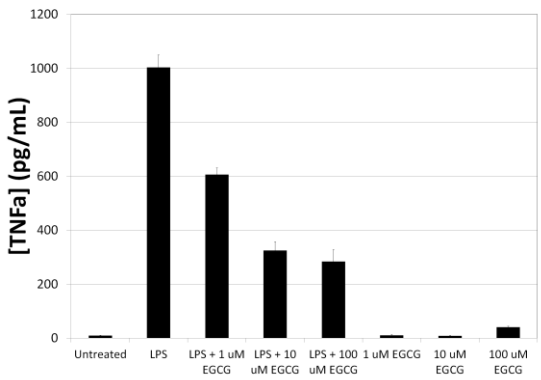

Figure 2. EGCG inhibits production of TNF-α. BMDM were seeded at 7.0 x 10^5 cells/well in 24 well dishes and pre-treated overnight with shown concentrations of EGCG or DMSO (carrier) prior to treatment with 100 ng/ml LPS. Supernatants were collected after 2 hours of treatment +/- LPS and subjected to TNF-α. This experiment was performed in triplicate; similar results were found when repeated.

Student attitudes and engagement were assessed by administering questionnaires in which students ranked their level of agreement/disagreement with several statements on a 5-point Likert scale (Figure 3). Large gains in student confidence were observed for discipline-specific skills. More modest gains were observed in the more general skills including designing and executing experiments. We suspect that this is due in part to the fact that most science labs at Merrimack College include experimental design and hypothesis testing into the laboratory curriculum. Since Immunology is populated by juniors and seniors, it is likely that they had all gained some level of competency in this realm through previous courses. This is supported by relatively high "pre" scores in response to the statement: "I am comfortable designing testable hypotheses and designing/executing my own

experiments." To assess student understanding directly, we administered an in-lab quiz based on analysis of data from the primary literature (this is available on the ACUBE Resources site). Over 90% of students performed well (score of 85% or higher)

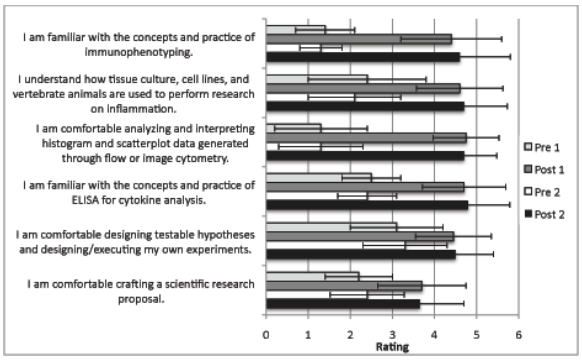

Figure 3. Survey of student learning and attitudes. Survey questions were administered to students prior to (pre) and following (post) the laboratory activities. Students were asked to rate their level of agreement with each statement on a 5-point Likert scale, with 1 indicating "strongly disagree" and 5 indicating "strongly agree". These surveys were given to students taking the course in two separate semesters (semester 1, n=14 students; semester 2, n=13 students).

on assessments that challenged them to analyze immunophenotyping and ELISA data.

Student surveys indicated a high degree of interest, a sense of ownership of their project, and a sense of accomplishment. A sampling of student comments supports this idea: "I felt independent to the point of being expert and accomplished."; "I was grossed out by the mouse bones at first, but once I successfully got my own macrophages, I felt really invested in the project."; "Although writing the proposal was hard, it helped me to organize my thoughts."; "I learned so much from this – great lab design! I really wanted to see what happened and didn't even mind coming in after-hours." Another sign of student engagement in the course was that students came up with the idea to hold an "anti-inflammatory lunch" at the same time as the final presentations. This idea had its genesis in an observation made by a student: that many of the compounds tested by students are found in edible items. Our lunch menu included sushi (containing fucoidan in sea weed), green tea (containing ECGC), grapes (containing resveratrol) and Neem leaf tea.

There are multiple opportunities to either expand upon or modify this laboratory module. In the absence of either image or flow cytometric analysis, expression of myeloid markers may be performed using standard immunofluorescence, western blotting, and/or quantitative real-time PCR. Our lab used BMDM, however, the above activities (minus the differentiation procedure itself) can easily be performed using a macrophage-like cell line such as RAW 264.7 or J77A.4. Instructors wishing to use real murine bone marrow may learn the technique on their own with the recent advent of video-based resources that focus on scientific methods (Trouplin et al., 2013). Instructors may also expand upon the immunophenotyping exercise to include examination of additional markers such as F4/80, CD115 (M-CSF receptor), and others. Additionally, there are plentiful opportunities to use image cytometry to experimentally examine factors that affect the bone marrow differentiation process itself, for example, the concentration of M-CSF added and/or the presence of additional growth factors and cytokines.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Thomas Fazzio at the University of Massachusetts Medical Center, Worcester, MA, for his donation of mice.

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Homework, Motivation, and Academic Achievement in a College Genetics Course

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Abstract: We conducted a mixed methods study in an upper-level genetics course exploring the relationships between student motivation, homework completion, and academic achievement at the college level. We used data from an open-ended questionnaire, homework grades and completion reports, and exam scores. We used these data sources to measure self-perceived motivating/demotivating factors and then tested these factors for correlation with homework completion and academic achievement. We found no significance in homework completion when considering credit or extra credit as a motivating factor. According to student reports they completed significantly more homework when considering reinforcement of content as a motivating factor. However, we found discrepancies between students' reported motivation and actual completion rates. Self-reported study style, self-perceived conscientiousness, intelligence, attitude, time commitments, and complexity of assignments had significant impacts on whether or not students completed homework assignments and impacted students' academic achievement. Overall, we found a positive relationship between homework completion and academic achievement within this upper-level college genetics course and provide implications for increasing student motivation.

Keywords: academic achievement; college student performance; genetics education; homework; motivation

INTRODUCTION

Homework is a well-established aspect of academic life. However, students sometimes question its validity and usefulness. As such, homework has often been the focus of academic researchers, who, responding to the concerns of students, parents, and teachers, have investigated the utility of homework at the K-12 level (Cooper et al., 1998; Cooper et al., 2006; Trautwein et al., 2006). Although there are some studies that highlight the benefits of homework completion among college students (Emerson & Mencken, 2011; Grodner & Rupp, 2013; Ramdass & Zimmerman, 2011; Trost & Salehi-Isfahani, 2012), further research into its utility at the college level is needed. This is especially true given the current push toward developing "flipped" classrooms. At the K-12 level, research shows that when students do not complete homework assignments, academic achievement is not beneficially affected (Cooper et al., 1998). Therefore, we think that it is important to understand why students do or do not complete their homework. We define homework as any academic, course-related task assigned by the instructor intended for students to carry out during non-class hours. Furthermore, we define academic achievement as the grade a student receives, either for a course or for a specific assignment. Previous studies performed at the college level suggest that credit and extra credit are strong motivators for homework completion (Carkenord, 1994; Ryan & Hemmes, 2005). Student characteristics may also influence homework completion and academic achievement (Trautwein et al., 2006). Some characteristics that are known to influence academic achievement include intelligence, conscientiousness, attitude, and study style (Busato et al., 2000; Laidra et al., 2007). Knowing why students do homework and how doing homework influences academic achievement can help instructors structure assignments to maximize completion, help students recognize and overcome demotivating factors, and provide evidence for the value of including homework assignments in college level curriculum.

THEORECTICAL FRAMEWORK

Trautwein et al. (2006) proposed a model of academic achievement in which various factors influence homework completion at the 8th grade level. We altered this model by replacing parental influence with social factors to better represent factors facing college students (Figure 1). We justify this change because the role of parents is typically less immediate for college students living away from home (e.g., in dorms). As such, the role that parents played in K-12 education (e.g., providing academic

expectations and assistance with homework) is transferred to students' peers in postsecondary education.

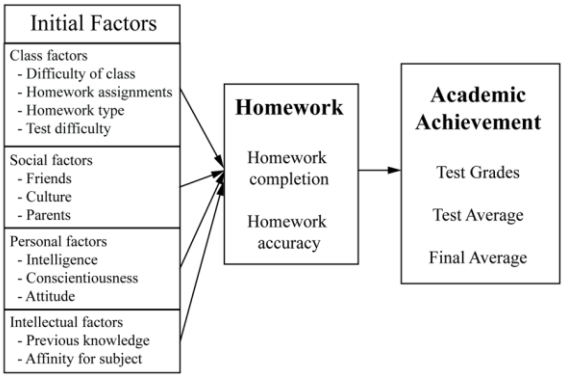

Figure 1. Theoretical framework of academic achievement and homework completion (adapted from Trautwein et al., 2006).

LITERATURE REVIEW

Homework is known to bolster student achievement at the K-12 level (Cooper et al., 1998; Cooper et al., 2006; Cooper & Valentine, 2001; Corno, 2000; Gurung, 2003; Keith, 1982; Keith & Cool, 1992; Paschal et al., 1984; Trautwein et al., 2002; Trautwein et al., 2006). Previous research on the benefits of homework completion on college students are linked to significant improvement in their test performance, higher retention rates in enrollment, and improved self-regulatory behaviors like motivation to study, self-efficacy, goal setting, and time management (Grodner & Rupp, 2013; Ramdass & Zimmerman, 2011). However, there have been limited investigations looking at homework and academic achievement in postsecondary settings. Cuadros et al. (2005) found that homework did not increase immediate achievement within an introductory chemistry lab setting. Student performance on homework assignments was not a good predictor for performance on practice tests issued randomly throughout the course. However, they found homework worked as an effective study tool for scheduled exams and that those who did well on assigned homework performed significantly better on those exams. Homework has also been documented as an important learning opportunity for college students (Leinhardt et al., 2007). While we know that the lack of completing homework does not offer any tangible benefit beyond freeing time commitments (Cooper et al., 1998), simply assigning homework does not guarantee either completion or improvements in student success.

Most investigations on student motivation for homework completion at the college level have focused on external incentives, including credit and extra credit (Carkenord, 1994; Ryan & Hemmes, 2005). In psychology, external incentives have been recognized as powerful motivating forces for some time (Chapman & Feder, 1917), and more recent research confirms their powerful role in human psychology (Flaro et al., 2007). As far as external incentives motivating homework completion go, credit has been shown to be one of the most powerful motivating forces. Ryan and Hemmes (2005) compared for-credit and no-credit homework completion across assignments and found that students are more likely to complete an assignment if it bears a credit contingency. Another study, by Tuckman (1998), reported that a required credit incentive of weekly quizzes caused greater improvements in achievement than the introduction of prescribed learning strategies. Michael (1991) argued that credit was the only feasible motivating factor that could be powerful enough to get college students to spend time away from friends, significant others, and unanticipated outings.

Extra credit is another potential motivator, but unlike required credit, its recorded effects vary considerably depending on the conditions in which it is applied (Boniecki & Moore, 2003; Carkenord, 1994; Junn, 1995; Walker et al., 2005). Carkenord (1994) found that by combining extra credit assignments with the ability to use those completed assignments as aids on tests, students completed extra credit assignments nearly 74% of the time. However, Walker et al. (2005) offered extra credit for students to participate in out of class research, yet this only yielded in a 38% participation rate. Although extra credit appears to be not as strong of a motivating factor as required credit, it has many other uses than merely getting students to complete assignments. Junn (1995) used extra credit to improve the achievement of students whose grades were poor, but not beyond the margin of redemption.

There could be many factors beyond credit and extra credit that influence student motivation toward homework completion. For example, Cote and Levine (2000) found a link between attitude and a positive academic experience. In their study, a student who comes to a university with the desire and motivation to learn is more likely to seek out and find positive academic experiences, triggering positive feedback loops between the student and the instructor or institution. In this way, students with a positive attitude are more likely to do well because they are more likely to discover, use, and continue to use the resources required to do so. This mindset could apply to homework as part of a positive feedback loop for students who use it as a study tool. Homework inculcates a mastery of the material, and a student benefits from that mastery, making them more likely to continue doing homework.

Laidra et al. (2007) found that intelligence is correlated with predicting high grades throughout all levels of K-12 education. However, Busato et al.

(2000) found that intelligence is not as strong of a predictor of academic achievement at the postsecondary level. Rather, conscientiousness is a better predictor of academic achievement than intelligence (Busato et al., 2000; Paunonen & Ashton, 2001), and is also positively correlated with effort for K-12 students (Laidra et al., 2007; Trautwein et al., 2006). While there are no studies linking intelligence and conscientiousness with homework completion, we expect that conscientious students will more likely complete homework assignments and perform better in a course.

Students choose to prepare for course exams in numerous ways. Bustato et al., (2000) identified four primary studying styles: a) undirected, a student does not distinguish the important material from the unimportant ones but rather tries to memorize all course content, b) reproduction directed, a student focuses on reproducing the content learned at the examination to obtain a good grade, c) application directed, a student focuses more on applying the content to real-world scenarios while studying, and d) meaning directed, a student focuses on understanding the meaning of the content and employs critical thinking to develop one's own views. Some research has found that choice of study style is positively correlated with academic achievement and positive attitudes about postsecondary schoolwork (Drysdale et al., 2001; Hong et al., 2004). However, Busato et al. (2000), found no positive correlation between any study style and academic achievement. Thus, more needs to be known about the relationships among motivation, intelligence, attitude, conscientiousness, study style, academic achievement, and homework completing at the postsecondary level.

Research Questions

The purpose of this study was to understand the role that homework plays in academic achievement at the college level. The research questions guiding our study were:

- What motivates/demotivates students to complete their homework?
- What is the relationship between homework completion and academic achievement?
- What student-reported characteristics are related to homework completion?

DESIGN

We conducted a mixed methods study with thirty one students from a four credit genetics course designed for upper-level life-science majors at a southern research university. All participants were assigned homework as part of the course and these assignments included graded and extra-credit Problem Packs and non-graded weekly homework. Problem Packs were comprised of problems similar to what students were provided on the exams. For the class, graded and extra-credit homework factored in students' course grades, but for this study we used only exam averages as our measure of academic achievement. We focused on test performance as our measure of academic achievement to correct for any influence homework scores may have had on student grades.

Data Sources

Our data came from student responses on an open-ended questionnaire and student grades on problem packs and five in-class exams.

Problem Packs

The instructor assigned seven problem packs; four were required for credit and three were for extra-credit. He wrote the assignments so the questions aligned with the current chapter assigned (Figures 2 and 3). Students had to complete each problem pack outside of class and sign a pledge stating that they completed the work individually. Problem pack due dates corresponded with exam dates.

One strand of a section of DNA isolated from E. coli reads

5'-GTAGCCTACCCATAGG-3'

(a) Suppose mRNA is transcribed from this DNA using the complementary strand as the template. What will be the sequence of the mRNA?

(b) What peptide would be made if translation started exactly at the 5' end of this mRNA? (Assume no start codon is required.) When tRNA ala leaves the ribosome, what tRNA will be bound next? When the amino group of alanine forms a peptide bond, what bonds, if any, are broken and what happens to tRNA ala?

(c) How many different amino acids are encoded in this mRNA? Would the same amino acids be made if the other strand of DNA served as the template for transcription?

Figure 2. Sample problem from Polyploidy and Transcription Problem Pack.

Imagine that the small polypeptide chain, Arg-Gly-Ser-Phe-Val-Asp-Arg, is encoded somewhere within the following segment of DNA taken from a prokaryotic cell:

```
                    ↓
AATCGCTGGCTAGCTGCTTCCTTGGGGATGGC
TTAGCGACCGATCGACGAAGGAACCCCTACCG
```

Which strand is the template strand? Draw a box around the appropriate 21 nucleotides on the coding strand corresponding to the 7 amino acids in the polypeptide. Label each strand with its correct polarity (5' and 3').

Hydroxylamine is a mutagen that achieves its effects by adding a hydroxyl (OH) group to cytosine which causes it to pair with adenine. If left unrepaired, it results in the replacement of a G-C base pair with an A-T base pair in the DNA. When hydroxylamine is applied to the above organism, a G-C to A-T conversion was fixed into the DNA at the 17th position of the DNA sequence (indicated by an arrow). What type of mutation does this produce, and what is the effect on the polypeptide?

Acridine orange is another mutagen that is an intercalator, i.e., it slides itself between bases of a DNA molecule and induces insertions of extraneous nucleotides into the molecule. Imagine acridine orange inserts itself between the C and T nucleotides at the 17th and 18th positions (immediately to the right of the C nucleotide indicated by the arrow) and results in the insertion of an adenine at that position in the template strand. What type of mutation does this produce, and what is the effect on the polypeptide?

Figure 3. Sample Problem from Exam 3.

Weekly Homework

The course instructor also assigned weekly homework to reinforce content covered in each chapter and prepare students for the problem packs and course exams. The questions came from the required textbook for the course (Klug et al., 2008). The instructor did not collect or grade these assignments; however, he often reminded and encouraged students to complete the homework each week. He also discussed and reviewed these questions in detail during weekly recitation sessions.

Questionnaire

We administered a questionnaire prior to the final exam on the last day of the course. The questionnaire consisted of 17 questions, eight multiple response and nine open-ended questions. We used this to identify student reported motivation for taking the course, their feelings about the course, homework and problem pack completion, how students viewed themselves compared to their peers in terms of motivation, intelligence, studiousness, responsibility, interest in school, value placed on school, and effort put into school, preferred study strategies, perceptions about the purpose of homework assignments, and personal motivating/demotivating factors for completing homework.

Data Analysis

We determined motivating and demotivating factors for homework completion by analyzing the questionnaire. We categorized students' top three motivating and demotivating factors for completing assignments in this course. Then, we ranked each categories based on the frequency of responses.

We compared student reported motivation with actual performance in terms of Problem Pack and homework completion, using a *t*-test to determine if there was a significant relationship between credit, extra credit, or reinforcement motivating factor and completion of the related assignment type. To determine this, we obtained the overall frequencies for credit and extra credit assignment completion by students from the instructor. When quantifying non-graded homework completion, we used student-reported completion rates provided on the questionnaire. We created a numerical scale that ranged from 0 (never) to 3 (always) based on student responses regarding how often they completed the weekly homework. We averaged student responses and divided by the maximum value, 3, reporting the result as a percentage.

Next, to address whether student homework completion related to academic achievement in this course, we ran a Pearson correlation comparing students' exam average against their completion of problem packs. We omitted student reported non-graded homework in order to minimize error.

We also ran a Pearson correlation measuring completion of problem packs as related to student reported intelligence, conscientiousness, and attitude. Conscientiousness and attitude were aggregate factors, comprising the mean student response across three composite characteristics. Conscientiousness included student reported studiousness, responsibility, and effort put forth in school. Attitude included student reported motivation, interest, and value for school. Intelligence was measured from students' response as an individual factor.

Finally, to measure the value of study style in promoting homework completion we first categorized student responses about how they studied for course exams into two study styles: whether students used homework as a study tool when preparing for exams or not. We tested each study style against completion of credit and extra credit problem packs using a *t*-test, omitting non-graded assignments.

RESULTS

Homework Completion

Student responses fell into a broad range of 12 categories for motivating factors and 13 categories for demotivating factors. Overall, students were most likely to complete homework assignments given for required course credit.

Motivating Factors

Students reported motivating factors that fell into 12 categories (Table 1). Out of all student responses, the top three motivating factors were reinforcement (28.75% of total responses), credit (18.75%), and extra-credit (11.25%). Responses placed into the "reinforcement" category were those that indicated that simply having to learn the material or wanting to master the material was a motivating factor, those placed into the "credit" category indicated that a credit contingency was a motivating factor, and similarly, those placed into the "extra credit" category indicated that extra credit was a motivating factor. Altogether, the top three factors composed 58.75% of all student responses.

Demotivating Factors

Students reported demotivating factors that fell into 13 categories (Table 2). The most common demotivating factors were "other commitments" (27.85%) and "unable to understand" (20.25%). Together, these two categories compose 48.1% of total student responses. They are followed up by both "too difficult" and "too long" (8.86%). This indicates that students' primary problems are not being able to find the time to complete their homework and being unable to understand the level of complexity required. And, while some students seemed to be easily distracted from homework, such as the student who listed "Good movie on HBO" as a demotivating factor, other students are very dedicated to completing their homework assignments, such as the student who listed "The only thing that would stop me is if there was some kind of emergency in my life . . ."

Responses placed into the "other commitments" category indicated that having other things to do was a demotivating factor. Those that were placed into the "unable to understand" category showed that the student literally could not comprehend the nature of the questions and that this was a demotivating factor, while responses placed in the "too difficult" category were those in which students indicated that they understood the nature of the questions, but the difficulty of the work was a demotivating factor. The "too long" category was for responses that showed

Table 1. Student reported motivating factors.

Motivating Factor	Percent n=80	Illustrative Responses
Reinforcement	28.75%	"To better educate myself and understand the material," "Information might be on exam"
Credit	18.75%	"If it's for a grade," "Required"
Extra Credit	11.25%	"Extra credit," "Extra credit for attempting problems"
Able to Understand	8.75%	"Can understand homework," "If I understood the material"
Free Time	7.50%	"Easy schedule," "Having free time"
Interest in Material	6.25%	"I like the material," "Can capture interest"
Concern for Grade	5.00%	"I need to maintain a high GPA and this is a 4 hour course," "Wanting to do well in the course"
Material Covered	3.75%	"We go over the material before it's due," "Going over more examples in class like those in the PP and homework"
Self-Assessment	3.75%	"To test my knowledge," "Determine what I know and don't know"
Not Too Long	1.25%	"Less problems"
No Distraction	1.25%	"No good movies on"
Other	3.75%	"We get ample time to do it and the teacher is willing to help," "It's against my work ethic to turn in an incomplete assignment"

students' feelings about excessive length of assignments or the time that it took to complete assignments was a demotivating factor.

Completion

Overall, students completed 79.03% of all credit assignments, 64.56% of all extra-credit assignments, and 56.99% of all non-graded assignments. This contrasts with student-reported motivation, which listed "Reinforcement" as the top motivator, "Credit" as the second, and "Extra-credit" as the third. Non-graded assignment completion was derived from student-reported data, and as such may be less accurate than completion levels for credit and extra-credit assignments, which were derived from student grades.

A *t*-test revealed the relationship between listing credit and completing problem packs as not significant ($t(29) = -.497$, $p = .623$). Within the same sample, we found no significant relationship between listing extra credit as a motivating factor and the actual completion of extra credit problem packs ($t(29) = -2.13$, $p = .83$). Students who listed reinforcement as a motivating factor tended to report that they completed more non-graded homework assignments than those who did not ($t(29) = -2.64$, $p < .05$).

Table 2. Student reported demotivating factors.

Demotivating Factor	Percent	Illustrative Responses
Other Commitments	27.85%	"Other class work," "Not having enough time"
Unable to Understand	20.25%	"I did not quite understand the material when given the problems," "Not understanding questions"
Too Difficult	8.86%	"Too hard; don't know how to do it," "Difficulty of subject matter"
Too Long	8.86%	"Length of problems," "Time it takes to work them"
Not for Credit	7.59%	"Not for a grade," "Counts little"
Boring or Redundant	3.80%	"Just not interesting," "Lots of very similar, repetitive problems"
Distraction or Procrastination	3.80%	"Good movie on HBO," "Procrastination/Avoidance"
Personal Feelings	3.80%	"Frustration after not understanding it," "Don't feel like it"
Extra Credit	3.80%	"It's extra credit," "Extra credit"
Material Not Covered	2.53%	"Material not focused on as much during lecture," "We don't go over the material in class before it's due"
No Outside Aid	2.53%	"Don't know who could help," "Not enough help from outside sources"
Extenuating Circumstances	2.53%	"The only thing that would stop me is if there was some kind of emergency in my life. I can't think of anything else that would stop me," "Severe illness"
Other	3.80%	"Redundant/Too difficult," "The lectures are not interesting"

Homework Completion and Academic Achievement

A Pearson correlation seeking 2-tailed significance revealed a strong correlation between homework completion to academic achievement, $r(29) = .633$, $p < .001$. Students who completed more homework did better on their exams than students who did not (Figure 4).

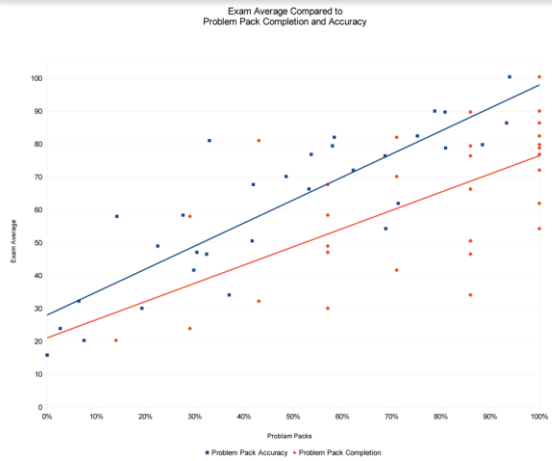

Figure 4. Relationship between average exam grade and homework completion versus homework accuracy.

Student Characteristics

We considered the relationship between intelligence, conscientiousness, attitude, and study style with homework completion and found conscientiousness ($r(29)=.62$, $p<.001$), attitude ($r(29)=.54$, $p<.01$), and intelligence ($r(29)=.36$, $p<.05$) were all significantly related to homework completion. In addition to having a strong correlation to homework completion, the three student characteristics correlated with each other significantly. Intelligence was significantly correlated with conscientiousness ($r(29) = .388$, $p < .05$), but not with attitude ($r(29) = .349$, $p = .054$). Conscientiousness and attitude were strongly correlated ($r(29) = .757$, $p < .001$).

We also measured the relationship between study style and completion of required and extra credit problem packs. Of 31 students, 14 did not report that they used their homework to study for exams, while 17 did. A *t*-test revealed that this relationship was significant ($t(29) = -2.24$, $p < .05$); students who used homework to study completed more homework overall.

Summary

Students have a wide variety of motivating and demotivating factors. Students in this class were motivated to do homework assignments due to credit value offered for the assignment, the utility of assignments in studying for exams, and a need to learn the material. Students who viewed themselves to be intelligent, conscientious, and have a positive attitude toward school were more likely to complete their assignments regardless of credit. Students were less likely to complete homework assignments if they were busy or if the assignments were perceived to be too complicated. Overall, we found a positive relationship between homework completion and academic achievement within this college genetics course.

DISCUSSION AND IMPLICATIONS

The purpose of this study was to understand the role homework plays in academic achievement in upper-level postsecondary courses. Our findings suggest that the relationship between homework and academic achievement established at the K-12 level (e.g., Cooper et al., 1998; Cooper et al., 2006; Trautwein et al., 2006) also holds true at the college level. In addition, we found credit to be a powerful motivator for college students (Ryan & Hemmes, 2005). We found most students were motivated to complete homework because they want to learn the course material and earn credit. In this course, reinforcement of course material was the primary goal of the homework. Thus, students' reported views were similar to the instructor's. However, we found that reinforcement alone was not as strong a motivator as student self-reports would suggest. Students whose goal was reinforcement should have been equally likely to complete any type of assignment. All assignments covered the same material and would have prepared students equally well for exams. However, we found discrepancies between students' reported motivation and actual completion rates. Students were pragmatic in practice given that they were more likely to attempt assignments that directly impacted their grade. It is more likely that most students are motivated by a combination of factors. Thus, even students who are motivated by reinforcement are more likely to complete credit assignments than non-graded assignments. Extra-credit is not as powerful a motivator as credit, but more powerful than no credit. Thus, an implication of this finding is that to better motivate students to complete homework, instructors should assign credit value for integral homework assignments and assign extra credit to less critical assignments.

We found students were less likely to complete homework when they felt overwhelmed by time demands, when assignments were perceived as too complex, or they thought the assignments would take too long to complete. An implication of this is that to maximize homework completion, an instructor may want take an honest evaluation of homework requirements. If assigning a problem of extreme difficulty, they may want to limit the number of tasks and try to set standards on expected completion times. Thus, students can then structure their

schedules with a precognition of how much time they need to regularly dedicate to assignments.

The positive relationship of conscientiousness, attitude, and study style on academic achievement observed by Busato et al. (2000) may be working through the mediating factor of homework completion. These findings suggest it is important for college instructors to craft homework assignments in such a way to maximize student completion. Positive attitudes about the course and studying is likely to be self-reinforcing, so ways to improve student attitudes about homework, such as emphasizing its utility in preparing for exams, should be explored. In addition, courses that help students improve their study skills should be considered a more essential part of college curricula. Instructors could also potentially create homework assignments that factor into a portion of the exam grade. Even without this factor, students who do homework tend to do better on exams than those who do not, but this credit assignment would increase student motivation to complete homework and make the assignments an integral part of the learning system. We still need to explore the issue if homework completion is what directly improves the exam performance or whether students who are motivated to complete homework assignments are also motivated to study well for the exam, increasing their overall performance. In the case presented, the academic end result remains that homework completion and exam performance are positively linked.

ACKNOWLEDGEMENTS

All research was conducted under the approval of the University of Southern Mississippi IRB (#10010403).

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Survey of Biology Capstone Courses in American and Canadian Higher Education: Requirement, Content, and Skills

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Abstract: Capstone experiences have high educational impact with various approaches available for biology. However, no information exists regarding the pervasiveness of capstone courses in Canadian and American biology programs. This study surveyed the prevalence and character of biology capstone courses in the USA and Canada. The survey included a majority of public institutions offering primarily undergraduate programs. Seventy percent of American biology degree programs required a capstone course vs 27% of Canadian schools. Large and graduate institutions were less likely to require a capstone course. Medium-sized institutions were more likely to deliver their biology capstone course as a seminar, whereas small institutions were more likely to include an undergraduate research experience. Sixty percent provided some review of biology's conceptual foundations, but most capstone courses devote little time considering the history or philosophy of biology. Most schools included the development of students' writing, speaking, and thinking as learning objectives. Research skills were significantly less likely to be a learning objective. Although biological capstone courses may intend to integrate students' biological knowledge, educators also need to consider how that knowledge could be synthesized into students' entire education.

Keywords: curriculum, research, communication, thinking, history, philosophy

INTRODUCTION

High impact educational practices positively impact student learning outcomes (Brownell and Swaner, 2010, Julien et al., 2012). Capstone experiences are one such practice which facilitate students' integration of their learning; students are able to construct a robust knowledge structure for themselves when their knowledge is interconnected and integrated into a whole rather than fragmented into a series of unconnected courses (Levine, 1998, Kuh, 2008). Different strategies for delivering capstone experiences exist (Smith, 1998): They may be delivered through the major discipline (Stanford and Duwel, 2013), as a general education requirement (Griffin and Burns-Ardolino, 2013, Kerrigan and Carpenter, 2013), or be an experience not tied to an individual course (Redman, 2013, Wenk and Rueschmann, 2013). In addition, capstone courses may not be restricted to students' final year of a degree program instead integrating students' associate degree program (Stubbs et al., 2013) or serving to introduce a discipline (Chaplin and Hartung, 2012).

A variety of approaches exist for providing capstone experiences within the biology major. They may involve journaling or writing courses that promote critical thinking (Lankford and vom Saal, 2012). Some develop students' communication skills (Obringer and Kent, 1998). Other possible approaches include a comprehensive final exam, literature review, research presentation, exit interview, or e-portfolio (Davis, 2011, Stanford and Duwel, 2013). Alternatively, honours theses delivered as an independent studies course (Levine, 1998) including an undergraduate research experience (Brownell and Swaner, 2010) may serve as a capstone experience. Capstone experiences do not need to be delivered as a course but could be a requirement for completing a major (Davis, 2011). However, if a capstone experience is not integrated into a particular course it may become extra workload within a full course load for students.

Although these many ideas for capstone experiences have been discussed in the published literature, nothing exists to indicate the degree to which capstone courses have been implemented in Canada and the USA and the content and skills that are taught within them. An earlier survey of the general biology curriculum indicated that students and faculty were interested in an integrative senior course, but was not widely considered at that time (Carter et al., 1990). The survey reported in the present paper fills this gap in our curricular knowledge by determining whether American and Canadian institutions of higher education require students to complete a capstone course in partial fulfillment of their biology degree program. The survey also reports on the nature of biology capstone courses, in addition to their mode of delivery.

METHODS

An online survey using Google Forms was constructed asking institutions to indicate the nature of their institution describing it as either public or private, and whether it was a two-year community college, undergraduate college, or research university. The survey also asked participants if a capstone course was offered as part of their biology major and whether that capstone was a required course. In addition, the survey asked respondents to indicate the nature of the course in terms of its delivery, the content being learned, and which skills were being developed in their students. The last question provided participants the opportunity to include any other noteworthy characteristics of their capstone course not captured in the survey.

The link to the online survey instrument was emailed to all institutions that offered a major in biology (as indicated by their website) that were members of representative American and Canadian organizations of higher education (N = 446) including: American Association of State Colleges and Universities (AASCU, 2014), Council of Public Liberal Arts Colleges (COPLAC, 2014), Association of Universities and Colleges of Canada (AUCC, 2014), and Alberta Introductory Biology Association (AIBA, 2014). These four organizations form a representative population of biology programs offered in Canada and the USA. The email was sent directly to a biology representative or administrator as indicated on the institution's website requesting that the survey be completed by whomever best understood the biology program or teaches the biology capstone course. An emailed follow-up was sent to each member who had not responded to the survey within four weeks. Some institutions were members of more than one organization but were only entered into the survey once. The surveyed population consisted of only those institutions offering a biology major as determined from each university and college's website. American protectorates and international members of AASCU were not included. Institution size was determined from 2013 enrollment data obtained from the AUCC website (AUCC, 2014) and IPEDS Data Center (US Dept of Education et al., 2013) using head counts which included the number of undergraduate, graduate, full time, and part time students. This enrolment data was used to categorize institutions into small (<5,001 students), medium (5,001-15,000 students), and large (>15,000 students) institutions. Primarily undergraduate institutions offered few, if any, graduate programs.

Survey confidence intervals were calculated at a confidence level of 95% when the total population size was known. Significant differences among regions and institutional sizes and types were determined using chi-square analysis. In some cases, however, an expected cell value in the contingency table was less than one, or more than 20% of the expected values were less than five producing an unreliable chi-square analysis. In these cases Fisher's Exact Test was used. Tukey's test determined the statistical significance among the ranked proportion of a biology capstone course which reviewed fundamental biological concepts, or considered the history and philosophy of biology.

RESULTS

The survey generated an overall 40% response (N = 177). There was no response bias by instituion size ($X^2 = 0.898$, $p = 0.638$), type ($X^2 = 0.658$, $p = 0.417$), or region ($X^2 = 1.987$, $p = 0.851$). The vast majority of responding institutions were public (98%) consisting primarily of undergraduate or research institutions offering four-year bachelor degrees. Two of the responding institutions were two-year colleges and were included in the survey analysis because associate degree programs may require capstone courses (Stubbs et al., 2013). Indicative of the differences between the Canadian and American context, the Canadian sample had a significantly greater proportion (73%) of research institutions responding to the survey relative to the American response (28%; $X^2 = 15.55$, 1 DF, $p < 0.0001$). The response sample had a greater proportion of public institutions from the US (99%) than from Canada (88.5%, Fisher's Exact Test $p = 0.0103$). There were no significant regional differences among Eastern or Western Canada or the American West, Midwest, Northeast or South. Large institutions were significantly less likely to be undergraduate (25%) institutions than medium (83%) or small (92%) institutions (Fisher's Exact Test $p = 0.0489$). In contrast, small institutions were less likely to be public (92%) than medium and large institutions (99% and 100%, respectively; $X^2 = 67.06$, 2 DF, $p < 0.001$).

Sixty-three percent of responding institutions indicated that their biology degree programs require students to complete a capstone course (Figure 1). However, only 27% of Canadian vs 70% of American biology degree programs require their students to complete a biology capstone course in partial fulfillment of the biology major ($X^2 = 15.55$, 1 DF, $p < 0.0001$), although another 23% of responding Canadian institutions offer a biology capstone course but do not require it. Less than 10% of all responding biology degree programs indicated either that: i) they offered a capstone course but did not require it; ii) had discontinued their capstone course; iii) their biology program was subdivided into different sub-fields (e.g. zoology, botany, ecology, wildlife biology, biotechnology, molecular biology) but that not each offered a capstone course; iv) students complete a capstone as part of their general education requirements but not necessarily as a biology course. Large and research institutions were significantly less

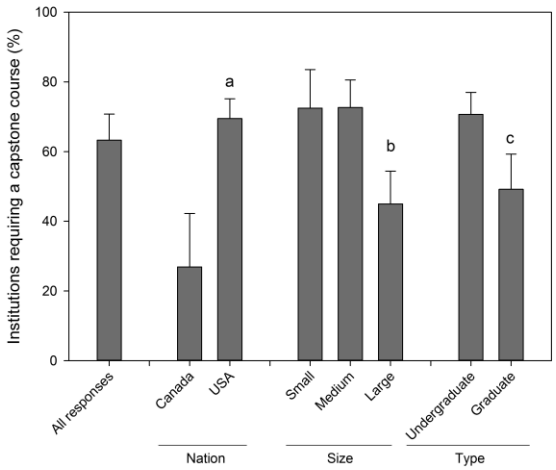

Fig. 1. Prevalence of biology capstone courses among responding institutions (N=177). Error bars indicate confidence interval at the 95% confidence limit. Chi-square analysis detected significant differences: [a]American institutions are more likely to offer a required biology capstone course (X^2 = 15.55, 1 DF, p < 0.0001); [b]Large institutions are less likely to require a biology capstone course (X^2 = 13.05, 2 DF, p = 0.0015); [c]Primarily undergraduate institutions are more likely to offer a required biology capstone course (X^2 = 7.06, 1 DF, p = 0.0079).

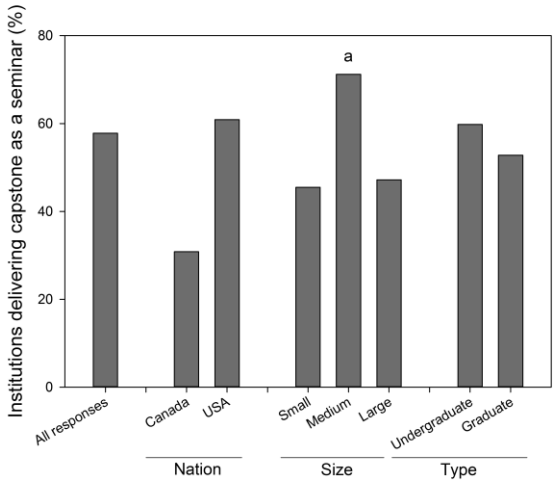

Fig. 2. Percentage of biology capstone courses offered as a seminar. Percentages are of those institutions (N=128) offering a capstone course in their biology major. Confidence intervals are not reported because the total population size of institutions offering a biology capstone course is unavailable. Chi-square analysis detected significant differences: [a]Medium sized institutions tend to deliver their capstone course as a seminar (X^2 = 8.05, 2 DF, p = 0.018).

likely (X^2 = 13.05, 2 DF, p = 0.0015 and X^2 = 7.06, 1 DF, p = 0.0079, respectively) to require the completion of a capstone course by their students majoring in biology.

The delivery of surveyed biology capstone courses was approximately split among all respondents between seminar (58%) and lecture (42%) (Figure 2). However, medium-sized institutions were significantly more likely than small or large institutions to deliver their biology capstone course as a seminar (71% vs 46% and 47%, respectively; X^2 = 8.05, 2 DF, p = 0.018). The inclusion of an undergraduate research experience (URE) in responding biology capstone courses was also split (54% included a URE) among all respondents (Figure 3). Small institutions, however, were statistically more likely than medium or large sized institutions to include a URE in their biology capstone course (73% vs 42% and 53%, respectively; X^2 = 7.83, 2 DF, p = 0.020; Figure 3). American biology capstone courses were twice as likely as Canadian courses to be delivered as a seminar (61% USA vs 31% Canada), and the inclusion of a URE was more likely in Canadian capstone courses than in American (69% Canada vs 51% USA) though these differences were not significant. In addition, there were no statistical differences between primarily undergraduate or graduate/research institutions: Both types of institution were approximately split between delivering the biology capstone as a seminar vs lecture, and between whether or not the capstone included a URE.

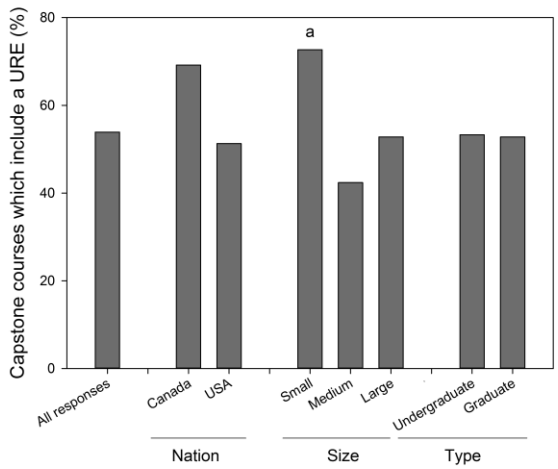

Fig. 3. Percentage of biology capstone courses which include an undergraduate research experience (URE). Percentages are of those institutions (N=128) offering a capstone course in their biology major. Confidence intervals are not reported because the total population size of institutions offering a biology capstone course is unavailable. Chi-square analysis detected significant differences: [a]Capstone courses at small institutions are more likely to include an undergraduate research experience (X^2 = 7.83, 2 DF, p = 0.020).

There were no significant differences between Canada and the US, between primarily undergraduate and graduate/research institutions, or among the three institutional sizes with regard to the content taught in biology capstone courses: Most do some review of biology's conceptual foundations (60%), while most do not consider history or philosophy of biology (55% do not). Canada, however, was less likely to review biology's conceptual foundations (39% vs 62% in the US) though this difference was not significant.

When the amount of time devoted to considering biology's conceptual foundations, history or philosophy, is more closely examined (Figure 4) it becomes clear that most Canadian and American institutions spend little to no time considering these issues. American and Canadian institutions were significantly less likely to consider the history and philosophy of biology (56% and 55% indicated no consideration whatsoever, respectively) than to review its conceptual foundations (40% indicated no review whatsoever; Tukey test, $p < 0.05$). Significant differences were not detected among nations, regions, or sizes and types of institution.

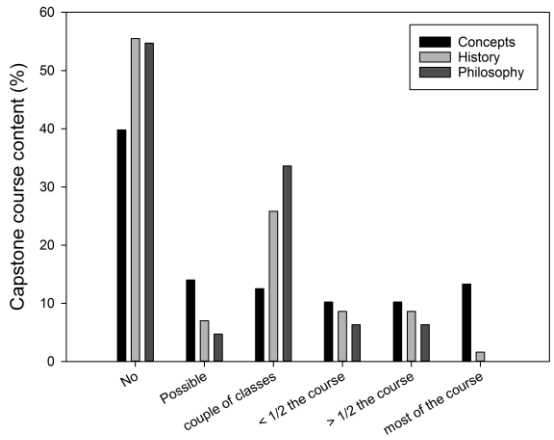

Fig. 4. Percentage of institutions offering capstone courses which consider the major foundational concepts, history, and philosophy of biology. Percentages are of those institutions (N=128) offering a capstone course in their biology major. Confidence intervals are not reported because the total population size of institutions offering a biology capstone course is unavailable. The Tukey Test indicated a significant difference ($p < 0.05$). The history and philosophy of biology are less likely than foundational concepts to be covered in biology capstone courses among all institutions (N=128) when the categories were converted into ranked values: No = 0, Possible = 1, Couple = 2, < ½ = 3, > ½ = 4, Most = 5.

Most responding institutions indicated that the development of students' writing, speaking, critical thinking, and research skills are learning objectives of their capstone course (Figure 5). The development

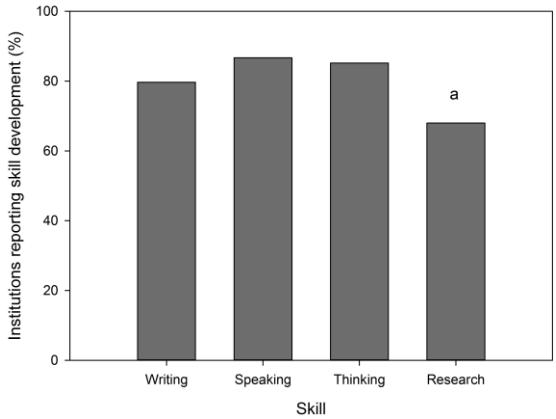

Figure 5. Percentage of institutions indicating that the development of a particular skill is a learning objective of their capstone course. Percentages are of those institutions (N=128) offering a capstone course in their biology major. Confidence intervals are not reported because the total population size of institutions offering a biology capstone course is unavailable. [a]Chi-square analysis detected significant differences among the different skills being developed in students among all responding institutions ($X^2 = 17.25$, 3 DF, $p = 0.00006$). The development of students' research skills was less likely to be a learning objective of the course than writing, speaking, or critical thinking.

of students' research skills (68%), however, was significantly less likely to be a learning objective of the course than the development of students' writing, speaking or critical thinking skills (80%, 87%, 85%, respectively; $X^2 = 17.25$, 3 DF, $p = 0.00006$).

The survey permitted participants to include any comments that would better clarify the nature of their capstone course. From these comments it appears that the most common capstone course of biology degree programs involves students researching a question, and reporting their results in a formal paper and public presentation. There were variations on this approach with some being embedded in a single course or spread over two courses. In addition, the research could involve the collection of lab or field data, or be restricted to the current literature to produce either a review paper or simulated grant application.

Other examples of capstone courses included those which involved either guest or student-led seminars discussing current topics in biology or applied students' biological knowledge to a term-long theme (e.g. global warming or the impact of agricultural practices) connecting to other disciplines with evolution as the most common unifying theme. A few institutions indicated that their capstone courses prepared students for the work world (e.g. résumé and cover letter preparation) including graduate school applications. In addition, some

capstone courses prepared students for externally administered assessments of student learning (e.g. Major Field Test in Biology, Area Concentration Achievement Test) as required for accreditation. One survey respondent commented that the external assessment for accreditation did not provide information different from what they had gathered from their own internal tests and student grades, and that the preparatory course was not of the same educational value as their other biology courses. One university indicated that their general education requirements include visual literacy which could be satisfied by the biology capstone course. Visual literacy in this capstone involved teaching students to understand scientific information presented in tables, graphs, and other visual representations. Three institutions reported that their capstone courses consider ethical issues in biology including research, biomedical, and environmental ethics. One institution commented that the communication learning objective of their capstone course involved everything necessary for networking in science including the use of technology that enables remote meetings, conferencing, sharing information, and accessing work groups. Finally, there was one institution that reported that their biology capstone course was run as a journal club with articles chosen by the instructor.

DISCUSSION

While department chair, I regularly discussed students' educational programs when they registered for their subsequent year of study. Their program planning form was a single page which organized their required courses into lists of boxes that needed to be checked off before being eligible to graduate. My difficulty was that students' learning seemed to be reduced to a series of disconnected courses with little integration or cumulative learning (Smith, 1998). In the courses I taught, students seemed to approach their education using a memorize-regurgitate-purge learning cycle with the result that their learning did not inform their next educational experience. Students busy themselves collecting courses but never take the time or are never granted the opportunity to build for themselves an integrated knowledge structure: The bricks (courses) remain in a pile in a corner of the lot never being used to construct a house (Smith, 1998). At some institutions the lack of integrative, cumulative, or reflective learning may be a result of the professionalization of the professoriate producing a greater commitment by faculty to the needs of the discipline rather than to the needs of student learning (Smith, 1998). To enable students to integrate their learning, undergraduate degree programs need to scaffold their educational experiences such that students are able to interconnect their knowledge gained from different courses and experiences, leading to deeper learning and a more robust knowledge structure (Ambrose et al., 2010). There are many possible learning structures that can integrate students' cumulative learning (Smith, 1998): learning communities, ability or skills based education, service and experiential learning, summative self-evaluations involving reflexive learning as might occur within an e-portfolio, and capstone courses.

Capstone courses are integrative experiences typically near the end of students' degree programs that tie together the disparate parts of an education (Smith, 1998) and have been shown to produce positive learning outcomes in physiology (Julien et al., 2012). Similar studies have not been published for biology degree programs. Other roles for capstones include providing breadth to move students beyond the narrow confines of their major, enabling students to apply their cumulative knowledge to solve or answer a problem or question, and finally to prepare students for their transition to a post-undergraduate reality (Levine, 1998). Courses are one vehicle of providing students a capstone experience. Others include comprehensive examinations and senior theses or projects (Levine, 1998, Davis, 2011, Kinzie, 2013).

The present survey suggests that American and Canadian capstone courses include many of these possibilities for integrating students' education but that the most prevalent form of biology capstone includes a research assignment which is presented as a formal paper and oral presentation similar to what has been previously reported for the academy at large (Kinzie, 2013) and for biology in particular (Stanford and Duwel, 2013). Some schools such as Allegheny College have strengthened students' performance in their senior capstone project by scaffolding the development of research skills throughout students' undergraduate years (Coates et al., 2014). Other examples from the current survey included preparing students for an accrediting assessment tool, preparation for the work world after graduation, and current biological topics seminars.

The advantages of seminars may include the introduction of students to a range of approaches to a discipline (de Pillis and Adolph, 2010) with student-led seminars developing students' communication, information literacy, and critical analysis skills (Obringer and Kent, 1998). The strength of student-led seminars is the active learning this entails as a result of students talking to, and teaching each other (Tanner, 2009) that can lead to deeper learning (Weimer, 2013) relative to lectures which have been documented to have less impact on student learning (Bligh, 1998). Some might argue that guest-led seminars are no different than lectures; however, it has been suggested that a passionate speaker who is invested in the material and who treats the subject matter with respect can have a deep impact on students' thinking (Palmer, 2007) exposing students

to current research and potential careers in the biological sciences. The present survey indicated that students were as likely to experience their capstone course as a seminar whether they were enrolled in an undergraduate college or in a research university, and whether they were attending a Canadian or American institution. Thus, from the results of the present survey there is clearly a diversity of approaches to capstone courses in biology degree programs which may reflect the different understandings of the learning outcomes for such a learning experience: application of biological research skills, integration of biological concepts, placing biological knowledge in its broader context to solve real-world problems, and to prepare students for life after their biology major. In addition capstone courses may facilitate institutional assessment of their own degree programs.

Undergraduate research is a high impact educational practice resulting in improved student learning outcomes (Fechheimer et al., 2011, Haave and Audet, 2013). However, many of the surveyed biology degree programs did not report the inclusion of an undergraduate research experience (URE) in their capstone course. If it is not part of the capstone, does it happen elsewhere in students' degree programs? At Augustana, for example, our biology capstone does not include a URE but students are required to complete another senior course in their fourth year: most of our fourth-year courses include a URE. Thus, our biology degree program does not ensure that all of our students have a URE. Promising students are directed to our independent studies courses which will provide them with a URE, but not all students are so directed. Is this an important consideration? Is it important that all biology students graduate with a URE even though most will not become practicing biologists? Or is it more important for biology students to graduate with a sense for how our biological concepts impact how we interact with each other and design our communities? For example is it important for students to consider whether our biological understanding indicates whether we are biologically, environmentally, and/or culturally determined and should that affect how we design and implement our community programs? These are integrating questions that biologically informed citizens need to consider. On the other hand, UREs develop skills important to any community leader: speaking, writing, critical thinking, and gathering and assessing data and information, in addition to producing self-reliance (Brownell and Swaner, 2010). Any assumption that research institutions provide more research opportunities for undergraduate students is not supported by the data presented here similar to what has been previously reported (Hu et al., 2007). A previous survey of the general biology curriculum had indicated that smaller colleges were providing greater opportunities for undergraduate research than at large universities (Carter et al., 1990). The current survey indicates that small institutions are more likely to provide a URE within their biology capstone course but that there is no difference when primarily undergraduate institutions are compared with graduate institutions. It seems that size does matter when it comes to providing students with a URE.

The current survey indicated that the vast majority of biology capstone courses do not review its conceptual foundations. Is it safe to assume that students have a sufficient grasp of biology's theoretical foundations? Typically students' freshman biology course(s) considers biology in its whole but never again throughout their program. Do students need another chance to integrate their biological knowledge after completing higher level studies in biology to avoid compartmentalization of their knowledge structure? If the central tenets of biology rest on the interdependence of biological function, development, and evolution (Haave, 2012) then perhaps students should be given the chance to integrate their deeper knowledge gains after the disciplinary focus that typically happens in students' sophomore and junior years.

Very few institutions included discussion of the historical and philosophical foundations of biology in their capstone courses possibly indicating that their understanding is not considered significant to the practicing biologist or informed citizen. However, without a grounding in the philosophical assumptions of modern biology, or a grasp of the historical contingencies that produced a biological science focused on molecules with little consideration of ontogeny may produce graduates with little understanding of the types of questions that have been the most productive in advancing our biological knowledge and why they have been successful in doing so (Hawke, 1983). A consideration of the history and philosophy of biology can also illuminate why particular biological fields languish as a result of having unclear questions or inappropriate tools for their investigation. In addition, placing our current biological understanding in the context of its history may increase students' engagement in the discipline. It has been argued that including the stories of biological researchers gives biology a human face to which students can relate and helps place biology in its social context; this enables students to consider the implications of biological research and how it is a social endeavour (Chamany et al., 2008). Doing so may provide students a sense of their own possible place in the biological sciences.

The literature suggests that capstone experiences have a positive impact on students (Brownell and Swaner, 2010), and the present survey indicates that most biological degree programs require them. It is unknown from this survey how students' undergraduate biology program is integrated beyond

their freshman year when a capstone is not offered. Capstone courses are one way to integrate a broad education, though if the capstone is embedded in the major its ability to do so may be limited (Kinzie, 2013). Attending to the historical and philosophical underpinnings of biology may be one way to achieve integration of the biology major into students' broader knowledge structure (Hawke, 1983).

To summarize, the present survey of Canadian and American biology degree programs produced a 40% response with most responding institutions being public undergraduate institutions. Most responding institutions required the completion of a capstone course by their biology students, though not so much in Canada or in large, research institutions. Biology capstone courses are as likely to be delivered as a seminar as they are as a lecture, though in medium-sized institutions the seminar predominates. In addition, an undergraduate research experience has an even chance of being a part of the capstone course though in small institutions this is more likely to happen. The majority of biology capstone courses have the development of students' communication and thinking skills as learning objectives including research skills to a lesser extent. Most capstone courses do not consider the history or philosophy of biology and spend little time reviewing the conceptual foundations of biology. It is apparent that biological capstone courses are primarily intended to integrate students' biological knowledge structure. Consideration of how that might be synthesized into students' entire education may be warranted.

ACKNOWLEDGEMENTS
Thanks to Alysha Wagner who assembled the data set as part of a CTL Summer Studentship from the University of Alberta. The data discussed in this paper were presented in part by NCH at the 58th annual meeting of the Association of College and University Biology Educators (ACUBE), University of Portland, OR, October 17, 2014

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INNOVATIONS

Generation of the Dimensional Embryology Application (App) for Visualization of Early Chick and Frog Embryonic Development

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Abstract: The study of embryonic development of multiple organisms, including model organisms such as frogs and chicks, is included in many undergraduate biology programs, as well as in a variety of graduate programs. As our knowledge of biological systems increases and the amount of material to be taught expands, the time spent instructing students about embryology is becoming more abbreviated. In addition, limitations in budget and resources make the laboratory components of embryology courses more difficult to support. We have generated a free mobile tablet application (App) called Dimensional Embryology (http://itunes.apple.com/app/id985989230) that can be used to quickly and easily teach students about the organization, shape and position of embryonic structures in both early chick and frog embryos. For each organism, we have highlighted different time points in early development, and have two-dimensional and three-dimensional images of the embryos. Using color enhanced images and images with the major structures isolated away from the background, students can make correlations between observations of serial sections and the three-dimensional shape and position of each internal structure. Students that used the Dimensional Embryology App found it to be very informative, easy and convenient to use.

Key words: Embryology, development, chick, frog, three-dimensional, computer-assisted learning

INTRODUCTION

The development of an embryo begins with a fertilized egg, which undergoes cleavage, gastrulation and organogenesis/ histogenesis. Knowledge of fundamental embryology is critical for understanding general organismal development, for comparing the ontogeny of different organisms, and for helping elucidate the establishment of different developmental disorders and diseases. However, exclusive embryology courses are no longer part of many curricula, due to a lack of time in which to teach the material, along with a lack of financial support for supplies, equipment, and instructional faculty (Scott et al., 2013). Portions of embryology are now incorporated into multiple courses, as students and faculty alike understand the importance of establishing a foundational knowledge of embryonic development (Burk et al., 2013; Hamilton and Carachi, 2014; Scott et al., 2013). Introductory biology, developmental biology, anatomy and physiology, histology, and vertebrate comparative anatomy courses, in pre-medical biology undergraduate programs, medical schools, veterinary and dental schools often teach about the anatomical structures in a range of organisms and how those tissues and organs develop in the embryo (Beale et al., 2014; Burk et al., 2013).

Many of the courses that include embryology use a traditional lecture style paired with a laboratory component and are required within major degree programs, or are part of a self-directed learning program. Within lectures on embryology in these courses, two-dimensional images of different times in development are often drawn or displayed on a screen. In the laboratory, both live and fixed fertilized embryos are observed. For early embryonic development, vertebrate model organisms such as frog and chick embryos are used to identify anatomical structures, to observe when structures develop in the embryo by looking at different time points, and to evaluate the differences and similarities in embryonic development among different organisms. Live frog and chick embryos allow students the ability to directly observe changes over time, however the live embryos present some difficulties. Students can observe gross, external anatomical changes but cannot visualize any internal characteristics. Furthermore, due to the cost of purchasing live organisms, the need for approval to use live vertebrate organisms for research and the necessity for available space for housing live organisms, laboratory experiences with live developing embryos are limited. Historically, preserved whole mount embryos and serial cross-sections of embryos that have been fixed at different times during development have been used for evaluation. While the prepared slides are useful to visualize some of the structures, this approach also

has limitations. The whole mounts do not allow for the observation of internal structures. Using traditional histological serial sections, the students do not appreciate what the structures actually look like and cannot distinguish the different parts of the embryo. Students have difficulty making the association between what is observed in serial sections and the three-dimensional shape and location of structures in the whole mounts. Students lack the ability to visualize the complexity of the structures and the relationships between the structures in the embryo. In addition, slide observation needs to be done in the lab where microscopes are available and an instructor is present to help guide the students as they visualize the sections of the embryo and compare them with the Atlas of Descriptive Embryology (Shchoenwolf, 2008) and websites such as Developmental Biology Online (Scadding, 1998) and DevBio: Vade Mecum[3] (Tyler and Kozlowski, 2010).

To address the problems with embryology, we have generated a mobile tablet application, an iPad App, called Dimensional Embryology that can be used to visualize chick and frog embryo serial sections, along with three-dimensional models of the embryos at different times in development. The App can be used as supplemental information, in web-based or self-study programs, and in programs that have limited funding and equipment. Furthermore, unlike many labs where the students cannot revisit the specimens or repeat the observations, by using a mobile tablet application the students can review the material at any time, allowing for more self-study time. To our knowledge, there is no resource that clearly demonstrates the three-dimensionality of the structures within chick and frog embryos. This is a critical component of student knowledge of embryonic development that is lacking. Websites and applications similar to ours have been created to visualize mouse, such as **EMAP eMouse Atlas Project (**http://www.emouseatlas.org**) (Richardson et al., 2014)** and human embryo serial sections and three-dimensional structures (Ecker et al., 2003; Museum of Health and Medicine, 2012; O'Loughlin, 2008; Sulik and Beam Jr., 2015) but not for other organisms. The Dimensional Embryology App uses different colors to label the structures in every serial section and uses the serial sections to create a three-dimensional, color enhanced model of the embryo that can be rotated on both the X and Y axes.

The Dimensional Embryology App can be downloaded for free to an iPad through the iTunes store, http://itunes.apple.com/app/id985989230. It is easy to use and provides a quick, convenient resource for the study of embryology.

METHODS

Image preparation

Standard sequential serial cross-section slides of 24 hour (hr), 48hr, and 72hr chick embryos, and hatched (4mm) and 10mm frog embryos were obtained from Carolina Biological Supply Company, (catalog #311532, 311604, 311652 for chick and special order for frog). To generate a library of images for each embryo at each developmental time point, the embryonic sections were imaged using a Leica microscope at 10X, with a digital camera. The images were transferred to a Dell computer with Leica Application Suite. For larger sections that were too large to be imaged in one frame, multiple images were photomerged using Adobe Photoshop. Background inconsistencies and excess embedding wax remaining from sectioning procedure was manually removed from the image in Photoshop (Fig 1A). The three-dimensional model was generated from the sequence of images in ImageJ, freeware from the National Institutes of Health (Fig 1B) (http://imagej.nih.gov/ij/index.html).

The major tissues and organs in the developing organisms were color enhanced in Adobe Photoshop, using the color replacement tool to pseudo-color the structures, to allow for easier identification within the embryo. The two-dimensional enhanced series of images and the subsequent three-dimensional model of the embryo were generated in ImageJ (Fig1C, 1D). To generate the isolated images, the enhanced, pseudocolored organs were further processed by removing all of the unlabeled tissue from the images of the embryo. Again the sequence of images was used to generate a three-dimensional model (Fig 1E, 1F). The scale bar is 200μm.

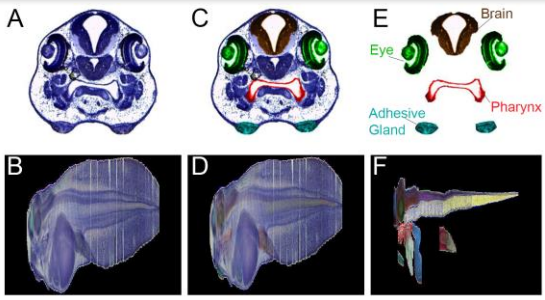

Fig. 1. 10mm Frog embryo with nonenhanced, enhanced and isolated two- and three-dimensional images. (A) nonenhanced serial section (B) single view of a nonenhanced three-dimensional model (C) enhanced serial section (D) single view of an enhanced three-dimensional model (E) isolated serial section showing the major structures with unlabeled, excess tissue removed (F) single view of an isolated three-dimensional model

iPad App Creation

The app was created for the iPad because of its high performance and its popularity in academics. The core challenge in writing the application was to enable the user to smoothly iterate through thousands of images using a scroll bar. The use of web technology was considered but would require a user to download thousands of images ultimately resulting in slow wait times and/or slow performance. The iPad App bundles all of the images with the App when it is originally downloaded and installed. The app works by allowing a user to select an animal, development time, and enhancement choice. The image selector slider position, along with the user choices, dictates the image that will be shown (Fig2A). The image files were named such the file name also represents the animal, development time, enhancement choice and slide number. An example file name was chick48hisol0257.jpg, indicating that the animal was chick; development time was 48 hours; enhancement choice was isolated, and slide number was 257.

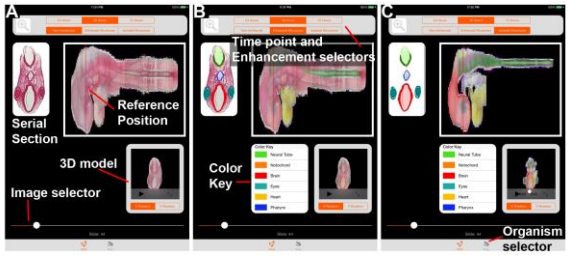

Fig. 2. Dimensional Embryology App screen with highlighted features. (A) Non-enhanced images for 48hr Chick (B) Enhanced images for 48hr Chick (C) Isolated images for 48hr chick

Assessment

Thirteen students in an undergraduate developmental biology course used the Dimensional Embryology App to first look at the non-enhanced two-dimensional serial sections and the generated three-dimensional model to see if they could identify structures in the embryo and describe the three-dimensional shape of the embryo. Then they used the enhanced images and the three-dimensional models, to begin to determine the position of the major structures in the embryo. Finally, using the isolated images and the three-dimensional model, students evaluated the relative positions and shapes of all major internal structures. During the use of the App, students were observed and were asked to qualitatively assess the usefulness and clarity of the App and to state their overall impressions in a survey.

RESULTS

The students evaluated the App for ease of use, clarity and ability to identify the three-dimensional structures within the embryos contained in the image library, including chick embryos that are 24hrs old, 48hrs old and 72hrs old, as well as hatched (4mm) and 10mm frog embryos. By showing the traditional, two-dimensional serial sections (Fig 1A), the students first saw the general histological sections with dark and light staining patterns, indicative of the tissue diversity. When they began with the non-enhanced serial sections, and the accompanying three-dimensional model that rotates in both X and Y to visualize the entire embryo (Fig 1A,B), they had difficulty determining where structures like the heart and eyes were, and found it near impossible to describe the three-dimensional shape of those structures. They were able to understand the relationship between the slide number (section) and its position in the embryo using the reference position on the App (Fig 2A).

The enhanced images with the color-coded structures allowed the students to visualize the anatomical positions and organization of the tissues and organs in relationship to the other structures in the embryo (Figs 1C, 1D, 2B), while the isolated images displayed some of the organs and tissues in the embryo without the presence of the connective tissue and other less emphasized structures in the embryo. By looking at the enhanced and isolated structures in both two- and three- dimensions (Fig 1E, 1F), students detect the shape, structure, size and detail of the embryonic components. When using the three-dimensional models of the isolated structures, the students quickly identified and described the three-dimensional nature of the structures (Figs 1F, 2C). For example, the lens and ocular cup of the eye were clear to the students in the enhanced and isolated images and the entire structure of the eye was clear in the three-dimensional model. In addition, the students found it very easy to see the looped heart of the chick in the three-dimensional model (Fig 2C), while in the serial sections the students only saw two separate circular structures and could not determine the final shape of the heart.

The Dimensional Embryology App also provided the students with an easy tool for evaluating how the embryonic structures change through development using the time point and enhancement selectors and differences between organisms using the organism selector (Figs 2B, 2C, 3). When they began to compare the neural tube across the chick developmental time points they were able to see the change in structure of the forming neural tube. By switching to the frog embryo, the comparable neural tube was apparent. Similarly, the progression of eye development and the commonality of development among species (Fig 3) were clarified by simply switching from one screen of the App to another. The students preferred to use the App and not the original images for making these types of comparisons.

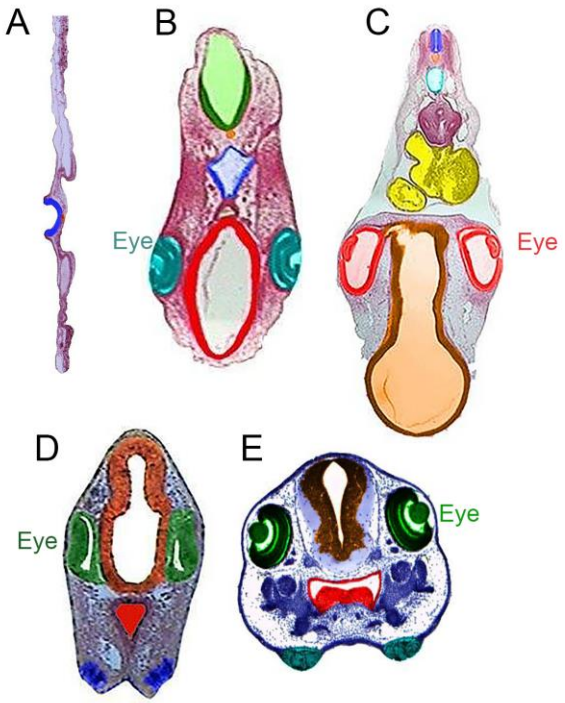

Fig. 3. Enhanced two-dimensional serial sections of Chick and Frog embryos at different developmental time points. (A) 24hr Chick (B) 48hr Chick (C) 72hr Chick (D) Hatched (4mm) Frog (E) 10mm Frog

DISCUSSION

Research has shown that the use of computer-assisted learning (CAL) has aided in an increase in student understanding and consequently has resulted in improved test scores (Burk et al., 2013; Khalil et al., 2005; Khalil et al., 2010). Additionally, CAL decreases the costs associated with teaching expensive anatomy, embryology and vertebrate biology laboratories, by replacing the need for purchasing live or fix embryos with virtual labs, websites, and applications (Beale et al., 2014; Nieder and Borges, 2012; Petersson et al., 2009). Furthermore, the use of CAL can decrease the amount of class or laboratory time spent on teaching a topic, as students can use the tools individually, spending time outside of the classroom to master the material. Over eighty percent of dental schools use CAL for embryology and anatomy (Burk et al., 2013). As embryology ceases to exist as a standalone course, the time spent teaching embryonic development is limited. There are a number of CAL resources currently available to support the study of embryonic development, including extensive support of understanding human development (Ecker et al., 2003; Hill, 2015; Museum of Health and Medicine, 2012; O'Loughlin, 2008; Sulik and Beam Jr., 2015). Model organisms, such as chicks and frogs, are often used to study development, however there are less resources available to evaluate their development (Muneoka, 2000; Scadding, 1998). The resources that are available do not clearly demonstrate the relationship between two-dimensional serial sections and three-dimensional embryonic structures for frogs or chicks, while indicating the different tissues in the images.

The library of images contained in the Dimensional Embryology App spans multiple times in development for chick and frog embryos (Fig 2, 3). The App uses a series of two- and three-dimensional images of embryos to help students gain a better understanding of embryonic structures and their relative positions in the embryo. By providing the students with a series of images for different time points in both chick and frog development, they can begin to compare the organisms and the timing of development of different structures in the embryos. The comparative anatomy of the developing eye in frog and chick embryos for example, demonstrates the developmental similarities between species and the progression of eye development within one organism over time (Fig 3).

When student learning of embryology and development of multiple species was qualitatively assessed, the students that used the enhanced or isolated images on the App found it easier to understand the structure and organization of the chick and frog embryos as compared to using the serial section slides. They commented that the App was easy to use, clear to understand and convenient. Our qualitative assessment suggests that the students support the use of the App and are more likely to learn about embryology quickly and easily. Further testing is needed to quantitatively assess student learning when using the App.

Future directions include adding additional time points for chick and frog embryos, other organisms such as pig embryos, and specific mouse organs, to allow a user to experience more exposure to embryology. Additional learning tools could be added to the Dimensional Embryology App, such as textual explanations, videos of specific slides and self-quizzes. We anticipate and encourage constructive feedback from users suggesting changes and new features.

ACKNOWLEDGEMENTS

K. Lee and the University of Pittsburgh at Johnstown Mentorship funded the grant. B. Robart who helped initiate the project. The numerous undergraduates at The University of Pittsburgh at Johnstown who contributed to the project - Jeri Baker, Gary Hoover, Clay Kubrick, Tien Lau, Colleen Maquire, Dudley McNitt, Brittney Miller, Michael O'Reilly, Ben Policchichio, Emily Pudliner, and Lee Quist.

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Laboratory Exercise in Behavioral Genetics Using Team-based Learning Strategies

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Abstract: In this paper, we describe a two-week learning module where students tested the role of the *fruitless* gene on aggression and courtship in *Drosophila melanogaster* via team-based learning (TBL) strategies. The purpose of this module was to determine if TBL could be used in the future as a platform to implement the course goals and teach scientific skills in two sections of a junior/senior-level college Behavioral Genetics laboratory. We utilized the TBL format: pre-class preparation, readiness assurance, and concept application. The first week students learned the concepts necessary to understand the role of the *fruitless* gene on behavior and were tested as individuals and teams during the Readiness Assurance Test (RAT). They practiced working with the organisms and observing their behavior before developing novel research experiments and engaging in an extensive peer review process of their experimental designs. The following week, each group re-designed and implemented their experiments. Student performance improved during the team RAT, they preferred TBL, and were more prepared for their final research projects. Therefore, we found that incorporating TBL in this laboratory module was a successful tool toward encouraging the development of scientific skills in this laboratory.

Key words: Team-based learning, student-centered learning, teacher-centered classroom

INTRODUCTION

Pedagogical research has shown that team-based learning (TBL) is an effective student-centered learning strategy for teaching in undergraduate science lectures (Metoyer et al., 2014; Nieder et al., 2005). TBL transforms teacher-centered classrooms with pre-class preparation, group work, and peer teaching to strengthen knowledge acquisition and application (Michaelsen & Sweet, 2008). This provides a learning environment that allows instructors to focus on the core competencies and disciplinary practices, as outlined by the American Association for the Advancement of Science Vision and Change Report (see Figure 1; Bauerle et al., 2009). McInerney & Fink (2003) found that the combination of challenging student projects and TBL improved student recall, as exemplified by increased final exam scores. Therefore, TBL may provide the opportunity for students to practice science authentically in laboratory settings, in the way that scientists implement research.

In this paper, we describe our implementation of TBL in one learning module over a two-week period in two sections of a majors only, junior/senior-level college Behavioral Genetics laboratory. Given that animal behavior requires a deep understanding of several disciplines of biology and that phenotypes can be widely variable, animal behavior can be difficult for students to grasp and test in laboratory settings. Therefore, we aimed to determine if TBL was suitable format to teach undergraduate students behavioral genetics. TBL was employed in an effort to reach our learning objectives for the course (see Table 1) in a learning module looking at the role of the *fruitless* gene on courtship and aggression in *Drosophila melanogaster*.

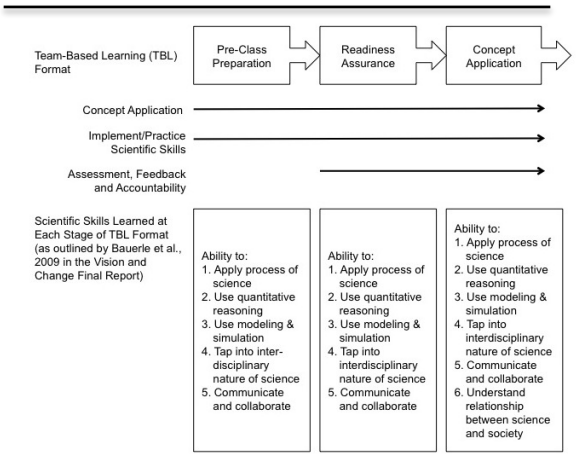

Fig. 1. Diagrammed representation of the potential for the team-based learning format to incorporate the core competencies and disciplinary practices (i.e. scientific skills) as outlined by the National Science Foundation Vision and Change Report (Bauerle et al., 2009).

General Theoretical Background

Drosophila melanogaster are an ideal model system to study intersexual and intrasexual selection in the classroom or laboratory. Many studies have found a range of aggressive behaviors in males, such as lunging and boxing (Yurkovic et al., 2006; Baier et al., 2002), and that males will establish hierarchal relationships and territories to attract females (Yurkovic et al., 2006; Hoffmann, 1987; Dow & von

Table 1. Learning objectives of the module and our application through the TBL format.

Learning Objectives	TBL Process Applicable
1. Understand the role of genetics on complex behavioral phenotypes in *D. melanogaster*.	Pre-class Preparation Readiness Assurance Concept Application
2. Identify, measure, and quantify specific behavioral phenotypes in *D. melanogaster*.	Pre-class Preparation Readiness Assurance Concept Application
3. Formulate hypotheses, design, and perform experiments following the scientific process.	Pre-class Preparation Concept Application
4. Detect and resolve procedural problems.	Readiness Assurance Concept Application
5. Develop and implement independent novel research.	Concept Application

Schilcher, 1975). Females, on the other hand, exhibit less aggressive behaviors and share resources, rather than commandeering them like males (Vrontou et al., 2006).

In an effort to mate with the female, *D. melanogaster* males perform an elaborate courtship dance for females (see Yamamoto and Koganezawa [2013] for a visual representation of the display). The courtship dance is a succession of genetically predetermined behaviors that generally follow the same order: 1) orientation: the male quickly "orients" himself in front of the female, 2) tapping: the male taps the female on her abdomen, 3) song: the male performs a courtship song by extending and vibrating his wing, 4) licking: the male licks the female's genitals, 5) mounting: the male attempts to mount the female to copulate (also called attempted copulation), and 6) successful copulation: the male successfully mounts the female (Yamamoto and Koganezawa, 2013). During courtship, both males and females emit pheromones to detect suitable and viable females (Dickson, 2008; Everaerts et al., 2010). Several genes, including the *fruitless* (*fru*) gene, regulate the neural loci influencing the individual stages of the courtship dance and aggression (Lee & Hall, 2000). The *fru* gene, which encompasses approximately 130 kb, encodes for 18 variable isoforms all belonging to the family of BTB-ZnF (Broad-complex tramtrack and bab zinc finger) transcriptional factors (Ito et al., 1996; Ryner et al., 1996). The different isoforms arise through initiation of transcription from one of four different promoters and the alternative splicing of the 5' and 3' ends (Heinrichs et al., 1998; Goodwin et al., 2000).

By modifying *fru*, male patterns of aggression can be feminized and female patterns made more masculine (Vrontou et al., 2006; Demir & Dickson, 2005). For example, males expressing the feminizing fru^F isoform will orient away from females, they will indiscriminately court males and females, or courtship can be completely blocked (Demir & Dickson, 2005). fru^F males left on food plates for several hours or days begin to form courtship chains in which each male courts the one ahead of him (Hall, 1978; Demir and Dickson, 2005). Females expressing the fru^M variant also exhibit significant changes in courtship behavior. fru^M females display male sexual instincts; they will court wild-type females, and when placed together on food plates, will also form courtship chains similar to the chains formed by fru^F males (Demir and Dickson, 2005).

PROCEDURE

This learning module was implemented in two sections over a two-week period. Each laboratory was 4 hours long; if needed, this laboratory could be condensed into one 4-hour long laboratory. Each section consisted of approximately 21-24 enrolled students. Because we utilized the team-based learning format, students worked both prior to lab as well as during lab, in large teams (7-8 students per group), small groups (2-3 students), and individually.

The laboratory exercise tested the role of the *fru* gene on aggression and courtship in *D. melanogaster*. A list of materials to implement this experiment can be found in the Lesson Plan, supplied in the Supplemental Materials. We used Canton-S wild type and *fruitless* mutants in this laboratory exercise. Canton-S is one wild type strain commonly used in *Drosophila* research laboratories. Wild type strains are ideal for both teaching and research purposes because they are genetically and phenotypically variable in their population, but Canton-S is not easily accessible for teaching. Another wild type strain, Oregon-R, is readily available via Carolina Biological (Item #172100). Alternative mutants, to address similar research questions for aggression and courtship, can be purchased through Carolina Biological (see Table 2).

Table 2. Additional strains readily available through Carolina Biological Supply Company to test similar behavioral genetics questions.

Strain	Supplier	Item No.	Behavioral Phenotype To Test
Ebony	Carolina Biological	172500	Disrupted circadian rhythm (Newby & Jackson, 1991) and courtship (Wang et al., 2008), and increased aggression (Jacobs, 1978)
Sepia	Carolina Biological	172575	Mating success (Stanić & Pavković-Lucic, 2005)
Wrinkled	Carolina Biological	172600	Mate choice and courtship song
Flightless	Carolina Biological	144455	Mate choice, courtship dance, and courtship song
Black	Carolina Biological	172330	Decreased aggression (Jacobs, 1978)

Prior to Experimentation

Each line was reared on standard fly medium and incubated at room temperature (~25º C) and a 12:12 light:dark photoperiod. Three to five days prior to experimentation, adults (Canton-S females and males, fru^C males and females, fru^F males, and fru^M females [identified using the serration on their wings]) were anesthetized using carbon dioxide, sexed by the presence of sex combs, and painted on their dorsal thorax with non-toxic acrylic paint to identify between sex and strains (see Figure 2). An alternative to painting is to rear newly-eclosed adults on medium dyed with food coloring; this will turn their abdomens the color of the food coloring (R. Yukilevich, personal communication, 19 September 2014). Each individual was housed separately in 23-mL plastic vials (capped with cotton balls) until testing. To optimize aggression, adults should be collected at eclosion and kept in isolation until testing following the methods of Vrontou et al. (2006).

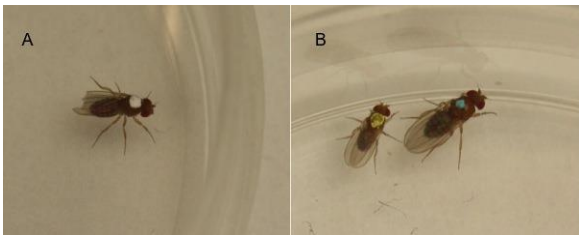

Fig. 2. A) A male painted with white non-toxic acrylic paint on its dorsal thorax. B) A male (left) painted yellow and a female (right) painted blue on their dorsal thorax with non-toxic acrylic paint.

Week 1: Learning to Work with Drosophila and Observe Behavior

To simulate TBL in the laboratory, we followed the format for team-based learning in the first laboratory session (see Figure 3). The TBL format includes opportunities for pre-class preparation, readiness assurance, and concept application in this sequence (Michaelsen & Sweet, 2008). For pre-class preparation, we posted the lab worksheet, three readings (previously published journal articles), and

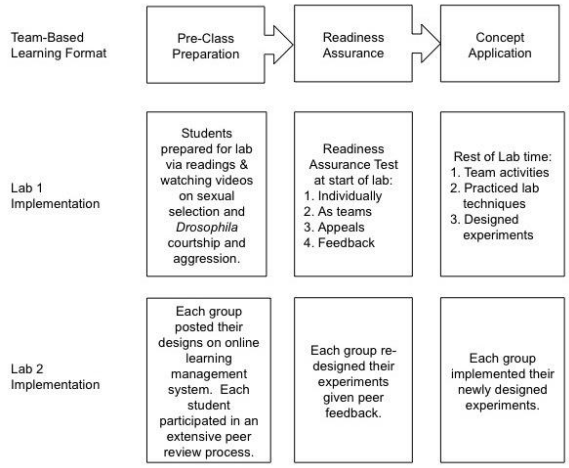

Fig. 3. A diagram representing our implementation of the team-based learning format during week one (Lab 1 Implementation) and week two (Lab 2 Implementation) of this laboratory module.

eight videos on *Drosophila* courtship and aggression on the online learning management system (see Lab Worksheet in Supplemental Materials)**.** Students individually prepared for the first week's laboratory by watching the videos and reading the materials supplied online.

At the beginning of the laboratory, students were individually given a Readiness Assessment Test (RAT); this is a short, but challenging, multiple choice assessment designed to test students' comprehension of the readings and critical thinking abilities (two versions are supplied in Supplemental Materials). Students were divided into teams of seven to eight individuals prior to the laboratory by distributing member resources equally and avoiding previously formed coalitions (see Michaelsen & Sweet, 2008 for further information). Each team took the same RAT again in their teams using test cards, called the Immediate Feedback Assessment Technique or IF-AT. They were encouraged to discuss the possible answers at length and come to a consensus on an answer before proceeding. One student in the team scratched the card to reveal a "*"

if the answer was correct. If they were incorrect, they were given the opportunity to change their answer for a diminishing score. At the end of the RAT, each team was allowed to write and submit an appeal if they felt that any of the questions were unfair, incorrect, or too ambiguous. Through discussion of the questions immediately following the RAT, we were able to identify and resolve students' misconceptions and answer any remaining questions.

To apply concept application, as well as practice laboratory skills and behavioral observations, students engaged in a series of activities as teams with the instructors (see Lesson Presentation in Supplemental Materials). These served to further engage students in understanding the role of the *fru* gene on behavioral phenotypes in *Drosophila*. These activities incorporated the 4S's: the students were asked a series of multiple-choice, specific questions addressing a significant problem (Parmelee and Michaelsen, 2010). The students were required to come to a team consensus on the answer in the allotted time and simultaneously report their answers. At this point, the instructors were able to address any additional misconceptions or gaps in understanding of the material.

The activities were followed by a short lecture and demonstrations (see Lesson Presentation in Supplemental Materials) to teach students how to handle, sex, and observe *Drosophila* behaviors. Teams were divided into smaller groups of two to three students to practice scientific skills and run experiments. Dividing the teams provided students with the opportunity for greater participation in application of the concepts and scientific skills, which would not be feasible with large teams in a laboratory setting.

In their smaller groups, students practiced aspirating, anesthetizing, and identifying phenotypes and sexes. Once students felt comfortable and were successfully performing the lab techniques and working with flies, they placed individual adults together in petri dishes to practice observing male courtship and female and male aggressive behaviors. We focused on male courtship behaviors because they are easier for students to observe and quantify without the use of expensive laboratory equipment.

While observing these behaviors, each group applied their novel observations and knowledge gained in the readings to formulate a unique research question, hypothesis and prediction. For the remainder of the lab period, each group developed hypotheses-driven experimental designs to independently test a novel research question, which uploaded onto the online learning management system's discussion board before leaving.

Week 2: Independent Experimentation

To employ the TBL format in the second laboratory (see Figure 3), students prepared for the laboratory by participating in an extensive peer review process of their peer's experimental designs. Each student provided feedback to at least one group using the online learning management system discussion board. In addition, the instructor provided additional feedback as support, where necessary. Prior to the laboratory, each group was responsible for reviewing their peers' and instructor's feedback.

At the beginning of the laboratory, each group re-designed their experiments given the provided feedback and independently carried out their experiments. Most experiments required trouble-shooting and each group was provided ample time and supplies to do so. If needed, students were allowed the entire lab period to fix problems with the experiment and obtain enough data to make logical, evidence-driven conclusions concerning their hypotheses. When the experiments were completed, each group analyzed and recorded their results in their lab notebooks, which were handed in and graded at the end of the semester.

RESULTS AND DISCUSSION

Results from Student Experiments

During Week 2, each group of students proposed novel hypotheses and tested independent research projects of one aspect of the role of the *fru* gene on either aggression or courtship. Our objective was to prepare students to independently run their final research projects at the end of the semester. Because of this, each group of students pursued different projects depending upon their interest.

The peer review process was a strong component of the TBL approach in this learning module. The students provided insightful feedback and reasoning using their understanding of animal behavior, behavioral genetics, the scientific process, and their readings. The peer review process was an opportunity for reinforcement of conceptual learning and procedural knowledge learned during the first week of this laboratory module.

Educational Outcomes of the Learning Module

Given that animal behavior is an interdisciplinary science that can be challenging to teach and for students to fully understand, we used TBL with this module to determine if this teaching format would enhance student comprehension of both the concepts and the techniques necessary to test behavioral genetics questions using *D. melanogaster*. Students were formally assessed at the beginning of week 1 with the Readiness Assessment Test (RAT) and were polled at the end of the semester with a survey created by Mennenga (2010) to determine the effectiveness of the team-based learning method.

Results from the Readiness Assessment Test

The Readiness Assessment Test (RAT) was used to encourage student preparedness, reinforce the concepts learned in the readings, and provide an opportunity for discussion, argumentation and peer instruction for students to work through their

misconceptions of the material. To test whether the RAT improved overall student performance, we compiled the scores for both sections and compared the averages of the individual RAT scores to the team RAT scores using a Paired-Samples T-Test (SPSS v. 22.0). We found that students performed significantly higher ($p < 0.0001$, see Figure 4A) on the team RAT, in comparison to the individual portion; students scored 24.9% higher on the team portion.

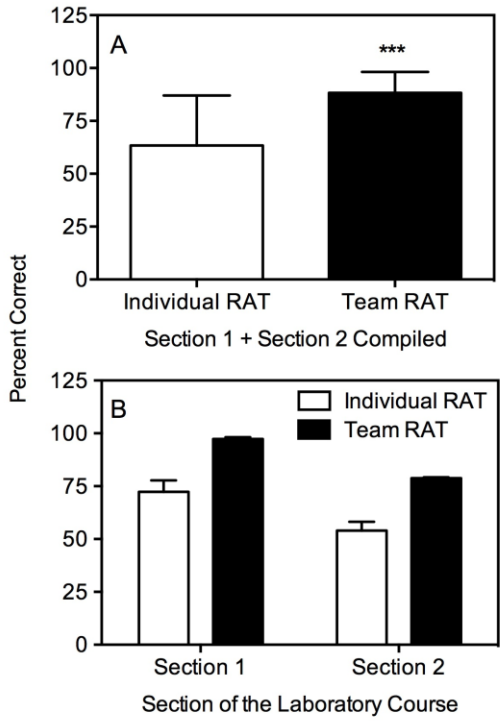

Fig. 4. Effect of the Readiness Assessment Test (RAT) on student performance. When scores were compiled for both sections of the course (A), the level of performance increased significantly (Paired-Samples T-Test, $p < 0.0001$) by 24.878%. Both sections showed considerable improvement in the team portion of the RAT. Means ± SEM in (A) and (B). *** = $p < 0.0001$.

Since students in the first section were provided the answers using the IF-AT cards and were allowed to take the RAT home, the second section of the course was given a different RAT that tested the same concepts. Therefore, we compared differences in scores between the two sections using Repeated Measures ANOVA (SPSS v. 22.0). We found a significant difference ($p < 0.0001$, see Figure 4B) between the two sections. However, each section followed the same trend: the students performed higher on the team portion of the RAT than the individual RAT. The difference between the sections is due to lower scores on both the individual RAT and team RAT; the trend that this section scored lower than the other was observed throughout the semester.

We found that the use of the RAT in this module provided students with the opportunity to be responsible and accountable for preparing for the laboratory and to work with the content at a higher level. By organizing the students into teams and giving them the same RAT to complete as a team, they were able to reinforce their knowledge via argumentation and peer teaching, as well identify and change their misconceptions of the content. We found that student misconceptions during the RAT were centralized to how the genotypes related to phenotypic outcomes in behavior, given that these strains exhibit complex behaviors controlled by a spliced variant. We recommend incorporating practical and procedural knowledge into the RAT (which we did not do in this module) because we noted that students struggled initially with sexing the flies, using the dissection microscopes to observe the behaviors, and incorporating environmental (i.e. sound, light, size of chambers, etc.) variables that alter behavior into their designs.

Our results are corroborated by other studies (Beatty et al., 2009; Wiener et al., 2009) that have found that either the RAT or similar classroom assessment techniques improve student performance. The success of the RAT is dependent on the incorporation of student preparedness with peer instruction and collaborative learning in the teams. Both peer instruction (Rao and DiCarlo, 2000; Ramaswamy et al., 2001) and collaborative learning (Tao and Gunstone, 1999; Springer et al., 1999) have been established as successful methods for fostering a deeper understanding of both conceptual and procedural knowledge in science education (Simon and Cutts, 2012).

Results of Experiments and Final Projects

We found that, although they gathered data for their experiments, the majority of students were not confident with their results because they either were unable to solicit the behaviors or their results were unexpected. This is because, until this laboratory, we used a more traditional "cookbook" laboratory format where students were given protocols to perform during the laboratory. This was the first inquiry-based laboratory where the students designed and implemented their own experiments. As a result, they were able to apply the concepts and use problem-solving skills developed in the module to see the flaws in their experimental designs. Although their experiments did not necessarily work, these lessons are the basis of scientific procedural learning.

As a result of this laboratory module, students were better prepared at the end of the semester to develop independent research projects. Instead of a final examination, students worked in their small groups to design and implement projects that addressed a novel research question in behavioral

genetics using either *D. melanogaster* or another invertebrate system used in the course. We found that students not only retained the conceptual knowledge learned in the module, but that they were practicing science, designing their experiments, problem-solving, and working with the behaviors and animals with more precision and confidence than before.

Results from a Student Survey

At the end of the course, students were polled using a survey established previously by Dr. Heidi Mennenga (Mennenga, 2010) to test the efficacy of team-based learning in nursing courses. The surveys consisted of three categories (called "subscales") to determine whether the implementation of TBL was effective in the course. These categories included a series of questions that measured if student accountability, their preference for TBL over traditional teaching methods, and their satisfaction were higher than neutral scores. The higher the score is over the neutral score indicates higher levels of student accountability, preference, or satisfaction.

Using a One-Sample T-Test (SPSS v. 22.0) the average for each category was compared to the neutral score for each category (as established in Mennenga, 2010). Results from the survey (see Figure 5) showed that the use of TBL in this laboratory module resulted in significantly higher: 1) accountability ($p < 0.0001$), 2) preference for TBL over our previous "cookbook" approach ($p < 0.001$), 3) student satisfaction ($p < 0.0001$), and 4) total, a.k.a. the sum of all three categories ($p < 0.0001$). In addition, students commented in the survey that TBL increased both their preparation and conceptual understanding of the material. In particular, students said team-based learning helped: "better prepare us in understanding the experiment before hand," and "[me] understand the material during class."

Regardless of our short implementation of TBL (over two weeks instead of the entire course), these results indicate that students felt that TBL improved their personal accountability during pre-class preparation and group work, they preferred TBL to our traditional laboratory format, and that their overall satisfaction improved. To improve student performance and "buy in" to the TBL approach, Michaelsen and Sweet (2008) suggest the implementation of TBL throughout the entire semester. This is important because team cohesion, cooperative learning, and student responsibility require extended periods of time for development.

Benefits of Approach to Students

Improved student achievement in this learning module coordinated with overall student satisfaction as seen in the student evaluations. We observed that students' understanding of animal behavior and the role of genetics on behavioral phenotypes, ability to design hypothesis driven experiments, and engagement in the class was heightened using this format. Student groups developed unique research questions, clear hypotheses, strong peer reviews, and were better at collaborating with their peers. Several students developed strong innovative final research projects using these experiments as a basis. In addition, this approach increased innovation, creativity in future research, problem solving, and practice with experimental design.

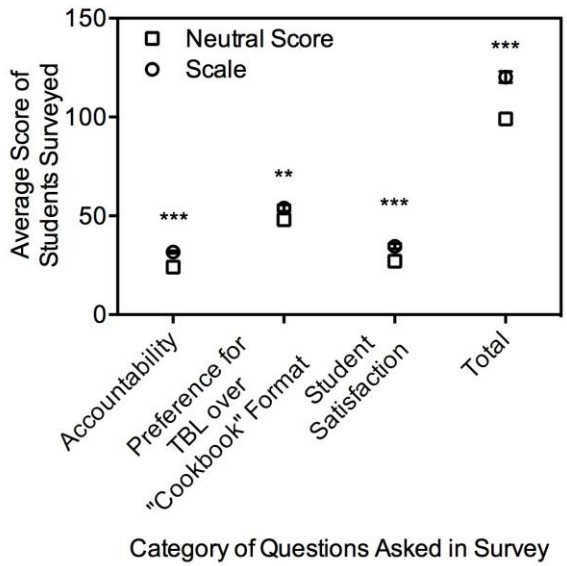

Fig. 5. Results from a student survey to determine the efficacy of the implementation of team-based learning (TBL). Student preference for TBL over traditional methods was significantly higher across all three categories: accountability ($p < 0.0001$), preference for TBL over traditional "cookbook" format ($p < 0.001$), satisfaction ($p < 0.0001$), and the total of all three scales ($p < 0.0001$). Means ± SEM. ** = $p \leq 0.001$, *** = $p < 0.0001$.

CONCLUSION

Many undergraduate laboratories employ "cookbook" laboratory exercises; this approach does not provide students with an authentic, research-driven experience. Educational outcomes of "cookbook" laboratories are limited because students do not develop the scientific or laboratory skills as intended. By combining the laboratory module with TBL, we were able to provide students with an inquiry-based, research experience in the laboratory. In addition, we successfully addressed the learning objectives of the laboratory module: both conceptual knowledge (Table 1 Objective 1) and scientific skills (see Table 1 Objectives 2-5) improved, as well as overall performance, both within the laboratory module and after. We have shown that combining TBL in a biology laboratory course may be an effective strategy in increasing student conceptual and procedural knowledge of science. Therefore, TBL has the potential to transform undergraduate laboratories from the traditional "cookbook" model

to a student-centered inquiry-driven model focused on acquisition of scientific skills. However, implementation of TBL in larger, controlled studies is necessary to determine if TBL is an option for inquiry-based teaching in biology laboratories.

ACKNOWLEDGEMENTS

We would like to thank Dr. Barry Dickson (Janelia, Howard Hughes Medical Center) for his continual advice and support in the use of his *fru* mutants of *D. melanogaster*. In addition, we would like to thank Dr. Gregory Lnenicka (for his use of his Canton-S strain of *D. melanogaster*) and Dr. Hedi Mennenga (for the use of her team-based learning surveys as published in Mennega, 2010). Specifically, we would like to thank Dr. Jennifer Mather (Universiy of Lethridge), Dr. Christine Gervasi (University at Albany-State University of New York), Dr. Billie Franchini (Institute for Teaching, Learning and Academic Leadership, University at Albany-State University of New York), Caroline Girard-Cartier (University at Albany-State University of New York), and Dr. David Tieman for the pictures in Figure 2 for us) and the Department of Biological Sciences (University at Albany-State University of New York) for their support in this endeavor.

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Detecting the Presence of Nora Virus in *Drosophila* Utilizing Single Fly RT-PCR

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ABSTRACT: A single fly RT-PCR protocol has recently been developed to detect the presence of the persistent, horizontally transmitted Nora virus in *Drosophila*. Wild-caught flies from Ohio were tested for the presence of the virus, with nearly one-fifth testing positive. The investigation presented can serve as an ideal project for biology students to gain relevant laboratory experience. The study can be easily adapted to best meet the needs of instructor and student, and provide exposure to PCR, work with *Drosophila*, data analysis, and molecular biology.

Key words: single fly RT-PCR, *Drosophila*, Nora virus, wild-caught flies

INTRODUCTION

Molecular biology is at the forefront of biology. Current students must have an understanding of basic laboratory techniques and practices to show competency in their field and be informed about innovations in molecular biology. Polymerase chain reaction (PCR) is one molecular technique that is an integral tool in the laboratory today. It is commonly used in a wide array of biological disciplines, including forensics, industry, and medicine (Jordan & Lynch, 1998; Bartlett & Stirling, 2003; Yue, 2014). Developed in the early 1980s by Kary Mullis and his colleagues at Cetus Corporation in California, the technique replicates sequences of DNA. This enables small samples of DNA to produce larger quantities, which in turn can be used for further research (Mullis et al., 1986; Jordan & Lynch, 1998; Yue, 2014). As a result of the frequency of use, ease, and potential that PCR has to offer, current biology students need to become familiar with the process and its applications. Additionally, an understanding of PCR at the molecular level is crucial and personal experience will enable students to continue to explore the expanding field of molecular biology.

PCR has a multitude of uses that are diverse and innovative (Jordan & Lynch, 1998). The combination of reverse transcription with PCR allows RNA sequences to be analyzed in a process called reverse transcriptase polymerase chain reaction (RT-PCR). RNA cannot be used directly in standard PCR, so RT-PCR first produces DNA copies (cDNA) of the RNA via reverse transcription of the RNA template, followed by amplification of product (Bustin, 2000). RT-PCR is a sensitive and flexible process, allowing one to get quick results from very small sample sizes (Freeman et al., 1999; Bustin, 2000). Not only should biology students become familiar with conventional PCR, they also need to perform applications like RT-PCR to understand some of the techniques alternate uses.

A RT-PCR protocol has recently been developed utilizing single female *Drosophila.* The protocol to detect the presence of Nora virus is discussed. The study that is presented is recommended for biology students as a tool to use and understand the process of RT-PCR, and the protocol can be easily adapted to serve other applications. Completion of this laboratory exercise gives students practice at catching and identifying *Drosophila* species, data analysis, and virology. Students will gain valuable field and laboratory experience, research Nora virus, work with the model organism *Drosophila*, and expand their molecular understanding of PCR.

This exercise enables students to gain first-hand experience with *Drosophila*. *D. melanogaster* has been used as a model organism for research since the early 1900s. Studies involving *Drosophila* have examined anything from the basis of heredity to physiological systems (Kounatidis & Ligoxygakis, 2012; Rämet, 2012; Teixeira, 2012). Due to *Drosophila's* conserved immune response pathways with humans in their response to viruses, *Drosophila* is commonly used as a model for innate immunity (Reiter et al., 2001; Hoffman, 2003; Hultmark, 2003). Unlike humans, who have innate and adaptive immune response systems to combat pathogens, *Drosophila* depends solely on an innate immune response (Hoffman, 2003). As a result, *Drosophila* is a successful model organism for understanding mechanisms of the innate immune system (Kemp & Imler, 2009; Sabin et al., 2010; Kounatidis & Ligoxygakis, 2012; Teixeira, 2012). Approximately 20 groups of viruses exist in natural populations of insects from 12 viral families including *Rhabdoviridae*, *Dicistroviridae*, *Birnavirdae*, and *Reoviridae* (Kemp & Imler, 2009; Sabin et al., 2010). More than twelve human viruses have been studied in *Drosophila* (Hughes et al., 2012).

Nora virus is the ideal virus for students to investigate. It is a picorna-like virus. The virions are naked icosahedral-shaped particles approximately 28

nm in diameter and contain a genome of single-stranded positive-sense RNA (Habayeb et al., 2009). The genome consists of four open reading frames (ORFs). The first two ORFs encode non-structural proteins and ORFs three and four encode the structural proteins of the virion.

Drosophila is frequently exposed to pathogens since they eat, lay eggs, and develop on decaying fruit or media. Nora virus infects the intestine and is excreted in the feces. Therefore, when *Drosophila* feed on contaminated media, the flies ingest the virus and become infected. Transmission occurs horizontally, passing the virus to other flies through the fecal-oral route, with continuous shedding of the virus at high rates. The chance of obtaining positive RT-PCR results is high, although there is variation in viral loads of individual flies (Habayeb et al., 2009). Nora virus is unique due to its ability to persist in its host without causing any pathogenic effects or influencing longevity (Habayeb et al., 2009; Ekström et al., 2011). The virus is also non-pathogenic to humans, so work with flies is safe for students.

Basic knowledge of *Drosophila* is necessary for completion of this exercise. The four stages of the life cycle progress from egg, larva, pupa, and finally to adult. Development times are dependent on rearing temperature and larval density. Flies reared at higher temperatures and lower densities develop more rapidly. Development time also varies with species. The species caught in this study, *D. melanogaster* subgroup species and *D. virilis*, have development times of 13 days and 20 days, respectively, at 18°C. *Drosophila* become sexually mature at different times, ranging from a few days to weeks in some species, and maturity differs between males and females. While adults thrive on decaying fruit and media, eggs are typically laid on ripening fruit so that larvae can feed as the food source begins to rot (Markow & O'Grady, 2006).

Only female flies were selected in this study since they are generally larger and increase the chance of having an adequate amount of genetic material for testing. *Drosophila* can be sexed by examining the genital organs under magnification. Female genitalia is not surrounded by dark bristles found in males, the tip of the abdomen is less rounded, and contains more sternites (Markow & O'Grady, 2006). Females also lack sex combs on their front legs. While they are typically larger than males, sexing should not be based on this criterion alone.

While it may seem straightforward to follow the methodology outlined in this investigation, it is important for students to gain practice following the techniques. The exposure to field and laboratory work in this exercise provides students the opportunity to gain firsthand knowledge about *Drosophila*, Nora virus, and practice with basic PCR procedures. Students must be able to show efficiency in technical problems that often arise (Freeman et al., 1999). The methods do not include molecular explanations about the temperatures specified or what the primers or RNA are doing (Jordan & Lynch, 1998). Thus, there are many areas in which this investigation can be expanded and modified to best fit the goals of the student and instructor and emphasize learning over rote performance.

MATERIALS & METHODS

Fly stocks and husbandry

Flies were collected the week of August 3, 2014 in Sylvania, Ohio. Fruit baits were made by placing overripe bananas and apples into plastic cups, placing plastic wrap over the cup, sealing the wrap with a rubber band, and poking holes in the wrap with a toothpick. Cups were placed outside for several hours in the evening. Larger species of flies were collected with sweeps over open fruit. Additionally, mushrooms were soaked for an hour in tap water and placed in plastic covered cups. These cups were placed on the ground in a shaded flower bed in the morning. Approximately 150 flies were collected, about half of which were female.

Flies were retrieved from the cups and placed in plastic vials with instant medium (Formula 424® Instant *Drosophila* Medium, Carolina Biological Supply Company,Burlington, NC). A plastic vial was placed over a hole in the plastic wrap to remove the flies, with some gentle tapping on the cup. The plastic vials were plugged with a foam stopper. Flies were anesthetized with FlyNap (Carolina Biological Supply Company) and examined with a dissecting microscope to identify the species. *Drosophila* species can be identified with the aid of Markow and O'Grady's field guide, which contains distribution maps and identification keys (Markow & O'Grady, 2006). Additionally, FlyBase (Dos Santos et al., 2015) has colored illustrations of many species. The female flies were kept in vials of approximately 40 individuals per vial and transported to the University of Nebraska at Kearney (UNK) in Kearney, Nebraska. The flies were kept together for transport until they were separated into individual microfuge tubes when they arrived at UNK, which was no longer than 5 days.

RNA extraction and RT-PCR

RNA extraction was performed on random single female flies a week after capture. Female flies were placed in 1.5 ml microcentrifuge tubes with 20 µl of TRIzol® reagent (Invitrogen, Carlsbad, CA) to extract RNA. Flies were homogenized with disposable Kontes® plastic pestles. An additional 20 µl of TRIzol® reagent was added to the homogenate and the tubes were shaken by hand. The tubes were incubated at room temperature for 5 minutes and centrifuged at 12,000 rcf for 10 minutes at 4°C to pellet the insoluble exoskeleton and debris. The supernatant fraction was transferred to new

microcentrifuge tubes and 8 µl of Chloroform was added to each tube. The tubes were shaken by hand, incubated at room temperature for 3 minutes, and centrifuged at 10,000 rcf for 15 minutes at 4°C. The upper aqueous layer was transferred to a new RNAse-free microcentrifuge tube and 20 µl of isopropanol was added. They were shaken by hand, and incubated at room temperature for 10 minutes. The tubes were centrifuged at 12,000 rcf for 10 minutes at 4°C. The supernatant fraction was removed and the pellet was washed with 40 µl of 75% ethanol. The tubes were centrifuged at 7,500 rcf for 5 minutes at 4°C. The supernatant fraction was removed and the tubes were briefly centrifuged so the remaining supernatant fraction could be removed with a micropipette. The tubes were air dried for 3 minutes and then the pellet was resuspended in 5 µl of RNase-free water by flicking the sides of the tube. Finally, 0.5 µl of RNase Out (Invitrogen) was added to each sample.

The samples were analyzed for the presence of Nora virus by RT-PCR using *Nora ORF 1* 54-844 (Forward 5'TGGTAGTACGCAGGTTGTGGGAAA3'; Reverse 5'AAGTGGCATGCTTGGCTTCTCAAC3') primers and FideliTaq RT-PCR Master Mix (2X) (USB®, Cleveland, OH). Fifty µl reactions were prepared by combining 19 µl of water, 25 µl of master mix, 2 µl (20 picomoles) of forward primer, 2 µl (20 picomoles) of reverse primer, and 2 µl (~200 ng) of the RNA samples. The tubes were thoroughly mixed by vortexing and centrifuged. The parameters for RT-PCR are as follows: 50°C for 30 minutes, 94°C for 2 minutes, followed by the amplification loop consisting of 30 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 68°C for 2 minutes, with a final cycle of 68°C for five minutes.

The samples were prepared for agarose gel analysis by adding 10 µl of the PCR product and 2 µl of 6X loading dye. The electrophoresis apparatus was set up and samples were loaded on a 0.8% agarose gel with the separation performed at 75 volts for approximately 1 hour. The results were analyzed with a confidence interval at 95%, indicating that one can be 95% confident that the population of *Drosophila* infected with Nora virus falls in the indicated range. This is consistent with a significance level of 0.05. The formula to calculate the confidence interval boundaries (Confidence Interval Boundaries = p_{avg} +/- Z score (1 - α) * s_{pavg}) is shown, where p_{avg} is the sample proportion, the Z score (1- α) is the standard score and was calculated by taking the inverse of the standard normal cumulative distribution when α = 0.05, and s_{pavg} is the sample standard error.

RESULTS

A positive reaction for Nora virus was observed for the control and laboratory stock flies with Nora virus, showing a product at approximately 800 bp (Figure 1; Lanes 5-10). The negative controls do not show a PCR product (Figure 1; Lanes 3-4). The positive control was Nora virus RNA. The laboratory stock flies utilized have been kept for many generations and are known to be highly infected with Nora virus. This indicates that single female fly RT-PCR was successful.

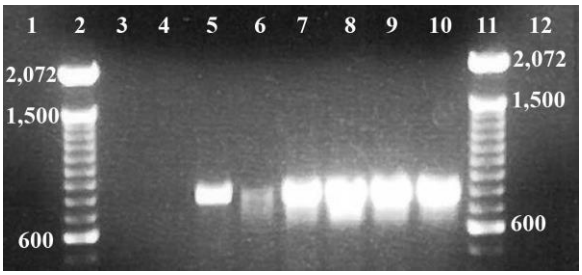

Fig. 1. Confirmation of Nora virus detection using single female flies via RT-PCR. Lane 1 = Empty; Lane 2 = 100 bp Ladder; Lanes 3 & 4 = Negative (water) control; Lanes 5 & 6 = Positive (Nora virus RNA) control; Lanes 7-10 = single female fly via RT-PCR; Lane 11 = 100 bp Ladder; Lane 12 = Empty. The product seen in Lanes 5-10 is approximately 800 bp, which is the expected size for the Nora virus product.

Female wild-caught flies tested for the presence or absence of Nora virus infection were predominantly from the *D. melanogaster* species subgroup, although *D. virilis* was also found. A total of 6 flies out of 32 tested positive for Nora virus (Figure 2a; Lanes 5, 7, 10, 13 and Figure 2b; Lanes 4, 7). Nora virus was not detected in the other 26 flies, as indicated by the absence of a PCR product (Figure 2a; Lanes 4, 6, 8-9, 11-12 and Figure 2b; Lanes 5-6, 8-13 and Figure 2c; Lanes 4-15). The results of this study show that 18.75% of wild-caught flies in Sylvania, Ohio have Nora virus. The 95% confidence interval suggests that 5.22% to 32.3% of the fruit fly population would be expected to be infected with Nora virus.

DISCUSSION

This study developed an RT-PCR protocol for single *Drosophila* and used the protocol to investigate the number of wild-caught flies infected with Nora virus. The need for single fly RT-PCR is two-fold. First, the exact number of flies with Nora virus infection can be revealed, as well as the viral titer levels. This is a more accurate approach, as opposed to conducting RT-PCR with groups of flies. Secondly, it exposes how widespread Nora virus is in different species and increases confidence in results since flies are independently examined. The single fly RT-PCR protocol provides experience for biology

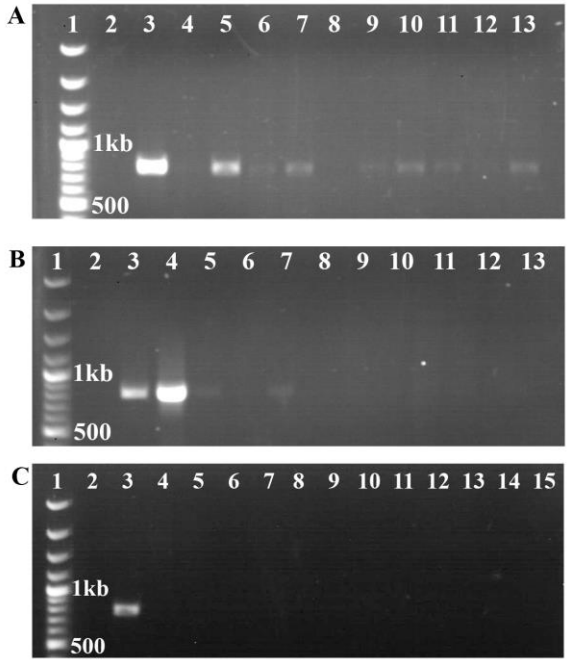

Fig. 2. Detection of Nora virus using wild-caught single female flies via RT-PCR. Panel A is samples 1-10: Lane 1 = 100 bp Ladder; Lane 2 = Negative (water) control; Lane 3 = Positive (Nora virus RNA) control; Lanes 4-13 = single female fly via RT-PCR. The product seen in Lanes 5, 7, 10, and 13 is approximately 800 bp, which is the expected size for the Nora virus product. Panel B is samples 11-20: Lane 1 = 100 bp Ladder; Lane 2 = Negative (water) control; Lane 3 = Positive (Nora virus RNA) control; Lanes 4-13 = single female fly via RT-PCR. The product in Lanes 4 and 7 is approximately 800 bp, which is the expected size for the Nora virus product. Panel C is samples 21-32: Lane 1 = 100 bp Ladder; Lane 2 = Negative (water) control; Lane 3 = Positive (Nora virus RNA) control; Lanes 4-15 = 1 female fly via RT-PCR. The product in Lane 3 is approximately 800 bp, which is the expected size for the Nora virus product.

students, even those who lack prior exposure to *Drosophila* work.

Nora virus appears to be widely distributed in laboratory stocks of *D. melanogaster*. The extent to which Nora virus infects different species of *Drosophila* in the wild is unknown. Studies have shown that organisms in the laboratory experience significant adaptation to the change in conditions within a short time period due to genetic bottlenecks and selection pressures (Gilligan & Frankham, 2003; Swindell & Bouzat, 2005; Gilchrist et al., 2012). The shift in genotype occurs even when population size is large and the environment is not stressful for the organisms (Gilligan & Frankham, 2003; Gilchrist et al., 2012). Thus, it was imperative to look for the presence of Nora virus in wild-caught flies. The results of this study indicate that Nora virus is present in *Drosophila* in their natural setting, with nearly one-fifth of flies in northwest Ohio infected.

There are several ways this study could be expanded and improved. Individual flies should be isolated immediately after capture to eliminate the potential for cross-contamination. Although the results do not seem to indicate that cross-contamination occurred since both positive and negative results were observed, contamination is possible if flies are reared together for any duration of time. Isolation can be achieved by storing wild-caught *Drosophila* in individual vials on dry ice. Additional locations and *Drosophila* species should be tested to better understand virus prevalence. Biology students can easily collect flies at different locations for testing, and will likely see a wider variety of species. Unfortunately, identification of subgroups of *D. melanogaster*, such as *D. simulans*, *D. melanogaster*, and *D. yakuba* is difficult based upon phenotype and morphology, hence the reason they were grouped in this activity. Fortunately, distinquishing between *Drosophila* species, such as *D. melanogaster*, *D. virillis*, and *D. pseudoobscura* is relatively easy, and was done in this study. By sampling a wide array of locations and *Drosophila* species, the average relative density of Nora virus RT-PCR products could then be tested to compare virus titer levels.

This study provides the opportunity for biology students to practice a new single fly RT-PCR protocol, with the potential for exposure to many other biological domains. The objectives and interests of the instructor and student should first be discussed. Then, a project can be created which introduces the student to PCR use and history, work with *Drosophila* in the laboratory and field, and data analysis, while enabling the student to learn about virology in a relevant way. Students must experience first-hand the advances that have taken place and are still taking place in molecular biology. The protocol outlined present an opportunity for students to create and complete a significant science project.

ACKNOWLEDGEMENTS

The project described was supported by grants from the National Center for Research Resources (5P20RR016469) and the National Institute for General Medical Science (8P20GM103427), a component of the National Institutes of Health, and a UNK Graduate Research Services Council Grant.

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PERSPECTIVES

Finding clarity by fostering confusion: Reflections on teaching an undergraduate integrated biological systems course.

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ABSTRACT: Undergraduate biology programs in smaller liberal arts colleges are increasingly becoming focused on health science fields. This narrowing of focus potentially decreases opportunities for these students to explore other sub-fields of biology. This perspectives article highlights how one small university in Connecticut decided to institute a required integrated biological systems course into their biology degree. The course focuses on empowering biology students to view science topics through multiple scales.

Keywords: undergraduate, liberal arts universities, integrated systems

An ongoing trend within many university biology departments is the narrowing of focus to health and biomedical science fields. Our students are receiving an extremely content focused education, but what is the impact on their comparative skills? With the trend to present course content in stand-alone units can our students understand, and more importantly articulate how subjects are interconnected? Over the course of my more than twelve years teaching college level biological science, I am continuously amazed and disturbed by the difficulty that many upperclassman biology majors have articulating connections between biological topics.

Striving to foster student learning by purposefully encouraging confusion is a daunting task, but if we can acknowledge the potential power that such confusion brings perhaps this is a valuable goal to work towards. Confusion in this context does not mean a lack of understanding of the topic. Instead, I view confusion as a powerful state that enables students to challenge their own understanding of the value, meaning, and context of the subject being studied. In their future academic and professional lives, it is highly likely that my students will experience these moments of confusion. Instead of viewing this state as the end of learning, I hope they would acknowledge the potential transformative opportunities present.

Students are often overwhelmed by the large amount of detailed information presented in their biology courses, and the trend is to fall into the "I will just memorize it now and understand it later" mentality. As we are all aware, later gets postponed indefinitely as students move on to the next class and the next dose of concentrated material. As educators, we are under our own pressures to make sure that our students have the necessary knowledge to move onto the higher level courses. How many of us have wanted to slow down a little and explain a concept more fully, but have realized that time constraints simply did not allow us the luxury?

Having completed my undergraduate degree at a fairly large state university, I was accustomed to the subject partitioning that often occurs in larger schools. My specific discipline, wildlife management, while housed in a school of life sciences and agriculture, was part of a much smaller college of natural resources. After having completed my required basic biology courses as a freshman, there was very little overlap with the traditional biology students. Whereas my curriculum moved from general biology to dendrology, wildlife ecology, silviculture, and water resources, the traditional biology students moved on to courses in cellular biology, microbiology, and genomics. We all graduated with life science focused degrees, but the fundamental learning processes involved in each discipline were very different. Ultimately I graduated with a specialized science degree that included many interdisciplinary courses that not only took my learning outside of the traditional biology student path, but allowed me to view my field in many different scales (from molecular to organismal to environmental).

When I began my teaching career, I thought that the smaller colleges I was teaching at would naturally have very diverse biology course offerings. As I soon discovered, career-focused degree programs seemed to push-out the more integrative courses,

often relegating them to the non-science major realm. While career preparation is certainly important, is our focus on such programs narrowing our students' knowledge base to a dangerous point?

What is the result of this information imperative? Students may acquire the knowledge base necessary to be successful in a focused career, but are they prepared for the complex interdisciplinary thinking that drives many fields today? This is especially true in smaller liberal arts colleges, where biology majors are challenged to meet the demands of a rigorous scientific plan of study, while simultaneously fulfilling their liberal arts requirements. In such an atmosphere biology students might be tempted to devalue their opportunities to explore the wide range of subfields within their discipline. It is truly a challenge for these students to fit in their schedules all the required biology courses needed for graduation, their general elective courses, and then all the other biology and science courses that their prospective graduate programs require. Unfortunately, this often results in students missing out on taking more interdisciplinary biology courses. In response to this, the biology department at the University of Saint Joseph decided to add another general biology course to our required list of courses. The purpose of this course was to provide a platform for students to explore the interconnections between the many different subfields of biology.

As I was developing this course I decided that the central idea I wanted to use was the concept of scales. The purpose of the course is to provide students with an awareness of how biological interactions occur both within and across multiple scale levels. The integrative systems approach to biology utilizes multiple techniques (experimental, theoretical, etc.) to study living organisms and systems. The approach considers all levels of organization, from the molecular and cellular levels to the larger organismal, community, ecosystem, and biosphere levels. The aim of such an approach is to reach an understanding that biological processes are parts of integrated systems instead of isolated parts. An integrative approach to viewing (and teaching about) biology includes investigation of systemic structures, systemic dynamics, and systemic control. When focusing on systemic structures, students can gain an understanding of how structure influences function, and vice versa. Systemic dynamics focuses on understanding how a system's behavior changes over time when faced with various conditions. Systemic control focuses on developing an understanding of the mechanisms that systems use to control their state. Every aspect of biology can also be viewed across a continuum scale, from coarse (large scale) to fine (small scale). For example, if a student were interested in learning about the structure of rainforests and how that structure makes these areas unique, they could focus on a fine scale, and look at individual species, or they could look at a coarse scale and investigate landscape patterns in tropical areas.

So began a semester of purposeful un-focusing, moving away from a central idea to a wider spectrum of interrelated and integrated topics. Instead of being designed around a series of discrete topics, the course topics were introduced as examples of specific scale levels. I introduced the concept of scales to my students on the very first day of class, and was not entirely surprised to find they were more comfortable with small scales than with larger more complex scales. Without ever acknowledging it, the students had been creating scale boundaries with each class they took. Temporal boundaries were defined by exams and semesters, while spatial boundaries were defined by the subject. Integration between temporal and spatial boundaries within the content of the subject being taught was tenuous at best.

Part of this may be due to the focus on health and biomedical careers, almost all the students in the class had already defined future career goals in the pharmacy or health fields. They were quite comfortable conversing in the language of those fields (i.e. about genes, molecules and cells), but had difficulty articulating the impacts of these fine scales on larger coarser scales such as organismal biology, population ecology, ecosystem science, and biosphere science.

Throughout the semester the students were given opportunities to investigate topics of interest to them. The majority of the students choose to focus on cellular or molecular topics as opposed to organismal or ecological topics. In our undergraduate biology program, students initially take a general biology course focusing on evolution and taxonomy. Following that course they progress onto cellular biology and molecular biology courses. The students were encouraged to combine their focus with a completely different scale. For example, a student who chose to focus on breast cancer, wrote about the basic biological composition of breast cancer, which cells were involved, which genes were active, etc. Since the first essay was at a very fine scale (cellular), the student's second essay needed to take the same topic, breast cancer, and address it through the lens of a larger scale (i.e. environmental). The student now had to discuss how the outside world influenced the cancerous cells.

One of the more successful projects, and certainly the most confusing and frustrating one for my students, was a project involving traditional literature review of a topic combined with an analysis of paintings and prints from our campus art gallery. For biology students, the idea of venturing into a perceived non-science field was certainly confusing if not daunting. One of the first hurdles they encountered was the task of interpreting meaning from a static object, a skill that is not unlike drawing

inferences from static sets of data. At first when I asked them to describe what types of scientific information they could gather from the image, they were very literal in their responses. They would talk about the colors of paint or ink in the image, they described what the image showed. Artists determine which aspects to record, and define the scales that they want to portray. The viewer then draws their interpretation of the visual data that is presented. Just as with a set of data points, multiple inferences can be drawn, and the inferences themselves depend upon the implied or explicit scale being used by the viewer. Within a single image, the viewer can derive information across multiple scales, or can choose to focus on a single scale. While some students struggled to see beyond the painted or printed work, others took the leap and drew connections between the literature they were researching and the ideas implied by the art.

You may wonder how my students responded to this purposeful blurring of both disciplines and scale. Some enjoyed the chance to explore biological scales, while others had difficulty seeing the value in the course. I am not sure what the long term impact of the integrated biological systems course will be. Some students will probably only remember specific examples from the content, but hopefully for most students, the effect will last longer. Perhaps the awareness of other scales, and the clarity that comes with contemplation, will continue on with them as they encounter new topics. Most importantly, I hope they discover that confusion can be a catalyst in opening up new paths of discovery.

Bioscene: Journal of College Biology Teaching
Submission Guidelines

I. Submissions to *Bioscene*

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

- Articles: Course and curriculum development, innovative and workable teaching strategies that include **some type of assessment** of the impact of those strategies on student learning.
- Innovations: Laboratory and field studies that work, innovative and money-saving techniques for the lab or classroom. These do not ordinarily include assessment of the techniques' effectiveness on student learning.
- Perspectives: Reflections on general topics that include philosophical discussion of biology teaching and other topical aspects of pedagogy as it relates to biology.
- Reviews: Web site, software, and book reviews
- Information: Technological advice, professional school advice, and funding sources
- Letters to the Editor: Letters should deal with pedagogical issues facing college and university biology educators

II. Preparation of Articles, Innovations and Perspectives

Submissions can vary in length, but articles should be between 1500 and 5000 words in length. This includes references and tables, but excludes figures. Authors must number all pages and lines of the document in sequence. This includes the abstract, but not figure or table legends. Concision, clarity, and originality are desirable. Topics designated as acceptable as articles are described above. The formats for all submissions are as follows:

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

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

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

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

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

Single Author:

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

Two Authors:

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

Multiple Authors:

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

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

(1) Articles-

(a) Single author:

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

(b) Multi-authored:

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

(2) Books-

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

(3) Book chapters-

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

(4) Web sites-

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

For references with more than five authors, note the first five authors followed by et al.

D. Tables

Tables should be submitted as individual electronic files in Word (2003+) or RTF format. Placement of tables should be indicated within the body of the manuscript. All tables should be accompanied by a descriptive legend using the following format:

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

E. Figures

Figures should be submitted as high resolution (≥ 300dpi) individual electronic files, either TIFF or JPEG. Placement of figures should be indicated within the body of the manuscript. Figures only include graphs and/or images. Figures consisting entirely of text will not be allowed and should be submitted as fables. All figures should be accompanied by a descriptive legend using the following format:

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

Color figures: When color is involved in a figure, it should be encoded as RGB and the resolution should be 300 dpi. Manuscripts that include color figures accepted for the May issue (online only) will appear in color at no charge to the author(s). For color reproduction in the December issue (print and online), there will be a page charge of $300. Author(s) will be notified of the costs and will have the option of either delaying publication until the May issue or paying the page charge. There is no fee for color in an image used on the cover of *Bioscene*.

III. Letters to the Editor

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

IV. Other Submissions

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

V. Manuscript Submissions

All manuscripts are to be sent to the editor electronically. *Authors must clearly designate which type of article they are submitting (see Section I) or their manuscript will not be considered for publication.* Emails should include information such as the title of the article, the number of words in the manuscript, the corresponding author's name, and all co-authors. Each author's name should be accompanied by complete postal and email addresses, as well as telephone and FAX numbers. Email will be the primary method of communication with the editors of *Bioscene*.

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

VI. Editorial Review and Acceptance

For manuscripts to be sent out for review, at least one author must be a member of ACUBE. Otherwise, by submitting the manuscript without membership, the corresponding author agrees to page charges. Charges will be the membership fee at the time of submission per page. Once the authors' membership or page charge status has been cleared, the manuscripts will be sent to two anonymous reviewers as coordinated through the Editorial Board. Authors' names will be withheld from the reviewers. The associate editors will examine the article for compliance with the guidelines stated above. If the manuscript is not in compliance or the authors have not agreed to the page cost provisions stated above, manuscripts will be returned to authors until compliance is met or the page cost conditions have been met. Reviewers will examine the submission for:

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

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

VII. Revision Checklist

Manuscripts will be returned to authors for failure to follow through on the following:

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

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