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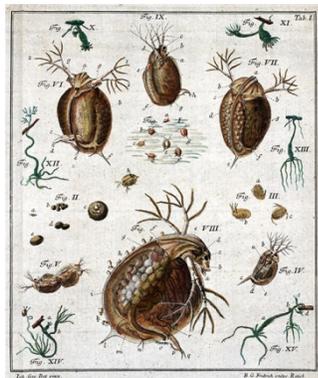
**Association of College  
and University Biology Educators**

Editor-in-Chief:

James W. Clack  
Indiana University –  
Purdue University

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# ***Bioscene: Journal of College Biology Teaching***

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The objectives of ACUBE are:

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

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## ARTICLES

# The effect of CGS21680 Treatment on Thioglycollate-Induced Peritonitis: An Introduction to Immunopharmacology

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**Abstract:** Inflammation occurs not only in response to infection, but also as a byproduct of many common diseases and pathologies. Because inflammation is an important modulator of human health, it is vital that students planning to pursue careers in biology, medicine or biomedical research are exposed to the topic as undergraduates. This laboratory exercise is appropriate for upper level undergraduate students and utilizes a murine model of non-infectious inflammation to illustrate both the principles of an innate immune response and the effects of an anti-inflammatory compound on inflammation. Thioglycollate, when injected into the peritoneal cavities of wild type, C57BL/6 mice, results in peritonitis, which is characterized by intraperitoneal leukocytosis, or elevated white blood cell count. The peritoneal exudates from thioglycollate-challenged mice are comprised predominantly of neutrophils and macrophages. The peritoneal leukocytosis elicited by thioglycollate challenge is significantly inhibited by treatment with the anti-inflammatory small molecule agonist of the adenosine A<sub>2A</sub> receptor, CGS21680. Additionally, treatment with CGS21680 modulates the white blood cell composition in the peritoneal cavities of thioglycollate-treated animals, resulting in elevated macrophage to neutrophil ratios. This exercise affords students the opportunity to observe inflammation in real-time and to modulate the progression and severity of inflammation using a pharmacological tool.

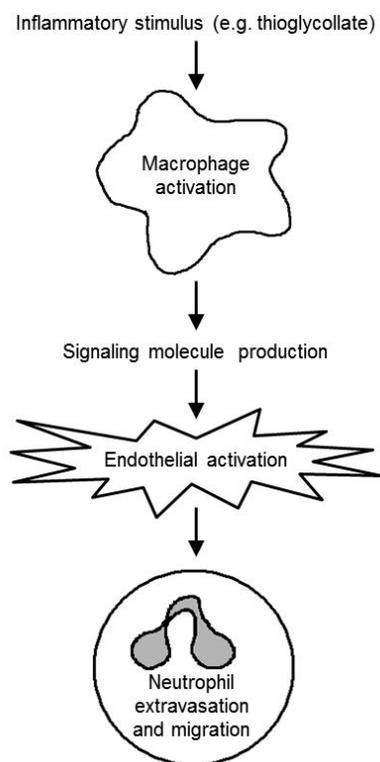
**Keywords:** inflammation, white blood cells, pharmacology, *in vivo* experimentation, flow cytometry

## INTRODUCTION

Immunology, in the broadest sense, is the study of an organism's defenses against infection. Although we are constantly surrounded by potentially pathogenic microorganisms, we rarely become ill because the cells and molecules of our immune systems have evolved complex systems of protection. The responses that our bodies mount against infection are termed immune responses, of which there are two main types, innate and adaptive immune responses. Innate immune responses are our first line of defense against infection, and are rapidly mounted, non-specific responses to "danger." Most cells involved in innate immunity are derived from the common myeloid progenitor cells found in bone marrow; these cells include the macrophages, neutrophils, dendritic cells, monocytes, eosinophils, basophils and mast cells. Unlike other inflammatory cells, tissue resident macrophages are cells that play several important roles in immune responses, including acting as our first responders to infection, effectively discriminating between "self" and "non self" and producing and secreting a variety of signaling proteins to induce inflammation. Inflammation is a response to infection traditionally defined by four Latin words: calor, dolor, rubor and tumor (heat, pain, redness and swelling; Murphy et al, 2008). Several important events occur during inflammatory responses that allow the cells of the immune system

to fight infection. The signaling proteins secreted by macrophages trigger a phenomenon known as endothelial activation, a process that results in blood vessel dilation, increased vascular permeability and increased adherence of circulating white blood cells to blood vessel walls. The macrophage-derived signaling molecules also recruit the adhered white blood cells out of circulation and into the tissue at the site of infection. The first class of white blood cells recruited to the submucosal tissue is the neutrophils, with the influx of neutrophils peaking by 6 hours after the initial pathogenic insult. The movement of neutrophils out of circulation and into the tissue in response to macrophage-derived signaling molecules is called extravasation and consists of four basic steps: rolling adhesion of neutrophils to the endothelium, tight binding of neutrophils to endothelial cells, diapedesis (migration of neutrophils between adjacent endothelial cells), and migration of neutrophils along a concentration gradient of macrophage-produced signaling molecules to the site of infection (Figure 1). Once in the tissue, neutrophils aid macrophages in the identification and clearance of the invading microorganism, and as the inflammatory process progresses, additional macrophages may also be recruited to the site of infection (Poher, 2002; Murphy, 2012).

While the activity of inflammatory cells is vital for host response to infection, inappropriately high or prolonged activity results also in host tissue



**Fig 1.** Schematic of thioglycollate-induced inflammation.

destruction. Furthermore, it has become increasingly clear that inflammatory responses occur not only in response to infection, but also may be inappropriately mounted against host tissue. Inappropriate and/or dysregulated immune responses are a major cause of morbidity and mortality in many common diseases including stroke, heart attack, sickle cell anemia, sepsis, colitis, and allergy. It is therefore necessary that inflammatory responses be tightly regulated, and for this reason, the development of novel anti-inflammatory agents is of the utmost significance. It has been demonstrated that the activation of the  $G_s$ -coupled adenosine  $A_{2A}$  receptor ( $A_{2A}R$ ), which is expressed on neutrophils, macrophages and T lymphocytes, as well as various other inflammatory cells including fibroblasts, monocytes, platelets and mast cells, plays a role in terminating inflammation via the regulation of cells involved in both innate and adaptive immunity. This makes it an interesting pharmacological target for the treatment of inflammatory conditions. Characteristic responses of activated neutrophils, including the generation of superoxide anion, the upregulation of adhesion molecule expression and the release of elastase are inhibited by  $A_{2A}R$  signaling. Similarly, the production of pro-inflammatory cytokines by stimulated macrophages and T lymphocytes is efficaciously inhibited by  $A_{2A}R$  activation, and T cell anergy can be induced by  $A_{2A}R$  agonist treatment (Lappas et al., 2005). In addition to inhibiting the

activity of individual cell types, the effects of  $A_{2A}R$  agonist treatment also modulate the interactions amongst inflammatory cells: the inhibition of IL-12 release from macrophages and IFN- $\gamma$  release from T lymphocytes serves to block the propagation of the positive regulatory loop facilitating the activation of both cell types. Along with modulating neutrophil, macrophage and T lymphocyte activity,  $A_{2A}R$  activation has also been suggested to inhibit the production of IL-6, IL-12 and IFN- $\gamma$  by plasmacytoid dendritic cells (Schnurr et al., 2004). Additionally, exposure of human dermal microvascular endothelial cells to TNF- $\gamma$  stimulates an upregulation in  $A_{2A}R$  expression, and  $A_{2A}R$  agonist treatment elicits a dose-dependent increase in cAMP levels in TNF- $\gamma$ -treated cells (Nguyen et al., 2003). The selective activation of the  $A_{2A}R$  with small molecule agonists such as 2-[4-(2-carboxyethyl)phenethylamino]-5'-*N*-ethylcarboxamidoadenosine (CGS21680) also effectively limits inflammation and injury in many pathologies with limited or no side effects:  $A_{2A}R$  agonists have significant protective effects in multiple models of ischemia-reperfusion injury, limit the progression of inflammatory bowel disease, protect against graft-versus-host disease following allogeneic hematopoietic stem cell transplantation, attenuate inflammation and injury in diabetic nephropathy, reduce stress-induced gastric lesions, and improve survival in murine models of endotoxemia and sepsis (Awad et al., 2006; Day et al., 2003; Lappas et al., 2006; Lappas et al., 2010; Linden, 2005; Naganuma et al., 2006; Odashima et al., 2005b; Odashima et al., 2005a; Sullivan et al., 2004). Several  $A_{2A}R$  agonists are currently in clinical trials for inflammation related indications.

Because of the prevalence of inflammation as a modulator of human health, it is vital that students planning to pursue careers in biology, medicine or biomedical research are exposed to the topic as undergraduates. The laboratory exercise described forthwith is designed for upper level undergraduate students and utilizes a murine model of non-infectious inflammation to illustrate both the principles of an innate immune response and the effects of an anti-inflammatory compound on inflammation. The objectives of the exercise are threefold: to introduce students to a murine model of inflammation; to provide an opportunity for students to observe an inflammatory response in real time, and to demonstrate the basic tenets of a pharmacologic intervention.

## MATERIALS AND METHODS

Thioglycollate, when injected into the peritoneal cavities of wild type, C57BL/6 mice, acts as a non-infectious irritant that triggers the initiation of an inflammatory response. Although the mechanism of thioglycollate-induced inflammation is not well

characterized, it is thought that thioglycollate acts as a chemoattractant. The inflammatory response is characterized by leukocytosis, or elevated white blood cell count, in the peritoneal cavity, resulting from the activation of tissue resident macrophages and the subsequent recruitment of neutrophils out of circulation and into the peritoneal cavity (Baron and Proctor, 1982). By 5 hours after thioglycollate injection, the total white blood cell (WBC) numbers in the peritoneal cavities of experimental mice (as compared to mice injected with a saline control) are significantly elevated, with the predominant WBC cell types found in the inflammatory exudates being macrophages and neutrophils. The infiltrating white blood cells can be collected via intraperitoneal wash, after which they can be counted and characterized according to cell type, illustrating the inflammatory cell infiltration that is characteristic of innate immune responses (McCarron et al, 1984). The use of C57BL/6 mice is recommended because this is a readily available, commonly used laboratory strain. However, thioglycollate injection elicits a similar progression of WBC infiltration, and resulting leukocytosis, in multiple other mouse strains including BALB/c and C3H/HeJ mice; these alternate strains would also be appropriate for use.

As an additional component of the exercise, a subset of the thioglycollate-treated mice is treated with the novel anti-inflammatory compound, CGS21680. CGS21680 is a small molecule agonist of the adenosine A<sub>2A</sub> receptor, which has been shown to modulate the activity of virtually all inflammatory cells, including macrophages, neutrophils, and lymphocytes (Hutchinson, et al, 1989). CGS21680 is safe, efficacious and readily available commercially. Students will evaluate the effects of CGS21680 treatment on the progression and/or severity of thioglycollate-mediated peritonitis. Not only does this exercise present undergraduate students with the opportunity to work (often for the first time) with research animals, but it also introduces them to several standard immunological and pharmacological techniques.

### **The Humane Care and Use of Laboratory Mice**

The use of laboratory animals in research and teaching has contributed to many seminal advances in science and medicine. Although many non-animal models have been developed for the study of inflammation, these models often cannot fully mimic the complex immunological processes that occur in the body; the use of research animals is therefore critical for many immunological studies. An important aspect of this laboratory exercise is to introduce students to the ethical and regulatory considerations that govern the humane care and use of laboratory animals. Before using laboratory mice for teaching and/or research purposes, every institution must establish an Institutional Animal Care and Use Committee (IACUC), which is tasked

with ensuring the proper care, use and humane treatment of all laboratory animals housed within the institution. Committee membership should include a doctor of veterinary medicine, at least one practicing scientist experienced in research involving animals, and at least one public member representing the interests of the general community (National Research Council, 1996). The committee must inspect all animal housing and activity areas and approve all animal protocols. Before introducing this laboratory exercise into a course syllabus, an animal use protocol for the procedure must be prepared and include the rationale and purpose of the proposed use of mice, a justification of the species and number of mice to be used, an overview of the training of all instructors, a description of the method of euthanasia to be utilized and the details of the animal housing conditions. The animal study described herein has been approved by the Lebanon Valley College Animal Care and Use Committee, but must be subsequently approved by the IACUC of each institution that adapts the protocol for its own use. Furthermore, instructors and students must be properly trained in basic animal handling techniques (National Research Council, 1996).

### **Laboratory Mice**

When designing an experimental study that utilizes research animals, careful consideration must be given to the number of animals to be used. In general, it is recommended that investigators use the minimum animals necessary to yield statistically significant results. When the experimental protocol described herein has been previously used in an instructional setting, it has been found that it is sometimes possible to obtain statistical significance when a minimum of 3 mice are used per experimental group. However, due to the variability in immunological response commonly observed between individuals, it is not unusual to require the use of a minimum of 6 mice per experimental group. To limit the number of mice required, while still achieving statistically significant results, instructors may consider pooling data from several lab groups or lab sections.

Female C57BL/6 mice, 8-12 weeks old, are purchased directly from a supplier, such as the Jackson Laboratory (Bar Harbor, ME). C56BL/6 mice are commonly used in immunological and pharmacological studies, and at the age of 8-12 weeks are generally accepted to be “adults” with sufficient body weight to tolerate the doses of thioglycollate and CGS21680 administered in this experiment. Although either male or female mice may be used successfully in this protocol, female mice are recommended because they tend to be easier to handle than males, which is an important consideration for use in a teaching laboratory. Mice are housed in autoclaved, polycarbonate cages with corncob bedding, and maintained in an environment

of 21°C and 55% humidity with a 14 hour light/10 hour dark cycle. All chow should be supplied by Harlan, or a comparable supplier.

### Thioglycollate-induced peritonitis

Using a 26 gauge needle, a 2 mL volume of a sterile 4% thioglycollate (Sigma-Aldrich) solution in PBS is injected into the peritoneal cavities of C57BL/6 mice (Jackson Laboratories). It is recommended that each laboratory group inject 6 mice with thioglycollate. Lab groups of 2-3 students are recommended. Additionally, each group injects a 2 mL volume of sterile saline into the peritoneal cavities of 3 control C57BL/6 mice. Three of the thioglycollate-treated mice receive a 1 µg/kg intraperitoneal bolus of 2-[4-(2-carboxyethyl)phenethylamino]-5'-N-ethylcarboxamidoadenosine (CGS21680) (Sigma-Aldrich) immediately after thioglycollate injection, 1.5 hours after injection and 3 hours after injection. The remaining 3 thioglycollate-treated mice receive intraperitoneal injections of saline vehicle following the same time course – immediately after thioglycollate injection, 1.5 hours after injection and 3 hours after injection. Intraperitoneal injections are an approved method of injection of laboratory mice and are commonly used because they require only the temporary restraint of mice and cause limited discomfort. The proper intraperitoneal injection technique is demonstrated in Video 1 ([www.lvc.edu/biology/video/1.mp4](http://www.lvc.edu/biology/video/1.mp4)). Mice are euthanized 5 hours after initial thioglycollate injection and intraperitoneal cells are harvested. There are multiple approved, humane, methods of euthanasia; individual instructors must select a method that is most suitable for their laboratory and institution (National Research Council, 1996). Although the optimal time period from initial thioglycollate injection to euthanasia is 5 hours, it is possible to harvest cells as soon as 2 hours post injection should time constraints require. This shorter time frame will result in a more minimal leukocytosis, but the expected trends in WBC infiltration will still be observed. Alternately, to accommodate a shorter laboratory period, students may inject the mice prior to the official start of lab, or the laboratory instructor may perform the initial thioglycollate and CGS21680 (or vehicle) injections prior to the start of the laboratory period. Because there are 3 experimental groups (thioglycollate + CGS21680, thioglycollate + saline vehicle, saline vehicle + saline vehicle), the minimum number of animals necessary for one complete experiment is nine. Therefore, if each laboratory group performs the complete experiment, the total number of animals utilized in this laboratory will be dependent upon the number of groups in the class, with each group requiring nine mice. To limit the number of animals needed for this exercise however, each group may be assigned a single experimental condition only, which

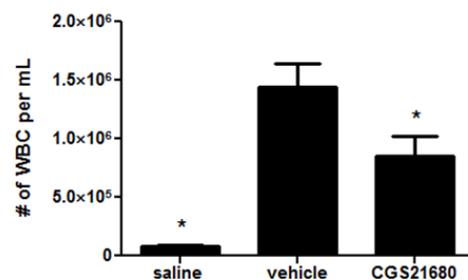
would require three mice. In this approach, three lab groups would work together to perform one complete experiment, thereby reducing the number of mice used.

### Intraperitoneal leukocyte harvest and counting

Intraperitoneal white blood cells are harvested via intraperitoneal wash. The intact anterior peritoneal surface of each mouse is exposed via midline incision, after which 6 mL of cold PBS is slowly injected intraperitoneally with a 26 gauge needle. The abdomens of the mice are gently massaged and the peritoneal fluid is reaspirated. The proper technique to be utilized when harvesting intraperitoneal leukocytes is demonstrated in Video 2 ([www.lvc.edu/biology/video/2.mp4](http://www.lvc.edu/biology/video/2.mp4)). A Hausser bright line hemocytometer (Fisher Scientific) is utilized to determine the total white blood cell (WBC) number in each mL of the peritoneal exudates. The peritoneal fluid is then centrifuged at 800g for 7 minutes and the pellets are resuspended in 2 mL PBS and kept on ice.

### Wrights stain of peritoneal exudates

A 20 µL sample of each resuspended cell pellet is smeared across a microscope slide and allowed to dry. The slides are covered with Wrights stain (Carolina Biologicals) for 3 minutes after which the slides are covered with an excess of Wrights stain buffer (Carolina Biologicals). After 1.5 minutes, the slides are tilted to drain the stain and buffer off the slide surfaces; the slides are again covered with Wrights stain buffer for 8 minutes after which the slides are flushed with distilled water until the runoff is clear. The stained slides are air dried and observed with a microscope. A minimum of 100 white blood cells are counted on each slide, and the percentages of macrophages and neutrophils in each of the peritoneal exudates are calculated.



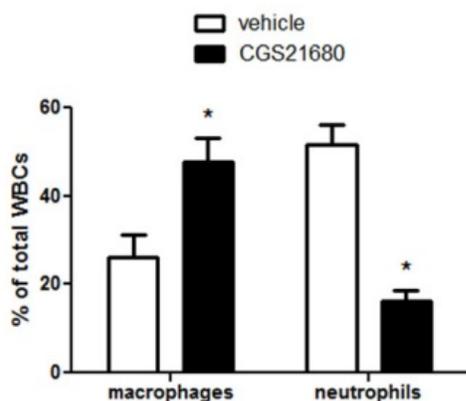
**Fig 2.** CGS21680 inhibits thioglycollate-induced peritonitis. Mice were euthanized 5 hours after thioglycollate injection and intraperitoneal cells were harvested. Thioglycollate-challenged animals were compared to vehicle-treated controls and CGS21680-rescued animals using a one way ANOVA followed by Dunnett's multiple comparison test (\*  $p < 0.05$ ). Results shown are representative of those expected when a minimum of 3 mice are used in each experimental group.

### Flow cytometry of cell surface markers (optional extension activity)

If a flow cytometer is available for use, the white blood cell populations in the peritoneal exudates may be further characterized (Shapiro, 2003). To further define the white blood cell types found in the peritoneal exudates, the remaining cells in the resuspended cell pellets are washed and resuspended at  $5 \times 10^6$  cells/ml in phosphate buffered saline (PBS). Aliquots (0.1 ml) are placed on ice and labeled for 30 min in the dark with fluorochrome-labeled anti-mouse F4/80, clone BM8 (a marker found on macrophages) and anti-mouse Gr-1, clone RB6-8C5 (a marker found on neutrophils) (eBioscience). Control samples are labeled with isotype-matched control antibodies. Stained cells are washed with 1 ml iced PBS and resuspended in PBS containing 1% paraformaldehyde. The fluorescence intensity is measured with a dual laser benchtop flow cytometer (FACSCalibur; Becton Dickinson) with a minimum of 20,000 events being collected. An excitation wavelength of 488 nm and an emission wavelength of 530 nm are used for FITC-stained cells, and an excitation wavelength of 488 nm and an emission wavelength of 585 nm are used for PE-stained cells. Analysis is performed with Flow Jo software (Tree Star, Inc.).

### Statistics

Unpaired t tests or one-way analysis of variance (ANOVA) with post-hoc Dunnett's multiple



**Fig 3.** CGS21680 treatment modulates thioglycollate-induced inflammatory cell recruitment. C57BL/6 mice were subjected to thioglycollate challenge via intraperitoneal injection. Animals received a 1 mg/kg i.p. bolus of CGS21680, or vehicle, immediately after thioglycollate injection, 1.5 hours after injection and 3 hours after injection. Mice were euthanized 5 hours after thioglycollate injection and intraperitoneal cells were harvested. Wright's stain analysis was utilized to determine the percentages of macrophages and neutrophils in peritoneal exudates from vehicle and CGS21680-treated mice. \*  $p < 0.05$  vs. vehicle treated, thioglycollate-challenged controls as assessed by unpaired t-test. Results shown are representative of those expected when a minimum of 3 mice are used in each experimental group.

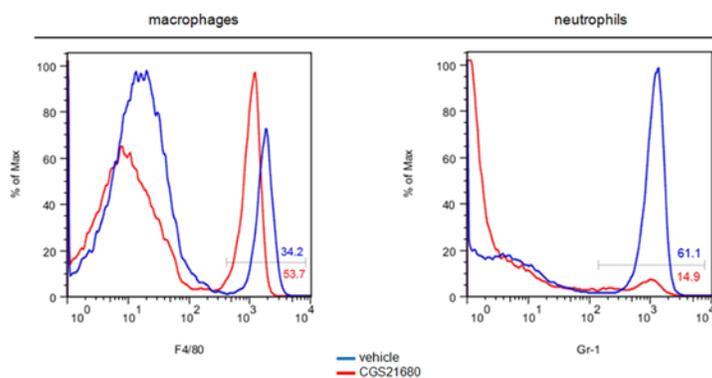
comparison should be used. Statistical software such as Prism GraphPad may be useful for statistical analyses.

### RESULTS

The intraperitoneal injection of sterile thioglycollate induces non-infectious peritonitis, with approximately 19 fold more WBCs found in the peritoneal exudates of thioglycollate-treated animals than in the peritoneal exudates of saline-treated controls (Figure 2). The peritoneal leukocytosis elicited by thioglycollate challenge is significantly inhibited by treatment with a 1  $\mu$ g/kg i.p. bolus of CGS21680 immediately after thioglycollate injection, 1.5 hours after injection and 3 hours after injection, with an approximate 40% decrease in the number of WBCs infiltrating the peritoneal cavities of drug-treated mice as compared to vehicle-treated controls (Figure 2). The peritoneal exudates from both vehicle-treated and CGS21680-treated mice are composed predominantly of neutrophils and macrophages. Interestingly, Wrights stain analysis reveals that CGS21680 treatment results in an increase in the percentage of macrophages and a decrease in the percentage of neutrophils present in the peritoneal cavity after thioglycollate challenge as compared to vehicle-treated controls (Figure 3). The altered white blood cell composition in the peritoneal cavities of CGS21680-treated mice is confirmed via flow cytometry, which illustrates that CGS21680-treated mice have a greater macrophage to neutrophil ratio in their peritoneal cavities 5 hours after thioglycollate challenge than do their vehicle-treated counterparts (Figure 4).

### DISCUSSION

This laboratory exercise has been utilized in an upper level undergraduate immunology course for several years with consistently good outcomes; more than 90 third and fourth year undergraduate students have participated in the laboratory module. The thioglycollate model of peritonitis is an excellent *in vivo* model of murine inflammation for use in undergraduate curricula because it is accessible to students unaccustomed to animal handling, is relatively inexpensive, and produces unusually reliable results. Furthermore, the model is advantageous because it triggers an innate immune response that is an accurate mimic of the response elicited by pathogenic microorganisms, but it does not require the use of any infectious organisms, thereby significantly improving the safety of the exercise as compared to other *in vivo* models. The inflammation initiated by thioglycollate challenge results in the extravasation of white blood cells from circulation into the peritoneal cavity in a manner that is predictable both in terms of timing and cell composition. Students consistently observe a significant elevation of white blood cell numbers in



**Fig. 4.** Flow cytometry confirms the elevated macrophage to neutrophil ratio in the peritoneal exudates of CGS21680-treated mice. C57BL/6 mice were subjected to thioglycollate challenge via intraperitoneal injection. Animals received a 1 mg/kg i.p. bolus of CGS21680, or vehicle, immediately after thioglycollate injection, 1.5 hours after injection and 3 hours after injection. Mice were euthanized 5 hours after thioglycollate injection and intraperitoneal cells were harvested. Cell surface expression of Gr-1 and F4/80 was assessed by flow cytometry. Histograms shown are representative of those expected when a minimum of 3 mice are used in each experimental group.

the peritoneal cavities of thioglycollate treated animals by 5 hours post-injection, with the white blood cell population in peritoneal exudates being comprised predominantly of neutrophils and macrophages. CGS21680 treatment has several interesting effects on this thioglycollate-induced peritonitis. First, and most obviously, the infiltration of WBCs into the peritoneal cavities of thioglycollate-treated mice is significantly inhibited by CGS21680 treatment. Additionally, the composition of WBCs in the peritoneal exudates of CGS21680-treated mice is noticeably altered as compared to their vehicle-treated counterparts, with an elevated ratio of macrophages to neutrophils. This trend likely is due to the overall decrease in the number of neutrophils recruited into the peritoneal cavities of CGS21680-treated mice (reflected in the lower WBC counts in the peritoneal exudates), resulting in the tissue resident macrophages comprising a greater overall percentage of WBCs present. Saline treated control mice can be expected to have very few white blood cells in their peritoneal cavities, with the majority of these cells being macrophages, as normal, healthy animals do not have tissue resident neutrophils.

If it is desired, this exercise can be extended, and additional subsets of thioglycollate-treated mice can be euthanized 1, 2, and/or 3 days after thioglycollate challenge. By extending the time course of the study, students will get a broader view of the inflammatory cascade; by 1-2 days after thioglycollate challenge, lymphocytes will have been recruited into the peritoneal cavities, demonstrating the connection between innate and adaptive immune responses. If these extended duration experiments are performed (i.e. longer than 5 hours between the initial thioglycollate injection and euthanasia), CGS21680 (or saline control) should be injected immediately after thioglycollate injection, 1.5 hours after injection, 3 hours after injection and then every 12 hours. As with all *in vivo* models, it is to be

expected that there will be variability between animals in terms of total number of WBCs in the peritoneal exudates and the macrophage:neutrophil ratios. For this reason, it is strongly encouraged that no fewer than three mice be used in each experimental group. To achieve even more statistically significant results, it is useful to combine the data from individual laboratory groups and analyze the results as a class. Students frequently cite this laboratory exercise as among the most beneficial of the course because it affords them the opportunities to observe inflammation in real time and to manipulate this inflammation using a pharmacologic tool. Additionally, students gain experience in animal handling, slide preparation and staining, flow cytometric analyses, pharmacologic dosing regimens, and statistical analyses: skills that will be invaluable as the students move forward in their scientific careers.

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## REFERENCES

- AWAD, A.S., HUANG, L., YE, H., DUONG, E.T., BOLTON, W.K., LINDEN, J., AND OKUSA, M.D. 2006. Adenosine A<sub>2A</sub> receptor activation attenuates inflammation and injury in diabetic nephropathy. *Am. J. Physiol Renal Physiol.* 290: F828-F837.
- BARRON, E. J., AND PROCTOR, R. A. 1982. Elicitation of peritoneal polymorphonuclear neutrophils from mice. *J. Immunol. Methods.* 49(3) 305-313.
- DAY, Y.J., HUANG, L., MCDUFFIE, M.J., ROSIN, D.L., YE, H., CHEN, J.F., SCHWARZCHILD, M.A., FINK, J.S., LINDEN, J., AND OKUSA, M.D. Renal protection from ischemia mediated by A<sub>2A</sub> adenosine receptors on bone marrow-derived cells. *J. Clin. Invest.* 112: 883-891.
- HUTCHINSON, A. J., WEBB, R. L., OEI, H. H., GHAI, G. R., ZIMMERMAN, M. B., AND WILLIAMS, M. 1989. CGS21680C, an A<sub>2</sub> selective adenosine receptor agonist with preferential hypotensive activity. *J. Pharmacol. Exp. Ther.* 251 47-55.
- LAPPAS, C. M., SULLIVAN, G. W., AND LINDEN, J. 2005. Adenosine A<sub>2A</sub> agonists in development for the treatment of inflammation. *Expert. Opin. Investig. Drugs* 14: 797-806.
- LAPPAS C.M., DAY Y.J., MARSHALL M.A., ENGELHARD V.H., AND LINDEN J. 2006. Adenosine A<sub>2A</sub> receptor activation reduces hepatic ischemia reperfusion injury by inhibiting CD1d-dependent NKT cell activation. *J. Exp. Med.* 203: 2639-2648.
- LAPPAS C.M., LIU P.C., LINDEN J., KANG E.M., AND MALECH, H.L. 2010. Adenosine A<sub>2A</sub> receptor activation limits graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. *J. Leukoc. Biol.* 87: 345-354.
- LINDEN J. 2005. Adenosine in tissue protection and tissue regeneration. *Mol. Pharmacol.* 67: 1385-1387.
- MCCARRON, R. M., GOROFF, D. K., LUHR, J.E., MURPHY, M. A., AND HERSCOWITZ, H. B. 1984. Methods for the collection of peritoneal and alveolar macrophages. *Methods Enzymol.* 108 274-284.
- MURPHY, K., TRAVERS, P., AND WALPORT, M. 2012. *Janeway's Immunobiology*. Garland Scientific. New York, NY.
- NAGANUMA M., WIZNEROWICZ E.B., LAPPAS C.M., LINDEN J., WORTHINGTON M.T., AND ERNST, P.B. 2006. Cutting edge: Critical role for A<sub>2A</sub> adenosine receptors in the T cell-mediated regulation of colitis. *J. Immunol.* 177: 2765-2769.
- NATIONAL RESEARCH COUNCIL. 1996. *Guide for the Care and Use of Laboratory Animals*. National Academy Press, Washington, D.C.
- NGUYEN, D. K., MONTESINOS, M. C., WILLIAMS, A. J., KELLY, M., and CRONSTEIN, B. N. 2003. Th1 cytokines regulate adenosine receptors and their downstream signaling elements in human microvascular endothelial cells. *J. Immunol.* 171: 3991-3998.
- ODASHIMA M., BAMIAS G., RIVERA-NIEVES J., LINDEN J., NAST C.C., MOSKALUK C.A., MARINI M., SUGAWARA K., KOZAIWA K., OTAKA M., WATANABE S., AND COMINELLI F. 2005a. Activation of A<sub>2A</sub> adenosine receptor attenuates intestinal inflammation in animal models of inflammatory bowel disease. *Gastroenterology* 129: 26-33.
- ODASHIMA M., OTAKA M., JIN M., KOMATSU K., WADA I., MATSUHASHI T., HORIKAWA Y., HATAKEYAMA N., OYAKE J., OHBA R., LINDEN J., AND WATANABE, S. 2005b. Selective adenosine A receptor agonist, ATL-146e, attenuates stress-induced gastric lesions in rats. *J. Gastroenterol. Hepatol.* 20: 275-280.
- POBER, J. S. 2002. Endothelial activation: intracellular signaling pathways. *Arthritis Res.* 4(Suppl 3) S109-S116.
- SCHNURR, M., TOY, T., SHIN, A., HARTMANN, G., ROTHENFUSSER, S., SOELLNER, J., DAVIS, I. D., CEBON, J., and MARASKOVSKY, E. 2004. Role of adenosine receptors in regulating chemotaxis and cytokine production of plasmacytoid dendritic cells. *Blood* 103: 1391-1397.
- SHAPRIO, H. M. 2003. *Practical Flow Cytometry*. John Wiley and Sons, Inc., Hoboken, N. J.
- SULLIVAN G.W., FANG G., LINDEN J., AND SCHELD, W.M. 2004. A<sub>2A</sub> adenosine receptor activation improves survival in mouse models of endotoxemia and sepsis. *J. Infect. Dis.* 189: 1897-1904.

# Laboratory Measures of Filtration by Freshwater Mussels: An Activity to Introduce Biology Students to an Increasingly Threatened Group of Organisms

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**Abstract:** Many aquatic organisms survive by filter feeding from the surrounding water and capturing food particles. We developed a laboratory exercise that allows students to measure the effects of filtering by fresh water mussels on water turbidity. Mussels were acquired from Wards Scientific and exposed to a solution of baker's yeast. Over a period of one to two hours, students measured changes in water clarity using miniature Secchi discs. The exercise has been used in a freshwater biology class at a state university. This exercise allows students to make hypotheses, gather data, and explore interactions between living organisms and their environment. Many North American species of freshwater mussels are threatened or endangered because of habitat changes and the introduction of exotic mussels. Therefore, students are also able to examine the potential effects of biodiversity loss in aquatic environments.

**Key words:** freshwater mussel, macroinvertebrate, filter feeding, ecology

## INTRODUCTION

Filter feeding organisms are a component of most aquatic ecosystems. The introduction of Dreissenid mussels, like the zebra mussels, into North American waters has elevated prominence of these organisms and their role in aquatic ecosystems. In spite of this increased awareness, little emphasis has been made in educational curriculum to demonstrate the potential impacts of filter feeders on aquatic ecosystems.

Lake Erie, one of the Great Lakes of North America, is the eleventh largest freshwater lake in the world. With a maximum depth of 64 meters and a length of 388 kilometers, it contains more than 484 cubic kilometers of water (Great Lakes Information Network, 2012). Lake Erie is the warmest of the Great Lakes and biologically the most productive. In the 1980s, Lake Erie was invaded by the zebra mussel, *Dreissena polymorpha* (Berkman et al., 1998; USGS, 2008). This species is native to Russia and is relatively small, reaching a maximum of about 5 centimeters in length. In the United States, it reproduces rapidly and reaches densities of 70,000 per square meter. These mussels live and grow by filter feeding by which they pass water through their gills and collect food particles from the water.

Today, it is estimated that zebra mussels filter the entire water volume of Lake Erie in less than one month. This filtration has removed many of the photosynthetic algal cells (phytoplankton), the aquatic producers that form the base of aquatic food chains, normally found in the lake. In the past twenty years, the zebra mussel has increased water clarity by up to 600 percent as a result of filtering out

phytoplankton, (UW Seagrant Inst., 2005; USGS, 2009).

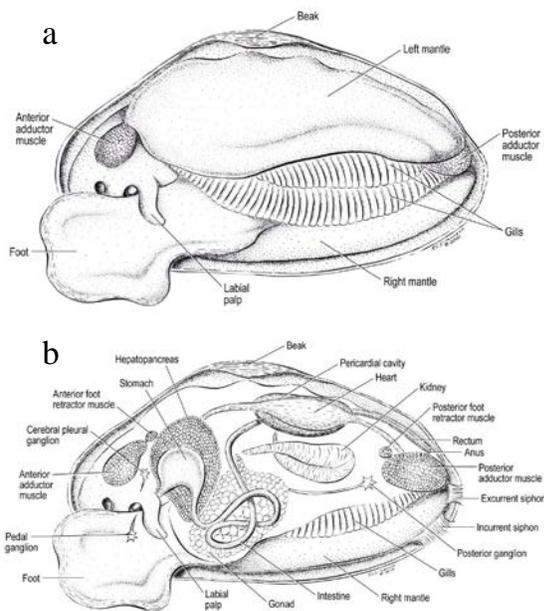
From this example, it is clear that filter feeding by aquatic organisms can have a large impact on aquatic food webs. A number of aquatic organisms, including microscopic rotifers, caddisfly larvae, paddlefish, and freshwater mussels rely on filter feeding to obtain energy. Among these filter feeding organisms, freshwater mussels live in the substrate of many freshwater streams and rivers, quietly filtering large volumes of water for most of their long lives, which in some cases can exceed 100 years (Bauer, 1992). As they filter the water, they extract nutrients and other suspended particles, changing the properties of the water around them. Because they are long lived, stationary, and sensitive to changes in the water quality, mussels are commonly used as bioindicators of water ecosystem health (Angelo et al., 2007; Jovic et al., 2011).

This laboratory exercise, developed and tested in a senior-level freshwater biology class at a state university, allows students to study live mussels and examine changes in water clarity as a result of their feeding behavior. It allows students to create hypotheses and collect quantitative data concerning filtration rates and can easily be used with both majors and non-majors as a laboratory exercise.

## Background

### *Mussel Anatomy*

Freshwater mussels have a two-part shell that is hinged on the posterior side (Cummings and Mayer, 1992) giving them the name "bivalves." Shell color is variable and generally ranges from yellow-green to black with green lines called rays that run



**Fig. 1.** Diagrams of mussel anatomy. a. Surface layers of the typical mussel. b. Internal structures of the typical mussel. Illustration by Rick L. Simonson ([www.RLSimonson.com](http://www.RLSimonson.com); used with permission).

perpendicular to the long axis of the shell found on the shell in some species. The interior lining of the shell (nacre) is usually white; however, the nacre of some species of freshwater mussels is highly iridescent and nearly purple. The exterior of the shell can be smooth as it is in the plain pocketbook mussel, *Lampsilis cardium*, or the shell can be bumpy as found in the pimpleback mussel, *Quadrula pustulosa* (USFWS, 2006; Cummings and Mayer, 1992). Shell shape ranges from elongate to round with many variations within and between species (USFWS, 2006). Freshwater mussels range in size from a few centimeters to 30 centimeters across the longest axis (Cummings and Mayer, 1992).

Freshwater mussels have strong adductor and retractor muscles (Figure 1a) that work together to open and close the shell. When threatened, the mussel will tighten its retractor muscles sealing itself inside the hard shell. Most predatory organisms are not strong enough to overcome the mussel's defense and pry the shell open. When the danger has passed, the mussel will extend a muscular foot that allows it to move slowly until it reaches an appropriate area of the substrate (Figure 1a). The mussel then extends its foot into the substrate, orients itself so that the hinge is dorsal and buries itself in the substrate so that it is anchored. If habitat conditions are sufficient, the mussel may not have to move for the rest of its life (Utterback, 1916).

#### *Mussel Feeding*

During feeding, the majority of freshwater mussels draw water through the incurrent siphon which is at the posterior end of the shell (Figure 1b).

The water passes through the gills in a U-shaped tube and then exits through the anal siphon at the anterior end of the shell. The gills of the mussel produce mucus that traps food particles. The food particles are then transported by ciliary action to the mouth where the food is consumed. In addition to capturing food, the gills conduct gas exchange with the surrounding water. Thus, the mussels feed and respire almost constantly. Their long sedentary lives and constant exposure to the water make freshwater mussels highly sensitive biological indicators of changes in water quality, including reductions in dissolved oxygen and the accumulation of metals and toxins (Strayer et al., 2004).

#### *Awareness of Freshwater Mussels*

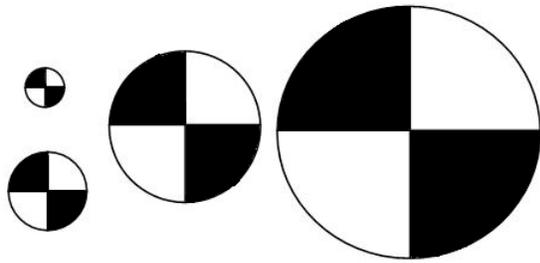
Historically 297 different species of freshwater mussels were native to North America (Williams et al., 1993). Nineteen of these species are currently listed as extinct or no longer occurring in nature, 62 species are federally listed as endangered, and 130 species are in need of conservation efforts. Thus, approximately 70 percent of the mussel species native to North America are now either extinct or threatened (Williams et al., 1993, Strayer et al. 2004).

Despite declines, freshwater mussels can be found in many streams, rivers, ponds, and lakes throughout the United States. In addition, conservation efforts have increased rapidly due to range expansion of invasive freshwater mussels, providing recent distribution maps and popular literature for many U.S. states (USGS, 2009). These resources can support informed classroom discussions of mussel lifecycle, ecology, and niche.

#### *Measures of Water Quality*

Many methods are used to assess water quality including chemical tests and biological integrity indices. One common measure of water quality measurement is turbidity, or the measure of water clarity caused by suspended solids. This is an important measure because murky water with little light penetration can indicate high levels of nutrients which may cause an algal bloom. The algal cells produce oxygen through photosynthesis during daylight hours if sunlight can reach them; however, at night or if the water becomes too clouded, the algal cells' respiration will be greater than photosynthesis, removing oxygen from the water and potentially leading to the death of aquatic organisms in the system.

Water turbidity can be measured through a number of methods. The simplest and least expensive method relies on a Secchi disc (Preisendorfer, 1986). This weighted disc is 20 cm in diameter and is divided into black and white quadrants (example in Figure 2). The water turbidity is determined by lowering the Secchi disc on a rope or tape which is marked with measurement increments. The disc is lowered into the water column until it disappears from sight. This depth is



**Fig. 2.** Mini-Secchi discs. Discs pictured are 10 mm, 15 mm, 35 mm, and 55 mm in diameter. Students should use the smallest possible disc for the experiment. Photo-reduction can also be used to adjust disc size as needed.

recorded. Then the disc is raised until it reappears. This depth is also recorded. The two measurements are averaged; this is the Secchi disc transparency measure (Wetzel and Likens, 2000), which is a standard water turbidity measure.

In this laboratory activity, miniature Secchi discs (Figure 2) are used to measure water turbidity to determine if the mussels are filtering the water and reducing water turbidity. Relative to control conditions in which mussels are not present, filtration of water by the mussels increased the water clarity by reducing the turbidity and allowed the Secchi disc to be seen over greater distances.

## MATERIALS AND METHODS

### Pre-laboratory Preparation

First, instructors need to obtain freshwater mussels. We purchased mussels from Ward Scientific. At the time of this work, 10 live mussel specimens of assorted species could be purchased from Ward Scientific for approximately \$23.00. Specimens obtained were generally small (Wards offer mussels ranging from 1.5-4 inches). Multiple mussels of 1.5 inches were placed into each aquarium.

If funding is not available to purchase freshwater mussels, they can be found in local ponds, streams, or rivers. State and Federal agencies must be consulted prior to field collection, not only to acquire the proper permits, but also to comply with state and federal laws and to avoid collection of any threatened species. Live mussels are often found in water less than one meter deep with their white hinge structure pointing up. If the water is relatively clear, mussels can be found by wading in the water and searching for the white structures. Mussels can be found associated with gravel, mud, and sand bottoms. Often, empty shells can be found on land or in the water and can be used to target areas in which to find live ones.

Housing for the mussels should be prepared ahead of time. The aquaria used in our study were 9.5 L (2.5 gallon); however, depending on the size of the mussels, different aquaria or plastic containers may be appropriate. Approximately 5 cm of sand

was placed in each aquarium to serve as substrate for the mussels. Water from the mussels' natural environment or tap water was then added until the aquaria were approximately two-thirds full. Tap water should not be used without conditioning to remove chlorine and other chemicals added during municipal water treatment.

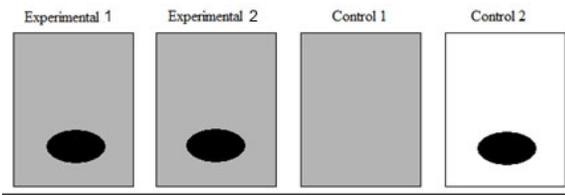
The aquaria need to be aerated to ensure that dissolved oxygen is available to the mussels. We accomplished aeration with Whisper aquarium pumps and a single airstone per aquarium. If the exercise is to be performed within 2 to 3 days of obtaining the mussels, food will not need to be added to the aquaria; however, if the mussels are obtained well in advance of the laboratory, food in the form of phytoplankton or single-celled algae should be placed in the aquaria. Phytoplankton can be found at pet supply stores. At the time of this work, Petco provided Two Little Fishies PhytoPlan Advanced Phytoplankton Diet for \$16.00.

In these experiments, yeast was used as the turbidity-producing agent. It was chosen because mussels can filter single celled organisms effectively and baker's yeast can be purchased from local grocery stores. One gram of baker's yeast was dissolved in 250 mL of pond water for each experimental aquarium. The suspension was added to all aquaria except Control 2(C2), and then the water was stirred with a large glass stir rod to ensure thorough mixing (Figure 3).

Miniature secchi discs were used to measure changes in turbidity. For our study, the discs were made using Microsoft Publisher. Four circles with diameters of 5mm, 10mm, 20mm, and 40mm were created (Figure 2). The circles were separated into four quadrants and two of the quadrants located diagonally from one another were colored black. These circles were then laminated and secured to small wooden dowels with a staple. The miniature secchi discs are inexpensive and simple to make. Standard 30 cm rulers were used to make the secchi measurements.

### Experiments

Depending on time available and student knowledge, a discussion with students should be conducted prior to setting up the experiment. Students should be asked about what affects water turbidity. Students may talk about nutrient loading, increase in phytoplankton, and water mixing effects on turbidity. An emphasis should be made on filter feeding and the significant reduction in turbidity that can result. Students should also define the controls that they will need for an experiment. At a minimum, students should be introduced to the concept of experimental versus control conditions in an experiment and should develop hypotheses to be tested by the experiment. For more advanced science students, instructors can teach students about sample



**Fig. 3.** Experiment design. The two experimental aquaria (E1 and E2) and one control aquarium (C1) contain the yeast suspension (gray fill). The other control aquarium (C2) contains no yeast. The experimental aquaria (E1 and E2) and the control 2 aquarium (C2) contain mussels (black ovals).

size, measurement and statistical analysis as part of the experimental design.

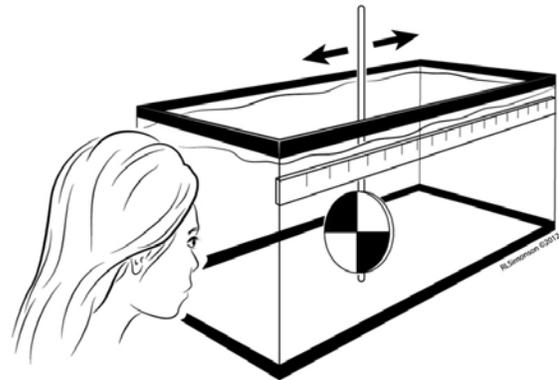
In this experiment, mussel filtration with 2 replicates (E1 and E2) was compared to two control aquaria (C1 and C2) (Figure 3). Control aquarium C1 received yeast but did not contain mussels. This allows students to measure if the yeast settles out of the water, making the water less turbid, or if the yeast replicate in the tanks, making the water more turbid. The second control tank, C2, contained a mussel but did not have any yeast. Because mussels are filtering the water and potentially expelling waste products, C2 should be measured as a negative control.

At the start of the experiment 250 ml of yeast suspension was added to C1, E1 and E2. To ensure that the yeast stay suspended in the water column, before taking a turbidity reading, all aquaria were stirred for 30 seconds with a stir rod, being careful not to disturb the substrate or the mussel if present. The turbidity of the water was then measured with the mini-Secchi discs. Although all four discs were tested, the smallest visible disc (5mm) was used for the 9.5 L (2.5 gallon) tanks in this experiment (Figure 2). Rulers were placed on the top of the aquaria, running parallel to the longest side of the aquaria, to allow students to measure their distances (Figure 4). The Secchi discs were placed in the aquarium by holding onto one end of the dowel and lowering the disc into the water column facing the short end of the aquarium perpendicular to the ruler. To determine a turbidity measurement, the dowel was moved along the longest side of the aquaria while looking through the short end of the aquarium until the disc was no longer visible. This distance was noted as the distance at which the disc disappeared. The disc was then moved back toward the viewer until it was visible again and that distance was noted. The Secchi disc transparency measure was determined by averaging the distance at which the disc disappeared with the distance when the disc reappeared.

Turbidity measurements were taken every 15 minutes thereafter in each aquarium, C1, C2, E1, and E2, for a period of 90 minutes using the same stirring procedure prior to conducting measures. At the conclusion of the experiment, mussels were immediately removed from the aquaria and placed

into fresh water. Note that mussels may die if left in the nutrient rich aquaria with the yeast.

Data analysis was performed on Microsoft Excel. Students input all Secchi disc transparency measures for each aquarium, C1, C2, E1, and E2. An experimental mean for each time period was found using the data from the replicates E1 and E2. Students created a scatter plot and tested the data with a linear regression for changes in water clarity over time.



**Fig. 4.** Diagram of experimental set up. Ruler is placed near the top of the tank. The Secchi disk is placed on the end of the dowel. One student can look through the narrow end of the tank at the Secchi disk to judge the point at which they can no longer see the Secchi disk, while another student moves the dowel. A third student uses the ruler to mark the point at which the dowel appears to the observer in the tank. This procedure is then repeated as the dowel moves the Secchi disk back into view and the second measurement marked. The students should then average the measurement to figure the Secchi disk transparency measure.

## RESULTS AND DISCUSSION

In this study, the experimental aquaria, E1 and E2, had a mean Secchi disc transparency measure of 3.5 cm immediately following the addition of the yeast suspension, and a Secchi disc transparency measure of 5.0 cm after 90 minutes (Table 1). Mussels reduced the turbidity in the experimental aquaria by 32%. Thus, water clarity, due to filter feeding, increased within a 90 minute period.

The turbidity readings for both controls demonstrated smaller changes over time. At zero minutes, the yeast only control, C1, had a Secchi disc transparency measure of 3.7 cm. After 90 minutes it had increased to 4.1 cm (Table 1). This indicated a 10% increase in water clarity. These data suggest that yeast numbers may change slightly even if mussels are not present so having this control is important for understanding if mussels are responsible for the increased water clarity. Students can hypothesize possible causes for the change in water clarity even if mussels are not present. Students may suggest that the yeast settled out of solution, that the conditions killed the yeast, or that

Time (minutes)	E-1 (cm)	E-2 (cm)	Experimental Average (cm)	C-1 (cm)	C-2 (cm)
0	3.4	3.4	3.5	3.7	18.1
15	3.5	3.4	3.5	3.7	18.1
30	4.0	4.6	4.3	3.8	18.0
45	4.1	4.7	4.4	3.7	18.0
60	4.4	4.8	4.6	4.0	18.1
75	4.7	4.9	4.8	4.0	18.0
90	4.9	5.0	5.0	4.1	18.0

**Table 1.** Representative Secchi disc transparency measures in cm reported over time. Experimental aquaria (E-1 and E-2) had three mussels each plus yeast, Control 1 (C-1) had only yeast added and control 2 (C-2) had only 3 mussels added.

other aquatic invertebrates were in the original pond water feeding on the yeast. Instructors may want to point out experimental variation and the concept of significance. Depending upon the level of the class, the instructor may want to have students collect more data that can be analyzed for statistical significance.

The mussel only control, C2, remained relatively constant. The Secchi disc transparency measure was 18 cm throughout the 90 minutes (Table 1). This is still an important control to have to introduce students to the concept of negative control.

Students were asked to graph their data as the percent change in Secchi disc transparency measures over time. This plot indicates an increase in water clarity produced by mussel filtration (Figure 5). After the graphs were made, students were asked to make comparisons of data they had collected and to draw conclusions about the experiment. Students were able to accept or reject their hypothesis and to offer possible reasons for why the experimental results occurred.

At the conclusion of the experiment, mussels must be removed from the experimental aquaria to keep them alive. This activity was completed several times, and after the first trial, mussels were kept in the experimental aquaria with the yeast suspension

overnight instead of removing them. As a result all four mussels died. Mortality could have resulted from oxygen limitation due to microbial growth or clogging of the mussels' gills as a result of the extremely high concentration of yeast. To ensure mussel survival, it is important to remove them from the mussel suspension and to place them in new pond water after the trial is complete.

Depending on the goals of the laboratory exercise, experimental mussels can be sacrificed to allow students to conduct a dissection and learn about mussel organs (Figure 1). A number of laboratory guides for mussel dissection are available, as well as videos placed on YouTube. It should be noted that mussels purchased from a commercial facility should not be released into local waters.

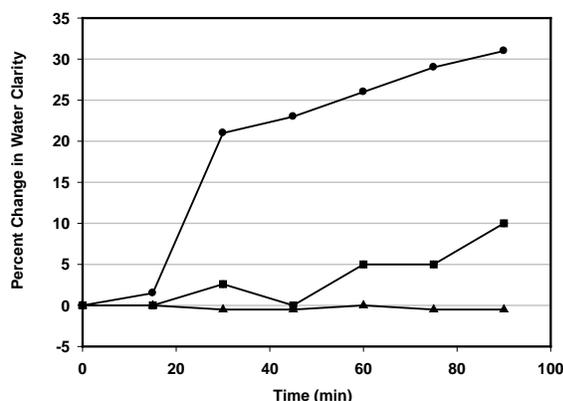
### Extensions

A number of extensions of this laboratory exercise are possible. For example, if the volume of water used in the experiment is known and the number of yeast cells is calculated, filtration rates can be determined (number of cells per unit volume through time). Alternatively, sub-samples of the experimental water can be collected and a hemocytometer can be used to estimate the number of yeast cells equivalent to a given Secchi disc transparency measure. Because freshwater mussels will filter any suspended material, the filtration of algal cells could be tested instead of yeast.

Outcomes of the laboratory exercise can be in the form of a formal laboratory write up or in the form of graphs or answers to questions. For example, students can be asked to predict water clarity after 3 hours based on regression equations generated from the experiment.

### CONCLUSIONS

Freshwater mussels are common members of the benthic community in most freshwater ecosystems of North America, although as a group the majority of their species are threatened. This laboratory exercise offers biology students a chance to physically interact with and collect data on the filtration behavior of these mollusks. This exercise also gives students the opportunity to calculate the amount of water that these animals can filter and offers a logical extension



**Fig. 5.** Observed mean percent change in water clarity as compared to time 0. Experimental aquaria, E1 and E2, had mussels and yeast (circles), C1 aquarium had mussels only (triangles), and C 2 aquarium had yeast but no mussels (squares). Positive numbers indicate increasing clarity, while negative numbers indicate reduced clarity.

to any course that currently incorporates dissection of mussels. The laboratory preparation for the instructor is simple, and costs are low. In addition, this laboratory may be of particular interest to instructors in regions infested with zebra mussels (*Dreissena polymorpha*), which have been shown to drastically change water quality in the Great Lakes (Holland, 1993).

#### ACKNOWLEDGEMENTS

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#### REFERENCES

- ANGELO, R.T., CRIGAN, M.S., CHAMBERLAIN, D.L., STAHL, A.J., HASLOUER, S.G., AND C.A. GOODRICH. 2007. Residual effects of lead and zinc mining of freshwater mussels in the Spring River Basin (Kansas, Missouri, and Oklahoma, USA). *Science of the Total Environment*. 384:467-496.
- BAUER, T. 1992. Variation in the life span and size of the freshwater pearl mussel. *Journal of Animal Ecology* 61:425-436.
- BERKMAN, P.A., HALTUCH, M.A., TICHICH, E., GARTON, D.W., KENNEDY, G.W., GANNON, J.E., MACKEY, S.D., FULLER, J.A., AND D.L. LIEBENTHAL. 1998. Zebra mussels invade Lake Erie muds. *Nature*. 393:27-28.
- CUMMINGS, K.S., AND C.A. MAYER. 1992. Field Guide to Freshwater Mussels of the Midwest. Illinois Natural History Survey Manual 5. 194p.
- HOLLAND, R.E. 1993. Changes in planktonic diatoms and water transparency in Hatchery Bay, Bass Island area, western Lake Erie. *Journal of Great Lakes Restoration*. 19: 617-624.
- GREAT LAKES INFORMATION NETWORK. 2012. <http://www.great-lakes.net>. Accessed January, 2009.
- JOVIC, M., STANKOVIC, A., SLAVKOVIC-BESKOSKI, L., TOMIC, I., DEGETTO, S., AND S. STANKOVIC. Mussels as a bio-indicator of the environmental quality of the coastal water of the Boka Kotorska Bay (Montenegro). *Journal of the Serbian Chemical Society*. 76:933-946.
- PREISENDORFER, R.W. 1986. Secchi disc science: Visual optics of natural waters. *Limnology and Oceanography*. 31: 909-926.
- STRAYER, D.L., DOWNING, J.A., HAAG, WENDELL, R., KING, T.L., LAYZER, J.B., NEWTON, T.J., AND S.J. NICHOLS. 2004. Changing perspectives on pearly mussels, North America's most imperiled animals. *BioScience*. 54: 429-439.
- UTTERBACK, W.I. 1916. The naiades of Missouri. *American Midland Naturalist*. 4: 41-53.
- UNIVERSITY OF WISCONSIN SEAGRANT INSTITUTE. 2005. [Http://seagrant.wisc.edu/zebramussels](http://seagrant.wisc.edu/zebramussels). Accessed January, 2009.
- UNITED STATES FISH AND WILDLIFE SERVICE. 2006. [Http://www.fws.gov/midwest/mussel/species.html](http://www.fws.gov/midwest/mussel/species.html). Accessed January, 2009.
- U.S. GEOLOGICAL SURVEY. 2008. [Http://www.glsc.usgs.gov/main.php?content=research\\_h\\_invasive\\_zebramussel&title=Invasive%20Invertebrates0&menu=research\\_invasive\\_invertebrates](http://www.glsc.usgs.gov/main.php?content=research_h_invasive_zebramussel&title=Invasive%20Invertebrates0&menu=research_invasive_invertebrates). Accessed January, 2009.
- U.S. GEOLOGICAL SURVEY. 2009. <http://nas.er.usgs.gov/taxgroup/mollusks/zebramussel>. Accessed May, 2012.
- WETZEL, R.G., AND G.E. LIKENS. 2000. *Limnological Analyses*. 3rd ed. Springer-Verlag. 21p.
- WILLIAMS, J.D., WARREN, M.L., CUMMINGS, K.S, HARRIS, J.L., AND R.J. NEVES. 1993. Conservation status of the freshwater mussels of the United States and Canada. *American Fisheries Society: Fisheries*. 18: 6-22.

## Using eBird to Integrate Citizen Science Into an Undergraduate Ecology Field Laboratory

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**Abstract:** Encouraging nonprofessionals to participate in ecological research through citizen science programs is a recent innovation and an effective strategy for gathering ecological information across broad geographical areas. In this paper, we demonstrate how reporting field-based observations through eBird, a citizen-based birding and data-recording program, can be used as a lab activity in an undergraduate ecology class. This exercise exposes students to worldwide data collecting networks in which non-scientific communities serve as major stakeholders. This lab activity also introduces basic field techniques in ornithology and allows students to answer inquiry-based research questions using a citizen science database.

**Key words:** citizen science, ecology teaching, eBird, participatory research

### INTRODUCTION

Citizen science provides an opportunity for members of the general public who do not have formal scientific training to contribute to scientific research (Cooper et al., 2007; Bonney et al., 2009). Here, individual volunteers or networks of volunteers perform or manage research-related tasks such as observation, measurement, data compilation and simple computation. The spatial scale of citizen science can be local, regional, national or global (Devictor et al., 2010). Citizen science programs are a venue for professional scientists to interact with non-scientific people who are interested in scientific aspects of nature. Furthermore, such programs allow the public to contribute to scientific research programs and to be an important stakeholder in scientific research studies (Schmeller, 2008). Such programs are an active and effective means of communication between professionals and laypeople, where scientific information is disseminated through educating the public and making them aware of scientific issues (Losey et al., 2007).

During the past few decades, citizen science programs have evolved to have more emphasis on scientifically sound practices and measurable goals for public education (Silvertown, 2009). Recent technologies, particularly the internet, have allowed citizen science data to be collected and accessed more efficiently. Moreover, increasing prevalence and use of user-friendly electronic devices that can record information, such as mobile phones, data loggers, personal digital assistants, and high resolution digital still and video cameras, have made data collection easy for the participants of citizen science programs (Sanford & Rose, 2007).

### Applications of citizen science in ecological research and biodiversity conservation

Citizen science programs are being used extensively in global environmental monitoring (such as climate and water resources) and biodiversity monitoring. Such continuous long-term monitoring is essential to understand the causes and effects of biodiversity loss in order to promote conservation efforts and curb species declines. Many citizen-based biodiversity monitoring programs assess the survival and reproductive success of wildlife (Lepczyk, 2005; Ries & Mullen, 2007) with many of these programs focusing on wildlife phenology (e.g., migration of birds, budburst in trees, or flowering of plants). Such investigations are important in assessing the effects of global warming and global climate change on ecosystems and biodiversity in different geographic areas (Lawrence, 2009; Mayer, 2010). Citizen science networks allow scientists to achieve research objectives more feasibly and cost-effectively than would otherwise be possible. For instance, employing well-trained professional scientists or skilled technicians to perform every step of a research project could be economically unfeasible and recruiting an accomplished task force, practically impossible (Ottinger, 2010).

In addition to long-term monitoring, citizen science is also being used as a means of public education and outreach to promote the science-based awareness of natural resources and wildlife (Jordan et al., 2009). Citizen science projects often generate enthusiasm among the general public and encourage the younger generation to be engaged in scientific research (Nerbonne & Nelson, 2008). Some programs may even provide extra benefits to the

community, such as the provision of specific materials specifically for use by primary or secondary school students. As such, citizen science is one form of informal science education. To encompass all these multilateral aspects of citizen science, this field is now frequently referred to as “participatory scientific research” (Raymond et al., 2010).

### **Limitations of citizen science programs**

Data collection in a citizen science program is performed by laypeople who may not have strong scientific backgrounds, training in field survey methods, or strong species identification skills. Therefore, there could be multiple errors in citizen science based datasets including species misidentifications as well as bias with regard to the independence of sampling events in time and space (Wagle, 2000). Moreover, complicated sampling methods and high-tech equipment that require special training cannot be used in citizen science programs. Similarly, citizen science programs are often only effective in monitoring charismatic species that are easily identified by laypeople and are not suitable to study taxonomically and ecologically cryptic species that require specialized skill for identification (Bonney et al., 2009). At times, sampling effort is inconsistent in citizen science programs and may vary within or between years (Ottinger, 2010). Furthermore, high inter-observer variability may exist among participants depending on their experience and science-based training. Therefore, it should be noted that data generated by citizen science programs need to be handled and interpreted carefully.

### **Examples of citizen science projects**

Following are examples of citizen science programs that have been used extensively in wildlife and environmental research that students can explore before conducting this lab activity. We recommend that lab instructors provide a brief introduction about other citizen science programs before the field activity.

**Christmas Bird Count:** A citizen science program implemented by the Audubon Society. This program aims to capture a snapshot of bird populations over many decades and to provide insight on the dynamics of bird populations across North America during the early winter. Volunteers gather information on birds over a three-week period at the turn of the year (December-January), and submit their observations to a review panel. Afterward, cumulative data are made available to the public and researchers for review and scientific study. Website: [www.audubon.org/bird/cbc](http://www.audubon.org/bird/cbc)

**NestWatch:** A nest-monitoring project developed by the Cornell Lab of Ornithology in collaboration with the Smithsonian Migratory Bird Center. NestWatch serves as a nest-monitoring scheme to record reproductive success for all North American breeding birds and provides useful information to the

general public about nesting biology. Website: <http://watch.birds.cornell.edu/nest/home/index>

**Monarch Watch:** An educational outreach program run by the University of Kansas that monitors the abundance, habitat use and migration of the Monarch butterfly. The Monarch Watch website provides detailed information on the biology and conservation of Monarch butterflies. This project involves capturing Monarch butterflies during the migratory season, tagging them, and attempting to recover the tags or to recapture tagged butterflies. The tagging program provides a great deal of information regarding Monarchs, their migration, and geographical range (Wells, 2010).

Website: <http://monarchwatch.org/>

**Journey North:** An internet-based citizen science database that tracks annual biological events, particularly how seasonality and climate change affect wildlife migration and ecosystem dynamics. Through field observations, participants record the migration patterns of wildlife in response to seasonality. Species of interest include Monarch butterflies, robins, hummingbirds, whooping cranes, gray whales, and bald eagles, along with other birds, animals, and plants. Using the nationwide data generated by participants, migration maps can be generated. Website: [www.learner.org/jnorth](http://www.learner.org/jnorth)

## **THE ACTIVITY**

### **Background information**

One of the largest and fastest growing global biodiversity data resources available is eBird. eBird is a real-time, online, freely-accessible, citizen science program coordinated by the Cornell Lab of Ornithology and the National Audubon Society (<http://ebird.org/content/ebird>). Launched in 2002, eBird has evolved a long way to enhance public participation, improve data validity, and widen data access to the research community. eBird is a rich database for bird abundance and distribution data on a variety of spatial and temporal scales. One strength of eBird is that it utilizes data collected by both professional and recreational bird watchers to generate enormous amounts of data.

eBird compiles bird sightings and abundance data from an international network of users and makes them available to the global community of educators, ecologists, land managers, landscape biologists, ornithologists, and conservation biologists. These data are currently being used in scientific analyses of global bird distribution and abundance (<http://ebird.org/content/ebird/about/ebird-publications>). Utilizing a user-friendly and intuitive website, eBird makes it easy for bird watchers to submit their observations and visualize all submitted eBird data via maps, graphs, charts and tables. eBird also provides users opportunities to network with other birders in their areas, search for the best places to see birds, and generate and catalogue bird lists.



world of recreational birding. An important goal of biology education is to allow students to interact with nature and assist them in understanding key elements of ecosystems; and in this lab students identified both birds and their associated habitats. Students performed traveling counts, a commonly used avian survey technique. We also used this opportunity to describe other survey techniques used to count wildlife such as transect surveys, aerial counts, and point counts.

### Assessment questions

Students were given the following assignment to reflect on their experience with citizen science:

1. Print the dataset and email it to yourself using eBird.
2. What are the advantages of citizen science programs to the public and the scientific communities? Limit your answer to two advantages to the general public and three advantages to the scientific community. List three potential limitations, drawbacks, or challenges of citizen science programs and briefly discuss them.
3. What kinds of biological studies could be developed using the eBird database? What kind of ornithological or ecological questions could be answered by analyzing information from the eBird database? Limit your answer to three different ecological questions/biological studies. Provide examples in your answer. Hint: climate change.
4. Think about one key ecological or environmental question that can be investigated using the eBird database. Then, through statistical and graphical analysis of eBird data, answer your question. Some sample questions are: "How are the distributions/abundances of common/rare birds changing in your home state/college town?" "Has intensification of the land-use activities in your home state affected the abundance of birds?" Use appropriate graphical and statistical analyses. Your answer should contain at least one graph/plot and the text should be limited to 200 words.
5. Briefly describe two additional citizen science programs that are not listed in this handout. Include program objectives, type of data collected, and inferences made with data collected.
6. It was emphasized that scrutinizing citizen science databases for accuracy of species identification is of high importance. Assume that you are in charge managing the eBird database. Discuss how you could test the validity of certain doubtful records such as isolated records of rare birds or species being recorded outside their natural ranges. Discuss how the scientific community can improve the accuracy of data

collected by citizen volunteers. Limit your answer to 200 words.

7. Write a brief reflection of your experience doing citizen science. What is your opinion about being a "citizen scientist" in an ecology lab?

### Student opinions about the lab

Citizen science turned out to be a completely new concept to most students. The majority of students were unaware of citizen science. The few who had heard about citizen science programs had never participated in them. For all the students, this was the first time that they realized how large-scale ecological information collected by citizen volunteers could be used to address global environmental issues. Overall, students found this activity informative, enjoyable, relevant to their lives, and they strongly recommended that this lab be continued in the future. This exercise made students feel that they were actually making a difference and contributing to something larger than themselves; this seemed to provide students with additional incentive (beyond simply earning a grade) to successfully complete this lab activity.

The following comments summarize student feedback from the lab:

"No one in our lab section had ever had any experience in birding, but with the species guide and binoculars, we were able to correctly identify about eight different species of birds. This gave us greater insight into the diversity of the bird populations in Clemson. Citizen science, in my opinion, is a great opportunity that I would be unaware of without this lab."

"In my opinion, this is a good lab for introducing students to citizen science programs. It is important for a class to relate to real life, and this lab definitely relates to realistic research. Before this lab, I had never heard about citizen science programs, and I was surprised at how often they are used."

"It really made me feel that I was actually making a difference and that there was more of a reason for me to be performing this lab rather than just for my educational gain. I really felt like I was benefitting a program and that there was more of a purpose for my actions and work. I almost feel as if citizen science should be taught more to the public and advertised more than what it already is."

The student feedback we received indicated that our lab effectively introduced students to citizen science and conveyed the importance of student participation in citizen science programs. It also demonstrated to students how they could contribute to an understanding of global ecological processes and use citizen science databases to develop and address research questions that were relevant to their lives. Based on our experience presented here, we strongly recommend using this lab in undergraduate ecology or general biology classes.

## REFERENCES

- BONNEY, R., COOPER, C.B., DICKINSON, J., KELLING, S., PHILLIPS, T., ROSENBERG, K.V., AND J. SHIRK. 2009. Citizen science: a developing tool for expanding science knowledge and scientific literacy. *BioScience* 59:977-984.
- COOPER, C.B., DICKINSON, J., PHILLIPS, T., AND R. BONNEY. 2007. Citizen science as a tool for conservation in residential ecosystems. *Ecology and Society* 12:11.
- DEVICTOR, V., WHITTAKER, R.J., AND C. BELTRAME. 2010. Beyond scarcity: Citizen science programmes as useful tools for conservation biogeography. *Diversity and Distributions* 16:354-362.
- DUNN, J., AND J.K. ALDERFER. 2008. *National Geographic field guide to the birds of western North America*. Washington, D.C., USA: National Geographic Society.
- JORDAN, R., SINGER, F., VAUGHAN, J., AND A. BERKOWITZ. 2009. What should every citizen know about ecology? *Frontiers in Ecology and the Environment* 7:495-500.
- LAWRENCE, A. 2009. The first cuckoo in winter: Phenology, recording, credibility and meaning in Britain. *Global Environmental Change-Human and Policy Dimensions* 19:173-179.
- LEPCZYK, C.A. 2005. Integrating published data and citizen science to describe bird diversity across a landscape. *Journal of Applied Ecology* 42:672-677.
- LOSEY, J.E., PERLMAN, J.E., AND E.R. HOEBEKE. 2007. Citizen scientist rediscovers rare nine-spotted lady beetle, *Coccinella novemnotata*, in eastern North America. *Journal of Insect Conservation* 11:415-417.
- MAYER, A. 2010. Phenology and Citizen Science. *Bioscience* 60:172-175.
- NERBONNE, J.F., AND K.C. NELSON. 2008. Volunteer macroinvertebrate monitoring: Tensions among group goals, data quality, and outcomes. *Environmental Management* 42:470-479.
- OTTINGER, G. 2010. Buckets of resistance: standards and the effectiveness of citizen science. *Science Technology & Human Values* 35:244-270.
- PETERSON, R.T., AND V.M. PETERSON. 2002. *Birds of eastern and Central North America*. Boston: Houghton Mifflin Harcourt.
- RAYMOND, C.M., FAZEY, I., REED, M.S., STRINGER, L.C., ROBINSON, G.M., AND A.C. EVELY. 2010. Integrating local and scientific knowledge for environmental management. *Journal of Environmental Management* 91:1766-1777.
- RIES, L., AND S. MULLEN. 2007. The biogeography of a mimicry complex is revealed by a citizen-science butterfly monitoring program. *Ecological Society of America Annual Meeting Abstracts*.
- SANFORD, C., AND J. ROSE. 2007. Characterizing eParticipation. *International Journal of Information Management* 27:406-421.
- SCHMELLER, D. 2008. European species and habitat monitoring: where are we now? *Biodiversity and Conservation* 17:3321-3326.
- SILVERTOWN, J. 2009. A new dawn for citizen science. *Trends in Ecology & Evolution* 24:467-471.
- WAGLE, U. 2000. The policy science of democracy: The issues of methodology and citizen participation. *Policy Sciences* 33:207-223.
- WELLS, C.N. 2010. An ecological field lab for tracking monarch butterflies & their parasites. *The American Biology Teacher* 72:339-344.

# A Web-based Computer-aided Learning Module for an Anatomy Course Using Open Source Image Mapping Software

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**Abstract:** Computer-aided learning (CAL) is used increasingly to teach anatomy in post-secondary programs. Studies show that augmentation of traditional cadaver dissection and model examination by CAL can be associated with positive student learning outcomes. In order to reduce costs associated with the purchase of skeletons and models and to encourage study outside of the laboratory period, interactive web-based CAL modules were developed for a comparative vertebrate anatomy course using skulls on hand, an open source image editor, and a simple text editor. Each module featured images of an animal skull in four orientations and allowed the user to identify individual bones and bony landmarks with a mouse. Study modules and practice quizzes were made available to students through the institution's learning management system for 24-hour access.

**Key words:** anatomy, computer-aided, dissection, interactive, web-based

## INTRODUCTION

Anatomy courses are commonly offered in post-secondary education programs and may serve as an elective toward fulfillment of degree requirements, be required for admission into a profession program, or be required as part of graduate or professional program curricula. At some undergraduate institutions, both human anatomy and comparative vertebrate anatomy are offered as separate courses within the same academic department.

The laboratory portion of anatomy courses is traditionally focused on dissection of preserved animal cadavers and examination of mounted skeletons and/or anatomical models. Models and skeletons used in these laboratories are quite expensive; for example, recent list prices from a biological supply catalog for a single mounted carp (fish) and dog skeleton were \$579.00 and \$939.00 respectively. Skulls are slightly less costly; the same catalog listed a rabbit skull at \$215.00 and a cow skull at \$562.00. The costs of procuring non-expendable specimens for a new course can be beyond budget limitations. For example, the purchase of seven rabbit skulls for a class with an enrollment of fourteen students (one skull per pair of students) would require an initial investment of \$1,505.00, excluding shipping costs. For labs featuring dissection, additional costs are associated with the use of preserved cadavers. Not only is there a cost to purchase and store the cadavers for the term, but also a significant cost for disposal. With strained course budgets, anatomy instructors may be faced with the decision of whether or not to replace old models or even include dissection as part of the curriculum (Winkelmann, 2007).

One alternative for reducing the use of preserved materials or models is computer-aided learning

(CAL) (Paalman, 2000). The use of CAL ranges widely from teaching human reproductive anatomy to elementary school students (Dalton et al., 1989), to teaching basic veterinary anatomy (Khalil et al., 2005) and complex segmental liver anatomy for radiology residents (Kuszyk et al., 1997). In addition to reducing the costs of materials, CAL provides instructors greater flexibility in dissemination of material and students with increased opportunities for learning (Paalman, 2000). Computer-aided learning appears to be as effective as traditional dissection in learning anatomy (Bukowski, 2002; Khalil et al., 2010; Hopkins et al., 2011). In some cases, CAL used with traditional methods has produced positive learning outcomes (Elizondo-Omaña et al. 2004). McNulty and colleagues (2004) found that as use of CAL increased so did medical students' anatomy exam scores. Studies also report that students readily and positively accept CAL (Allen et al., 2008; Khalil et al., 2010).

I was faced with reinstating a comparative vertebrate anatomy course which had not been taught at my institution for more than ten years. Because of a limited budget for course supplies, incorporating CAL modules into the laboratory portion of the course became a viable alternative to purchasing a number of models and skeletons. Unfortunately, software featuring 3D interactive images can be expensive and writing code to generate complex programs can require specialized skills and equipment (Petersson et al., 2009). To avoid extra costs and using a modest programming skill set, I created a series of web-based interactive modules for the study of mammalian skull anatomy using skulls already on hand, an open source image editor, and a text editor. The modules featured pointer-over identification of bones and bony landmarks with

color delineation of the borders of targeted bones and landmarks. An accompanying label identifying the targeted item was also highlighted when the pointer moved over it. By housing the modules on our institution's learning management system (LMS) (Jenzabar v. 7.4.2), students had instant access to the images and accompanying practice quizzes whenever they wished.

## METHODS

Creation of each module required three separate steps: the photography and image touch-up, the image mapping and HTML (Hyper Text Markup Language) code generation, and finally, writing the JavaScript functions that do the actual real-time user interaction. HTML image mapping with JavaScript was selected for the following reasons: it has better cross-platform (Windows/Mac/Linux/Unix/other) compatibility, better low-bandwidth performance than Flash or specialty CGI (computer-generated imagery) coding, easier integration with existing server software and LMS engines, few browser or Flash version compatibility issues, and it requires minimal web coding.

An Olympus E-500 8.0 megapixel digital camera with an Olympus 14 - 45 mm lens was used to photograph the individual skulls featured in the modules. With a square yard of black broadcloth serving as a background, each specimen was positioned on a one quart bag of sand placed under the cloth. In addition to the camera's flash, a fluorescent shop light and goose neck LED desk lamp provided back lighting. Skulls were photographed in separate frontal, dorsal, ventral, and lateral views. The initial images were saved in .tiff format at 3264 x 2448 pixels per inch resolution.

Image maps delineating the borders of individual bones and bony landmarks within the images were created using the GNU Image Manipulation Program v. 2.6 (GIMP) (<http://gimp.org>, 2011) for Linux operating systems. GIMP is an open source program for image composition, photo retouching, and other types of image manipulations. The program will also run on Microsoft Windows and Mac OS X. I found the program to be very user friendly and well supported by tutorials and user group blogs.

Image mapping of individual bones and landmarks within an image can be easily accomplished using the steps I followed. First, the image was cropped using the auto crop function  
 <Image> -> Autocrop Image -> Edit -> Copy -> Edit -> Paste as -> New Image  
 then scaled to 640 x 480 pixels at 72 pixels per inch resolution using the scale image function  
 <Image> -> Scale Image  
 in order to fit the viewing area of the LMS. The cropped image was saved in .png format for browser compatibility. I next selected the Web Image Map tool under Filters in the main toolbar

<Filters> -> Web -> Image Map  
 and using the polygon or circle selection, defined the area of an individual bone or landmark by tracing its boundaries or changing the size and position of the circle. GIMP automatically generated the associated HTML code defining the map. The following is a simplified example for a polygon drawn over a bone:

```
<map name="mymap">
  <area shape="poly"
  coords="202,240,250,238,273,350,193,313">
</map>
```

Note that the map tag <map> ...</map> encapsulates an "area" tag. It is the area tag that defines the size and shape of a portion of the target image, that is, the specific bone or bony landmark. The comma-separated decimal values in the "coords" property are x,y pixel coordinates of angle points for the polygon. Put simply, the x,y coordinates are the "dots to be connected" in drawing the polygon. There may be multiple area tags for each image, as there were in the skull images.

Each resulting set of area coordinates was identified in the Area Settings screen with the appropriate name of the bone or landmark mapped before mapping the next item in the image. The final image map containing multiple area coordinates was saved with a .map extension (Figure 1) (See Appendix 1 for the GIMP HTML snippet for Figure 1).

The next step in the process was to create a web page featuring the image and an accompanying legend with the name of each bone or bony landmark to be identified. I used a plain text editor to code the web page, but any standard web-page creation tool (e.g. Microsoft® Office FrontPage®) would work as well. The HTML in the .map file was copied and pasted into the web page HTML code following the image tag <img> that was modified to include a "usemap" property referencing the name of the skull image map (see Appendix 2).

HTML image map area tags support a variety of JavaScript functions that allow user interactivity. In this case, when the cursor is moved into the target



**Fig 1.** Example of image-mapped bones and bony landmarks generated by the image mapping function of the Gnu Image Manipulation Program (GIMP) on a coyote skull.

area, the legend color of the target bone or bony landmark changes from green to yellow. The following functions were used in the modules:

OnMouseOver() – The mouse pointer is over a section of a particular mapped segment.

OnMouseOut() – The mouse pointer has moved off the mapped segment previously reported.

OnClick() – The mouse button has been clicked indicating a selection.

The color of the text that changes is controlled by a style segment written in CSS (cascading style sheet) format. The style segment defines divisions (<div></div>) or “chunks” of the web page and defines each particular division’s general display attributes. A separate division was defined for each legend line that can control the display attributes, such as text color, for each legend line individually. Three similar JavaScript action functions were written to manipulate colors of the text descriptions of the mapped areas. This is an example of the “ramus” polygon area of Figure 1 modified to include three JavaScript action functions:

```
<area shape="poly"
coords="202,240,250,238,273,350,193,313"
alt="ramus"
onMouseOver="makeYellow('text1')"
onClick="makeGreen('text1')"
onMouseOut="restoreColor('text1')"
/>
```

The addition of this code in each of the area tags is identical except for the designation of the bone name representing the mapped segment of the image (See Appendix 2). This method allowed me to not only create a multi-area mapped image but to use a single area for object level flexibility. The resulting web page was integrated into the LMS portal for the course.

I created online practice quizzes within our LMS portal using its Test Builder function. The quizzes consisted of 20 multiple choice questions based on the four image views of each skull, reusing the mapped areas previously defined for the study



**Fig. 2.** Example of an online practice quiz question to identify a bony landmark. Color delineation of the ramus was generated using the image mapping function of the Gnu Image Manipulation Program (GIMP).

modules. Individual questions featured one of the images with a specific area highlighted to be identified (Figure 2). The mouse-related functions were not used in quiz questions.

Students were given each of the skulls featured in the modules and a laptop computer to access the images during laboratory. They were allowed to work at their own pace for up to one hour of the laboratory period. In order to encourage them to use the module and quizzes for study, students received a handout containing instructions on how to access the materials (a copy of the handout may be requested by emailing the author). Practice quizzes were allotted a 10 minute completion time but allowed unlimited attempts.

## RESULTS

I made a preliminary assessment of the modules using practical exam scores for questions based on each skull, LMS usage statistics, and our standard student course evaluations. The skull modules consisted of 12 area-mapped images of three mammal skulls (coyote, deer, and human) in four orientations. Once familiar with the steps required, I was able to complete an image with up to 20 mapped bones or landmarks and create the associated web page in less than four hours. A demonstration of one of the modules is available at: <http://facultyweb.berry.edu/rcarleton/skulldemo>.

Student use of the modules outside of laboratory varied. Five of the six students enrolled in the course accessed the study modules an average of 6.2 times (range 1 to 18 times). Four students accessed one or more module quizzes prior to the midterm exam. The amount of time students spent on quizzes and number of quiz attempts varied by skull type and by student. Quiz scores ranged from 11 of 20 correct (55.0%) to 20 of 20 correct (100.0%) depending on skull type and number of attempts. One student, who did not access the modules or quizzes outside of lab, missed five of the six questions pertaining to skull anatomy on the midterm examination; all other students answered the questions correctly. Each of the four students who completed the online course evaluation included positive comments about the modules.

## DISCUSSION

Computer-aided learning can be easily incorporated into an anatomy course using good quality digital photography, an open source image editor, a fairly basic set of programming skills, and a little innovation. Creating my own CAL modules allowed me to customize the software to my course curriculum and to the specimens I had on hand. It also allowed me to work within my course budget by negating the need to purchase expensive materials and commercially-available software. Although not enough data were available to analyze learning

outcomes, it was determined that most of the students enrolled in the course voluntarily used the modules for study and generally did well on exam questions. Although it may seem daunting to develop CAL tools, I found it relatively easy, requiring minimal assistance, and well worth the effort.

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## REFERENCES

ALLEN, G.A., WORTH, E., AND L.E. HARDIN. 2008. An Internet-supported delivery system for basic science education. *Journal of Veterinary Medical Education* 25:24-28.

BUKOWSKI, E.L. 2002. Assessment outcomes: Computerized instruction in a human gross anatomy course. *Journal of Allied Health* 31:153-158.

DALTON, D.W., HANNAFIN, M.T., AND S. HOOPER. 1989. Effects of individual and cooperative computer-assisted instruction on student performance and attitudes. *Educational Technology Research and Development* 37:15-24.

ELIZONDO-OMAHÑA, R.E., J.A. MORALES-GÓMEZ, S.L. GUZMÁN, I.L. HERNÁNDEZ, R.P. IBARRA, AND F.C. VILCHEZ. 2004. Traditional teaching supported by computer-

assisted learning for macroscopic anatomy. *The Anatomical Record (Part B: New Anat.)* 278B:18-22.

HOPKINS, R., G. REGEHR, AND T. WILSON. 2011. Exploring the changing learning environment of the gross anatomy lab. *Academic Medicine* 86:883-888.

KHALIL, M.K., LAMAR, C.H., AND T.E. JOHNSON. 2005. Using computer-based interactive imagery strategies for designing instructional anatomy programs. *Clinical Anatomy* 18:68-76.

KHALIL, M.K., MANSOUR, M.M., AND D.R. WILHITE. 2010. Evaluation of cognitive loads imposed by traditional paper-based and innovative computer-based instructional strategies. *Journal of Veterinary Medical Education* 37:353-357.

KUSZYK, B.S., CALHOUN, P.S., SOYER, P.A., AND E.K. FISHMAN. 1997. An interactive computer-based tool for teaching the segmental anatomy of the liver: Usefulness in education of residents and fellows. *American Journal of Roentgenology* 169:631-634.

MCNULTY, J.A., HALAMA, J., AND B. ESPIRITU. 2004. Evaluation of computer-aided instruction in the medical gross anatomy curriculum. *Clinical Anatomy* 17:73-78.

PAALMAN, M.H. 2000. New frontiers in anatomy education. *The Anatomical Record (New Anatomist)* 261:47.

PETERSSON, H., SINKVIST, D., WANG, C., AND O. SMEDBY. 2009. Web-based interactive 3D visualization as a tool for improved anatomy learning. *Anatomical Sciences Education* 2:61-68.

WINKELMANN, A. 2007. Anatomical dissection as a teaching method in medical school: a review of the evidence. *Medical Education* 41:15-22.

## APPENDIX 1

Example of HTML code generated by the GIMP image mapping function to define bones and bony landmarks of the coyote skull featured in Figure 1.

```
<map name="mymap">
<!-- #$.:Image map file created by GIMP Image Map plug-in -->
<!-- #$.:GIMP Image Map plug-in by Maurits Rijk -->
<!-- #$.:Please do not edit lines starting with "$" -->
<!-- #$.VERSION:2.3 -->
<!-- #$.AUTHOR:Renee Carleton -->
<area shape="poly" coords="202,240,250,238,273,350,193,313" alt="ramus" />
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<area shape="poly" coords="26,270,61,235,73,242,45,299" alt="occipital condyle" />
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<area shape="poly" coords="179,189,265,178,271,207,206,206,176,218" alt="zygomatic arch" />
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<area shape="poly" coords="543,228,567,276,598,283,556,231" alt="premaxilla" />
<area shape="poly" coords="105,104,226,99,244,169,85,190,66,140" alt="parietal" />
</map>
```

## APPENDIX 2

Complete web page code to generate interactive mouse action identification of individual bones and bony landmarks of the coyote skull featured in Figure 1.

```
<html>
<head>
<script language="javascript" type="text/javascript">
function makeGreen(object) {
document.getElementById(object).style.color = "green";
}
function makeYellow(object) {
if (document.getElementById(object).style.color == "green") {
document.getElementById(object).style.color = "orange";
} else {
document.getElementById(object).style.color = "yellow";
}
}
function makeWhite(object) {
document.getElementById(object).style.color = "white";
}
function restoreColor(object) {
if (document.getElementById(object).style.color != "green") {
if (document.getElementById(object).style.color == "orange") {
document.getElementById(object).style.color = "green";
} else {
document.getElementById(object).style.color = "white";
}
}
}
function resetAll() {
for (i=1; i < 25; i++) {
var obj = "text" + i;
makeWhite(obj);
}
}
</script>
<style type="text/css">
div#main
{ width: 780px;
height: 675px;
margin-top: 0;
margin-left: 0;
margin-right: 0;
margin-bottom: 0;
padding: 0;
background-color: black;
overflow: hidden;
}
div#image
{ width: 640px;
height: 480px;
float: right;
margin-top: 0;
margin-left: 0;
margin-right: 0;
margin-bottom: 0;
padding: 0;
overflow: hidden;
}
div#label
{ position: absolute;
top: 0;
left: 0;
width: 155px;
margin-bottom: 0;
margin-left: 0;
margin-right: 0;
margin-top: 0;
padding: 0;
}
div#text0,div#text1,div#text2,div#text3,div#text4,div#text5,div#text6,div#text7,div#text8,
div#text9,div#text10,div#text11,div#text12,div#text13
{ font-family: arial;
font-size: 13px;
font-weight: bold;
```



## PERSPECTIVES

# Integrating Functional, Developmental and Evolutionary Biology into Biology Curricula

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**Abstract:** A complete understanding of life involves how organisms are able to function in their environment and how they arise. Understanding how organisms arise involves both their evolution and development. Thus to completely comprehend living things, biology must study their function, development and evolution. Previous proposals for standardized post-secondary biology curricula have relied upon surveys of current practice, producing a curriculum that omits development and conflates evolution with ecology. To produce undergraduate biology programs that focus on the core essence of biology, curricula must address these three pillars undergirding biology: function, development, and evolution. Focusing the curriculum in this way may ease the difficulty of squeezing the burgeoning growth of biological knowledge into biology degree programs. A number of different approaches are possible, ranging from ensuring that these three principles are woven into the core biology courses to having specific required courses for each. Whichever approach is taken, it is imperative that biological function, development and evolution are integrated with each other such that students graduate with an understanding that these three concepts are inextricably entwined with, and dependent upon, each other.

**Key words:** curriculum design, functional biology, evolutionary biology, developmental biology, integrated biology

During his long and productive career, Ernst Mayr advocated that biological phenomena had two sources of causation: proximate and ultimate (Mayr, 1996). Proximate causation involves the mechanisms of immediate utility and explains how organisms are able to live in their immediate surroundings. This is the focus of functional biology (e.g. cell biology, physiology, genetics, and metabolism). Ultimate causation, in contrast, considers how species are produced. A full explanation of any given organism, Mayr argued, had to address both proximate and ultimate causation: the left and right hands of biology are function and evolution. Mayr's thesis had its origins in the mid-twentieth century when he was involved in the construction of the modern evolutionary synthesis in which genetics was wedded with evolution (Smocovitis, 1992). Gene expression provided the proximate explanation and evolution the ultimate explanation of how organisms and species arise.

Scott Gilbert et al. (1996) have suggested that Mayr's dualistic view of biology misses a key aspect of how organisms come into existence: ontogeny. Evolution results from changes in the developmental program. Changes in development produce different structures, functions, and behaviors in individual organisms which affect their ability to reproduce. Evolutionary theory explains how the fittest organisms are selected by their environment to have increased reproductive success. In contrast, development explains how the fittest organisms are produced. Evolutionary biology considers the

survival of the fittest whereas developmental biology considers the arrival of the fittest. The mechanisms by which development and evolution occur are dependent upon how cells and organisms function in their respective environments. Differing functional abilities determine which individuals survive to reproduce. Changes in the developmental program lead to differences in the functional abilities of reproducing organisms. Thus, evolutionary biology is derived from developmental biology which is derived from functional biology: ontogeny mediates between the ultimate and proximate causations of organisms (Gilbert et al, 1996). Some may consider developmental biology simply a result of gene expression. Ontogeny, however, cannot be completely reduced to genetics because of the presence of morphogenetic fields: the modular entities of cells and their secreted proteins responsible for the production of identifiable functional structures within an organism (Gilbert et al, 1996). Biology curricula need to attend to this re-synthesis which incorporates development into the modern evolutionary synthesis of evolution and genetics (Hlodan, 2009); development bridges the gap between proximate and ultimate causations in the evolutionary synthesis of the mid-twentieth century (Carroll, 2008).

Previous papers which proposed standardized curricula for post-secondary biology degree programs have relied upon surveys of current practice, producing a curriculum that omits development and conflates evolution with ecology (for examples see

Heppner et al, 1990; Cheesman et al, 2007). Surveys may produce an accepted standard or consensual curriculum but do not consider the theoretical foundations of biology. They assume that the biological principles will be adequately covered and integrated across the biological curriculum. Such an approach produces a curriculum based on disparate facts rather than on integrated theory and produces a representation that is current *status quo* but does not necessarily advance biology education.

Any academic discipline, however, must focus its education of the next generation of practitioners on its practice and principles not simply on subject matter. A discussion of the practice of biological science and its place in biology curricula is beyond the scope of the present article which addresses only the theoretical aspect of biology curricula. Making principles the focal point gives biology educators a means of focusing curricula despite the exponential growth of biological knowledge. Currently, educators are faced with a difficult choice when considering how to include growing current knowledge into the finite time of existing required courses. By attending to principles rather than to memorized facts, educators may instead choose from among the wealth of current biological knowledge to exemplify how the principles of biology are manifested in the living universe. Others have also suggested that a conceptual rather than content approach to course design may be a better way of enhancing students' understanding of biology (Sundberg and Dini, 1993). This conceptual approach, moreover, may be extended to the design of biology curricula (Bybee, 2002) as exemplified by the recent report published by the AAAS (Brewer and Smith, 2011). This most recent call for curriculum change in biology undergraduate education (Brewer and Smith, 2011) is excellent in its focus on core competencies (ability to apply the process of science, use quantitative reasoning, modeling and simulation, tap into interdisciplinary nature of science, communicate and collaborate, and understand the science-society relationship) and concepts (evolution, structure-function, information storage and retrieval, energy and matter transformation, and systems). However, similar to previous calls for curriculum change, it also omits one of the three conceptual pillars of biology; developmental biology is limited to a list of sub-disciplines which only provide supporting evidence for evolution.

To produce undergraduate biology programs that focus on the core essence of biology, curricula must address these three pillars undergirding biology: function, development, and evolution. Rather than having degree programs emphasize sub-disciplines and courses they could instead consider the principles and then require students majoring in biology to complete whichever courses are necessary to convey

the principles. Thus, rather than degree programs requiring courses in fields such as genetics, physiology, cell biology, ecology, biochemistry, microbiology, evolution, or molecular biology, they could instead require that majors graduate with an understanding of how prokaryotic and eukaryotic organisms function, develop, and evolve. The reductive (e.g. genetics and molecular biology) and holistic (e.g. behavior and ecology) biological sciences have their place within this scheme as different approaches to investigating the three fundamental biological pillars. For example, genetics considers the role that genes play in biological function, development and evolution. Similarly ecology considers the role of the environment in how life functions, develops and evolves.

In essence, to understand life, to study life, students need to comprehend how organisms work; how they overcome the problems of gas exchange, reproduction, waste removal, and energy conversion. The solutions employed by cells and organisms to overcome the challenges presented by their internal and external environments require an understanding of functional biology (metabolism, molecular biology, gene expression, anatomy and physiology). This would enable students to explain how organisms manage to maintain themselves while their environments change.

Students also need a foundation in understanding how life comes into existence. This must address two inter-related questions whose answers are dependent upon proximate mechanisms. First, how do organisms function while developing? This can occur through simple cell division in unicellular organisms in addition to the more complicated self-assembly of multicellular organisms. The study of these phenomena includes a consideration of cell communication, genetic control, cell motility, and developing structure/anatomy. Second, how do organisms evolve? What mechanisms produce Earth's biodiversity? This includes a consideration of selection theory, population genetics and ecology. These two aspects of how life arises are intimately entwined as evolutionary change necessarily derives from changes in developmental programs. Conversely, developmental programs evolve by natural selection of the procreating adults that development has constructed.

Both aspects of how organisms come into existence (development and evolution) are dependent upon how organisms function. Developing organisms must necessarily live/function in their developmental environments. Organisms are selected to develop and procreate based upon their relative ability to function.

Historical contingency clearly plays a role in evolution but, other than serendipity, the developmental program is the major constraint of organisms' ability to evolve over generations

(Gilbert, 2006); it limits what is available to be selected by the environment. The developmental history determines what features are available to be modified over generations. The history of an organism's evolution is written into its developmental program determining its future possible evolutionary paths.

Post-secondary biology curricula must ensure that graduates have a firm foundation in its principles and thus necessarily consider how organisms function, assemble (development) and are selected (evolution). However, developmental biology is missing from the AAAS list of major topics to be included in producing biologically literate students (AAAS, 1989). It is included in the BSCS (1993) guide but its integration with functional and evolutionary biology is only made explicit in the penultimate paragraph of the development essay by John Tyler Bonner. In addition, the most recent recommendation for renewal of biology curricula (Brewer and Smith, 2011) omits ontogeny in its list of core concepts required by all biology students.

If the three themes of function, development and evolution undergird a coherent theory of biology, then they must be woven across the curriculum of undergraduate degree programs in the biological sciences. Students must be introduced to these three conceptual pillars in any freshman biology course providing students with a rudimentary base of our current understanding of how life is possible. Currently, courses often treat the biological principles such as evolution as separate topics rather than as an integrated system (Musante, 2008). This may be appropriate in subsequent more advanced courses in which the focus is on their detailed mechanisms. A senior capstone course could then reconsider how all three themes are related and inextricably tied to each other. Alternative curricular sequences of biological concepts could be developed that best utilize the particular faculty expertise in any given biology department. Evolution might be consistently addressed in all courses negating the necessity for a specific evolution course. Similarly, function may be addressed in anatomy, cell or evolution/diversity courses negating the need for a specific course in biological function. I suggest, however, that a senior course that specifically integrates all three themes may be required to emphasize the point for students before they graduate. I have found that such integration may occur very well in third- or fourth-year developmental biology courses, and in fourth-year seminar and history and philosophy of biology courses.

Interestingly, the latest survey of US undergraduate biology curricula suggests that functional biology is well represented at the cellular and molecular level but not at the organismal level in the consensual biological core of required courses (Cheesman et al, 2007). In addition, a good

percentage of biology degree programs do not require their students to complete courses in both embryology (developmental biology) and evolution. Thus the de facto core curriculum excludes development and lists evolution interchangeably with ecology. It is unclear from the surveys whether the omission of development and evolution in institutions' list of required core courses is because these themes are adequately addressed in other required courses, is simply a result of the historical contingency of their degree program, or if the particular biology programs still subscribe to Mayr's dualist philosophy which inadequately addresses the role of development in how organisms are assembled to become procreating adults.

If ontogeny does bridge the gap between proximate and ultimate causation in biology (Carroll, 2008) then it is critical that undergraduate biology curricula reflect current research and teach biology as an integrated discipline (Futuyma, 2007) which would include the explicit synthesis of biological function, development and evolution. Surveying biology degree programs to determine the required core courses is interesting to assess current common practice but does not consider this growing understanding of how life is integrated. Revising biology curricula to reflect this current understanding is possible via many different course combinations but would require a first year course which introduces how these three concepts are interdependent upon each other in addition to concerted effort at the department level to provide the broad overview of the entire major. Regardless of how it is achieved, Gilbert et al's (1996) resynthesis of development with evolution and function needs to be an important aspect of an integrated biology curriculum.

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## REFERENCES

- AAAS: AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE. 1989. *Science for all Americans: Project 2061*. The Association, Washington, DC. Accessed from <http://www.project2061.org/publications/sfaa/online/sfaatoc.htm> on 10 August 2011.
- BREWER, C., AND D. SMITH (eds). 2011. *Vision and Change in Undergraduate Biology Education: A Call to Action*. American Association for the Advancement of Science, Washington, DC. Accessed from <http://visionandchange.org/finalreport> on 2012 May 14.
- BSCS: BIOLOGICAL SCIENCES CURRICULUM STUDY. 1993. *Developing Biological Literacy*. Kendall/Hunt Publishing Company, Dubuque, IA. 128 p.
- BYBEE, RW. 2002. Biology education in the United States: the unfinished century. *BioScience* 52: 560-567.
- CARROLL, SB. 2008. Evo-devo and an expanding evolutionary synthesis: a genetic theory of morphological evolution. *Cell* 134: 25-36.
- CHEESMAN, K., FRENCH, D., CHESSMAN, I., SWAILS, N., AND J. THOMAS. 2007. Is there any common curriculum for undergraduate biology majors in the 21<sup>st</sup> century? *BioScience* 57: 516-522.
- FUTUYMA, D.J. 2007. Unifying biology. *BioScience* 57: 915.
- GILBERT, S.F., OPITZ, J.M., AND R.A. RAFF. 1996. Resynthesizing evolutionary and developmental biology. *Developmental Biology* 173: 357-372.
- GILBERT, S.F. 2006. *Developmental Biology*, 8<sup>th</sup> ed. Sinauer Associates, Inc., Sunderland, MA. pp 3-24, 721-751.
- HEPPNER, F., HAMMEN, C., KASS-SIMON, G., AND W. KRUEGER. 1990. A de facto standardized curriculum for US college biology and zoology. *BioScience* 40: 130-134.
- HLODAN, O. 2009. Illuminating biology: an evolutionary perspective. *BioScience* 59: 368-370.
- MAYR, E. 1996. The autonomy of biology: The position of biology among the sciences. *Quarterly Review of Biology* 71: 97-106.
- MUSANTE, S. 2008. Critical conversations: the 2008 Biology Education Summit. *BioScience* 58: 685-689.
- SMOCOVITIS, V.B. 1992. Unifying biology: the evolutionary synthesis and evolutionary biology. *Journal of the History of Biology* 25: 1-32.
- SUNDBERG, M.D., AND M.L. DINI. 1993. Science majors vs nonmajors: is there a difference? *Journal of College Science Teaching* 22: 299-304.

# A Field Guide to Constructivism in the College Science Classroom: Four Essential Criteria and a Guide to their Usage

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**Abstract:** This field guide provides four essential criteria for constructivism as well as a guide for using these criteria to identify and assess the level of constructivism being used in an educational experience. The criteria include: 1) prior knowledge, 2) cognitive dissonance, 3) application with feedback, and 4) metacognition. This guide provides timely, valuable information and “best practices” for science educators, especially faculty in higher education.

**Key words:** constructivism, prior knowledge, biology teaching, cognitive dissonance, metacognition, science pedagogy.

## INTRODUCTION

Constructivism has become one of the most important learning theories in modern education. It is the basis of inquiry teaching methods, and consequently it is the primary learning theory underlying the AAAS “Vision and Change in Undergraduate Biology Education: A Call to Action” (AAAS, 2011), the newly released framework for K-12 science educators (“A Framework for K-12 Science Education: Practices, Cross-cutting Concepts, and Core Ideas”, National Research Council, 2011); and the research and findings that support them. Constructivism is also used as a theoretical framework for many educational research studies in biology (e.g. Anderson, 2002; Banet & Ayuso, 2003; Burrowes, 2003; Donovan, Bransford, & Pellegrino 1999, Eshel, 2007; Herron, 2009; Llewellyn, 2005; Shields, 2006; Yager, 2000).

In our practices, we have encountered numerous people who would benefit from a concise primer on the essential criteria of constructivism, but do not otherwise have a background in educational theory. We have worked with college and university instructors and faculty in biology and other sciences who were trained only in the STEM fields, as well as students, new teaching assistants, pre-service science teachers, and others who are being introduced to (and frequently asked to understand or even use) constructivism or constructivist teaching methods such as inquiry. This article provides an introduction to the vocabulary of constructivism, a concise, easy to understand, distillation of the theory of constructivism, as well as a portable rubric that can be used when trying to identify the constructivist elements of a real-life educational activity. This article thus meets the challenge of a recent *Bioscene Perspectives* article (Jensen, 2011) that called for

enhancing the pedagogical knowledge of college and university biology educators.

Just as a mushroom hunter needs to be able to identify mushroom species without becoming a mycologist, a practicing science educator needs to be able to identify and assess constructivism to be able to promote its use. This article, based on the authors’ previously published theoretical review article (Baviskar et al., 2009), serves as a field guide for identifying constructivism in the habitat of a college science classroom. So grab your checklist and hone your powers of observation while we describe the characteristics that will help you identify constructivist teaching strategies.

### How to Recognize Constructivist Look-Alikes!

No field guide for mushroom hunting is complete without comparing edible Morels to deadly False Morels. Likewise, we first need to compare and thereby remove the confusion that arises from similar terms for different theories, specifically: “cognitive” or personal constructivism vs. “social” constructionism. Cognitive constructivism is a theory that describes learning as taking new ideas or experiences and fitting them into a complex system that includes the learner’s entire prior learning. In other words, students arrive with pre-existing ‘constructs,’ and in order to learn, must modify these existing structures by removing, replacing, adding, or shifting information in them. Social constructionism is a sociological theory that describes how the facts that a society believes to be true are ‘constructed’ through social interactions (Baviskar et al., 2009, Longino, 1990; Marin, Benarroch, & Gomez, 2000; Richardson, 1984; Rodriguez, 1998). While *social constructionism* is an interesting theory and happens to share a similar name to constructivism, it is *cognitive constructivism* that can help us understand how our students learn.

Another confusion associated with constructivist teaching is the idea that constructivism requires the use of ‘group-work.’ Because of the importance of group work in many constructivist teaching methods, along with the confusion between social and cognitive constructivism, it is easy to equate constructivism with any kind of ‘group-work’ or ‘talking amongst peers’ (for an example of this confusion, see Straits, 2007; Straits & Wilke, 2007). Although cognitive constructivism can be effective in group-work, groups are neither necessary nor sufficient to make an activity constructivist.

One final ‘look-alike’ warning is the idea that constructivism is merely ‘students doing whatever they want’ in a completely unstructured classroom or lab. Constructivism is a student centered learning theory. It assumes that learning can only take place when students are actively engaging with the topic and ‘constructing’ their own knowledge bases. Because of this need for engagement, many constructivism-based teaching methods, like inquiry, use a lot of student directed activities. However, in order to be based in constructivism, a lesson must engage the motivations to build on the prior knowledge of the students (Bybee, 2002; Llewellyn, 2005), which involves much more than simply ‘letting the students do what they want.’

#### **Key “Field Marks” or the Identifying Criteria of Constructivism**

The four criteria essential to identifying and assessing constructivism are as follows: 1) eliciting prior knowledge, 2) creating cognitive dissonance, 3) applying new knowledge with feedback, and 4) reflecting on learning (metacognition). Any given activity or lesson plan can be considered more or less

constructivist depending on how many, and to what extent, the four criteria have been incorporated. Table 1 provides a summary of the four criteria, identifying characteristics, and an exemplar lesson from the published literature.

#### *Eliciting Prior Knowledge*

Learners ‘construct’ knowledge by modifying and contributing to their existing mental constructs. In the constructivist literature, this existing mental construct is succinctly referred to as ‘Prior Knowledge.’ Eliciting prior knowledge refers to any activity that both describes the students’ prior knowledge for the teacher and also focuses the students’ attention on those aspects of their mental constructs to be modified by the subsequent lesson. The teacher can use this description of their students’ prior knowledge to fine-tune the lesson. Maybe one class needs to focus on a single basic concept, while another class may be able to skip ahead to applications of the concept. Also, if the students don’t know where the lesson is supposed to ‘fit’ into the rest of their knowledge, they may simply memorize the lesson and then forget it after the quiz, or worse, try to fit the information improperly into the wrong topic, creating misconceptions.

When observing a lesson to identify signs of constructivism, look for opening activities that emotionally and cognitively engage students in the topic at hand. The activity should encourage them to think about what they already know or to attempt to solve a problem in the relevant topic. Be wary of activities that simply check whether students have read their text, or done homework. Likewise, be wary of high stakes assessment tools or activities that contribute significantly towards a grade. Readings or

**Table 1.** Field Guide to Constructivist Teaching and Learning.

<b>Four Essential Criteria</b>	<b>Field Marks: Expected methods &amp; learning activities</b>	<b>Exemplar: Leaf decomposition in streams (Hopkins &amp; Smith 2011)</b>
<b>1. Eliciting prior knowledge</b>	Demonstration, problems, focused listing, surveys, quizzes, interviews, discussions, concept mapping. Emphasis on eliciting student ideas.	Present students with a fresh and a decomposed leaf. Have students draw and list the processes that they think contribute to the change in the leaf.
<b>2. Creating cognitive dissonance</b>	Uncover misconceptions, compare lists, discuss missing information, demonstration, create discomfort. Pose a controversial question, or state/write a surprising or counterintuitive statement	Compare student drawings and lists. Explore relevant variables in decomposition. Reveal gaps in knowledge of the process.
<b>3. Application of new knowledge with feedback</b>	Formative assessments, feedback on new constructs, hypothesis testing, gain of new knowledge. Focus on process of gaining new knowledge, solving problems, design & logic of analysis and presentations.	Generate testable hypotheses, design and carry out experiments to manipulate variables related to the process of decomposition. Data analysis and summary of results, with feedback.
<b>4. Metacognition (reflection on learning)</b>	Repeat Step 1 and have students reflect on their own learning. Assignments should have students explain variables, processes, or derive conclusions from evidence. Reflective paper, presentation, field report, peer teaching.	Repeat initial leaf exercise & compare with initial drawings. Have students reflect on their new knowledge through presentations in a scientific format.

vocabulary quizzes usually do not provide enough motivation for students to explore their prior knowledge and high stakes assessments often distract students by focusing on techniques for acquiring points. The most constructivist activities are going to force the students to access and apply their prior knowledge in a way that can be observed and interpreted by the teacher. Demonstrations can be useful if they actually engage the students. Having students try to solve a problem or explain some data that has not yet been covered; conducting open-ended discussion of background knowledge, focused listing or informal surveys about students' concepts of the topic, and short discussions about the topic through current events or applications to the students' lives all can help elicit prior knowledge (Donovan, Bransford, & Pellegrino, 1999; Leamson, 1999).

#### *Creating Cognitive Dissonance*

If the students' constructs are different from the teacher's and the students do not realize it, or do not try to change them, no learning will take place regardless of how the lesson is taught. Only when the students realize that their prior knowledge is insufficient or inappropriate to understand something will the students become motivated to modify their constructs. The realization that their current constructs do not match their needs is called cognitive dissonance, and it is often as uncomfortable as it sounds.

To identify cognitive dissonance, look for wrinkled brows. When students are presented with information or puzzles that their current constructs cannot account for, they often look confused. Misconceptions are another sign that students' constructs are inappropriate for the problem at hand. Constructivist lessons often seek out misconceptions and then present problems that the misconception cannot address. If the teacher presents information that doesn't match the students' prior knowledge and then says something similar to, "How do you account for this?" or "What is the evidence for this observation?" he or she is likely trying to create cognitive dissonance in the students.

Modifying a mental construct is difficult. In order to learn, neural connections must be broken and remade which takes time, uses energy, and requires effort. Cognitive dissonance is an emotional discomfort intended to motivate the physical effort required for learning (Leamson, 1999; Zull, 2002). Too much cognitive dissonance, however, and the student will stop focusing on the lesson and instead focus on removing the emotional discomfort. Too little, and the student will not be motivated to modify the erroneous prior knowledge. Therefore, constructivist lessons tend to have variable activities and constructivist teachers tend to shape their lessons to find an optimum level of dissonance for each particular class.

#### *Application of New Knowledge with Feedback*

Creating cognitive dissonance and motivating students to modify their constructs does not guarantee that the students' new constructs match the goals of the teacher, only that the students have reconciled a single challenge to their constructs. Next, the students need to apply their new constructs to a variety of other puzzles or information to find out if the new constructs really work. Application of new knowledge has two main functions. First, it is a test and fine-tuning of the new construct. Second, it is repetition using multiple perspectives that helps to reinforce the learning. To accomplish these functions, it is important that the students receive both appropriate learning activities and feedback for their work. Grades by themselves usually don't provide enough detailed or timely feedback to serve as formative assessment. Formative feedback, in a constructivist sense, requires explicit directions on the next misconception to be dealt with, as well as detailed explanations of past performance.

To identify application of new knowledge in a college science classroom, look closely at the learning activities. Proper application of constructivist principles will take place if students are given a series of problems addressing a topic from several angles, the topic of the problem series is related to a prior misconception, and the problems create cognitive dissonance. Questions that can be classified in the upper end of Bloom's Taxonomy (Bloom et al., 1956), critical thinking questions, case studies, and other more complex assessments are often used in 'application of knowledge' activities (Lord & Baviskar, 2007).

Appropriate, constructivist feedback is often found in detailed comments by the teacher for assignments or other assessments, but appropriate feedback can come in many forms. The teacher can give feedback by presenting one solution for a problem to the class in general. This presentation would give students something to compare their own constructs to. More importantly, truly effective feedback often comes from the students' peers or from the assignment itself. One of the places where small group interactions are very effective is in providing timely and relevant feedback. If the assignment involves performing a self-correcting activity, the feedback can come from the assignment itself.

#### *Reflection on Learning or Metacognition*

Metacognition is the act of thinking about your own thinking. Because constructivism is student-centered, students are ultimately responsible for their own learning. The more students recognize both what and how they are learning, the more efficient their future learning will become. Because learning takes time and effort in several topics or from several perspectives, the process may not be self-evident to the students. It is especially easy for students (and

teachers) to misdiagnose a complex process of constructivist learning as simply inefficient memorization of another small fact. Reflection and metacognition will not only help the students understand the extent of what they have learned, but also help them to approach new learning in a more knowledgeable, and therefore efficient, way.

Metacognition can often be recognized when students are required to explain what they have done, how they did it, and why it was important. Reports, papers, presentations, and other discussions are a good sign that metacognition might be asked for. Look for questions or objectives that ask the students to explain a logical sequence or derive a conclusion from evidence, rather than to simply report what they have seen or done.

## CONCLUSION

Our primary objective in writing this field guide is to provide the theoretical, research-based, essential criteria of constructivism in a way that can be used in an applied setting, such as a biology classroom. By presenting the four essential criteria of 1) eliciting prior knowledge, 2) creating cognitive dissonance, 3) applying new knowledge with feedback, and 4) reflecting on learning or metacognition, we hope to provide college and university biology educators and educational practitioners in general with an easily accessible guide to identifying and evaluating the use of constructivism in educational activities.

Our secondary objective is to open a dialog among educators, theorists, and researchers who wish to use and discuss constructivism, especially as they move to implement the new Visions and Frameworks called for by AAAS and the National Research Council. By using these four essential criteria, college and university science teachers will be able to evaluate their own and their colleagues' lessons, review curricula, and plan and evaluate educational research according to the principles of constructivism. They will also be able to use terms (like 'constructivism' itself) properly and to open a broader multidisciplinary dialog in the literature to discuss what constructivism really means from theoretical, experimental, and applied perspectives.

Finally, college science teachers, educational theorists, and educational researchers can all communicate about constructivism from their own perspectives, while using common language and ideas. Educational terminology is a lot like common names for species. Any terms can work if used by a small group of practitioners who understand each other's perspectives, but to cross disciplines and effectively read the literature, we need to have commonly held definitions and theories. So with field guide in hand, you can now examine and explore diverse publications and classrooms for your own glimpse of the constructivist lesson.

## REFERENCES

- AAAS. 2011. Vision and Change in Undergraduate Biology Education: A Call to Action. AAAS. Washington D.C., USA.
- ANDERSON, R. 2002. Reforming science teaching: What research says about inquiry. *Journal of Science Teacher Education*, 13(1): 1-12.
- BANET, E., & G.E. AYUSO. 2003. Teaching of biological inheritance and evolution of living beings in secondary school. *International Journal of Science Education*, 25(3): 373-407.
- BAVISKAR, S.N., R.T. HARTLE, & T. WHITNEY. 2009. Essential criteria to characterize constructivist teaching: derived from a review of the literature and applied to five constructivist-teaching method articles. *International Journal of Science Education*, 31(4): 541 - 550.
- BLOOM, B.S., M.D. ENGELHART, E.J. FURST, W.H. HILL, & D.R. KRATHWOHL (EDS.). 1956. *Taxonomy of Educational Objectives: The Classification of Educational Goals, by a committee of college and university examiners. Handbook I: Cognitive Domain*. New York: David McKay Company, Inc. .
- BURROWES, P.A. 2003. Lord's constructivist model put to a test. *American Biology Teacher*, 65(7): 491-501.
- BYBEE, R. 2003. *Learning science and the science of learning*. Arlington, VA: NSTA Press.
- DONOVAN, M., J. BRANSFORD, & J. PELLEGRINO (EDS.). 1999. *How people learn: Bridging research and practice*: National Academies Press.
- ESHEL, N. 2007. *The science inside learning*. Washington, DC: Education and Human Resources Programs, of American Association for the Advancement of Science.
- HERRON, S. 2009. A curious thing happened on the way to constructivism.... *Journal of College Science Teaching*, 38(6): 8-11.
- HOPKINS, J. & R. Smith. 2011. An inquiry-based field & laboratory investigation of leaf decay: a critical aquatic ecosystem function. *American Biology Teacher*, 73(9) 542-546.
- JENSEN, J. 2011. Higher education faculty versus high school teacher: does pedagogical preparation make a difference?. *Bioscene*, 37(2): 30-36.
- LEAMNSON, R.N. 1999. *Thinking about teaching and learning: Developing habits of learning with first-year college and university students* Sterling, VA: Stylus Publishing.

- LLEWELLYN, D. 2005. *Teaching High School Science Through Inquiry: A Case Study Approach*. Thousand Oaks, CA: Corwin Press and NSTA Press.
- LONGINO, H.E. 1990. *Science as social knowledge: values and objectivity in scientific inquiry*. Princeton, New Jersey: Princeton University Press.
- LORD, T.R., & S. BAVISKAR. 2007. Moving students from information recitation to information understanding: Exploiting Bloom's Taxonomy in creating science questions. *Journal of College Science Teaching*, 36(5): 40-44.
- MARIN, N., A. BENARROCH, & E.J. GOMEZ. 2000. What is the relationship between social constructivism and Piagetian constructivism? An analysis of the characteristics of the ideas within both theories. *International Journal of Science Education*, 22(3): 225-238.
- NATIONAL RESEARCH COUNCIL. 2011. *A Framework for K-12 Science Education: Practices, Crosscutting Concepts, and Core Ideas*. Washington D.C., U.S.A. (prepublication copy).
- RICHARDSON, R.C. 1984. Biology and Ideology: The interpenetration of science and values. *Philosophy of Science*, 51(3): 396-420.
- RODRIGUEZ, A.J. 1998. Strategies for counterresistance: Toward sociotransformative constructivism and learning to teach science for diversity and for understanding. *Journal of Research in Science Teaching*, 35(6): 589-622.
- SHIELDS, M. 2006. *Biology Inquiries: Standards-based labs, assessments, and discussion lessons*. San Francisco, CA: Jossey-Bass Teacher, Wiley.
- STRAITS, W.J. 2007. A literature-circles approach to understanding science as a human endeavor. *Science Scope*, 31(2): 32-36.
- STRAITS, W.J., & R. WILKE. 2007. How constructivist are we? Representations of transmission and participatory models of instruction in the "Journal of College Science Teaching". *Journal of College Science Teaching*, 36(7): 58-61.
- YAGER, R.E. 2000. The constructivist learning model. *Science Teacher*, 67(1): 44.
- ZULL, J.E. 2002. *The art of changing the brain: Enriching the practice of teaching by exploring the biology of learning*. Sterling, VA: Stylus Publishing.

# Bringing History and Philosophy of Biology into the Lab

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**Abstract:** This project brings the historical and philosophical study of science into the lab. We explore the possibility of studying scientific methods and objects of investigation using philosophical and historical analysis rather than framed in their usual research in terms of hypothesis testing, data collection and scientific analyses. Focusing on the classification of the water flea, we began by studying the descriptive writings and illustrations based on the use of the compound microscopic studies of the early naturalists, Jan Swammerdam and Jacob Christian Schäffer. We then applied these now-discarded classification schemas, techniques, and methods to the study of and classification of samples of locally collected microplankton from our university's ponds. Next, we used current classificatory methods of investigation to key-out these specimens. We then considered the different methodologies of observation and classification and discussed the suitability of these practices for classifying highly phenotypically plastic and strongly endemic clonal species. Lastly, we used these investigations to revisit perennial problems surrounding the understanding of the concept and category of species and the possible impact our investigations of the early study of the water flea may have on these.

**Key words:** philosophy of science in practice, species problem, *Daphnia*, Jan Swammerdam, Hasok Chang, complementary science, undergraduate research

## INTRODUCTION

Philosophers of science frequently discuss the nuanced set of problems surrounding the definition and conceptualization of the basal unit of biological classification. These discussions often focus on the problems concerning the concept and category of *species*—widely referred to in both biological and philosophical writings as the “species problem” (Claridge, Dawah, & Wilson, 1997; Wilson, 1999; Richards, 2010<sup>1</sup>). While exploring these ongoing debates within philosophical research is no doubt a valuable enterprise, my intent is to explore a viable approach to undergraduate research that would fully engage biology and philosophy students so that they could examine both theoretical and conceptual problems and investigate the practical impacts of these.

### Motivation for the project:

Frequently philosophy is understood as a discipline that asks general and abstract questions in contrast to the specific and concrete questions of specialist science (Chang, 2009). Specialist science has a narrow focus by design. Certain assumptions must be made allowing scientists to get on with their research. As a consequence, some avenues of investigation are left unexplored. Although they could investigate these avenues if they wanted, there are good reasons why specialist scientists do not address all interesting questions. These questions may not be considered appropriate for specialist

science investigation, other research is considered more pertinent, other questions may be more likely to be solved, or time and resources may be limited (Chang, 2009). These questions are not neglected because they are uninteresting. They are neglected so that specialists can get on with the project of *doing science* (Chang, 2009).

Hasok Chang suggests that despite this common view that philosophy's focus is generalities; philosophy needn't always ask general questions. Philosophy can also ask specific questions. In fact, it can aim to investigate those questions neglected by specialists. This mode of philosophical investigation Chang calls “complementary science” (Chang, 2004; 2009; see also 2011). Complementary science is the role that philosophy can take in investigating some of the questions/avenues not explored in the specialist science. In doing so, philosophical inquiry can augment our knowledge of the world and complement the knowledge acquisition of specialist science (Chang, 2009).

In pursuit of a different path of philosophical investigation suitable for undergraduate students in philosophy and biology, I began thinking about how to adopt Chang's novel suggestion. Keen to construct a project appropriate for undergraduate students that enabled them to explore philosophical problems in biological classification, I began speaking to Melissa Daggett, a member of the biology department, about the possibility of interdisciplinary projects.

Daggett and I developed separate but related sister projects. My interest was to develop a project

<sup>1</sup> For criticisms of Richards' approach see Kendig, 2012.

that facilitated the kind of philosophical thinking about conceptual problems of classification that challenged students and made use of both their lab skills and philosophical skills of analysis by exploring the role of philosopher of science as complementary scientist.

This project provided a conceptual space for students to actively use knowledge and skills from disparate disciplines and enabled them to utilize these in solving conceptual problems in history, philosophy and biology—both in the library and in the lab.<sup>2</sup>

### The current state of *Daphnia* research

A wealth of *Daphnia* research spanning 342 years has produced a substantial store of knowledge concerning its phenotypic plasticity, predator-induced behavior, diel vertical migration, various aspects of its ecology and physiology, as well as its ability to reorganize its life cycle stages (Lampert, 2011). However, the diverse phenotypic, behavioral, life history adaptations and the strong endemism of the clonal species of the genus *Daphnia* also introduce particular challenges to its species classification.

Although genomic data has increased the knowledge of these organisms and their possible relatedness, the sequencing of the *D. magna* and *D. pulex* genomes has proved to be only partially useful and has not (as originally hoped) succeeded in fully resolving the phylogeny and classification of many species. Even using new genomic data, many species classifications of the genera remain unresolved (Edwards, 1980; Schwenk, Ender & Streit 1995; Kotov & Taylor, 2010). Despite frequent use of *D. pulex* and *D. magna* as common research organisms in classrooms around the world and by intensive research by *Daphnia* specialists, the taxonomic diversity of the genus *Daphnia* and the family Daphniidae is not widely agreed upon even within specialist science. Korovchinsky (1997) cites estimates that range from 129-146. The classification of many species of the genus *Daphnia* and the family Daphniidae remains unresolved.

### Recovery of lost ideas and classifications

What initially made *Daphnia* exciting research subjects for our project was their long (but forgotten) history of unruly and unresolved taxonomy. We used the history of water flea classification as a tool to investigate forgotten scientific techniques and to explore possible reasons for its apparent species liminality.

*Daphnia* have been and continue to be extensively studied by scientists, students, and naturalists alike. Although it continues to be used as a popular research organism in ecotoxicology studies and with undergraduate students, the long history of

*Daphnia* research and classification is, at best, sparse. Swammerdam's contributions are occasionally mentioned but rarely discussed even in limnology and ecotoxicology textbooks. Photographs of the differing head shapes of *Daphnia* are popular exemplars used in textbooks on developmental biology but tend to be unaccompanied by any extended discussion. *Daphnia* are only given a brief mention in Mary Jane West-Eberhard's 749-page tome, *Developmental Plasticity and Evolution* (West-Eberhard, 2003).

In one of the longest and most referenced recent historical reviews on *Daphnia* classification, there is a mere two paragraphs detailing the contribution of naturalists from 1669-1763 (Korovchinsky, 1997). In his new 250-page volume *Daphnia: Development of a Model Organism in Ecology and Evolution*, Winfried Lampert, 2011, takes a disappointingly short paragraph to discuss this same time period in a section entitled "the long history of a model organism." Over the course of our research we found this abridgement of history very common with very few exceptions (notably Fryer, 2008).

Research into the history of *Daphnia* classification proved to be a challenge. We found some secondary texts in addition to much current research, and some historical reviews. However, the primary texts were not widely available—namely Swammerdam's *Historia insectorum generalis / Algemeene verhandeling van de bloedeloose diertkens* (an English translation was published posthumously in 1758).

An exhaustive search resulted in finding this rare volume. We studied the first published illustrations and descriptions of water fleas included in Swammerdam's work from 1669. Swammerdam drew his own illustrations in the original 1669 work. Although we utilized the English translation for the text, we relied on Swammerdam's original illustrations for our later study. We noted slight but distinct differences in the illustrations posthumously published in the English edition in 1758. The



Fig. 1. Swammerdam's original 1669 illustrations. Permission for this and all other reprinted materials obtained from the Linda Hall Library of Science, Engineering & Technology.

<sup>2</sup> Two undergraduate students, one majoring in chemistry (Austin Anderson), and the other in philosophy (Joshua Swindler) collaborated on research for this project. Both had taken courses in biology and in philosophy.

publisher did not include Swammerdam's original drawings but instead included those drawn by an illustrator. These modified Swammerdam's original illustrations making them arguably less accurate and more stylized (see also Fryer 2008).

Swammerdam made liberal use of analogy in his descriptions of the behavioral and morphological characteristics of the water flea, frequently referring to the comparative structures of known organisms and machines. Describing the movement of his live subject, he reports:

"...it is continually seen to jump in the water,...like the whirling or turning about of that kind of pigeons [sic]...or may be compared to the turning of a wheel about the axle-tree of a chariot" (Swammerdam, 1758).

He describes the tree-like morphological structure of the upper appendages as such:

"At the first and second joint, reckoning from the single trunk, there arise on each side a little branch...; and at the third or extreme point, three such buds or shootings are placed, which also seen to be again divided into other joints" (Swammerdam, 1758).

This observed structural analogy lead him to dub them "Arborescent Fleas" (Swammerdam, 1758).

After Swammerdam's initial illustrations, it was



**Fig 2.** Close up of Swammerdam's 1669 illustration, C.

not until Schäffer's monograph *Die grünen Armpolypen* (1755/1763) that another detailed study of the water flea was published. It was actually Schäffer who first referred to what we now know as *Daphnia* by its colloquial name, "die geschwänzten zackigen Wasserflöh" (the tailed, branched water flea). Using a compound microscope, Schäffer's detailed descriptions and illustrations of water flea microanatomy, function of the parts, locomotion, and behaviors improved upon Swammerdam's original investigations. Schäffer most likely used a Culpeper type microscope (Fryer, 2008).

Illustrations in Schäffer's *Die grünen Armpolypen*<sup>3</sup> were much more numerous, in color and included a high level of detail of many of the smaller characteristics of the water flea. Among these, he depicts a different kind of water flea which

<sup>3</sup> Schäffer's illustrations were drawn by a professional artist which was common practice at the time (Fryer, 2008).



**Fig. 3.** Table one from Schäffer's 1755.

he names "ungeschwänzten zackigen Wasserflöh" due to the lack of tail spine (this is most likely a water flea of the family Daphniidae, *Simocephalus vetulus*) (Fryer, 2008; Ashworth, 2009).

After Swammerdam's *Historia insectorum generalis* and Schäffer's *Die grünen Armpolypen*, many naturalists did not continue to use microscopic investigations as the basis of their illustrations but instead simply used Swammerdam's and Schäffer's illustrations as reference guides, often relying on re-drawing Swammerdam's original illustrations (Fryer, 2008).

We hoped that critical re-examination of the work of the early naturalists' depictions and analogical descriptions of the behavior of the water flea and their repeated attempts to resolve various species complexes might result in an augmented understanding of the process of classifying phenotypically plastic clonal species. To test this hypothesis we needed to go into the lab and use this recovered knowledge to investigate and attempt to identify the species of a number of water fleas.



**Fig 4.** Close up of Schäffer's Figs. IV & VI.

## IMPLEMENTATION

After studying the early naturalists' writings and illustrations we were interested in staying as true to the kinds of collection techniques and procedures of these early naturalists as possible. We opted to investigate our own local microfauna—the water

fleas present in two of our university campus ponds—instead of ordering stocks of *D. pulex* or *D. magna*. We collected four samples—one from the top and one from the bottom of each pond.

In the lab we isolated 3 individuals from each sample at random. Each flea was named and labeled with its collection data. In our initial observations we utilized the discarded historical techniques of microscopic investigation, analogical descriptions, and detailed illustrations recovered from our research of Swammerdam and Schäffer. Despite research into their use and operation, we had no access to Culpepper type microscopes. We instead used low magnification dissecting microscopes set from 7x to 35x to observe and draw the live water fleas. The aim was not to attempt to replicate the historical investigations but to best approximate the techniques and methods of description whenever possible.

In each sample, the following questions were addressed: Is this the same kind of organism that is described by Swammerdam or Schäffer? How is this judgment made? What characteristics/ behaviors are used in making this decision? What is my level of confidence that these are the same (or different) kinds of organisms?

To answer these questions, we used Swammerdam and Schäffer's illustrations and analogical descriptions as our field guide. Following the analogical style of Swammerdam, we described what we saw in terms of the structures our subjects had in common with known organismal and mechanical parts and processes. Each organism was then identified as either being similar to or dissimilar to those individuals described and illustrated by Swammerdam and Schäffer. A representative sample of analogical descriptions recorded in the students' notebooks follows:

*"The arm of J-3 is very thick and powerful with a claw that is only partially hooked. The claw is shaped like a cat's claw, curved but not a severe hook"...*  
*"Both the back and front edges of J-3's carapace are serrated including the tail spine, which is long and narrow. The serrations are similar to the serrations you would see on a hand saw"* (Swindler, 2011).

*"Viewing the specimen (A-4) from the ventral side it appeared as if there were many internal structures running the length of the organism that were pulsating in a rhythmic fashion. It was seen that the foot would occasionally kick. It also appeared as if the organism was encased in a structure that appeared shell like"...* *"The head of the organism did not appear like a bird but more elongated and flat near the bottom. The head and the eye structure appeared like a miners helmet with a light affixed to the front of it. The dorsal section of the body did not run smooth form the rostral to the caudal end but instead was indented in on itself near the back of the head"* (Anderson, 2011).



Fig. 5. *Daphnia* collection by one of the students.

After completing these initial observations, relying on recovered illustrative and descriptive techniques, we then used up-to-date classificatory theories, methodology, techniques, and equipment. We monitored the water fleas for morphology and behavior using a stereomicroscope (Leica M165 FC). We captured pictures using a QI imaging camera and Q capture Pro 6.0 imaging software. Our investigations produced a data set of 15 drawings, 60 photographs, and detailed descriptions based on 12 specimens from diverse ecologies (top and bottom of the 2 different university ponds). We keyed-out each of our 12 specimens by combining the morphological methods and recording techniques recovered from Swammerdam and Schäffer and the morphological observations and recorded images using the stereomicroscope and QI imaging camera. Using these data, we identified the genus and species of the water fleas using a current image-based key to zooplankton (Haney et al., 2010). We found that we had 3 *Daphnia rosea*, 3 *Simocephalus serrulatus*, 1 *Daphnia schødleri*, 2 *Daphnia catawba*, 1 *Daphnia ambigua* (?), 1 *Simocephalus vetulus*, and 1 unknown.

### Philosophical discussions of classification and the history of scientific practice

Investigating forgotten historical techniques of observation and lost classificatory names like those of Swammerdam and Schäffer highlights the problems associated with classification that are absent from the majority of university biology textbooks. The recovery of these historical observations by students themselves introduces them to these conceptual and operational difficulties in a very direct way.

Many classic experiments used to instruct and inform students of the importance and purpose of biological classification often make assumptions about the methods and techniques that must be used. This project attempted to examine these parts of the process of scientific knowledge acquisition thought to be beyond criticism—those methods and techniques of observation (e.g. such as those stated in lab protocols) through the use of alternative methods

of observation and recording recovered from the early naturalists.

We found that current protocols in *Daphnia* research/classification suggest that microscopic investigations are used or complemented with photos but not illustrations. These protocols also suggest the use of preserved dead specimens (Standard Operating Procedure for Zooplankton Sample Collection and Preservation and Secchi Depth Measurement Field Procedures, 2005 and Balcer et al., 2009/2011).

Through our active investigation of the recovered methods and discarded techniques used by the early naturalists, Swammerdam and Schäffer, we found that these recovered methods may provide additional knowledge relevant to the study of *Daphnia* morphology that may help resolve their classification.

In the process of creating visual representations of live specimens, we found that the practice of illustration utilized by Swammerdam in particular, led us to identify crucial morphological characteristics among our own illustrations. Upon reflection, we realized this was due at least in part to the concentrated attention required to produce accurate illustrations. For each illustration, it was necessary to observe the live specimen under low magnification for an average of 90 minutes. We found that these techniques increased our ability to make fine-grained discriminations necessary for morphological classification. But we also found drawing from life improved our ability to identify certain behaviors and characteristics that helped us better identify morphological features in our updated observations. Knowledge gained through the close study required to produce faithful representations of the specimens in our illustrations informed our decisions as to which features we focused on when using the stereomicroscope and capturing images. In the process of drawing the water fleas we became aware of the differences in body shape, size, and composition and were then better able to identify the characteristics that changed from individual to individual.

Reflecting on our use of both recovered and

updated techniques, we concluded that while high magnification photos provide much more detail, and often more accurate representation of an organism than a free-hand drawing, the *process* of drawing out the individuals observed reveals a lot about their morphology that goes otherwise unnoticed. Much of the knowledge gained in attempting to produce accurate representations came from close attention to the function and movement of certain structures.

Although it was sometimes difficult to draw and photograph them, observing the movement and functioning of live specimens provided more information about their morphology and structure than of dead ones. Information about how the various parts of the organism fit together allowed for a more accurate rendering of the specimen under investigation by revealing the modularity of the parts of the organism as well as the integration and organization of these parts within the whole organism. We also found that, once Daphnids die, they change color and muscle disintegration occurs which compromises one's ability to make finer discriminations between similar specimens. This leads us to suggest that some photographic evidence used for image-based morphological classification may be compromised.

Through our investigations as "complementary scientists" using discarded techniques and descriptions from the early naturalists, we found that some of these reclaimed practices and processes may still be useful. We modestly suggest that these reclaimed practices and processes of classification should not in fact have been discarded and may perhaps be a useful augmentation to current *Daphnia* research and classification.

Throughout the project, the students and I discussed the philosophical implications of the techniques and practices used in the early naturalists' research and in current research. We then examined how these have shaped (and continue to shape) the classification of water fleas. In particular, we considered how these have affected the conception(s) of species used, the problematic classification of clonal water fleas, and why the classification of



Fig. 6. A selection of our illustrations next to the stereoscopic images taken of organisms observed.

species is important.

Knowing what kind of living being an organism is greatly informs what other information we can infer about it, e.g. knowing what kind something is means we know what inferences we can make about it and what generalizations apply to it as a member of that kind. Understanding if it is of one kind rather than another requires close and careful comparative observations of behavior and morphology.

In our preparatory research we found that the genome of *D. pulex* was already sequenced (Colbourne et al., 2005) and the *D. magna* genome sequencing project is now in its fourth year, so far compiling a beta gene set (Colbourne, 2011). Although genomic data has increased our knowledge of these organisms and their possible relatedness with other sequenced model organisms, the sequencing of the *D. magna* and *D. pulex* genomes has not (as originally had been hoped) succeeded in fully resolving the phylogeny and classification of many species. Because of this, morphology is still widely accepted as a key feature used in microcrustacea taxonomy despite their heritable polymorphisms, geographic variability, and phenotypic plasticity (Dodson and Lee, 2006). Although this reliance on morphology may appear to be an odd typological throwback in the age of phylogenomics, it is one that has been gathering support over the last decade.

While many mainstream biology textbooks (e.g. Futuyma, 2006) continue to characterize evolution as changes in the gene frequency of populations, opponents of this exclusively gene-centered view are becoming more numerous.

The ecological endemism and phenotypic plasticity of *Daphnia* made it a particularly pedagogically useful model organism for this interdisciplinary lab project. Observational research and the keying-out of water fleas furnished unique learning opportunities for the students to consider and use early pre-Linnaean morphological notions of species classification as well as investigate the history and philosophy of classification of the water flea. Students from diverse academic disciplines worked alongside each other. Interaction was frequent and fecund. Each day, students shared knowledge from their own disciplinary skill sets that they believed was pertinent to the project. Through this exchange, each was able to benefit from a robust multi-disciplinary understanding. Each was able to collect and record data using a variety of techniques, each was able to philosophically study and reflect on the techniques used to represent and classify the *Daphnia*, and each was able to critically discuss how these intervene on the conceptions of species used.

## FUTURE PROJECTS

We offer this project summary as an example of how active collaborative research activities for students and faculty may bring together a variety of

disciplines less frequently considered for interdisciplinary research. The project described in the present paper focused exclusively on morphological and behavioral features of the water flea. This focus made it an accessible project for both science and non-science majors. Further interdisciplinary projects relying on different foci can be modeled on the approach discussed herein. For example, related projects could combine early cellular or molecular techniques with those currently in use. These may be especially pedagogically useful for students studying developmental biology, embryology, and genetics and the history and philosophy of these.

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## REFERENCES

- ANDERSON, J. 2011. Summer Research Institute (SRI) project notebook. Classification and Complementary Science. *Missouri Western State University: Saint Joseph*.
- ASHWORTH, W., JR. 2009 The Grandeur of Life. Linda Hall Library of Science, Engineering, and Technology: Kansas City. [printed exhibition catalog].
- BALCER, M., MAYS, N., CANGELOSI, A. 2009/2011. Standard Operating Procedure for Zooplankton Sample Analysis. Great Ships Initiative. Available at <http://www.nemw.org/GSI/GSI-SOP-LB-RA-SA-2.pdf>
- CHANG, H. 2004. *Inventing Temperature: Measurement and Scientific Progress*. Oxford University Press New York.

- CHANG, H. 2009. Philosophy as complementary science. *The Philosophers' Magazine* 40. Accessed from <http://www.philosophypress.co.uk/?p=375> on 31 Oct 2010.
- CHANG, H. 2011. How historical experiments can improve scientific knowledge and science education: The cases of boiling water and electrochemistry. *Science & Education* 20: 317-341.
- CLARIDGE, M., DAWAH, H., WILSON, M. 1997. *Species: The Units of Biodiversity*. Chapman and Hall: London.
- COLBOURNE, J., SINGAN, V., AND D. GILBERT. 2005. wFleaBase: the Daphnia genome database. *BMC Bioinformatics* 6:45.
- COLBOURNE, J. 2011. Daphnia magna genome sequencing project. The Center for genomics and bioinformatics collaboration wiki. <https://wiki.cgb.indiana.edu/display/grp/Daphnia+magna+Genome> Accessed May 1, 2011.
- DODSON, S., LEE, C. 2006. Recommendations for taxonomic submissions to *Hydrobiologia*. *Hydrobiologia* 556: 1-5.
- EDWARDS, C. 1980. The anatomy of Daphnia mandibles. *Transactions of the American Microscopical Society* 99(1):2-24.
- FRYER, G. 2008. Jacob Christian Schäffer FRS, a versatile eighteenth-century naturalist, and his remarkable pioneering researches on microscopic crustaceans. *Notes and Records of the Royal Society* 62: 167-185.
- FUTUYMA, D. 2006. *Evolutionary biology*. Massachusetts: Sinauer.
- HANEY, J. et al. 2010. *An-image-based Key to the Zooplankton of the Northeast, USA. version 4.0. University of New Hampshire Center for Freshwater Biology. Accessed from <http://cfb.unh.edu/cfbkey/html/index.html> April-August 2011.*
- KENDIG, C. 2012. Review of The Species Problem by Richard Richards. *The Philosophical Quarterly* 62:2, 405-408.
- KOROVCHINSKY, N. 1997. On the history of studies on Cladoceran taxonomy and morphology, with emphasis on early work and causes of insufficient knowledge of the diversity of the group. *Hydrobiologia* 360: 1-11.
- KOTOV, A., TAYLOR, D. 2010. A new African lineage of the Daphnia obtuse group (Cladocera: Daphniidae) disrupts continental vicariance patterns. *Journal of Plankton Research* 32(6):937-949.
- LAMPERT, W. 2011. *Daphnia: Development of a Model Organism in Ecology and Evolution*. International Ecology Institute: Oldendorf/Luhe Germany.
- RICHARDS, R. 2010. *The Species Problem: A Philosophical Analysis*. Cambridge University Press: Cambridge.
- SCHÄFFER, J. 1755/1763. Die grünen Armpolypen, die geschwänzten u. ungeschwänzten zackigen Wasserflöhe, und eine besondere Art kleiner Wasseraale.
- SCHWENK, K., ENDER, A., STREIT, B. 1995. What can molecular markers tell us about the evolutionary history of Daphnia species complexes? *Hydrobiologia* 307: 1-7.
- STANDARD OPERATING PROCEDURE FOR ZOOPLANKTON SAMPLE COLLECTION AND PRESERVATION AND SECCHI DEPTH MEASUREMENT FIELD PROCEDURES 2005. Revision 10. EPA. Available at [http://www.epa.gov/greatlakes/monitoring/sop/chapter\\_4/LG402.pdf](http://www.epa.gov/greatlakes/monitoring/sop/chapter_4/LG402.pdf)
- SWAMMERDAM, J. 1669. *Historia insectorum generalis* or *Algemeene verhandeling van de bloedeloose dierkens*. t'Utrecht: Meinardus van Dreunen, ordinaris Drucker van d'Academie.
- SWAMMERDAM, J. 1758. *The Book of Nature*. C.G. Seyffert: London.
- SWINDLER, J. 2011. Summer Research Institute (SRI) project notebook. Classification and Complementary Science. Missouri Western State University: Saint Joseph.
- WEST-EBERHARD, M. 2003. *Developmental Plasticity and Evolution*. Oxford University Press: New York.
- WILSON, R. 1999. *Species: New Interdisciplinary essays*. MIT Press: Cambridge.

# A Role for History and Philosophy of Biology in Exploring New Questions in Biology

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**Abstract:** A number of current reports are challenging educators of undergraduate biology students to increase the role, interactions and approaches of other disciplines. The goal stated in these reports is to produce a college graduate with the skills and competencies to solve pressing global problems such as producing ample food, fuels, and making health care available, while also preventing the degradation of the Earth's renewable resources. This perspectives essay presents the inspiration and motivation that resulted from the interactions and collaborations with faculty in philosophy. My goal is to describe how these interactions occurred and how it has helped to inform and guide me in the development and implementation of new biological questions that can be addressed by undergraduate researchers. It has also allowed reflection on how scholarly endeavors in biology and philosophy can complement each other.

**Key words:** interdisciplinary, undergraduate research, history and philosophy of Biology, *Daphnia*

## INTRODUCTION

Recent reports have highlighted the importance of interdisciplinary and interconnected approaches with an increased focus on competencies in educating the next generation of biologists (American Association for the Advancement of Science, 2011; National Research Council, 2003 and 2009; Woodin, 2010; Labov, 2010). These reports also emphasize the interrelatedness of science, technology, engineering, and mathematics (STEM) courses as being critical for producing a college graduate with the ability to solve pressing global problems such as producing ample food, fuels, and making health care available, while also preventing the degradation of the Earth's renewable resources (NRC, 2009).

This increasing recognition of and emphasis on facilitating and promoting innovative collaborations between institutional academic departments, referred to as interdisciplinary collaboration, often implies expanding on and promoting the interrelatedness of questions and problems within the STEM disciplines. An appreciation and broader expansion of interdisciplinary collaborations into non-STEM fields should be supported and emphasized as well. Greater inclusion of non-STEM disciplines such as philosophy, history and literature, offers opportunities and insight into the exploration of even more questions and approaches. In addition, the influx of a wider range of novel ideas and approaches can encourage and enhance creative and critical thinking in ways that are applicable to solving problems that have the potential for highly advantageous societal impact.

This perspectives essay presents the inspirations and motivations I gained through interacting and collaborating with faculty in philosophy. These interactions have resulted in the initiation and

development of a research project based on questions in biology that I was peripherally aware existed. In addition, the questions formulated and their natures lend themselves to the types of projects approachable to undergraduates. My hope is that through communicating an example of how my interactions and collaboration with faculty in a non-STEM discipline such as philosophy have enhanced ideas and opportunities for developing new undergraduate research projects, such interactions can be sought and applied by faculty at other institutions.

## Initial Inspiration

During the spring of 2010, Missouri Western State University had begun a search to hire a new faculty member specializing in philosophy of biology. This individual would reside in the Department of History, Philosophy and Geography (currently the Department of Philosophy and Religion) and would be expected to actively interact and collaborate with faculty in the Department of Biology. In this situation, the promotion of interdisciplinarity began through a general invitation to biology faculty members to serve on the search committee for hiring this faculty member in a department outside of biology. This was a genuine committee appointment, not a mere token representative. The biology faculty participated in the candidate interviews, attended their seminars, and provided legitimate input regarding which candidate to hire.

This was a beneficial interdisciplinary experience from my perspective, in that it was during one such candidate's seminar that I found myself reevaluating my understanding of the biological species concept. Prior to this presentation, I had taken for granted that there was one broadly accepted species concept (Mayr, 1999). In most of the courses

I teach, I spend the most time at the cell and molecular level of the biological hierarchy and my assumption was that biologists had a fairly uniformly accepted definition of a species, especially in the era of genome sequencing. Catherine Kendig's talk revealed that the species concept is a major area of discourse with over 27 competing definitions of a species (Claridge et al., 1997; Wilson, 1999; Wilkins, 2009).

## IMPLEMENTATION

### The *Daphnia* Project

After Kendig was appointed to the faculty position in Philosophy, we engaged in subsequent discussions of possible research projects that could be done during the following summer to highlight and examine the species concept in collaboration with our students, as well as for our own professional understanding and scholarship. Kendig and I discussed the use of a well-studied organism, *Daphnia*, by developing an approach to the taxonomic problem that could complement the methods traditionally used in a cell and molecular laboratory. Throughout the academic year, we collected and read references and practiced culturing *Daphnia*. I continued to learn how philosophers of biology looked at scientific questions and Kendig learned the details of properly performing procedures in the biology laboratory, including bringing her cross-listed class, BIO/CHE/PHL 308 History and Philosophy of the Natural Sciences into the lab to "do science" several times.

Our first approach was to establish clonal populations of laboratory *Daphnia* and see if we could expose them to different kairomones, the chemical cues released by predators that induce beneficial changes in daphnid phenotype. We then considered having students observe for changes in phenotype following which they could then question how changes in morphology without a change in genomes could influence their interpretation of species identification (Tollrian and Harvel, 1999, Tollrian and Leese, 2010, Hanazato and Dodson, 1995).

Following multiple somewhat disappointing attempts, we developed an alternative plan that turned out to be what ecologists refer to as a common garden experiment (Mettelbach et al., 1999; Thorpe et al, 2005). We would collect *Daphnia* from one or more of the nine ponds we have on campus; key out the *Daphnia* morphologically using available taxonomic keys; and then attempt to culture clonal populations over the summer in a common controlled media and see if they would morphologically change back into some preexisting form. Learning the methods to collect and culture the *Daphnia* and to determine the chemical structure and mechanisms of kairomone action relied significantly on the more traditional form of interdisciplinary STEM

collaborations, in that we invoked the help of an organismal biologist in culturing the *Daphnia* and organic chemists in determining which molecular structures and chemicals could potentially induce phenotypic change in our *Daphnia*.

While Kendig and I viewed the *Daphnia* project as a complementary interdisciplinary project, we recruited undergraduates to participate in two distinct undergraduate summer research teams. The biology team would question the identification of *Daphnia* species by focusing on techniques and procedures traditionally used in a cell and molecular laboratory, while the history and philosophy of biology team would pursue the question using more interdisciplinary methods as described in Kendig et al.(this issue). This sister team approach demonstrated and reinforced our view that interdisciplinary collaborations do not have to completely merge disciplines in order for the faculty and student participants to benefit from exchanging ideas and pursuing answers to questions specific to their own discipline. Both teams received institutional funding in terms of salaries, tuition, and supplies in order to carry out their projects.

Kendig et al. provide a concise description and review of the use of *Daphnia* in proposing new approaches to answering questions associated with phylogenetic classifications as carried out by the history and philosophy of biology team. Their recovered methods of observing living organisms supplemented the biology team project in which we applied current specialist science methods for analyzing the *Daphnia* proteome by combining a common garden experimental approach with the cell and molecular techniques of isolating and analyzing nucleic acids and proteins (Frohlich, 2009; Tautz, 2011; Colbourne et al. 2011).

### The Biology Team

The biology team consisted of three undergraduates, all seniors, graduating with degrees in biology the following fall semester. The students were provided with a one page set of instructions at the beginning of the summer (Appendix 1). The requirements were simple and concise in order to increase focus and yet provide flexibility. The specific aims of the biology team were: 1. to collect, identify, and photograph representative *Daphnia* from local aquatic habitats, 2. to develop standardized culturing methods for maintaining isolates in the laboratory, and 3. to develop and perform various cell and molecular techniques to characterize each species. These aims were designed to complement the methods and techniques being applied by the history and philosophy of biology team.

The students on the biology team were able to complete all three specific aims and were able to present a poster of their results at a University-wide undergraduate research symposium (data not shown).

Procedures for using new equipment were developed and student-tested including one for a microtiter plate protein assay that was used as a lab practical for the introductory BIO 106 Principles of Cell Biology students. Another procedure developed and modified for student use was a method for collecting protein samples from individual *Daphnia* that could be used in monitoring changes in the *Daphnia* proteome through SDS-polyacrylamide gel electrophoresis (Frohlich et al. 2009). The new student-tested procedures are currently being used in an assessment of student gains in laboratory skills in an introductory cell biology course (unpublished results).

Many published reports point out that undergraduate research experiences be relevant and accessible to a broad array of students and that they be based on scientific evidence for how people learn (National Research Council, 2000). Projects should also include interdisciplinary and interconnected approaches. While both the biology and the history and philosophy of biology research projects were carried out alongside each other in the same lab space, my interest and focus was mostly on the complementary role the biology project had on the history and philosophy of biology project. My students engaged in an authentic undergraduate research experience. They also benefited from the interconnected approaches provided by the two different disciplines, while increasing their competency in performing specific laboratory skills. In addition, I believe the experience I had in working with a faculty member outside my own specific discipline has given me the awareness and opportunities to explore new questions that I may not have otherwise thought to ask.

In conclusion, this perspective described the inspiration, motivation and the undergraduate research projects that resulted from an interdisciplinary collaboration between faculty from two different disciplines one in biology, a STEM discipline and one in philosophy, outside of STEM. I would like to encourage faculty at other undergraduate institutions to explore possible opportunities to strengthen the interdisciplinary connectedness with non-STEM courses and to consider the benefits such interactions have on us, the faculty, and on the students we educate. A greater emphasis on interdisciplinary approaches will continue to be critical for helping our students become able to solve the pressing global problems facing us today and in the future.

#### ACKNOWLEDGEMENTS

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#### REFERENCES

- AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE. 2011. Vision and Change in Undergraduate Biology Education: A Call to Action. AAC&U, Washington, DC (Accessed from <http://visionandchange.org/finalreport.pdf>. on 30 December 2011.)
- CLARIDGE, M., DAWAH, H., WILSON, M. 1997. *Species: The Units of Biodiversity*. Chapman and Hall: London.
- COLBOURNE, J. K., PFRENDER, M. E., GILBERT D, THOMAS WK, TUCKER A, et al. 2011. The ecoresponsive genome of *Daphnia pulex*. *Science*. 331: 555-61.
- EBERT, D. 2005. Ecology, Epidemiology, and Evolution of Parasitism in *Daphnia* [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); Chapter 2, Introduction to *Daphnia* Biology. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK2042/>
- FRÖHLICH, T., ARNOLD, G.J., FRITSCH, R., MAYR, T., AND C. LAFORSCH. 2009. LC-MS/MS-based proteome profiling in *Daphnia pulex* and *Daphnia longicephala*: the *Daphnia pulex* genome database as a key for high throughput proteomics in *Daphnia*. *BMC Genomics*. 10: 171.
- HANAZATO, T., AND S. I. DODSON. 1995. Synergistic Effects of Low Oxygen Concentration, Predator Kairomone, and a Pesticide on the Cladoceran *Daphnia pulex*. *Limnology and Oceanography*. 40: 700-709.
- HANEY, J.F. et al. 2010. An-image-based key to the zooplankton of the northeast, USA version 4.0. University of New Hampshire Center for Freshwater Biology. Accessed from <http://cfb.unh.edu/cfbkey/html/index.html> on 6 Jun 2011.
- KENDIG, C., SWINDLER, J.T., AND J. A. ANDERSON. 2012. Bringing History and Philosophy of Science of Biology into the Lab. (submitted to *Bioscene*).
- LABOV, J. B., REID, A. H., AND K. R. YAMAMOTO. 2010. Integrated biology and Undergraduate science education: a new biology education for the 21<sup>st</sup> Century? *CBE-Life Sciences Education*. 9: 10-16.

- LAMPERT, W. 2006. *Daphnia*: model herbivore, predator and prey. *Pol. J. Ecol.* 54: 607-620.
- MAYR, E. 1999. *Systematics and the Origin of Species from the viewpoint of a zoologist*. Harvard University Press.
- MITTELBACH, G.G., OSENBURG, C. W., AND P. C. WAINWRIGHT. 1999. Variation in feeding morphology between pumpkinseed populations: Phenotypic plasticity or evolution? *Evolutionary Ecology Research*. 1: 111–128.
- NATIONAL RESEARCH COUNCIL. 2000. How people learn: brain, mind experience, and school: Expanded edition. *Washington, DC: National Academies Press*.
- NATIONAL RESEARCH COUNCIL. 2003. BIO 2010: Transforming Undergraduate Education for Future Research Biologist. *Washington, DC: National Academies Press*.
- NATIONAL RESEARCH COUNCIL . 2009. A New Biology for the 21<sup>st</sup> Century: Ensuring the United States leads the Coming Biology revolution. *Washington, DC: National Academies Press*
- TAUTZ, D. 2011. Not just another genome. *BMC Biology*. 9: 8.
- THORPE, R. S., REARDON, J. T., AND A. MALHOTRA. 2005. Common garden and natural selection experiments support ecotypic differentiation in the Dominican anole (*Anolis oculatus*). *The American Naturalist*. 165: 495-504.
- TOLLRIAN, R., AND C. D. HARVELL. 1998. *The Ecology and Evolution of Inducible Defenses*. Princeton University Press. 395p.
- TOLLRIAN, R., AND F. LEESE. 2010. Ecological genomics: steps towards unraveling the genetic basis of inducible defenses in *Daphnia*. *BMC Biology*. 8: 51.
- WILKINS, J. S. 2009. *Species a history of the idea*. Berkeley, University of California Press.
- WILSON, R. 1999. *Species: New interdisciplinary essays*. MIT Press: Cambridge.
- WOODIN, T., CARTER, V., AND L. FLETCHER. 2010. Vision and Change in Biology Undergraduate Education, A Call for Action—Initial Responses. *CBE-Life Sciences Education* 9: 71-73

## APPENDIX

### Initial references to read and look over:

#### Basic Daphnia Biology

<http://www.ncbi.nlm.nih.gov/books/NBK2042/>

#### Identifying zooplankton:

An Image-Based Key To The Zooplankton Of The Northeast (USA)

<http://cfb.unh.edu/cfbkey/html/index.html>

#### Summary procedure (may vary slightly for each individual)

In the field:

- Pick a minimum of one body of water (on campus or elsewhere) in which to collect your daphnia sample.
- Collect a minimum of one water sample with daphnia using a plankton net.

In the lab:

- Set up 10 small petri dishes with daphnia water (see recipe below).
- Place one isolated daphnia/dish.
- Use the on-line key and other resources to learn the terminology associated with daphnia anatomy and to identify the Genus and species of the daphnia in your sample(s).
- Take pictures and make multiple drawings of your samples to document and support your identification.
- Continue to observe and document the life cycle and population growth of each of your daphnia "clones". Pay special attention to any changes in morphology throughout the summer.
- Learn and perform at least one specialized cell and molecular technique as your cultures grow and more individuals within a clone become available. Possibilities include the analysis of gene expression through PCR, SDS-PAGE, Western blotting and immunofluorescent microscopy.

## EDITORIAL

### Teaching and Coaching

*“The coach is first of all a teacher... I believe that next to parenting, teaching and coaching are the two most important professions in the world”.* John Wooden

*“All right, listen up! I'm Coach Boone. I'm gonna tell you about how much 'fun' you're gonna have this season... This is no democracy. It is a dictatorship. I am the law.”*

Coach Boone (played by Denzel Washington) in Remember the Titans

I'm a big sports fan. As a kid I played any number of sports-football, baseball, ice hockey, and, because I grew up in Indiana, basketball. Later in life, I would also play volleyball and soccer. Along the way, I had the opportunity to coach a number of these sports. The athletes that I coached ranged from ages 7 to 23.

Having been both a coach and an educator, I have often thought about the differences and similarities between the methods of instruction and motivation used in each. Of one thing I'm certain – I had much more control over my athletes than I've ever had over my students. As you can imagine, I had much more latitude in trying to get my athletes motivated than I have ever had in the classroom. Let me point out some examples.

One advantage that coaches have that instructors do not is that they can use punitive means in order to motivate their athletes. For example, if I felt my soccer players were not putting forth enough effort during practice, I would stop the practice and make them run “suicide drills,” where they would sprint between the goal line and each of the other lines on the soccer field. This was usually enough to regain their attention and make sure that they were focused during practice. Heaven help the instructor who, when they find students are not paying attention or being disruptive in class (texting and performing e-commerce on their laptops comes to mind), actually attempts to make their students attend to their classroom duties! Indeed, with the current atmosphere of consumerism in higher education, there are few behaviors short of complete classroom disruption that a student can be disciplined for.

Another significant difference that I have noticed between athletics and academics is that coaches can make demands of their athletes that involve supplementary effort well outside of their normally scheduled practices. This supplementary effort often involves weightlifting and/or other types of crosstraining activities that are expected of the athletes in addition to their normal practices. These sorts of supplementary activities are also expected to

continue in the “off-season.” For example, my son is a high school swimmer. During his high school swimming season, he is expected to participate in three-hour afternoon practices every day of the week. In addition, he attends two hour practices that begin at 5:15 AM three days a week. On top of this, there is a single three-hour practice on Saturdays. When he is not actively swimming during the high school season, he is expected to continue working out and, in fact, swims twice a day for a local AAU swim team. I have previously talked about the amount of effort that students admit to in studying and preparing for classes. It is going down. Can you imagine asking your students to attend an extra series of classes starting at, say, 6 AM Monday through Friday? Can you imagine expecting your students to devote even three hours a week to supplementary study outside of the semester? In my experience, it is extremely hard to just to get my students to come to my office hours, even intermittently. I recently performed an experiment with a group of senior students who were signed up to take my fall biochemistry course. I contacted each of them individually during the summer and arranged to provide them reading materials and asked if they would take some time to read them over the summer. I reasoned that, as seniors and (90%) preprofessional students, they would at least begin the readings. At the beginning of the semester, I asked each student if they had done any of the reading during the summer and, to a student, they admitted that they had not. If I were a coach, I might have dismissed them from the team...

Another interesting difference between coaches and instructors is that coaches can make demands of their athletes regarding their other time commitments. For example, many coaches have the power to determine what extracurricular activities, their high school and college athletes are allowed to pursue, in order to i) ensure that their athletes do not get injured playing other sports and, ii) ensure that their athletes will have enough time to devote to that particular sport and do well. As educators, we often have

students who have so many time commitments – work, families, and other activities – that they find it almost impossible to allot enough time for study outside of class. I have actually tried to persuade my students that they can only do so much and that their college education is an important investment that they should protect by allotting enough time in order to do well. I have done so largely to no avail – many of my students still work full-time while attempting to take on a full (12 or more contact hour) class load; many of these same students complain that my course contains too much material. In addition, I'm sure we all have experienced the reaction of a student when we recommend that they take a course that, while not required, might prepare them better for one of their requisite courses. Wind sprints, anyone?

Interestingly, I was never, ever asked by an athlete who I coached why we performed a certain drill, why they had to do wind sprints, or any other activity connected to the sport. For that matter, none of my players ever filled out an evaluation of my coaching, either. Of course, as we all know, the only indicator of a coach's quality is their win loss record!

There are obviously cultural differences that have led to the differences in which athletes and students view their commitments to sports and academics, respectively. How did this happen? I can honestly say that I do not know. However, we can get a glimpse of the way we view sports and academics. If we simply review and compare how sports and academics are portrayed in popular media. There are hundreds if not thousands of fictional books, television shows, and films that tend to glorify sports and the efforts that their participants put forth to excel in the sports. Films such as Rocky, Chariots of Fire, Rudy, Remember the Titans, and many, many other films come to mind. In each of these, one part of the film focuses on the travails of the athlete as they strive to excel in their chosen sport(s), appropriately so. I'm sure that we have all marveled at the dedication and athletic skills that elite athletes exhibit in their various sports. At the same time, I'm sure that we have all marveled at the abilities of the "rocket scientists" who successfully prepared and landed the Mars rover, Curiosity. I'm sure if you asked most people about the dedication and hard work of people in medical fields, and of those

performing basic research in science, that they would express the same level of admiration for these people. However, there is a serious disconnect between that admiration and the average person's understanding as to how that level of medical and/or scientific expertise is achieved. For whatever reason, the average person seems to think that only "smart" people can excel in the medical and scientific fields, and that this excellence ultimately results from similar levels of hard work and dedication as those displayed in athletics. Somehow, in our culture we perceive excellence in sports to be attainable through hard work and dedication, while excellence in an academic or professional field is perceived to be linked to one's IQ. This is also mirrored in popular media. The vast majority of feature films that have dealt with schools and/or academia have either been comedies (Fast Times at Ridgemont High, Ferris Bueller's Day Off) or have dealt with gang violence in the schools. I can probably count the number of feature films that celebrate the pursuit of excellence in academics on one hand. The only films that come to mind are Stand and Deliver starring James Edward Olmos and, perhaps, Conrack starring Jon Voight. Even the acclaimed film, Good Will Hunting, starring Matt Damon and Robin Williams focused on the protagonist's ability in mathematics as a "gift;" you never see Mr. Damon study in the movie. I can understand why this might be. A film about students studying for hours on end would probably be much less entertaining than watching someone do one-handed push-ups. However, those mental push-ups that we are doing when we study are good for us. They provide us with a foundation essential for a solid education. Somehow this needs to be communicated to our students.

How do we accomplish this? How do we get students to recognize that being successful in academics, rather than resulting from a combination of a magic gift of IQ and pointless drudgery, requires the same dedication and dogged determination (i.e., work) that is necessary to be successful in athletics? I wrestle with this quite a bit because I believe that, sooner or later, we must come to grips with the way in which we as a society view learning at every level of education. If you find out how, please let me know.

Happy Holidays to All,  
Jim Clack

# ***Bioscene*: Journal of College Biology Teaching**

## **Submission Guidelines**

### I. Submissions to *Bioscene*

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

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

### II. Preparation of Articles, Innovations and Perspectives

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

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

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

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

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

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

Single Author:

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

Two Authors:

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

Multiple Authors:

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

\*\*\*\*\*

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

(1) Articles-

(a) Single author:

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

(b) Multi-authored:

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

(2) Books-

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

(3) Book chapters-

GLASE, J.C., AND M. ZIMMERMAN. 1991. Population ecology: experiments with Protists. 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 ( $\geq 300$ dpi) individual electronic files, either TIFF or JPEG. Placement of figures should be indicated within the body of the manuscript. Figures include both graphs and images. All figures should be accompanied by a descriptive legend using the following format:

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

III. Letters to the Editor

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

IV. Other Submissions

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

V. Manuscript Submissions

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

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

## VI. Editorial Review and Acceptance

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

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

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

## VII. Revision Checklist

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

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

## VIII. Editorial Policy and Copyright

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