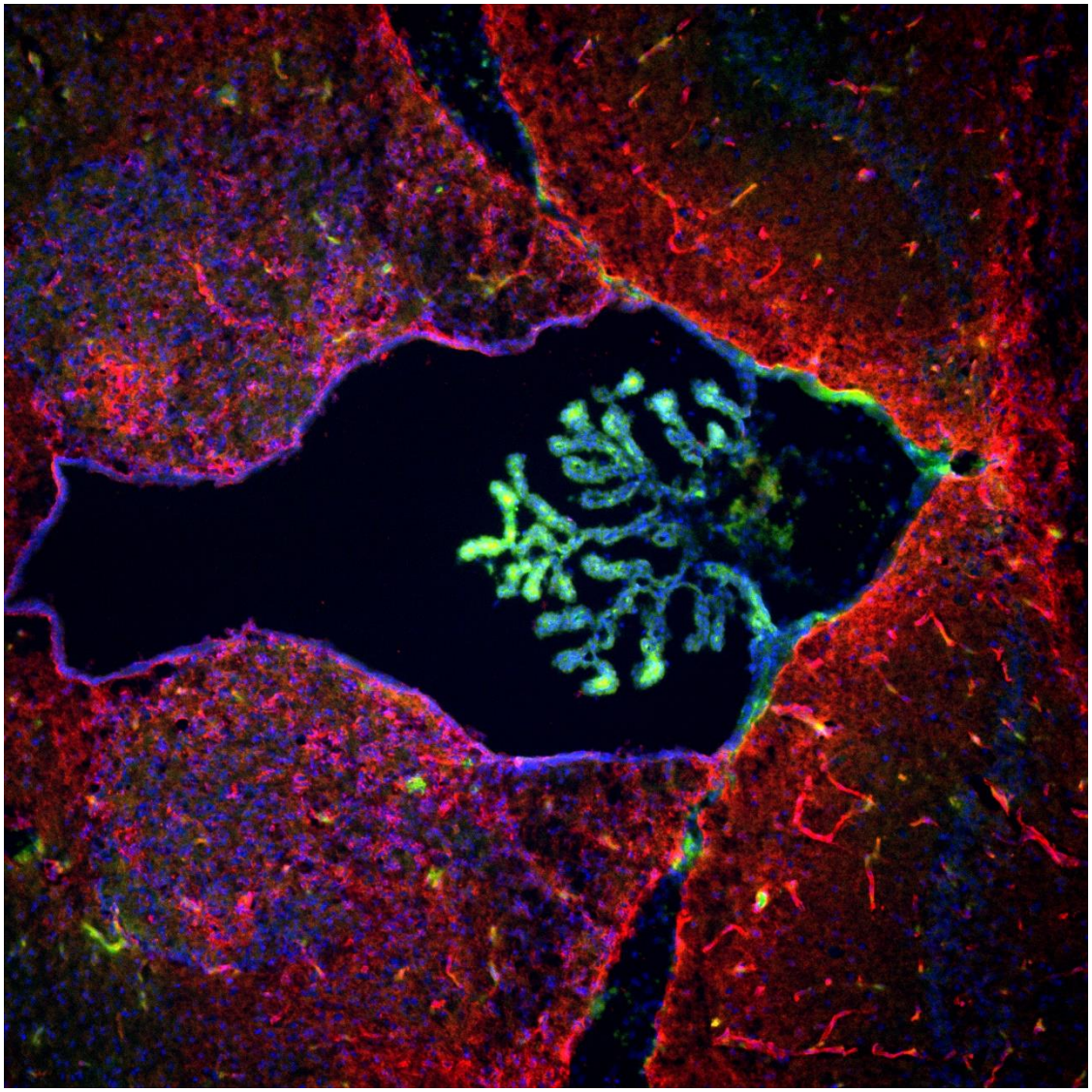
Journal of College Biology Teaching
Bioscene



Volume 47(2) December 2021

Journal of College Biology Teaching
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Volume 47(2) **December 2021**

ISSN 1539-2422

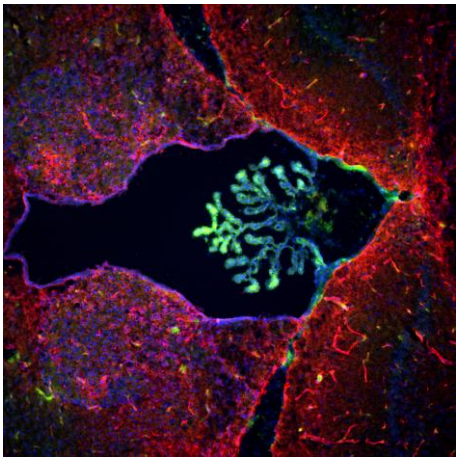
A Peer-Reviewed Journal of the

Association of College and University Biology Educators

Editor-in-Chief:
Robert W. Yost
Indiana University – Purdue University Indianapolis

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

Bioscene is published in May (online) and December (online and in paper format). Manuscripts submitted for consideration by Bioscene should follow the guidelines for submission.



Cover image:
Picture of the choroid plexus from a Wistar rat (*Rattus norvegicus*). The nuclei are stained blue. You can see the ependymal cells lining the ventricle with the choroid plexus extending into it. Aquaporins are stained red.

Photographer:
Makenna Reed
Bonnie Blazer-Yost Lab,
Indiana University-Purdue-University Indianapolis

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66th ANNUAL MEETING

The 66th Annual ACUBE meeting is being planned for Portland, OR. Please check the May 2022 issue for more details.

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A Publication of the Association of College and University Biology Educators

ACUBE Mission Statement

Members of ACUBE share ideas and address the unique challenges of balancing teaching, research, advising, administration, and service.

We are a supporting and mentoring community that provides professional development opportunities to:

- develop and recognize excellence in teaching;
- incubate new and innovative teaching ideas;
- involve student research in the biology curriculum;
- advise and mentor students in and out of the classroom;
- enhance scholarship through our international, peer-reviewed journal, *Bioscene*.

Articles

Mitigating Student Resistance to Active Learning by Constructing Resilient Classrooms

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Abstract: Shorter lectures punctuated with activities to engage students in the learning process can increase student understanding, critical thinking, and overall learning. However, some students have negative responses to active teaching strategies. Here we explore the topic of student resistance to active learning, including reasons for this opposition and strategies to prevent or respond to it. Recognizing factors that lead to students' resistance to active learning is important to mitigating these barriers to learning. Equally critical to mitigating student resistance is the promotion of student resilience. Structuring classrooms to promote resilience includes community building, structured activities, and policies that recognize student diversity, and the complexity of learning processes.

Keywords: faculty learning community; independent learners; student anxiety; intellectual development; instructional design; student support; marginalized students

Introduction

In Fall 2018 and Winter 2019, our Centre for Teaching and Learning hosted a Faculty Learning Community, or FLC (Cox, 2004), to explore student resistance to the active learning techniques our participants had implemented in their courses. Through our discussions and review of the literature, we learned some of the reasons for student resistance and considered strategies to mitigate their response to our active teaching approaches. We learned that implementing active learning requires that our classrooms be designed for resilience and that how instructors facilitate active learning is key to ensuring students are open to what can initially seem counterintuitive to their lived learning culture.

What is an FLC?

A Faculty Learning Community is a small group of instructors engaged in a collaborative, yearlong program that builds a sense of community and includes activities that enhance the members' understanding of and scholarly approach to teaching and learning (Cox, 2004). An FLC achieves these outcomes by providing a structured and goal-oriented program that promotes the learning, development, and scholarship of the members. Our FLC was composed of a small group of instructors from several departments and disciplines across the University of Alberta who were at different stages of their teaching careers. Our common ground was that we all practice, to a greater or lesser extent, some form of active learning with our students. We developed our community by sharing our strategies and experiences about a variety of active learning practices.

A surprising outcome of this sharing among our FLC members was the realization that our assumptions about the uniqueness of our experience with students' resistance to active learning were unfounded. All FLC members, regardless of discipline, had experienced similar instances of student resistance to active learning. This paper is a result of that common realization, resulting in suggestions for mitigating students' resistance that transcend disciplines.

Resistance to Active Learning

Research has shown that shifting from a teacher-centered emphasis to a learner-centered classroom environment that includes peer collaboration and application can result in improved learning outcomes for students (Freeman et al., 2014; Haak et al., 2011; Prince, 2004; Umbach & Wawrzynski, 2005), such as increasing student understanding and critical thinking (Lumpkin et al., 2015). Our FLC's working definition of active learning included activities that encourage students to participate and engage with the course material and each other in a more meaningful way (Lumpkin et al., 2015). Even though the evidence demonstrates that active learning improves student learning outcomes, students often resist active learning. Instructors intuit this resistance from students' attitudes, body language, and direct or indirect comments heard in the classroom or read on student ratings of instruction (Smith & Cardaciotto, 2011; Van Sickle, 2016). Sharing these experiences in our FLC initiated a critical analysis of resistance to active learning and a scholarly exploration of the literature to identify research that may help us to overcome student, instructor, and institutional resistance to active learning.

A key objective of our FLC was to tackle the issue of resistance to active learning. Resistance can be defined as those attitudes and behaviors that are in opposition to the desired outcome (Richmond & McCroskey, 2012), or as "any observable behavior that makes an instructor less likely to use an instructional strategy" (Prince & Weimer, 2017). We largely focused on resistance from the student's perspective but also realized that similar challenges exist from the instructor and institutional perspectives. Student resistance can present itself in four forms: passive, non-verbal, partial compliance, and open (active complaints or refusing to do a task or project) (Shekhar et al., 2015). Our FLC members all experienced one or more forms of resistance in our classrooms when undertaking active learning strategies. Although we perceived student resistance as "prevalent," research suggests that the majority of students appreciate active learning strategies and understand their educational value (Finelli et al., 2018; Prince & Weimer, 2017).

Conceptualizing resistance as "barriers to learning" may better empathize with the challenges both students and instructors face when engaging in or facilitating active learning (Seidel & Tanner, 2013). A student's resistance to active learning is commonly shaped by factors internal and external to the student:

- Environmental forces. Work, family, racism, sexism, academic pressure from other courses and institutional culture are all forces that can erode or promote students' readiness for learning (Tolman & Kremling, 2017);
- Level of intellectual development. At earlier stages of intellectual development, students are more likely to perceive learning as something that is done to them while at more advanced stages of intellectual development, students are more likely to perceive learning as something they actively do (Perry, 1981);
- Self-perceptions as students. When students see themselves as good at being students, for example, they may expect the university to validate this perception rather than see learning activities as an opportunity to develop their ability to think (Tolman & Kremling, 2017);
- Anxiety level. A student's anxiety about achieving a particular grade may lead the student to feel unprepared or experience pressure to obtain scholarships or admission to competitive programs (Weimer, 2013);
- Feeling marginalized. A student's uneasiness in participating in active learning may result from being stereotyped, a visible minority, or having divergent views from the instructor or the majority of students (Tolman & Kremling, 2017).

Recognizing these root causes of resistance for students is the first step in preventing or responding to it (Tolman & Kremling, 2017; Weimer, 2013). Instructors can mitigate students' resistance by being patient with the student's intellectual development, explaining the purpose of assignments or class structure, affirming the role of student autonomy, ensuring that students' personhood is respected, and facilitating in-class learning activities in ways that respond to the student's needs at that time in their learning (Finelli et al., 2018). The antidote for student resistance to learning is to construct an educational environment that supports student resilience for learning.

Resilience and Learning

It has been established that resilient learners tend to succeed in learning while non-resilient learners often indicate that they have more difficulty in their coursework and are less engaged (Waxman et al., 2003). Our FLC defined resilience for learning as the attitudes and behaviors that enable learners to respond positively to new or challenging learning situations and take risks in learning (Cassidy, 2015). The literature supports the idea that academic resilience has numerous components, including confidence level, ability to understand and manage stress in adverse conditions, and motivation (Beri & Kumar, 2018). Although resilience can result from internal fortitude, it is also largely a consequence of the social and institutional structures that support us (Ungar, 2019). These structures have implications for the classroom environment and, specifically, for active learning.

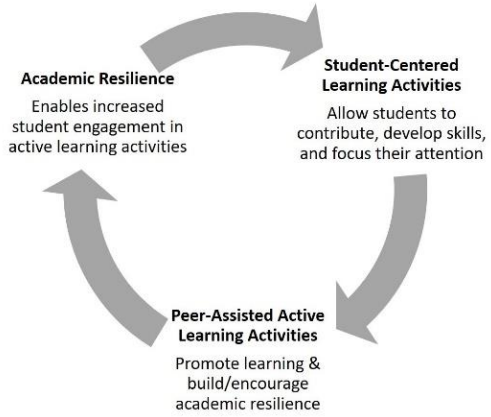
Evidence suggests that resilience enhances active learning and that active learning enhances resilience (Figure 1). Academic resilience has been associated with increased participation in active learning (Waxman et al., 2003). Additionally, the implementation of student-centered learning strategies seems to allow all learners to have an increased level of perceived control in their learning, allowing them to contribute, develop skills, and focus their attention (Edwards et al., 2016). Even students with lower resilience tend to do well in this type of environment. However, less-resilient learners will require more help to become engaged and control their attention.

Active learning strategies that enable students to interact with their peers not only promote learning but also help to build resilience by strengthening peer relationships (Cassidy, 2015). Peer relationships are a positive source of encouragement and resilience for those who are experiencing challenges and adversity. Encouraging and preserving these relationships is important to foster a learning environment that builds academic resilience and promotes active learning.

In addition to improvements in student achievement, active learning strategies in the form of team-based learning improve the performance and retention of underrepresented minority students (Snyder et al., 2016). In the study by Snyder et al. (2016), for example, all students who participated in peer learning activities in an introductory biology course demonstrated improved grades with a significant reduction in failing grades and course withdrawals for underrepresented minority students. Similarly, in his review of the evidence related to active learning, Prince (2004) found that active learning strategies that encouraged students to work collaboratively resulted in higher achievement, improved student attitude and self-esteem, and improved retention, particularly for students from underrepresented groups.

Being flexible rather than rigid about expectations is also critical to promoting resilience (Ungar, 2019) and enhancing overall learning. Student resilience for learning is promoted with learner-centered teaching in which students' self-efficacy is enabled by giving them a choice in how and what they learn within the structure of the curriculum and discipline (Weimer, 2013). Structure can promote resilience, but too much structure can be stifling and hinder creativity

Figure 1: Increased resilience will increase the likelihood that students will participate in active learning and active learning can help build student resilience.



(Ungar, 2019). Because creativity is central to learning, we argue there exists a sweet spot for providing sufficient structure to nurture resilience but not so much structure that students are unable to make choices that promote their learning. Knowing how much structure is appropriate will depend upon instructors knowing their students, which may be different for each course and student cohort. For example, is this students' first interaction with the course material or is the course at an advanced level? If it is an introductory course, students may need more guidance (lecturing) from the instructor. In contrast, if it is an advanced course, it may be more appropriate to provide more application activities that enable students to explore the course material on their own. Ultimately, the key is tuning the course to find the correct balance of lecturing and active learning, which is dependent upon the discipline, year level, and student cohort, among other factors (Haave, 2019).

Implications

When creating a resilient classroom to mitigate student resistance to active learning, instructors need to 1) be mindful of fostering learning communities within safe and inclusive learning environments and 2) prepare and design courses such that the rationale and expectations for active learning are made clear to students and actively promoted by instructors. Active learning is best implemented when instructors are actively engaged with their students during the learning activity.

Learning Communities

To promote students' resilience for learning, classrooms need to develop into learning communities, one of the high-impact practices proposed by the Association of American Colleges and Universities (Kuh et al., 2017). Learning communities can be fostered by structuring classroom environments with course policies that make it safe for students to risk failure. Instructors create a safe space for learning from one's mistakes by creating and sustaining a learning community where students experience a sense of belonging. The creation of learning communities and safe learning spaces reinforce each other. For example, Chávez (2007) offered findings from a semester-long qualitative study of classroom environments facilitated by instructors identified as "multiculturally empowering." These teachers "worked with all students to create collective, empowering learning experiences that utilized and honored multicultural realities within a shared and rigorous academic experience." Six elemental dynamics aimed at empowering or liberating individuals in learning communities were identified and are summarized in Table 1 These dynamics are associated with classroom management policies that created spaces in which a diversity of students felt valued and able to contribute and grow.

Course Preparation and Design

Course preparation and design are critical to the successful implementation of active learning. By focusing on preparation strategies that set the stage for successful active learning, we engage our students and mitigate resistance. A review of select literature led to several tips for preparing resilient classrooms (Brookfield, 2015; Mohamed, 2008;

Table 1: Elements of an empowering multicultural learning environment and associated classroom management policies (summarized from Chávez, 2007).

. Dynamic	Example Classroom Management Policies
Climate of Safety	● Develop guidelines for respectful interaction and hold students to them ● Encourage students to take responsibility for the safety of themselves and others ● Invite students into discussions ● Acknowledge that each person is in a different place with the subject ● Encourage students to challenge ideas and assumptions
Spirit of Risk-Taking	● Acknowledge that an appropriate level of discomfort indicates that risk-taking and safety are well balanced ● Remind students that discomfort and uncomfortable situations do not necessarily translate into harm ● Facilitate and reward an atmosphere of risk-taking at the outset
Congruence	● Maintain congruence in behavior to be trusted and effective ● Create inclusive environments that allow students to see themselves represented in readings, case studies and assignments ● Demonstrate, by example, the ability to sincerely listen to others
Proactivity	● Take action as a community of learners together by taking risks, facilitating respectful conflict, acting as allies for each other, and showing personal vulnerability ● Take a diversity of ideas and turn them into practice
Multiplicity	● Challenge one-dimensional perception and introduce contrasting ideas, knowledge, and experiences ● Utilize a diversity of knowledge, methods, styles, and relationships in various processes and activities
Reciprocity	● Create an environment in which the diverse strengths of students are incorporated and valued ● Create parity among groups of people by power, idea sharing, and reciprocal validation of each other's ideas

Owens et al., 2020; Tharayil et al., 2018; Toven-Lindsey, 2018). Resilience-promoting strategies for mitigating students' resistance to active learning may be divided into three broad categories: preparation, explanation, and facilitation [See figure 2].

Preparation

As instructors, we need to create a positive and inclusive environment that allows students to take ownership of their education. Course policies must empower students to be active agents in their learning. When students have a perceived sense of control and develop their identities as independent learners, they are more likely to be resilient learners. For instance, classroom policies need to make clear what students can expect from each other and their instructor. Instructors might frame students' post-secondary education as being a journey or a process of becoming life-long learners (Grow, 1991). Moreover, if instructors ensure students know what learning resources are available to them, students can choose to engage in learning as circumscribed by their lived context. Enabling students to have control over how they will learn and be assessed can strengthen their academic resilience. However, there are important limits imposed by the structure of the course. Course syllabi need to indicate the curriculum to be learned and the activities that will promote its mastery. Instructors should use a variety of teaching methods and assessment strategies. We suggest starting small and creating situations in which students can succeed. In general, gradually moving toward a learner-centered emphasis is more effective for both teachers and students (Felder & Brent, 1996), as is the continuing use of at least some class time (20-60% seems to be the best balance) for lectures (Henderson et al., 2018). Additionally, thinking ahead about the physical space and room set up may facilitate active and collaborative learning. A room with moveable desks and chairs will allow for better small group work than a small lecture hall and will encourage active participation (Tharayil et al., 2018).

Instructors need to be reflective and purposeful in their selection of activities and be open to student feedback with a willingness to make changes to assignments that improve the learning activity (Tharayil et al., 2018; Weimer, 2013). Because active learning can be more challenging for instructors and some activities do not work out as well as expected, instructors are encouraged to regularly solicit student feedback on the learning activities used. The Critical Incident Questionnaire is one method of gathering anonymous student feedback on learning activities using structured and specific questions (Brookfield, 2017). Comments are summarized and issues requiring clarification are addressed during the next class. Any changes made to teaching as a result of student feedback need to be highlighted so it is clear to students that their voices are being heard. Additionally, learning should be assessed incrementally to help students know if their learning strategies are working or not before it is too late.

Explanation

Our teaching strategies must be well explained to students, a facilitation strategy that is widely supported in the literature with many specific explanation strategies available (Cooper et al., 2017; Tharayil et al., 2018; Toven-Lindsey, 2018; Weimer, 2013). Explaining course expectations for student participation, engagement, and peer interactions should be done early. It is critical that instructors further communicate their rationale for using active learning and do so explicitly and frequently. By describing the purpose of each activity and providing a rationale for how each activity relates to learning, instructors can help increase students' perception of the value of active learning. Because students are more likely to participate if they perceive a low cost and a high-value return from active learning, instructors need to connect each activity to learning objectives and describe the relevance to industry or clinical practice expectations. Each learning activity requires explicit instructions, including explanations of what

Figure 2: Strategies for mitigating student resistance to active learning and building academic resilience.

preparation is required and how each activity will be assessed. Finally, instructors need to ensure students have a clear understanding of the task at hand, which will allow students to complete the activity on their own. Although these suggestions may seem obvious, each of us in our FLC noted how often we did not articulate to students the reasons we were implementing an active learning strategy. We tended to assume the reasons were self-evident to students, but this is not the case.

Facilitation

To ensure that students value active learning, instructors must meaningfully facilitate in-class learning activities (Mohamed, 2008; Tharayil et al., 2018; Weimer, 2013). There are a wide variety of facilitation strategies. If active learning is a regular part of the course, students will come to expect it, so it is important to develop a routine early. During learning activities, instructors need to walk around the classroom with a smile, being and appearing approachable. However, in addition to walking around their classroom, instructors need to encourage and engage with their students, asking and answering questions, and guiding students if they get stuck or start down a wrong path. As instructors, we need to approach and engage with non-participating students. Rather than confront students not participating, we can instead ask if they are stuck, whether they have any questions, or if they need more time to work through a problem. Instructors need to invite questions and look for creative ways to encourage students to ask questions. Calling on students to answer questions can heighten anxiety. Instead of cold-calling on individuals, teachers can assign students to teams at the beginning of the class or the semester and have teams of students respond to questions, or allow students to confer with their classmates before asking for volunteers. Additionally, active learning can be facilitated by grading for participation, assigning marks for completing activities in groups, and using group exams to encourage collaborative learning.

Scaffolding is also a good strategy to employ. This can be done by starting with smaller activities and building up to larger, more complex projects. Another method is giving a short lecture followed by a simple activity and then progressing to providing material ahead of the class and dedicating the whole class to active learning activities. Above all, to facilitate active learning, instructors must create an environment in which it is okay to make mistakes. This means creating a safe environment and learning community where students feel respected and valued for their unique lived experiences and contributions.

These strategies are not prescriptive nor are they exclusive. Instructors have implemented these strategies in a variety of ways in their classes (Tharayil et al., 2018). For example, some instructors might explicitly explain the purpose of an assignment or exercise while others use reflection and discussion of the assignment or activity to help students understand its purpose. The strategies presented herein are also connected and interrelated. Walking around the room has been suggested as a way to invite students' questions of the instructor and engage in discussion about the topic, allowing instructors to interact with non-participating students (Tharayil et al., 2018). This is a different instructor presence than many of us in our FLC experienced as students when our instructors would assign an in-class or lab task and then simply wait at the front of the room to receive completed assignments. The literature advocates for instructors to be active in the classroom in addition to students.

Conclusion

The design of our classes can promote students' resilience by explicitly building in community and policies that acknowledge students as whole and complex human beings with diverse lived experiences. By designing courses in which structured activities promote student interaction with ample scope for formative evaluation, as well as incorporating policies that enable students to have some control within that structure over how they will learn and be assessed, we create classroom environments that take into account that learning is a human developmental process. In this context, initial failures should be expected and accepted, if not encouraged. By structuring classrooms to promote students' resilience, instructors can mitigate students' resistance to learning. Many of the strategies used to ameliorate student resistance to active learning also build academic resilience. We think that active learning experiences embedded in a resilient learning environment will produce an orientation toward lifelong learning that will reward students beyond their post-secondary academic years.

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A Field Project Investigating the Influence of Urban Noise on Eastern Gray Squirrel Behavior

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Abstract: Many science departments encourage students to gain experience conducting research. However, finding ecological research projects that allow students to test a hypothesis in the field, over the relatively short time span of a semester, can be challenging. This article describes an inquiry-based research activity examining the influence that urban noise has on the behavior of gray squirrels. One of the consequences of urbanization is increased noise from automobiles and other human activities. This research project allows students to pose hypotheses regarding how squirrel behavior changes in response to human noise and then test those hypotheses. This activity is well suited for students in ecology, animal behavior, or vertebrate biology classes. It allows students flexibility in the hypothesis they test and the methods they use, while giving students a framework that lets them successfully complete a field research project. Students gain experience developing hypotheses, designing a field experiment, writing a research proposal, collecting field data, conducting data analysis, and presenting the results of their project.

Keywords: Ecology, urban ecology, animal behavior, squirrels, behavioral ecology, foraging behavior, student research, research projects, teaching methods, undergraduate research, flight initiation distance, flight distance, escape distance, alert distance, noise pollution, urban noise, vertebrate biology

Introduction

There has been an increasing desire among many science departments to encourage students to gain experience conducting scientific research. When conducting laboratory experiments, it is relatively easy to control variables. However, field projects can be extremely time-consuming, and it can require tremendous amounts of time and effort to conduct experiments on animals in the field. This article describes an inquiry-based research activity examining the influence that urban noise has on the behavior of gray squirrels. Students gain experience developing hypotheses, designing an experiment, writing a research proposal, conducting data analysis, and presenting the results of their project

One of us, the first author, is an instructor for a course that uses this activity at our institution. The other two authors are students that have conducted this research project and have assisted in revising the activity.

Introduction to Eastern Gray Squirrels

Eastern gray squirrels (*Sciurus carolinensis*) are common in North America and range throughout much of the central and eastern part of the United States and the southern regions of Canada (Koprowski, 1994; Whitaker 1996). These squirrels are generally grayish or brownish in color, although there is a black (melanistic) morph that is found in certain regions, particularly in Canada. They are medium-sized tree-dwelling squirrels and build large nests (dreys) of twigs and leaves high up in trees. When the leaves of deciduous trees drop in the fall, it is easy to spot the conspicuous dreys. In addition to dwelling in dreys, gray squirrels frequently have a den in a hollowed-out tree trunk (Koprowski, 1994; Whitaker 1996). The dreys are frequently used in warmer months while cavity dens are often used in winter (Koprowski, 1994). Eastern gray squirrels are not territorial, and squirrels have overlapping home ranges. Their home ranges vary from 0.5 hectares to over 20 hectares (Koprowski, 1994).

Gray squirrels are diurnal (are most active at dawn and dusk). Their peak activity occurs about two hours after sunrise, and two-five hours before sunset (Koprowski, 1994). They have a broad diet that includes seeds, nuts, flowers, fruit, tree bark, buds, and mushrooms as well as occasional eggs and nestlings of small birds (Koprowski, 1994; Whitaker, 1996). Females consume more food than males and food consumption peaks in summer or fall, while declining in the winter (Knee, 1983). During periods when food resources are abundant, squirrels collect and store food. They are scatter hoarders and may have thousands of stored caches. Eastern gray squirrels do not hibernate, but during the cold winter months they will venture out of their nests on warm days and dig up some of their stored caches (Whittaker, 1996).

Introduction to Influence of Urban Noise on Squirrel Behavior

Urbanization has resulted in an increase in noise from automobiles, businesses, and human interactions. Common urban noise includes not only automobiles but also mowers and other yard equipment, recreational vehicles, construction work, sirens, alarms, barking dogs, and music. Noise can influence wildlife in many different ways. Often noise can cause wildlife to move away from an area, to increase their time spent being vigilant (examining their surroundings for potential threats), and decrease the time spent foraging (Dertien & Larson, 2021; Engelhardt & Weladji, 2011; Cooper et al., 2008). Dertien and Larson (2021) found that small mammals tend to avoid areas within 50-100m of trails or people. Squirrels that were exposed to predator calls showed increased vigilance and decreased foraging (Jayne et al., 2015).

Flight initiation distance is a common behavioral measurement examining how close a person or object can get to an animal before the animal flees. Flight initiation distance is also referred to as flight distance, escape distance, or alert distance. Engelhardt and Weladji (2011) and Cooper et al.

(2008) found a difference in how squirrels react to humans in environments with different levels of human disturbance. They found flight initiation distance in squirrels significantly increased with decreasing human disturbance. Thus, in areas where squirrels are frequently exposed to humans (areas of increased human disturbance), squirrels let humans approach more closely, perhaps due to squirrels learning to adjust their antipredator behavior with experience to humans (Engelhardt & Weladji, 2011; Cooper et al., 2008).

This study allows students to examine how noise affects the behavior of squirrels. Students can pose hypotheses regarding how the presence of noise will influence squirrel behavior, design experiments to test their hypotheses, analyze their results and draw conclusions about the influence of noise on squirrel behavior.

General Methods

General Format of Research Projects

Rather than providing students with a hypothesis to test and methods to follow, I (the first author and course instructor) prefer to have students pick their own hypothesis and develop their own methods, as it provides students with valuable experience developing a hypothesis and designing their own experiment. Also, this makes the project truly "theirs" and they become more invested in their projects. I have found that student research projects work best when students collaborate in groups of three or four because it enables students to divide up the work so that they can collect sufficient data to allow them to conduct statistical analysis and draw meaningful conclusions. Because these projects are field projects, for safety reasons, I like to have a minimum group size of two so that students always go out to the field with a partner. I generally let students form their own groups based upon which students are interested in pursuing similar hypotheses. If there are students that haven't found partners, I try to help them find a group to work with. It is also preferable to avoid large groups; I have observed that groups of more than four students tend to have considerable problems fairly dividing the work among group members. Having students work in groups, means that the instructor has a more reasonable number of projects to oversee.

First, students are provided with background information on squirrel foraging behavior and the influence of urbanization on wildlife. Next, groups conduct a literature search and write a two-to-three-page summary of squirrel behavior and the influence of urban noise disturbances on squirrel behavior. Students are required to cite a minimum of five sources, three of which must be scientific journal research articles. After going over their summaries with each group, I ask groups to come up with a hypothesis that they could test regarding the influence of noise on squirrel behavior. If students have a difficult time developing a hypothesis to test, we discuss how they think that noise might influence squirrels, and I help guide them toward possible hypotheses that they could test. Examples of possible hypotheses that students could test (ones that my classes have examined) include:

• Squirrels presented with loud noise (e.g., music from a speaker, or the sound of a siren being played from a speaker) will demonstrate a greater flight initiation distance than when they are not exposed to loud noise.

• The type of noise that squirrels are exposed to will influence their flight initiation distance (students could use their cell phone to play different sounds at a set volume such as a siren, car alarm, motorcycle, barking dog, leaf blower, mower, or loud music).

• Increasing the level of noise (none, low, medium, high) results in squirrels showing greater flight initiation distance.

• Squirrels exposed to higher levels of human disturbance (e.g., in regions closer to trails or areas in parks that are more frequently visited) will be less likely to respond to human noise (will have a lower flight initiation distance) than squirrels exposed to lower levels of human traffic.

Once students have developed a hypothesis that they plan to test, and their hypothesis has been approved by me, each group designs an experiment to test their hypothesis. Next, groups submit a scientific research proposal which should include an introduction with supporting background literature, and a detailed methods section including a discussion of proposed statistical analysis (Darling, 2020 includes a handout on writing a research proposal). I also ask students to include a list of required items, and a detailed calendar outlining what the group will do each week (preparation/set-up, data collection, data analysis, and preparation for presentation of their results). I have found that requiring a formal, graded, research proposal helps students structure their ideas and prevents students from jumping ahead and making major mistakes in their experimental design.

After reading their proposals, I meet with each group to discuss their projects and make suggestions regarding their hypothesis and experimental design. I then ask groups to revise their proposals based upon that discussion. After groups rewrite their proposals, I then meet with groups again. Students do not begin data collection until their proposals have been reviewed and approved. Generally, it takes several revisions before students have refined their experimental design. Their revised proposal is graded, and their final proposal grade is an average of the grades on their proposals.

Before students begin their projects, I ensure that they have background knowledge on how to conduct their projects, understand that they must work with a partner when they are out in the field, have worked out the details of their experimental design and know what data they will record, I also make certain that they have an idea of how they will analyze their data, understand what results will support their hypothesis, and what results will cause them to reject their hypothesis. I then ask each group to provide me with a data collection sheet detailing what data they will record and how often data will be collected. If necessary, I also ensure that groups have submitted Institutional Animal Care and Use Committee (IACUC) proposals required by the university and that their IACUC proposals have been approved.

Suggestions and Helpful Hints for Projects

While students should develop their own hypothesis and experimental design, they may need some assistance. Below I outline some helpful suggestions to consider when discussing experimental design with each group.

- Students should design an experiment to examine the influence of noise on squirrel behavior. There are many ways that they could do this. Exactly how they do this will depend on what hypothesis they choose to address.

- Students should conduct their projects in field sites that squirrels frequent. This could be locations on campus or nearby local or state parks. One of the advantages of using squirrels as a study animal is that they are prevalent; they frequent urban and suburban areas. Exactly which locations students use may depend upon the hypothesis they test. To have success encountering squirrels, students should be certain to pick areas where squirrels are common.

- When conducting experiments, students can attract squirrels using plates of food (e.g., sunflower seeds or peanuts). However, they should be certain to keep the type and quantity of food constant for all their trials.

- "Seeding" the area with nuts or seeds prior to conducting experiments is essential. Without seeding the area, students waste a tremendous amount of time in the field waiting for animals to come. However, if they go out and seed the area, each day for 4-6 days before they begin their experiments, they will have a much easier time attracting squirrels to the area.

- It is preferable to conduct trials during mornings and/or late afternoons when squirrels are most active.

- Students should try to control for everything except the variable that they are testing. For example, if they are testing the hypothesis that noise (e.g., music from a speaker) will result in squirrels showing a greater flight initiation distance, then during each trial, squirrels should always be given the same quantity and type of food and the only thing that should be changed is the presence or absence of music from a speaker. If students are not comparing the influence of squirrel exposure to human traffic, then they should try to control for exposure to human traffic by choosing sites that have similar levels of human traffic. Students can use their cell phones to manipulate noise. For example, they could choose a particular song to play and then manipulate the volume on their cell phone. Alternatively, they could test a hypothesis about how squirrels respond to different types of sounds by using their cell phones to play different sounds (car alarm, siren, music) at a set volume.

- Students can measure flight initiation distance by waiting for a squirrel to begin foraging at the food plate and then starting to slowly walk toward the squirrel. The flight initiation distance is recorded as the distance from the student to the squirrel when the squirrel begins to flee as approached. Students can drop markers (e.g., small colored markers) at the beginning and ending locations and then use a measuring tape to measure the flight initiation distance. This distance represents how close the researcher was able to get to the squirrel.

- Students should conduct sufficient replicates, from a variety of locations, and over many different days to allow for statistical analysis. Generally, it is useful to have a minimum of 20 replicates for each treatment.

- This exercise allows students to statistically analyze data. I conduct this project in an upper- division course and students have previously been exposed to some statistics in introductory biology. However, I provide students with a brief overview of statistical analyses, and I meet with each group to discuss appropriate data analysis.

Results and Discussion

Graphing the data will help students visualize their results. The data analysis will depend upon what hypothesis was tested. For example, students can graph the results to determine if the average flight initiation distance (FID) increases with the presence of noise. Then, they can conduct appropriate statistical analyses. For example, students could compare the mean flight initiation distance between treatments by using t-tests or ANOVA (depending upon the number of means that they are comparing).

Examples of student results from this exercise are shown in Figures 1 and 2. In general, my students have found that as noise increases, FID significantly increases (Figure 1). Students in my class have also found that FID varies among locations that have different levels of foot traffic. Overall, FID was highest in parks that had the lowest foot traffic (Figure 2). Mt. Tom (a more remote location in Massachusetts) had the highest overall average FID whereas, busy Ray Ash Park (MA) had the lowest FID. This is consistent with the results from Engelhardt & Weladji (2011) that found squirrels would flee faster, creating a higher flight distance in areas that were around many humans or are in areas of decreased foot traffic.

As in any science experiment, students do not always find what they predict, and this is a great opportunity to discuss with students all the reasons why they may find results that differ from their predictions. After analyzing their data, I have students present their results in an oral presentation to the class, in a poster presentation at our collegewide poster symposium, and as a full written report.

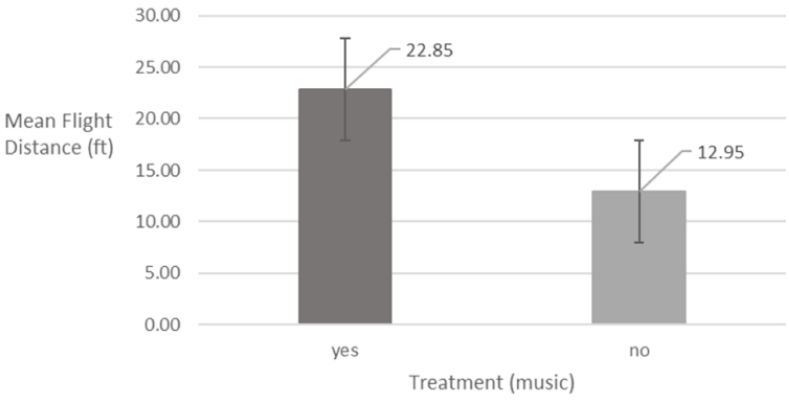

Figure 1: An example of one group's results for the mean flight initiation distance (FID) for squirrels exposed to loud noise (music playing from a speaker) compared to squirrels not exposed to loud noise. Treatment "yes" is the experiment group that had music playing while treatment "no" was the control group that had no music playing. There was a significant increase in FID with exposure to loud noise.
(t-test: t= 10.9, df=118, p= 6.91x E-20)

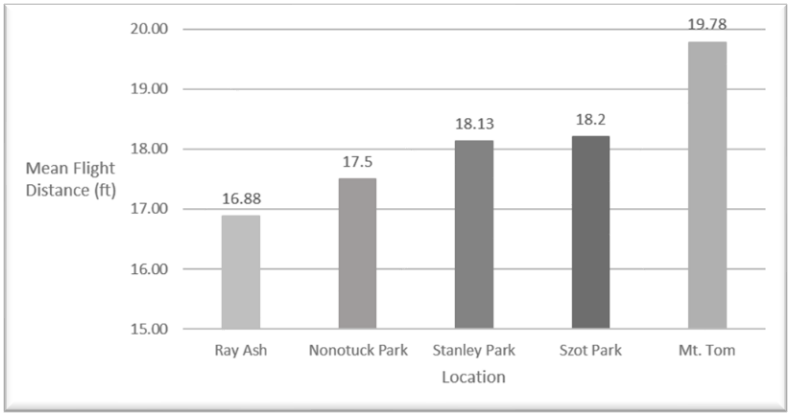

Figure 2: An example of one group's results for the mean flight initiation distance (FID) for five different locations. The five locations are different parks ranked from high (Ray Ash, MA) to low (Mt. Tom, MA) foot traffic. FID was significantly higher in regions that had lower foot traffic (Mt. Tom) than for regions (Ray Ash) that with high foot traffic (ANOVA p=0.033).

Conclusion

It can be very challenging to find ecology and animal behavior field research projects that can be easily and inexpensively conducted. This exercise provides an opportunity for students to gain experience developing hypotheses, designing a field experiment, collecting field data, writing a research proposal, conducting data analysis, and presenting the results of their project. This exercise provides students with flexibility regarding what hypothesis they test, and what methods they use, while providing a framework that helps them successfully complete a behavior project.

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Teaching CRISPR-Cas9 Genome Editing Through Novel Research in a Multi-week Lab Module

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Abstract: CRISPR-Cas9 is revolutionizing how we conduct scientific research, treat disease, and develop new crops. The widespread impact of this genome-editing technology makes it critical for undergraduate students to understand and engage with this new tool. In this article, we describe a multi-week lab module that teaches undergraduates how to design CRISPR-Cas9 constructs and screen for CRISPR-modified genotypes. The module is conducted through the lens of independent research; students conduct a genotype screen for novel knockout mutations. In our module, students screen Zea mays (maize) seedlings for mutations in the MAD2 gene, which assists our ongoing investigation of meiotic chromosome segregation. This module can be adapted to knockout any gene in any organism, and thus align with an instructor's research program. Engaging in original research helps undergraduate students develop independence and initiative in the lab as well as the molecular techniques of CRISPR-Cas9.

Keywords: CRISPR-Cas9, sgRNA, vector, genotype screen

Introduction

The revolutionary genome editing technology CRISPR-Cas9 is changing our world by providing new tools for scientific research, medicine and agriculture. The pioneers of CRISPR-Cas9 were recently awarded the Nobel Prize in recognition of its impact (Ioannidis et al., 2020). The power of CRISPR comes from its precision; unlike previous techniques, CRISPR enables direct editing of the genetic code at one specific location in the genome. With this precision, scientists can delete, insert or modify any gene in any organism. This powerful tool is now a standard tool in the molecular toolkit for scientific experimentation (Adli, 2018). The ability to use CRISPR is critical for students considering a career in the biological sciences. However, understanding the technology allows all students, regardless of career path, to participate in informed discussions of its ethical usage. This article describes a 5-6 week lab module that teaches undergraduate students how to use CRISPR by engaging them in a novel research project. The module is broken into five units, and each unit can be performed in a single 2-3 hour lab section (Table 1).

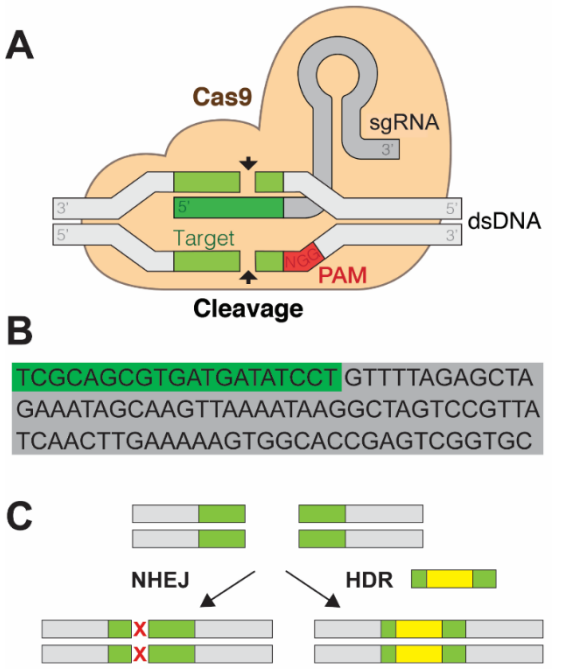
The module will span five weeks if a unit is performed each week. We typically execute this module over six weeks, building in a buffer week after Unit 3 to allow students to troubleshoot and repeat failed protocols. This module was designed for our intermediate-level genetics course, Biology 248: Genes and Genomes, a course taken primarily by sophomores and juniors who have completed a pre-requisite introductory biology course. We have implemented and iterated this module over three years with 40-60 students per year (lab sections of 20 students).

The precision of CRISPR-Cas9 is based on complementary base pairing. Scientists can target a specific genomic location for modification by introducing a single stranded RNA molecule, called a single guide RNA (sgRNA). The sgRNA is designed to complement the target DNA sequence; it scans the genome and anneals to the sequence like primers in a PCR reaction (Fig. 1A). The sgRNA consists of two components, the crRNA (CRISPR RNA) and the tracrRNA (trans-activating CRISPR RNA). The crRNA is approximately 20bp long and complementary to the target sequence (Fig. 1B). The tracrRNA is 75bp long and contains a hairpin loop that binds Cas9 nuclease and recruits it to the target sequence (Fig. 1A). While there are now multiple types of Cas9, the original nuclease creates a double-stranded DNA break. Cleavage occurs a few bases away from a specific sequence called PAM (proto-spacer adjacent sequence), a three base pair sequence, 5'-NGG-3' where N is any base (Fig. 1A). Because NGG is only three bases long, there is a high frequency of these sites in any genome. The sgRNA therefore confers specificity, bringing the Cas9 nuclease to one specific PAM sequence within the genome (Doudna & Charpentier, 2014). After Cas9 induces a double strand break, the organism's repair machinery is recruited to the cleavage site. There are two repair pathways, non-homologous end joining (NHEJ) and homology-directed repair (HDR) (Fig. 1C). NHEJ is more error-prone, often deleting or adding nucleotides, which results in frameshift mutations that can render a gene

Table 1: Chronology of CRISPR lab module units. One unit can be performed each week in a 2–3-hour time block for a total of five weeks. We prefer to build in a buffer lab during week 4 to allow students to troubleshot and repeat any failed steps. Optional advanced activities are not required for the basic CRISPR module but offer students further exploration of the content and development of their laboratory skills

Week	Lab Activity	Optional Advanced Activities
Week 1	Unit 1: CRISPR construct design	Design sgRNA and Cas9 expression cassettes and cloning scheme
Week 2	Unit 2: Genomic DNA isolation	
Week 3	Unit 3: PCR of targeted gene & gel electrophoresis	Design primer sequences, PCR program, make buffers and pour gels
Week 4	Buffer week	Troubleshoot and repeat PCR or DNA isolation if failed in previous weeks
Week 5	Unit 4: Sequencing	
Week 6	Unit 5: Analysis	

Figure 1: A) The sgRNA consists of a crRNA (dark green) that is complementary to the target genomic sequence (light green) and a tracrRNA (dark grey) that forms a hairpin loop. The Cas9 nuclease (buff) binds the tracrRNA and cleaves upstream of the PAM sequence (red), creating a double-stranded break within the target genomic sequence. Graphic created by Marius Walter, reproduced with his permission. B) Sequence of the sgRNA: the crRNA (green) is customizable to bind any genomic sequence; the tracrRNA (gray) is a standard sequence that forms the hairpin and binds Cas9. The crRNA sequence shown here anneals in exon 1 of MAD2. C) After CRISPR-induced cleavage, cells use the non-homologous end joining pathway (NHEJ) or the Homology-directed repair pathway (HDR) pathways to repair the break. NHEJ is error-prone and often causes frameshift mutations during repair while HDR is more accurate but requires a template DNA for repair (green-yellow box).



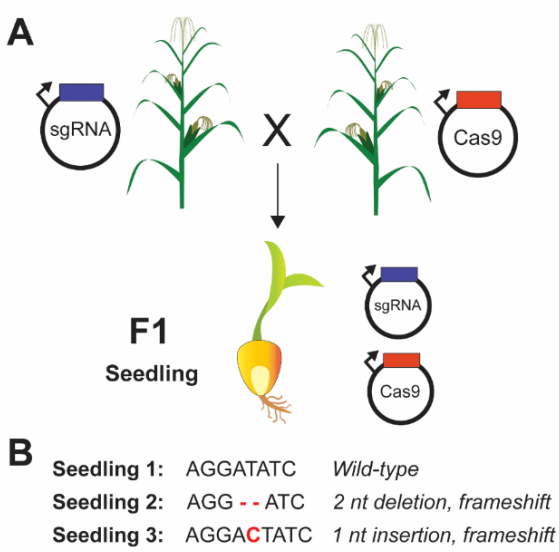
non-functional (Lieber, 2008). HDR uses a template to repair the break, which can swap new DNA sequences into the genomic location (Thompson & Schild, 2001). Between these two repair types, CRISPR can induce gene knockout (NHEJ) or gene editing (HDR) (Doudna & Charpentier, 2014). In this lab module, we induce a double-strand break within a gene called MAD2, and screen individuals for a NHEJ-produced frameshift that renders the gene nonfunctional.

The module uses the model organism Zea mays (maize) and contributes to a research program investigating the role of MAD2 in meiotic chromosome segregation (Sun & Kim, 2012). Here, we will describe how to design sgRNAs to the MAD2 gene, create in silico the CRISPR-Cas9 vectors, and screen maize seedlings for MAD2 knockouts (Fig. 2). These activities can be adapted to the instructor's specific research interests and model system to allow students the opportunity to conduct novel research. In addition to learning the tangible molecular skills related to CRISPR-Cas9, engaging in original research offers students the opportunity to develop their independence and initiative in a laboratory setting.

Methods and Materials

This lab module consists of five units: 1) CRISPR construct design, 2) DNA isolation 3) PCR of the targeted gene and 4) sequencing and 5) analysis. In theory, one module can be done each week in a 3 hour lab section for a total of 5 weeks, but in practice, buffer weeks are necessary to allow students multiple attempts in case of protocol failure. Typically, the full module takes 6 weeks to complete. One of the strengths of this lab module is that students experience novel research; the results are unknown and contribute to an active research project. The other strength is that while the research is novel, all steps are technically easy with controls that allow students iterative practice.

Figure 2. A) Students design the sgRNA sequence (blue box), link it with a promoter (black arrow) making an expression cassette, and in silico clone it into a vector (black circle). The same steps are taken to produce the Cas9 (red box) vector. These vectors were transformed into parental maize lines and crossed to produce F1 seeds containing both vectors. B) Students screen the F1 seedlings; each one is genetically unique and may contain no mutation (wildtype) or a different frameshift mutation.



1 *CRISPR construct design*

As described above, the power of CRISPR lies in a sgRNA that directs precise edits. In this unit, students learn how to design sgRNAs and develop a cloning scheme to insert them into transformation vectors. All construction is done in silico with a free software program. After students understand the DNA design concepts, the following units continue with previously synthesized constructs for time consideration.

To begin the unit, students should download ApE (A Plasmid Editor) created by M.W. Davis. This software program allows users to view and edit DNA sequences, design sgRNAs, etc. Many programs offer this molecular functionality, but ApE is our preferred choice due to its simplicity for beginners, compatibility with multiple operating systems, and free cost. ApE can be downloaded at https://jorgensen.biology.utah.edu/wayned/ape/. This unit can be modified depending on student skill level and desired pedagogical depth. At the simplest level, students will design the sgRNA (Fig. 1B); at the most advanced, students can design both the sgRNA and Cas9 expression cassettes and a

cloning scheme to insert them into the transformation vectors (Fig. 5).

To create the sgRNA, students need to design the 20bp crRNA targeted to the gene of interest and combine it with the 75bp tracrRNA in ApE (Fig. 1B). In our module, we are attempting to knockout MAD2, thus we need to target an early exon to maximize the disruption of a frameshift mutation (Fig. 3A). Students should retrieve both the genomic MAD2 sequence and the exon-only cDNA sequence from NCBI (https://www.ncbi.nlm.nih.gov/gene/542621, LOC542621). While students should use the cDNA sequence to design the sgRNA (creating a frameshift mutation in an intron will not knockout gene function), the genomic sequence is required in Unit 3. After pasting the cDNA sequence into ApE, students identify a crRNA sequence by selecting "Tools>sgRNA Analysis" (Fig. 3B). The resulting window (Fig. 3C) displays potential crRNA sequences ranked by their efficiencies (Doench et al., 2014).

Students should select a crRNA sequence that anneals early in the sequence to maximize the disruption caused by a frameshift. We selected the first sequence highlighted in Fig. 3C, as it is the earliest and most efficient crRNA. To view where a crRNA sequence is located, double click on the sequence and it becomes highlighted in the main ApE window. The selected crRNA sequence should be pasted into a new ApE window upstream of tracrRNA sequence; this is the completed sgRNA sequence (Fig. 1B). To distinguish the crRNA and tracrRNA within the sgRNA, the sequences are visually annotated in ApE by highlighting the sequence, selecting Features> New Feature, selecting a color and naming the sequence (Fig. 4).

For more advanced in silico work, students can design the sgRNA and Cas9 expression cassettes and a cloning scheme to place them in the transformation vector. In maize, sgRNAs are typically expressed using the polymerase III promoter (ZmPolIII U6-6); this sequence can be obtained in Qi et al., 2018, supplemental Table 2 (Qi et al., 2018). Students should paste the promoter sequence upstream (in front) of the sgRNA sequence to create the sgRNA expression cassette (Fig. 4A). Similarly, the Cas9 cassette can be created by pasting the ubiquitin promoter sequence (available at https://www.addgene.org/64402/) upstream of the Cas9 sequence (accessed at NCBI; GenBank ID: MT221180.1). Both cassettes must be "cloned" into the pTF101.1 transformation vector (available at https://www.addgene.org/134770/). Rather than performing the actual cloning, students design a cloning scheme and create vector maps of the final plasmids. Using ApE, students select unique restriction sites (Enzymes>Enzyme Selector>Graphic Map +U to see where the sites are located, Fig. 4D) and insert the cassette sequences into two separate vectors. Students generate a plasmid map to visualize the final vector sequence by selecting circular display option (Fig. 4A) and generating the map by selecting Enzymes> Graphic Map (Fig. 5). If students perform the entire exercise, they will be able to generate a map of sgRNA and Cas9 in their respective vectors as shown in Fig. 5.

2 Genomic DNA Isolation

After Unit 1, students should understand how to construct CRISPR vectors ready for transformation into the desired organism. Our model organism is Zea mays, which has a transformation and regeneration time of approximately 9-12 months. To make the logistics feasible, the vectors were transformed in advance of the course and students screen the resulting transgenic seedlings. More specifically, we transformed the two vectors into separate maize lines and crossed these transgenic lines to produce F1 progeny for students to screen (Fig. 2A). The NHEJ repair of CRISPR-induced double stranded breaks causes random insertions and deletions, which means each seedling represents a potentially unique genetic alteration to the targeted MAD2 gene (Fig. 2B). Each student receives a seedling and is thus responsible for screening a unique individual. The goal of Unit 2 is to isolate genomic DNA seedlings for genotype screening in upcoming Units. F1 seeds are germinated in advance of the Unit 2 lab, and are approximately 4-6 inches tall at the time of sample collection. Students remove and weigh 1-2 leaves, obtaining approximately 100mg of tissue for extraction. Students maintain the seedling through the end of the lab module so identified mutants can be used for future experiments. A Quick-DNA Plant/Seed Miniprep Kit (Zymo Research, catalog no.D6020) is used for genomic DNA extraction, and the protocol is followed per manufacturer instruction. Successful isolation of genomic DNA is confirmed and quantified using Qubit fluorescence quantification assays (ThermoFisher Scientific, catalog number Q33230). DNA can also be quantified using a UV spectrophotometer. Students should obtain approximately 10-25µg of genomic DNA (100-250ng/µL). If the concentration is below this range, students can still proceed to PCR in Unit 3, but they will need to increase the template volume to equal 100-250ng of total genomic DNA in the reaction.

3 PCR of targeted gene:

In Unit 3, students PCR amplify the MAD2 gene sequence targeted for CRISPR modification. PCR is an essential tool in molecular biosciences; while it may seem technically simple, it is critical that students understand how the process works. In our module, we focus on the importance of primer design and highlight how the annealing of primers sets the boundaries of amplification. To reinforce these concepts, we provide students with several primer sequences and ask them to: 1) determine which primers are forward and which are reverse; 2) annotate the primers on the MAD2 sequence in ApE; and 3) determine how large the resulting PCR product will be (Fig. 6). Students set up the PCR reaction using 2xHigh-Fidelity PCR Master Mix (ThermoFisher Scientific, catalog number F531S) according to manufacturer's protocol, along with 0.5µM of each primer and 100-250ng of genomic DNA. Thermocycling conditions are 98°C for 30 seconds, then 30 cycles of 98°C for 10 seconds, 60°C for 30 seconds, and 72°C for 30 seconds, followed by a final extension of 72°C for 10 minutes. During PCR amplification, students pour their own 2% agarose gels containing SYBR Safe DNA Stain (ThermoFisher Scientific, catalog number S33102). Expected sizes for the PCR product depend on the student's selected primers, but range from 420-600bp. Students confirm successful amplification by gel electrophoresis. Only a small aliquot (~5-10µL) of the reaction should be run on the gel; the remaining volume (15-20µL) is saved for sequencing. The sample is mixed with loading dye (New England Biolabs, catalog number B7021S) and run with a DNA ladder (New England Biolabs, catalog number N0550S) for approximately 30 minutes at 100V. The gel can be imaged with a blue light box.

Figure 3. A) MAD2 gene diagram: exons are shown as numbered blue boxes, introns are shown as red lines, and the promoter is denoted with an arrow. The sgRNA is designed with a crRNA sequence (green box) that anneals in exon 1 to maximize frameshift disruption. B) MAD2 cDNA sequence (exons only) is pasted into ApE, and sgRNA analysis is performed by selecting Tools>sgRNA Analysis to identify crRNA sequences. C) Results from crRNA identification analysis showing sequence, location, directionality and efficiency ("score") as described in (Doench et al., 2014).

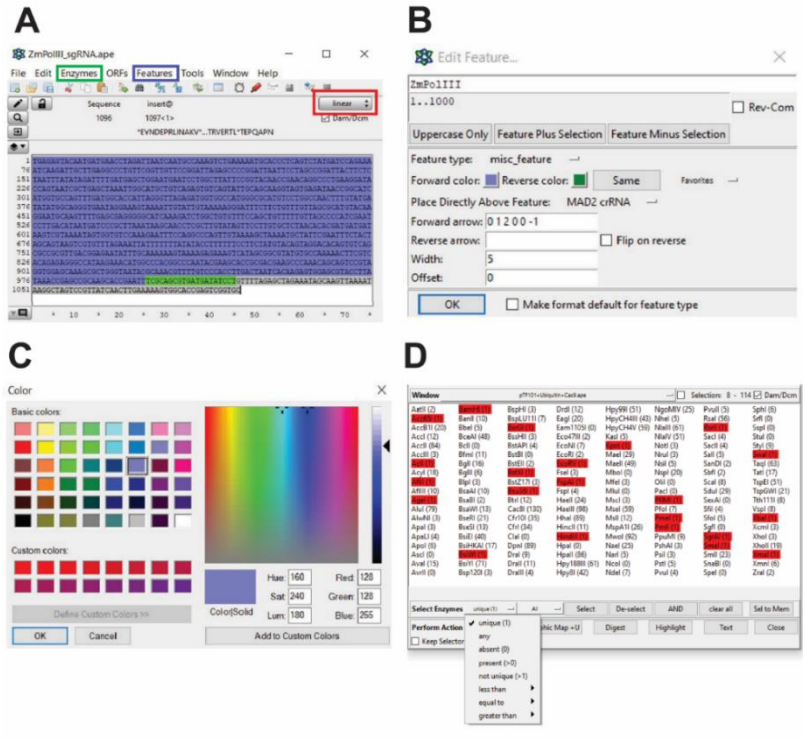


Figure 4. A) Main ApE window with annotated sgRNA expression cassette sequence. ZmPolIII promoter is shown in purple, crRNA is shown in green, tracrRNA is shown in gray. The Enzyme dropdown menu (green box) contains important functions such as Enzyme Selector (shown in D), Graphic Map, and Graphic Map +U, which display a map of the annotated sequences (+U contains unique restriction sites). The Feature dropdown menu (blue box) contains functions to annotate sequences (see B). The red box highlights the button to toggle between linear and circular sequence displays. B) To annotate sequences, highlight the specific region then click Features>New Feature and the displayed window will appear. The sequence can be named in the top box and color selected by clicking on "Forward color" or "Reverse color". C) The color window allows customization of color; purple was selected for the promoter. D) To find enzyme restriction sites in a sequence, select the Enzyme dropdown menu (green box in A)>Enzyme Selector and the displayed window will appear. All possible enzymes are listed with the number of cut sites in parentheses. To find unique sites suitable for cloning, select "Unique" from the dropdown menu and click "Select". The unique sites are shown in red. If the Graphic Map +U button is clicked, a map is generated to show where these sites fall within the sequence.

A representative gel is shown in Fig. 6C, where each lane holds a student's sample. If PCR fails, students can repeat the reaction; see Discussion section for details. Occasionally PCR will yield multiple bands (lane 2) due to non-specific amplification; multiple products will interfere with subsequent sequencing so students should either repeat the reaction with a higher annealing temperature or excise the desired band from the gel and purify using the same kit described below in Unit 4.

Students set up the PCR reaction using 2xHigh-Fidelity PCR Master Mix (ThermoFisher Scientific, catalog number F531S) according to manufacturer's protocol, along with 0.5µM of each primer and 100-250ng of genomic DNA. Thermocycling conditions are 98°C for 30 seconds, then 30 cycles of 98°C for 10 seconds, 60°C for 30 seconds, and 72°C for 30 seconds, followed by a final extension of 72°C for 10 minutes. During PCR amplification, students pour their own 2% agarose gels containing SYBR Safe DNA Stain (ThermoFisher Scientific, catalog number S33102). Expected sizes for the PCR product depend on the student's selected primers, but range from 420-600bp. Students confirm successful amplification by gel electrophoresis. Only a small aliquot (~5-10µL) of the reaction should be run on the gel; the remaining volume (15-20µL) is saved for sequencing. The sample is mixed with loading dye (New England Biolabs, catalog number B7021S) and run with a DNA ladder (New England Biolabs, catalog number N0550S) for approximately 30 minutes at 100V. The gel can be imaged with a blue light box.

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4 Sequencing

If PCR reactions are successful, students are ready to proceed to Unit 4: sample clean up and preparation for sequencing. Unsuccessful PCR reactions can be run again, and they offer an excellent opportunity for iterative practice and discussion of controls. A more in-depth discussion of PCR troubleshooting can be found in the Discussion section. The remaining PCR sample is cleaned up with either ethanol precipitation or a column-based kit, such as the NucleoSpin Gel and PCR Clean-up kit (Takara, catalog number 740609.250). Students follow the manufacturer's protocol, and the eluted DNA is quantified using Qubit fluorescence quantification or UV spectrophotometry. PCR clean up in Unit 4 will yield approximately 1-20ng/µL of purified MAD2 PCR product, more than sufficient quantities for sequencing. The samples are sequenced by Sanger sequencing; we outsource our sequencing to a company such as GeneWiz or GenScript. Samples are diluted and mixed with a sequencing primer per company preparation guidelines. Students should use their selected forward primer (Fig. 6A) as the sequencing primer. Most companies are able to return sequencing results in a few days, so sequences will be available for the following week's lab.

5 Analysis

In the final unit, students analyze their sequencing data to determine if their plant carries a CRISPR-induced MAD2 frameshift mutation. Sanger sequencing results are returned from GeneWiz, our preferred vendor, as both seq and .ab1 files. The .seq file is a sequence file containing the nucleotide text and the .ab1 file is a file containing the raw chromatogram trace data, with colored peaks representing each nucleotide (Fig. 7A). These files can be opened using ApE. Students will need to analyze their chromatogram to confirm nucleotides are appropriately assigned; irregular peaks at the beginning and end of the sequence should be excluded as they are not reliable reads (see Fig. 7A for an example) (Koh et al., 2021). Students then compare the wild-type MAD2 sequence with their sequencing results to determine if there is a mutation. Alignment programs such as BLAST: Basic Local Alignment Search Tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) allow students to paste both sequences and easily view alignment results (Fig. 7B). While Sanger error rates are very low (Shendure & Ji, 2008), discrepancies can occur between the two sequences that are not caused by CRISPR. Students should critically examine the location of any sequence differences, successful CRISPR-induced mutations will be located 3-6bp from the PAM sequence and include addition or deletion of a few nucleotides (Fig. 7C). Point mutations or differences at other locations are likely sequencing errors. If the two sequences are identical, the student's plant contains wild-type MAD2 and no CRISPR-induced NHEJ mutation has occurred.

The number of students who find a CRISPR-induced mutation can vary widely. In maize, the CRISPR mutational rate ranges from 10-85% depending on the target gene and the expression of CRISPR components (Jaganathan et al., 2018). Due to the variability in finding a mutant, we pool the class data after students individually perform their alignment analysis. Students write a final lab report using all sequence data, and are thus able to report the CRISPR mutational rate as well as the sequence(s) of CRISPR mutants.

Discussion

CRISPR-Cas9 has become a critical technique in the molecular toolkit. It is used in basic experimental science, agricultural crop improvement, and medical therapies. In this lab module, students have the opportunity to design CRISPR-Cas9 constructs and screen organisms for CRISPR-altered genotypes. In our version of the module, we target the MAD2 gene in Zea mays as it is part of an ongoing research program investigating chromosome segregation. Instructors can adapt the module to target any gene in any organism to assist with an active project. With this adaptation, students have the opportunity to engage in original research.

In addition to learning the technical skills of CRISPR construct design and genotype screening, another pedagogical goal of this module is to develop lab independence, initiative and confidence. Students often begin their scientific training expecting smooth protocols and clear results; when experiments do not proceed flawlessly, they view the experience as a failure. However, true scientific research is full of setbacks, repeated experiments, and unknown results. This module allows students to experience

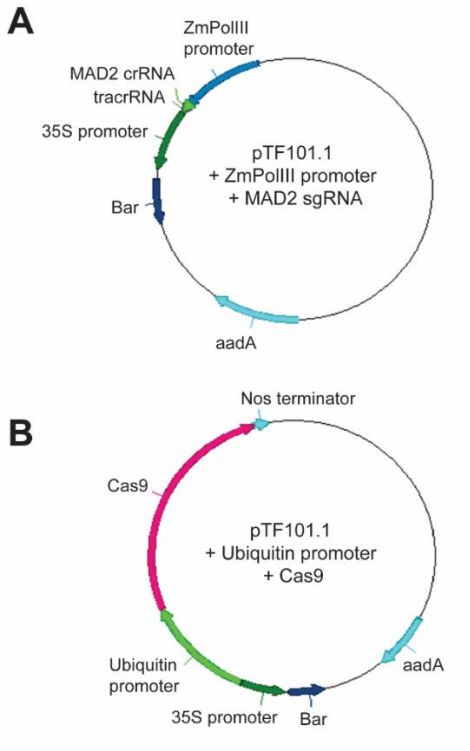
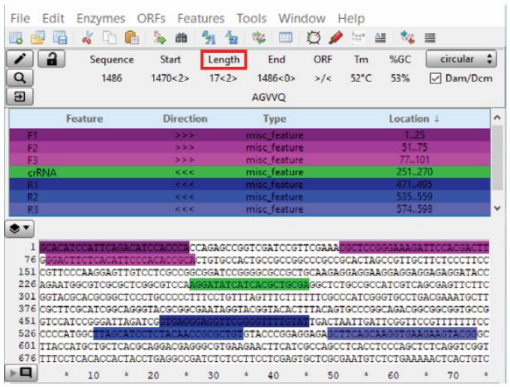


Figure 5. A) Map of sgRNA vector: completed vector contains the sgRNA expression cassette with the ZmPolIII promoter expressing the MAD2 crRNA+tracrRNA. The pTF101.1 backbone also contains selective markers to aid transformation into maize: the Bar gene to confer herbicide resistance and the aadA gene to confer spectinomycin/streptomycin resistance. B) Map of Cas9 vector: completed vector contains the Cas9 expression cassette with the ubiquitin promoter expressing the Cas9 gene.

A

Primer	Sequence
F1	GCACATCCATTCAGACATCCACCCA
F2	CGCTCCGGGAAAGATTCCACGACTT
F3	GGACTTCTCACATTCCCACACCGCA
R1	ATACAAAACCCCGAACCTCCCTCAC
R2	ACAGCGCGGTTGTAGAGGATGCTAA
R3	CCGTACTTCTTCACCTTGCTGAAGC



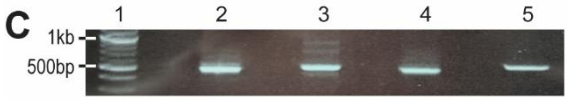


Figure 6. A) Table of primers provided to the students. Students are provided the primer sequences and asked to determine which are forward and reverse. Shown here are the three forward primers (F1-3) and three reverse primers (R1-3). Any forward primer can be used with any reverse primer. B) Students find (Edit>Find) and annotate the primer sequences on the MAD2 genomic DNA. It is critical that students annotate primers on the MAD2 genomic sequence rather than the MAD2 cDNA sequence because several of the primers anneal in introns. Forward primers are annotated in pink and reverse primers are annotated in blue. The target crRNA sequence is shown in green. After picking a forward and reverse primer for PCR, students determine the length of the product by highlighting the intervening sequence. Length is displayed in the main ApE window (red box). C) Sample student gel: lane 1 is a ladder and lanes 2-5 are student reactions. All reactions were successful with MAD2 bands around 500bp, but the sample in lane 3 has additional larger bands due to non-specific priming.

the challenges and rewards of original research, while using proven techniques that will succeed. Building in extra weeks beyond the five units allows students the opportunity to repeat and troubleshoot protocols.

Unit 3 is an excellent opportunity for troubleshooting, iterative practice, and discussion of controls. PCR can be challenging for students due to the required pipetting accuracy and multiple reaction components. To address PCR difficulties, we include a positive control (instructor-pipetted reaction with confirmed genomic template) and negative control (no polymerase) with each thermocycle run. Students often struggle with identifying which specific aspect of the experiment could have failed. A discussion of controls helps them distinguish between possibilities and rule out potential causes.

This lab module also builds student independence by removing much of the typical "behind the scenes" instructor assistance. We stress to the students at the beginning of the module that they are equal partners in this research project. They are responsible for keeping track of and properly storing their samples and reagents from week to week. They continue to care for their seedlings throughout the module until its genotype is determined. Students program their own thermocycler program, prepare their own buffers and gels for electrophoresis, and assist one another with challenges. By engaging in all aspects of research: design, preparation, execution, troubleshooting, analysis, and lab citizenship, students gain a fuller understanding and appreciation of the scientific process in addition to learning the valuable skill of CRISPR genome editing.

Figure 7.

A) Chromatogram displaying Sanger sequencing results. The first 10-15bp are unreliable reads (yellow box), identified by their irregular shape and spacing, and should not be included in the alignment. Beginning around 15bp, the peaks become regularly shaped with a single color peak rising above the baseline corresponding to the nucleotide identity. B) After trimming the sequence, students align their sequence with the MAD2 wild-type sequence using BLAST. Select "blastn" (red box), paste the wild-type sequence in the Query box, select the "Align two or more sequences" box (red arrow), paste the Sanger results in the Subject box, and hit "BLAST" (black box). C) Sample BLAST alignment results. There is 99% homology between the two sequences with a single nucleotide addition in the sample sequence. This addition falls within the target sequence (red box), where CRISPR-induced mutations will occur, thus this sequence reveals a MAD2 knockout mutant as a single nucleotide addition causes a frameshift.

Acknowledgements

The author is supported by a National Science Foundation grant MCB-1925546.

Special thanks to the many Hamilton College students who participated in this lab module through BIO248: Genes and Genomes, as well as teaching assistants Jodi Weiss, Michael Goldstein, Andre Burnham and Rylie Mainville who helped make this module a reality. Thanks to Hamilton College and the Biology Department for supporting the development and implementation of the module. Thanks to Marius Walter for permission to use his CRISPR-Cas9 graphic displayed in Fig. 1A.

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Connecting interdisciplinary research and place-based learning: Using a local ecosystem as the focus for an undergraduate research group

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Abstract: Undergraduate research experiences benefit students' scientific skills, and recent trends in undergraduate research and education include focusing on interdisciplinary projects and on place-based learning. Here we describe a semester-long pilot interdisciplinary undergraduate research program focused on local aquatic ecosystems, discuss perceived benefits of this research approach, and show how this program's structure could be modified for use at other undergraduate institutions. The program included nine undergraduate students and five faculty from multiple scientific disciplines (geography, virology, ecology, vertebrate and invertebrate zoology) and involved approximately half a day of research per student per week and weekly hour-long lab meetings of the entire group, followed by end-of-semester poster presentations to the university. Student self-assessment data indicated that the program improved students' perceptions of their scientific ability, as well as their comfort level at performing scientific skills. We suggest that bringing together faculty from multiple disciplines with projects focused on the same local ecosystem is a valuable technique for providing undergraduate students with not only hands-on research experience, but also with (1) exposure to a diversity of research areas and methods and (2) a better understanding of the ecosystems in and around their campus.

Introduction

Research experiences for undergraduate students have been shown to have many benefits, including increasing the likelihood of students pursuing STEM graduate degrees (Eagan et al. 2013). Among students that choose to attend graduate school, those that participated in research experiences as an undergraduate possessed stronger research skills than those that have no undergraduate research experience (Gilmore et al. 2015). A survey of undergraduates participating in research at liberal arts colleges showed that 91% of students reported professional and/or personal gains from their experience (Seymour et al. 2004), and Lopatto (2004) found similar positive results in a larger survey of >1100 undergraduate researchers. Most participating students appreciate the benefits of research experiences.

In the past two decades, some research experiences for undergraduates have shifted from a single-discipline focus to an interdisciplinary approach, which helps to prepare students for the interdisciplinarity inherent in many STEM careers and can increase diversity in research applicant pools (Raicu and Furst 2009). Another recent emphasis in some STEM disciplines has been a focus on place-based science teaching, defined by Semken and Freeman (2008) as a style of teaching that "focuses on local and regional environments and synthesizes different ways of knowing them." Place-based education may increase diversity in educational programs (Semken 2005) and increase both the students' academic performance and feeling of belonging (Johnson et al. 2020).

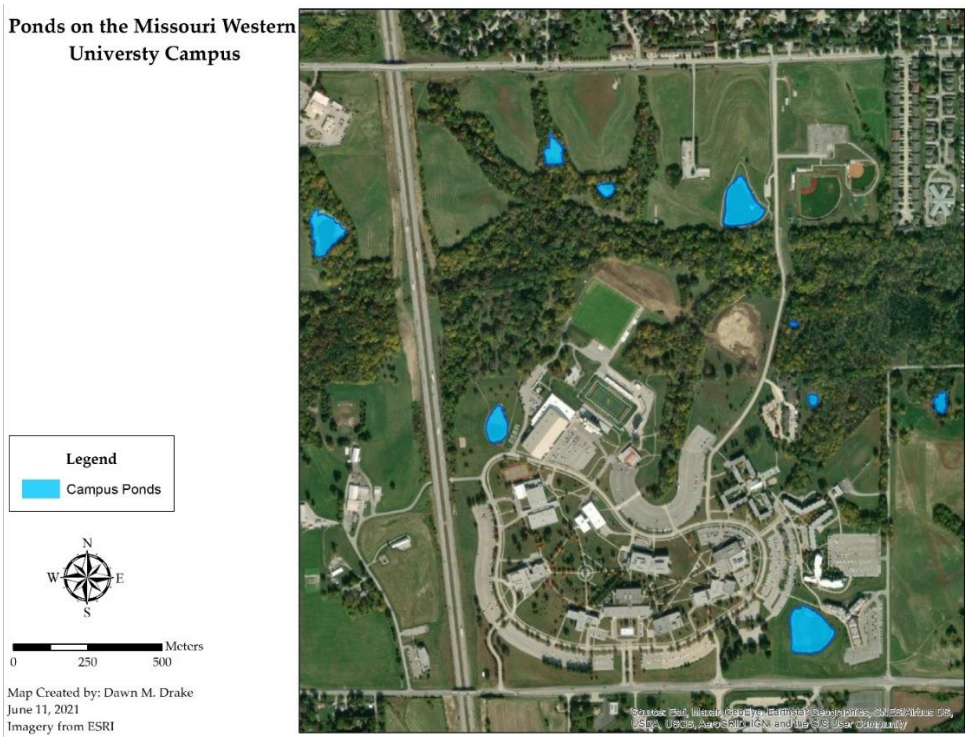
Some recent undergraduate research experiences have focused on combining an interdisciplinary approach with place-based learning (e.g., Montgomery 2020). Here we describe our development of a pilot interdisciplinary semester-long undergraduate research program focused on a specific local ecosystem, the Missouri Western State University campus ponds. Some faculty in the MWSU biology department have conducted research in the campus ponds for years, while others began pond-focused projects in the 2020-2021 academic year, and we determined that working together as a research group would allow us to address larger questions than our individual labs could address. The goal of this collaborative project was to begin to address the question "What biotic and/or abiotic factors drive diversity in these campus ponds?" The desired outcomes were (1) to provide students with a hands-on research experience focused on a piece of the group's overarching research question, (2) to give students the experience of participating in a collaborative, interdisciplinary research group with weekly lab meetings, and (3) formal student presentations of their research results in a poster symposium at the end of the semester.

Procedure

The large 740-acre (300-ha) Missouri Western State University campus, located in St. Joseph, includes nine former farm ponds with a range of sizes (0.05-0.97 ha), depths (0.65-2.2 m mean depth), and watershed characteristics; four ponds are in areas that receive heavy human use – e.g., in the middle of campus or beside roads/sidewalks – and five are in more remote wooded areas (Figure 1). These ponds provide an on-campus "natural laboratory" ideal for research projects from diverse disciplines.

Five faculty from different scientific disciplines (geography, virology, herpetology, ichthyology, and invertebrate zoology) led pond-focused research projects with the overarching research question of "How does watershed land use affect the biota of urban ponds?" We advertised for undergraduate research students via the Biology Department's Program of Research, Teaching, and Applied Learning (PORTAL) website, posters advertising the research interests of faculty, a department email listserv, announcements in geography classes, and a Powerpoint slide shown in freshman- and sophomore-level majors biology classes at the beginning of the semester. Nine students signed up to work with individual faculty on project topics including aquatic turtle population ecology, isolation of viral DNA from pond water, developing DNA markers for fish species identification, zooplankton population dynamics, and using GIS to map the ponds and their watersheds. Each faculty-student team determined their own research schedule and goals, which varied from developing new laboratory protocols to learning to use specific GIS and GPS applications, to directly testing hypotheses by collecting data in the field. In some cases, students helped to develop the initial project goal(s); in other cases, the faculty member had already selected a goal, but encouraged the student(s) to help determine how to address the goal.

Figure 1. Ponds on the Missouri Western University Campus.



Weekly lab meetings of the "Pond Team" students and faculty lasted 45-60 minutes. During the initial meeting, all members introduced themselves, learning objectives (Table 1) were explained, and the group toured four of the more accessible ponds to learn about their history and ecology from a faculty member. Subsequent meetings were organized, in rotation, by participating faculty labs and provided opportunities for students to present their project ideas and/or preliminary data to the group and for faculty to briefly introduce the group to their areas of expertise (which was eye-opening for students, as well as other faculty). Meetings also offered an opportunity for a brief weekly report from each student on challenges faced and progress made during the preceding week. Near the end of the semester, one lab meeting was devoted to a discussion of how to construct a conference-style scientific poster and prepare an "elevator speech" to go with it. In the last week of the semester, students' posters were presented to the rest of the university and community as part of a formal PORTAL undergraduate research symposium.

Development of this research program format was facilitated by our institution's transition to a four-class-days-per-week schedule that leaves Fridays open for applied learning. However, a similar format could fit within a more typical five-class-day-per-week schedule, as long as a set weekly lab meeting time could be established for the entire group and individual student/faculty groups could set aside time dedicated to research.

The program described here took place during the spring semester (January-May) but could be carried out during any semester, although fieldwork for some projects (e.g., trapping turtles) cannot feasibly occur during winter. Several individual faculty continued their pond-focused fieldwork over the summer, and this program will also be conducted in the fall semester.

Assessment

We requested that students complete a pre- and post-program self-evaluation of their comfort level in performing scientific skills as well as a post-program SALG URSSA (validated) assessment of their perceived gains during the program (available at salgsite.net/instrument/92544). Surveys were anonymous except for identifying codes used on the self-evaluations to ensure that students' pre- and post-program responses could be matched; one faculty member assigned the codes to individual students, and another faculty member handled the survey data.

Eight students completed the SALG URSSA assessment. Students perceived that they had experienced moderate to good gains in skills related to the program's learning objectives over the course of the semester, as indicated by a selection of responses to SALG URSSA questions (Table 2).

While there is high overlap in standard deviations for mean response scores, the two highest means suggest that students perceived great improvement in their ability to explain their research to others and in their understanding of what everyday research is like. Student responses also showed that the program had favorably impacted their attitudes toward research: they felt that they had engaged in real-world research and felt like scientists who were responsible for their own work, were able to think creatively about their projects, and were part of a scientific community (Table 3). Finally, open-ended responses indicated that students were positively impacted not only by the research experience, but also by the weekly interdisciplinary lab meetings (Table 4).

Table 1. Major learning objectives for the MWSU Pond Team.

Identify a gap in knowledge in their area of research
Develop a research question
Formulate hypotheses
Identify and demonstrate the ability to use appropriate research methods
Troubleshoot research methods
Work collaboratively with others
Work autonomously
Understand work in the diverse areas of the Pond Biodiversity Project and describe how their own projects fits in
Communicate effectively to explain their research project to (1) other people working on the Pond Biodiversity Project and (2) people who are not working on this project
Reflect on their research to identify strengths and opportunities for improvement

Table 2. Selected SALG URSSA scores for student self-evaluation of perceived gains in scientific skills (1=no gain, 2=a little gain, 3=moderate gain, 4=good gain, 5=great gain).

Scientific skill	Mean score (±SD)
Formulating a research question that could be answered with data	3.9 ± 0.8
Figuring out the next step in a research project	4.2 ± 0.5
Understanding the theory and concepts guiding my research project	4.1 ± 0.6
Identifying limitations in research methods and designs	4.1 ± 0.8
Conducting observations in the lab or field	4.2 ± 0.7
Comfort in working collaboratively with others	4.2 ± 0.9
Ability to work independently	4.1 ± 1.0
Understanding the connections among scientific disciplines	4.2 ± 0.7
Explaining my project to people outside my field	4.5 ± 0.5
Confidence in my ability to contribute to science	4.1 ± 0.6
Understanding what everyday research work is like	4.5 ± 0.8

Table 3. Selected SALG URSSA scores for student self-evaluation of extent of engagement in attitudes and behaviors (1=none, 2=a little, 3=some, 4=a fair amount, 5=a great deal).

Activity	Mean score (±SD)
Engage in real-world research	4.6 ± 0.5
Feel like a scientist	4.6 ± 0.5
Think creatively about the project	4.0 ± 0.5
Feel responsible for the project	4.5 ± 0.5
Feel a part of a scientific community	3.9 ± 0.8

Table 4. Selected SALG URSSA student comments.

I enjoyed meeting every week and being part of a team.
I think that this group meeting with multiple disciplines was a big step forward.
It really helped me to understand what it would be like to work hands on.
It promoted my interest in working with animals and studying in labs and conducting research to better understand our environment.
I just gained a lot of field experience and leadership skills.
I am very likely to enroll in a graduate program after doing these multiple research projects.

Discussion

The key points of our semester-long research program were (1) a research focus on an easily accessible local ecosystem, (2) a broad overarching project topic/research question that allows participation of faculty and students from multiple scientific disciplines, (3) hands-on research experience for undergraduates, (4) regular lab meetings that familiarize students with their colleagues' work and allow students to update the group on their own work, and (5) formal public presentation of research results by individual students that allows them to practice professional skills. These key points resulted in student perceived benefits, such as improved scientific skills and attitude towards research. By applying these key

points, this research program's structure could be modified for use at other undergraduate institutions.

Focusing on an easily accessible local ecosystem, as was done here with the Missouri Western State University campus ponds, could be readily modified for use at other undergraduate institutions. Place-based education can provide important benefits such as increasing diversity in educational programs (Semken 2005) and students' academic performance and feeling of belonging, as was seen in a place-based learning program for freshmen (Johnson et al. 2020). The size of the group could also be scaled to meet the needs of the institution. In our program the overall student: faculty ratio was 1.8 (nine students, five faculty), but individual faculty mentored one to five students, modifying their project plans based on the number of interested students. A higher student:faculty ratio in a program like this could be workable as long as projects are feasible for groups of students; for example, sampling water chemistry, macroinvertebrates, and turtles are research activities conducive to teams of students working together to collect data. Other teams could have small groups of students working on interrelated parts of the project. For instance, with the geography students, one group focused on mapping the ponds while another was completing a literature review in preparation for the next step in the watershed mapping portion of the project.

Having a broad overarching program goal that allows for the participation of faculty and students from multiple scientific disciplines, reinforced by regular lab meetings, provides important exposure for students to a diversity of research areas and methods. This certainly contributed to the students' perceived gains in scientific skills (their ability to explain their research to others and understanding what everyday research is like) and attitude towards research (engaging in real-world research, feeling like a scientist, feeling responsible for the project, and feeling a part of a scientific community). The application of an interdisciplinary approach for undergraduate research experiences helps to prepare students for the collaborative nature of many STEM careers and can increase diversity in research applicant pools (Raicu and Furst 2009). This year's Pond Team research group was a pilot program, and student comments and evaluations (both formal and informal), coupled with faculty experience, have given us indications of how this program can be modified in future semesters. Student evaluations were overwhelmingly positive, but a few metrics on the self-evaluation - for example, skill in data analysis - received lower scores, suggesting that these aspects could be more heavily emphasized in future semesters, to the extent possible for individual projects (some are more focused on development of methodology). Faculty were pleased to have an opportunity to learn more about their colleagues' research and to provide and receive feedback from peers in slightly (sometimes very) different areas of expertise. In addition to the benefits to personal research for the faculty, many of us feel better, prepared to discuss the different areas of research within the department with prospective students and their parents.

We feel that this pilot program was a success, and we plan to continue with the interdisciplinary Pond Team in the future. Plans for the upcoming year(s) include continuing with the research that was already started, moving into new areas, and inviting new disciplines. For example, we have added a geologist and feel the addition of a mathematician and/or statistician would also be helpful. This project has also served as a model for the developing an additional interdisciplinary, place-based team centered around a long-term on-campus prairie restoration project on 40 acres of former hay fields.

The typical undergraduate research experience was enhanced here by the application of an interdisciplinary approach and place-based learning, to create a program that demonstrated notable student perceived gains. In addition to facilitating hands-on research experience for undergraduates, this type of program results in public presentations by the students. Both of these have well-acknowledged benefits, such as increasing the likelihood of students pursuing STEM graduate degrees (Eagan et al. 2013) and students possessing stronger research skills (Gilmore et al. 2015). Most participating students appreciate the benefits of research experiences (Lopatto 2004 and Seymour et al. 2004). Participating in a coordinated multidisciplinary research program requires notably more time and effort than conducting research with undergraduates as a solo endeavor, but we found that students (and faculty) benefited greatly from this combined effort focused on a familiar local ecosystem.

Acknowledgements

Funding for this program was provided by the Missouri Western State University Department of Biology. Assessment data were collected under Missouri Western State University IRB protocol 3455.

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Combating the Negative Exam Score Impact of Online Human Physiology Laboratories via Cognitive and Structural Strategies: A Preliminary Analysis

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Abstract: The COVID-19 pandemic forced higher education to develop new strategies to meet the needs of students. One of the most critical issues was delivering an online experience in undergraduate Biology laboratories aimed at meeting hands-on outcomes, specifically within the health sciences. Hybrid lab group models in which students rotated between in-person and online attendance represented one such option. A student population of 71 at Minnesota State University, Mankato was studied to observe the effects on student exam scores. Negative student perceptions were made clear in course feedback surveys, and students that attended 2+ labs via Zoom preceding an exam were associated with a lower Exam I score compared to peers that only attended 0-1 labs via Zoom. Three class strategies were introduced before Exam II, meant to improve online learning. The Exam II scores saw no overall significant difference between groups, with Multi-Zoom students closing the gap from Exam I. Importantly, Multi-Zoom students that utilized the class strategies significantly outperformed Multi-Zoom students that that did not use them. Although any classroom results represent a mix of variables, the risks of online labs intended to assess hands-on and data interpretation outcomes of Human Physiology can be detrimental when targeted strategies are absent.

Keywords: Hybrid, Online, Physiology, Lab, Students, Pre-health

Introduction

The emergence of COVID-19 in 2020 demanded new strategies for a return to higher education institutions ("COVID-19: 20 Countries' Higher Education Intra-Period Digital Pedagogy Responses" 2020). While many educators favored a fully online approach, others adapted a hybrid (FlexSync, HyFlex) method. This challenge was felt particularly hard by the life sciences, as most laboratory sections require students to demonstrate hands-on outcomes to succeed. This is extremely true for the health sciences. Classes such as Anatomy, Physiology, and Microbiology are especially important for success as a healthcare professional since they represent students' first opportunity to collect and analyze physiological data. Human Physiology courses are typically enriched for student pursuing a career in the health sciences, although a more diverse array continues to join the population (Griff 2016). The key conceptual outcomes from lab include Homeostasis, Cellular Physiology (Diffusion and Osmosis), Action Potential, Skeletal Muscle, Cardiac Muscle, White and Red Blood Cells, Lung Physiology, and Kidney Physiology. Moreover, these classes are typically some of the largest on a campus with 10,000+ enrolled students. Meeting the demands of a large student population and COVID-19 space guidelines represents a unique challenge. Another downfall of hybridized, online learning is that the majority of students do not prefer this classroom approach (Adnan 2020). Students cite the lack of hands-on experience and classroom connection as factors. The typical challenges of teaching were exacerbated by a slew of new factors. However, these poor perspectives are not reflected in other primary literature documenting the success of virtual laboratories (Mahaffey 2018; Brinson 2015; Lombardi et al. 2014). Specifically, students cite that the use of structured, asynchronous modules helped keep them on track and improved their experience (Lima et al. 2020). It would appear that exchanging in-person opportunities for a hybridized schedule where students occasionally attend online should only be made when supported by targeted learning structures. This appears especially true for Organismal Biology laboratories that involve gathering live heath data from participants or animal tissues, as is done in Human Physiology.

The contents of this report focus on the effects of online participation in 3 hybridized Human Physiology laboratory sections at Minnesota State University, Mankato. A hybrid approach was utilized in BIOL 330 Human Physiology to facilitate laboratory sections of 24 students to fit in a room with capacity for 16 due to COVID-19 regulations. The lab curriculum was reflective of a typical Human Physiology course (Supplemental Table 2). Lab partner groups of 3 were randomly designated at the beginning of the semester, resulting in 8 groups per section. Each week, 1 member would attend lab via Zoom and 2 would attend in-person. The lab member designated for Zoom would rotate each week. Each group would complete and restart the cycle every 3 weeks.

The hybrid approach facilitated 3 primary benefits. First, labs composed of 24 students could meet COVID-19 space regulations. Second, students that were sick or exposed had a means to attend lab with the online structures in place. Lastly, in-person students received a higher percentage of instructor attention, allowing for unique in-depth learning opportunities. However, the detriment of this structure was clear, with the primary downfalls felt most by students attending online. These pitfalls included technology issues, lack of teamwork/communication, managing the hybrid schedule, and the obvious loss of hands-on learning, the final being most impactful since most labs relied on live experiments or animal tissues. The report herein seeks to document the effects of a hybrid approach on student lab

exam scores, analyze the results by levels of online participation, and highlight steps for the future.

Materials and Methods

Study Population and Data Collection

A total of 71 Human Physiology student exam scores were included in this study, from a course of 253 enrolled students. The 71-student population within this study was divided between 3 laboratory sections. All 3 lab sections followed the same hybrid protocol. One additional lab section of 21 student exam scores were used as a supplemental cohort, and these students were attending a lab with the same hybrid protocols. The population of 71 was split into 3 lab sections – two sections of 24, and one section of 23. 3 students dropped between Exam I and Exam II, reducing the Exam II population to 68. All students received the same study resources, which include access to professors, practice exam questions, guiding questions, their lab notebooks, and lab instruction. The only difference between section exams was the order of questions. Some student took advantage of more resources than others, specifically utilizing professor office hours to discuss guiding questions, but these variables do not represent isolatable categories. Composition of student majors are shown in Figure 1A, and integrated data is available in Supplementary Table 1. Data was collected with course feedback surveys, tracked use of learning platform resources, and examination results – all non-identifying information.

Data Collection

Student majors were documented through the student portal system. Post-exam course feedback surveys were administered to students and were optional with consent. Some students did not complete these. In-person vs. online attendance was tracked by typical attendance procedures. Online participants that chose not to turn on their camera were encouraged to do so. If an online student did not respond to multiple inquiries, they were considered absent from that point and did not receive credit for the lab. These regulations were built into the course to adhere to COVID spacing regulations. Exam scores were collected from the course learning system.

Hybrid Schedule and Normal vs. Multi-Zoom Designation

A typical student schedule would only have a student attend 1 lab via Zoom per every 3 weeks. Each group member would rotate weekly. However, students were required to utilize Zoom in case of sickness or quarantine (likewise, this meant that their lab partners were consequently eligible to attend more in-person labs). At times, this resulted in a student attending more than the planned numbers of labs via Zoom. These students were classified as Multi-Zoom. Any student that attended 2-3 of the first 3 labs via Zoom preceding Exam I was classified as Multi-Zoom, and any student that attended 2-4 of the 4 labs preceding Exam II was classified as Multi-Zoom. Any student that attended 0-1 labs via Zoom preceding Exam I or II was classified as normal. Students were not permitted to Zoom for the sake of convenience.

Laboratory Examination Format and Scoring

Lab exam scores were utilized as a measurement of student success. Exams were based on lab learning outcomes, reinforced with guiding questions at the beginning of each section in the lab notebook. The questions are based on a mixture of laboratory concepts, data interpretation, and application to clinical situations. Both exams were comprised of 100% multiple-choice questions that totaled 75 points. The course total was 825 points, so the 2 exams analyzed in this manuscript represent 18.1% of the course grade. Correct answers earned 100% of a questions value while wrong answers earned 0%. Exam I covered content from 3 labs across 18 questions. Exam II covered content from 4 labs across 24 questions. Each exam was completed in 50 minutes or less. Scoring statistics and information can be found in Table 1. All students marked that enough time was allotted for each exam, and none failed to finish or failed to attempt any questions. Exam format statistics, concepts tested, and example questions can be found in Supplementary Table 2.

Classroom Structure and Cognitive Learning Strategies

The primary strategies were introduced to labs with the intention of closing the gap between normal and Multi-Zoom students (Supp. Table 3). First, Zoom cameras were to be turned on to increase engagement. Second, groups were instructed to work together to create a plan for lab and designate roles, including for the Zoom participant. For instance, one member could be the subject of the experiment, another member could run the data collection program, and the Zoom partner could read along instructions and identify how they related to assignment questions. Lastly, those who participated over Zoom were highly encouraged to fill out a short cognitive reflection survey administered through Microsoft Forms. These strategies were created with the intention of increasing engagement, teamwork, and reflection (Coulson and Harvey 2013; Huitt, Killins, and Brooks 2015).

Statistical Analysis

For the identification of statistical trends between categorical groups of students, raw and percentage test scores were measured. GraphPad Prism software and GenePattern tools were used to plot and format figures, analyze data, and calculate statistical significance (Reich et al. 2006). Comparison of quantitative data between two groups was done by Welch's t-test or a Brown-Forsythe ANOVA test (Table 1) if multiple groups were measured. Parts of a whole statistics were evaluated with Expected vs. Observed analyses, with the null expected ratios being 50/50. When an additional 21 students from another lab section were added to the 71-student cohort to generate a larger study population, all 92 student Exam I scores were normalized to unified Z-score metric and measured. The Welch's t-test comparing these groups used this normalized Z-score value in favor of raw exam scores to correct for the different exam. All reported P values were two-sided. P values less than 0.05 were considered statistically significant. All student data can be found in Supplementary Table 1 and is available upon request.

Results

Student Population and Exam Landscape

The population and course structure were representative of large, non-R1 university Human Physiology courses. There was an enrichment of nursing majors within the population (Figure 1A). The hybrid format – 1 week on Zoom for every 2 weeks in-person – was not well-received by students, with a

substantial 82.5% of respondents indicating that joining a hands-on Human Physiology lab through Zoom had a negative impact on their learning (63/71 feedback responses submitted) (Figure 1B). These results represented a significant difference from the expected 50/50 null hypothesis if students had no clear preference ($P < 0.0001$).

Laboratory exams were utilized as a metric of student success. Exam statistics and student success are presented in Table 1. Notably, Exam II saw a significant reduction in scores from Exam I (Figure 1C), dropping by an average of 16%. Exam I covered 3 labs of material, and Exam II covered 4 (Supplemental Table 2). The difference between Exam I and Exam II was calculated for each student, a measurement of their growth, and data was normalized when noted to account for the 16% decline.

Attending Multiple Labs Over Zoom was Associated with Lower Exam I Scores

The vast majority of the 71 students indicated that attending lab via Zoom was detrimental (Figure 1B). These perspectives were reinforced by the results of Exam I, which documented a lower exam score for those that had to attend 2-3 of the 3 assessed labs via Zoom (Multi-Zoom) vs students that only had to attend 0-1 via Zoom (Figure 2: Normal). Data from an additional lab section of 21 students following the same hybrid protocol was collected and similarly categorized for Zoom frequency. Combined analysis of the 92 normalized student Z-scores reinforced the possible disadvantage faced by Multi-Zoom students (Figure 2) ($P = 0.052$). A Z-score difference of -0.160 ± 0.079 points existed between the groups. Notably, there was a 0.317 Z-score difference in Quartile 1 (25th percentile) between the two groups. No specific questions were enriched for poor responses in the Multi-Zoom group after multiple t-test FDR-corrected analysis with GenePattern Marker Analysis (Supplemental Figure 1).

Classroom Changes were Associated with Lessened Impact of Multiple Online Labs

A set of class changes were implemented after Exam I due to the lower scores of Multi-Zoom students (Supplemental Table3). These were intended to help students before Exam II, which was larger and more thorough since concepts from 4 lab periods were being assessed. Exam II nonetheless proved significantly more difficult than Exam I (Table 1). As an additional measure of student understanding, the difference between Exam I and Exam II % score was measured. The class average was a 16% drop. Notably, having 4 labs preceding Exam II caused an increase in students that had to attend 2 or more via Zoom. Exam II had 27 Multi-Zoom students, an increase from the 12 on Exam I. Whether the Multi-Zoom group made up ground or the normal group lost it, the scores between both groups were remarkably similar (52.78 vs. 51.56; $P = 0.6931$) (Figure 3A). The gap that existed between these groups on Exam I did not emerge in Exam II. For normal vs. Multi-Zoom groups, Quartile 1 (60.5 vs. 63.0) and Quartile 2 (both 53) remained very similar between groups, and a lower Quartile 3 was once again present in the Multi-Zoom group (40 points vs 49.5 in the normal group). One caveat is that 3 of the 71 students dropped the course before Exam II. These students scored an average of 34/75 (45.3%) on Exam I, and all 3 represented Multi-Zoom individuals. The difference in % between Exam I and II was also used as a metric of success, and normal vs. Multi-Zoom students did not produce a significantly different result (Figure 3B). Neither normal (-17.43%) or Multi-Zoom students (-13.71%) were significantly divergent from the class average decline of -16.0%.

The 27 Exam II Multi-Zoom students were isolated to study patterns of improvement. Of these 27 students, 16 (59.3%) chose to utilize the cognitive strategies suggested after the Exam I gap was found (Supplemental Table 3).

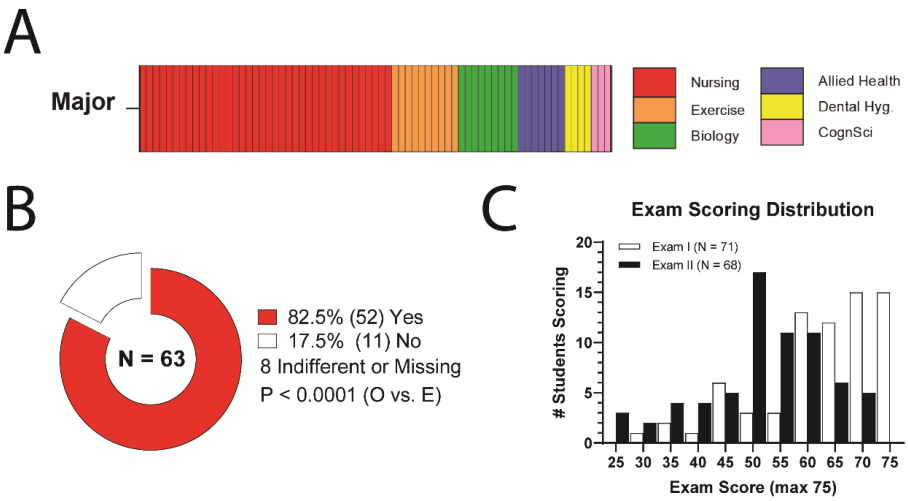

Figure 1: Student Population and Exam Statistics. The study population is comprised of 3 laboratory sections of Human Physiology assessed across 2 exams. (A) Student majors are summarized in a waterfall plot. A single student equals a single column. A color legend designates majors. (B) Proportions of student responses to the question "Does attending lab over Zoom negatively impact your learning?". The results are reported in a pie graph and analyzed for significance with an Observed vs. Expected analysis, which assumes a null hypothesis of 50/50 preference. (C) Exam I and II score distributions are represented by a histogram. Raw exam scores out of 75 points are reported. Exam points and the difference between Exam I and II (in %) were used as a measure of student success.

Table 1. Human Physiology Exam Characteristics

	Exam I (N = 71)	Exam II (N = 68)	P-value
# Questions	18	24	
Points	75	75	
Time	50 minutes	50 minutes	
Labs Assessed	3	4	
Score Average	63.0 (84.0%)	51.6 (68.8%)	< 0.0001*
Score Standard Deviation	11.3 (15.1%)	11.4 (15.2%)	0.907*
Quartile 1	58 (77.3%)	44.5 (59.3%)	0.584†
Quartile 2 (median)	67 (89.3%)	52 (69.3%)	
Quartile 3	71 (94.7%)	61 (81.3%)	
Minimum	29 (38.7%)	25 (33.3%)	
Maximum	75 (100%)	71 (94.7%)	
Range	46 (61.3%)	46 (61.3%)	

* Unpaired t test
† 2-way ANOVA

Table 1. Statistical Comparison of BIOL 330 Human Physiology Lab Exams. Cumulative data was collected from students in all 3 sections to be analyzed together. All sections were led by the same instructor, aimed to meet the same outcomes, and were assessed with the same exams. Exam I occurred on week 4 of the semester and Exam II occurred on week 9 of the semester. Labs did not meet on weeks with a lecture exam.

Multi-Zoom students that utilized the strategies significantly outperformed their counterparts that didn't, scoring an additional 11.87 ± 4.77 points (15.8% ± 6.36%) (P = 0.0266) (Figure 3C). Interestingly, Multi-Zoom students that utilized the strategies (N = 16; 55.69) had a higher point average than normal students (N = 41; 52.78), although this result was not statistically significant

When the difference in % grade between Exam I and II (example: 70% - 65% = -5%) is used as the success metric, there is still a significantly large difference between Multi-Zoom students that used the strategies (-8.08%) between those that didn't (-22.06%) (P = 0.0098) (Figure 3D). These groups were polar between the class average of -16% difference. A dot plot visualizes these rates of decline, with strategy users marked in green and non-users in grey (Figure 3E). These results display students that started with a high Exam I score and declined sharply on Exam II vs those that experienced more stable declines (or in some cases rises). Lastly, Exam II data was divided into 4 groups based on 2 designations: Normal vs. Multi-Zoom and Cognitive Strategies Used vs. Unused and analyzed using a Brown-Forsythe ANOVA test. Although the Multi-Zoom + Strategies Used group displayed the highest average, the overall results were not significant (P = 0.0826) (Supplemental Figure 2). Only 3 students were designated as Multi-Zoom for both Exam I and II. These students did not reflect any statistical trends. In contrast, 35 students were normal participants for both Exam I and II, but again, these students did not display a statistically significant advantage in terms of averaged Exam I/II score. Interestingly, the 11 students that marked that Zoom does not inhibit their learning (Figure 1B) dropped an average of 21.3% between Exam I and Exam II, more than the class average 16.0% drop. Only 5 of these 11 students had been on multiple Zooms for either Exam I or Exam II.

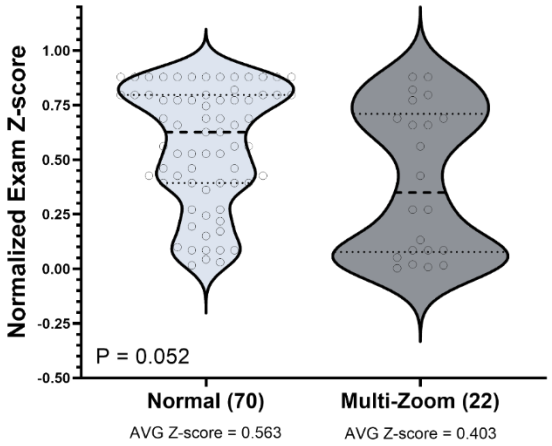

Figure 2: Students that Attended Multiple Labs Over Zoom were Non-significantly Associated with Lower Exam I Scores. Exam I scores are used as a measurement of student success (75 points). Three labs worth of material were tested on Exam I, so students were designated as Multi-Zoom if they attended 2 or 3 of these online. An additional 21 students from one additional section were added to the population, with all 92 scores normalized to Z-scores. These scores are used as the metric between normal and Multi-Zoom students, each represented by a clear bubble. Violin plots demonstrate the population differences and Exam I Z-score values between normal (cyan) and Multi-Zoom (red) students. Clear bubbles represent raw student exam scores out of 75 points. Large, dashed lines within each group represent the average. Small, dashed lines represent Quartile 1 (lower) and 3 (upper) ranges. Differences were measured using a Welch's t-test.

Discussion

Performing hands-on laboratories intended to prepare students for hands-on health science fields over an online

platform was expected to come with negative consequences. This was bolstered by low student opinions of hybrid lab models. The main issues cited in class discussions included alack of hands-on experience with material, insufficient inclusion by in-person partners, and the ability for students to ignore/miss important concepts due to the online format. This report measured the statistical differences in terms of laboratory exam performance, as these exams were intended to measure outcomes associated with patient and tissue data and its interpretation. We are the first to report that while a trend towards poorer exam performance was observed, it can be somewhat mitigated through the implementation of targeted class structures and cognitive strategies.

This report followed a representative group of students in a Human Physiology course during the fall of 2020 at Minnesota State University, Mankato. During the COVID-19 pandemic, hybrid models were utilized to facilitate large enrollment courses, including laboratories. Attending via online platforms was predictably disdained by a large percentage of students. The resources do not exist for many high-enrollment schools to host 100% in-person labs given COVID-19 regulations, so the ability to participate in labs via Zoom proved vital for keeping students connected, especially when they had to quarantine. Altogether, while Zoom was not perfect, it was a much better solution than static approaches, despite student wishes for a full in-person lab experience.

The results of Exam I demonstrated the detrimental effect of attending too many of the labs via the online Zoom platform, with a nearly significant gap emerging between students that attended 0-1 of the labs via Zoom (normal) vs those that had to attend 2-3 of the labs (Multi-Zoom) online. While this result was suspected, it had yet to be observed in a controlled setting with a large number of students. Several Multi-Zoom students informally expressed concern preceding the exam, admitting that in-person experiences were their preferred method of learning.

While it is unscientific to speculate, it should be noted that several students within the cohort cited repeated but variable impasses that prevented them from attending scheduled in-person labs, often unrelated to COVID. Given the supportive structure necessary to maintain class throughout the pandemic, these online attendances were allowed, as well as when absolutely any indication of sickness or contact was cited. Students were also not strongly encouraged to participate online with cameras on during the first 3 weeks, so the effects of attending via Zoom but not being present behind the camera could have exacerbated the detriment. Circumstances aside, the data aligns with student and faculty perspectives that hands-on, Organismal Biology laboratories conducted over an online platform do not predict success on assessments. Virtual labs in place of hands-on counterparts have been observed to meet learning outcome criteria, but these results indicate that traditional labs may still have more to offer most healthcare-oriented students (Hensen et. al., 2020).

Given the steep fall between groups, new strategies were highly encouraged for all 3 lab sections. Although these steps were simple, they represented evidence-based strategies that could buoy learning despite the circumstances

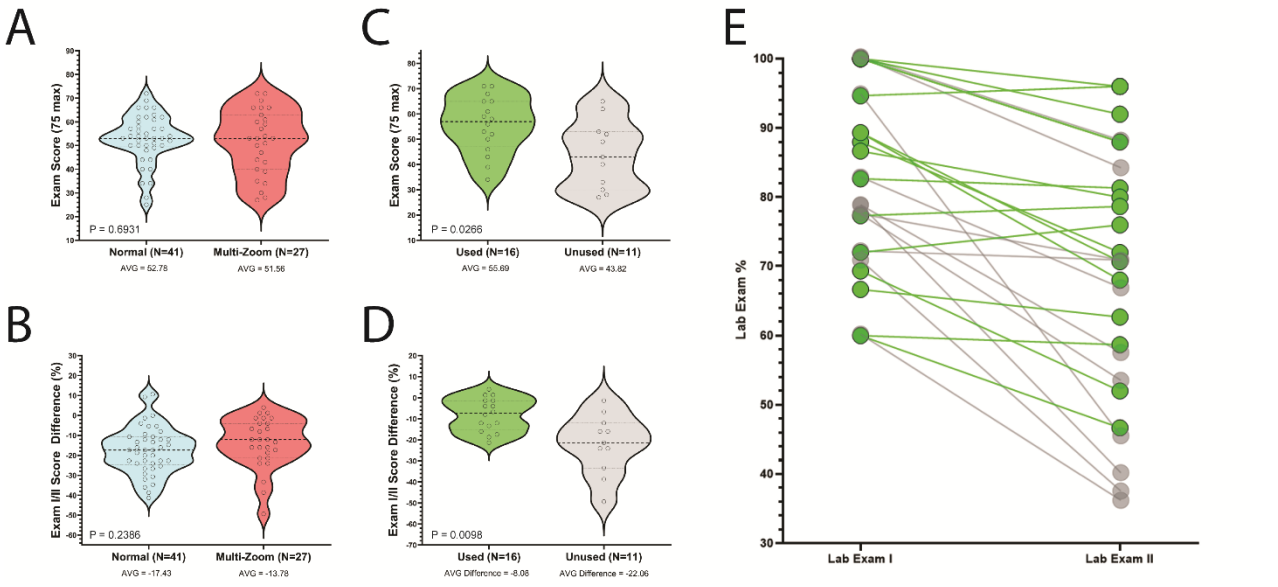

Figure 3: Higher Multi-Zoom Exam II Scores are Associated with Utilization of Classroom Strategies. Three classroom cognitive strategies were introduced to the class after the trend between normal and Multi-Zoom students emerged. (A) Violin plots demonstrate the Exam II scoring differences between normal (cyan) and Multi-Zoom (red) students. Each clear bubble represents a raw student exam score out of 75. Large, dashed lines within each group represent the average. Small, dashed lines represent Quartile 1 (lower) and 3 (upper) ranges. Differences were measured using a Welch's t-test. (B) Violin plots demonstrate the difference in score (%) between Exam I and Exam II. (C) Raw Exam II scores are measured between Multi-Zoom students that utilized the cognitive strategies (green) vs. those that did not (grey). Quartiles and average are displayed similarly between A and B. (D) The difference between Exam I and II scores (in %) are measured between Multi-Zoom students that utilized the cognitive strategies (green) vs. those that did not (grey). (E) A dot plot displays the movement in Exam score (%) between Exam I and II of the 27 Multi-Zoom students. Those that used the cognitive strategies are displayed green. Those that did not are displayed grey.

(Supp. Table 3). Other classrooms have found success implementing similar strategies in STEM (Peng et al. 2020; Villanueva et al. 2020). Exam II assessed concepts from 4 laboratories, so the exam itself was larger, more comprehensive, and caused more of the class to attend 2+ labs online due to the alternating 3-week rotation schedule. The results of Exam II showed a marked improvement for students that attended multiple Zooms, with the performance gap closing. The overall class average decreased significantly though, most likely due to the added content.

Because the Multi-Zoom group erased their deficit in Exam II, factors for success were examined. Among the factors was the use of the cognitive strategies mentioned above, which students marked "Used" or Unused" on the survey questions after Exam II. The data made it very clear: Multi-Zoom that utilized the strategies earned a significantly larger number of raw points and saw a superior average difference in the Exam I to II % scores. Those that used Zoom for multiple labs preceding Exam II and did not decide to utilize the strategies were associated with a significantly lower point total and average difference between Exam I and II, earning lower than class average marks in both categories. Although the strategies were shown to likely influence a more positive outcome, other factors preceding Exam II need to be accounted for. For instance, 3 of the 71 students dropped the course before Exam II. All 3 were Multi-Zoom for Exam I. Additionally, it makes sense that more prepared students would take the time to read and utilize the cognitive strategies, hence why that Multi-Zoom group may have done better no matter what. The other factor is that students may have acclimated better towards the online format. Teamwork likely improved between partners as well, but equally, other groups may have worsened, all depending on context. That said, confounding factors associated with the reporting of these data remain caveats that require consideration.

This work provides an exploratory statistical evaluation of the performance of hybrid classroom approaches for hands-on Human Physiology laboratories. While obvious weaknesses to the approach exist, with some modifications to the group/class structure, some of the detriment incurred by online participation can be remedied. As the COVID-19 pandemic subsides in the future, the hybrid laboratory approach may still carve out a refined role at larger institutions looking to engage more students.

Authors' Contributions

KTH was responsible for collecting data, and analyzing data, creating figures, and authoring the manuscript. Dr. Michael Minicozzi and Dr. Brittany Smith are acknowledged for their contributions of data sharing and supportive consultation, respectively.

Funding

No external or internal funding support was provided.

Disclosure of Conflicts of Interest

The are no conflicts of interest to report.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article [and its supplementary information files]. Requests for additional data may be sent to the author.

Consent Statement

The data herein is archival since it is de-identifiable information that was collected for normal class purposes with the hopes of being used to make beneficial adjustments to the course during the semester.

Ethical Statement

All data collected will remain strictly confidential. All participants were assigned randomized, unique identification numbers used throughout data analysis and reporting. No key linking these identifiers to the students exists.

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Hartert Supplemental Table 1: Population Statistics

Student #	Major	Exam I Points	Exam I %	Multi-Zoom (Exam I)	Zoom bad?	Exam II Points	Exam II %	Exam I/II Difference	MultiZoom (Exam II)	Zoom strats. used	Avg Exam %
1	ALLIED HEALTH	54	72.0	1	1	56	74	2.7	1	1	73.3
2	ALLIED HEALTH	75	100.0	1	0	62	82	-17.3	1	0	91.3
3	NURSING	67	89.3	1	1	50	66	-22.7	1	1	78.0
4	EXERCISE SCI	45	60.0	1	1	43	57	-2.7	0	0	58.7
5	NURSING	75	100.0	1	1	52	69	-30.7	0	1	84.7
6	NURSING	70	93.3	1	0	61	81	-12.0	0	0	87.3
7	NURSING	59	78.7	1	1	27	36	-42.7	0	0	57.3
8	NURSING	71	94.7	1	0	52	69	-25.3	0	1	82.0
9	BIOLOGY	67	89.3	1	1	56	74	-14.7	0	1	82.0
10	EXERCISE SCI	67	89.3	0	1	52	69	-20.0	1	1	79.3
11	NURSING	58	77.3	0	1	58	77	0.0	1	1	77.3
12	NURSING	52	69.3	0	1	39	52	-17.3	1	1	60.7
13	ALLIED HEALTH	50	66.7	0	1	46	61	-5.3	1	1	64.0
14	EXERCISE SCI	62	82.7	0	1	61	81	-1.3	1	1	82.0
15	EXERCISE SCI	58	77.3	0	0	40	53	-24.0	1	0	65.3
16	BIOLOGY	75	100.0	0	1	65	86	-13.3	1	1	93.3
17	NURSING	59	78.7	0	X	43	57	-21.3	1	0	68.0
18	NURSING	62	82.7	0	1	49	65	-17.3	1	0	74.0
19	ALLIED HEALTH	58	77.3	0	X	52	69	-8.0	1	0	73.3
20	BIOLOGY	75	100.0	0	1	65	86	-13.3	1	0	93.3
21	NURSING	66	88.0	0	1	53	70	-17.3	1	1	79.3
22	COGN SCIENCE	53	70.7	0	0	28	37	-33.3	1	0	54.0
23	NURSING	75	100.0	0	1	68	90	-9.3	1	1	95.3
24	COGN SCIENCE	71	94.7	0	X	33	44	-50.7	1	0	69.3
25	NURSING	45	60.0	0	1	43	57	-2.7	1	1	58.7
26	NURSING	71	94.7	0	1	71	94	0.0	1	1	94.7
27	DENTAL HYGIE	75	100.0	0	1	65	86	-13.3	1	1	93.3
28	NURSING	45	60.0	0	1	27	36	-24.0	1	0	48.0
29	NURSING	59	78.7	0	1	30	40	-38.7	1	0	59.3
30	ALLIED HEALTH	65	86.7	0	1	59	78	-8.0	1	1	82.7
31	EXERCISE SCI	45	60.0	0	1	34	45	-14.7	1	1	52.7
32	BIOLOGY	75	100.0	0	1	71	94	-5.3	1	1	97.3

33	NURSING	54	72.0	0	X	53	70	-1.3	1	0	71.3
34	NURSING	75	100.0	0	1	52	69	-30.7	0	1	84.7
35	NURSING	62	82.7	0	1	51	68	-14.7	0	1	75.3
36	NURSING	63	84.0	0	1	62	82	-1.3	0	0	83.3
37	BIOLOGY	70	93.3	0	0	49	65	-28.0	0	0	79.3
38	NURSING	70	93.3	0	0	65	86	-6.7	0	0	90.0
39	EXERCISE SCI	71	94.7	0	X	56	74	-20.0	0	0	84.7
40	NURSING	67	89.3	0	1	53	70	-18.7	0	0	80.0
41	NURSING	63	84.0	0	1	34	45	-38.7	0	1	64.7
42	DENTAL HYGIE	75	100.0	0	1	68	90	-9.3	0	1	95.3
43	ALLIED HEALTH	45	60.0	0	1	33	44	-16.0	0	1	52.0
44	ALLIED HEALTH	75	100.0	0	1	71	94	-5.3	0	1	97.3
45	EXERCISE SCI	41	54.7	0	1	49	65	10.7	0	0	60.0
46	BIOLOGY	75	100.0	0	0	65	86	-13.3	0	0	93.3
47	EXERCISE SCI	75	100.0	0	1	59	78	-21.3	0	1	89.3
48	NURSING	71	94.7	0	0	52	69	-25.3	0	0	82.0
49	NURSING	59	78.7	0	0	40	53	-25.3	0	0	66.0
50	BIOLOGY	62	82.7	0	1	50	66	-16.0	0	1	74.7
51	NURSING	71	94.7	0	1	61	81	-13.3	0	0	88.0
52	DENTAL HYGIE	71	94.7	0	1	56	74	-20.0	0	1	84.7
53	NURSING	71	94.7	0	0	53	70	-24.0	0	0	82.7
54	BIOLOGY	58	77.3	0	1	49	65	-12.0	0	0	71.3
55	EXERCISE SCI	70	93.3	0	1	56	74	-18.7	0	0	84.0
56	EXERCISE SCI	67	89.3	0	1	50	66	-22.7	0	0	78.0
57	NURSING	67	89.3	0	1	40	53	-36.0	0	1	71.3
58	NURSING	46	61.3	0	1	52	69	8.0	0	1	65.3
59	NURSING	75	100.0	0	1	61	81	-18.7	0	0	90.7
60	NURSING	49	65.3	0	1	25	33	-32.0	0	0	49.3
61	BIOLOGY	70	93.3	0	1	65	86	-6.7	0	0	90.0
62	COGN SCIENCE	70	93.3	0	1	61	81	-12.0	0	0	87.3
63	NURSING	58	77.3	0	1	50	66	-10.7	0	0	72.0
64	NURSING	66	88.0	0	1	53	70	-17.3	0	0	79.3
65	NURSING	65	86.7	0	1	48	64	-22.7	0	1	75.3
66	NURSING	75	100.0	0	1	55	73	-26.7	0	1	86.7
67	NURSING	70	93.3	0	1	44	58	-34.7	0	0	76.0
68	DENTAL HYGIE	75	100.0	0	1	58	77	-22.7	0	1	88.7
69	NURSING	36	48.0	1	X	X	X	X	X	X	X
70	NURSING	37	49.3	1	X	X	X	X	X	X	X
71	NURSING	29	38.7	1	X	X	X	X	X	X	X

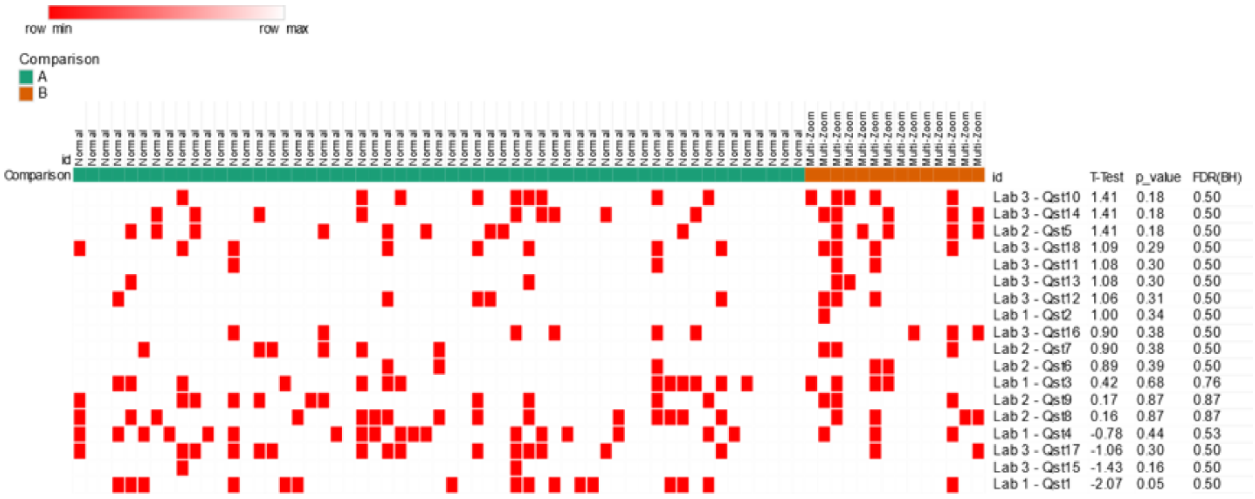
Hartert: Supplemental Table 2: Exam Content

Lab #	Concepts	Exam
1	Homeostasis, Feedback	I
2	Diffusion, Osmosis, Transport	I
3	Action Potential	I
4	Electromyogram (muscles intro)	II
5	Skeletal Muscle (frog muscle)	II
6	Cardiac Muscle (turtle heart demo)	II
7	Electrocardiogram	II
8	Red and White Blood Cells, Immunology	III
9	Lung Volumes	III
10	Kidney (online)	III
	Question Examples	
	Exam I: (5 points) You measure a patient's heart rate and record these readings in BPM: 62, 64, 62, 60, 68, 64. What is the setpoint of this patient's heart rate, and what is their range?	
	A. 63.3 BPM setpoint, 4 BPM range	
	B. 63.3 BPM setpoint, 8 BPM range	
	C. 68.0 BPM setpoint, 4 BPM range	
	D. 68.0 BPM setpoint, 8 BPM range	
	Exam I: (4 points) A sub-threshold stimulus to a single neuron's axon will produce which of the following?	
	A. Due to recruitment, a small action potential will be produced	
	B. Since threshold was not reached, no action potential will be produced (neurons are all or nothing)	
	C. Since action potentials are graded, a very weak stimulus will produce a very small action potential	
	D. A and C	
	Exam II: (3 points) In terms of skeletal muscle cell contraction, which of the following statements is NOT true?	
	A. Myosin forms cross-bridges with actin	
	B. Ca++ enters the muscle cell from the motor neuron	
	C. The muscle cell shortens during contraction	
	D. Myosin is a "motor" protein	
	E. Tropomyosin covers the myosin binding sites on actin	
	Exam II: (4 points) Which of the following do skeletal and cardiac muscle have in common?	
	A. Both experience summation	
	B. Both experience the length tension relationship	
	C. Both experience variable recruitment	
	D. Both can increase contractility	
	Exam II: You would expect the S-T segment to shift during a heart attack because:	
	A. The AV node becomes diseased	
	B. Blood is pumped more slowly	
	C. An ectopic source has taken over the heart rate	
	D. Ventricle tachycardia	
	E. The damaged area slows electrical conduction through the ventricle	

Hartert: Supplemental Table 3: Cognitive Strategies

New Zoom Strategies for Exam II				
1) Cameras on				
2) Designate roles for each group member, involve Zoom member				
3) After Zooming, fill out the cognitive reflection form:				
	Top 3 concepts you learned			
	Top concept you are unclear with and need to work on			
	How to apply concepts from today to your future field			
	Comments and at least 1 direct question for instructor			

Hartert: Supplemental Figure 1: Question Statistics



Supplemental Figure 1. No Specific Questions from Exam I Were Enriched for Either Normal or Multi-Zoom Students. A Marker Selection test was used to detect if questions from Exam I were associated with incorrect (red) or correct (white) responses from Normal or Multi-Zoom students. Questions are identified by number and what lab they assessed. T-tests were conducted for each question. P and FDR values for each question represent the likelihood of association. No significant relationships were observed.

A Longitudinal Study of iPads on Undergraduate Learning in an Ecology Laboratory

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Abstract: Technology has long been implemented in education and iPads are increasingly used in undergraduate courses. This study was conducted to evaluate the effects of using iPads on undergraduate student learning in the ecology laboratory in 2013-2015. Two sections of the ecology laboratory were studied each year: one group used iPads and the other used conventional paper manual. Pre- and post-quizzes and a post-laboratory survey were conducted to assess student attitudes toward iPad use as well as learning outcomes. The pre- and post-quiz scores didn't show significant differences between the iPad group and the conventional paper group. The survey results indicated students generally had positive attitudes towards using iPads: iPads made labs more interesting and convenient, saved time, and simplified data transfer and processing. Students who used iPads were more likely to recommend their use and embrace new technology. However, the students didn't agree that iPad use encouraged them to learn, promoted participation, or improved knowledge comprehension. We concluded that use of new technology should depend on learning outcomes and instructional strategies, suggested openness to adopt a new technology by both teachers and students and recommended appropriate implementation and clear assessment of using technologies in undergraduate learning.

Keywords: educational technology, iPads, science laboratory, undergraduate education, learning assessment

Introduction

The seven principles of good practice in teaching have been one of the most popular guidelines for teaching and learning in higher education. The principles stipulate that good practice should encourage contact between students and faculty, develop reciprocity and cooperation among students, encourage active learning, provide prompt feedback, emphasize time on task, communicate high expectations, and respect diverse talents and ways of learning (Chickering & Gamson, 1987). These principles have been shown to be effective in undergraduate education since they were first mentioned in publication in late 1980s (Armstrong et al., 2007; Hiltz, 1988; Johnson et al., 1991; Levesque, 2011; Ryu & Zhu, 2021; Uno et al., 2013). For example, collaborative learning, academic learning communities, and use of technologies such as clickers in the classroom have many attributes of the seven principles such as encouraging contact between students and faculty, promoting collaboration among students, and giving prompt feedback. Armstrong et al. (2007) showed that cooperative working among students in small groups improved student outcomes (e.g., the knowledge improvement in course material) in a large biology course. A study on an academic learning community between ecology and statistics courses revealed this high-impact practice improved students' knowledge in statistics and their understanding of ecological problems (Ryu & Zhu, 2021). In another biology course, genetics, students were found to improve problem-solving skills by using clickers and class discussions (Levesque, 2011). Use of technologies like clickers therefore can encourage applications of the seven principles and could promote student learning.

Technology has long been implemented in education (Alavi & Leidner, 2001; Bates & Poole, 2003; Chickering & Erhmann, 1996). Currently students live in a world filled with new and advanced technologies and they embrace the digital technologies in the digital age (Roberts, 2020). Teachers should also embrace new technologies and utilize new methods to teach more effectively and help student learn effectively (Guenther et al., 2021; Menges, 1994). Apple's innovation - iPads offer an exciting platform for teaching and learning in a collaborative and interactive way (Melhuish & Falloon, 2010). The original iPad was released in 2010. Since then, it has become increasingly popular in K-12 and higher education (e.g., Hargis et al., 2014; Hilton, 2018; Moon et al., 2017; Stark, 2012; Tay & Wang, 2016; Ulery et al., 2020; Ward et al., 2013) and was even more widely used during the COVID pandemic (Bursztynsky, 2020).

Many studies have been conducted to use iPads in the lectures of undergraduate courses of various subjects (e.g., human anatomy in Scibora et al., 2018; writing in Sullivan, 2013; and education in Wakefield & Smith, 2012). Wakefield and Smith (2012) found iPads in the multicultural education course helped students improve problem-solving skills compared to the traditional class. Sullivan (2013) indicated iPads reinforced students to guide their writing process and encouraged collaborative working. In contrast, Scibora et al. (2018) studied iPad use in human anatomy course and reported the iPad group had lower attainment of course objectives and less class engagement. However overwhelming studies on iPad use demonstrated positive effects on undergraduate teaching and learning (Cavanaugh et al., 2013; Hargis et al., 2014). Compared to the lectures, iPad use in the science laboratories were studied less frequently and the results were mixed. Eid and Al-Zhuhair (2015) studied iPad use in a general chemistry laboratory and found students using iPads were more satisfied with the course although iPads did not improve the students' overall quantitative performance. Chakraborty and Cooperstein (2017) investigated the use of iPad in the anatomy and physiology laboratory for 300 students and reported that students improved their grades and felt they learned more content.

The purpose of this study was to evaluate the effects of a relatively new technology, iPads, on undergraduate student learning in an ecology lab (2013-2015). Integrating iPads in teaching should be welcomed and appreciated by students because they have grown up in the age with advanced technologies and have been using new technologies. The use of iPads will make them feel connected to the real world and become more interested in the subject (Melhuish & Falloon,

2010). Therefore, they will be more likely to enjoy their learning experience and improve learning. More importantly, using iPads will develop cooperation among students, promote greater participation, encourage active learning, and provide instant feedback, thereby promoting effective learning (Chickering & Erhmann, 1996). Use of iPads in the laboratories is expected to save students time on data transfer and processing. The students will therefore have more time to reflect upon why they conducted the particular laboratory, which will reinforce the concepts and knowledge learned in the lecture, and the teachers can devote more class time to helping them do so. Additionally, students can use iPads to take photos in the field to aid further comprehension of environmental problems and preparation for their lab reports. Consequently, it is expected that using iPads will enhance collaboration among students, encourage active learning, and increase time efficiency, which are three important principles of best teaching practices (Chickering & Gamson, 1987).

Methods

Ecology course

The iPads (the 4th generation released in November 2012) were incorporated as a new educational technology into a biological science lab course, BIO260W ecology in 2013-2015, to evaluate their effects on undergraduate student learning. BIO260W is required for all students majoring in biology (both B.A. and B.S. degrees) and is offered only in the fall semester because the weather conditions during early spring in the Northeast U.S. makes it difficult to collect ecological data outside. The course includes two 75-minute lectures and one 2.5-hour laboratory each week. The approximately 25-40 students attend the same lecture section and break into two or three lab sections at different times, each of which is enrolled by 10-22 students. Students in this course are a mix of sophomores, juniors, and seniors with sophomores typically representing the largest percentage (49.2%, 38.7%, and 64.9% in 2013, 2014, and 2015 respectively). They voluntarily enrolled in the sections based on their own class schedules.

The studied ecology laboratory

The selected experiment in the ecology laboratory for testing the iPad use was "Dispersion of Lawn Plants". Distribution pattern is one part of population studies in ecology, and it is impacted by the organisms and the environment. The goal for this experiment was to ask students to collect data in order to quantify the distribution pattern, identify the three types of patterns, and create a histogram to demonstrate data. Specifically, students form a group of 3-4 persons to use one regular adult-size hula hoop as a small sampling quadrat and randomly throw the quadrat on the lawn on the university campus to count how many individuals of broadleaf plantain Plantago major are in the quadrat. They also need to record the growth environment for those plants and repeat the procedure 20 times. In addition, they use three hula hoops together as a big sampling quadrat and repeat the procedure another 20 times. After collecting the two sets of data, students process the data using statistics to calculate variance and mean. Then they apply the index of dispersion - variance/mean ratio to quantify the distribution pattern. When the ratio is approximately 1, the plant population distribution pattern is random; when it is less than 1, the pattern is regular (also called even distribution); and when it is greater than 1, the pattern is clustered (also called aggregated distribution). Students then compare the two sets of data to see whether the size of sampling units makes a difference. Finally, students use EXCEL to create of a histogram for one set of data. The procedure of how to create a histogram is introduced in the lecture before the lab.

Use of iPads and Institution Review

In the studied period of 2013-2015, there were two sections each year in the ecology laboratory. One section was randomly selected to be the control group and the other group was selected to be the treatment. In the control group (paper group), students carried the traditional laboratory manual and pen to the lawn to conduct the experiment, recorded data on paper, and brought data back to the lab to share with partners; then individually they input data into EXCEL and analyzed data. In the treatment group (iPad group), students were given instructions on how to use the iPads to record and transfer data. They took a picture of instruction in the laboratory manual, carried iPad to conduct experiment, and record data; after coming back to laboratory, they emailed data to each partner and individually analyzed data.

This study has been approved by the Human Subjects Committee at the University of Hartford according to conditions set forth in federal regulation 45 CFR 46.101(b) and was determined to be exempt from further committee review. All students were given an informed consent form to review and sign. This form summarized the purpose of the study, indicated that there were no known risks or benefits, and informed them that they could withdraw from the study at any time without penalty.

Assessments using pre- and post-quiz scores and a post-survey

To assess whether the new technology of iPads aided student learning in the laboratory, two types of assessments were used. A quiz (total 10 points) was taken by both groups of students (using iPads and paper manual) immediately before introducing this experiment (pre-quiz, see Appendix I) and another was taken one week after this experiment (post-quiz). Both quizzes had the same questions and were not previously announced. The quizzes were collected, coded, and mixed together by Dr. Levesque and were graded blindly by Dr. Zhu at the same time to avoid bias.

A post-experiment survey was also given to students to evaluate the effects of technology in education (Bangert, 2004); it consisted of 10 ranking scale statements and one open-end question about opinions of using iPad in this experiment. The 10 statements included:

S1. The iPad use makes the laboratory more interesting/fun; S2. The iPad use encourages me to learnin this laboratory; S3. The iPad use makes the laboratory more convenient (e.g., without carrying a laboratory manual); S4. The iPad use saves my time in the laboratory; S5. The iPad use makes it easy to record, transfer, and share data; S6. The iPad use makes it easy to process the data or create graphs; S7. The iPad use encourages my participation and interaction with group members; S8. The iPad use helps me learn the contents better; S9. I recommend using iPad in the laboratory

when possible; S10. I like new technology to be used in the laboratory in general; Students were asked to rate the statements on a scale of 1-10 where 1 is "strongly disagree" and 10 is "strongly agree" for the statements S1-S10. Students who didn't use iPads in the experiment were asked to complete the survey as if they had used the device. The open-ended question in the survey was: What are your other comments (what aspects you like or what aspects you don't like)?

Statistical analyses

The Shapiro-Wilks test on assumptions of normality and the Levene's test on homogeneity of variance were conducted before data analysis (Allen et al., 2014). Results showed all data met those assumptions with significance level greater than 0.05. We then used Mixed-ANOVA to compare the quiz scores between the iPad and paper groups in both pre- and post-quizzes (Ryu & Zhu, 2021). There were three variables: 1) the dependent variable was quiz score; 2) the between-subjects factor was group, which had two categories: iPad and paper; and 3) the within-subjects factor was time, which also had two categories: pre- and post-quizzes. Results of η^2 (Eta squared) were reported to show effective size. Values of 0.01, 0.06 and 0.14 were considered small, median and large effective size (Schäfer & Schwarz, 2019; Ryu & Zhu, 2021). ANOVA was used to compare the quiz scores among groups in different studied time periods (Kuehl, 2000). Multivariate analysis of variance (MANOVA) was used to compare the post-survey results in S1-S10 for the two groups (iPad vs. paper) and Tukey HSD for post hoc tests was used to compare groups in each question in the survey (Cohen, 1988). All statistical analyses were conducted using IBM SPSS Statistics26 (Allen et al., 2014).

Results

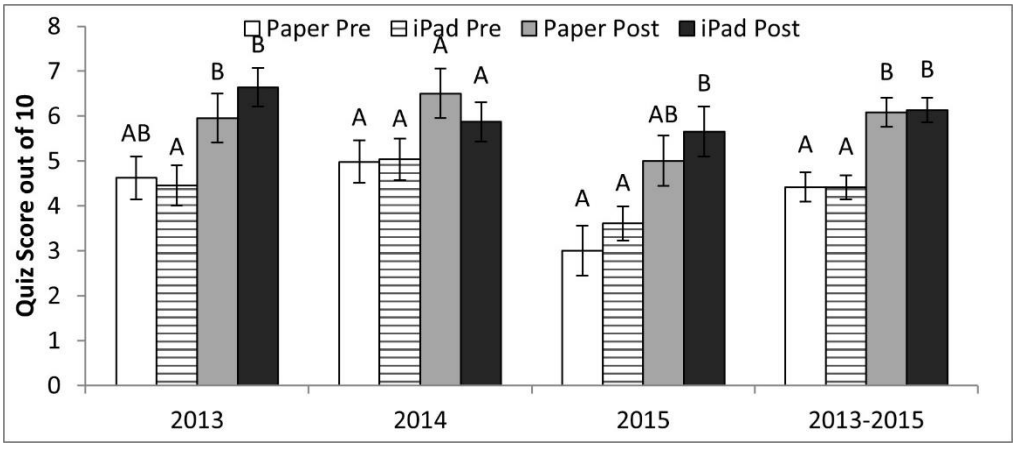
Assessment from pre- and post-quiz scores

There were 90 total students who participated in pre- and post-quizzes in 2013-2015 (9-16 each year in the paper group and 13-21 each year in the iPad group, Table 1). The pre- and post-quiz scores didn't show significant differences between the iPad group and the conventional paper group in each year (Figure 1; all $p>0.05$; all effective size $\eta^2 <0.01$ except $\eta^2=0.05$ in 2015, see Table 1). When data of three years were combined, the result was the same: there were no significant differences in either the pre- or post-scores between the two groups. However, there was a significant time effect when pre- and post-scores were compared (for year 2013, 2014, 2015, and 2013-2015, all $p<0.01$; all $\eta^2>0.30$, see Table 1) and it showed students in both groups consistently increased in post-quiz scores (Figure 1). The trends were slightly different in the three years studied. In 2013, the pre-quiz score for the iPad group was 4.45 ± 0.45 (mean ± SE) out of 10, which was not significantly different from 4.62 ± 0.48 in the paper group. Students in the iPad group increased 49.2% in the post-quiz score to 6.64 ± 0.43 whereas those in the paper group increased 28.7% to 5.95±0.54 although the post-quiz scores didn't differ between the two groups. In 2014, the increases of scores were 16.5% in the iPad group and 30.6% in the paper group whereas in 2015 the increases were 66.7% and 56.7% respectively. When all the three years combined, the increases were nearly the same (37.5% vs. 38.8%), which again presented no differences between the iPad group and the paper group.

Assessment from the survey

There were 41 total surveys (10-16 each year) collected in the paper group and 50 total in the iPad group (13-21 each year, Table 1). The results of S1 to S10 statements in the survey between the two groups showed significant differences in levels of their opinions (i.e., the scaling value, Figure 2). The two groups differed in 2013 (df=10, F=2.827, p=0.016), 2014 (df=10, F=2.938, p=0.019), and 2013-2015 (df=10, F=3.334, p=0.001) whereas 2015 revealed no differences (df=10, F=2.476, p=0.07) However, the comparisons between groups varied each year. In 2013, the group using iPads had more positive views (5.5 is the value for a neutral opinion; positive view is >5.5 and negative view is <5.5) on the use of iPads than the group who used paper manual. There was a consistent trend that the average score for each of the 10 statements was lower in the conventional paper group than those in the iPad group (Figure 2A). Scores in five statements were significantly different between the two groups: S3 iPads make the laboratory more convenient; S7 iPads encourage participation and interaction; S8 iPads help learn better; S9 I recommend use of iPads in the laboratory; and S10 I like the new technology used in the laboratory in general (all $p<0.05$).

Figure 1. Pre- and Post-Quiz scores for two groups of students using traditional paper manuals and using iPads in the ecology laboratory experiment.



Data were shown as mean ± SE. Different letters above the columns denote significance at the level α=0.05.

Table 1. Sample size for quiz and survey for two groups of students using traditional paper manuals and using iPads in the ecology laboratory experiment and effective size η^2 in quiz

	Year	2013	2014	2015	2013-2015
Quiz sample size	Paper	16	14	9	39
	iPad	21	17	13	51
Survey sample size	Paper	16	15	10	41
	iPad	21	16	13	50
η^2 for group in Quiz		<0.001	0.007	0.048	<0.001
η^2 for time in Quiz		0.378	0.382	0.307	0.340

Among the five, four statements (S3 and S7-9) were shown opposite views (the iPad group >5.5 and the paper group <5.5). In 2014, no clear trend was observed. Four statements S5-S8 showed higher average scores in the paper group than the iPad group although they were not significantly different (Figure 2B). In 2015, there was no clear trend either but all the views were positive (>5.5) in both groups (Figure 2C). When data of 2013-2015 were combined and compared, they revealed significant differences in three statements between two groups (the iPad group had higher values and more positive views): S3 iPads make the laboratory more convenient; S9 I recommend use of iPads in the laboratory; and S10 I like new technology to be used in the laboratory in general (Figure 2D).

The survey data also demonstrated consistently low scores in three statements in each year: S2 The iPad use encourages me to learn in this laboratory, S7 iPads encourage participation and interaction, and S8 iPads help learn better. In the combined 2013-2015 data, both groups presented relatively neutral views on S7 (5.90±0.39 in the iPad group vs. 5.56±0.51 in the paper group). Both groups had negative views on S2 (5.42±0.38 vs. 5.12±0.42) and S8 (5.20±0.38 vs. 4.49±0.41, the lowest scores).

Summary of student comments

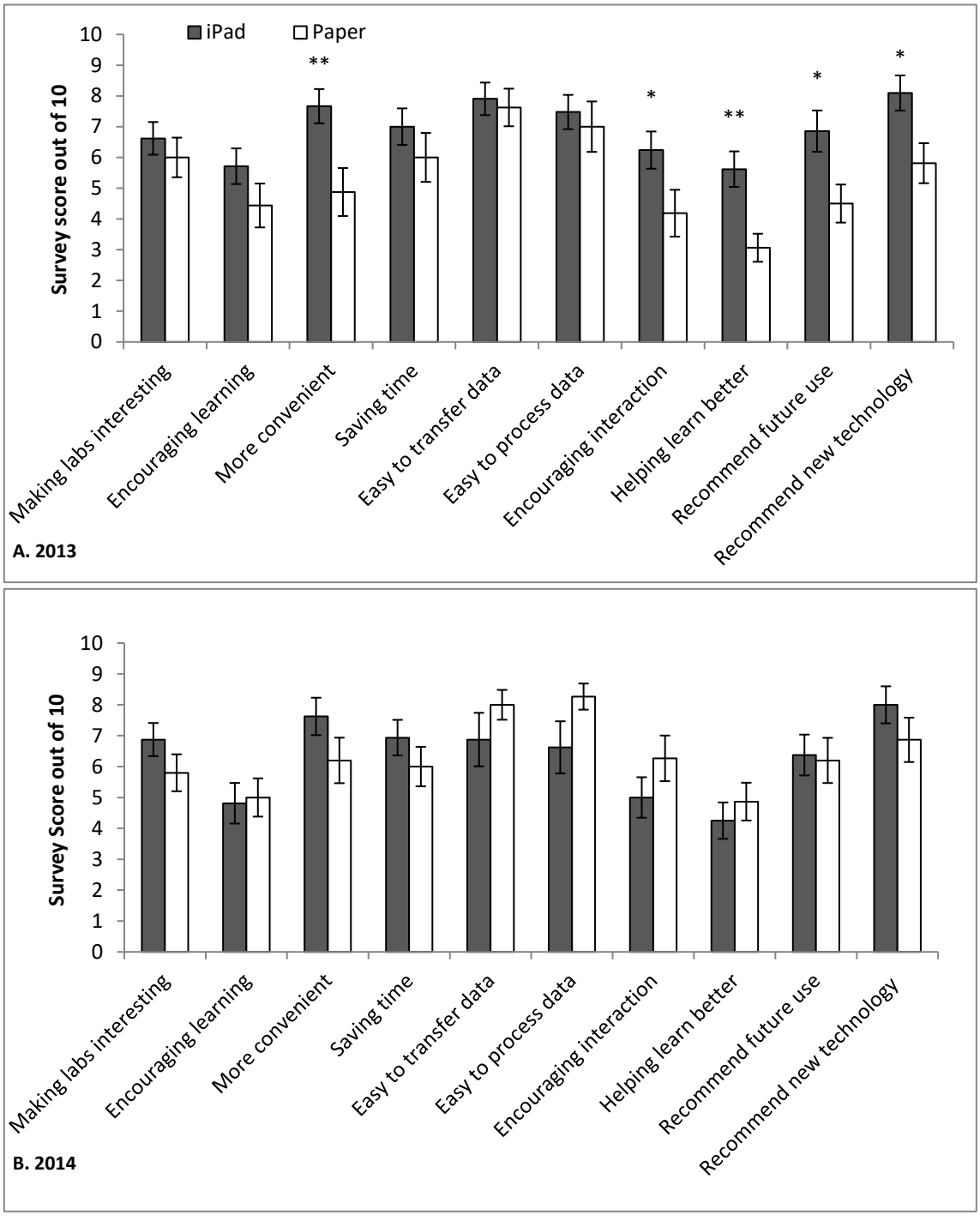
A total of 39 student comments were provided by students in the three-year study period (17 in 2013, 16 in 2014, and 6 in 2015; Appendix II). The iPad groups had 7, 12, and 5 comments and the paper groups had 10, 4, and 1 comments in 2013, 2014, and 2015 respectively. In 2013, 3 comments in the iPad group stated iPad use was helpful and made it easier whereas 4 comments talked about the difficulty of transferring data from iPads to individual students. In the paper group, 2 students thought it would be good, 6 were worried about the iPad use (e.g., distracting, lack of knowledge to use the device, and damage), one was neutral, and the other one didn't show his/her opinion on iPad use. In 2014, 9 students in the iPad group thought iPad use was good (convenient and easy to record and transfer data), 2 complained about data transfer, and one recommended use of iPad mini because iPad was heavy. In the paper group, one supported the idea of iPad use, one had a neutral view, one worried lack of knowledge to use, and the other one had a mixed view - it was good, but it might be a distraction and hinder personal interactions. In 2015, 4 out of 5 comments in the iPad group had positive views on iPad use and one student preferred paper manual. In the paper group, the only comment expressed concern about whether the iPad could function smoothly.

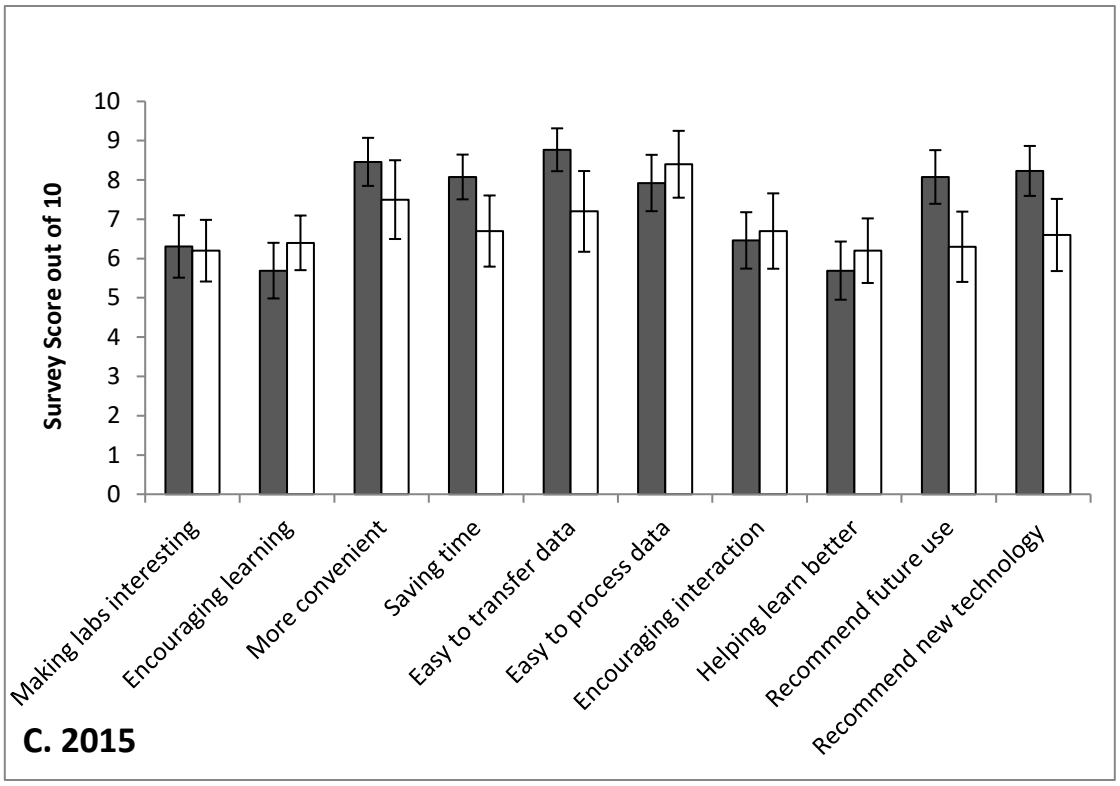
Discussion

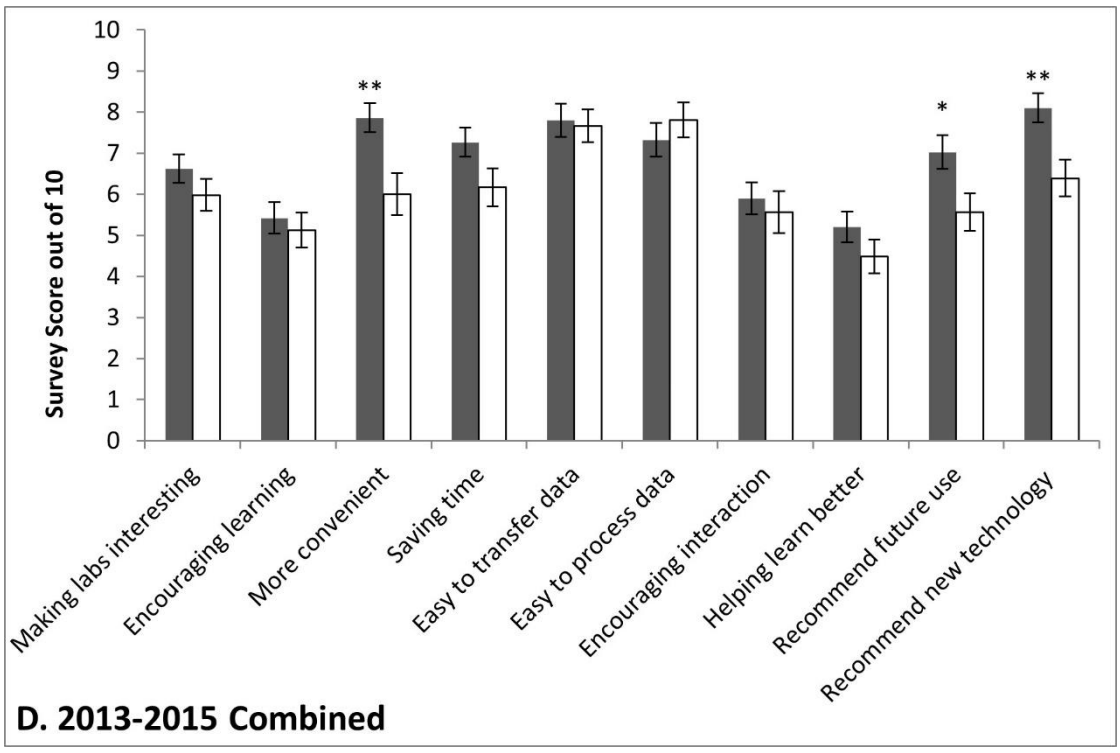
Our study demonstrated students in both groups had positive views on iPads (score>5.5): making labs more interesting, more convenient, saving time, easy to transfer data, and easy to process data. However, the students didn't think use of iPads encouraged them to learn and participate or improve learning outcomes. These latter attitude results were contradictory to our expectations and many other studies that demonstrated technology like iPads encouraged learning and promoted collaborations in undergraduate education (Bates & Poole, 2003; Melhuish & Falloon, 2010). They were also not consistent with studies in several science laboratories. For example, in the anatomy and physiology laboratory (Chakraborty & Cooperstein, 2017), students felt more positively about learning using iPads and improved their grades; in a chemistry laboratory (Eid & Al-Zhuhair, 2015), it was also found that students felt more satisfied with learning content with iPads despite no improvement in grades. The quantitative data from the pre- and post-quiz scores also confirmed that iPads didn't help students gain more knowledge in the ecology laboratory. Different results in attitudes and quantitative data might be due to the length of use of iPads. In the other studies, iPads were used for a semester or longer whereas our study only used iPads once in one experiment. More use of iPads would help students get more familiar with the technology and application of iPads in the whole laboratory course might produce different results. Another reason might be that iPads were more like a tool for recording and transferring data in this study instead of a technology of learning in the whole semester.

The trend of changes in views toward iPads from 2013 to 2015 indicated that students were reluctant to changes in learning and familiarity of the technology could ease the reluctance. There were five opposite statements (iPad group >5.5) in 2013, only one in 2014 (paper group >5.5), and zero in 2015 (all positive views). In 2015, iPads were ubiquitous and considered a common device in the society. Therefore, student attitudes changed due to familiarity. However, in 2013 (only three years after the first iPad release), students who didn't use iPads had strong reservations about this technology whereas those using iPads welcomed it despite the fact that a number of students in the iPad groups had technical difficulties (which were reflected in student comments). By viewing the comments in the survey

Figure 2. Survey ranking scores of ten categories for two groups of students using traditional paper manuals and using iPads after the ecology laboratory experiment was completed. A. data from Fall 2013; B. data from Fall 2014; C. data from Fall 2015; and D. combined data for 2013-2015. Data were shown as mean ± SE. * indicates significance at the level α=0.05 and ** indicates significance at the level α=0.01.




Survey Score out of 10
10
9
8
7
6
5
4
3
2
1
0
Making labs interesting
Encouraging learning
More convenient
Saving time
Easy to transfer data
Easy to process data
Encouraging interaction
Helping learn better
Recommend future use
Recommend new technology
C. 2015


Survey Score out of 10
10
9
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Making labs interesting
Encouraging learning
More convenient
Saving time
Easy to transfer data
Easy to process data
Encouraging interaction
Helping learn better
Recommend future use
Recommend new technology
D. 2013-2015 Combined

from students who did not use iPads, we found the negative attitudes might be due to the following reasons: some were afraid of using iPads due to lack of knowledge; some worried that using them can be a distraction because students may be interested in the technology itself instead of the laboratory; some thought iPads might break or could not function well; and some just didn't like Apple products. These concerns were reasonable and therefore it was not surprising to see students were afraid of changes in learning at the beginning. However, they may change their view if they are provided with appropriate instructions and have an opportunity to try using the new technology as shown in this study: students who used iPads were more likely to recommend their use and embrace the use of new technology (e.g., King et al., 2014).

There are many barriers to adopt new technology in education and attitudes of teachers and students are important factors (Rogers, 2000). To foster positive attitudes and clear the misperceptions of new technology, structured guidance and positive teachers' attitudes are highly recommended. Christensen (2002) revealed that positive teacher attitudes toward information technology foster positive attitudes in their students. Other studies also reported teacher's openness to change may influence the technology integration and the technology impact on content acquisition (Baylor & Ritchie, 2002). Therefore, teachers should be open for change and have positive attitudes toward integration of new technology in undergraduate education. Following these, students can have positive attitudes toward new technology, which in turn may improve learning. This is particularly important for current undergraduates, who are considered as the Net generation. They expect any technology used in education should be customized for their needs and should not require them to change (Roberts, 2020).

Adopting and applying technology is not guaranteed to fulfill all the learning outcomes. It is highly recommended an appropriate implementation should be executed and a clear assessment about the advantages and limitations of new technology should be conducted after the experimentation or implementation of new technology (Melhuish & Falloon, 2010). Not a single new technology will promote every aspect of the learning outcomes (e.g., the seven principles of good practice). Adoption and choice of new technology should depend on learning outcomes and instructional strategies (Chickering & Erhmann, 1996). For any given instructional strategy, some technologies are better than others. No matter what technology is used, it is important to utilize consistent instructional methods in undergraduate education (Scibora et al., 2018).

There were several limitations in this study. Like many studies, the sample size was relatively small in this study (e.g., Ryu & Zhu, 2021; Wakefield & Smith, 2012). Larger sample size would make data more powerful and results more convincing. Also, the study lasted three years (which was better than a one-time study) but the results varied each year. A longer term would make it easier to interpret data. The variability from year to year could be due to familiarity of iPads by students as explained before. It could also be due to other reasons such as the academic abilities of students each year, different academic years (sophomore through senior), and different levels of knowledge and accessibility to technology among students. The average GPAs for the three years of the study (2013, 2014, and 2015), determined by recording the GPA of each student as of the semester prior to entering the ecology course, were 3.15, 2.87, and 2.79 in the iPad sections, respectively, while the paper sections had GPAs of 3.22, 3.21, and 3.25, respectively. It was significantly different in two groups in 2015 and 2013-2015, reflecting the differences in student academic abilities. Also, it was true that sophomores represented the largest group, but the percentages were different (49.2%, 38.7%, and 64.9% in 2013, 2014, and 2015 respectively), meaning the composition of sophomore, juniors, and seniors were different each year. It is possible that differences in student academic years and/or academic abilities can impact student attitudes about using new technologies as well as the learning outcomes associated with the technologies. In addition, the iPad application was only used in one laboratory experiment; more repeated uses of iPads in the whole course would make it easier to compare with other studies. Lastly more assessments in addition to pre- and post-quizzes and a post-survey might provide more accurate and holistic views (Ryu & Zhu, 2021). Despite these limitations, our study added another case study of using iPads in a science laboratory and provided useful information about technology use in higher education such as calling for openness to adopt a new technology by teachers, reducing student reluctance of using new technology by providing guidance and more practices, and recommendations of various assessments.

Conclusions

This study revealed students generally had positive attitudes towards using iPads in an ecology laboratory: they made labs more interesting and convenient, saved time, and simplified data transfer and processing. Students who used iPads were more likely to recommend their use and have positive attitude towards new technology use in the laboratory than students who did not. However, the students didn't agree that use of iPads encouraged them to learn and participate or improve learning outcomes. This study also showed no improvement in the knowledge comprehension for students. Further investigation is necessary to evaluate whether the new technology such as iPads can promote effective teaching and learning in undergraduate science laboratory courses. Adoption of new technology should depend on learning outcomes and instructional strategies. It is highly recommended that appropriate implementation and use of technologies should be executed and clear assessments about the advantages and limitations of new technology should be conducted.

Acknowledgements

We thank the Educational Technology Grant from the Provost's Office and the Department of Biology at the University of Hartford for providing financial support to conduct this study. We also thank all the students who participated in this study. Conflict of interest

The authors declare no conflict of interest.

Author contributions

Bin Zhu: Conceptualization; Formal analysis; Investigation; Validation; Writing-original draft; Writing-review & editing. Aime A. Levesque: Conceptualization; Investigation; Validation; Writing-review & editing.

Institutional Review Board

This project was approved by the Human Subject Committee at the University of Hartford and was exempt for further review.

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Perspectives

Descriptions of Centrifugation Manipulations in the Literature Illustrate the Need for Better Laboratory Training of Biologists

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Abstract: Students in environmental sciences, ecology, or wildlife management are often reluctant to acquire training in basic laboratory techniques. To advocate the importance of this training, we reviewed literature in the fields of biological and environmental science for one frequently used technique—centrifugation—and evaluated whether centrifugation parameters are properly expressed. Centrifugation is used to extract and purify different types of biomolecules for further characterization or quantification. The repeatability of the procedure depends on the proper identification of parameters defining the gravitational force applied to a solution, for example the duration, distance from central axis, and angular velocity. Correctly expressing rotation velocity—the "speed"—is therefore crucial to ensuring repeatability and the possibility of using the same protocols in different laboratories. When scrutinizing the materials and methods sections of publications in ecology, zoology, botany, or general biology journals, we noticed that velocity is expressed in different ways and essential information is often missing. We sampled 2000 articles in different fields of biological sciences that recorded centrifugation as a technique in the materials and methods section. We found centrifugation velocity to be properly expressed in gravitational force "g" in only 47.8% of the papers. The score dropped to 40.4% in journals specialized in ecology. We use this analysis to advocate for a minimum of training in the techniques of biochemistry that are of common use in environmental sciences. Better training would allow higher reproducibility of results in scientific publications.

Keywords: Centrifugation, Laboratory training, Analytical biochemistry, Ecology, Wildlife management, Biology program

Introduction

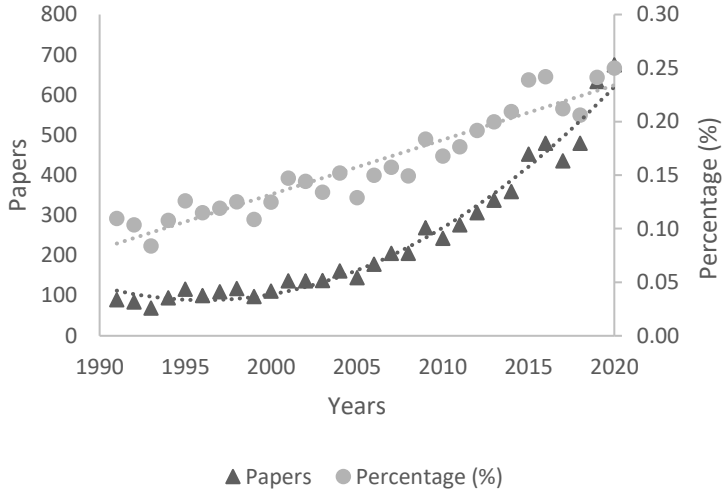
While ecosystems face major treats (global changes, pollution, plastic wastes, invasive species, loss of biodiversity and habitats), environmental scientists are increasingly required to objectively evaluate the scale of deterioration, to suggest strategy to alleviate their impact and to adapt to new environment. Environmental sciences are multi-disciplinary and may require skills from different disciplines (for example chemistry and ecology in eco-toxicological studies). By tradition, training in colleges and universities has been compartmentalized according to these disciplines often leading to highly specialized professions. For example, in biology, students interested in environmental sciences are often oriented to specialize in ecology, wildlife management or conservation biology. More importantly, these students might have a narrow perception of environmental sciences and do not realize the importance of transdisciplinary training. After teaching for close to three decades in a bachelor program of biological sciences to students mostly interested in ecology, marine biology or wildlife management, it is clear for us that they usually do not realize the importance of other disciplines like chemistry, biochemistry, genetics and the relevance of a minimum of literacy and competence in these disciplines.

Ecological sciences have been using basic biochemical techniques for many decades. For example, proximal analysis or protein content analysis has been routinely performed to evaluate the physiological conditions of individuals or wild populations (e.g., Dutil et al., 2008). With the development of molecular ecology and physiology conservation, the use of biochemical or molecular techniques in ecology, zoology, botany, and their allied sciences has significantly progressed in the last 30 years. As an example, the number and percentage of articles containing "enzymatic activit*" as a topic in these fields of study have increased steadily between 1991 and 2020 (Fig. 1).

A good knowledge of laboratory techniques is therefore essential in the training of biologists or ecologists outside the fields that traditionally use these techniques (e.g., biochemistry, clinical laboratory science). Nevertheless, this training in undergraduate or graduate programs might be inadequate or even absent. To illustrate this, we surveyed the literature for reports of centrifugation speed. Centrifugation is a common and simple technique for the separation, preparation, or purification of cells, structures, or biomolecules, and this technique can be useful in a wide range of biological sciences and applications. We therefore used this technique and the proper expression of its parameters as a proxy of the knowledge and proper training of researchers.

A quick consultation of different textbooks on biochemistry or laboratory techniques revealed that the easiest way to express centrifugation velocity to ensure reliability is in "g" (gravity) or RCF (relative centrifugal force) rather than in revolutions per minute (rpm). The force of sedimentation depends not only on the velocity of rotation, but also on the distance from the rotation's axis (rotation radius). Therefore, knowing the rpm with no information on rotation radius (or rotor) cannot be repeated in laboratories equipped with different centrifuges. On the other hand, gravity can be repeated on any centrifuge when we control the velocity and know the distance of the solution or homogenate from the central axis (Basha, 2020; Boyer, 2012; Farrell & Taylor, 2006; Gallagher, 2012; Holtzhauer, 2006; Juban & Barkley, 1996; Kamoun, 1999; Katoch, 2011; Lo, 2019;

Figure 1. Publications with "enzymatic activit*" as a topic in peer-reviewed journals in the following Web of Science categories: Ecology, Behavioral sciences, Zoology, Marine biology and Freshwater biology, Limnology, Oceanography, Fisheries, Forestry, Plant sciences, Mycology, Environmental sciences, and Environmental studies (Web of Science, 2021)



Percentage (%): the proportion of the total number of publications in these fields that have "enzymatic activit*" in the keywords or abstracts.

Ohlendieck & Harding 2018; Robyt & White, 1987; Rosenberg, 2005; Wilson & Walker, 2010).

Most modern centrifuges can be programmed to directly select the correct gravity "g" If this is not possible, a simple nomogram may be used to determine the relative centrifugal field (Boyer, 2012; Burtis & Ashwood, 1999; Gallagher, 2012; Hofmann & Clokie, 2018; Kaneko et al., 1997; Ohlendieck & Harding, 2018; Rosenberg, 2005). The equation

$$RCF = 1.118 \times 10^{-5} \times r \times n^2$$

gives equivalent results, where 1.118×10^{-5} is an empirical factor, r is the horizontal distance (i.e., the radius in centimeters) from the center to the bottom of the tube in the rotor cavity or bucket, and n is the rotation velocity of the rotor in revolutions per minute (rpm) (Basha, 2020; Bermes & Young, 1999).

We observed that the improper utilization of velocity is particularly frequent in publications in ecology, zoology, and related sciences. Owen (2011) observed the same trend in the materials and methods sections of ornithological publications. In this paper, we examined the expression of centrifugation velocity (g vs. rpm) in peer-reviewed papers appearing in publications related to ecology, aquatic science, environmental science, botany, and zoology. Our objective is not only to promote proper descriptions and terminology for centrifugation protocols to make the appropriate expression of velocity, but more importantly to highlight the importance of good training in basic laboratory techniques for biologists in any field.

Methods

Using the Google Scholar, Web of Science, and Scopus databases, we sampled 2000 peer-reviewed articles published between 2000 and 2018 in which the centrifugation technique was mentioned in the methodology. Papers came from journals in five categories (Table 1). We used the expression centrifug* with a category name to find references. We were then noting the way that centrifugation procedures were expressed and the units that were used in the section "Material and methods". For example, RPM, g, etc. (Table 2)

For comparison, we sampled 200 papers published between 2000 and 2018 from a biochemical journal. We chose Comparative Biochemistry and Physiology A, B, C, or D because the field of comparative physiology and biochemistry involves scientists with training in both biological and biochemical sciences. This makes these journals a good control group for comparison with biologists mostly involved in environmental sciences.

To check if the article by Owen (2011) published in the Journal of Field Ornithology had an impact on the way of expressing centrifugation velocity in studies in ornithology, we selected publications in this field of zoology from our database. We compared 119 papers before (2000–2011; N=74) and after (2012–2018; N=45) it was published. We also validated whether centrifugation step descriptions were more rigorous based on the reputation of the journal. We compared publications based on their impact factors as a proxy of their importance and how centrifugation was described in the material and methods sections.

We used exact binomial tests (Dorai-Raj, 2014) to compare the differences between categories, the conditions of centrifugation before and after the Owen's paper (2011) and according to the journal impact factors. Statistical analysis performed with R version 4.1.0 (R Core Team 2021)

Results and Discussion

Table 2 illustrates the strong inconsistency in the way that velocity is expressed in all fields covered by this study except in comparative biochemistry and physiology. More than 52% of the sampled references from the different journals showed an inappropriate description (rpm or missing value of velocity). The phenomenon is particularly and significantly marked in ecological ($p<0.001$), environmental ($p=0.014$), and zoological sciences ($p=0.009$), where appropriate units were used only 40%

Table 1: Categories used for the search.

Categories
Aquatic Sciences, including water research, limnology, and marine sciences
Botany and mycology
Ecology, including behavioral ecology, wildlife management, and conservation biology
Environmental sciences, including ecotoxicology and pollution research
Zoology

of the time, while this proportion reached in a very highly significant way with 83% in Comparative Biochemistry and Physiology publications ($p<0.001$). Our results appear to show critical training gaps in the different fields of biology, which can lead to poor repeatability of methodological protocols. The reviewers and editors of these peer-reviewed journals should therefore pay more attention to the use of appropriate units.

Velocity is still often expressed in rpm with no mention of the rotor, potentially rendering centrifugation procedures among laboratories difficult to reproduce. On many occasions, speeds were vaguely defined like "centrifuged at high speed, low speed, maximum speed or highest level" or even gentle, mild or brief centrifugation. (Ahyong & O'Meally, 2004; Aroca et al., 2006; Collins et al., 2008; Cureton et al., 2011; Morris et al., 2002;Cleveland et al., 2010; Dietz et al., 2013; Helm et al., 2008; Mills & Sebens, 2004; Ziegler and Wittwer, 2005; Straubinger-Gansberger et al., 2014); Bellstedt et al., 2010; Potts et al., 2003;(Fujishige et al., 2000; La Terza et al., 2004; Weir et al., 2012). In some publications, velocity is expressed with unusual units, or no units at all: for example, pm (Shoresh & Harman, 2010), tpm (Heulin et al., 2008), rps (Li et al., 2011), 12 000 with no speed unit (Kori-Siakpere et al., 2006), and 1 g (Pride, 2005) among others. Without the centrifuge and rotor models, or without proper expression and units of the velocity, it is just impossible to precisely repeat the experimental conditions.

Some authors have also noted the same lack of uniformity in their field of research. For example, Pendleton (2006) published a paper (study of pollen contained in honey) on the standardization of centrifugation speeds by using RCF or g. This study gave many examples of papers where speed was expressed in rpm without the size of the rotor and argued for the correct use of the centrifugation unit to obtain reproducible results. In a letter to the editor, Ata et al. (2016) promoted the use g force instead RPM in medicine, arguing that gravitational force is standard while RPM does not represent the same force in different centrifuge models. The same inconsistency had already been noticed in ornithology. Owen (2011) deplored the use of rpm in articles specialized in ornithology. In her paper, he proposed that blood needs to be generally centrifuged for 5–20 minutes at 10000–15000 g. To validate whether Owen (2011) had any impact on methods using this technique, we sampled our dataset for publications in which they mentioned blood centrifugation in ornithological journals before and after its publication.

The use of g as a unit doubled—to a still-low 40%—between 2012 and 2018 (Table 3) and this increase is highly significant ($p<0.001$). The recommended speed of10000 to 15000 g to obtain plasma or serum in birds has almost never been respected. The way that centrifugation velocity has been expressed in the ornithological literature has improved, but it remains below 50% of proper expression.

We evaluated to what extent the impact factor of the journal in which the studies were published could influence the rigor of defining proper protocol procedures (Table 4). In journals for which the impact factor is unknown or low (< 1), recording of centrifugation details is particularly inappropriate (70.7% and 60.5%, respectively). These both levels were very highly significantly lower than the average ($p < 0.001$). However, for journals with impact factors higher than 1, the results appear to be the same with approximately 50% having an

Table 2: Units of centrifugation velocity used in publications separated by field of study.

Category	N	g	Rpm and g	rpm	Missing	Inconsistent	No unit	Appropriate	Inappropriate
Aquatic sciences	386	54.1	2.8	32.1	8.8	2.1	0.0	57.0	43.0
Botany and mycology	349	56.4	1.7	28.9	11.7	1.1	0.0	58.2	41.8
Ecology	515	38.4	1.9	36.9	22.1	0.6	0.0	40.4	59.6
Environmental sciences	349	41.8	1.4	44,7	8.3	3.7	0.0	43.3	56.7
Zoology	401	41.4	2.0	37.9	16.2	2.2	0.2	43.4	56.6
Total	2000	45.8	2.0	36.15	14.1	1.85	0.05	47.8	52.2
Comp. Biochem. Phys	200	80.5	2.5	9.5	5.0	2.5	0.0	83.0	17.0

N: number of publications; g, rpm and g, rpm: percentages of publications specifying centrifugation in these units; Missing: no mention of centrifugation speed; Inconsistent: methods with several centrifugation steps where speeds are indicated in g or rpm without uniformity; No unit: centrifugation speed indicated but no unit specified; Appropriate: centrifugation method described correctly (i.e., g or rpm+g); Inappropriate: centrifugation method described incorrectly (i.e., rpm, Missing, Inconsistent, No unit). Values are expressed as a percentage

Table 3: Conditions of centrifugation of avian blood in ornithological journals before and after the publication of Owen (2011).

	N	g	rpm	Missing speed	10000–15000×g	Appropriate	Inappropriate
2000–2011	74	18.92	39.19	41.89	2.70	18.92	81.08
2012–2018	45	40.00	33.33	26.67	8.89	40	60

2000–2011: papers before the publication of Owen (2011); 2012–2018: papers after the publication of Owen (2011)

N: number of papers; g, rpm: percentages of publications specifying centrifugation in these units; Missing: no mention of centrifugation speed; 10000–15000×g: percentage of papers using Owen's recommended centrifugation speed to obtain plasma or serum in birds; Appropriate: centrifugation method specifies g; Inappropriate: centrifugation method specifies rpm or speed is missing.

Table 4: Publications where centrifugation was used classified by journal impact factor.

Impact factor	n	g	rpm and g	rpm	Missing	Insconsistent	0 unit	appropriate	Inappropriate
Unkown	82	29.3	0.0	53.7	13.4	2.4	1.2	29.3	70.7
0-0.99	263	39.2	0.4	42.6	14.4	3.4	0.0	39.5	60.5
1-1.99	479	49.1	2.3	34.7	12.1	1.9	0.0	51.4	48.6
2-2.99	491	45.6	3.7	35.0	14.3	1.4	0.0	49.3	50.7
3-3.99	210	51.9	0.5	34.8	11.9	1.0	0.0	52.4	47.6
4-4.99	228	43.4	1.8	36.8	15.4	2.6	0.0	45.2	54.8
5 and +	247	49.4	2.0	29.1	18.6	0.8	0.0	51.4	48.6

N: number of publications; g, rpm and g, rpm: percentages of publications specifying centrifugation in these units; Missing: no mention of centrifugation speed; Inconsistent: methods with several centrifugation steps where speeds are indicated in g or rpm without uniformity; No unit: centrifugation speed indicated but no unit specified; Appropriate: centrifugation method described correctly (i.e., g or rpm+g); Inappropriate: centrifugation method described incorrectly (i.e., rpm, Missing, Inconsistent, No unit).

Table 5: Conditions of centrifugation from 291 papers in biology education journals.

n	g	rpm and g	rpm	Missing	Inconsistent	Appropriate	Inappropriate
291	38.83	4.12	26.46	23.02	7.56	42.96	57.04

N: number of publications; g, rpm and g, rpm: percentages of publications specifying centrifugation in these units; Missing: no mention of centrifugation speed; Inconsistent: methods with several centrifugation steps where speeds are indicated in g or rpm without uniformity; Appropriate: centrifugation method described correctly (i.e., g or rpm+g); Inappropriate: centrifugation method described incorrectly (i.e., rpm, Missing, Inconsistent).

accurate velocity unit ($p>0.05$) which remains low. We wondered if the inappropriate recording of centrifugation speed was due to the way this technique is taught. To answer this question, we found 291 articles in the laboratory activities section of seven journals dedicated to teaching biology with centrifugation in the methods section (Advances in Physiology Education, American Biology Teacher, Biochemistry and Molecular Biology Education, Bioscene, CBE - Life Science Education/ Cell Biology Education, Journal of Biological Education, and Journal of Microbiology and Biology Education). We were surprised at the results: only 42.95% of speeds were correctly expressed (Table 5).

Conclusion

We report here on the lack in scientific rigor in the expression of centrifugation velocity in publications from different fields of environmental sciences: ecology, zoology, aquatic sciences, botany, and mycology. Our objective was to illustrate the importance and necessity of better basic training in analytical techniques in biochemistry and physiology for biologists involved in these different fields. In addition, proper descriptions of the methods used are of paramount importance to ensure the repeatability of all studies and the associated methodological protocols. The editorial committees and reviewers of peer-reviewed journals in these disciplines should also be more attentive to the accuracy of these descriptions; their difficulties in identifying these pitfalls may also result from inadequate training.

The aim of this study was to highlight the transdisciplinary nature of environmental and life science in general and the importance of good training in basic laboratory techniques for biologists in any field. This demonstration through the survey of good and bad expression of the units of velocity while using a simple and widely use technic (centrifugation), clearly exhibit the importance to develop skills often perceived as outside our defined field of expertise. Considering the future environmental challenges, we advocate that strong and wide basic education in science preclude efficient transdisciplinary communication and approaches, essential to face these challenges.

Conflicts of Interest

There are no conflicts of interest to report.

Acknowledgments

The authors are grateful to Laure Devine and Serge Goyette for helpful comments. We thank Dr. Alain Caron for help with the statistics.

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Innovations

The Last Lab - Unanswered Questions

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Abstract: How should an instructor use the last lab period in a human physiology or any other biology course? It is impossible to answer all the questions that arise during a semester course. The activity described below helps students see, pose, and answer some unanswered questions that remain after a full semester human physiology course. Students investigate a claim about a course topic, then read experimental literature to find evidence to support or not support their chosen claim. Information gained connects learned course topics with everyday issues and questions. This activity can be used by any college biology instructor in their own course.

Keywords: human physiology, inquiry-based learning, capstone lab, last lab period

Introduction

After several iterations of making hypotheses, planning procedures, measuring, and collecting data, analyzing results, and drawing conclusions during a semester-long lab section of a course, sometimes that pesky, last lab period looms at the end of the semester. Some instructors use it as a capstone experience bringing together topics and techniques into a 2–3-hour culmination of the lab experience in their course (Haave, 2015; Davis, 2011). Others use it as a student report period where students learn from each other about conclusions and reasons for their conclusions from separate, weeks-long, small group lab investigations. (Parent et al., 2010) Another activity is to have student teams present Concept Maps that they have worked on for several weeks, then describe their thought processes about how they constructed them (Martin-Culver, 2019). What is another activity that can be used in this last lab period to tie topics together but also extend learning and boost curiosity beyond your course?

The classroom activity described below was used successfully by the author during the last lab period of a one-semester human physiology course for majors. It is an opportunity to address some of the many questions that remain unanswered from course material. It could be modified by any instructor for almost any biology course to help students connect the semester's topics with everyday issues. Students find updated information, ask additional enticing questions, and embrace new curiosity for these issues.

Activity Procedure

A list of topics and questions that were not addressed in the course are first shown to the students (Table 1). Students have worked in two- or three-person lab teams all semester utilizing the Student Designed Lab format as described by Davis (2002). There are usually 12-16 students or 5-7 teams per lab section. The instructor reads down the list adding important, timely connections or additional questions to some of the topics but emphasizes the large amount of information that remains to be learned for all these topics. A list of untouched chapters in the textbook is another resource that might be skimmed through at this time.

Next, the instructor asks if any student team has any new topics not on the list that they would like to investigate. Teams talk among their members for a few minutes to check each other's interests in any of the Table 1 topics.

The instructor then presents some Claims (Table 2) that connect to some of the topics in Table. 1. The object is to challenge students to be skeptical, read critically and find current research evidence that supports or does not support one of the claims. Student teams then choose their Claim from the list that they will investigate. Teams are given 30-40 minutes to use the internet to find at least 3 pieces of evidence from credible research journals or websites that support or do not support their chosen claim. Multiple key search words should be split up and used by 2-3 person teams. Teams should talk and share their research findings in their teams as the process plays out. The instructor should encourage teams to investigate supportive and contra-supportive evidence for their chosen claim.

The last hour of lab is used for student teams to share their discovered evidence that supports their claim or not. This can be done formally by students constructing a short PowerPoint showing diagrams or data that support their claim. As student teams talk, the instructor can write notes to summarize their findings on a whiteboard or via computer on a screen. The reports may also be done informally without a PowerPoint but with important visual aids or data sent to the instructor who then shows these on a front screen. Student teams lead the discussion from their seats. Other student teams are encouraged to ask questions, take some notes, and think critically about the evidence presented by each team.

At the end of the session, students are asked to reflect and answer two questions on a sheet of paper or in an email to the instructor: 1) What are two new things that you learned about human physiology today? 2) What are 2 remaining questions you have about any of the topics discussed today? These responses have been used by the instructor to update and modify this activity for subsequent semesters. Many students have their eyes opened to new or updated topics and seem to enjoy the outcomes of this last lab section.

Table 1: Possible Topics for student teams to choose from.

Physiology of Weight Gain/Weight Loss?
Physiology of Obesity? Android vs. gynoid obesity? Leptin? Adopokines? Visceral Fat Inflammation?
Why does regular exercise keep you healthy? Myokines? Improves brain plasticity? Long-term studies and their effects? Evidence? What specific changes occur or don't occur?
Physiology of Sleep? How does sleep improve immune function specifically?
Physiology of Aging? Prevention of anorexia of aging? Stem cells involved? Brain plasticity? Role of insulin growth factor? An aspirin a day? Reducing influence of antioxidants? Experimental evidence for antioxidant effects?
Physiology of Acupuncture? Involvement of the nervous system? Pressure points?
Physiology of Stroke? Timing of nervous tissue repair? Lasting effects on gray matter vs. white matter?
Physiology of Macular degeneration? Use of stem cells to help with other eye disorders?
Physiology of Diverticulitis, Crohn's Disease or Irritable Bowel Syndrome?
Physiology of Thyroid Cancer vs. Goiter?
Physiology of Cell Suicide (=Apoptosis)? Does Apoptosis stimulate stem cell recruitment?
Physiology of Cholesterol Statin Drugs ? Pros and Cons? Reduced Immune function specifics?
Other student-generated topics?

Table 2: Claims to Accept or Reject! Evidence Needed!

Android obesity causes less visceral fat inflammation than gynoid obesity, thus it is less of a health risk.
Certain female athletes who show symptoms of female athlete triad have leptin insensitivity that contributes to their triad symptoms and problems.
It is impossible to prevent ACL tears in female athletes with regular menstrual cycles.
Regular aerobic, not anaerobic, exercise, promotes beneficial brain plasticity.
Aerobic-trained athletes produce more muscle myokines than anaerobic trained athletes. Thus aerobic athletes are sick less and can expect to live longer.
Increased incidence of certain brain waves during sleep results in a stronger immune system. Too much sleep or too little sleep results in a less effective immune system. Clear connection between immune system function and sleep.
An aspirin a day slows aging of the brain.
By lowering the active levels of insulin-like growth factor in the blood after age 60, a person will age more slowly.
There is no measurable data that shows increased levels of antioxidants prevent cancer or decrease overall body inflammation.
Abdominal visceral fat levels result in increased cancer in any abdominal organ.
Statin drugs (used to reduce cholesterol) suppress the immune system and are more of a health threat than their health benefit.
Or student groups can make their own Claim, get it approved by the instructor first, then find out if it is supported or not.

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Bioscene: Journal of College Biology Teaching
Submission Guidelines

I. Submissions to Bioscene

Bioscene: Journal of College Biology Teaching is a refereed publication of the Association of College and University Biology Educators (ACUBE). Bioscene is published online in May and in print and online in December. Submissions should reflect the interests of the ACUBE membership

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

II. Preparation of Articles, Innovations and Perspectives

Submissions can vary in length, but articles should be between 1500 and 5000 words in length, including references and tables but excluding figures. All pages and lines of the document should be sequentially numbered including the abstract but excluding figure and table legends. Concision, clarity, and originality are desirable. 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. It should 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. If the study required institutional approval such as an Institutional Review Board (IRB), the approval or review number should be included in this section. For example, this study was approved under IRB number 999999. The editor will delete disclaimers and endorsements (government, corporate, etc.)

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. Other than heading titles, the first word in a sentence or a proper noun, authors should not use capitalization, underlining, italics, or boldface within the text. Authors should not add extra spaces or indentations, nor should they use any hidden editing tools. All weights and measures must be given 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 should appear alphabetically by the author's last name at the end of the manuscript text under the heading references. All references must be from published materials in the literature or the Internet. Authors should use the current APA style when formatting the reference list.

(1) Articles-

(a) Single author:

DeBuhr, L. E. (2012). Using Lemna to Study Geometric Population Growth. The American Biology Teacher. https://doi.org/10.2307/4449274

(b) Multi-authored three to seven authors:

Green, H., Goldberg, B., Schwartz, M., & Brown, D. D. (1968). The synthesis of collagen during the development of Xenopus laevis. Developmental Biology, 18(4), 391–400. https://doi.org/10.1016/0012-1606(68)90048-1

(c) Mutli-authored more than seven authors

List the first six authors than an ellipsis followed by the last author.

(2) Books-

Bossel, H. (1994). Modeling and Simulation (1st ed.). New York, NY: A K Peters/CRC Press. https://doi.org/10.1201/9781315275574

(3) Book chapters-

Glase, J. C., & Zimmerman, M. (1993). Population ecology: Experiments with Protistans. In J. M. Beiswenger (Ed.), Experiments to Teach Ecology (pp. 39–82). Washington, DC: Ecological Society of America. Retrieved from https://tiee.esa.org/vol/expv1/protist/protist.pdf

(4) Web sites-

McKelvey, S. (1995). Malthusian growth model. Retrieved November 25, 2005, from https://www.stolaf.edu/people/mckelvey/envision.dir/malthus.html

D. Tables

Tables must be submitted as individual electronic files in Word (2013+) or RTF format. Tables should not be submitted in Excel. Placement of tables should be indicated within the body of the manuscript. The editor will make every effort to place them in as close a proximity as possible. All tables must be accompanied by a descriptive legend in the format:
Table 1. A comparison of student pre-test and post-test scores in a non-majors' biology class.

E. Figures

Figures must be submitted as high resolution (≥ 300dpi) individual electronic files, either TIFF or JPEG. Figures submitted should not be layered as in Photoshop or similar program. No cut and paste figures will be accepted. Placement of figures should be indicated within the body of the manuscript. The editor will make every effort to place them in as close a proximity as possible. Figures only include graphs and/or images. Figures consisting entirely of text will not be accepted and must be submitted as tables instead. All figures should be accompanied by a descriptive legend in the format: **Fig. 1: or Figure 1:** Polytene chromosomes of Drosophila melanogaster.

III. Letters to the Editor

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

IV. Other Submissions

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

V. Manuscript Submissions

All manuscripts are to be sent to the editor electronically and must comply with the preparation as described above. 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. Telephone and FAX numbers are optional. Email will be the primary method of communication with the editors of Bioscene.

Communicating authors will receive confirmation of the submission. Manuscripts should be submitted either as a Microsoft Word 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 a manuscript to be sent reviewers, at least one author must be a member of ACUBE. Otherwise, by submitting the manuscript, the corresponding author agrees to page charges that are equivalent to the current membership fee assessed per printed 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. Reviewer names and affiliation will be withheld from the authors. 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, manuscripts may be returned to authors

Reviewers will examine the submission for:

- Suitability: The manuscript relates to teaching biology at the college and university level.
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- 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 an 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. To be considered for a resubmission and acceptance, the author must address all of the reviewers' comments and suggestions using the original document and track changes. Manuscripts resubmitted beyond a six-month window will be treated as a new submission. Should manuscripts requiring revision be resubmitted without corrections, the article will be returned until the requested revisions have been made. Upon acceptance, the article will appear in Bioscene and will be posted on the ACUBE website. Time from acceptance to publication may take between twelve and eighteen months.

VII. Revision Checklist

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

A. Sending the editor an email that includes a copy of the revised article using track changes showing any text changes made in the resubmission that address the comments/concerns of the reviewers. An explanation should be given for why any of the reviewers' comments/concerns were not addressed.
B. Making sure that references are formatted appropriately in APA style format shown above.
C. Sending the final figures, tables, appropriate titles, and legends in the format describe above (**editable** JPEG or Word format) as separate attachments with their placement in manuscript clearly indicated.

VIII. Editorial Policy and Copyright

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