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Cover image: Yellow-bellied marmot
Marmota flaviventirs
Sequoia National Park near Takopah Falls
Taken by John C. Watson Ph. D.
IUPUI

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ANNUAL MEETING

In 2019, the 63rd Annual ACUBE meeting will be held at Syracuse University Friday October 18th – Saturday Oct 19th in Syracuse, NY.

NEW SUBMISSION GUIDELINES FOR AUTHORS

Future authors should review the changes to the submission guidelines contained in this edition. One key change is that we are moving to APA formatting for references.

CORRECTIONS

The cover photo credit in the December 2018 issue was incorrect. The photo was taken by the Dr Doris Audet, not Neil Haave. Neil's name was also spelled incorrectly.
The editors of Bioscene apologize for these errors.

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A Method to the Midterms: The Impact of a Second Midterm on Students' Learning Outcomes

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Abstract

Midterm exams are a multi-use tool, providing evaluation of students for professors but also acting as a learning tool for students. Midterms may improve learning outcomes by contributing to the testing effect: the phenomenon in which retrieval of learned material (i.e., testing) produces improvements in long-term retention beyond those produced through additional rehearsal or re-exposure (i.e., studying or re-reading). Additionally, increased frequency of testing may impact student behaviors and attitudes (e.g., spaced practice, self-efficacy), increase the testing effect, or impact both, which ultimately improves learning outcomes. This study considered the differential impact of one versus two midterm exams on students' exam difference scores (final exam score minus first midterm exam score). We also considered whether two midterm exams differentially impacted low- and high-achieving students. Results suggest that two midterm exams benefit freshmen but not junior students.

Keywords: testing effect, frequency effect, midterm exam, student learning outcomes

Introduction

Midterm and final exams are common forms of assessment implemented in undergraduate university courses to determine the degree of students' mastery of course material. However, midterms can act as a multi-use tool, providing evaluation of students for professors but also acting as a learning tool for the students. Usually, courses will have one or more midterm exams spaced throughout the semester in addition to a final exam; these midterm exams may or may not be cumulative (Myers & Myers, 2007). Although there are anecdotal preferences for the number of midterms a course should have, there is limited research on the benefits of one versus two midterm exams on the outcome of students' final exam scores. Our study was designed to fill this gap in the research by considering whether a second midterm could improve student learning outcomes. Studies supporting midterm exams as a learning tool cover two broad areas of research: testing effects and frequency effects.

Testing Effects

Interest in the testing effect has generated significant research both in labs and classroom settings. The testing effect occurs when retrieval of learned material (i.e., testing) produces improvements in long-term retention beyond those produced through additional rehearsal or re-exposure (i.e., studying or re-reading) (Brame & Biel, 2015; Carpenter, 2012; Roediger & Butler, 2011). Early laboratory research on the testing effect was predictably structured (Carpenter, 2012). A learning phase allowed participants to encode the material. This was followed by a testing phase or re-study (control) phase allowing participants to either retrieve or re-read the material. Finally, a second test phase was used to determine retention of the material. The positive impact of testing in early work implied that testing should be introduced into educational settings to improve achievement (Spitzer, 1939; Wheeler & Roediger, 1992). However, laboratory conditions do not adequately mirror educational settings, therefore, substantial work has now been done to ensure that the testing effect holds true in classroom settings.

A plethora of classroom research suggests that the testing effect is robust. The testing effect occurs despite differences in test materials (e.g., words, prose, pictures, spatial locations), test formats (e.g., multiple choice, short answer, free recall, quiz), and timing (e.g., minutes versus weeks between testing phases) (Bae et al., 2018; Carpenter, 2012; Carpenter & Kelly, 2012; McDaniel et al., 2007; Rowland, 2014). Additionally, the testing effect has been duplicated across multiple disciplines (e.g., psychology, biology, chemistry) (Bailey et al., 2017; Pyburn et al., 2014; Schwieren et al., 2017) and different populations (e.g., primary school, university) (McDaniel et al., 2007; Roediger & Butler, 2011; Spitzer, 1939). Furthermore, the testing effect is not limited to retention of learned material (i.e., rote memory); the testing effect has been shown to improve application of material, improve

knowledge-based inferences, promote transfer of rules to novel contexts or knowledge to a different knowledge domain, and facilitate learning of new material (Brame & Biel, 2015; Carpenter, 2012). Finally, the testing effect can be increased when tests are combined with feedback (Bailey et al., 2017; Brame & Biel, 2015; Foss & Pirozzolo, 2017; Roediger & Butler, 2011; Schwieren et al., 2017) and when multiple tests are offered (i.e. three or more) (Bailey et al., 2017; Foss & Pirozzolo, 2017; Roediger & Karpicke, 2006; Wheeler & Roediger, 1992).

Two recent meta-analyses provide strong evidence for the testing effect based on laboratory research (Rowland, 2014) and classroom research (Schwieren et al., 2017). Rowland (2014) suggested two theoretical frameworks that may explain the testing effect: retrieval effort theories and the bifurcation model. Retrieval effort theories suggest that the difficulty and effort during the initial testing phase impact the intensity and depth of processing leading to a testing effect (Rowland, 2014). Whether difficulty increases retrieval routes, supports specific types of processing (i.e., item-specific processing), or allows for elaboration of memory traces remains unclear. The bifurcation model suggests that tests produce non-normal distributions of memory strength over time (Kornell et al., 2011; Rowland, 2014). Specifically, successfully tested (i.e., retrieved) items receive a large boost in memory strength, un-retrieved items receive no boost, and re-studied material receives a small boost. Thus, testing does not reduce the speed of forgetting, but increases memory strength for successfully tested items and makes them more likely to remain above a recall threshold during the final testing phase, thereby bifurcating the distribution.

Despite significant research, there has been limited consideration of whether the testing effect is equally powerful in various student subpopulations. Pyburn et al. (2014) argued that learning tools do not affect all students equally and specific attention should be focused on whether the testing effect as a phenomenon is equally apparent in disadvantaged populations. They examined whether a pre-test differentially influenced low- and high-skilled English language comprehenders. They found that a multiple-choice pre-test was more beneficial to low-skilled English comprehenders; additionally, the pre-test closed the achievement gap between these two groups. There is also a small selection of research suggesting that a negative testing effect (i.e., when a testing phase causes a decline in learning outcomes) is due in part to the cognitive ability of the participants. Mulligan et al. (2018) suggested differences in encoding might explain why there are only a few inconsistent instances of a negative testing effect. Briefly, the negative testing effect is potentially tied to the type of processing that occurs during the testing phase versus the requirements of the final test. Item-specific processing during the testing phase reduces a participant's ability for inter-item processing (and vice versa). Item-specific information helps distinguish one target from another and improves the odds of retrieval (e.g., the ground finch Geospiza conirostris can eat cactus-flowers). Inter-item relational information is categorical or grouping information; that is, common features of targets (e.g., all ground finches are seed-eaters). Inter-item relational information is tied to successful free recall. Therefore, when the testing phase forces one type of processing but success on the final test requires the other type of processing a negative testing effect may result. For example, if the testing phase includes a multiple-choice question asking a student which finch eats cactus flowers, inter-item processing leads to the answer Geospiza conirostris. However, in the re-study condition, a student may recognize that the given list of finches all eat seeds and are therefore ground finches. If the final test is a free recall test in which students are asked to list ground finches, inter-item processing is more useful to access the categorical information that all ground finches are seed eaters than the specific exception that can also eat cactus flowers. More importantly, Mulligan et al. (2018) found that manipulating the type of processing interacted with the cognitive ability of the student, particularly in the re-study control condition. A student's cognitive ability limits their ability to recognize and process categorical information during the re-study phase (i.e., the fact that the list of birds given in the re-study condition are all seed eaters and thus ground finches). Therefore, high-achieving students in the re-study condition could outperform low-achieving students in the testing condition when the test forces them to encode item-specific details and miss inter-item details that are more useful for a final exam that requires categorical knowledge. The testing effect research supports the use of a midterm as a useful learning tool, and limited research on frequency also suggests two midterms may be more beneficial than one (Bailey et al., 2017; Foss & Pirozzolo, 2017; Roediger & Karpicke, 2006; Wheeler & Roediger, 1992). Additionally, research on the negative testing effect and disadvantaged student subpopulations suggests that the number of midterms may differentially impact low and high achievers (Mulligan et al., 2018; Pyburn et al., 2014).

Frequency Effects

It is difficult to separate a phenomenon like the testing effect from other aspects of testing, such as frequency because a single test can potentially impact students across various theoretical frameworks. As already noted, increasing frequency has been shown to

increase testing effects (Bailey et al., 2017; Foss & Pirozzolo, 2017; Roediger & Karpicke, 2006; Wheeler & Roediger, 1992). However, frequency research makes novel predictions regarding subpopulations and potential limits on the impact of frequency. The frequency research suggests different underlying causes for the impact of increased frequency; for example, spaced or distributed practice, improved self-efficacy, reduced procrastination, or student-instructor relations (Bailey et al., 2017; Myers & Myers, 2007). Increasing test frequency has been shown to improve individual test scores as well as final exam scores (Bailey et al., 2017; Myers & Myers, 2007). Unfortunately, each of these studies used multiple cumulative exams (6-10 midterms); therefore, whether educators will see an increase in performance using a second non-cumulative midterm remains unclear. There is some suggestion that the expectation of a cumulative exam is enough in itself to increase student performance (Lawrence, 2013). Lawrence (2013) specifically tested differential impacts of cumulative exams on low and high achievers. While all students benefited from cumulative exams (versus non-cumulative exams), she found that the benefits were greater for low-achieving students. Due to the limited research on student subpopulations, Lawrence's work supports considering low- and high-achieving students separately in the present study, even though our second midterm exam is non-cumulative.

When considering what level of frequency is necessary to create improvements, a meta-analysis by Bangert-Drowns et al. (1991) suggests that extremes are unnecessary. Frequency varies substantially and while they concluded that increasing frequency of tests improved student achievement on final exams, they also noted that students are only at a serious disadvantage when they receive no tests at all. Furthermore, they determined that improvements in student learning diminish as test frequency increases: having one midterm exam benefits student learning more than no exams but having four exams will not produce a four-fold improvement in final exam results. These findings suggest that a second midterm may be a sufficient increase in frequency to produce a positive impact on student achievement.

Our project had two objectives: to determine if changing the frequency of midterm exams from one to two improves student learning outcomes and to consider whether testing influences low- and high-achieving students differently. We hypothesized that students in courses with two midterm exams would show greater improvement on their final exam score relative to their first midterm exam score than students in courses with a single midterm exam. Additionally, we predicted that low-achieving students would disproportionately benefit from two midterms.

Methods

Courses analyzed in our study were selected from the courses taught by one of the co-authors (NH) between 1990 and 2018, and syllabi were compared for their assignment breakdown and the number of midterm exams. The courses included in our study were selected based on whether the types of assessments and year of implementation were similar, except for the number of midterm exams. In total, four iterations of freshman cell biology and two iterations each of junior cellular biology and junior biochemistry I and II were selected for analysis. Freshman cell biology courses selected for inclusion in this study were offered in fall 2000 (1 midterm), 2003 (1 midterm), 2001 (2 midterms), and 2002 (2 midterms). Selected junior cell biology courses were offered in fall 1992 (2 midterms) and 1993 (1 midterm), junior biochemistry I courses were taught in winter 2010 (1 midterm) and fall 2010 (2 midterms), and the junior biochemistry II courses were from winter 2013 (1 midterm) and 2011 (2 midterms).

The one- and two-midterm cohorts for freshman cell biology and junior biochemistry I and II were similar in course structure: lab component (30-40%), quizzes (5-10%), midterm (20-30%), and cumulative final exam (35%). The one- and two-midterm cohorts for junior cell biology both had a lab component (40%), term paper (15%), and similar weighting for the midterm exams (one midterm = 20%; two midterms = 15% + 10%) and final exam (one midterm = 30%; two midterms = 35%). In all courses, the second midterm exam in the two-midterm condition was not cumulative, but each would contribute to the material on a cumulative final exam. All lectures were taught by the same instructor (author NH) and so were taught in a similar style. While course structure was similar, individual course elements occasionally differed from year to year (e.g., different textbooks or lab manual editions, different lab instructors, fresh quiz and exam questions). Therefore, the potential exists for confounding variables because the classes were not absolutely identical. The freshman biology courses used the same syllabus, and each of the junior cell biology, biochemistry I, and biochemistry II courses used the same syllabus for the same course. But clearly, the syllabi differed between courses (the syllabi were different for each of freshman biology, junior cell biology, junior biochemistry I, and junior biochemistry II). Student marks and class demographics from the selected courses were collected from the instructor's grade books, and students' identities were anonymized with a study ID before data analysis. Students who did not fulfill the assessment requirements of the study (i.e., did not complete one of the midterm exams or the final exam)

were removed from the dataset before analysis. This study was approved by the University of Alberta Research Ethics Board (Project #82145).

Our study had a 2 (midterm: one or two) x 2 (achievement level: high or low) x 2 (course level: freshman or junior) between-subjects factorial design. To assess improvements in final exam scores we chose to compare difference scores (i.e., final exam score minus midterm one exam score) rather than raw scores. Difference scores are better able to tell us how each students' performance changed across the semester and act as our dependent variable. To determine if there were differential impacts on weaker students, students were split into high- versus low-achieving cohorts based on whether they fell in the upper or lower 50% of the course, as determined by the median score of the first midterm exam. Finally, because we collected data from courses aimed at two different year levels, freshman and junior, course level became an additional factor. Rather than compare individual classes (e.g., cell biology vs biochemistry), we combined students into a single freshman cohort (N = 118) and a single junior cohort (N = 84). There were no significant differences between the first midterm scores of the freshman one- and two-midterm cohorts and between the junior one- and two-midterm cohorts indicating that students in the one- and two-midterm cohorts started out academically similar.

Results

The 2 x 2 x 2 analysis of variance (ANOVA) showed a main effect for achievement, $F(1,188) = 5.555$, $p = .019$. High-achieving students (mean exam score difference = -4.635, SEM = 1.135) had significantly different mean difference scores than low-achieving students (mean exam score difference = -.761, SEM = 1.188). There was no main effect for midterm exam score or course level.

There was an interaction effect for midterm exams and course level, $F(1,188) = 4.137$, $p = .043$, in which freshman students were impacted by the number of midterms while junior students were not (Figure 1). Specifically, freshmen who received one midterm performed significantly poorer on their final relative to their midterm exam (mean exam score difference = -5.885, SEM = 1.566) than freshmen who received two midterms (mean exam score difference = -4.635, SEM = 1.135).

There was no interaction effect between the number of midterm exams and achievement level: low-achieving students did not differentially benefit from a second midterm exam relative to high-achieving students.

Discussion

Our primary goal was to consider whether increasing midterms from one to two exams would improve learning outcomes in undergraduate biology courses. Within the testing effect research, there is a strong consensus that retrieval practice leads to better long-term retention than re-study alone (Rowland, 2014; Schwieren et al., 2017). There is also evidence to suggest that increasing the frequency of testing will lead to greater improvements in learning outcomes (Bailey et al., 2017; Bangert-Drowns et al., 1991; Foss & Pirozzolo, 2017; Myers & Myers, 2007; Roediger & Karpicke, 2006). Whether frequency improves the testing effect, alters student attitudes and behaviors (e.g., spaced studying), or impacts both, remains unclear. Regardless of the mechanism, we expected that two midterm exams would result in improved final exam scores relative to their first midterm exam score. Our results partially support this prediction. An ANOVA found a significant interaction effect between course level and number of midterms indicating that freshman students were positively impacted by a second midterm while junior students were not. This is similar to the impact that an e-portfolio assignment can have on student learning (Haave, 2016). Freshmen who received a second midterm exam did not perform as poorly on their final exam relative to their first midterm exam compared to those who completed only one midterm exam: a second midterm exam rescued freshman students from a significantly poorer final exam result. Freshmen are a unique student population as they are transitioning from high school to university while learning to become self-directed learners. Having freshmen practice retrieving their learning in the classroom (something they typically do not incorporate into their own study regime, Brown et al., 2014) is beneficial in the short-term, but may also benefit their ongoing development as learners. In contrast, juniors may be sufficiently self-directed learners that there is no additional impact from a second midterm. Therefore, junior students may require other kinds of learning interventions to continue their development as self-directed learners.

We were also interested in considering the subpopulation of low achievers. We believed that low achievers would see a greater benefit from two midterms than high achievers, but our results do not support this prediction. While we saw a main effect for achievement (i.e., there was a difference in how high- versus low-achieving students performed on their final vs their first midterm exam), we found no interaction effect to suggest that low or high achievers benefited from the second midterm in a unique way. Both low and high achievers did worse on the final compared to the midterm. Low achievers had a significantly smaller difference score, meaning their midterm and final

marks remained more similar than those of high achievers. This result is contradictory to other research on disadvantaged populations. For instance, Pyburn et al. (2014) found that a multiple-choice pre-test led to improved exam performance, but low-skilled English comprehenders benefited more than high-skilled English comprehenders. It appears that initial learning

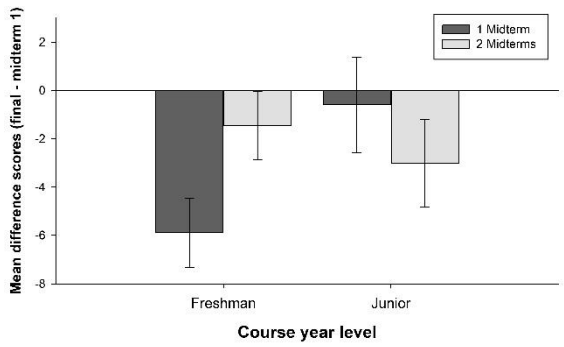

Fig. 1. The impact of course level and number of midterms on difference scores (final minus the first midterm exam score). ANOVA results indicate a significant interaction effect between course level and number of midterm exams, $F(1,188) = 4.137$, $p = .043$. Error bars represent standard error of the mean.

ability may not impact the influence of a second midterm exam. This result is unexpected as it could be argued that freshmen are not as experienced learners as juniors which is why freshmen benefit from a second midterm exam whereas juniors do not. Clearly, initial achievement level and learning ability/experience have a more complicated relationship than we anticipated.

Conclusion

Our results suggest that a second midterm exam may improve learning outcomes for students enrolled in a freshman but not a junior biology course. Additionally, a second midterm exam did not differentially improve the final exam scores relative to the midterm exam scores for low-achieving students. A primary limitation to our study is that it only analyzes biology courses. In addition, we were able to match only a handful of course iterations for analysis which limited our sample size. The small sample negatively impacted the effect size and power of the statistical test. Furthermore, while differences in course structure were minimized by using courses offered close in year and with similar course structuring external to the additional midterm exam, we were not able to account for all variations, such as students' prior GPA, relying instead on the first midterm exam score as an indicator of academic ability or preparation. A possible confounding factor is that the junior cell biology course had a term paper rather than in-class quizzes which our statistical analysis could not address. More robust conclusions will require future research with access to a larger campus population as well as additional disciplines. Future testing of sophomores and seniors may also provide additional information about the impact of course level. One obvious question is whether sophomores and seniors will show a similar pattern; that is, will additional midterm exams impact sophomores but not seniors? Finally, we cannot make any claims regarding the mechanism by which two midterm exams improved student learning outcomes. One future direction for research is to attempt to make distinctions between the testing and frequency effects. Distinguishing between these two mechanisms remains problematic. However, in terms of useful interventions, it is sufficient to recognize that regardless of why, testing in the classroom acts as a beneficial learning tool, not simply a necessity for program assessment purposes.

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References

BAE, C.L., THERRIAULT, D.J. AND J.L. REDIFER. 2018. Investigating the testing effect: Retrieval as a characteristic of effective study strategies. Learn. and Instr 60: 206-214.

BAILEY, E.G., JENSEN, J., NELSON, J., WIBERG, H.K. AND J.D. BELL. 2017. Weekly formative exams and creative grading enhance student learning in an introductory biology course. CBE—Life Sci. Educ. 16(1): 1-9.

BANGERT-DROWNS, R.L., KULIK, J.A. AND C. L.C., KULIK. 1991. Effects of frequent classroom testing. J Educ. Res. 85(2): 89–99.

BRAME, C.J. AND R. BIEL. 2015. Test-enhanced learning: The potential for testing to promote greater learning in undergraduate science courses. CBE- Life Sci. Educ. 14(2): 1-12.

BROWN, P.C., ROEDIGER, H.L. AND M.A. MCDANIEL. 2014. To learn, retrieve. Pp. 23–45. Make it stick: The science of successful learning. The Belknap Press of Harvard University Press, Cambridge, MA.

CARPENTER, S.K. 2012. Testing enhances the transfer of learning. Current Directions in Psychol. Sci. 21(5): 279–283.

CARPENTER, S.K. AND J.W. KELLY. 2012. Tests enhance retention and transfer of spatial learning. Psychonomic Bulletin and Review 19(3): 443–448.

FOSS, D.J. AND J.W. PIROZZOLO. 2017. Four semesters investigating frequency of testing, the testing effect, and transfer of training. J Educ. Psycho.l 109(8): 1067–1083.

HAAVE, N. 2016. E-portfolios rescue biology students from a poorer final exam result: Promoting student metacognition. Bioscene: J. Coll. Biol. Teach.. 42(1): 8–15.

KORNELL, N., BJORK, R.A. AND M.A. GARCIA. 2011. Why tests appear to prevent forgetting: A distribution-based bifurcation model. J Mem. Lang. 65: 85–97.

LAWRENCE, N.K. 2013. Cumulative exams in the introductory psychology course. Teach. Psychol. 40(1): 15–19.

MCDANIEL, M.A., ANDERSON, J.L., DERBISH, M.H. AND N. MORRISETTE. 2007. Testing the testing effect in the classroom. Eur. J Cogn. Psychol. 19(4–5): 494–513.

MULLIGAN, N.W., RAWSON, K.A., PETERSON, D.J., AND K.T. WISSMAN. 2018. The replicability of the negative testing effect: Differences across participant populations. J Exp. Psychol. Learn. 44(5): 752–763.

MYERS, C.B. AND S.M. MYERS. 2007. Assessing assessment: The effects of two exam formats on course achievement and evaluation. Innov. High. Educ. 31(4): 227–236.

PYBURN, D.T., PAZICNI, S., BENASSI, V.A. AND E.M. TAPPIN. 2014. The testing effect: An intervention on behalf of low-skilled comprehenders in general chemistry. J Chem. Educ. 91(12): 2045 2057.

ROEDIGER, H.L. AND A.C. BUTLER. 2011. The critical role of retrieval practice in long-term retention. Trends Cogn. Sci. 15(1): 20–27.

ROEDIGER, H.L. AND J.D. KARPICKE. 2006. Test enhanced learning: Taking memory tests improves long-term retention. Psychol. Sci. 17(3): 249–255.

ROWLAND, C.A. 2014. The effect of testing versus restudy on retention: A meta-analytic review of the testing effect. Psychol. Bull. 140(6): 1432-1463.

SCHWIEREN, J., BARENBERG, J. AND S. DUTKE. 2017. The testing effect in the psychology classroom: A meta-analytic perspective. Psychol. Learn. Teach. 6(2): 179–196.

SPITZER, H.F. 1939. Studies in retention. J Educ. Psychol. 30(9): 641–656.

WHEELER, M.A. AND H.L. ROEDIGER. 1992. Disparate effects of repeated testing: Reconciling Ballard's (1913) and Bartlett's (1932) results. Psychol. Sci. 3(4): 240–2.

Learning Strategies to Initiate and Motivate Students of an Introductory Microbiology Laboratory Class to Perform Cooperatively an Inquiry-based Project.

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Abstract

Pre-nursing students of an introductory Microbiology laboratory class, having learnt typical microbiological techniques during the semester, gained the confidence of conducting an inquiry-based project as part of their lab course work. Students cooperatively performed a microbiological analysis, to evaluate safety of sushi. This paper presents learning strategies and assessment methods to prepare and motivate the students for undertaking an investigative project. Approaches are discussed that were taken to continually assess the students during the performance of the project in order to ensure harmonious group activity and transition through the various stages of the investigative project. Students commented that their investigative experience had increased their thinking and analytical skills and heightened their awareness of the process of scientific discovery.

Key words: Introductory Microbiology laboratory course; Pre-Nursing majors; Inquiry; Cooperative Learning

Introduction

For quite some time, education experts and many biology teachers have been urging critical thinking exercises, hands-on experimentation, and inquiry-based science education in conducting undergraduate biology courses. Human society has become rapidly technological and science has assumed a major presence in the everyday functioning of an increasing number of people. It is, therefore, imperative for the young student to gain an understanding of the process of science and how researchers make discoveries in order to make informed decisions in today's world (American Association for the Advancement of Science, 2011; Somres & Ham, 2009; National Academies of Sciences, Engineering, and Medicine, 2015, 2017). Numerous reports of successful inquiry-based undergraduate laboratory teaching in biology have been reported (Mitchell & Garziano, 2006; Marshall, 2007; Madhuri & Broussard, 2008; Spiro & Knisely, 2008; Walker et al., 2008; Hurd, 2008; Lu et al., 2008; and, Zhang, 2008). Further, it has been shown that when investigative approaches are performed by biology students in a cooperative manner working together to achieve a common goal, higher levels of student achievement are attained compared to traditional methods of teaching (Goyette & DeLuca, 2007; Goldberg & Dintzis, 2007; Seifert et al., 2009; Weisman, 2010; Premo et al., 2018). One of the key findings in cooperative learning has been that student's self-esteem is significantly enhanced. Cooperative learning also provided the students with an opportunity to acquire interpersonal communicative skills, enhance their motivation for learning, and to discover and exercise their critical thinking skills (Weiman, 2009). It is, therefore, important that biology and other science courses should be taught based on inquiry, research, and teamwork.

Typically, inquiry-based projects in an undergraduate laboratory course are conducted for biology juniors and seniors who have previously completed a set of biology courses including rigorous laboratory courses and students work on the project throughout the semester (Seifert et al., 2009). At the University of Guam, pre-nursing majors are required to take an introductory one semester-long microbiology laboratory class. The students have limited science background and have not had the opportunity to engage in investigative projects in science lab classes. As part of efforts at improving the laboratory course experience for pre-nursing majors, an inquiry-based cooperative learning approach was tested. After ensuring that students acquired the skills in experimental microbiology in par with national standards for such an introductory course, the students were motivated to utilize these skills in conducting an inquiry-based project in a cooperative manner, in the last three weeks of the semester.

In this paper, are presented: (i) the steps taken to initiate and motivate the students into conducting an inquiry based cooperative project; (ii) the food microbiology investigative project decided upon by the students and general design of the project; (iii)

student microbiological skills assessment and evaluating student preparedness for undertaking the project; (iv) learning outcomes; (v) approaches taken to enhance cooperative learning; (vi) assessment of student's performance of the inquiry-based project; (vii) conclusions and discussion that students arrived at by consensus among the class; and (viii) post-project feedback from students.

The successful completion within a limited time and budget of an inquiry-based project by pre-nursing students clearly shows that such an investigative, cooperative learning approach can be introduced in an introductory microbiology lab course. It was also found that at the end of the semester, the students had a heightened awareness of the process of scientific discovery and the significance of basic science in providing breakthroughs in understanding disease, in medical diagnostics and in developing therapeutics. Students exhibited a high level of excitement and enthusiasm for microbiology and the molecular life sciences.

The Inquiry-based Cooperative Learning Strategy and Student Assessment

The conceptual strategy for initiating and conducting the inquiry-based food microbiology analysis project in a cooperative manner, the evaluation design, learning outcomes, and assessment of student performance of the project are discussed below. Students were assessed prior to the start of the project and during the performance of the project to test the student's knowledge of microbiological techniques required for the food analysis, their preparedness for undertaking the project, and, efficient conduct of the project in collaboration with their peers. This was followed by a post-project analysis of student's experience of the classroom investigation.

A. Initiation of Inquiry project

Earlier in the semester, students isolated from their own skin surface resident bacteria using typical culturing methods and were surprised to learn that they harbor the potential human pathogen Staphylococcus aureus. Students also learnt of the immense diversity of microbes present in the surrounding environment. Students identified potential problems that microbes may cause on the island of Guam, ranging from those in hospitals and clinics, to the drinking water supply, sewage treatment plant and release of raw sewage and garbage directly into the coral reef areas. Some students voiced their concern over the safety of salads and sushi that is served in food store outlets. Students also remembered, from their lecture class, that many bacteria produce toxins which can be introduced into food during processing, preparation, and handling. Students agreed to undertake, as part of their lab course work, an inquiry project to evaluate microbiological contamination if any present in the sushi.

B. Microbiological Analysis Project Design

Eighteen microbiology students formed three groups of six students each, to determine the levels and nature of microbial contamination of sushi. The three groups planned on testing three sushi samples of the same variety, essentially to obtain results in triplicate. Students agreed to perform the project in a cooperative manner sharing their observations, data, and thoughts. Students within each group agreed to monitor each others methodology to ensure that the correct steps were being taken and all data observed were collected. The students also felt that it would be important for the three groups to interact with each other to comment on experimental procedures, observations, and data collection to ensure uniformity while conducting the project, for statistical validity. Finally, all three groups agreed to share their data with each other to arrive at a consensus with the instructor moderating the discussions.

Based on their learning of typical microbiological principles and methods during the semester, students reasoned that they would be able to investigate four important aspects in their microbiological evaluation, namely:

i. quantitate levels of bacterial contamination using the standard plate count (SPC) and coliform count methods.

ii. isolate and identify the bacteria using selective/differential culture plates, wet mount analysis, and Gram staining.

iii. determine if the contaminating bacteria form spores.

iv. antimicrobial testing to determine the effectiveness of selected typical antibiotics on the recovered contaminating bacteria.

C. Pre-project Evaluation Design and Assessment

In order to incorporate an investigative cooperative learning approach in an introductory microbiology lab course, it was essential to ensure that students had acquired skills in: performance of microbiological techniques and experiments using appropriate scientific controls; collection and organization of results; drawing conclusions; and, in interacting with fellow students. Students were assessed for the following important learning components prior to embarking on the project:

i. Student Learning of Microbiological Techniques: Students learnt a set of seven standard microbiological techniques that are required for analyzing the contamination levels of food. The techniques are: (a) light microscopy analysis using wet mounts; (b) Gram staining; (c) aseptic & pure culture techniques & culturing

methods; (d) spore analysis test; (e) antimicrobial sensitivity testing (Kirby Bauer method); (f) standard plate count method (SPC); and, (g) identification of unknown bacteria using selective/differential culture media. Students also learnt general microbiology safety guidelines and universal precautions as described in their microbiology lab manual (Brown, 2009).

ii. Laboratory Notebook: Students were required to maintain a logbook of their lab class activities. The log book notes of all students were inspected, and comments provided on the format of journal entry.

iii. Data Collection & Organization: Students were required to organize all data obtained from the experiments that they had performed in the lab class, in the form of tables and graphs. The students also learnt the importance of statistical validity and therefore tested three sushi samples of the same variety, essentially to obtain results in triplicate.

iv. Collaboration with Peers: Students were familiarized with the cooperative learning approach by requiring all students to share their data with the rest of the class. This was achieved by drawing data tables on the blackboard. Each student recorded his or her data on the blackboard followed by an interactive discussion on the observations. This exercise taught the students how to arrive at conclusions by consensus, taking into account all the pros and cons.

v. Theoretical Knowledge: An exam was conducted to test the student's basic theoretical knowledge associated with the microbiological techniques to be used, as well as familiarity with lab equipment, culture media, and reagents. A post-exam review ensured that all students learnt the concepts forming the basis of each microbiological procedure.

vi. Laboratory Report: Earlier in the semester, the students were required to present their results for the "bacterial unknown identification" experiment in a concise and well-organized laboratory report. This exercise prepared the students for writing lab reports that would form the final part of the investigative project.

D. Pre-project Questionnaire - Evaluating Student Preparedness for Undertaking Project

In the first ten weeks of the semester, students had completed a series of microbiological experiments acquiring skills that would be required for successfully completing the food analysis project and attended lecture classes on essential microbiological concepts. Students were provided a questionnaire to determine their comfort level with microbiological concepts and skills. The questions and response data are provided in Table 1. The results indicated that all students in the class had acquired fundamental microbiological skills and had gained the confidence in continuing with the project.

E. Learning outcomes

The main student learning outcomes of the inquiry-based investigative project in the Introductory Microbiology course are specified below:

1. Enhancement of student's curiosity levels and thinking ability.
2. Application of microbiology techniques and approaches in investigating a scientific question, relevant to public health.
3. Designing experiments, collecting and organizing data in the form of tables & figures, photo documentation, and preparation of scientific reports.
4. Development of collaborative and communication skills.
5. Inculcate awareness and enthusiasm for the scientific discovery process.

F. Project performance assessment

At every step of the multi-stage investigative project, each student groups methodology was monitored to ensure that the correct microbiological procedures were being used. The groups were advised not to proceed to the next stage until clearance was obtained, ensuring harmonious group activity and transition through the various stages of the project.

To enhance the cooperative approach, the three groups were asked to share their experiences at the end of every stage of the investigative project. This allowed each group to comment on and critique each other and ensure that all three groups were maintaining uniformity in their experimental methods for statistical validity. The strategy of monitoring the students themselves allowed detection of any unexpected mistakes that were made and to correct them or to account for them while drawing conclusions from the results obtained. For example, at the very first stage of the project the three groups blended their sushi sample in a sterile blender, prepared appropriate dilutions and plated on a rich nutrient medium for culturing bacteria. During the discussions, the students found that one of the groups had peeled the sushi wrapping and the rice away from the raw fish contents and did not include them in the blender while preparing food dilutions. A dialogue ensued, and the students discussed the consequences and the results that they could expect for the three food samples. The students agreed that not including the rice and wrapping would mean that they essentially would be performing duplicates instead of in triplicates as originally planned. However, the students reasoned that this mistake could be used to their advantage. They hypothesized that the major source of bacterial contamination would come from

Table 1. Student's comfort level with microbiological concepts and skills required to perform a microbiological evaluation of raw fish containing ready-to-eat food preparations ("sushi".)

Concepts & Skills	Average Score*
General Microbiological Principles	4.62 ± 0.62
Light Microscopy analysis using wet mounts	4.81 ± 0.40
Staining and observation of microorganisms	4.75 ± 0.45
Aseptic & Pure culture techniques and culturing methods	4.62 ± 0.62
Spores and spore analysis test	4.37 ± 0.62
Antimicrobial sensitivity testing (Kirby Baeur Method)	4.56 ± 0.63
Standard Plate Count Method (SPC Method)	3.56 ± 0.89
Identification of unknown bacteria using selective/differential culture plates	4.68 ± 0.48
Writing lab reports and presentation of data in clear and succinct format	4.37 ± 0.80

*Students were asked to indicate their comfort level in nine areas, on a scale of 1-5 (1, Not at all; 2, Very Little; 3, Somewhat; 4, Quite a bit; 5, Very much). n =16

the raw fish content of the sushi sample and not from the cooked rice and wrapping. They reasoned that if indeed this were true, then the level of bacterial contamination for all the three samples would be similar. If the contamination levels for the sample where the rice and wrapping were not included were lower, then that would indicate that the cooked rice and wrapping also were contaminated. During the observations and collection of data, the students found lower levels of contamination when the rice and wrapping were not included. The students concluded that the rice and wrapping used were also contaminated. The students found significant contamination of Staphylococcus aureus and Staphylococcus epidermidis based on selective/differential culture plate test. Both these species are present abundantly on the surface of the human skin. The students concluded that the sushi samples were prepared under unhygienic conditions, where the food preparer probably did not wear gloves and rolled the sushi with bare hands. However, some students argued that the contaminants could very easily have been introduced by the students themselves during the food analysis project. The interaction between the three groups at every stage of the project allowed students to build consensus regarding conclusions. This exercise would play a very important part in the end stage of the project when students came together to arrive at a summary conclusion regarding the safety of sushi.

An important element of involving students in cooperative discussions is the fact that students spontaneously start thinking critically. This was evident during the identification of yeast contaminants using selective culture plates - the chloramphenicol antibiotic in the plate prevents the growth of bacteria, thus any colonies detected would be that of yeast. The students did not observe growth of any colonies on the plates for the three food samples and concluded that the sushi samples did not contain any yeast contamination. However, on one of the plates, one colony was found growing at the edge of the plate and the students of the group after discussion among themselves remarked that the colony may be a bacterial contaminant that is resistant to the chloramphenicol antibiotic. This raised concern among students about the potential for spreading of antibiotic resistant strains of bacteria through sushi. However, students counter-argued that the contaminant could very well have come from the teaching lab while analyzing the food sample. It was evident that the students were able to utilize the important microbiological concepts that they had learnt in the lecture and laboratory class for their investigative project.

During the final discussion session, it was evident that students were actively engaged and had realized the significance of the investigative project. The students debated the conclusions to be arrived at regarding the contamination levels of the sushi samples. Students agreed that that the precise rules of food safety testing, including statistical analysis, was not performed. Some students argued that the bacterial contaminants they recovered may have very well come from a breach of aseptic procedures in the lab while evaluating the sushi samples. Others commented that there is a possibility that sushi sold at stores may exhibit some levels of non-pathogenic bacterial contamination which did not pose a serious threat to humans, especially since no case of food poisoning was reported from any of the food outlets. The students also reasoned that if indeed the sushi samples exhibited some levels of contaminating bacteria, then the presence of these contaminants did not indicate that the food was spoiled, rather there may be a potential for rapid spoilage of food. Coliform counts

using the selective culture plate did not reveal any fecal contamination of the food samples – the students heaved a sigh of relief! The colonies found on the plates were non-lactose fermenting species and students commented that these bacteria may potentially be pathogenic since gram negative bacteria are known to secrete toxins (Tortora et al., 2009). The students expressed their concern that the gram-negative bacteria contaminating the sushi samples that did not respond to any of the antibiotics tested in their antimicrobial testing analysis, could potentially be harmful if ingested. The students reasoned that these bacteria might represent resistant strains whose genome codes for enzymes responsible for inactivating the effects of the antibiotics tested as learnt from their microbiology textbook (Tortora et al., 2009).

The scientific argumentation and data analysis by all three groups provided strong evidence that student's curiosity, thinking ability, and enthusiasm were enhanced as a result of collaborative project participation. The classroom discussions generated among students, collection of quantitative and qualitative data, organization of data in the form of tables & figures, nature of the conclusions arrived at by consensus among the students, and preparation of final report, provides strong evidence that the main student learning outcomes were achieved in the inquiry-based project in the introductory microbiology course.

G. Post-project Student Feedback and Student's Experience of the Investigative Project

Post-project feedback from the students was obtained via: questionnaire, spontaneous student comments made verbally during the progress of the project in the classroom to each other, verbal comments provided by some students to the Instructor outside of the classroom, and, official course and instructor evaluation by students.

a. Post-project Questionnaire:

Sixteen of the eighteen students present during the last laboratory class for the semester provided feedback on the investigative project – the students were asked to not include their names in their responses to the questionnaire. The questions that were asked of the students are given below:

i. Did you find the investigative project interesting and important?
ii. Do you think that being able to apply microbiological techniques learned in the lab class to an investigative project enhances the lab experience of students?
iii. Did you feel comfortable performing the project using the microbiological techniques that you learnt earlier in the semester?
iv. Do you think that it is important to learn to work collaboratively with your fellow students?
v. Do you feel that performing an investigative project enhances your ability to think and analyze data compared to performing experiments directly from the lab manual?
vi. Do you feel that an investigative project should be included as an important component of the microbiology lab course conducted at the university?

The students unanimously answered in the affirmative for all six questions. One student further commented that this cooperative approach was good training that would help them prepare for a career in nursing. The positive feedback from the students was further borne out by the spontaneous student comments as described in the next section. In official student evaluations, only one of fourteen students commented that the investigative project performed in the laboratory was a distraction with regards to preparation for the final examination for the microbiology course. Since the sample size of the class was small (eighteen students), it is conceivable that not all students taking an introductory microbiology course would be in favor of an inquiry-based project as part of laboratory course work. However, the largely favorable response from students indicate that the investigative project indeed helped students learn the real-world applications of microbiology.

b. Spontaneous Student Comments:

A very strong indication that the investigative project was viewed favorably by the class is the spontaneous comments on the project made by the students to each other during the performance of the lab work, and verbally to the Instructor outside the classroom. Six students informed the Instructor that they enjoyed the investigative project commenting that they had been used to "learning chapter by chapter straight from the lab manual". One student remarked "You should introduce it as a regular part of the lab course work in future micro lab classes". Another student went on to comment "We never realized that biology can be so interesting. If we had known, we would have become biology majors".

In the final project report submitted by group # 3, the following comment was included: "This project has enlightened our group and put many questions on our table. The project conducted has many implications as to how exactly food is handled and what steps food handlers are taking to minimize food contamination. The project can serve as a helpful resource and educate the food industry as to approximately how many microbes can contaminate food if the proper techniques are not practiced."

c. Official Student Evaluations and Student Performance in Final Exams:

Only one of fourteen students who participated did not favor the idea of an investigative project in an introductory course, citing that it distracted from preparing for the final exam. There was no significant difference in student ratings of the Instructor received for the project-based microbiology course and the scores that were received in earlier microbiology courses. The students of the project-based microbiology course did not perform better on their end-of-course final exam compared to students in earlier Microbiology courses. There seems to be no correlation between participation in an investigative project and increased success in the final exam on microbiology.

Discussion

Here, is reported the outcomes of an inquiry-based project performed cooperatively by pre-nursing students in an introductory microbiology laboratory class. These students had a limited background in the sciences and none of them had participated in a research type project for any of the earlier courses that they had taken. It was indeed remarkable to observe the collaborative nature of the students in undertaking a project. The intensity of the classroom discussions reflected the ability of the students to think and integrate concepts learnt in the microbiology lecture and laboratory class. The overall impression was that such an investigative project enhanced the learning experience of pre-nursing students and created a general sense of confidence in their academic work. Students felt much more aware of their capabilities, which would be very important in their future careers in the health professions. Students felt quite thrilled that they were able to interact with each other in a critical yet harmonious manner and accomplish the goals set for the project.

The main aim of this inquiry-based project was to test if pre-nursing students of an introductory microbiology course were able to utilize and integrate microbiological concepts and experimental skills; to test the collaborative capability of the students; and ability to communicate effectively and arrive at conclusions by consensus. The success of the students in fulfilling these aims clearly shows that inquiry-based projects using a cooperative learning approach can be effectively utilized in an introductory microbiology lab course to enhance student learning in a limited time and budget format.

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References

American Association for the Advancement of Science. 2011. Vision and change in undergraduate biology education: A call to action. Washington, DC.

Brown, A. 2009. Microbiological Applications by Bensons, 11th Edition. McGraw Hill Publishers, New York.

Goldberg, H.R. and Dintzis, R. 2007. The positive impact of team-based virtual microscopy on student learning in physiology and histology. Adv. Physiol. Educ. 31, 261-265.

Goyette, S.R. and DeLuca, J. 2007. A Semester-long student-directed research project involving enzyme immunoassay: Appropriate for Immunology, Endocrinology, or Neuroscience Courses. CBE Life Sci. Educ. 6, 332-342.

Hurd, D.D. 2008. A Microcosm of the biomedical research experience for upper-level undergraduates. CBE Life Sci. Educ. 7, 210-219.

Lu, F., Eliceiri, K.W., Squirrell, J.M., White, J.G., and Stewart, J. 2008. Student learning of early embryonic development via the utilization of research resources from the nematode Caenorhabditis elegans. CBE Life Sci. Educ. 7, 64-73.

Madhuri, M. and Broussard, C. 2008. "Do I need to know this for the exam?" Using popular media, inquiry-based laboratories, and a community of scientific practice to motivate students to learn developmental biology. CBE Life Sci. Educ. 7, 36-44.

Marshall, P.A. 2007. Using Saccharomyces cerevisae to test the mutagenicity of household compounds: an open-ended hypothesis-driven teaching lab. CBE Life Sci. Educ. 6, 307-315.

Mitchell, B.F., and Garziano, M.R. 2006. From organelles to protein gel: a 6-wk laboratory period on flagellar proteins. CBE Life Sci. Educ. 5, 239-246.

National Academies of Sciences, Engineering, and Medicine. 2015. Integrating discovery-based research into the undergraduate curriculum: Report of a convocation. Washington, DC: National Academies Press.

National Academies of Sciences, Engineering, and Medicine. 2017. Undergraduate research experiences for STEM students: Successes, challenges, and opportunities. Washington, DC: National Academies Press.

Premo, J, Cavagnetto, A, and Davis W. B. 2018. Promoting collaborative classrooms: The impacts of interdependent cooperative learning on undergraduate interactions and achievement. CBE—Life Sciences Education, 17:ar32, 1–1.

Seifert, K., Fenster, A., Dilts, J.A., and Temple, L. 2009. An investigative, cooperative learning approach to the general microbiology laboratory. CBE Life Sci. Educ. 8, 147-153.

Somres, B., and Ham, B. 2009. Experts urge bold new undergrad biology courses for the 21st century. Science, Vol 325, 1637.

Spiro, M.D. and Knisely, K.I. 2008. Alternation of Generations and Experimental Design: A guided-inquiry lab exploring the nature of the her1 developmental mutant of Ceratopteris richardii (C-Fern). CBE Life Sci. Educ. 7, 82-88.

Tortora, J.G., Funke, B.R., & Case, C.L. 2009. Microbiology, 10th Edition. Pearson Benjamin Cummings Publishers.

Walker, D.E., Lutz, G. P., and Alvarez, C.J. 2008. Development of a cross-disciplinary investigative model for the introduction of microarray techniques at non-R1 undergraduate institutions. CBE Life Sci. Educ. 7, 118-131.

Wieman, C. 2009. Galvanizing sciences departments. Science, Vol 325, 1181.

Weisman, D. 2010. Incorporating a collaborative web-based virtual laboratory in an undergraduate bioinformatics course. Biochem. Mol. Biol. Educ. 38, 4-9.

Zhang, S. 2008. A research project-based and self-determined teaching system of molecular biology techniques for undergraduates. Biochem. Mol. Bio. Educ. 36, 181-188.

Identifying the breakdowns in how students and faculty interpret course objectives

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Abstract

Because students and professors place different values on syllabi components, perceptions of course objectives vary. Previous studies investigated the relationship between students' and instructors' expectations and syllabi content, but do not address the role of explicitly stated course objectives in syllabi. Our study used qualitative methods to investigate relationships among student-reported perceptions of course objectives, professor-reported intended course objectives, and explicitly stated course objectives from syllabi. We used interviews from two professors who taught introductory biology courses for non-majors, course syllabi, and student responses to an open-ended questionnaire about course objectives. After using a deductive approach to code students' responses, we found only 21% of students accurately identified a course objective listed in the syllabus. We identified three main themes in student reported course objectives: Knowledge (n=539), Practice (n=30), and Performance (n=41). Two of these (Knowledge and Practice) aligned with professor intended course objectives but did not align with explicitly stated course objectives. Based on our findings, we conclude that students poorly identified explicitly stated course objectives but correctly identified their professors' intended objectives. Therefore, we recommend professors better connect their intended course objectives with those explicitly stated in the syllabus.

Keywords: Syllabus, course objectives, communication, undergraduate biology

Introduction

A traditional communication tool between students and professors is the course syllabus. Syllabi serve as a classroom contract between students and professors by presenting professor expectations, assignments, and anticipated learning outcomes (Griffith et al., 2014). However, students and instructors value different syllabi components, making syllabi alone an inadequate communication tool (Becker & Calhoon, 1999; Smith & Razzouk, 1993). Defective communication via syllabi highlight disconnections between teachers' and students' interpretations of course objectives (Aggar & Shelton, 2015; Mitchell & Manzo, 2018).

Traditionally, syllabi fulfill one or more of four primary roles: as a contract, a permanent record, a learning/teaching tool, and/or a communication medium (Albers, 2003; Parkes & Harris, 2002; Thompson, 2007). As contracts, syllabi present expectations, rules, and responsibilities to which faculty and students are expected to adhere (Matejka & Kurke, 1994; Parkes & Harris, 2002), as well as act as a permanent record of teacher performance by documenting the scholarship of the course, course concepts, expectations for students, and evaluation techniques (Albers, 2003; Parkes & Harris, 2002). Documentation of course content through syllabi can assist administrators or reviewers in determination of a course's alignment with a department and/or institution's mission (Albers, 2003). Instructors design and use syllabi as learning/teaching tools to motivate students and positively influence their attitudes (Bain, 2004; Parkes & Harris, 2002). When used as a learning/teaching tool, syllabi place increased emphasis on resources and practices students can utilize throughout the course to become better learners (Davis & Schrader, 2009). Syllabi also communicate procedural and logistical information regarding due dates for assignments and exams, grading criteria, and anticipated learning outcomes (Parkes & Harris, 2002).

Students place significant value on parts of syllabi, such as exam and course assignment due dates, they believe will contribute to their success in the course (Becker & Calhoon, 1999). This suggests students approach syllabi as a course contract for

success (Davis & Schrader, 2009; Marcis & Carr, 2004). In contrast, faculty tend to place more value on parts of syllabi components related to expected student conduct (Davis & Schrader, 2009; Wolf et al., 2014), suggesting faculty utilize the document as a teaching tool. In some instances, such as large enrollment courses with multiple sections, faculty are expected to share a syllabus and have little control of the components and learning objectives that go into the document (Mitchell & Manzo, 2018). In instances where the same syllabus is shared across different course sections, faculty typically make fewer attempts to clearly communicate syllabi elements, resulting in less student use (Mitchell & Manzo, 2018). This leads to a feedback loop where the syllabus is further devalued.

Collier and Morgen (2008) further investigated these differences and found that instructors grew increasingly frustrated when students expected syllabi with more explicit content, as instructors felt the syllabi were already highly explicit. This disagreement between students and instructors can result in negative impacts on student performance, as some students fail to understand the expectations instructors have about students' coursework commitments (e.g., time spent on studying and assignments) (Collier & Morgen, 2008). Additionally, Aggar and Shelton (2015) investigated syllabi across private and public higher education institutions and found students at public institutions encounter more authoritarianism in their syllabi than at private institutions. Although Aggar and Shelton (2015) studied syllabi from a labor contract perspective for classroom and behavior management, they found high syllabi diversity between institution type and class size. On a larger scale, this higher diversity among syllabi can contribute to student confusion and miscommunication as students must navigate varying syllabi across their undergraduate career. Existing literature continues to highlight how students and instructors value and view components of syllabi.

While approaches to syllabi differ between students and instructors, a common attribute of most syllabi is the inclusion of course and learning objectives. It is possible that the terms course objective and learning objective are used interchangeably in the extant literature, but as we focus on course objectives for this study, we feel the need to clarify the differences between the two. In this study, we use the term course objective to mean a goal to be achieved by the student after completion of the course, whereas our operational definition of learning objective is informed by Mitchell and Manzo (2018) as, "...a commonly used metric with which students can be assessed" (p. 456). Furthermore, we posit that course objectives may also include less measurable goals put in place by instructors, such as developing an appreciation for a specific topic. Most higher education institutions require course objectives for each class, but in Texas specifically, each course taught at the university level has state-mandated course objectives. Course objectives can guide syllabi development and highlight what students should know and be able to do after being instructed on a topic (Allan, 1996; Hartel & Foegeding, 2004). Mitchell and Manzo (2018) state that a well-developed and clear learning objective includes a verb that contains an observable action item, conditions for when the action should be carried out, and the associated performance level. Clear learning objectives allow students to know exactly what is required of them (e.g., contractual) and what they will learn as a result of completing requirements (e.g., teaching tool) (Mitchell & Manzo, 2018). Instructors can also provide additional instruction about how students can use learning objectives to track the trajectory of their learning throughout a course (Osueke et al., 2018). For example, in writing-intensive courses, instructors might communicate learning objectives through examples of exam questions and descriptions of answers to communicate performance expectations (Yule et al., 2010). Students can track their learning trajectory by comparing their answers on previous exams to determine potential improvement strategies to achieve higher performance expectations. In this way, instructors can help bridge the gap between differing valuations of learning and possibly course objectives, making syllabi more useful to students.

A common theme in the extant literature is the exploration of differences and relationships between students' and instructors' views of syllabi and learning objectives. For example, past research has explored the relationship between students' and instructors' expectations of syllabi content in fields such as nursing (Davis & Schrader, 2009), psychology (Becker & Calhoon, 1999), political science (McCrea & Lorenzet, 2018), management (Mitchell & Manzo, 2018), and introductory biochemistry courses (Osueke et al., 2018). However, what the literature fails to explore is the role explicit syllabus-stated course objectives play in fragmented communication between students and instructors. Additionally, research that explores the relationship between explicit syllabus-stated course objectives, teacher reported intended course objectives, and student perceptions of intended course objectives in biology courses is lacking. Students might perceive course objectives differently than how the professor intends for them to be interpreted and/or how they are expressed in the course syllabus, therefore, addressing differences in perceptions of course objectives could provide insight for improving communication between students and

instructors. The purpose for this study was to investigate the relationship among student reported perceptions of course objectives, professor reported intended course objectives, and explicit syllabus-stated course objectives. This project was guided by the following research questions (Figure 1):

1. In what ways do professor reported intended course objectives compare to explicitly syllabus-stated course objectives? (Fig. 1A)
2. In what ways do student reported perceptions of course objectives compare to professor reported intended course objectives? (Fig. 1B)
3. In what ways do student reported perceptions of course objectives compare to explicitly syllabus-stated course objectives? (Fig. 1C)

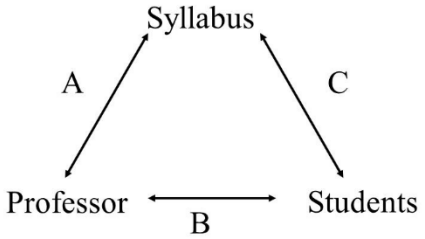

Fig. 1: Illustration of communication triangle for research questions.

Theoretical Framework

Instructional communication theory classifies the professor as a communicator. The professor's success in this enterprise relies on: 1) their communication conduct and 2) their opinions and views on communication (Staton-Spicer & Marty-White, 1981). Three paradigms comprise instructional communication theory: process-product paradigm, student-mediated paradigm, and culture-of-the-school paradigm. Our project focuses on process-product paradigm of instructional communication theory, which assumes teacher behaviors precede, and are most responsible for, student learning and achievement (Morreale et al., 2014). In our study, the usage of explicit syllabus-stated course objectives by professors represents the process, and accurate (as defined and described by professors) student perception of course objectives represent the product. It is important to note that in this case the term accurate is entirely derived from the perspective of the professor, as they create and communicate the course objectives throughout the semester.

Previous studies of the process-product paradigm have explored three stages of instruction: preoperational, process, and product (Staton-Spicer & Marty-White, 1981). The preoperational stage typically involves measuring teacher characteristics (such as their opinions of and methods for communication), the process stage typically includes observation of teacher classroom behaviors, and the product stage assesses teacher effectiveness by measuring student outcomes.

For this project, since we are more interested in students' understanding of course objectives rather than student learning outcomes, we framed the preoperational stage as determining how teachers display course objectives in their classrooms. Our process component consisted of course syllabi and interviews to assess how the objectives were displayed (explicit vs. implicit). The product component of our study was students' ability to correctly remember and identify course objectives (Fig. 2).

Methodology

Context

In this study, we investigated an introductory biology course designed for non-science majors. In accordance with Texas House Bill 2504, all undergraduate course syllabi in Texas are required to have explicitly stated course objectives for each course

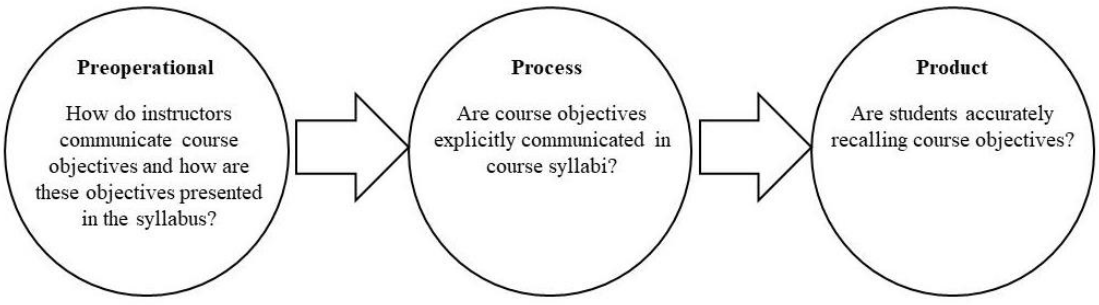

Fig. 2: Process-product paradigm of instructional communication theory

which are published on a university's website for public access (Kolkhorst, 2009). Student outcomes from taking the course should include the ability to demonstrate understanding of basic biology principles, have at least a conversational knowledge of modern biological science, and be able to make wise decisions regarding health and nutrition based on metabolism, physiology, and genetics.

At the university of this study, department policy dictates that introductory courses with multiple sections, taught by multiple professors have identical course objectives to ensure continuity of content for students across sections. While the course objectives for both sections of the course were identical (Table 1), each instructor created their own syllabus and determined how to incorporate the course objectives into their course.

Table 1. Course objectives for an undergraduate non-majors biology course.

Course Objectives
To examine the nature of science, the scientific method, & hypothesis testing.
To examine cell diversity, structure, & function.
To examine basic chemical principles, the nature of organic molecules, & the function of chemicals within cells.
To examine the role of energy in maintaining life & learn how cells acquire & use energy.
To examine the structure & function of DNA especially as it pertains to protein synthesis.
To examine the principles of inheritance (genetics) & explore patterns of inheritance in humans.
To examine the principles & regulation of cell division, & the consequences of malfunctions in the regulation of cell division (e.g. cancer).
To examine aspects of biotechnology & discuss the role that biotechnology plays in our world, including an exploration of the ethics & consequences of emerging technologies.
To examine the anatomy & physiology of the human reproductive system.

Participants

Participants for this study included two professors, Professors Richards and Kommala (pseudonyms), who taught three sections of the same introductory biology course for non-science majors at a large university in Texas and their undergraduate students. We asked undergraduate students enrolled in each professor's course to voluntarily take part in an online, open-ended questionnaire wherein we asked students to describe their ideas about course objectives and how these course objectives were communicated in the course. Per IRB approval (2017319), we obtained participant consent, administered the questionnaire, and conducted semi-structured interviews near the end of the course.

We collected data from student responses (n=424), as well as individual semi-structured interviews with each participating professor (n=2) to establish intended course objectives and identify how each professor conveyed those objectives within and beyond their course syllabus. We also used the course syllabus from each professor to verify the course objectives were explicitly stated for each course section.

Data Analysis

We examined responses and identified common themes that emerged across all participants and data sources using an inductive approach to coding. We transcribed data verbatim and then applied descriptive codes to each student-identified objective. We then used an inductive approach to coding to sort student responses based on themes which naturally arose from the data and reflected student perceived course objectives. We then used a deductive approach to categorize responses as either "accurate" or "inaccurate" based on a comparison to explicit syllabus-stated course objectives. Then we examined responses not aligned with explicit syllabus-stated course objectives and compared them to the professor's interview response.

At least two members of our research team coded each data source. When discrepancies arose between researchers, differences were discussed until a consensus over conflicting ideas was reached and a final coding was agreed upon. Consistency in this approach was high with an inter-rater reliability of 96%. We employed member checking with each professor to ensure our interpretations of their course objectives were consistent with their intended objectives. We also generated frequency counts of student response accuracy by counting responses that further evidenced our interpretations of the data. Multiple student responses required separation into two categories. These instances account for the higher number of total coded responses than the total number of students. For example, we coded the student response, "To understand the basic biology behind an organism. Such as cell structure, and DNA and how it all shapes living organisms and its functions" for both general biology content and genetic biology content.

Results

Course Objectives

Professor reported course objectives:

During our individual interviews with each professor, both discussed at length the importance of showing

students that science is approachable and relevant in everyday life. Professor Richards recognizes the science content-based course objectives outlined in her course syllabus, "the course objectives...because of the way the state of Texas is and the requirements are…" but did not focus on them. Instead, Professor Richards discussed her implied course objectives that centered around themes of science perception and life skills. "My learning objective in a non-majors course is not so much sciencey… I want them [students] to leave class feeling good about science…and just have better critical thinking skills." These themes continued throughout the interview, as Professor Richards described the importance of leaving non-science majors, "feeling like science is approachable," and teaching them to be, "a little more skeptical about what they read and what they hear and what they believe." Similarly, Professor Kommala also stressed the need to make science approachable for non-majors, as evidenced in her interview: "…the course objective is to do the applied measures of biology without making the students hate biology." Additionally, Professor Kommala attempted to relate biology to, "daily life" as evidenced in her interview:

> "humans are affected or benefited by the microorganisms… I extract the main concepts that apply to daily life, like what makes you sick and why you have less immunity to a disease when you have cancer and when you go through chemo."

Both professors provided similar course outcomes for their students but did not convey these objectives into their syllabi.

Student reported course objectives.

While coding student responses to the questionnaire, we found some students reported multiple course objectives (n=610). Three themes emerged from these reported course objectives: Knowledge (n=539), Practice (n=30), and Performance (n=41) (Table 2). These themes were further subdivided to gain a more in-depth understanding for student perceptions of course objectives.

We coded student responses that described an act of learning or acquiring new knowledge as Knowledge (n=539). We then further subdivided these responses: Biology-Based Knowledge (n=403), Nature of Science (n=82), Directly from the Syllabus (n=13), Reflective (n=15), and Personal (n=26). Most student perceived course objectives (n=403) identified biology-based knowledge (e.g., "to gain a better understanding of the world around me from an atomic level to a biological level" and "to understand what biology really means"). Although most student responses under this theme were generic in nature, it does illustrate that students recognize that the course objective is to learn biology content.

Table 2. Themes and subthemes that emerged from student responses to questionnaire.

Theme	Subtheme	Example
Knowledge (539)	Biology Based Content (403)	Learning the basics of modern biology, such as how organisms grow, work, and reproduce.
	Nature of Science (82)	Basic understanding of scientific theory, to know what science is.
	Directly from Syllabus (13)	To examine cell diversity, structure, and function; to examine basic chemical principles, the nature of organic molecules, and the function of chemicals within cells.
	Reflective (15)	Ensuring that students gain a stronger sense of the world around them and how each living thing comes to be.
	Personal (26)	My goals for this course is to become more knowledgeable about the study of living things.
Practice (30)	Science Specific Skill (14)	Learning how to apply content from the course in a practical/objective manner.
	Non-Science Specific Skill (16)	To be able to think more critically.
Performance (41)	Grade Driven (41)	Getting an A so my grade doesn't drop.

Student responses categorized as Practice (n=30) centered around gaining critical thinking skills. Examples of this category included "the ability to demonstrate critical thinking skills," and "to apply the information I know to real world situations." We further subdivided these responses into science specific skills (n=14) (e.g., "using the scientific method to test out biological functions"), and non-science specific skills (n=16) (e.g., "to be able to think more critically"). These responses showcase that students identify skills that are applicable both within and outside the field of biology.

We coded the remaining student perceived course objective responses (n=41) as Performance based goals that centered upon passing or making a good grade in the course (e.g., "I just want to pass; I need to get an A in the class"). This theme was unrelated to the course and centered around the individual students' performance in the course.

Comparison of Course Objectives

Professor to syllabus. Both professors stressed course objectives which differed from those objectives listed in their course syllabi during their interviews. For example, both professors' explicitly stated course objectives focused on specific Biology topics intended for the course (Table 1), whereas their implied objectives focused on themes of science perception, life skills, and making biology more approachable. As these implied course objectives were not included in the syllabi, the variability between implied and explicit syllabus-stated course objectives highlights how professors can not solely rely on their syllabus to communicate all course objectives to students, but also must rely on classroom actions to present learning objectives.

Student to professor. Student questionnaire responses of perceived course objectives (n=569) aligned with professor intended objectives Knowledge (n=539) (e.g., "…I really focus on concept and applications in the teaching and in the class.") and Practice (n=30) (e.g., "the ability to demonstrate critical thinking skills). These findings suggest students recognized the professors' intended course objectives regarding skills and familiarity with science rather than the explicitly-stated course objectives outlined in the syllabus. This could suggest an influence of the instruction practices used by the professors. Both professors indicated their daily use of various classroom activities (e.g., lectures, active learning activities, etc.) to reinforce their intended course objectives for their students. While the intended objectives appeared to be the target of each professors' daily lessons and were recognized by the students, neither professor transferred them to the syllabus.

Student to syllabus. Student reported perceptions of explicit syllabus-stated course objectives were largely "inaccurate" (n=480). Very few students identified an actual course objective (n=130), and fewer (n=13) students copied their response word for word directly from the syllabus. This suggests that few students know where to find information about course objectives. Our findings show many students believed the course objectives to be "to learn modern biology" or "the fundamentals of science." However, given the highly-specific nature of the state-mandated policy, these perceived course objectives are considered inaccurate.

Summary

Both professors acknowledged the required course objectives mandated by the State of Texas, but reported similar implicit course objectives which included making science more accessible and relatable. We identified three themes when we asked students to report their perception of course objectives - knowledge, practice, and performance. Most student responses support the knowledge theme (n=539) as students can identify the objective is to learn biology content, but other responses support the practice (n=30) and performance (n=41) themes.

We found both professors stressed intended course objectives (making science more approachable, critical thinking/life skills) when we compared the explicit course objectives in the syllabus to their responses in their respective interviews. When we compared students' perception of course objectives to professor course objectives, students aligned more with the professor intended objectives rather than those explicitly stated in the syllabus. Specifically, students' perceptions of knowledge and practice aligned the most with the professor objectives. Lastly, we found few students could correctly identify the explicit course objectives in the syllabus, which suggests many do not know where to access information about their course objectives as clearly stated in their syllabi.

Discussion

Our findings highlight the breakdown of communication between professors and students regarding explicitly stated course objectives in syllabi. Students' inaccurate identification of course objectives explicitly stated in the syllabus provides evidence towards a disconnect between professors' intended course objectives and those explicit syllabus-stated course objectives. Given students incorrectly identified explicit syllabus-stated course objectives, but did correctly identify their professors' implied course objectives, it is evident that instructors should spend additional time and effort discussing and addressing course objectives presented solely in the syllabus (Mitchell & Manzo, 2018). Our findings provide support for the extant literature on the disconnect between course syllabi and students, as our

participants were unable to recall the course objectivesstated in their syllabus (Aggar & Shelton, 2015; Becker & Calhoon, 1999; Collier & Morgen, 2008; Osueke et al., 2018).

It has also been shown that students have difficulty recalling information presented in the syllabus throughout the semester (Smith & Razzouk, 1993), and prefer having a syllabus that focuses more on assignment details and grading policies (Appling et al., 2012). Our findings align with these conclusions, as students had difficulty accurately recalling course objectives, but reported their desire to have the information they need to succeed in the class. In contrast, instructors believe a course syllabus should serve to describe the course's purpose, academic honesty policies, and student conduct policies (Wolf et al., 2014). Creating course syllabi that meets the needs of both students and instructors is ideal but can be challenging and time consuming for an instructor.

While both our research and previous research suggest listing course objectives in the syllabus alone is not an effective form of communicating the instructor's goals for the course, simply removing course objectives is not a viable option due to a variety of administrative requirements (Albers, 2003). It is important for instructors to reflect on what outcomes they want students to achieve and craft course objectives that meet both the instructors' personal goals, state- or department-mandated expectations (Rubin, 2016; Schaub et al., 2017), and student expectations and requirements.

Within the framework of Instructional Communication Theory (Morreale et al., 2014) we found the process of explicitly stating course objectives in syllabi is ineffective, as most students (n=480) could not correctly identify course objectives from the syllabus. However, the product of students' accurate interpretation of course objectives as stated by the professor does work when the professor uses other ways to communicate their course objectives (e.g., using active learning activities in class). This is evident through the professors' reinforcement of course objectives at the start of lecture and in assignments (Appling et al., 2012). Given students could accurately identify implied course objects based on the professors' daily teaching practices, we recommend professors use other methods to communicate course objectives to their students.

If students are not accurately interpreting the intended course objectives that are outlined in course syllabi, they may not achieve personal, professor, department, or even University-desired outcomes for the course. However, further research is needed to determine how student performance is influenced by their ability to accurately interpret course objectives. Understanding how students use syllabi could be insightful when planning instructional methods, thus increasing the chances of student success in the course (Bain, 2004; Becker & Calhoon, 1999). Our findings indicate that students recognized course objectives the professors identified in their interviews over those explicitly stated in the syllabus. This is most likely due to the frequency and manner in which these ideas were covered and re-enforced through classroom activity. Therefore, we recommend professors clearly tie the intended course objectives covered in class back to those explicitly stated in the course syllabus to ensure re-enforcement of the ideas covered through classroom activities and assignments.

Conflict of Interest

The authors declare no affiliation or involvement with any organization or entity with any financial interest, or non-financial interest, in the subject matter discussed in this manuscript.

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References

AGGAR, B., AND B.A. SHELTON. 2015. Time, motion, discipline: The authoritarian syllabus on American college campuses.

Crit. Sociol., 43: 355-369.

ALBERS, C. 2003. Using the syllabus to document the scholarship of teaching. Teach. Sociol., 31: 60-72.

ALLAN, J. 1996. Learning outcomes in higher education. Studies in Higher Education, 21: 93-108.

APPLING, J., GANCAR, J., HUGHES, S., AND A. SAAD. 2012. Class syllabi, general education, and ePortfolios. International Journal of ePortfolio, 2: 199-206.

BAIN, K. 2004. What the best college teachers do. Cambridge, MA: Harvard University Press.

BECKER, A.H. AND S.K. CALHOON. 1999. What introductory psychology students attend to on a course syllabus. Teach. Psychol., 26: 6-11.

COLLIER, P.J. AND D.L. MORGAN. 2008. "Is this paper really due today?": Differences in first-generation and traditional college students' understandings of faculty expectations. Higher Education, 55: 425-446.

DAVIS, S. AND V. SCHRADER. 2009. Comparison of syllabi expectations between faculty and students in a baccalaureate nursing program. J. Nurs. Educ., 48: 125-131.

GRIFFITH, S.M., RODRIGUEZ-DOMENECH, M.M., AND A.J. ANDERSON. 2014. Graduate ethics education: A content analysis of syllabi. Training and Education in Professional Psychology, 8: 248-252.

HARTEL, R.W. AND E.A. FOEGEDING. 2004. Learning: Objectives, competencies, or outcomes? J. Food Sci. Educ., 3: 69-70.

KOLKHORST, L.W. 2009. Texas House Bill 2504. 81st Texas Legislature. Accessed from http://www.legis.state.tx.us/billlookup/Text.aspx?LegSess=81R&Bill=HB2504 on 14 Nov 2016.

LUDY, M., BRACKENBURY, T., FOLKINS, J.W., PEET, S.H. AND S.J. LANGENDORFER. 2016. Student impressions of syllabus design: Engaging versus contractual syllabus. IJ-SoTL 10: 1-23.

MARCIS, J.G., AND D.R. CARR. 2004. The course syllabus in the principles of economics: A national survey. Atlantic Economic Journal, 32: 259.

MATEJKA, K., AND L.B. KURKE. 1994. Designing a great syllabus. College Teaching, 42: 115-117.

MCCREA, E.A., AND A.J. LORENZET. 2018. Mind mapping: An experimental approach to syllabus review. Organization Management Journal, 15:1, 35-43.

MITCHELL, K.M., AND W.R. MANZO. 2018. The purpose and perceptions of learning objectives. Journal of Political Science Education, 14:4, 456-472.

MORREALE, S., BACKLUND, P., AND L. SPARKS. 2014. Communication education and instructional communication: Genesis and Evolution as field of inquiry. Commun. Educ. 63: 344-354.

OSUEKE, B., MEKONNEN, B., AND J.D. STANTON. 2018. How undergraduate science students use learning objectives to study. J. Microbiol. & Biol. Educ., 19(2): 1-8.

PARKES, J., AND M.B. HARRIS. 2002. The purpose of a syllabus. College Teaching, 50: 55-61.

RODRIGUEZ N.N., DISANTO, J., VARELAS, A., BRENNAN, S., K. WOLFE, et al. 2017. Building understanding of high school students' transition to college. International Journal of Teaching and Learning in Higher Education, 29: 402-411.

RUBIN, L. 2016. Six-word memoirs: A content analysis of first-year course learning outcomes. International Journal of Teaching and Learning in Higher Education, 28: 395-403.

SCHAUB, G., CADENA, C., BRAVENDER, P., AND C. KIERKUS. 2017. The language of information literacy: Do students understand? College and Research Libraries, 78: 283-295.

SMITH, M.F., AND N.Y. RAZZOUK. 1993. Improving classroom communication: The case of the course syllabus. Journal of Education for Business, 68: 215-221.

STATON-SPICER, A.Q., AND C.R. MARTY-WHITE. 1981. A framework for instructional communication theory: The relationship between teacher communication concerns and classroom behavior. Communication Education. 30: 354-366.

THOMPSON, B. 2007. The syllabus as a communication document: Constructing and presenting the syllabus. Communication Education, 56: 54-71.

WOLF, Z.R., CZEKANSKI, K.E., AND P.M. DILLON. 2014. Course syllabi: Components and outcomes assessment. Journal of Nursing Education and Practice, 4: 100-107.

YULE, J.V., WOLF, W.C., AND N.L. YOUNG. 2010. Emphasizing the "literacy" in "scientific literacy": A concise blueprint for integrating writing into biology classes. Bioscene, 36(2): 15-21.

Selection of an Optimal Cytotoxicity Assay for Undergraduate Research

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Abstract

Undergraduate research is a valuable tool to demonstrate both the dedication and time required to be a successful biologist. One area of research that has intrigued students over the last several years is cytotoxicity. However, at smaller undergraduate institutions, the time, training, and funding available for these research studies may be limited. Direct counting of cells is tedious and leads to mistakes, and although there are now several colorimetric toxicity assays, some have several steps and require near-perfect pipetting skills. To identify the most reproducible and affordable method(s) for undergraduate students to perform cell-based toxicity studies, we compared three colorimetric assays to counting viable cells directly. Using a breast cancer model system, students applied cantharidin to two different breast cancer cell lines, MCF-7 and MDA-MB-231, and performed MTT, resazurin, and crystal violet colorimetric assays or counted viable cells directly. We hypothesized that the MTT assay would be the most reproducible assay. Our results indicate that the crystal violet assay was not as reproducible as direct counting of cells, and therefore, not the best assay to use for toxicity tests. In contrast, the MTT and resazurin assays were highly reproducible and relatively low cost, and thus ideal assays for student research.

Key words: biology education; comparative study; higher education; cell viability

Introduction

Breast cancer is the second most common cancer in the United States, with about 230,000 new cases being discovered annually (www.cancer.org). Several breast cancer cell lines exist for research studies, including the well-characterized MCF-7 and MDA-MB-231 cells (Berthois, Katzenellenbogen, & Katzenellenbogen, 1986; Gupta & Kuperwasser, 2006; Harrell et al., 2006; St-Hilaire, Mandal, Commendador, Mannel, & Derryberry, 2011). We have used these cells in the past for testing of the toxicity of chemotherapeutics as well as pesticides (Kern & Schroeder, 2014; Jesionowski, Gabriel, Rich, & Schroeder, 2015; Florian, Mansfield, & Schroeder, 2016; Waszczuk & Schroeder, 2017) Additionally, other research has been published utilizing these as model systems for toxicity testing (Reardon et al., 1999; Ukpebor, Llabjani, Martin, & Halsall, 2011; Voborilova et al., 2011; Gurunathan, Han, Eppakayala, Jeyaraj, & Kim, 2013; Han et al., 2013; Gong, Goy, Olivo, & Yong, 2014).

Although a model system may be simple to select, the determination of the proper assays to monitor responsiveness can be difficult. There are several published and advertised cytotoxicity assays, examining both basic viability as well as metabolic activity. Henriksson et al. (2006) compared the amount of cell death observed using several assays, including cell counting, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tretrazolium bromide (MTT), crystal violet, and AlamarBlue (Henriksson, Kjellen, Wahlberg, Wennerberg, & Kjellstrom, 2006). MTT assays quantify the conversion of a yellow tetrazolium salt into purple formazan crystals using mitochondrial enzyme succinate dehydrogenase, which will only occur in viable cells (Riss et al., 2004; Niles, Moravec, & Riss, 2009; Sylvester, 2011). The AlamarBlue assay uses resazurin, a dye that shows both a colorimetric and a fluorometric change depending on cell metabolism, converting a blue dye to a pink color in the presence of active cells (Henriksson et al., 2006). Crystal violet is often used in microbial studies as a Gram stain. Due to the complexity of the staining process, it should detect only living cells. Dead cells are rinsed away through several washing steps, and the dye only stains the living cells after they have been fixed to a microplate. While this assay removes the chance of misconstruing increased or decreased metabolic activity as a direct consequence of increased or decreased cell number, it has several additional steps that can result in user error, especially for a student inexperienced in pipetting. These errors include washing away adherent live cells or not thoroughly washing away excess dye. By comparing the results of these differing assays, Henriksson et al. found that the observed cell viability in cell line LU-HNxSCC-7, which originated from a head and neck squamous epithelia carcinoma, was dependent on

which assay was used (Henriksson et al., 2006).

To compare these assays, we wanted to utilize both healthy, untreated cells, as well as cells exposed to a toxin; thus, we would be comparing the assays in a method similar to how undergraduates would be using them for data collection in a toxicity-style assay. The chemotoxin used in this study was cantharidin, which is produced by the blister beetle and is known for its anti-tumor affinity (Efferth et al., 2005). Cantharidin induces apoptosis through the p53 mechanism either intrinsically by causing mitochondrial release of cytochrome C, or extrinsically via activation of the caspase cascade (Chang et al., 2008). Cantharidin also causes oxidative stress that provokes DNA damage (Li et al., 2010). We have previously shown that cantharidin is a more potent activator of cell death than other common chemotherapeutics using an MTT assay (Kern & Schroeder, 2014).

Historically, crystal violet and MTT assays have been well-published, with fewer studies using AlamarBlue. Based on past experiences in our research group, we hypothesized that there would be issues with reproducibility in the more complex assays (crystal violet), but those requiring minimal pipetting would show fewer differences between replicates. Additionally, our goal was to determine which colorimetric assay best represented the number of viable cells determined by direct counting.

Materials and Methods

Cell Culture and Treatment

MCF-7 cells were maintained in MEM media with 5% calf serum, whereas the MDA-MB 231 cells were maintained in DMEM media with 10% newborn calf serum. During plating, cells were removed from a T-75 flask using trypsinization. Cells were washed twice with HBSS to remove residual serum proteins, then treated with 1 ml 0.05% trypsin for 5 minutes at 37ºC. Cells were removed from the flask using physical perturbation. Media (10 ml) was added to the suspended cells, and cells were evenly transferred into a 96-well plate with 100 µl suspended cells per well or to a 6-well plate with 1.5 ml of suspended cells per well. Cells were allowed to adhere to the microplate for approximately twenty-four hours before treatment with toxin began. For the cytotoxicity studies, cells were treated with either 500 nM to 50 µM cantharidin or 1 µM to 100 µM cantharidin. Untreated cells were replenished with fresh media on the day of treatment. After the treatment exposure for 48 hours, the viability of the cells was quantified by cell counting or by using colorimetric assays with MTT, resazurin, or crystal violet.

Viability Assays

As a control assay, we counted viable cells directly without colorimetric staining procedures. Cells were treated in a 6-well plate. Following treatment, cells were washed with twice with HBSS. Trypsin (0.05%, 0.5 ml) was added to each well and cells were incubated for 5 minutes at 37ºC. Detachment from the wells was determined visually and cells were pipetted into a 15-ml conical. HBSS (4.5 ml) was added to dilute residual trypsin and live cells were counted immediately on a hemocytometer. Eight squares of cells were counted for each treatment and averaged within each individual experiment.

In the crystal violet staining method, media was removed and 100 µL of 50% v/v ice-cold methanol was added to each well for 10 minutes to fix cells. After 10 minutes, the methanol was removed, and 50 µL of 1% w/v crystal violet was added to each cells for staining. After 10 minutes, the dye was removed and cells were rinsed twice with water to wash away the excess dye and any poorly-adhered cells. The dye was dissolved in 1% SDS, and the amount of stain absorbed by the live cells was quantified with a microplate reader at a wavelength of 540nm. Viable cells were quantified by normalizing the absorbance readings to the untreated control cells, set at 100% viability.

For the MTT assay, 10 µL of 5 mg/ml MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium) was added to the wells and placed in the incubator for 3 hours. After 3 hours of growth, the media was removed and 200 µL of DMSO was added to each well to dissolved the crystals (Riss et al., 2004). The plate was then read on a microplate reader at 570nm. Viable cells were quantified by normalizing the absorbance readings to the untreated control cells, set at 100% viability.

For the resazurin assay, 20 µL of 0.15 mg/ml resazurin was added to each well. After three hours of incubation, the plate was read at 570 and 595 nm on a microplate reader. Viable cells were quantified by subtracting the absorbance reading at 595 nm from the reading at 570 nm, and normalizing to the untreated control cells, set at 100% viability.

To ensure consistency during direct comparisons, all three colorimetric assays were run by the same undergraduate student, together on a single microplate. All experiments were run in triplicate on each plate, and three plates on different days were used for each cancer cell line. Results were normalized to the control within each replicate. Differences in viability for compiled data were confirmed using a between-subject test and ANOVA with a Fisher's Least Significant Difference post-hoc test using SPS (IBM SPSS Statistics 21, IBM Corp., Aramonk, NY, USA). Significant variation from controls was .

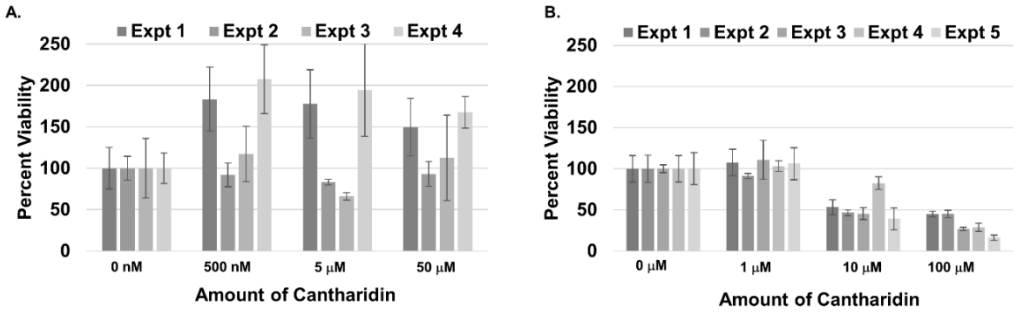

Fig. 1. Crystal Violet or MTT detection of viability of MDA-MB-231 breast cancer cells after toxin treatment. All results were normalized to the media control (0 nM/μM treatment) for each individual experiment (Expt). Error bars represent standard deviation amongst replicates in a single experiment. A. Cells were treated in quadruplicate with 500 nM to 50 μM cantharidin for 48 hours followed by staining with crystal violet. Stained cells were quantified by reading absorbance at 540 nm in a microplate reader. Results represent four separate experiments. B. Cells were treated in triplicate with 1 μM to 100 μM cantharidin. After 48 hours, cells were stained using MTT, and the formazan crystals were dissolved in DMSO prior to reading absorbance at 570 nM on a microplate reader. Results represent five separate experiments.

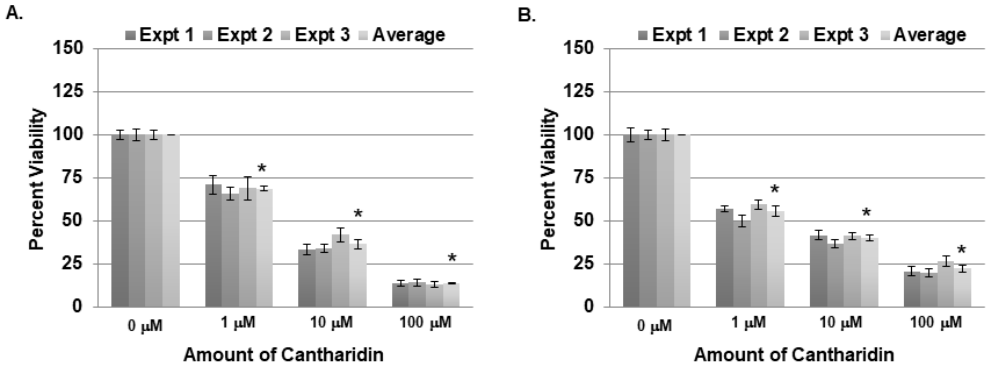

Fig. 2. Cell counting assay to determine viability of breast cancer cells after toxin treatment. MCF-7 (A) or MDA-MBA-231 (B) breast cancer cells were treated in triplicate with 1 μM to 100 μM cantharidin. After 48 hours, cells were removed from the wells using trypsin, and eight sets of viable cells per well were counted manually using a hemocytometer. All results were normalized to the media control (0 nM/μM treatment) for each individual experiment (Expt). Error bars represent standard deviation amongst replicates in a single experiment. Results represent three individual experiments run in triplicate; error bars indicate SEM. Statistically different viability compared to the control is indicated (*, $p<0.05$).

indicated with a $p<0.05$.

Results

One of the main focuses of our research lab is studying the toxicity of natural and man-made chemotherapeutics using a human breast cancer cell model. However, one of the challenges with undergraduate research is being able to discern when discrepancies from hypothesized results are due to true scientific data versus user error. Consistency with pipetting can be a difficult skill for undergraduate researchers to master; we have previously had confusion about the validity of data sets when repetitions of assays look completely different (Figure 1A). Early student researchers in our lab utilized crystal violet-based colorimetric assays (Rich et al, 2012), but results were sometimes contradictory from week to week. For example, during the time that one student showed large variations in viability (Fig. 1A), a second student was achieving high reproducibility using an MTT assay (Fig. 1B, individual data sets from

averaged data previously published in (Kern & Schroeder, 2014)). Subsequent research using MTT or resazurin assays were much more reproducible for all students involved (Waszcuk & Schroeder, 2017; Siegfried & Schroeder, 2018).

Due to these large variations between replicates in many crystal violet assays, we wanted to determine if there was an optimal assay that undergraduate students could utilize with both reproducibility and reliability. Thus, a single undergraduate student compared three colorimetric assays to a direct counting of viable cells. Since we had recently published on the high toxicity of cantharidin using an MTT-based assay (Kern & Schroeder, 2014), we utilized that same toxin in this comparison study and expanded our work to compare these assays in two distinct breast cancer cell lines.

As our assay control, the undergraduate researcher performed a cell counting assay. Two different breast cancer cell lines were plated into a 6-well microplate to facilitate easier removal than from a 96-well plate. After treatment with cantharidin for 48 hours, cells were removed by trypsinization and counted using a hemocytometer. Figure 2 shows the reduction in live cells for cantharidin-treated MCF-7

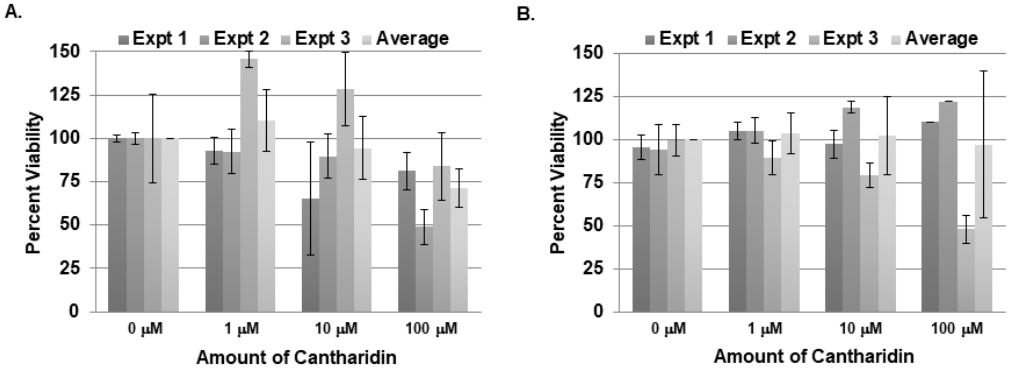

Fig. 3. Crystal violet colorimetric assay to determine viability of breast cancer cells after toxin treatment. MCF-7 (A) or MDA-MBA-231 (B) breast cancer cells were treated in triplicate with 1 µM to 100 µM cantharidin. After 48 hours, cells were fixed and stained with crystal violet. Stained cells were quantified by reading absorbance at 540 nm in a microplate reader. All results were normalized to the media control (0 nM/µM treatment) for each individual experiment (Expt). Results represent three individual experiments run in triplicate; error bars indicate SEM.

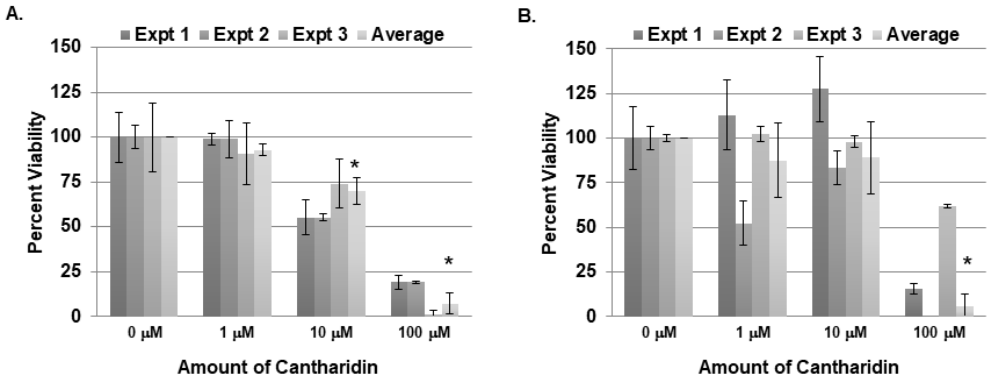

Fig. 4. MTT assay to determine viability of breast cancer cells after toxin treatment. MCF-7 (A) or MDA-MBA-231 (B) breast cancer cells were treated in triplicate with 1 µM to 100 µM cantharidin. After 48 hours, cells were stained using MTT, and the formazan crystals were dissolved in DMSO prior to reading absorbance at 570 nM on a microplate reader. All results were normalized to the media control (0 nM/µM treatment) for each individual experiment (Expt). Results represent three individual experiments run in triplicate; error bars indicate SEM. Statistically different viability compared to the control is indicated (*, $p<0.05$).

We then tested the colorimetric assays in a single microplate. As we had previously observed during initial (and less reproducible) studies, the crystal violet assay did not indicate cell death (Fig. 3A). As the concentration of cantharidin increased, no consistent reduction in viability was observed in either breast cancer cell line tested. Results from individual experiments showed large variations with over a 60% range in viability in some treatments in MCF-7 cells, such as for the 10 µM cantharidin treatment (Fig. 3A). Only one of the 100 µM cantharidin treatments in MDA-MB-231 cells showed cell death (Fig 3B).

In contrast, both the MTT and resazurin assays (Figs. 4 and 5) showed decreases in viability after treatment with cantharidin. For MTT assays, low variations between experiments were observed in MCF-7 breast cancer cells (Fig. 4A). Significantly less viability was observed in the averaged data for both the 10 µM and 100 µM cantharidin treatments. Less consistent results were observed for MDA-MB-231, although all three individual experiments showed lower viability with the 100 µM cantharidin treatment, and the averaged data was statistically less than the control for the 100 µM cantharidin treatment (Fig. 4B). Similar results were observed with the resazurin assay (Fig. 5). However, both cell lines showed more reproducible results for all three individual experiments.

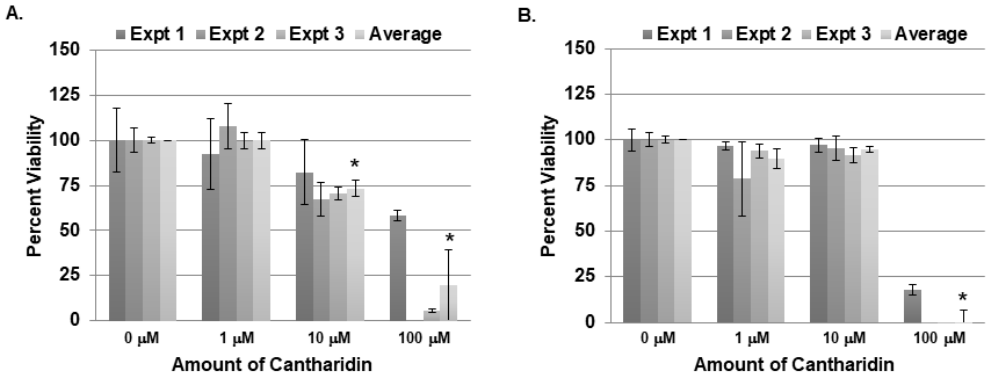

Fig. 5. Resazurin assay to determine viability of breast cancer cells after toxin treatment. MCF-7 (A) or MDA-MBA-231 (B) breast cancer cells were treated in triplicate with 1 µM to 100 µM cantharidin. After 48 hours, cells were stained using resazurin, and viable cells were quantified by reading absorbance at 570 nm and 595 nm. All results were normalized to the media control (0 nM/µM treatment) for each individual experiment (Expt). Results represent three individual experiments run in triplicate; error bars indicate SEM. Statistically different viability compared to the control is indicated (*, $p<0.05$).

Discussion

When determining whether compounds affect cell viability or cause cell death, several methods are available. One can test for the structural integrity of the nucleus or mitochondrial membrane, examine ATP and ADP levels, or stain cells using a variety of methods. However, in many research labs funding can be difficult to obtain, especially when performing research at smaller institutions, where performance earning is still as highly valued as at larger, Tier 1 research institutions. In these situations, it may not be feasible or affordable to purchase a cell counter, fluorescence microscope, or tritiated thymidine, much less train undergraduate students in their use. In these cases, other options must be examined.

Although a direct counting of cells may be the most accurate, there are drawbacks to this method. First, counting of the cells requires either counting of the entire well, or removal of cells via trypsinization. This can result in some damage to the cells, reducing the amount of counted cells. While this may be accounted for in normalization to a control (assuming the same amount of damage occurs in all wells), it does require more media and disposable plasticware, adding a cost increase to the experiment. It would take sixteen 6-well microplates to test the same number of wells as in a 96-well plate. For stock-brand tissue-culture treated disposable plates from many suppliers, this can increase the cost of a single experiment from less than $2.50 to greater than $20. Additionally, trypsinization may not yield adequate dissociation for all cell types and may, in fact, alter the cells in the subculture (Chaudhry, 2008; Park et al., 2008; Zhang et al., 2012). Beyond this, there is a 29-fold increase in surface area in a 6-well plate; this requires more cell culture resources per treatment. We have routinely assisted colleagues in assessing chemical constructs made in very low quantities that we would be unable

to perform replicates on were we to use only the cell counting method (unpublished data).

Thus, colorimetric assays have become a go-to method for testing cell proliferation and death. throughout the years, several experiments have used crystal violet as an effective method for testing of toxins and chemotherapeutics (Henriksson et al., 2006; Geserick et al., 2009; Feoktistova, Geserick et al., 2016a; Feoktistova, Wallberg et al., 2016b). Our data refutes those experiments. In our experience, the crystal violet assay may not be as effective for toxicity testing, since when using the crystal violet assay, much less cell death was quantified than with the MTT and resazurin assays in adjacent wells. Possible sources of error, with the crystal violet assay in particular, may include scraping or blowing the cells off the bottom of the plate with the pipette tip. Another possible source of error may include overpopulation of cells in the flask while growing. Also, the crystal violet assay tests how many viable cells are left after treatment, but staining is done with filtered dye after first fixing the cells. Rinsing is a key step to accurate readings. If proper rinsing of debris and dead cells was not conducted before dyeing the cells, or any precipitated dye remained in the wells after staining, an overestimation of cell viability would have resulted.

To eliminate some of the sources of variation (pipetting and rinsing errors), MTT and resazurin-based assays could be utilized. Mueller et.al. indicated that MTT may, in particular, serve as a preferred method for high-throughput screening of cytotoxic agents (Mueller, Kassack, & Wiese, 2004), while Borra et.al. showed that resazurin use can provide accurate assaying of mitochondrial activity at a low cost to the researcher (Borra, Lotufo, Gagioti, Barros Fde, & Andrade, 2009). In our own work, the cost of an MTT assay is approximately $4 per plate, with over half of that cost due to the disposable plasticware. Resazurin assays are even less expensive, as the resazurin salt is low cost (less than $30 per gram) and less than 3 mg is used per plate. However, there are several limitations to these protocols. While the resazurin assay does not require any rinsing of cells and thus may be able to be used for suspension cultures, both the crystal violet and MTT assays do require at least one rinse step. For a suspension culture, this would require repeated pelleting of the cells, which would risk damage or loss of cell material. Thus, these assays are better suited for adherent cells. As an additional complication, the type of toxicant being studied may also interfere with these reagents. Angius et. al. demonstrated that MTT has an affinity for lipids, and thus any toxicants applied through a liposome method may interfere with proper absorbance of MTT by the cells (Angius & Floris, 2015). MTT has also been shown to interact with fat-soluble compounds such as flavonoids and the vitamin E isomer α-tocopherol (Peng, Wang, & Ren, 2005; Lim, Loh, Tring, Bradshaw, & Allaudin, 2015). Free thiol groups can also reduce MTT to formazan (Shoemaker, Cohen, & Campbell, 2004).

Unlike the crystal violet assay, both the MTT and resazurin assays in both MCF-7 and MDA-MB 231 cell lines were able to indicate a reduction in metabolic activity, and this was attributed to a concurrent reduction in viability. This was highly reproducible, especially within MCF-7 cells. As an added benefit to undergraduate research, the resazurin assay method has fewer steps than the MTT assay method. For inexperienced pipettors, the resazurin assay may be ideal. However, there is one drawback to this protocol. Since the dye is added directly to the media, any components present in the media or in any toxins being tested could interfere with the colorimetric assay (O'Brien, Wilson, Orton, & Pognan, 2000; Simeonov & Davis, 2004). This includes coloration within the additives as well as pH variations that could alter the resazurin dye. In these cases, a more optimal assay may be the MTT assay, which still exhibited high reproducibility. We were faced with this issue within our own recent research, where the toxins being studied (essential oils) were colored and created a color artifact that interfered with the resazurin assay (Siegfried & Schroeder, 2018). Thus, an MTT-assay was utilized as all of the colored oils were removed from the wells prior to the addition of the MTT dye.

Although these methodologies are generally accepted as reflecting the number of live cells present, they do this through an assumption that metabolic activity remains constant across that cell population. Both resazurin and MTT assays rely on the conversion of the dye through increased redox activity. An increase in the amount of conversion may be accomplished not only through the presence of more cells, but also through a rise in the metabolic activity of a stable cell population. The majority of the redox activity involved in the conversion is attributed to mitochondrial NADH and NADPH, but these coenzymes are also able to reduce the dyes extracellularly as well as outside the mitochondria or even external to the cell itself (Bernas & Dobrucki, 2002; Uzarski, Divito, Wertheim, & Miller, 2017). Likewise, the presence of redox inhibitors can result in a drop in formazan production even if cellular levels remain constant (Stepanenko & Dmitrenko, 2015; Shenoy et al., 2017). Resazurin has been indicated previously as a more reproducible, and thus more accurate, determinant of cell viability (van Tonder, Joubert, & Cromarty, 2015). However, nonlinear growth of cells can result in inaccurate resazurin

correlations (Mallick, Scutt, Scutt, & Rolf, 2009; Quent, Loessner, Friis, Reichert, & Hutmacher, 2010; Rampersad, 2012). Thus, while these colorimetric assays can still be utilized as a general means of determining viability, conclusive changes in cell number may be determinant upon the assay conditions. We have observed these differences in our own study, where a direct counting of cells identified a reduction of viability under much lower cantharidin concentrations than was indicated by the metabolic assays (compare Figure 2 to Figures 4 and 5). Thus, we recommend that both resazurin and MTT are still feasible and economically preferential options for determination of cell viability in undergraduate research projects, as much of the literature and company advertisements claim. However, if financially practical, a concomitant counting of cell number would add to the study, and might allow students to tease out the differences between viable and metabolically active cells.

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References

ANGIUS, F. AND A. FLORIS. 2015. Liposomes and MTT cell viability assay: an incompatible affair. Toxicol In Vitro, 29: 314-9.

BERNAS, T. AND J. DOBRUCKI. 2002. Mitochondrial and nonmitochondrial reduction of MTT: interaction of MTT with TMRE, JC-1, and NAO mitochondrial fluorescent probes. Cytometry, 47: 236-42.

BERTHOIS, Y., KATZENELLENBOGEN, J. AND B. KATZENELLENBOGEN. 1986. Phenol red in tissue culture media is a weak estrogen: Implications concerning the study of estrogen-responsive cells in culture. P Natl Acad Sci *USA*, 83: 2496-2500.

FEOKTISTOVA, M., GESERICK, P. AND M. LEVERKUS. 2016. Crystal violet assay for determining viability of cultured cells. Cold Spring Harb Protoc, 2016: pdb prot087379.

FEOKTISTOVA, M., WALLBERG, F., TENEV, T., GESERICK, P., LEVERKUS, M., ET AL. 2016. Techniques to distinguish apoptosis from necroptosis. Cold Spring Harb Protoc, 2016: pdb prot070375.

FLORIAN, C.P., MANSFIELD, S.R. AND J.R. SCHROEDER. 2016. Differences in GPR30 regulation by chlorotriazine herbicides in human breast cells. Biochem Res Int, 2016: 1-7.

GESERICK, P., HUPE, M., MOULIN, M., WONG, W.W., FEOKTISTOVA, M., ET AL. 2009. Cellular IAPs inhibit a cryptic CD95-induced cell death by limiting RIP1 kinase recruitment. J Cell Biol, 187: 1037-54.

GONG, T., GOH, D., OLIVO, M. AND K.T. YONG. 2014. In vitro toxicity and bioimaging studies of gold nanorods formulations coated with biofunctional thiol-PEG molecules and Pluronic block copolymers. Beilstein J Nanotechnol, 5: 546-53.

GUPTA, P.B. AND C. KUPERWASSER. 2006. Contributions of estrogen to ER-negative breast tumor growth. J Steroid Biochem Mol Biol, 102: 71-8.

GURUNATHAN, S., HAN, J.W., EPPAKAYALA, V., JEYARAJ, M. AND J.H. KIM. 2013. Cytotoxicity of biologically synthesized silver nanoparticles in MDA-MB-231 human breast cancer cells. Biomed Res Int, 2013: 535796.

HAN, W., WANG, S., LIANG, R., WANG, L., CHEN, M., ET AL. 2013. Non-ionic surfactant vesicles simultaneously enhance antitumor activity and reduce the toxicity of cantharidin. Int J Nanomedicine, 8: 2187-96.

HARRELL, J.C., DYE, W.W., ALLRED, D.C., JEDLICKA, P., SPOELSTRA, N.S., ET AL. 2006. Estrogen receptor positive breast cancer metastasis: altered hormonal sensitivity and tumor aggressiveness in lymphatic vessels and lymph nodes. Cancer Res, 66: 9308-15.

HENRIKSSON, E., KJELLEN, E., WAHLBERG, P., WENNERBERG, J. AND J.H. KJELLSTROM. 2006. Differences in estimates of cisplatin-induced cell kill in vitro between colorimetric and cell count/colony assays. In Vitro Cell Dev Biol Anim, 42: 320-3.

JESIONOWSKI, A.M., GABRIEL, S.M., RICH, J.D. AND J.R. SCHROEDER. 2015. Failure of pesticides to alter migration of cancerous and non-cancerous breast cell lines in vitro. Toxicol Res, 4(1): 99-105.

KERN, K.M. AND J.R. SCHROEDER. 2014. Comparison of cantharidin toxicity in breast cancer cells to two common chemotherapeutics. Int J Breast Cancer, 2014: 423059.

LI, W., XIE, L., CHEN, Z., ZHU, Y., SUN, Y., ET AL. 2010. Cantharidin, a potent and selective PP2A inhibitor, induces an oxidative stress-independent growth inhibition of pancreatic cancer cells through:

2/M cell-cycle arrest and apoptosis. Cancer Sci 101 1226-33.

LIM, S.W., LOH, H.S., TING, K.N., BRADSHAW, T.D. AND Z.N. ALLAUDIN. 2015. Reduction of MTT to purple formazan by vitamin E isomers in the absence of cells. Trop Life Sci Res, 26: 111-20.

MALLICK, E., SCUTT, N., SCUTT, A. AND C. ROLF. 2009. Passage and concentration-dependent effects of Indomethacin on tendon derived cells. J Orthop Surg Res, 4: 9.

MUELLER, H., KASSACK, M.U. AND M. WIESE. 2004. Comparison of the usefulness of the MTT, ATP, and calcein assays to predict the potency of cytotoxic agents in various human cancer cell lines. J Biomol Screen, 9: 506-15.

NILES, A.L., MORAVEC, R.A. AND T.L. RISS. 2009. In vitro viability and cytotoxicity testing and same-well multi-parametric combinations for high throughput screening. Curr Chem Genomics, 3: 33-41.

O'BRIEN, J., WILSON, I., ORTON, T. AND F. POGNAN. 2000. Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. Eur J Biochem, 267: 5421-6.

PARK, Y.B., KIM, Y.Y., OH, S.K., CHUNG, S.G., KU, S.Y., ET AL. 2008. Alterations of proliferative and differentiation potentials of human embryonic stem cells during long-term culture. Exp Mol Med, 40: 98-108.

PENG, L., WANG, B. AND P. REN. 2005. Reduction of MTT by flavonoids in the absence of cells. Colloids Surf B Biointerfaces, 45: 108-11.

QUENT, V.M., LOESSNER, D., FRIIS, T., REICHERT, J.C. AND D.W. HUTMACHER. 2010. Discrepancies between metabolic activity and DNA content as tool to assess cell proliferation in cancer research. J Cell Mol Med, 14: 1003-13.

RAMPERSAD, S.N. 2012. Multiple applications of Alamar Blue as an indicator of metabolic function and cellular health in cell viability bioassays. Sensors (Basel), 12: 12347-60.

REARDON, D.B., CONTESSA, J.N., MIKKELSEN, R.B., VALERIE, K., AMIR, C., ET AL. 1999. Dominant negative EGFR-CD533 and inhibition of MAPK modify JNK1 activation and enhance radiation toxicity of human mammary carcinoma cells. Oncogene, 18: 4756-66.

RICH, J.D., GABRIEL, S.M., AND J.R. SCHROEDER. 2012. In vitro effects of herbicides and insecticides on human breast cells. ISRN Toxicol. . 2012 (232461): 1-9.

RISS, T.L., MORAVEC, R.A., NILES, A.L., DUELLMAN, S., BENINK, H.A., ET AL. 2004. Cell Viability Assays. In: Sittampalam GS, Coussens NP, Brimacombe K, editors. Assay Guidance Manual. Bethesda (MD): Eli Lilly & Company and the National Center for Advancing Translational Sciences.

SHENOY, N., STENSON, M., LAWSON, J., ABEYKOON, J., PATNAIK, M., ET AL. 2017. Drugs with anti-oxidant properties can interfere with cell viability measurements by assays that rely on the reducing property of viable cells. Lab Invest. 97: 494-497.

SHOEMAKER, M., COHEN, I. AND M. CAMPBELL. 2004. Reduction of MTT by aqueous herbal extracts in the absence of cells. J Ethnopharmacol, 93: 381-4.

SIEGFRIED, S.A. AND J.R. SCHROEDER. 2018. Toxicity of Thieves oils to MCF-7 and MDA-MB- 231 breast cancer cells. Amer J Essent Oil Nat Prod. 6(1): 1-8.

SIMEONOV, A. AND M.I. DAVIS. 2004. Interference with Fluorescence and Absorbance. In: Sittampalam GS, Coussens NP, Brimacombe K, editors. Assay Guidance Manual. Bethesda (MD): Eli Lilly & Company and the National Center for Advancing Translational Sciences.

ST-HILAIRE, S., MANDAL, R., COMMENDADOR, A., MANNEL, S. AND D. DERRYBERRY. 2011. Estrogen receptor positive breast cancers and their association with environmental factors. Int J Health Geogr, 10: 32-40.

STEPANENKO, A.A. AND V.V. DMITRENKO. 2015. Pitfalls of the MTT assay: Direct and off-target effects of inhibitors can result in over/underestimation of cell viability. Gene, 574: 193-203.

SYLVESTER, P.W. 2011. Optimization of the tetrazolium dye (MTT) colorimetric assay for cellular growth and viability. Methods Mol Biol, 716: 157-68.

UKPEBOR, J., LLABJANI, V., MARTIN, F.L. AND C.J. HALSALL. 2011. Sublethal genotoxicity and cell alterations by organophosphorus pesticides in MCF-7 cells: implications for environmentally relevant concentrations. Environ Toxicol Che*m*, 30: 632-9.

UZARSKI, J.S., DIVITO, M.D., WERTHEIM, J.A. AND W.M. MILLER. 2017. Essential design considerations for the resazurin reduction assay to noninvasively quantify cell expansion within perfused extracellular matrix scaffold Biomaterials, 129: 163-175.

VAN TONDER, A., JOUBERT, A.M. AND A.D. CROMARTY. 2015. Limitations of the 3-(4,5dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay when compared to three commonly used cell enumeration assays. BMC Res Notes, 8: 47.

VOBORILOVA, J., NEMCOVA-FURSTOVA, V., NEUBAUEROVA, J., OJIMA, I., ZANARDI, I., et al. 2011. Cell death induced by novel fluorinated taxanes in drug-sensitive and drug-resistant cancer cells. Invest New Drugs, 29: 411-23.

WASZCZUK, O.G. AND J.R. SCHROEDER. 2017. Cell-specific reduction in viability of two breast cancer cell lines after exposure to gold nanoparticles. J Pharml Res Int, 18: 8.

ZHANG, B., SHAN, H., LI, D., LI, Z.R., ZHU, K.S., et al. 2012. Different methods of detaching adherent cells significantly affect the detection of TRAIL receptors. Tumori, 98: 800-3.

Introverts are not disadvantaged in group-based active learning classrooms

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Abstract

Evidence of the effectiveness of active learning has resulted in a shift in post-secondary classrooms towards student-centred teaching, often relying heavily on peer-to-peer interactions. While the overall benefit of these teaching methods is established, it remains unclear whether all sub-populations of students benefit similarly. Given the intensive peer-to-peer nature of group-based active-learning approaches, we questioned whether introverted students are at a disadvantage in these active-learning classrooms. To explore this question, we examined how course performance, peer-evaluation scores, and affective measures of course experience differ for introverts, ambiverts, and extroverts in two active-learning classrooms over two years. Our results show no disadvantage in any of the measures explored for introverted students; introvert, ambivert and extrovert students performed equally well, received comparable ratings by peers, and reported similar affective attitudes towards our courses. Despite the intensive use of peer discussion, with permanent groups that were highly integrated into each class, our group-based, active-learning classrooms did not favor extroverts nor disadvantage introverts. We explore reasons why our results differ from other studies that find introverted students enjoy group work less.

Introduction

Overwhelming evidence of the benefits of active learning across disciplines and contexts in higher education has been well established (Freeman et al., 2014; Hake, 1998). Active learning is a broad term capturing any teaching method that involves students in their learning by doing more than listening and taking notes (Bonwell & Eison, 1991; Felder & Brent, 2009). In active-learning classrooms, students are often expected to construct their knowledge through discussion and debate with peers (Crouch & Mazur, 2001). While interactions with peers are often a crucial component of active learning, the extent of these interactions can vary from a quick 'pair-share' with a neighbour, to working extensively in permanent student groups for a semester. Evidence of the benefits of active learning have resulted in a widespread movement towards incorporating active learning, including peer-to-peer learning, into post-secondary classrooms.

While the importance of active learning is well known, it is not clear whether certain types of students are privileged over others in active learning classrooms, particularly given the importance of peer-to-peer interactions. Most studies examining the impact of active learning measure average learning gains, or average increases in class performance measures. With an average increase, some groups of students might experience smaller increases than others, placing them at a disadvantage. Alternatively, it is possible that certain students may have reduced performance, but the success of other students overwhelms this response when averaged. This disparity may be particularly true if the decrease in performance occurs for those students who are in the minority. For example, Eddy et al. (2015) examined the roles of gender and race/ethnicity/nationality in the preferred roles of students in peer discussions in active-learning classrooms. Both gender and race/ethnicity/nationality impacted the preferred roles students selected in peer discussions, with females preferring not to take leadership roles in groups and minorities preferring to be listeners in group discussions. Additionally, increased focus on working with peers can be challenging for LGBTQIA students, for whom the active-learning classroom may not be a welcoming or accepting place (Cooper & Brownell, 2016). Now that we understand the overall positive impact of active-learning approaches, it is important to explore the nuances of learning within these contexts to ensure that some students are not being disadvantaged while other students thrive. In our own teaching, we have questioned whether introverted students may be at a disadvantage in active, peer-discussion-based learning environments. Like many other post-secondary instructors, we have both recently "flipped" our classes away from a lecturing-intensive approach to an active-learning approach in which students spend much of their class time working in permanent small groups. We wondered whether this change would disadvantage

introverts relative to extroverts. Introverts tend to prefer less social stimulation, require more time to think and reflect before contributing ideas, and would often prefer to write rather than speak (Cain, 2012; Condon & Ruth-Sahd, 2013; Davidson, Gillies, & Pelletier, 2015). Introversion is not the same as shyness or social anxiety, and it is important to understand that introverts are not necessarily unwilling to talk, but typically need more time to process the information and formulate what they want to say. Extroverts, on the other hand, are characteristically comfortable with sharing their thinking spontaneously and making quick decisions. Given a choice, extroverts usually prefer speaking with others rather than working independently (Cain, 2012, Condon and Ruth-Sahd, 2013), seemingly making extroverts suitably "adapted" to the active-learning classroom and potentially placing introverts at a disadvantage. Certainly, recent coverage in mainstream media reveals a concern around introverts in classrooms. Articles such as, Why Introverts Shouldn't be Forced to Talk in Class, and Participation Penalizes Quiet Learners, express the concern that the increased use of peer discussion may be placing introverts at a disadvantage. There is evidence that introverts may find group work less enjoyable and may feel isolated and/or participate less (Hennessy & Evans, 2006). Introverts may have more negative views of group work (Walker, 2006), and may prefer more independent work over interactive teaching methods (Chamorro-Premuzic, Furnham, & Lewis, 2007; Pawlowska, Westerman, Bergman, & Huelsman, 2014). However, it is less clear whether introverts' performance in active group-based courses is negatively (or less positively) impacted, or whether students perceive the contributions of introverted peers differently from those of their more-extroverted peers. In courses where peer evaluation is a component of the grading system, it is important to understand how introversion may influence the value that their peers may place on a student's contributions, to ensure that students do not "overvalue" contributions of extroverted students relative to introverted students. As with any approach to learning, we need to understand the impact of group-based active learning on diverse students within our classrooms, to ensure we are not under- nor over-privileging certain types of students.

In this study, we compared course performance, peer-evaluation scores, and affective attitudes of self-identified introverts, ambiverts (students in the middle of the introvert-extrovert continuum) and extroverts in two active-learning classrooms over two years. To measure affective attitudes towards experiences in our active learning classrooms, we used a validated survey tool (Experiences of Teaching and Learning Survey, Entwistle, Mccune, & Hounsell, 2002) at the end of the semester. This tool measures four factors: perceived peer support; engagement with course material; and perceived learning gains in ability to work with other students, and ability to communicate knowledge and ideas effectively.

Methods

Our courses

We conducted this research in two courses, Quantitative Biology and Biology of Fungi, in 2015 and 2016 at a research-intensive Canadian university. Quantitative Biology I is an upper-level course aimed at introducing undergraduate biology students to statistics. The topics of the course include: sampling, statistical populations, statistical inference, t-tests, ANOVA, Linear Regression, Analysis of Frequencies, Permutation tests, and Transformations. In addition to three 50-minute classes a week, students attend weekly three-hour computer-based Labs where they learn how to conduct statistical tests in the statistical software R. In 2015 and 2016, there were 129 and 168 students enrolled in this course, respectively. Between 26-27% of students were in their second year in 2015/2016, 43-46% of students are in their third year, 20-30% are in their fourth year or above. This course is a pre-requisite for less than half of the class; others take this course as an elective.

Biology of Fungi is a third-year course that provides an introduction and overview of fungal biology, a topic that most students have not learned about prior to this course. The course deals with fungal diversity, evolution and ecology, and ends with a section on medical mycology. As for the Quantitative Biology course, there are three 50-minute classes each week and one three-hour lab. The course is an option for five of the six programs offered by the department but is not required by any program. The course typically fills to capacity (96 students) soon after registration opens. The majority of the students in the course are in their final year of studies.

Prior to the start of term, we send students a welcome email in which we outline the structure of the course and explain that they will be working in permanent groups of 5-6 students during class time. To help us form heterogeneous groups, we ask the students to complete a brief group-forming survey, with questions relating to previous course history, gender and year of program. We also ask to self-identify as an introvert, extrovert or ambivert and provide a link to a quick 'Introvert Test' on the Quiet website (www.quietrev.com/the-introvert-test/), with

the recommendation to take the quiz if they are unsure how to categorize themselves.

Once the groups are formed, our classes follow a format based on the Team-Based Learning approach developed by Michaelsen (2004). The courses are divided into modules, each of which begins with students preparing outside of class by completing assigned readings and videos. In the first class of each module, students write a quiz based on this background preparation first as individuals and then as a group. Using the results of the quiz, we follow with lectures for one or two classes, in which we clarify any points of confusion and provide any additional foundational knowledge necessary for the group assignments, which make up the rest of the module. The group assignments are designed to increase in complexity and require students to work together to apply the material in novel scenarios. Students work collaboratively during class time for more than 50% of classes. The module is wrapped up with a reflection/summary class and then the next module begins.

Course performance

We measured course performance as the student's final percentage grade, without the incorporation of the peer evaluation score. The final grades for each student were converted to a z-score to allow for meaningful comparison between courses and years.

Peer evaluation score

A student's mark in our courses is determined by both individual work on exams and assignments and group work on in-class assignments and quizzes. To promote accountability to the group, the group work component is weighted by a student's final peer evaluation score. Peer evaluations are completed using ITP Metrics (www.itpmetrics.com) mid-way through the semester and again at the end of the semester. ITP Metrics is a free, research-based online teamwork-assessment platform with a peer-feedback tool assessing individuals based on key teamwork competencies (O'Neill et al., 2018). The peer evaluation score is the student's average score (from all individuals in the group) divided by average score of all group members. The peer score is bounded at a minimum of 0.60 and a maximum of 1.05, and scores between 1.00 and 0.95 are rounded up to 1.00. Therefore, when the score falls below one, the student is assessed by the group as having done less than expected, if the peer score is equal to one, the student meets expectations, and above 1 (capped at 1.05), the student was assessed as having done more than expected. The score is not calculated as a zero-sum game, so that if one student does more than expected, that does not necessitate that another student has done less than expected. The student's overall score on the final peer evaluation is applied as a multiplier for the total group work component of their grade. The group components of the course count for 11% of the Quantitative Biology course and between 15- 20% of the Biology of Fungi course.

Affective measures of course experiences

At the end of the term, students completed the Experiences of Teaching and Learning (ETL) Survey (Entwistle et al., 2002) electronically through SurveyMonkey. This survey was developed by the Enhancing Teaching-Learning Environments in Undergraduate Courses Project of the University of Edinburgh and has been validated for several student populations (Hounsell & Mccune, 2002), including Canadian students (Fall, 2012). We examined two Experiences of Teaching and Learning sub-scales that addressed student perceptions of a) Peer Support ("Support from other Students": items 21, 24 and 29 of the Perceptions of the Teaching-Learning Environment component) and, b) Engagement with the course material ("Interest, enjoyment and relevance": items 8, 11, 19, 22 and 26 of the Perceptions of the Teaching-Learning Environment component) (Table 1).

For all items, ratings were made on a 5-point Likert-type scale (agree = 5, agree somewhat = 4, unsure = 3, disagree somewhat = 2, disagree = 1). The scores on each response were summed within each subscale to produce subscale scores for each student out of 15 and 25 for Peer Support and Engagement, respectively (ETL user guide).

We also examined two individual measures on the Experiences of Teaching and Learning survey that explored the perceived learning gains on aspects associated with group work. Students responded to statements about how much they felt they gained from this course (on a scale of "a lot", "quite a lot", "unsure", "not much", "very little"), with respect to: "ability to work with other students", and "ability to communicate knowledge and ideas effectively".

Statistical analyses

All analyses were conducted in RStudio version 1.1.143 using the base, lme4 and Psyc packages. To examine whether (1) student performance in terms of the final grades (z-scores), (2) Peer Evaluation Score and (3) the affective measures (Engagement and Peer Support) differed for introverts, ambiverts or extroverts in our classes, we produced general linear models with predictor variables a) introversion b) course and c) year and all higher order interactions. If variables were non-normal, they were arc-sine transformed (because they are proportions). In some cases, this transformation did not completely fix the

Table 1. Experiences of Teaching and Learning (ETL) sub-scale items examined (Peer Support and Engagement), with the ETL item number and statements associated with each sub-scale. Peer support is a 3-item sub-scale with a maximum possible score of 15. Engagement is a 5-item sub-scale with a maximum possible score of 25.

Sub-scale item	ETL item number and statement
Peer support – Support from other students (3 item scale)	21. Students supported each other and tried to give help when it was needed
	24. Talking with other students helped me to develop my understanding
	29. I found I could generally work comfortably with the other students on this unit
Engagement – Interest, enjoyment and relevance (5 item scale)	8. I can imagine myself working in the subject area covered by this unit
	11. I could see the relevance of the most of what we were taught in this unit
	19. This unit encouraged me to relate what I learned o issues in the wider world
	22. I found most of what I learned in this course unit really interesting
	I enjoyed being involved in this course unit

non-normality. However, with sample sizes above 50, we suspect the non-normality is not impacting our statistical conclusions.

Student responses about how much they learned with respect to ability to work with others, and ability to communicate knowledge and ideas effectively, with only single item responses for each, meant the data could not be treated as a continuous numerical variable and therefore were analyzed using Contingency analysis for frequency data. We then tested whether the frequency of student responses to the statements differed for introverts, ambiverts and extroverts in a given course and year. A non-significant result ($p>0.05$) would indicate that introverts, ambiverts and extroverts are not responding significantly differently to this statement, whereas a significant result ($p<0.05$) would indicate differences in how the three groups of students responded.

Results

For two active-learning classrooms over two years, we collected measures of course performance, peer evaluation scores and affective measures of course experiences. We used general linear models and contingency analyses to explore whether self-identified introverts, ambiverts and extroverts differed significantly for any of these measures, with the aim of determining whether active-learning classrooms place introverted students at a disadvantage relative to their peers.

A total of 266 students participated in this study. The proportion of introverts in each class ranged from 29-69%, the proportion of ambiverts ranged from 33-59% and extroverts ranged from 12-29% (Table 2). To measure overall course performance, we examined the mean final grades without the incorporation of the peer score for introverts, ambiverts and extroverts. The final percentage grades were converted to z-scores for

Table 2. The number and percentage (brackets) of students who self-identified as Introverts, Ambiverts* or Extroverts in Quantitative Biology or Biology of Fungi in 2015 and 2016 (N=266).

*in Fall 2015, Biology of Fungi students were not given the option of ambiverts on the initial survey. NA indicates the absence of data

	2015		2016	
	Quantitative Biology	*Biology of Fungi*	*Quantiative Biology*	*Biology of Fungi*
Introverts	24 (29%)	37 (69%)	26 (32%)	18 (38%)
Ambiverts	49 (59%)	NA	28 (35%)	16 (33%)
Extroverts	10 (12%)	17 (31%)	27 (33%)	14 (29%)

Table 3. Mean Peer evaluation score, mean score for the Experiences of Teaching and Learning survey sub-scale for Peer Support, and Engagement. Means shown for Introverts, Ambiverts* and Extroverts in Quantitative Biology and Biology of Fungi in 2015 and 2016.*in Fall 2015, Biology of Fungi students were not given the option of ambiverts on the initial survey, blacked out area indicates the absence of data

	2015					
	Quantitative Biology			*Biology of Fungi*		
	Introverts	Ambiverts	Extroverts	Introverts	Ambiverts	Extroverts
Mean Peer evaluation score (max. score 1.05)	1.02	1.01	1.03	1.02		1.01
Mean Peer Support (max. score 15)	13.79	14.16	14.30	13.89		13.94
Mean Engagement (max. score 25)	19.88	19.06	21.30	20.86		21.65
	2016					
	Quantiative Biology			*Biology of Fungi*		
	Introverts	Ambiverts	Extroverts	Introverts	Ambiverts	Extroverts
Mean Peer evaluation score (max. score 1.05)	1.01	1.01	1.02	1.02	1.01	1.00
Mean Peer Support (max. score 15)	14.00	14.36	14.26	14.28	14.81	14.86
Mean Engagement (max. score 25)	20.85	21.21	19.56	21.56	21.94	22.43

meaningful comparison across courses and years. The mean final grade (z-score) was not significantly different for introverts, ambiverts and extroverts in either course in either year (Fig 1, GLM, F=0.4812, df= 10, 255, p=0.9015).

To determine if peers evaluate the contributions of introverted vs. extroverted students differently, we examined the final peer evaluation scores and compared these scores for students in both courses and year. The mean peer evaluation scores varied little between the groups, ranging from 1.00 -1.03 (Table 3). The peer evaluation scores were not significantly different for the three groups of students across courses and years (GLM, F=0.9818, df=10,255, p=0.4597)

Mean Peer support scores from the Experiences of Teaching and Learning survey sub-scale were high, relative to the maximum score of 15. The scores ranged from 13.79 to 14.16 (Table 3). There was no significant difference between introverts, ambiverts or extroverts (GLM, F=1.026, df=10, 255, p=0.4218).

Mean Engagement scores from the Experiences of Teaching and Learning survey sub-scale ranged from 19.06 - 22.43 (Table 3). There was no significant

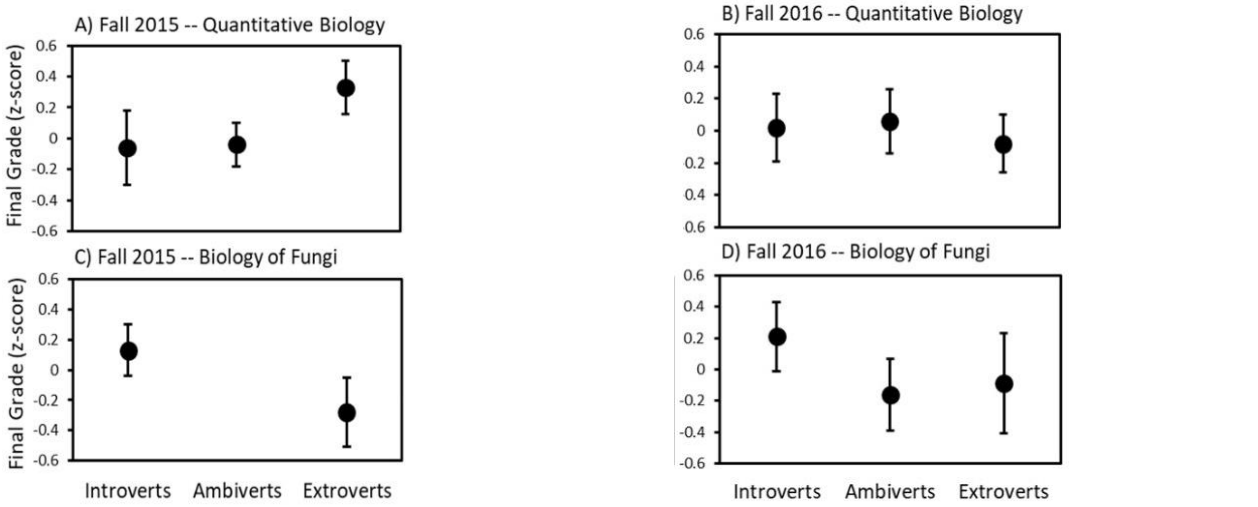

Figure 1. The mean final grade (percentage grade converted to a z-score) for Introverts, Ambiverts* and Extroverts in Quantitative Biology (panels A & B) and Biology of Fungi (panels C & D) in Fall 2015 (panels A & C) and Fall 2016 (panels B &D). Standard error of the mean bars shown. *in Fall 2015, Biology of Fungi students were not given the option of ambiverts on the initial survey, therefore no ambivert mean is shown in panel c

difference between introverts, ambiverts or extroverts (p=0.4821), but there were significant differences in engagement between the courses (p=0.0042) and years (0.0482). Engagement was higher in Biology of Fungi and went up between 2015 and 2016. There were no significant interactions between introversion, year and courses.

In evaluating the measures on the Experiences of Teaching and Learning survey that explored the perceived learning gains with respect to "ability to work with other students", and "ability to communicate knowledge and ideas effectively", most students responded as having learned "quite a lot" or "a lot" (Fig 2 & Fig 3). There was no significant

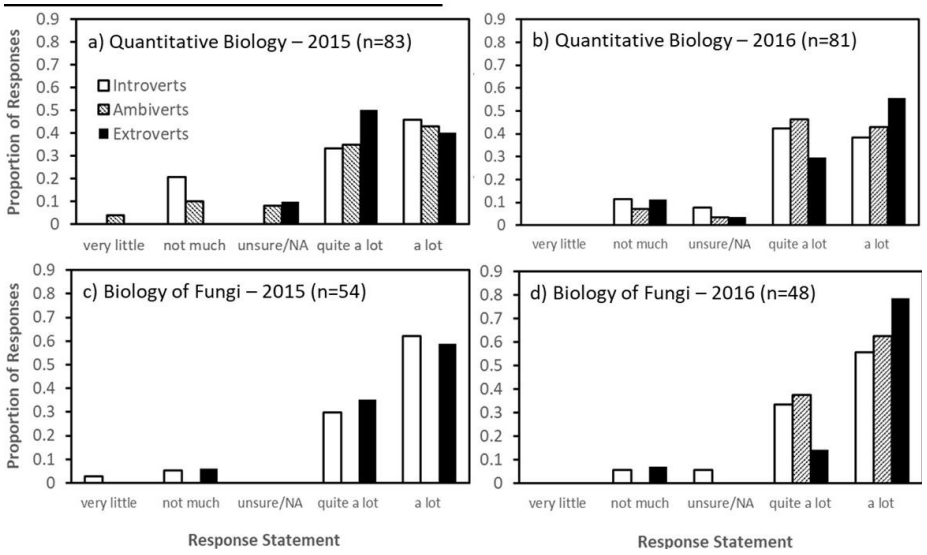

Figure 2. Proportion of student responses to the Experiences of Teaching and Learning survey item, how much did you learn in this course with respect to "ability to work with others". Reponses shown for Quantitative Biology (panels A & B) and Biology of Fungi (Panels C & D) in 2015 (panels A & C) and 2016 (panels B & D). Introverts = white bars, Ambiverts = cross-hatched bars, and Extroverts = black bars

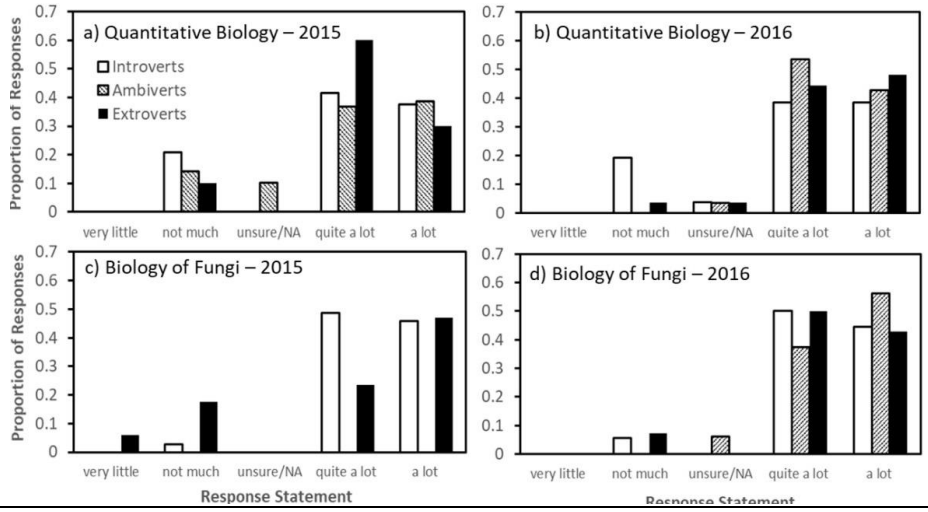

Figure 3. Proportion of student responses to the Experiences of Teaching and Learning survey item, how much did you learn in this course with respect to "ability to communicated knowledge and ideas effectively". Reponses shown for Quantitative Biology (panels A & B) and Biology of Fungi (Panels C & D) in 2015 (panels A & C) and 2016 (panels B & D). Introverts = white bars, Ambiverts = cross-hatched bars, and Extroverts = black bars.

difference in the proportion of student responses for introverts, ambiverts or extroverts for Quantitative Biology in Fall 2015 (p=0.5431) or Fall 2016 (p=0.9222). There were also no differences in Biology of Fungi in Fall 2015 (p= 0.934) or Fall 2016 (p= 0.6071).

Discussion

Our results show no disadvantage in any of the measures explored for introverted students in our active-learning classrooms. Introvert, ambivert, and extrovert students performed equally well in terms of final grades, received comparable peer evaluation scores, and reported similar affective attitudes towards our courses. Students reported high levels of both peer support and engagement with the course materials and these levels did not differ for introverted students. Additionally, reported learning gains in ability to communicate knowledge and understanding, and ability to work with others were not significantly different for introverts, ambiverts, and extroverts. Despite the intensive use of peer discussion, with permanent student groups that were highly integrated into all classes, our courses did not favor extroverted students nor disadvantage introverts. Our results contribute to the understanding of student experiences and performance in active-learning classrooms and provide evidence that active-learning classrooms can be positive experiences for introverted students. We are both introverts and have both thought deeply about how we ourselves would feel entering the courses we have designed. We suspect our initial reaction to reading the course syllabus and arriving at class on the first day would be dread-filled anxiety. But what we witnessed, and documented in this research, was a very different experience than we anticipated for introverted students.

Our findings also differ from several studies showing that introverts have more negative experiences in courses relying heavily on peer interactions (Chamorro-Premuzic et al., 2007; Pawlowska et al., 2014; Persky, Henry, & Campbell, 2015; Walker, 2007; Webb, 1982). For example, Webb (1982) reported that introverted students were more likely to be ignored by group mates when they asked questions to clarify misunderstandings than were extroverted students, and not receiving answers was correlated with lower achievement on tests based on group work topics. In this course, students were assigned to a group of three based on alphabetical order, with adjustments made to group composition to avoid placing friends on the same team and to ensure that highest or lowest achieving students were not on the same group. Walker (2007) specifically investigated whether introverts were at a disadvantage in post-secondary courses relying heavily on group work. Students were divided into groups of five or six students to complete a group research project; students were able to select their own group although some students asked the instructor to put them into groups. Introverted students reported having a more negative group work experience but there were no differences in grades between introverted and extroverted students. Similarly, Persky et al. (2015) reported that while extroverts and introverts had same final exam grade performance, introverts expressed a lower preference for team-based learning. In this study, students were divided into teams of six people, balanced for gender. In all of these studies, students worked in groups but the authors do not describe any specific steps taken to develop teamwork skills and does not appear that there was any intentional focus on team-building strengths. In contrast, our courses included approaches that we speculate facilitated a more-positive group-work experiences particularly for introverted students, as outlined below:

1. We target successful collaboration with peers as an important course outcome and work with students to intentionally develop these skills.

The ability to work successfully with peers is essential for our courses and in students' future careers (Kivunja, 2014) and we make this goal transparent for our students. While many instructors assume students will develop group work skills as they work together, we have found it important to allocate time, in and out of class, to build these skills. We incorporate into our curriculum readings on working successfully with peers (for example: https://www.nytimes.com/2016/02/28/magazine/what-google-learned-from-its-quest-to-build-the-perfect-team.html) and incorporate activities early in the term to help students recognize, value, and develop these skills. We build in time for students to discuss and explore what is working well in their groups, to set goals for how to work together and for themselves as group members, and to monitor these goals through the semester. At the end of the semester, we provide opportunities for reflection on group experiences, and time to celebrate the success of the groups. Throughout the semester, we also meet with groups or individuals who are struggling to discuss strategies for success in collaboration. Dedication of class time and course content to activities that help students work well with peers communicates the importance of group-work skills and gives students tools, resources, and support to develop these skills.

2. We discuss diverse ways individuals can contribute to peer groups and highlight the value of contributions other than talking

Given that peer evaluation is a contributor to student grades in our courses, we want to ensure that students recognize and value diverse ways in which individuals contribute to groups. One strategy we use is to provide distinct roles for students to take within a group during class time (note taker, time keeper, facilitator, etc.), which are rotated among group members during the term. Having a clearly defined task to perform for the group (e.g. keep track of time) can help students contribute to the group, when they might otherwise be unsure of how they can participate (Jacobs, 2014). Additionally, as part of discussions relating to peer evaluation, we emphasize that contributions will be different for different students, given diverse strengths and personalities within a group. We encourage students to recognize that participation does not equal how much someone talked during group discussion and to value contributions that may occur beyond peer discussions, such as organizing a Google Doc for shared notes, emailing meeting minutes, or organizing a time/space to study. For introverted students who may struggle to contribute during peer discussions in class, we offer these ideas as alternative ways they can contribute to the group. Class discussions about the diverse ways individuals can contribute to the group work are important for helping students recognize and value the contributions of others. We believe that all these measures are important to ensure that peer evaluation is not biased against introverted students.

3. We create the groups and aim for diversity in introversion in groups.

We create the student groups rather than let students form their own groups, to minimize anxiety around group formation. In a study where groups were formed by students, introverts' rating for "trusted each other", "enjoyed group work", and "felt valued" were significantly lower than ambiverts and extroverts (Walker, 2006). We also try to make the groups diverse in term of introversion - extroversion. While some studies have shown that groups that are more homogenous in term of introversion – extroversion have higher levels of satisfaction (French & Kottke, 2013), at least one study indicates that groups with an extroverted leader have increased group satisfaction and productivity (Rodríguez Montequín, Mesa Fernández, Balsera, & García Nieto, 2013). In the absence of a clear consensus about the composition of groups relative to introversion, we value creating groups that are diverse in terms of introversion-extroversion because such diversity is likely to be representative of the groups in which students will work during their careers.

Study Limitations

We recognize there are limitations to the generalizability of this research. Our study was conducted in only two classrooms (our own), with two instructors over two years at a single post-secondary institution. Our students self-identified on the introversion/ extroversion scale, potentially leading to some individuals incorrectly identifying themselves as an introvert or extrovert. Similar studies in different contexts need to be conducted to capture more broadly the range of experiences of introverts in active-learning classrooms. Future studies would be strengthened by incorporating follow-up interviews with students to explore which aspects of instruction were most important for student success and engagement with the course. Despite these limitations, our results indicate that, for many introverted students, the active-learning classroom was not a negative experience.

Conclusion

This study can be used to inform instructors who are concerned about the potential for active-learning techniques, particularly those that rely heavily on peer discussion, to negatively impact introverted students. We have shown, under the classroom conditions we describe, introverted students are not placed at a disadvantage in terms of performance, evaluation by peers, or in affective measures of course experience. When consideration is given to development of group work skills, recognition of diverse contributions to groups, and careful construction of groups, introverts can have positive experiences in active-learning classrooms that rely heavily on peer interactions.

Acknowledgements

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References

BONWELL, C., AND EISON, J. 1991. Active Learning: creating excitement in the classroom. ASHE-ERIC Higher Education Report. https://doi.org/ED340272

CAIN, S. 2012. Quiet: The power of introverts in a world that can't stop talking. New York: Crown Publishers. https://doi.org/10.1016/j.jaac.2014.04.007

CHAMORRO-PREMUZIC, T., FURNHAM, A., AND LEWIS, M. 2007. Personality and approaches to learning predict preference for different teaching methods. Learn. Individ. Differ. 17(3): 241–250.

CONDON, M., AND RUTH-SAHD, L. 2013. Responding to introverted and shy students: Best practice guidelines for educators and advisors. Open J. Nurs. 3(7): 503–515.

COOPER, K. M., AND BROWNELL, S. E. 2016. Coming out in class: Challenges and benefits of active learning in a biology classroom for LGBTQIA students. CBE Life Sci. Educ. 15(3): 1–19.

CROUCH, C. H., AND MAZUR, E. 2001. Peer Instruction: Ten years of experience and results. Am. J. Phys. 69(9): 970–977.

DAVIDSON, B., GILLIES, R. A., AND PELLETIER, A. L. 2015. Introversion and Medical Student Education: Challenges for Both Students and Educators. Teach. Learn. Med. 27(1): 99–104.

EDDY, S. L., BROWNELL, S. E., THUMMAPHAN, P., LAN, M. C., AND WENDEROTH, M. P. 2015. Caution, student experience may vary: Social identities impact a student's experience in peer discussions. CBE Life Sci. Educ. 14(4): 1–17.

ENTWISTLE, N., MCCUNE, V., AND HOUNSELL, J. 2002. Teaching-Learning Environments : Concepts, Measures and Preliminary Findings. Occasional Report 1, ETL Project, Universities of Edinburgh, Coventry and Durham, Retrieved from: http://www.ed.ac.uk/etl

FALL, U. L. 2012. Confirmatory Factor Analysis of the Short Revised Experiences of Teaching and Learning Questionnaire (SR-ETL-Q): Examining the Internal Structure within a Canadian Undergraduate Population. Thesis Master of Education, University of Alberta.

FELDER, R., AND BRENT, R. 2009. Active Learning: An Introduction. ASQ Higher Education Brief 2(4) https://doi.org/10.1007/978-1-4419-9863-7_605

FREEMAN, S., EDDY, S. L., MCDONOUGH, M., SMITH, M. K., OKOROAFOR, N., JORDT, H., AND WENDEROTH, M. P. 2014. Active learning increases student performance in science, engineering, and mathematics. PNAS 111(23): 8410–8415.

FRENCH, K. A., AND KOTTKE, J. L. 2013. Teamwork satisfaction: Exploring the multilevel interaction of teamwork interest and group extraversion. Active Learn. High. Educ. 14(3): 189–200.

HAKE, R. R. 1998. Interactive-engagement versus traditional methods: A six-thousand-student survey of mechanics test data for introductory physics courses. Am. J. Phys. 66(1): 64–74.

HENNESSY, D., AND EVANS, R. 2006. Small-group learning in the community college classroom. ProQuest Education Journal 12(1): 93–110.

HOUNSELL, D., AND MCCUNE, V. 2002. Teaching-Learning Environments : Concepts, Measures and Preliminary Findings. Occasional Report 2, ETL Project, Universities of Edinburgh, Coventry and Durham

JACOBS, G. 2014. Introverts Can Succeed With Cooperative Learning. Parole 4(1): 83–93.

KIVUNJA, C. 2014. Do You Want Your Students to Be Job-Ready with 21st Century Skills? Change Pedagogies: A Pedagogical Paradigm Shift from Vygotskyian Social Constructivism to Critical Thinking, Problem Solving and Siemens' Digital Connectivism. Int. J. High. Educ. 3(3): 81–91.

MICHAELSEN, L., BAUMAN KNIGHT, A., AND FINK, L. D. 2004. Team-Based Learning A Transformative Use of Small Groups in College Teaching. Stylus Publishing.

O'NEILL, T. A., DEACON, A., GIBBARD, K., LARSON, N., HOFFART, G., SMITH, J., AND DONIA, M. 2018. Team dynamics feedback for post-secondary student learning teams. Assessment and Evaluation in Higher Education 43(4): 571-585.

PAWLOWSKA, D. K., WESTERMAN, J. W., BERGMAN, S. M., AND HUELSMAN, T. J. 2014. Student personality, classroom environment, and student outcomes: A person-environment fit analysis. Learning and Individual Differences 36:180–193.

PERSKY, A. M., HENRY, T., AND CAMPBELL, A. 2015. An Exploratory Analysis of Personality, Attitudes, and Study Skills on the Study Skills on the Learning Curve within a Team-based Learning Environment. Am. J. Pharm. Educ. 79(2): 1-11.

RODRÍGUEZ MONTEQUÍN, V., MESA FERNÁNDEZ, J. M., BALSERA, J. V., AND GARCÍA NIETO, A. 2013. Using MBTI for the success assessment of engineering teams in project-based learning. Int. J. Technol. Des. Educ. 23(4): 1127–1146.

WALKER, A. (2007). Group- work- in- higher-education:-- are introverted- students- disadvantaged? Psychol. Learn. Teach. 6(1): 20–25.

WEBB, N. M. 1982. Group composition, group interaction, and achievement in cooperative small groups. J. Educ. Psychol. 74(4): 475–484.

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 only in May and in print in December. Submissions should reflect the interests of the membership of ACUBE. Appropriate submissions include:

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

II. Preparation of Articles, Innovations and Perspectives

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

A. Abstract: The first page of the manuscript should contain the title of the manuscript, the names of the authors and institutional addresses, a brief abstract (200 words or less) or important points in the manuscript, and keywords in that order.
B. Manuscript Text: The introduction to the manuscript begins on the second page. 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 the 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 from view 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 within the text should appear alphabetically by the author's last name at the end of the manuscript text under the heading references. All references must be cited in the text and come from published materials in the literature or the Internet. Authors should use the current APA style when formatting the reference list.

D. Example citations are below.

(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

E. Tables

Tables should be submitted as individual electronic files in Word (2013+) or RTF format. 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 using the following format:

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

F. Figures

Figures should be submitted as **high resolution** (≥ 300dpi) individual electronic files, either TIFF or JPEG. Placement of figures should be indicated within the body of the manuscript. 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. No figures put together using a cut and past method will be accepted. All figures should be accompanied by a descriptive legend using the following format:

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

III. Letters to the Editor

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

IV. Other Submissions

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

V. Manuscript Submissions

All manuscripts are to be sent to the editor electronically and must comply with the same guidelines for text, figure and table 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, as well as telephone and FAX numbers. Email will be the primary method of communication with the editors of *Bioscene*.

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

VI. Editorial Review and Acceptance

For manuscripts to be sent out for review, at least one author must be a member of ACUBE. Otherwise, by submitting the manuscript without membership, the corresponding author agrees to page charges. Charges will be the membership fee at the time of submission per page. Once the authors' membership or page charge status has been cleared, the manuscripts will be sent to two anonymous reviewers as coordinated through the Editorial Board. 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 stated above, manuscripts will be returned to authors until compliance is met or the page cost conditions have been met. Reviewers will examine the submission for:

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

Once the article has been reviewed, the corresponding author will receive a notification of whether the article has been accepted for publication in *Bioscene*. All notices will be accompanied by suggestions and comments from the reviewers. The author must address all of the reviewers' comments and suggestions using the original document and **track changes** for any consideration of a resubmission and acceptance. Revisions and resubmission should be made within six months. Manuscripts resubmitted beyond the six-month window will be treated as a new submission. Should manuscripts requiring revision be resubmitted without corrections, the associate editors will return the article until the requested revisions have been made. Upon acceptance, the article will appear in *Bioscene* and will be posted on the ACUBE website. Time from acceptance to publication may take between twelve and eighteen months.

VII. Revision Checklist

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

A. Send a copy of the revised article using track changes for text changes back to the associate editor, along with an email stating how reviewers' concerns were addressed.
B. Make sure that references are formatted appropriately in APA style format.
C. Make sure that recommended changes have been made or a clear explanation as to why they were not.
D. Figures and legends sent separately, but placement in manuscript should be clearly delimited.

VIII. Editorial Policy and Copyright

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