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What do Animal Physiology Students Learn From a CURE Investigating the Effects of Septicemia on Cardiac Function: Frog and Larval *Drosophila* Models

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Course-Based Undergraduate Research Experiences (CUREs) create a novel approach for undergraduate students to experience scientific research while offering faculty pathways for connecting their research to in-course experiences. This poster presents a CURE implemented in an undergraduate animal physiology laboratory course that investigated the effect of lipopolysaccharides (LPS) on frog and larval *Drosophila* cardiac function, research that is built on a recent study (Anyagaligbo et al., 2019). This study aimed to add to the research literature on how a CURE investigation influences students' cardio physiology content knowledge, affects changes in their perceived self-efficacy with content knowledge and laboratory skills, and identifies surprising and challenging aspects of the CURE culminating from the experience. The hypothesis of this study was that the CURE would lead to increased student learning outcomes regarding content knowledge and self-efficacy. Learning outcomes were measured using pre- and post-assessments from matched pair responses (n=42). The assessments included content and survey tasks to quantitatively and qualitatively measure students' content understanding, self-efficacy related to LPS and septicemia, and cardiac physiology. The results demonstrated statistically significant increases in students' content knowledge and self-efficacy related to LPS/septicemia and cardiac physiology. Student-reported surprises and challenges also are presented

Keywords: course-based research experiences, inquiry, liposaccharide, biology laboratory

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Introduction

Authentic undergraduate scientific research experiences can build skills and experience in applying scientific practices while engaging students in a scientific community of discovery and collaboration (Auschencloss et al., 2014; Lopatto, 2007; National Research Council [NRC], 2003; Shapiro et al., 2015). These experiences can foster excitement about science as students conduct scientific research and increase motivation to pursue careers in scientific research through feelings of recognition of being a scientist (Starr, 2020). Such experiences also develop critical reasoning skills and science practices, including collaborating with peers, arguing from evidence, and perseverance in problem-solving (i.e., Auchincloss et al., 2014; Ditty et al., 2013; Miller et al., 2013). Unfortunately, many undergraduate science courses they complete are offered in large, lecture style formats while laboratories often present standardized laboratory exercises that offer little opportunities for setting up equipment, constructing deeper understanding of the topic, arguing from evidence, and collaborating in sense-making activities to interpret the data (NRC, 2003; AAAS, 2010; Holt, 1969).

Course-based undergraduate research experiences (CUREs) derived from ongoing faculty research articulates the calls for change within undergraduate biology education echoed in *Vision and Change in Undergraduate Biology Education* (American Association for the Advancement of Science [AAAS], 2010). The Initiative recommended use of student-centered pedagogies in instruction and a trajectory of scientific research experiences for students beginning early in their undergraduate academic careers. Likewise, the NRC (2003) has recommended the use of project-based laboratories in undergraduate biology instruction to foster science practices and enculturate students in scientific research.

A challenge for college laboratory instructors is garnering sufficient time and materials to support such experiences. Examples of challenges laboratory instructors report include the increased amount of time to develop and implement a CURE, the need for increased funds to purchase necessary materials, anticipating what supplies will be required, arranging additional laboratory time that allows for project completion, the expanded role of an instructor that includes mentorship, and determining research projects that can be successfully completed in a course laboratory setting (Shortlidge et al., 2016). Additionally, the development of authentic experiences requires revision and re-implementation upon student feedback. Consequently, curriculum development is a time-consuming process (Marbach-Ad & Rietschel, 2016).

In this study, we sought to describe the initial stage of a transformative process in which a physiology laboratory classroom moves from a standardized lab to an authentic experience. This presentation describes the results of a pilot study on the first iteration of a short-term CURE and discusses future efforts to continue the course's transformation into an authentic research experience. This study is part of a larger study that explored student outcomes from a bench science investigation on the effects of bacterial endotoxin on cardiac tissue. The purpose of this paper is to report on the successes and challenges of implementing a unique authentic laboratory investigation in an animal physiology course on the effects of lipopolysaccharide (LPS) on cardiac tissue. The investigation was an extension of research conducted by Dr. Robin Cooper and his colleagues (Anyagaligbo et al.,2019).

Methods

This quasi-experimental study used a pre-post test design. The overall question that guided the study was: How does student participation in the frog cardiology laboratory activities affect students' understandings of the anatomy and physiology of the heart under the effects of a bacterial endotoxin?

Three supporting questions guided the exploration of students' views resulting from their participation in the laboratory:

- How do the frog cardiology laboratory experiences affect students' understandings of the anatomy and physiology of the frog heart? The *Drosophila* heart tube?
- How does the frog cardiology laboratory experiences affect students' knowledge about bacterial endotoxin and its effect on animal models?
- What were students' perceptions of the frog cardiology laboratory?

Intervention

Students enrolled in the course were majoring in biology or a related discipline and were taking the course as a requirement for their major. The intervention was derived from Anyagaligbo et al. (2019) and adapted for use in with the undergraduate students. There were three major parts: pre-lab activities that prepared students for the laboratory, bench lab research, and post-lab analysis and write up. A brief summary of each part of the intervention follows. Refer to Bernard et al., in this Proceedings for a detailed account of the laboratory intervention.

Pre-lab Activity

The pre-lab activity consisted of students drawing visual representations of the frog and human hearts, and the *Drosophila* heart tube, diagramming physiological changes of a single pacemaker cell, and diagramming the voltage changes in cardiac muscle cell excitation. In addition, students conducted a brief literature review on cardiac tissue in animal models. This latter exercise was designed to inform students on the extant research while highlighting the limited knowledge in science on this topic.

Laboratory Protocol

At the beginning of the laboratory, the lab instructor briefly provided an overview of the lab and presented a slide to review basic anatomy of the frog heart that was included in the pre-lab, and review the anatomy of the frog heart to demonstrate where to insert the fish hook that was part of the experimental set up: The fish hook was inserted in the apex of the heart with a fishing line extending from it — the line would be connected to the force transducer to measure the deflections of the heart and strength of heartbeats. The instructor then directed partners to select their role in the investigation: (1) dissect the frog and administer the solutions; or (2) set up the force transducer, observe patterns in the heart deflections, and mark the times on the transducer chart when the solution was applied.

Next, concurrently one partner dissected the frog to prepare it for set up with the force transducer as the other partner set up and prepared the force transducer for data collection.

Once the frog was set up for the study (see Figure 1), students were provided one of two solutions in a blind study: A) LPS dissolved in saline; or B) saline. Before applying the unknown solution, partner groups collected two minutes of data to record heart deflections, administer saline solution to keep the heart moist, and confirm they were recording clear deflections in the heart. Then one partner administered the unknown solution as the other partner digitally marked the chart where when the treatment was applied and then observed the resulting graph. They observed the heart rate as long as needed to observe any changes in deflections or strength of the heartbeat. Some groups observed 15-20 minutes while others observed over 30 minutes, and one or two groups per session had to restart their computers and their investigation when the computer crashed.

In a second smaller study, students measured the effects of an unknown solution (LPS in saline or saline) on the larval *Drosophila* heart tube. The faculty researcher set up the larval *Drosophila* for observation. Then students moved the set up to their lab space to observe the beating heart tube under the microscope as the unknown solution was administered.

Figure 1. Frog set up for LPS study. Not pictured here is the hook inserted in the apex of the heart with a piece of fishing line attached to the force transducer to measure the force of the beats of the heart as solution is applied to the heart.

Post-lab Activity

Data from all four sections of the lab were combined to create one large data set. Students analyzed the data using an ANOVA to determine whether observed differences were statistically significant at the 0.05 level. In this first running of the laboratory, the differences between the two treatments: saline and LPS diluted in saline were not statistically significant. Students were also provided a case of a septic patient for which they were to apply their new knowledge to explain how sepsis occurred. They also developed a write up to present and explain their results. The posttest was administered three weeks later (after the university shut down due to the pandemic) once all lab reports were submitted. See the Appendix for the pre and post assessments.

Data Collection and Analysis

A pre and post survey were developed for this study to connect directly to the content and experiences of the laboratory research experience. Topics included: basic questions about LPS and septicemia, cardiac anatomy and physiology, nature of science, and conducting experiments. The instrument was modeled after previously designed and tested instruments (e.g., Pomeroy, 1993; Lobatto, 2004) to learn about students' views.

Study participants included 42 undergraduate students enrolled in the undergraduate animal physiology lecture and laboratory courses taught by Dr. Robin Cooper in the spring semester of 2020. Sixty-eight students volunteered to participate in the study, but only 42 completed the pre- and post-test.

Students completed the pre survey before engaging in the pre-lab activity, the laboratory exercise, and the post-lab activity. The pre and post survey were identical and consisted of the following sections: self-efficacy related to LPS/septicemia and cardiac physiology, the nature of science and conducting experiments, content questions related to LPS/septicemia, and attitudes towards physiology laboratory. Wilcoxon sign ranked test was used to analyze differences in self-efficacy and nature of science (Taheri & Hesamian, 2013). Pre and post content scored were analyzed using a McNemar test (Adedokun & Burgess, 2012).

Findings

The data collected from the pre and post assessment were used to identify changes in students' knowledge about the anatomy and physiology of the heart and *Drosophila* heart tube and affective outcomes students gained from the experience. In addition, there were four open ended questions in the post test designed to gather information on students' experiences in completing the research study. The questions included: What was most helpful to you in the lab? What surprised you? What challenges did you experience? How did you solve the challenges?

Results from the pre and post survey demonstrated significant learning gains related to physiology within frog hearts and larval *Drosophila* heart tubes (Table 1), along with a significant increase in self-efficacy in students' perceived understanding about cardiac physiology within these models (Table 2). Additionally, a significant increase in learning gains and self-efficacy was observed in topics related to LPS (Tables 3 and 4).

Answers students provided about helpful aspects of the lab and surprises they encountered reflected similar topics. They most often reported being amazed to see the beating frog heart and *Drosophila* heart tube since many of them had never observed a live heart beating. Similarly, many of them had never dissected an organism before. Twenty-four percent (21 of 89 responses) of the responses to helpful aspects of the lab, and 25% (18 of 72) responses) to what surprised you described seeing the beating frog heart. Often the response would state that seeing the heartbeat help them make sense of the anatomy and physiology of the heart more than any textbook or diagram could. The wonderment they noted illustrated the curiosity and motivation the research study had instilled in them.

Another surprise reflected in the response to 'What surprised you?' was not finding statistical significance in the observed difference between the two treatments: saline and LPS and saline. Over 40% of the responses (29 out of 72) reflected surprise or frustration that their hypotheses that LPS would affect the heart rate was not supported by the data collected across the four laboratory sections. The incongruence between their hypothesis and the findings created cognitive disequilibrium for some students as they tried to make sense of the class findings when they believed they had observed a change in the frog heart rate when their unknown substance was applied. Even though their solution was unidentified, they were sure it was LPS. One student admitted to having no idea how LPS might affect the heart after the investigation and remained frustrated that their 'right' answer was not the outcome.

Students also noted challenges in setting up the lab, including dissecting the frog, inserting the hook in the heart, and setting up the force transducer. Other challenges included one or two computers that crashed during the data collection, which led to two groups moving to another station. The difficulty measuring the frog heart rate, particularly when other students bumped the table or the graph shown an anomaly was difficult, but most difficult was trying to measure the beats of the larval *Drosophila* because of its small size tube and its sporadic rhythm. Partners bumping the table during data collection also made observing the heart tube difficult. When ask about how they solved the challenges they faced, their responses were nearly unanimous: from the support of the instructor and teaching assistants. The comments across the four sections reflected active mentoring that occurred in the lab to support the students in their work.

Conclusions

Significant positive changes were demonstrated in students' knowledge of the anatomy and physiology of frog heart and *Drosophila* heart tube. Students' responses showed significant improvements in self-efficacy for all measured categories which broadly included the following areas: human, frog and *Drosophila* heart/heart tube anatomy; cardiac neurophysiology; and measuring changes in heartrate. Students demonstrated learning gains on understanding the origins of LPS about how LPS directly effects organs.

Students' responses demonstrated largest gains in self-efficacy when describing what happens when LPS is introduced to the *Drosophila* heart tube and frog cardiac tissue. Furthermore, students reported the benefits of directly observing live hearts from frogs and *Drosophila.* This manuscript also reports cognitive disequilibrium reported by students when hypothesizes were not congruent with their data and challenges with this particular CURE, such as technical issues and difficulties with visual measurement.

Future Directions

We are continuing to compare qualitative and quantitative findings to characterize students' understanding and perceived self-efficacy in relation to CURE dimensions (i.e., science practices, discovery, broadly relevant/important work, collaboration, iterative). We also are reviewing the data to make modifications to the lab and implement its next iteration in spring 2022.

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Jate Bernard is a medical student at the University of Kentucky. He received a B.S. in Chemistry from the University of Virginia.

Appendix

Table 1 Comparison of Pre- and Post-test Results on Physiological Knowledge about Frog Heart and Larval Drosophila Heart Tubes

Question	Frequency of Correct Pre-Test Responses (*n*=42)	Percentage of Correct Pre-Test Responses by Students	Frequency of Correct Post-Test Responses (*n*=42)	Percentage of Correct Post-Test Responses by Students
Does the larval (maggot) stage of Drosophila (Fruit fly) have some form of a heart?	13	30.95%	28	66.67%*
How many chambers does a frog heart have?	17	40.47%	36	85.71%***
Which of the following best describes the larval Drosophila heart?	4	9.52%	15	35.71%*
Which of the following best describes the human heart?	15	35.71%	34	80.95%***
What is the neurotransmitter released from the tenth cranial nerve on the frog or human heart?	12	28.57%	25	59.52%*
In larval Drosophila, which of the following can increase the heart rate?	3	7.14%	4	9.52%

**indicates significant change at 0.05 confidence level.*
****indicates significant change less than 0.001 confidence level.*

Table 2 Pre- and Post-Test Scores Assessing Students' Self-Efficacy Regarding Frog Heart and Larval *Drosophila* Cardiac Anatomy and Physiology

Survey Item	Average Pre-Test Response	Average Post-Test Response	Effect Size
I can describe the anatomy of the frog heart	1.71	3.07	-.542***
I can describe how the anatomy of the frog heart differs from the human heart.	1.62	3.19	-.569***
I can describe the anatomy of the heart tube of a Drosophila larva.	1.38	2.62	-.560***
I can explain how the autonomic system controls heart function in a frog.	1.76	3.07	-.558***
I can explain how the autonomic system controls heart function in larval Drosophila.	1.55	2.79	-.532***

I can compare the cardiac physiology of insects and amphibians.	1.74	2.86	-.507***
I can calculate a change in heart rate.	2.52	3.52	-.526***

****indicates significant change less than 0.001 confidence level.*

Table 3 Comparison of Pre- and Post-test Results on Lipopolysaccharide (LPS) and physiological effects of LPS

Question	**Frequency of Correct Pre-Test Responses (*n*=42)**	**Percentage of Correct Pre-Test Responses by Students**	**Frequency of Correct Post-Test Responses (*n*=42)**	**Percentage of Correct Post-Test Responses by Students**
Where does Lipopolysaccharide (LPS) come from?	22	52.39%	39	92.86%***
Does LPS have any direct effect on the physiology of organs?	19	45.24%	32	76.19%*

**indicates significant change at 0.05 confidence level.*
****indicates significant change less than 0.001 confidence level.*

Table 4 Pre- and Post-test Scores Assessing Students' Self-efficacy Regarding Bacterial Endotoxin and its Effect on Animal Models

Survey Item	**Average Pre-Test Response**	**Average Post-Test Response**	**Effect Size**
I can explain how septicemia develops in humans	1.5	2.83	-.549***
I can define bacterial LPS	2.07	3.19	-.482***
I can explain what happens when LPS is introduced to frog cardiac tissue	1.42	3.05	-.589***
I can explain what happens when LPS is introduced to the *Drosophila* heart tube	1.33	2.88	-.589***
I can identify substances that are released from tissues in the body when LPS is in the blood of an animal	1.52	2.71	-.522***
I can explain the cascade of the events that occur in a human example from the introduction of LPS in the blood to sepsis	1.48	2.95	-.584***

****indicates significant change less than 0.001 confidence level.*

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