Exploring the Leech Gut Microbiome as a Course-based Undergraduate Research Experience

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Course-based undergraduate research experiences (CUREs) engage students in scientific discovery and facilitate authentic learning experiences. To better understand the influence of CUREs on undergraduate biology majors, we developed a CURE for students enrolled in a general microbiology course (Micro-CURE). The Micro-CURE taught students the same skills as in a traditional microbiology laboratory course but in a setting that promoted engagement with real-world problems. Students were given a pre- and postsemester questionnaire to gauge both their self-reported perceptions of and actual gain of microbiological skills and content knowledge. After participation, Micro-CURE students reported an increase in familiarity with microbiology skills, demonstrated significant improvement in the use of microbiological technical skills, and exhibited an improved understanding of microbiology concepts.

Education researchers in the science, technology, engineering, and mathematics (STEM) fields have long called for transformation by promoting student engagement in active investigation (Holt et al., 1969). A growing body of research documenting the benefits of undergraduate research experiences (UREs) has intensified this call to action (e.g., American Association for the Advancement of Science, 2011). Studies on UREs' effectiveness have documented improvements in independent and critical thinking, research and communication skills, and the likelihood that students will pursue post-undergraduate research activity and careers in STEM fields (Corwin et al., 2015; Hathaway et al., 2002; Kardash, 2000; Laursen et al., 2010; Lopatto, 2004). Traditional apprenticeship URE models provide many benefits, but opportunities are limited and largely made available on an inequitable basis (Bangera & Brownell, 2014; Intemann, 2009).

Alternatively, course-based undergraduate research experiences (CUREs) engage all students enrolled in a class in the process of discovery (Auchincloss et al., 2014). CUREs utilize valid scientific practices with the principles of collaboration and iteration to explore a research question that is broadly relevant to the scientific community (Auchincloss et al., 2014; Laungani et al., 2018). Additionally, CUREs can help students develop a better understanding of science content in addition to science processes (American Association for the Advancement of Science, 2011; Bakshi et al., 2019; Blanton, 2008; Brownell et al., 2012). Utilizing CUREs rather than the traditional URE model promotes content learning (Jordan et al., 2014), provides research experiences to more students more equitably (Bangera & Brownell, 2014; Corwin et al., 2015; Rodenbusch et al., 2016), introduces students to existing research opportunities and the benefits of participation, and removes the perceived cultural barriers to interacting with faculty (Bangera & Brownell, 2014). By removing the barriers typically associated with the URE model, we can encourage more students of diverse backgrounds to become involved in STEM research.

For a CURE to be an effective research opportunity, have similar outcomes to a URE, and be differentiated from a course-based laboratory component, it must include scientific practices beyond data collection, an unknown outcome, relevance to the real world, collaborative components, and the principle of iteration, wherein multiple approaches are used to address the research question (Auchincloss et al., 2014). Numerous resources have been developed to assist in the implementation of CUREs, including drop-in models ready for immediate adoption (e.g., CUREnet) and guides for creating a CURE based on a faculty member's expertise and interests (Bell et al., 2015). After considering the objectives of the experience and choosing an appropriate project, an assessment method is then identified or developed to measure the ability of the CURE to meet the stated learning objectives (Shortlidge & Brownell, 2016). The efficacy of the course in reaching the stated objectives is commonly gauged using student feedback and self-assessment surveys (Brownell et al., 2012; Gasper & Gardner, 2013; Kloser et al., 2013), tests of scientific literacy skills (Gormally et al., 2012), critical thinking assessment tools (Laungani et al., 2018; Moore, 2012), alumni surveys (Hathaway et al., 2002; Russell et al., 2007), and faculty surveys (Hunter et al., 2007; Lopatto, 2003; Shortlidge et al., 2017). Although students' self-assessment of their skill development is important, the potential exists for their ratings of their own abilities and performance to differ from ratings provided by instructors or research mentors (Atwater & Yammarino, 1997; Kardash, 2000). Due to this potential disconnect, the ability to externally assess the growth of a student's research skills is a crucial part of the analysis.

One of the objectives of the CURE model is to help students gain a better understanding of research practices (American Association for the Advancement of Science, 2011). Traditional microbiology classes are typically taught solely within the laboratory to permit the use of specialized equipment and to maintain aseptic conditions, but this has the potential to distort students' impressions of microbiological research. The ideal microbiology CURE introduces students to the research experience, starting with field collection, so they can begin to see how their work relates to a real-world question. Additionally, promising results arising from class projects should be included in future publications to show students their contributions are relevant.

Our CURE for a general microbiology course (Micro-CURE) was designed to teach basic principles of microbiology while also providing an authentic research experience. Students were taught the same laboratory skills they would learn in a traditional microbiology laboratory class (e.g., aseptic technique, microscopy, and microbiological assays) within the context of a novel ecological research project. The purposes of this study were as follows:

1. Determine if participation in the Micro-CURE influenced students' understanding of course-specific content knowledge (course-specific content).
2. Determine how participation affected students' perceptions of their own research skill development (skills perceptions).
3. Assess the development of students' microbiology research skills (skills development).

Methods

We conducted our study in an undergraduate, general microbiology laboratory course intended for sophomore- and junior-level students at a public, intermountain-west, primarily undergraduate institution. The laboratory and lecture courses were taught by a single instructor. Students completed pre- and postsemester questionnaires and a final research report (see Online Appendix A for questions).

Research setting

The laboratory course met for 2 hours each week throughout a 15-week semester, divided into four phases: introductory, experimental design, isolation and characterization, and genetic analysis (Table 1). Students were provided with a laboratory manual that included background information and laboratory protocols. During Phase 1 (the first four lab sessions), students were instructed in laboratory safety using the principles outlined in the ASM Guidelines for Biosafety in Teaching Laboratories (Byrd et al., 2019). Students were then required to demonstrate competence and uphold all Biosafety Level 2 standards. For the research portion, we chose to explore leech gut microbiomes for three reasons: (i) Previously published studies indicate leeches possess simple microbiomes; (ii) several species of leeches thrive in nearby, accessible locations; and (iii) experts are available on campus to assist with locating and identifying leeches.

To prepare for Phase 2 (the fifth lab session), students performed a literature search by identifying a minimum of two leech microbiome papers; they then outlined the relevant stepwise methods and developed their own protocols for studying novel leech microbiomes. In the fifth laboratory session, all students met to discuss the protocols they developed. The conversation started with predetermined, guided questions (see Online Appendix B); students talked about the stages of the research protocols, including leech collection, surface sterilization, microbe isolation, and bacterial characterization and identification. As a class, students discussed each step and developed a standardized protocol. An optional field trip was planned prior to the sixth laboratory session; all students were invited to help collect the study leeches.

Over the ensuing 10 weeks, students followed their standardized protocol to isolate and characterize the gut bacteria using morphological techniques (e.g., standard staining protocols), biochemical techniques (e.g., catalase and oxidase tests), and genetic assays (e.g., DNA isolation, PCR, and DNA sequencing). Students used the ABIS Online dichotomous keys (https://www.tgw1916.net/ABIS/abis_maps.html) to obtain a putative identification for their bacterial isolates and conducted NCBI BLASTN searches of their 16S rRNA sequences to support their identification.

Participants

We recruited 167 undergraduates to participate in our study, which took place during the spring 2019 (n = 87) and fall 2019 (n = 80) semesters. After removing 13 students who completed the preassessment but not the postassessment, the final participant pool included 154 students. The majority of students were co-enrolled in the laboratory and lecture course; nine were taking the laboratory course for a second time (spring 2019, n = 4; fall 2019, n = 5).

Data collection

Data sources include a pre- and postsemester questionnaire and species characterization questions. We developed a set of open-ended questions about students' attitudes toward science and research practic-

TABLE 1

Outline of activities and desired outcomes.

Weeks	Activities	Desired outcomes
1–4	Learn the following: • Laboratory safety • Aseptic technique • How to keep a laboratory notebook • How to use a bright-field light microscope	Demonstrate proficiency in the following: 1. Perform inoculations, streaking for isolation, dilution series, and spread plating. 2. Write protocols, record results, provide evidence, and draw conclusions. 3. Set up, use, and care for microscopes.
5	Practice: • Performing a literature review • Designing a research project (experimental design)	1. Locate research papers on a given topic. 2. Demonstrate ability to read and discuss primary literature. 3. Develop an experimental plan.

6–10	• Isolate bacteria from leech gut.
	• Characterize bacterial isolates using morphological and biochemical assays.
	  • Negative stain
	  • Gram stain
	  • Hanging drop motility test
	  • Catalase test
	  • Oxidase test
	  • Nitrate reduction test*
	  • Urease test*
	  • Carbohydrate fermentation tests*
	  • Endospore staining*
	• Identify culture using dichotomous keys.
		1. Obtain pure isolates.
		2. Demonstrate ability to carry out and interpret standard microbiological assays.
		3. Use dichotomous keys to identify bacterial isolates.

11–15	• Perform molecular biology techniques.
	  • DNA isolation
	  • Polymerase chain reaction
	  • Agarose gel electrophoresis
	• Carry out DNA sequence analysis and BLAST search.
	• Draw conclusions and report final results.
		Demonstrate ability to do the following:
		1. Isolate DNA.
		2. Use precise pipetting skills.
		3. Run PCR and analyze results via agarose gel.
		4. Interpret trace data and BLAST results.
		5. Communicate data and draw appropriate conclusions.

Note. *Tests performed as needed to identify specific isolates using dichotomous keys at ABIS Online (https://www.tgw1916.net) es, microbiological techniques, and research objectives that we administered through a pre- and postsemester questionnaire. The questionnaire consisted of 15 questions, which were developed based on the Draw-a-Scientist Test (Chambers, 1983) and course learning objectives. The questions were divided across five sections: (i) Perceptions of Scientists, (ii) Perceptions of Science/Research Skills, (iii) Perceptions of Research, (iv) Perceptions of Microbiological Skills, and (v) General Content Knowledge (see Online Appendix A). We provide the full questionnaire even though we used only the questions about perceptions of microbiological skills and general content knowledge for this study.

In spring 2019, we administered the pre- and postsemester questionnaire to the students only at the end of the semester due to Institutional Review Board approval dates. The questions in each section were identical, with the exception of the question prefix (e.g., "Prior to taking this course" or "After taking this course"). In fall 2019, the presemester questionnaire was administered during the first week of class, and the postsemester questionnaire was given during the last week of class; the instrument was identical in both administrations. As the 2 semesters were statistically different from each other ($t(152) = 3.94$, $p = 0.00$), we did not combine data for analysis.

Data analysis

Course-specific content

Quantitative analysis was used on responses to both pre- and postsemester questionnaires. Trends in and differences between students' pre- and postsemester understanding of course content knowledge, students' perceived development of skills, and actual development of skills were identified by descriptive statistics and paired *t*-tests.

Skills perceptions

For the qualitative portion, we coded the data by question using both deductive and inductive approaches (Saldaña, 2013). The best approach for each question was determined by question type, and for some questions, we utilized both approaches. For questions that elicited a "yes," "no," or "maybe/none/some" response, we used a deductive approach, but questions that prompted descriptive responses required an inductive approach. Finally, some questions were two-tiered, meaning that they asked students if they had a specific skill set or had heard of a specific term; if the response was a "yes," students were asked to elaborate on their response, thus eliciting answers that required both coding approaches.

For the deductive approach (Questions 11–14), we first developed categories that matched the question syntax. Next, we read through student responses and sorted them into predetermined categories. For questions meant to gauge student understanding (Questions 11, 12, and 14; see Online Appendix A), responses were assigned a numerical score for analysis: Responses stating that students have no knowledge or that include incorrect or ambiguous descriptions were assigned a score of 0; responses that provided a basic definition were assigned a score of 1; those that described the purpose or provided a specific example were assigned a score of 2; and responses that described the purpose and provided a specific example were assigned a score of 3 (Table 2). After grouping responses into categories, we identified patterns to describe themes in student responses.

TABLE 2

Example responses for student understanding questions.

Score	Example of a response to the question "What do you know about microbiomes?"
0	"I really don't know anything about this."
1	"They consist of many different kinds of bacteria."
2	"A microbiome is the total number of microbes that live on and inside of the human body. There are many different ones such as the oral cavity and the skin."
3	"Microbiomes are environments where millions of microbes are found living in the same location and surviving with the help of one another. For example, the gut microbiome in a leech is full of extremely specific microbes that have their own functions and relationships with the host. All of these microbes working together in this microbiome provides health benefit to the leech."

For those questions where we used an inductive approach (Questions 10–15), we created open codes based on student responses, using the word choices of the students to capture their language. Next, we grouped student responses by similarity, then condensed the responses into themes (Saldaña, 2013). For instance, in the question asking students which microbiology techniques they had experience utilizing prior to taking the course (Question 10 in Online Appendix A), the listed skills were grouped into technique categories (Table 3).

Two of the authors independently categorized and reviewed the student responses to establish inter-rater reliability and assure trustworthiness. The course instructor was one of the author-raters due to the essential nature of the expertise surrounding the interpretation of the microbiology techniques; although this has the potential to introduce bias to the results, any discrepancies in codes, categories, or themes were discussed between the two raters by describing their coding processes for each instance and discussing different coding options until 100% consensus was reached (Saldaña, 2013).

TABLE 3

Themes of skills students reported having prior to taking the course.

Technique categories	Examples of skills listed
Aseptic technique	Sterilization, use of the Bunsen burner, etc.
Culturing	Spread plating, streaking for isolation, etc.
Microscopy	Microscopy
Basic lab skills	Pipetting, lab notebooks, centrifugation, etc.
Staining	Gram staining, negative staining, etc.
Dilutions	Dilution calculations, etc.
DNA-related skills	DNA isolation, PCR, electrophoresis, DNA sequencing, etc.
Species characterization	BLAST searching, dichotomous key
Experimental design	Design, planning, etc.
Biochemical assays	Oxidase test, catalase test, nitrates reduction, etc.

Skills development

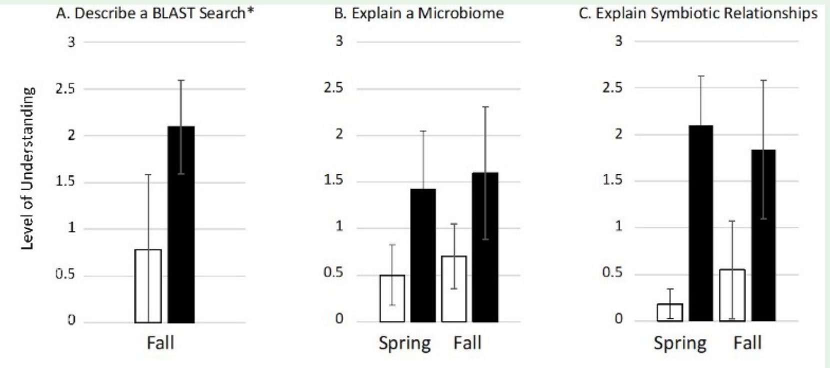
Mastery in microbiological techniques, as defined for the study, means there is agreement between the student-reported and published values for each assayed characterization trait. We ran an independent BLAST search using the 16S rRNA gene sequence obtained by each student to confirm the student's reported identification, and we used the identified species as a standard to verify the correct interpretation of each morphological and biochemical assay. If the BLAST search identified a Gram-positive species, for example, the Gram-status reported by the student for their isolated bacterial culture was checked for agreement; 1 point was assigned for answers that agreed with published characteristics for that species, and 0 points were assigned for disagreements. The number of students who successfully obtained a PCR product, successfully carried out DNA sequencing, and correctly interpreted and reported each morphological and biochemical assay was reported as the percentage of students demonstrating mastery of each technique.

Student assessment

Students were assessed on weekly prequiz performance, weekly lab reports, the experimental design assignment, and their laboratory notebooks. The weekly prequizzes were administered at the start of each class; these consisted of 10 to 15 multiple-choice and short-answer questions that were designed to encourage students to prepare for each lab period (e.g., by reviewing laboratory protocols and safety precautions). The weekly lab reports consisted of approximately 10 to 20 multiple-choice and short-answer questions designed to gauge students' understanding of the principles of each microbiological technique and assay. The experimental design assignment required students to identify a minimum of two leech microbiome papers, outline relevant methods, and develop their own research protocol. Students were assessed on the relevance of the papers selected, their analysis of the methods, and the quality and completeness of their developed protocol. The laboratory notebook assignment required students to maintain a complete record of all protocols, personal observations, documented evidence (e.g., photographs, sequencing trace files, research partner observations), and conclusions for each technique and assay performed. In their laboratory

FIGURE 1

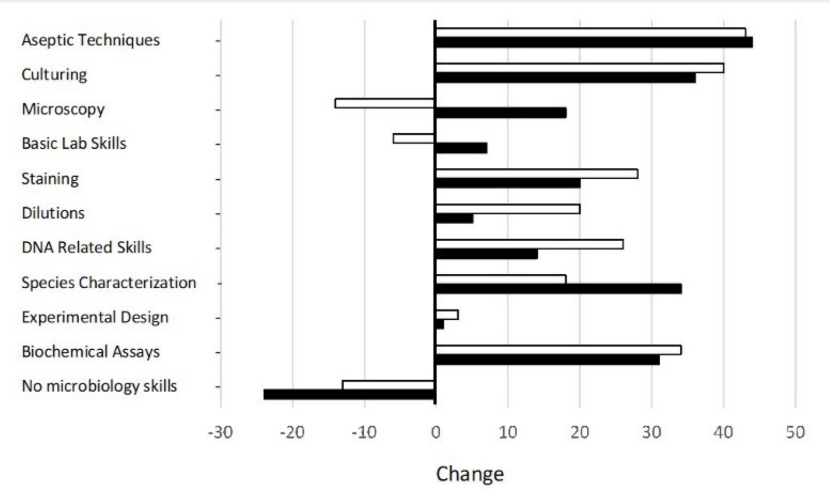
Student responses on course-specific content questions for presemester and postsemester questionnaires.



Note. Presemester responses are shown as white bars, and postsemester responses are shown as solid black bars. Each difference shown is significant ($p < 0.0001$). *Students were only asked this question during the fall 2019 semester.

FIGURE 2

Pre- to postcourse change in spring and fall in response to the question "What skills in microbiology techniques do you have experience doing?"



Note. Spring data are shown as white bars, and fall data are shown as solid black bars. Data were collected from open-ended responses; a negative change may indicate loss or failure to provide that category answer.

notebooks, students wrote an overall project conclusion in which they summarized all assay results, presented a well-constructed argument for the identification of their bacterial isolate, and proposed a hypothesis for the role that their identified bacterial species may play in the leech based on a literature review of the identified species.

Results

Course-specific content

Three questions gauged the depth of student understanding on concepts related to the research project. The first question asked students to describe a BLAST search (Figure 1A), the second asked students to explain the concept of the microbiome (Figure 1B), and the third asked students to explain symbiotic relationships (Figure 1C). There was a significant increase in the level of understanding reflected in students' descriptions of all three concepts (Table 4).

Skills perception

Students were asked via an open-ended question (Question 10) to report on their pre- and postcourse experiences with microbiology techniques; as expected, many students listed multiple skills. The pre- to postcourse change in skill theme frequency was determined (Figure

TABLE 4

Pre- and postquestionnaire statistics.

Concept	*t*-statistic	Presemester	Postsemester
BLAST (fall 2019)	$t(73) = 10.40,\ p < 0.0001$	M = 0.78, SD = 0.80	M = 2.09, SD = 0.50
Microbiome (spring and fall 2019)	$t(79) = 9.57, p < 0.0001$ $t(73) = 8.67, p < 0.0001$	M = 0.50, SD = 0.32 M = 0.70, SD = 0.35	M = 1.43, SD = 0.63 M = 1.59, SD = 0.71
Symbiotic relationships (spring and fall 2019)	$t(79) = 21.01, p < 0.0001$ $t(73) = 11.19, p < 0.0001$	M = 0.19, SD = 0.15 M = 0.55, SD = 0.52	M = 2.10, SD = 0.52 M = 1.84, SD = 0.74

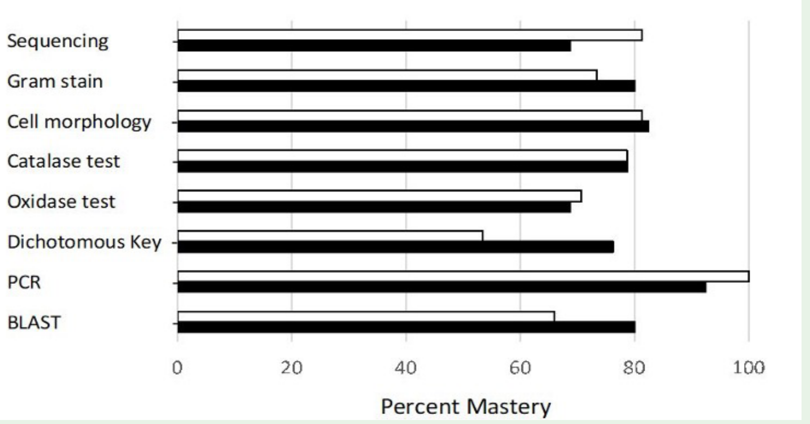
2). For each semester, aseptic technique and culturing were the most commonly listed microbiology skills that students gained from the course. In the fall 2019 semester, there were no skills listed that resulted in a negative change. In the spring 2019 semester, microscopy and basic lab skills were listed fewer times on the postcourse questionnaire.

Skills development

As part of the postsemester questionnaire, students were asked to answer a series of species characterization questions (Online Appendix C), report their 16S rRNA gene sequence, and list the results of each characterization assay. By comparing published characteristics of each identified species, as determined through an independent BLAST search using the student's reported 16S rRNA gene sequence, we sought to verify each student's ability to perform and interpret each characterization assay. Students correctly carried out and interpreted the majority of the characterization assays. Overall, students were most successful in mastering PCR (92.5% and 100% of students in spring and fall 2019, respectively, successfully obtained a PCR product of the expected size) and distinguishing cell morphology (82.5% and 81.3% for spring and fall, respectively). Students struggled the most with using dichotomous keys: 76.3% (spring 2019) and 53.3% (fall 2019) of students either misread the keys or failed to complete all the assays required to identify their cultures using these keys (Figure 3).

FIGURE 3

Percentage of students demonstrating mastery of microbiology skills by the completion of the CURE in spring and fall.



Note. Spring percentages are represented with white bars, and fall percentages with solid black bars.

Discussion

We observed improvements in students' understanding of concepts related to the research project, such as definitions of microbiomes and the BLAST search. Although the improvement observed in the description of the BLAST search is not unexpected due to students' hands-on experience with running BLAST searches of their personally generated DNA sequence data, the increased understanding of the microbiome is more surprising. Participation in the Micro-CURE has had a positive effect on students' abilities to explain microbiomes, even though they have spent limited time directly discussing the concept; anecdotal conversations with participants confirmed the influence of the project on this point. Even though there were improvements in both semesters, however, there were slight differences between the spring 2019 pilot semester and the fall 2019 semester. Students in the fall semester started with a slightly better prior understanding of microbiomes and symbiotic relationships. In terms of skills listed, the differences between spring 2019 and fall 2019 may be due to the timing of when the assessment was given. In spring, the precourse questionnaire was given as a retrospective immediately before students took the postcourse assessment. After listing microscopy and other basic lab skills on the precourse questionnaire, students may have simply failed to mention these skills on the postcourse version.

We wanted objective evidence that students had developed laboratory skills and the ability to reason through, and interpret, the various assays they performed as part of the research project. By having students report the 16S rRNA gene sequence they obtained on their bacterial isolates and the results of each characterization assay, we were able to confirm their results and verify their conclusions. The agreement of student-generated characterizations to literature-reported values for each species putatively identified through 16S rRNA gene sequencing varied across multiple characteristics. However, the assays were unknown to these students prior to the start of the course, and the interpretation of these characteristics can be subject to great variability even among professional researchers. Cell shape, for example, is not as simple as coccus or bacillus but is rather a continuum in which such intermediate shapes as coccoid, coccobacillus, vibrioid, and other such descriptions commonly appear in the published literature (Caccamo & Brun, 2018). Other parameters, including Gram status, are not always as clear-cut as might be expected, with Gram-variable cultures commonly reported in the literature, as well as cultures that appear Gram-positive in early growth phases but Gram-negative as they age (Beveridge, 1990). It must also be acknowledged that some of the assay failures may not have been due to student error; for example, although "universal" primers were used for PCR, it is very likely that the 16S rRNA gene sequences of some bacterial isolates were divergent enough that the primers failed to anneal (Marchesi et al., 1998). Likewise, there exists the possibility that some bacterial isolates were distinct species that, although closely related to the reference species included in the nucleotide databases, may differ in specific characteristics reported in the literature (e.g., oxidase and catalase status). Even given these shortcomings, students demonstrated a high degree of mastery after having worked on the research project; overall, student values agreed with literature values more often than not.

While providing many of the same benefits as traditional undergraduate research experiences, CUREs are scalable and make research experiences available to a larger and more diverse group of students (Bangera & Brownell, 2014). By engaging in CUREs, students gain content knowledge in an active process of discovery that helps them develop discipline-specific technical skills and allows them to experience the process of science as researchers (Bakshi et al., 2019; Bauer & Bennett, 2003; Dolan, 2017; Gasper & Gardner, 2013). These experiences not only result in cognitive gains but also help students better explore career options and increase their intentions to pursue careers in STEM fields (Brownell et al., 2012; Rodenbusch et al., 2016). We undertook this study to determine if participation in the Micro-CURE project influenced students' understanding of course-specific content knowledge and their perceptions and mastery of laboratory skills. We observed strong improvements in all three areas.

Our results demonstrate that students effectively learned discipline-specific technical skills and exhibited improved content knowledge after participating in a microbiology-based CURE. By adding to the overwhelming evidence that CUREs benefit students through active engagement in the process of discovery, we encourage universities to consider adopting CUREs as part of majors curricula. By adding our example to the growing list of STEM-based CUREs, we hope to stimulate exploration into additional projects that match with faculty interests and abilities as a means of engaging and inspiring the next generation of STEM researchers.

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