

Integrating CUREs in Ongoing Research: Undergraduates as Active Participants in the Discovery of Biodegrading Thermophiles

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Research-based courses are a powerful way to engage undergraduates in the scientific process while simultaneously teaching participants relevant laboratory, analysis, and scientific communication skills. In most programs, students conduct a simulated project which effectively improves student conceptions of scientific thinking but does not produce research-quality data. The course described here delivered an authentic research experience by assigning undergraduates an objective from an active grant-funded project. Participants contributed to research aimed at culturing biodegrading thermophiles from hot springs in Yellowstone National Park. Students participated in a backcountry field experience, collecting environmental samples of their choosing and determining appropriate culturing conditions. Following high-temperature incubations, 16S rRNA gene sequencing identified enriched microbial populations, with analytical and microscopy methods tracking degradation and growth. Importantly, several teams successfully cultivated thermophilic plastic-degrading consortia. Student learning was assessed using several methods, including grade distributions on assignments and statistical comparisons of pre- and posttests. A consistent and, in most cases, statistically significant increase was observed in the students' posttest scores. The grade distribution on summative assessments also suggests that students achieved the desired learning outcomes. Student perceptions of their learning and experience gains were high, with participants reporting improvements in components emphasized in the research activities. Overall, the findings highlight how involving undergraduates in real-world research projects can enhance student interest and ownership of scientific research, along with contributing quality data that inform active studies.

KEYWORDS CURE, ongoing research, undergraduate, field experience, high-throughput sequencing, cultivation, microbiology, microbial community analysis, biodegradation

INTRODUCTION

Many higher-education institutions engage undergraduates in scientific research through undergraduate research experiences (UREs) and course-based undergraduate research experiences (CUREs). These discovery-based programs improve student scientific thinking by actively engaging participants in the scientific process (1–5), expanding their expertise in collaboration, data assessment, and experimental design (2). Additionally, notable learning gains are

seen in students' ability to analyze data and draw conclusions from experiments (2, 6). Many undergraduates also give high ratings to UREs and CUREs, saying the programs increase their interest in pursuing science, technology, engineering, and math (STEM) careers and seeking advanced degrees (7, 8). Though beneficial to undergraduates, research experiences require significant time and effort from instructors (9) and often need outside financial support (3). Historically, research experiences are also only loosely tied to the instructor's research interests (10–13), with few steeped in questions being investigated by the faculty member. This leaves the functional utility of research conducted by URE and CURE students in question.

One solution is to bring these programs into the fold of ongoing scientific research. In this way, the courses would promote student scientific thinking and laboratory abilities while contributing meaningful results to ongoing projects. Due to limited time and resources, it is often unrealistic for faculty to burden graduate students and post-docs with high-risk experimental objectives. Such laboratory personnel are focused on building strong peer-review publication

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records. By giving URE or CURE students the opportunity to work on difficult research questions, they can potentially meet short- to medium-term objectives. This could advance difficult research questions or provide valuable preliminary data for future proposal calls. The programs themselves would thus be more effective and viewed by faculty instructors as a worthy endeavor.

Limited studies exist where CURE students have contributed to an active grant-funded project (14). Work here developed a CURE which engaged STEM undergraduates in research focused on culturing thermophilic microorganisms capable of depolymerizing plastic wastes. Students were challenged to overcome known difficulties in cultivating novel microorganisms. The course fostered interdisciplinary and group collaborations, pairing students across STEM disciplines. The course was also novel in its combined field and laboratory work, with students seeing a multistep project through from sample collection to enrichment and microbial identification. Our goal was to provide students with an understanding of what it means to think like a scientist, along with allowing participants to experience the excitement of scientific discovery. The course also contributed fundamental findings and method development related to the instructors' research program.

Intended audience

Students enroll in this theme-based three-credit-hour course independent of an introductory microbiology or biology class (no prerequisites). It is offered annually through Montana State University's Honors College, which limits enrollment to 12 students. The content is intended for freshman- and sophomore-level undergraduates within a 4-year STEM program and pulls from disciplines including microbiology, chemistry, engineering, environmental sciences, and ecology.

Learning time

Students meet weekly for one 50-min lecture and one 3-h lab. The lecture is taught by a Ph.D.-level instructor and introduces fundamental topics related to thermal biology and laboratory methods. The lab is dedicated to conducting experiments and is instructed by the same faculty member. Each laboratory module begins with a 10- to 15-min introduction, emphasizing important skills and concepts. The research-based laboratory skills develop students' competencies in enrichment cultivation, molecular methods, microbial species identification, aseptic technique, microscopy specimen preparation, and best practices for recording observations (15). Student teams are assigned by the instructor, with participants paired across disciplines and experience levels. Each team is assigned a plastic waste for which they are tasked with enriching biodegrading thermophiles. Student work outside the classroom is staggered, with short reading and writing assignments (<1 h) in the early weeks and a buildup toward the comprehensive

manuscript and presentation. These require ~5 to 10 h for writing, data analysis/display, and presentation framing.

Prerequisite student knowledge

Most participants will have had high school biology and perhaps a university-level laboratory course. A college-level general microbiology course is encouraged, though not required. No prior laboratory or fieldwork experience is needed. Because most students do not have a fundamental understanding of the scientific method or aseptic technique, meaningful up-front instructional time is dedicated to laboratory demonstrations.

Learning objectives

The learning objectives for this course are as follows:

1. Explain how and why microorganisms are important in thermal systems.
2. Demonstrate the ability to apply the scientific process.
3. Understand how microbiological and molecular techniques are used to study environmental microorganisms.
4. Communicate the results and implications of a research study to scientific audiences.
5. Develop effective teamwork and communication skills.
6. Keep a detailed record of scientific findings.
7. Read and interpret primary literature information.

PROCEDURE

Materials

Target waste materials were chosen by identifying recycling streams which would benefit from thermophilic biodegradation. Such materials include polyolefins (PO), natural rubber, and polycarbonates, with many available from vendors like Goodfellow USA (Coraopolis, PA). The cultivation approach used liquid microbiological media, with students selecting published medium recipes or using filtered hot spring site water. Participants can test various headspaces, creating either aerobic, anaerobic, or low-oxygen conditions. For anaerobic or low-oxygen headspaces, medium was dispensed under N₂ gas, with oxygen added to desired concentrations. A detailed list of all required materials, including reagents, supplies, equipment, and example media recipes (16–20) is provided in Appendix I in the supplemental material.

Student instructions

Module 1 (blue in Fig. 1; also see Table 1). During the first 3 weeks, students are assigned to a research team and introduced to the project background and experimental

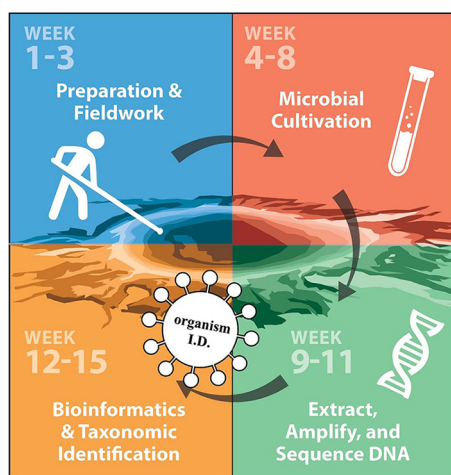


FIG 1. Workflow and timeline of the CURE course. Course segments are color coded by module. The week-by-week activities are described in Table 1.

objective. Our research focuses on cultivating thermophiles capable of degrading recalcitrant materials. Over the last several offerings, students were tasked with cultivating Yellowstone National Park (YNP) thermophiles capable of depolymerizing plastics. Initial laboratory sessions introduce students to the primary literature and how to analyze journal articles. Teams develop an enrichment strategy based on their literature search and using other novel culturing techniques of their choosing (Appendix 2). They determine the material's glass transition temperature, which helps establish hot spring sites to target for sample collection. Initial sessions also cover relevant safety training. In week 3, students participate in the YNP backcountry sampling trip and collect microbiological samples.

Module 2 (pink). Students prepare microbiological media (Appendix 3). Teams are allotted 50 culture tubes and must prepare media for transfers and abiotic/biotic controls. The microbiological media can be prepared under various headspaces, which necessitates benchtop anaerobic gas lines and degassing stations. Enrichment cultures are established in triplicate by inoculating 9 ml of sterilized medium with 1 ml of environmental inoculum (10% [vol/vol]) and incubating at elevated temperatures (40 to 90°C) (Appendix 4). An initial measurement of the material weight or medium headspace composition is made and used to track biodegradation activity. Hot-spring inoculum and culture samples are also collected and preserved for microbial community analysis and initial cell densities. The enrichments are incubated for 2 weeks, after which a 10% (vol/vol) sample of culture is transferred to fresh medium and incubated for an additional 2 weeks. During incubations, laboratory work focuses on measuring the geochemistry of collected field samples (Appendix 5), measuring cell densities (Appendix 6), and tracking biodegradation activity.

Module 3 (green). Groups extract DNA from each transferred culture (including the controls) (Appendix 7), amplify the V3V4 region of the 16S rRNA gene via PCR, and

interpret gel electrophoresis results. Successful amplicon products are purified and quantified (Appendix 8) and given to the instructor for barcoding and sequencing on an in-house Illumina MiSeq platform. In the four semesters of teaching this CURE, each student team has detected amplified DNA in a subset of enrichment cultures.

Module 4 (yellow). Students quality trim and refine their sequences using the mothur pipeline following standard operating procedures (SOP) (21) (Appendixes 9 and 10). Using computer coding in a command-line interface, they assemble overlapping contigs and identify high-quality sequences. Duplicates are removed, and unique sequences are aligned to the SILVA 16S rRNA reference. Poorly aligned sequences and chimeras are removed. Sequences present at less than 1.0% abundance are removed, with the remainder binned into operational taxonomic units (OTUs). Students identify representative OTUs in GenBank using BLASTn (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) (Appendix 11). Genus-level matches are determined using the percent identity, query coverage, E value, and environmental source data sets.

Faculty instructions

Module 1 (blue in Fig. 1; also see Table 1). Prior to the beginning of the course, instructors must make the following preparations.

1. Reserve pertinent camping equipment, campsites, transportation, and horse-packing crews. Make reservations in advance (conditional on the reservation type). Depending on the project and location, research permits may be required, necessitating coordination with government agencies.
2. Ask students to bring a sleeping bag, backpack, and headlamp with them to campus. Provide students a list of required supplies and state that the instructor can rent or borrow equipment the students do not possess. Also obtain emergency contact information, phone number, dietary restrictions, and medical conditions for each participant and encourage students to practice hiking to prepare for the 30 to 40 miles of trekking during the field sampling. Inform students that the field experience is optional and that there is no penalty if they do not participate. Ask students with disabilities to contact the instructor privately to discuss access needs and establish necessary accommodations. Due to this project's remote field location, equitable hiking and camping accommodations may not always be possible; however, the instructor should make every attempt to offer an alternative field experience for disabled participants. Satellite technology could also be used to help on-campus participants interact live with the field team.
3. Develop a schedule for staff participating in the field sampling. Staff are needed to drive and hike with

TABLE I
CURE design and preparation instructions

Week	Topic	In-Class Activity	Assignments & Reminders	Skills/Methods	Instructor Prep Notes
1	Intro. to research question; discuss cultivation options & field sampling	Class decoding of project significance, research questions, & exp. design	Complete content pre-assessment and pre-course CURE surveys		Prepare experimental design guide
2	Field safety training & trip preparation; finalize expt. design	Interactive group discussion of exp. Design matrix; bear and backcountry safety training; trip discussion	Geothermal safety training due; informal scientific literature search due; gear drop off	1. How to read the primary scientific literature 2. Develop a hypothesis	Reserve student camping equipment
3	Multi-day backcountry sampling trip in YNP	Research teams select hot spring sampling sites; cultivation and geochemical sample collection	Reminder about meeting location & food/gear requirements	1. Field Safety 2. Sample Collection 3. Group Collaboration	Prepare field sampling equipment and consumables
4	Microbiological media preparation	Prepare anaerobic/aerobic media for microbial enrichments	Informal reading assignment; Complete online BSL2 safety training	1. Safely work with pressure/puncture hazards 2. Use aseptic techniques 3. Use a microbalance	Prepare target materials and distribute into sterile hungate tubes; degas anaerobic water
5	Enrichment inoculation & incubation	Inoculate media; collect baseline microbial community sample & initial pH measurements	Research article response due (#1)	1. Label specimens 2. Maintain a lab notebook	Prepare anaerobic stock solutions
6	Aqueous geochemical analyses	Prepare & perform aqueous chemical analyses	Informal reading assignment	1. Safely work with chemical hazards 2. Prepare a dilution series	Reserve equipment; submit samples to core facilities
7	Enrichment transfer & activity measurement	Culture observations; transfer enrichments; measure pH change	Research article response due (#2)	1. Examine microbial growth and activity	
8	Microbial enumeration	Prepare promising enrichments for microscopic evaluation	Lab notebook collection (#1)	1. Sterilify filter microbes for enumeration	Autoclave glass chimneys; aliquot staining reagents
9	DNA extraction	Perform DNA extraction on enrichments	Research article response due (#3)	1. Use micropipettes and centrifuges 2. Extract genomic DNA	Aliquot DNA extraction reagents
10	PCR & gel electrophoresis	Understand key components of PCR & perform a PCR setup	Group draft manuscript due	1. Set up a PCR reaction	Aliquot PCR reagents; reserve laminar flow hood
11	PCR purification/quantification; high-throughput sequencing	Interpret gel electrophoresis results; purify & quantify positive samples	Informal reading assignment	1. Interpret electrophoresis gels 2. Calculate & prepare working solutions	Aliquot purification and Qbit reagents; conduct Illumina sequencing
12-13	Bioinformatics & sequence identification	Implement command-line code to trim and refine sequences; Use BLASTn to compare sequences and identify closely related microorganisms	Install and initialize remote access software; Research article response due (#4)	1. Learn to use computer coding to operate open-source mothur software 2. Interpret BLAST results	Generate student accounts for remote desktop connection; create folder of input files and programs for each student
14-15	Scientific communication	Interactive team meetings for evaluating raw datasets and developing manuscript figures and tables; group discussion of data interpretation	Individual final manuscript due; Group oral presentation; Complete content post-assessment and post-course CURE surveys	1. Analyze, interpret, and display datasets 2. Develop technical writing skills 3. Build oral communication skills	Identify a panel of faculty members, research staff, and graduate students willing to evaluate student presentations

students from campus to the backcountry site (and vice versa). Advanced preparation is needed to coordinate meal planning and camping arrangements.

- Order necessary consumables; ensure that the equipment is suitable for use and that procedures are optimized in advance.
- Select 10 to 12 scientific articles relevant to the course content. During initial sessions, students also benefit from instructor-provided articles related to their biodegradation pathway. These seed papers can help guide student literature searches.

Because student teams are heavily involved in the experimental design, we recommend implementing an in-class activity which helps students consider appropriate enrichment matrix variables (Appendix 2).

Module 2 (pink). Instructors must prepare autoclaved Hungate tubes containing a piece(s) of sterilized plastic material. Hungate tubes are prepared and sealed in a biosafety cabinet and maintained at room temperature. Teams may need autoclaved degassed water for preparing anaerobic or low-oxygen microbiological media. Initiate degassing several hours before class, to allow adequate cooling. Students using hot-spring water as their basal medium may request additional supplemental nutrients. We suggest preparing anaerobic stock solutions of each constituent (Appendix 1) so that it can be added to any medium, regardless of headspace.

During microbial enumeration, samples are filtered onto black polycarbonate membranes (GE Healthcare, Chicago, IL) and stained for 15 min with 25× Sybr Gold (Thermo Fisher Scientific). Filters are covered in a mountant and imaged by the instructor on an epifluorescence microscope. We recommend

limiting the number of samples students filter and view. Teams can select promising cultures based on turbidity or signs of activity; 12 filters can be reasonably prepared in a 3-h lab period.

Module 3 (green). For DNA extraction and marker gene amplification, instructors aliquot reagents to reduce cross-contamination. These include DNA extraction, PCR reagents (Appendix 1) (bovine serum albumin [BSA; NEB]; KAPA HIFI HotStart ReadyMix [Kapa Biosystems, Inc.], and 515F-Modified/806R-Modified primers [22, 23] with Illumina overhang sequences for multiplexing), PCR cleanup reagents, and Qubit components. Following DNA extraction, the instructor cleans, concentrates, and quantifies the DNA with a fluorometer. Concentrations of recovered DNA will vary; however, in general, there is enough DNA for PCR. Students will need access to a biosafety cabinet and thermocycler for PCR preparation. Due to time constraints, the instructor conducts gel electrophoresis. Students are provided a gel image for analysis. Following gel interpretation, students conduct PCR purification and quantification.

Module 4 (yellow). Before the bioinformatics lab, instructors must barcode and sequence the samples using an in-house sequencer or outsource to a sequencing facility. Provide students access to the resulting .fastq files and relevant software/reference files for downstream analysis. Coordinate with information technology personnel to provide students remote access to a high-performance computer. We recommend beta-testing the bioinformatic commands in advance. If the mothur pipeline is used, students should follow the SOP until representative OTU sequences are generated. BLASTn can then compare the representatives with cultivated organisms and type strains. Following sequence identification, the instructor meets with student teams to discuss data analysis and data display.

Suggestions for determining student learning

Several evaluation methods assessed each student's ability to apply the scientific process, use quantitative reasoning, and communicate findings (15). The methods included analyzing pre- and posttest scores, assessing lab notebook skills, examining primary literature comprehension, and appraising scientific communication abilities. The students' ability to design experiments, record findings, and interpret and communicate results (15) was measured by assessing grade distributions on several assignments. Students were provided a guide on the importance of keeping a good lab notebook and best practices for recording observations, with an annotated example highlighting strong record-keeping practices (Appendix 12). In the manuscript, students discussed the project's societal relevance, experimental design, and methodology, displayed graphical and tabular data, and interpreted findings (15). Students also provided a path forward for the research, offering specific suggestions for future experiments. A guide on preparing scientific manuscripts was provided (Appendix 13). Finally, student teams orally communicated their findings in a

presentation to a panel of faculty and staff (15). Importantly, the students interacted with these same panelists during the sampling trip and thus hopefully viewed them as members of their STEM community.

Scientific literature comprehension was assessed by assigning weekly articles and prompting students with a low-stakes writing assignment to encourage active reading. Formal writing-to-learn assignments were completed for four research articles, with students writing a 2-page response to a topic question. Rubrics for the literature exercises, draft/final manuscript, and oral presentation are available in Appendix 14. To determine comprehension of lecture content and laboratory modules, pre- and posttests were designed. The assessment consisted of eight lecture questions and seven laboratory concept questions (see questions and answers in Appendix 15), with score comparisons assessing learning gains. Pre- and postcourse surveys with Likert-scale questions were also administered. The survey (24) examined students' perceptions of how their scientific thinking abilities changed (or not) during the course. The study was approved by the Montana State University, IRB (no. DS031021).

Sample data

The cultivability of plastic-degrading thermophiles was generally low, with one or two student teams successfully growing a depolymerizing consortium each semester. Cultivability is dependent on material properties, with features like elevated glass transition temperatures ($>60^{\circ}\text{C}$) resulting in fewer positive enrichments. The cultivability is, however, equivalent to (or greater than) that resulting from successful isolations from other environmental systems (16, 25, 26). Taxonomic diversity and abundance were identified by 16S rRNA gene sequencing, allowing students to compare cultivability across the design matrix. By collecting and freezing inoculum samples, teams also compared baseline microbial communities to enriched consortia, discerning enrichments that showed signs of growth versus those with merely persistent populations. This comparison allows all student teams to successfully sequence and analyze thermophilic community composition, irrespective of significant plastic biodegradation activity. The high-throughput sequencing typically uncovers low microbial diversity, and importantly, many populations are from thermophilic clades. A microbial community data set from a team who successfully cultivated thermophilic PO-degrading consortia is shown in Appendix 16.

Safety issues

The course requires a unique suite of safety training. Prior to field sampling, students review a SOP for working in thermal areas. They are required to wear rubber boots and reflective vests when working in thermal areas, bring adequate amounts of food and water, and wear clothing appropriate for spending long days in unsheltered environments. An on-site SOP review is conducted, so students

remember to work in pairs, recognize unsafe terrain, and minimize inhalation of exsolving volcanic gas. Students also review YNP's rules for traveling in bear country and backcountry camping guidelines. The instructors conduct on-site training on using bear spray and best practices for handling bear encounters. The field work was conducted in Yellowstone National Park under the terms of permits YELL-2016-SCI-5480, YELL-2017-SCI-5480, YELL-2018-SCI-5480, and YELL-2019-SCI-5480.

All laboratory activities are conducted in biosafety level 2 (BSL2)-certified facilities. Standard BSL2 precautions, such as isolate storage and biohazard waste disposal, are observed when handling unknown organisms, and standard laboratory protocols and procedures required for teaching laboratories are strictly followed (27). Before working in the laboratory, students complete an institution-specific online BSL2 biosafety and chemical safety course and become familiar with biosafety guidelines for handling microorganisms (27). Biosafety precautions and BSL2 competency are emphasized throughout the laboratory activities, especially when working with unknown organisms. Students always wear lab coats and nitrile gloves, with protective eye equipment required for handling pressurized materials, hazardous chemicals, or puncture hazards. Students are taught to properly dispose of biohazard, glass, and sharps wastes, along with attending to open flames. None of the thermophiles enriched to date presents a known human health risk, and the risk of encountering BSL2 organisms in thermal environments is low; nonetheless, standard BSL2 precautions are always employed.

DISCUSSION

Field testing

Here, we report pre- and posttests and survey results from our CURE course offered during the fall 2018 and fall 2019 semesters and grade distributions across the 2016–2019 semesters. Because student data is contributing to an active project, the methods often shift between offerings, requiring the instructor to implement new laboratory modules and lecture content. The fluid modules relate to tracking microbial growth and biodegradation activity. The laboratory sessions examining microbial community composition are consistent and have been used in their current form for the past three semesters. The course is offered through the Honors College, which restricts enrollment to honors students and limits the class size to 12. The small size allows students to participate in the backcountry sampling trip, where YNP research permit requirements, campsite size limitations, and travel expenses all necessitate low enrollment. Access to high-tech laboratory equipment and supply costs will likely restrict scalability; however, some modules could work well in larger classrooms. The laboratory setup time is often extensive, notably during modules involving microbiological

medium preparation. Variations in the enrichment media require the instructor to customize available reagents and atmospheric conditions.

A total of 42 students completed the CURE course between 2016–2019, with 23 students completing the assessment and survey activities. Student demographic data are shown in Table S1 in the supplemental material, with the population predominantly comprising freshman-level students majoring in either ecology, engineering, or microbiology. Student diversity is low, with no foreign or minority students in the study population. The lack of diversity is reflective of the overall Honors College, where only 9.5% of undergraduates identify as a racial or ethnic minority and only 1.3% are international (L. Schultz, personal communication). Over the years, student CURE data sets have been leveraged to bolster specific aims within instructor-initiated grant proposals. In addition, several students have continued their CURE research work, transitioning to research positions and generating results for conference presentations and individual grant proposals. Although the CURE-generated results have yet to generate a scientific publication, any such manuscripts would include each annual student cohort participant as a co-author.

Student attitudes and perceptions were assessed using a published survey (24) and compared to national CURE averages. Overall, students self-reported experience gains in numerous elements and, importantly, large gains in components emphasized in scientific research (Fig. S1). Students noted significant experience gains with working on a project with an unknown outcome, contributing to the experimental design, collecting and analyzing data, gathering information from the primary literature, and writing scientific manuscripts (Fig. S1). Elements which are common to undergraduate courses but which were de-emphasized here saw neutral or even negative gains, including listening to lectures and working individually. Like other CURE courses (10, 28), experience gains were equivalent to or, in many instances, higher than the national average (29), though the population size here is significantly smaller.

To assess whether students thought our CURE improved their scientific thinking, communication abilities, and laboratory skills, we also surveyed a variety of learning gains, asking whether course components shifted student abilities from novice to more expert-like. Students reported either large or very large gains on >50% of the 21 surveyed items (Fig. S2), including understanding how scientists work on problems, analyzing data, and understanding the need for accurate data interpretations (Fig. S2). Students reported low gains in their tolerance toward research obstacles, suggesting that our CURE does not allow problem-solving abilities to adequately develop. In addition to engaging in an authentic scientific process, students also noted improvements related to scientific communication, particularly in understanding the literature and communicating findings (Fig. S2). Finally, the fostering of community relationships was evident, where students became part of a learning community and improved their self-confidence in science (Fig. S2).

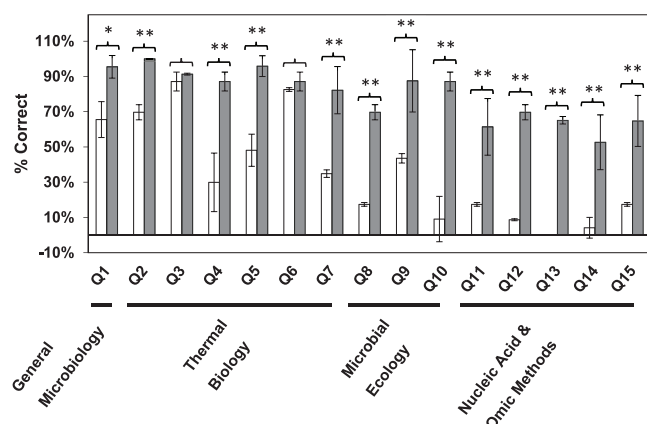


FIG 2. Learning gains evident from student pre- and posttests administered at the start and end of the semester. Students completed a pretest during the first week of class to assess baseline knowledge on the topics of microbiology, ecology, and thermal biology, and well as testing their familiarity with common molecular biology techniques (white bars). The same test was given at the end of the semester (gray bars), and the results highlight students' newfound knowledge, particularly with respect to understanding concepts related to molecular techniques and the types of information that can be learned from DNA-based methods. Single asterisks indicate a statistically significant learning gain ($P < 0.05$) in either 2018 or 2019, while double asterisks indicate a statistically significant learning gain in both years.

Evidence of student learning

The course evaluation strategy measured student learning gains using several methods. The scores of pre- and posttests were compared, providing evidence of gains for learning objectives 1 and 3. The average percent correct was calculated for each question, with a two-tailed proportion test determining statistical significance ($P < 0.05$). The results show that while students were familiar with environmental microbiology, thermal biology, and molecular techniques (Fig. 2), their knowledge was limited. There was a consistent and, in most cases, statistically significant increase in the posttest scores, with students showing full understanding of how and why microorganisms are important in thermal systems (Fig. 2). The most substantial learning gains (all statistically significant) were seen for questions probing understanding of the laboratory techniques (Fig. 2), though a lower proportion of students reached a mastery level. Students understood advantages of the methodological approach but struggled to explain the purpose of marker gene and metagenomic sequencing. Metagenomics is a complex topic, particularly for novice learners. Perhaps additional explanation during lecture sessions is needed. The lower comprehension of marker gene sequencing (questions 13 and 14) is confounding, as both the oral presentation and scientific manuscript required students to interpret 16S rRNA gene sequencing data. It is possible that the collaborative team format failed to promote individual understanding and that certain team members interpreted the concept without explaining their understanding to the group.

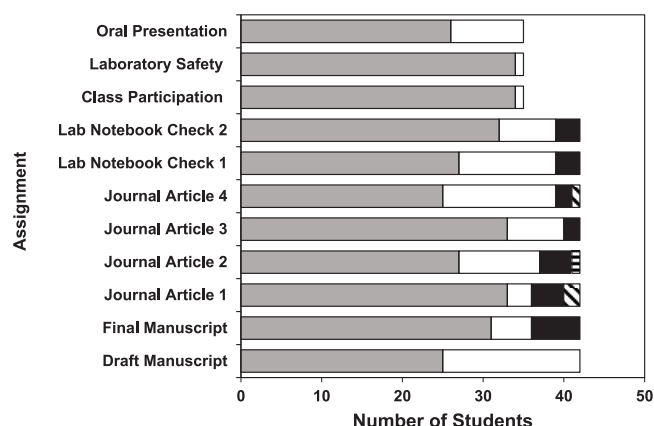


FIG 3. Grade distributions for CURE assignments during the 2016–2019 school years. The following scoring criteria were used: A– to A = 90% to 100% (gray bars); B– to B+ = 80% to 89% (white bars); C– to C+ = 70% to 79% (black bars); D– to D+ = 60% to 69% (horizontally hatched bars); F = <60% (diagonally hatched bars). Note that the oral presentation, laboratory safety, and class participation were not included as grading components in the 2016 course offering.

Students demonstrated their ability to design and implement an experimental approach (learning objective 2) during the initial laboratory sessions. Teams searched the literature to determine appropriate cultivation conditions for growing biodegrading thermophiles and implemented the approach during field sampling and hands-on laboratory sessions. Participants coordinated sampling plans before the backcountry trip, carefully discussing their target thermal sites and desired environmental conditions. Upon return to the lab, students initiated their cultivations during scheduled laboratory sessions. As a performance assessment, the draft manuscript assignment tasked teams with including a schematic of either their cultivation matrix or the experimental process. Teams generally performed well on the draft manuscript (Fig. 3) and often included a detailed schematic (example in Appendix 16). The schematic highlighted students' comprehension of the experimental design and how the methods target the growth of biodegrading thermophiles.

High performance on the group draft manuscript also indicated that students worked collaboratively to communicate their scientific knowledge (learning objectives 4 and 5). The draft manuscript was accompanied later by a group presentation, where student teams communicated the complete project to a panel of professionals. The panelists individually assessed the oral presentations, providing personalized feedback. Student audience members also provided constructive criticism, commenting on the clarity, organization, and technical content. The panelists were generally impressed with the presentations, which is reflected in the grade distribution (Fig. 3). Over the years, the panelists continually comment on the students' strong ability to interpret complex data sets and field questions.

In addition to team assignments, several assessments provided evidence of individual learning. Students maintained

a lab notebook to track discussions, protocols, observations, and analysis of results (learning objective 6). Student performance on the laboratory notebook checks was strong (Fig. 3), with most producing a detailed and organized record. A small student segment failed to compile a detailed notebook (Fig. 3). Most often these students were not “recording as they go,” which resulted in missing, inaccurate, or incomplete information. Along with record-keeping, our CURE increased students’ exposure to the primary literature (learning objective 7). Students completed four article assignments, responding to an instructor-prompted focus topic question. The article assignment grades were typically in the A and B range (Fig. 3). Variable grade distributions perhaps reflect the difficulty of the article content or the focus topic question. Questions targeted different article components, building up from engaging with the experimental approach to determining data significance. Some participants struggled with higher-order cognitive questions that required analyzing and interpreting the results.

The culmination of students’ individual comprehension came from the final scientific manuscript, where participants communicated their research ideas and interpretations. Revised components of the group draft were included in the final manuscript; however, each student individually outlined the results and discussed their significance. The assignment helped students understand the fundamental role writing plays in professional science. Most students received a grade of either A or B (Fig. 3), highlighting their proficiency at creating a written model of their knowledge (learning objective 4).

Possible modifications

While many may wish to implement our CURE, we realize that the methodological approach must relate to the research of each instructor. Instructors may wish to alter the medium preparation, cultivation timeline, methods used to track growth and activity, and target environments. For those without access to an anaerobic gas system, aerobic media can be used. If high cell densities are expected, inexpensive and quick spectrophotometric methods can track growth. In lieu of geothermal systems, environments such as municipal recycling and landfill facilities (26, 30), marine and freshwater habitats (31–33), and wastewaters (34) may serve as easily accessible sampling sites that are known to harbor plastic-degrading bacteria and fungi. The ubiquity of these environments suggests that the activity could be implemented at other institutions and would likely entail fewer resources, fewer site access issues, and lower overhead costs. Involving students in the field sampling process may be difficult, though we find that their participation is highly worthwhile. It is a phenomenal team-building experience, helping students build their research community and instilling the fun and challenge of working in environmental systems. The field sampling also helps contextualize the study, allowing participants to understand how the environmental setting connects to

the research objective. If field sampling is not an option, instructors should consider “virtual” samplings or live interactions with scientists using cellular or satellite technology. Finally, though this course targets lower-division undergraduates, upper-division undergraduates and graduate students may also benefit from the content and laboratory techniques. Instructors can accommodate these senior groups by increasing the complexity of reading assignments, lecture content, and laboratory modules.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, DOCX file, 8.2 MB.

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We have no conflicts of interest to declare.

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