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A CURE for Physiological Characterization of Bacterioplankton in Liquid Culture

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Bacterial characterization is an important aspect of microbiology that includes experimentally determining growth rates, environmental conditions conducive to growth, and the types of energy sources microorganisms can use. Researchers use this information to help understand and predict an organism's ecological distribution and environmental functions. Microbiology students generally conduct bacterial characterization experiments in their coursework; however, they are frequently restricted to model organisms without ecological relevance and already well-studied physiologies. We present a course-based undergraduate research experience (CURE) curriculum to involve students in characterization of previously untested, ecologically relevant aquatic free-living bacteria (bacterioplankton) cultures to identify the usable nutrient substrates, as well as the temperature and salinity ranges conducive to growth. Students use these results to connect their organism's physiology to the isolation environment. This curriculum also exposes students to advanced microbiology methods such as flow cytometry for measuring cell concentrations, teaches them to use the programming language R for data plotting, and emphasizes scientific communication through writing, speaking, poster creation/presentation, and social media. This CURE is an attractive introduction to scientific research and was successfully tested with 187 students in three semesters at two different universities. Students generated reproducible growth data for multiple strains across these different deployments, demonstrating the utility of the curriculum for research support.

KEYWORDS CURE, course-based undergraduate research experience, bacterial physiology, bacterioplankton, undergraduate research

INTRODUCTION

Providing undergraduate students with real research opportunities is a key component of enhancing undergraduate STEM education (1–5). However, traditional research positions at colleges and universities are limited in number, are usually highly intensive and require considerable time commitment, and therefore cannot be scaled to accommodate the majority of science majors (5). Course-based Undergraduate Research Experiences (CUREs) provide the opportunity for students to participate in real research under the aegis of the requisite coursework for attaining a degree and can thus reach considerably more students than standard research positions (5). CUREs can be incorporated into any lab-based course and have been shown to result in superior learning outcomes for all students (5–7), as well as improved retention in STEM for underrepresented minority students (8) compared to traditional sections with previously known outcomes, making them a valuable pedagogical option for improving undergraduate STEM education. Here, we describe a CURE curriculum for introductory biology students that involves them in real research to characterize the ubiquitous microbial denizens of aquatic systems, while also teaching advanced data analysis techniques and scientific communication skills.

Aquatic systems host robust free-living bacterial communities averaging cell densities of 10^6 cells mL^{-1} (9). Due to their vast numbers, these bacterioplankton strongly influence their surrounding environments, making them important elements in a system's ecology. The isolation of bacterioplankton from their natural environment into pure culture allows for physiological characterization of these organisms—an important experimental facet of environmental microbiology that links physiology to ecology through growth characteristics and metabolic capabilities (10, 11). Such experiments

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occur in other published curricula (12, 13) that frequently use common model laboratory isolates such as *Escherichia coli*, *Pseudomonas fluorescens*, or *Beneckea natriegens* (14–16). These organisms are simple to work with due to their short growth cycles, high cell density, and wealth of data with which to compare results. However, their use in a classroom laboratory setting does not provide students with the opportunity to generate new results or interact with microorganisms with wider environmental relevance.

In this CURE curriculum, students work to characterize growth ranges and optima for salinity and temperature, as well as testing a range of possible growth compounds, for bacterioplankton isolates that are not yet characterized. The novelty of this curriculum is that, in addition to students generating new results for undescribed bacterioplankton in liquid culture, students are exposed to some of the most up-to-date techniques in the field, such as flow cytometry to track bacterial growth, data analysis using the programming language R (and an Integrated Development Environment [IDE]; RStudio) (http://www.R-project.org/; http://www.rstudio.com/), and scientific communication via oral, written, and social media. This CURE curriculum can be adapted to match the flexible framework indicated in reference 17 and can also be utilized in series with other published courses on high throughput dilution-based isolation of bacteria (18) or the genomic characterization of bacterial isolates (19).

Intended audience

The CURE curriculum teaches students the necessary skills to use modern cultivation techniques for the characterization of BSL1 aquatic bacterial isolates in liquid culture. The intended audience for this course is first- or second-year college students majoring in a STEM field.

Learning time

The curriculum is divided into 7 parts and can be completed in 12 weeks with one 3-h lab period per week, but this may vary depending on the growth rates of the organisms being characterized. We recommend this project be completed in an ~15-week semester format to allow for break weeks and flexibility in scheduling. Table 1 contains the curriculum schedule without breaks.

Prerequisite student knowledge

All necessary training for students is included in the curriculum, so no prerequisites are required. However, we recommend that students have taken high school biology and chemistry.

Learning objectives

Upon completion of this course, students will be able to:

1. Find, read, and interpret relevant primary scientific literature.
2. Use sterile technique for proper handling of BSL1 bacterial isolates in liquid media.
3. Determine physiological traits of aquatic bacterioplankton.
4. Complete basic computer scripting with R to plot growth data.
5. Link research results to publicly available ecological and environmental data.
6. Communicate research methods and results to scientific and nonscientific audiences using posters, writing, and social media.

PROCEDURE

Materials

Cryostocks or live cultures needed for this curriculum can be obtained from a number of public culture collections, such as the American Type Culture Collection (ATCC). Pertinent cultures are also available from the Louisiana State University Culture Collection (LSUCC) and University of Southern California Culture Collection (US3C) housed in the Thrash Lab at the University of Southern California. To obtain cultures, please contact the corresponding author (JC Thrash). The artificial seawater medium used throughout the project has been previously published (20), and the recipe is detailed in Appendix 1 in the supplemental material. An order list for the course is found in Appendix 2, and many of the items would stock the course for multiple semesters. All media creation, experimental setup, and culture handling should be done in a biosafety cabinet or laminar flow hood to avoid contamination. Cell density is measured using flow cytometry.

Student instructions

The major parts of the semester and their corresponding week(s) and goal(s) are shown in Table 1. This schedule and description of lab activities and assignments can be used in conjunction with the detailed instructions for students reported in the student lab manual (Appendix 3).

Faculty instructions

A timeline for instructor lab preparation is found in Appendix 25. Below, we highlight the general activities according to their color-coded categories, as found in Table 1. Note that some experiments overlap, and thus the categories are interleaved in places. Please use Table 1 and Appendix 25 as the primary guides. The instructor organizes a poster symposium to highlight the students' work to take place at the end of the course. The planning includes reserving a space, arranging printing services, setting up

TABLE 1
Course schedule without break weeks

Week	Topic	Quiz Topic	In-Class Activity	Assignments	Supporting Documents	Due
1	Introduction	Syllabus	Pipette practice	Informal writing 1 Social media assignment	Informal writing 1 Social media assignment	
2	Nutrient Stocks		Create nutrient stocks used for minimal media experiment	Nutrient presentation	Media sheet Nutrient presentation	Informal writing 1
3	Minimal media plate #1	Pipettes and Nutrients	Set up and inoculate minimal media plate #1, science communication example	Elevator Pitch Writing 1	Media sheet, Elevator pitch assignment Writing1	
4	Temperature		Temperature inoculation	Writing 2	Flow cytometry parameters, media sheet	Nutrient presentations Writing 1
5	Minimal media plate #2	Temperature	Transfer minimal media plate #1 to plate #2, elevator pitch	Homework 1	Flow cytometry parameters, Nutrient stock preparation Homework 1	Elevator pitch
6	Growth curves		Plot temperature growth curves	Homework 2	R code, Homework 2, **Writing 2	Writing 2 Homework 1
7	Minimal media plate #3	Bacterial Growth	Transfer minimal media plate #2 to plate #3 Poster evaluation	Homework 3	Flow cytometry parameters, Nutrient stock preparation, Homework 3	Homework 2, Writing 2
8	Growth rates		Plot temperature growth rates	Homework 4	R code, Homework 4	Homework 3
9	Salinity	Growth rates	Salinity inoculation	Poster, Final writing	Media sheet, Flow cytometry parameters, poster assignment WA#3 assignment	Homework 4
10	Data round-up and poster drafts	Salinity	Review data, poster drafts,		Elevator speech assignment	
11	Exam review and posters		Poster presentation and review	Informal writing 2	Informal writing 2	Final Writing
12	Final exam		Final Exam		Final Exam Example	

tables and poster boards, and providing a participation worksheet (such as Appendix 17) to guide students' interactions as presenters and spectators. Instructors should begin planning the poster symposium at the start of the semester.

Week 1: Introduction to the Course. In preparation for week 1 (dark blue; Table 1), the instructor orders materials 1–2 months before the course begins (Appendix 2) to ensure that all materials arrive in time for the semester. A syllabus, assignments, and readings for the semester are placed on the course website prior to the first day of class if possible or at least 1 week before the class period in which the materials will be discussed. Approximately 1–2 weeks before class begins, the instructor receives or reviews training with dishwashing (Appendix 18), media creation (Appendix 1), sterile technique in a biosafety cabinet, and operation of a flow cytometer (Appendix 19) by growing the organism that will be used for the course. To be better prepared for the social media assignment (Appendix 5), the instructor familiarizes the students with how social media is used for scientific purposes. In class on week 1, the instructor reviews the relevant institutional lab safety rules with students and demonstrates proper pipetting and sterile techniques. Lastly, the instructor assigns an informal writing assignment to gauge student expectations/ideas of the nontraditional course (Appendix 4) and assigns the social media assignment for the course (Appendix 5).

Week 2: Begin Minimal Media Experiment. Before class in week 2 (yellow; Table 1), the instructor picks nutrient sources to test. We recommend using primarily sources that will already be contained in the complete medium (Appendix 1). In class on week 2, the instructor assigns nutrients to students with a maximum of 96 total wells across all students so that the counts can be done in a single 96-well plate for flow cytometry. Instructors scale the number of wells and nutrient sources per student based on the number of students in the course. The instructor supervises students while they create assigned nutrient stocks for their minimal media experiment in class. The instructor also assigns the minimal media presentation in Appendix 6 to students.

Week 3: Continue Minimal Media Experiment. Before class in week 3 (yellow; Table 1), artificial seawater medium (ASM; Appendix 1) is prepared if the students did not complete that task in class in week 2 (Appendix 3). The ASM is prepared without any organic carbon, nitrogen, or sulfur sources other than vitamins (Appendix 1; e.g., JW1, excluding amino acids, miscellaneous carbon and nitrogen [C&N] mix, and fatty acids). In class, to ensure sterile technique is practiced, the instructor supervises the students' work in the biosafety cabinet as they distribute ASM and nutrients into wells and inoculate the minimal media experiment. Afterwards, the instructor provides an example of scientific communication (such as a podcast or TED Talk) and guides the students in a discussion about whether the communication was effective using part 1 of Appendix 7. The instructor then assigns part 2 of Appendix 7 and the first writing assignment (Appendix 8) to students.

Week 4: Temperature Experiment. Before class in week 4 (green; Table 1), the instructor creates additional growth medium for the organism (containing all components) and ensures that all flasks have gone through the dishwashing protocol (Appendix 18) and are filled with 50 mL medium in preparation for the temperature experiment. Lastly, instructors set incubators at least 24 h before class at various temperatures for the upcoming temperature experiment. In week 4's class period, the instructor evaluates student minimal media presentations. Afterwards, the instructor leads a discussion about which temperatures would be ecologically relevant to test based on the organism studied. Students are assigned to temperature experiments so there is replication in the data. The instructor supervises the students' work in the biosafety cabinet as they inoculate the experiments to ensure sterile technique is being practiced. The instructor assigns the second writing protocol (Appendix 9) then obtains a t0 cell count sample from each flask and stores the flasks at discussed temperatures. After class, the instructor then obtains cell counts from the flasks at regular time points (e.g., once per day for organisms with a 7–10-day growth curve). The instructor fixes each sample in 3% glutaraldehyde and counts once at the end of the growth curve. At the end of the growth period, the instructor gathers the data into a comma-separated file (.csv) for students to plot the data in Rstudio.

Week 5: Continue Minimal Media Experiment. Immediately before class in week 5 (yellow; Table 1), the instructor performs cell counts on the minimal media plate 1 and prepares medium without added nutrients as noted above. In class, the instructor supervises the students' work in the biosafety cabinet to ensure sterile technique is practiced as the students transfer cultures from minimal media plate 1 to plate 2. After the transfers, the instructor arranges students into groups in which they present their elevator pitches to each other (Appendix 7). If possible, students are recorded so they can better evaluate their own communication style. After class, the instructor provides instructions on installing the programming language R and Rstudio (Appendix 10). They also provide the temperature growth cell count data and a basic structure of the Growth Curve Graphing Code with annotations of the functionality of each line such as that found in Appendix 21 to use the following week.

Week 6: Bacterial Growth. In class on week 6 (light blue; Table 1), the instructor displays Rstudio with an example code and plots the measured temperature data with students. This should include a line-by-line explanation of what the code does and real-time troubleshooting with students as they follow along. They also assign the second homework of plotting growth curves (Appendix 11).

Week 7: Continue Minimal Media Experiment. Immediately before class on week 7 (yellow; Table 1), the instructor uses flow cytometry to count the minimal media plate 2 and prepares minimal media plate 3 as above. In class week 7, the instructor supervises the students' work in the biosafety cabinet to ensure sterile technique is practiced as they transfer from minimal media plate 2 to plate 3. The instructor leads a discussion on physical or electronic examples of posters for students to evaluate in preparation of their own poster

TABLE 2
Student learning outcomes and their respective assessments

Learning outcome	Assessments
1. Find, read, and interpret relevant primary scientific literature	Presentation 1, Elevator pitch, Final writing (Appendices 6, 7, 14)
2. Use sterile technique for proper handling of bacterial isolates in liquid media	Successful completion of the protocols, Final exam (Appendix 24)
3. Determine and display physiological traits of aquatic bacterioplankton	Successful completion of the protocols, Homework #2, Homework #4 (Appendices 11 and 13)
4. Complete basic computer scripting with R to plot growth data	Successful completion of the protocols, Homework #1, Homework #2, Homework #4, Poster (Appendices 10, 11, 13, 15)
5. Link research results to publicly available ecological and environmental data	Final writing, Poster, Lab Report (Appendices 14, 15, 26, 27)
6. Communicate research methods and results to scientific and nonscientific audiences using posters, writing, and social media	Poster, Elevator pitch, Writing assignments, Presentations, Twitter participation (Appendices 15, 7, 8, 9, 14, 6, 5)

design and assign the third homework assignment of poster critique (Appendix 12).

Week 8: Bacterial Growth Continued. In class on week 8 (light blue; Table 1), the instructor guides students to calculate growth rates from the temperature experiment and review the basic structure of the Growth Rate Graphing Code using annotations for each line of code in Appendix 22. The instructor displays Rstudio with an example code and plots the provided data with students as previously described under week 6. They also assign the fourth homework assignment of plotting growth rates (Appendix 13).

Week 9: Salinity experiment. Before class on week 9 (purple; Table 1), media of varying ecologically relevant salinities to the organism being studied are created. The instructor assigns students to salinity experiments so that there is replication in the tested conditions. Our media is extremely adaptable—we used salinities 34.8, 23.2, 11.6, and 5.8 corresponding to medium recipes MWH 1–4 (or JW1–4) (Appendix 1), respectively, for isolates from the Gulf of Mexico. Ahead of class, the 125 mL cleaned and autoclaved flasks should be filled with 50 mL of the different media types to match the number of replicates per condition. In class during week 9, the instructor supervises the students' work in the biosafety cabinet to ensure sterile technique is being observed as the students inoculate the salinity experiment. Once the experiment has been inoculated, the instructor immediately obtains a t0 cell count, then again at regular time points matching that of the corresponding temperature experiment in week 4. At the end of the growth period, the instructor gathers the data into a comma-separated file (.csv) for students to plot in RStudio. The instructor assigns the final writing and poster assignments (Appendices 14 and 15) to students.

Weeks 10–12: Data Communication and Semester Wrap-Up. Before class on week 10 (gray corresponding to weeks 10 to 12; Table 1), the instructor provides students the salinity growth data and assigns them to plot growth and calculate growth rates. The instructor also gathers a list of student generated data that includes all tables, growth curves, and growth rate plots. In class week 10, the instructor allows students the class time to make and receive feedback on poster outlines and figures. In week 11, the instructor evaluates student poster presentations in class using a rubric such as the one in Appendix 15. Note: Although a physical poster presentation symposium would be ideal, in practice we did not print student posters for in-class presentations and instead displayed posters on a projector. Ideally, students focus their presentation on the discussion and future direction sections of their posters since they generally have the same experimental data with varying interpretations and connections to larger literature. After presentations, the instructor allows questions for a final exam review and gives students time for informal writing 2 (Appendix 16). Before class on week 12, the instructor prepares the final exam and optional practical stations (see example final in Appendix 24), then proctors the final exam in week 12. The poster symposium should happen after the final exam week and after students are given a chance to edit and print their posters postfeedback from presentations. In our case, multiple types of CURE courses joined together in a symposium in which 2–3 posters from each section were chosen to print and present while other students attended to give and receive presentation feedback.

Suggestions for determining student learning

Student learning can be determined through both traditional methods such as in-class quizzes (Appendix 23) and a final exam (Appendix 24), but also through communication-based assessments of learning that were highlighted and included in informal and final writings (Appendices 4, 14, 16), presentations (Appendices 6, 7, 15), a final lab report (Appendices 26–27), which can be used in conjunction with or in place of a final exam, and a final poster (Appendix 15). Relationships of assessments and course learning objectives can be found in Table 2, and answer keys and rubrics are

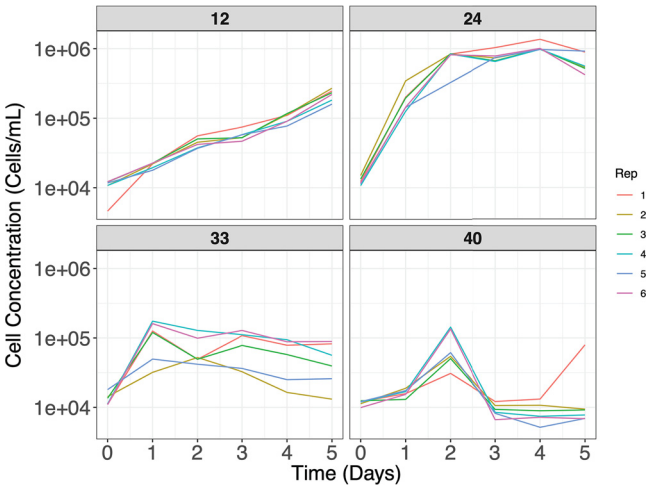

FIG 1. Growth data for strain LSUCC0135 at different temperatures, indicated in the strip above each plot in °C. Cell concentration is plotted against time and each replicate has a different color.

provided in the appendices. There are multiple sets of quizzes, broken down by semester. There were five postlab quizzes in 2018 that tested students on material covered in the previous lab section. In 2020 and 2021, there was a mix of 10 pre- and postlab quizzes (Appendices 26–27).

Sample data

Some combination of the following elements: (i) minimal media table, (ii) temperature growth curves/rates, and (iii) salinity growth curves/rates were produced for five isolates: LSUCC0112, LSUCC0117, LSUCC0135, LSUCC0713, and US3C007 (Appendix 28). All isolates remained axenic except LSUCC0112, which became contaminated sometime throughout the semester (see Discussion). Although the students had never worked with bacterial cultures prior to this course, the growth curve data exhibited good reproducibility evidenced by the overlapping growth data that was produced by different students and different sections. Example results for each data type can be found in Fig. 1 and Appendix 28. For instance, LSUCC0135 had a growth temperature range of 12°C–40°C with an optimum near 24°C. Its salinity range was 5.8–23.2, and the optimum salinity was undetermined but somewhere between 5.8 and 11.6. LSUCC0135 could grow on all carbon sources after two transfers. LSUCC0117 had a temperature range of 12°C–33°C with an optimum at 24°C. Its salinity range was 5.8–34.8 and the optimum was 11.6. LSUCC0117 could use the following carbon sources: leucine, lysine, methionine, glutamate, succinate, sucrose, serine, and folic acid (Appendix 28).

Safety issues

There are no biological safety issues in this laboratory if BSL1 strains are selected for investigation. If completely unknown strains are used, BSL2 protocols should be followed according to the *JMBE* Biosafety Guidelines for Handling Microorganisms in the Teaching Laboratory (21), which would also require that students be proficient in handling BSL1 strains first. Faculty should be careful with the use of glass and diluted acid for dishwashing protocols and glutaraldehyde if used for cell fixation.

DISCUSSION

Field testing

We deployed this CURE during the fall 2018 semester at Louisiana State University with three graduate teaching instructors for six sections, totaling 147 students. The two sections that characterized LSUCC0135 were in Biology 1207 Honors: Biology Laboratory for Science Majors and had 46 students, while the other four sections working with LSUCC0112 and LSUCC0117 were in 1208 Biology Laboratory for Science Majors and had 101 students in total. We also deployed this course at the University of Southern California during spring 2020 and fall 2021 semesters of BISC 221 and 121 Advanced General Biology, in two sections each that had 18 and 22 students enrolled total, respectively. spring 2020 students characterized LSUCC0713 and fall 2021 students characterized US3C007. Thus, the curriculum has been utilized with a total of 187 students across 10 sections of two different biology courses at two universities.

Data produced by these deployments produced mixed results. Example outcomes are detailed in Appendix 28, which shows growth rate data for multiple strains across the different deployments, and one of the minimal media experiments, which in this deployment was restricted to testing carbon sources only. In some cases, e.g., growth optima data for isolates, minimal media experiment for LSUCC0117, results were very reproducible across students and yielded publishable quality outcomes (Fig. 1, Appendix 28). Alternatively, the sections culturing LSUCC0112 contaminated the culture (detected via post course 16S rRNA gene PCR [20]), and the minimal media experiments for US3C007 failed (no growth in the positive control). Thus, as in all real research, some of the experiments worked, whereas others did not and will need to be repeated. Failed experiments offer just as much, if not more, teaching opportunity since instructors can involve students in determination of whether and how the experiment failed, discuss the value of controls for evaluating experimental success, and contextualize this more broadly in the utility of the scientific method. More importantly, the field testing demonstrated that the curriculum engages students to produce reproducible, publishable data in multiple different settings.

Evidence of student learning

We provide evidence of student learning through multiple means: grade distributions that reflect major assessments and overall course progress (Fig. 2), experimental results from the students showing successful completion of the protocols and execution of R code (Fig. 1, Appendix

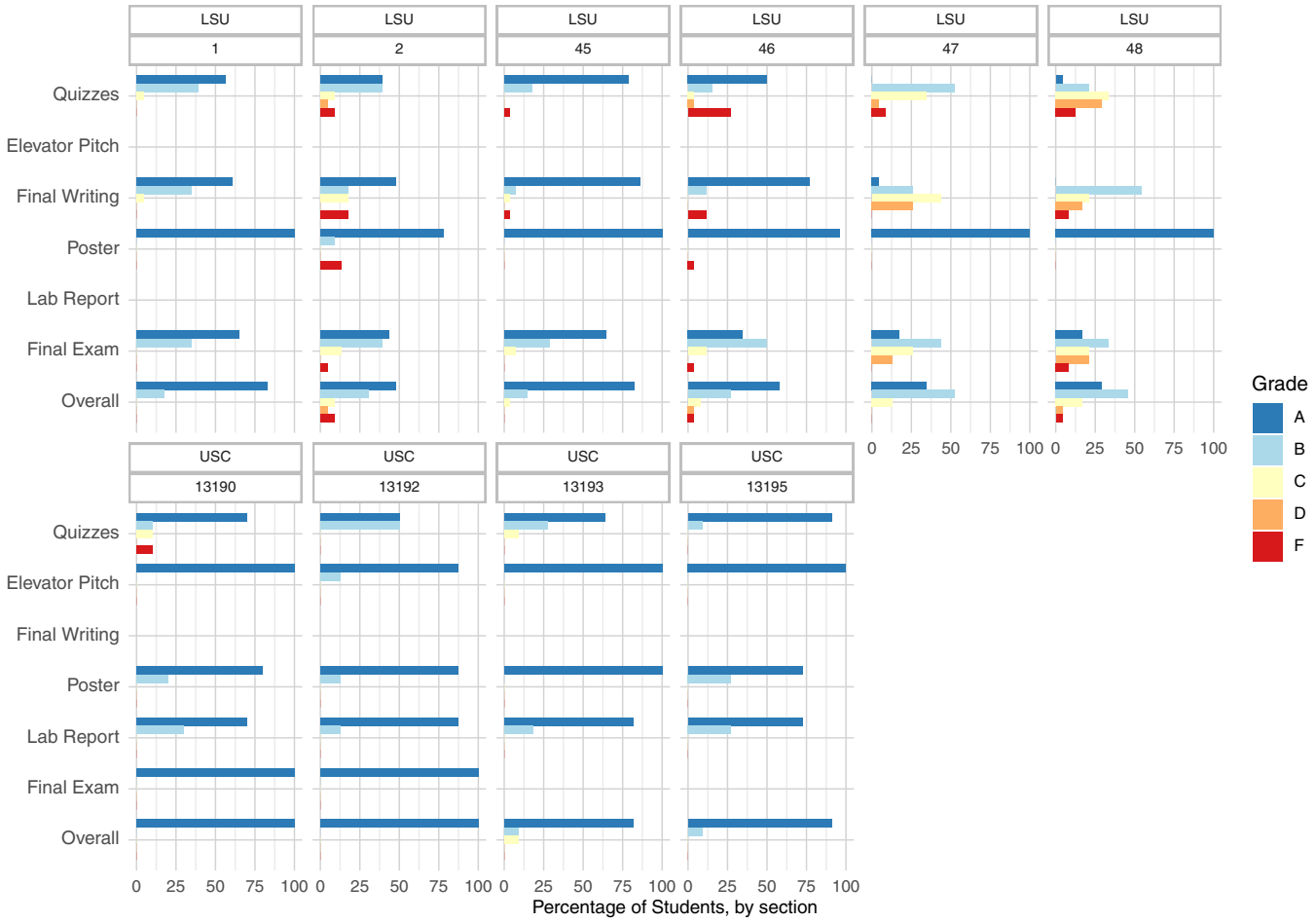

FIG 2. Grade distributions for major assignments and overall course scores. Each section is plotted separately by university and is denoted below the university designation. Grades according to an A, B, C, D, F scale are colored according to the key. The number of students by section were as follows: 1, $n = 23$; 2, $n = 23$; 45, $n = 28$; 46, $n = 26$; 47, $n = 23$; 48, $n = 24$; 13190, $n = 10$; 13192, $n = 8$; 13193, $n = 11$; 13195, $n = 11$.

28), and examples of outcomes from student assignments (Table 3, Appendices 28–30).

The grade distributions across the 10 sections reflect the variations in success of students in multiple different settings. Quizzes at LSU tested the major concepts covered in a previous lab, whereas those at USC were either pre- or postlab quizzes, testing either conceptual preparation for the upcoming lab or knowledge gained in the previous lab, respectively. Most students did very well on these quizzes; most students in the majority of labs had aggregate scores of As or Bs. Scores on the final exams, which were comprehensive tests of the skills learned during the semester, including protocols, calculations, experimental design, fundamentals of microbiology, reading comprehension, and data analysis, also reflected strong student performance in most sections. Grades from the elevator pitch and poster demonstrated that most students did very well learning to communicate their science effectively in an oral or multimedia format. Similarly, students achieved success with written communication of lab findings and contextualization of their research, as evidenced by the strong grades in most sections for the Final Writing (LSU) or Lab Report (USC). Excerpts of writing and associated scoring can be found in Table 3, and a representative poster is provided in Appendix 30. We also provide qualitative examples of student reflections on their learning experience (resulting from informal writing assignments; Appendices 4 and 16) that self-report several skills gained from the course (Appendix 29).

The research outcomes also demonstrate student learning since the successful completion of the protocols yielded reproducible experimental outcomes. In addition, the data visualization depicting the experimental results demonstrated learning because the students had to develop skills in the programming language R to manipulate and execute scripts using real data as input. Examples of these research products, which also contain the underlying results and demonstrate the reproducibility of student work, are in Appendix 28.

Possible modifications

Since the course was deployed at different universities, it has already been tested with a few modifications with regard to activities and assessments (Appendices 26–27). For example, a lab report was added for the USC deployments in

TABLE 3
Examples of student writings

	Excerpts about the cultured organisms from students' final writing
Excellent	"24°C acted as the optimum temp for the organism to survive in, as multiple replicates of [LSUCC0]117 tested in the environment showed exponential growth in a short period of time, as well as a short lag phase prior to experiencing its log phase. This is more than likely due to the fact that 24°C is relatively close to the temp of the Gulf of Mexico throughout a yr. 40°C was too hot for the organism to survive in, so its cells either did not replicate or died due to the temp causing the microbe's enzymes to denature, eventually killing its bodily functions and it in the process." *Mentions features of graphed data; relates physiology to ecology; explains why physiological tolerances have limits; student uses qualifying terms when data is not absolute.*
Good	The temp expt showed that LSUCC0117 can grow in temperatures ranging from 12°C, 24°C, and 33°C. The temp of the Gulf of Mexico is usually around 20°C to 23°C off the coast of Louisiana. It is important that LSUCC0117 can grow in temperatures far below and far above the norm because if the water ever suddenly had a spike or drop in temp the bacteria would still be able to grow and thrive. The salinity expt showed that LSUCC0117 can grow in a large variety of salinities and this is an important characteristic of this organism because it is what allows it to survive right off the coast of louisiana. The salinity of the water is continously changing as it rains and the Mississippi River flows into the Gulf of Mexico." *Briefly, but accurately, describes organism physiology; explicitly connects physiology to ecology; several spelling errors; does not cite source for Gulf of Mexico temp.*
Needs Improvement	"The optimal temp was 24 degrees with 33 being acceptable as well. The salinity levels all proved to be acceptable, but JW3 was the best option. There were a few bacteria wells that died off regardless. This can be blamed on the change in location as some bacteria death can be caused by significant intra- and interannual chemical fluxes, thereby creating 'vintages' from specific sample collections that can prevent reproducible growth or repeated transfers (Henson et al.). The results, nonetheless, supported the hypothesis of the bacteria growing optimally in temperatures and salinity levels that mimic the Gulf of Mexico." *Relates physiology to ecology; accurately references collected data; does not utilizes correct unit symbols; uses an incorrect reference to explain a phenomenon not relevant to the expt.*

exchange for the Final Writing assignment that was done at LSU. The Lab Report was used in conjunction with a Final Exam in spring 2020, but we have since dropped the Final Exam in favor of only using the Lab Report beginning in fall 2021. Fall 2021 also saw implementation of a phytoplankton microscopy lab (Appendix 27) that can add another perspective for students and potentially enrich their experience. Details for all course modifications between the USC and LSU deployments are available in Appendices 26 and 27.

We can envision several other modifications. We have so far only tested the course on a semester schedule, but the curriculum could be adapted to a quarter or a trimester system by combining the temperature and salinity growth experiments into a single lab period, or combining the data round-up lab for the temperature experiment with the salinity inoculation lab. In addition, the time for all experiments is dependent on the doubling time of the culture, so using a strain that completes a growth curve in <7–10 days could shorten the incubation periods for the minimal media experiment.

Another attractive modification would be to teach students how to use the flow cytometer for growth measurements. This could only be done with a smaller class size due to the setup and run times, the expense of the equipment, and the close supervision required for undergraduates. If the institution does not have access to a flow cytometer, cell counts could be conducted with a plate reader, via direct cell counts (microscopic counts), or via viable plate counts if the cells will grow on agar plates. If the institution does not have access to a full biosafety cabinet, a clean laminar flow workstation or a portable PCR hood could work for sterility.

This course could also be modified to involve the students in more of the preparation steps. Each section/instructor could take responsibility for one portion of the characterization (minimal media, temperature, or salinity) so that students could complete such tasks as making media, making stocks, performing cell counts, providing input on experimental design, dishwashing, etc. This would allow for more robust data-sharing and collaboration between students. Lastly, students could be exposed to the bioinformatic side of the workflow in a more comprehensive way by using techniques and surveys highlighted in recent publications (22).

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 5.1 MB.

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