CREARE: A Course-Based Undergraduate Research Experience to Study the Responses of the Endangered Coral Acropora Cervicornis to a Changing Environment†

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There is mounting evidence to support that students who participate in scientific research experiences are more likely to continue on to advanced degrees and careers in science, technology, engineering, and mathematics (STEM). To introduce more students to the benefits of research, we have drawn on an ongoing project aimed at understanding how the Caribbean staghorn coral Acropora cervicornis responds to environmental fluctuations to develop a semester-long course-based undergraduate research experience (CURE), entitled CREARE (Coral Response to Environment Authentic Research Experience). The main mode of instruction in CREARE is through topic modules, and course evaluation is achieved through writing assignments. Students in CREARE perform experiments in the laboratory to measure the abundance of photo-protective proteins in coral tissue from samples collected at different depths and at different times of the year and analyze environmental data using the R programming language. CREARE participants have contributed to the progress of the research project by generating novel data and making improvements to experimental protocols. Furthermore, pre- and post-course assessment of content knowledge revealed that students perform significantly better on a written exam after participating in CREARE, while also displaying appreciable shifts in attitudes toward science in student perception surveys. In addition, through qualitative analysis of focus group interviews, we gathered evidence to suggest that mediating variables that predict students’ persistence in science are bolstered through our application of the CURE modality. Overall, CREARE can serve as a model for developing more research-based courses that successfully engage students in scientific research.

INTRODUCTION

Undergraduate research experiences (UREs) have been prescribed by many as the means to improve student performance and increase the likelihood of a student pursuing advanced degrees and a career in STEM (1–3). The reported impacts attributed to UREs disproportionately benefit students from historically underrepresented groups in science, a segment of the population whose enrollment in STEM programs has been steadily increasing over the past decade but who also abandon STEM degrees at a higher rate than any other group (4, 5). Traditionally, the engagement of undergraduate students in research has been through one-on-one apprenticeships, which are limited in their offering due to a finite amount of research faculty. More recently, higher inclusion of students into research has been achieved through the integration of research into courses through the practice of course-based UREs, or CUREs. This modality provides opportunities to a larger number of students enrolled in a course who subsequently report many of the same benefits associated with research internships, such as increased self-efficacy, enhanced scientific identity, and career clarification (6–9).

CUREs offer ample opportunities for learning and skill development, thereby fostering beliefs in students that they can do well and obtain a positive outcome for engaging in research and therefore continue a career in science (10–11). These benefits are partly explained by self-efficacy theory, which posits that an individual’s beliefs in their capabilities to execute a
course of action and reach a specific goal arise from participation in activities where they view themselves to be efficacious and in which they anticipate positive outcomes (12–14). In addition to research self-efficacy, scientific identity and the endorsement of values associated with the scientific community have been shown to mediate the integration of students into science (3, 4, 15–18). The concatenation of the participant’s sense of belief in her/his capacities with their identification with and internalization of the collective values of a community has been termed the tripartite integration model of social integration (TIMSI) and has been successfully used for describing how students become more engaged with science, particularly students from groups historically underrepresented in STEM fields. According to TIMSI, when a student assumes the identity of a scientist and reports feeling like a scientist, she or he is more likely to pursue a career in science (15). Hence, we set out to develop a CURE where students are presented with opportunities to successfully engage in scientific research and to internalize the values of the scientific community through participation in a research project that is both locally relevant and of global importance.

Global climate change is having a profound and potentially devastating effect on many marine environments, including coral reef ecosystems. Caribbean reef corals, including the once-common scleractinian coral Acropora cervicornis, have suffered unprecedented mortality due to climate change–related stressors, such as increasing sea surface temperature (SST) and solar radiation (SR) (19, 20). Rising SST and increased SR are physiological stressors to marine organisms, which when severe might jeopardize vital functions such as growth, reproduction, and the capacity to fight diseases. Since 2006, A. cervicornis has been listed as a threatened species throughout all of its range (the Caribbean Sea and the Atlantic Ocean near Bahamas and Florida) under the International Union for Conservation of Nature (IUCN) Red List and the U.S. Endangered Species Act (ESA); thus, its recovery has become a major priority for tropical Atlantic nations and conservation groups (21, 22). Corals rely on multiple responses to thermal and irradiation stress, including the increased synthesis of photoprotective pigments such as fluorescent proteins (FPs) and melanin (23–26). Therefore, measuring the abundance of FPs and the activity of enzymes involved in the synthesis of melanin from the tissue of corals growing under different environmental conditions serves as an indirect measurement of the stress endured by individual colonies. Furthermore, correlating proxy measures of stress to long-term environmental monitoring data of SST and SR will provide insights into understanding how A. cervicornis colonies are responding to a changing environment to ultimately help guide future coral propagation and restocking efforts.

In an effort to introduce more undergraduate students at a Hispanic-serving institution (HSI) to the benefits of research experiences, we have drawn upon an ongoing long-term research project aimed at understanding how the Caribbean staghorn coral A. cervicornis responds to seasonal fluctuations of SST and SR to develop a CURE. The semester-long course is entitled CREARE, an acronym for coral response to environment authentic research experience, and also because of the Latin verb creare, meaning “I create, make, produce,” which reflects the intention of having students create new knowledge to address a scientifically relevant question through participation in the course. Here, we describe the consensus design of CREARE after three iterations and share assessment data for one of the three cohorts of students who have participated.

INTENDED AUDIENCE AND PREREQUISITE STUDENT KNOWLEDGE

CREARE is offered as an elective course for students in the College of Natural Sciences who have completed a two-course introductory sequence in Biology, a two-course introductory sequence in Chemistry, and an introductory course in Statistics. Students are expected to have university-level knowledge of biodiversity and its importance, natural selection, the impact of biotic and abiotic factors on populations, structure and function of macromolecules, and enzymes and their activity. Both the introductory biology and chemistry courses at our institution have a laboratory component; therefore, students are also expected to have experience with the application of the scientific method, handling biological specimens and chemical compounds and the proper execution of protocols. In addition, participants are expected to know how to make measurements and observations accurately, analyze and summarize quantitative data using graphs, differentiate between dependent and independent variables and use descriptive statistics such as measures of central tendency. Most of the students who participate in the course are sophomores and juniors with varying levels of exposure to scientific research outside of laboratory courses, from no prior research experience to one full year of research experience.

LEARNING TIME

All students and faculty meet formally once a week for 3 h during a 16-week semester (Table 1). After the first introductory meeting where laboratory safety guidelines are discussed (Appendix 1), students are expected to engage in course-related activities for an additional 3 h per week. These activities include additional time in the laboratory to perform experiments and analyze data, review literature, and/or work on writing assignments. Additional meeting times are coordinated with the instructional staff based on availability.

LEARNING OBJECTIVES

The learning objectives and supporting activities are presented in Fig. 1. Upon completion of CREARE, students are expected to:

1. Describe the relationship between fluctuations in environmental conditions and changes in the physiology of the stony coral A. cervicornis.
2. Develop skills and competencies to record data and analyze results.

3. Demonstrate fluency in data processing and analysis of large data sets and its application to uncover relationships in biological systems.

4. Effectively communicate the results of research to peers and members of the scientific community, through written reports and oral presentations.

5. Report increases in self-efficacy, enhancements in scientific identity, and clarification of career intentions.

**PROCEDURE**

The workflow of experiments from sample collection to data analysis is provided in Fig. 2. Prior to the beginning of the course, members of our research team collected coral samples from coral nurseries located 3 and 12 m of depth during the spring and fall (Appendix 2). The study site is in Punta Soldado, Culebra, Puerto Rico (N18° 16'50.7 W65° 17'14.6) and is managed by the local nonprofit organization Sociedad Ambiente Marino. Collected coral fragments are placed in tubes and frozen in liquid nitrogen immediately upon retrieval, transported to the laboratory, and stored in a −80°C freezer until further analyses. The Department of Natural and Environmental Resources of Puerto Rico approved the sampling protocol under permit number DRNA: 2016-IC-175 issued to CTH. Small groups of students (two to three students per group) are assigned a set of coral fragments collected at a specific date coming from one of two depths (3 and 12 m) for analysis. Students are instructed to extract tissue from frozen coral fragments...
(Appendix 3) and use the extracts to perform biochemical assays to measure the amount of photoprotective fluorescent proteins (FPs) in the extracted tissue (Appendix 4) and also measure the activity of the enzyme phenoloxidase (PO), which is involved in the synthesis of melanin (Appendix 5). In parallel, students perform statistical analysis on environmental data (Appendix 6) that is provided by the instructors. These data are for SST and SR in the collection sites in the months prior to sample collection. Finally, students compare the biochemical parameters between their samples at different depths and seasons and the corresponding environmental conditions. To gain fluency and practice in the techniques required to perform all the analyses described above, students participate in a series of modules.

**Biochemistry module**

Through the biochemistry module (Appendix 7), students were introduced to basic coral physiology and the responses of these organisms to temperature stress and solar radiation. The main focus was on the role of the enzyme PO in the production of melanin (25, 26) and the photoprotective qualities of fluorescent pigments in corals (23, 24). Herein, laboratory rules were discussed and instruction given on the use of basic laboratory equipment and handling of reagents.

**Data analysis module**

The two-part data analysis module (Appendix 8) provided guided practice on the application of statistics to large data sets of environmental data using R: A Language and Environment for Statistical Computing (27). In addition to becoming familiar with the use of R programming language, the module included general discussion on topics of statistical analysis techniques used in population ecology and the integration of mathematical and statistical analysis to biological research. Within R, students learned about environment setup, basic commands, script syntax, uploading and
reading files with different extensions, performing descriptive statistical analyses with small data sets, and data visualizations. This module was initially offered as a single 3-h session. Based on student feedback from the first iteration of the course and perceived difficulties with R, we found it necessary to extend the module to two 3-h session offered in successive weeks.

Science writing module

The science writing module's main objective (Appendix 9) was to develop the tools to communicate the results obtained to stakeholders outside of the classroom. These stakeholders are local coral reef researchers, members of community-based conservation groups, and government agencies. Topics discussed include sentence construction, organization of ideas within a paragraph, scientific wording and the fundamental parts of a research manuscript. The module was centered around an in-class activity where students write a paragraph using given keywords followed by peer-to-peer critique of anonymized paragraphs produced in the initial exercise.

Ecology module

The ecology module (Appendix 10) aimed to introduce students to the basic ecology of coral reefs, reef-building corals, associated organisms, and their importance to humankind. The main focus was on the major stressors, both human-derived and natural, that are currently threatening the existence of coral reef. The module is intended to give students a broader context of the work that they performed throughout the semester. This information is key for subsequent communication of research findings.

STUDENT INSTRUCTIONS

Students are provided access to a folder on a cloud-based sharing service with the required reading material and also instructed to download and install R (Appendix 8). Through the shared folder, students receive experimental protocols and submit all course deliverables. Students work together in small groups (two to three students per group). Collaboration and co-working among the groups, especially for data analysis, are strongly encouraged.

FACULTY INSTRUCTIONS

During the first meeting with students, project objectives were discussed, both in terms of educational outcomes and research objectives. During this first meeting, pre-course surveys and evaluations, later used to measure learning gains and shifts in attitudes toward science, are administered. During the second week, students begin work related to the project through participation in the first of four topical modules. Table 1 presents how topical modules and other supporting activities are distributed throughout the semester. The topical modules are designed to expose students to the underlying theoretical principles of the experimental design and to reinforce foundational concepts that are important for successful execution of experiments and interpretation of data (Fig. 1 and Appendices 7 to 10). Active learning methodologies, such as peer-to-peer learning and guided inquiry, are used in the topical modules to explore, explain, and evaluate topics ranging from overarching principles to specific details relevant to the project (28). Instructors devote at least an additional 6 h per week for collecting samples, preparing laboratory reagents, supervising student experiments, answering student questions, reading and providing feedback to students on writing assignments, and preparing and analyzing data from student assessment. Establishing a defined time period during the week to handle these additional tasks was critical to minimize the burden on instructor's time.

SUGGESTIONS FOR DETERMINING STUDENT LEARNING

Student achievement of the established learning objectives are evaluated mostly through a semester-long process of writing and revision, in which students progressively construct a report modeled after a research article (Table 2). This gradual crafting of a report mirrors the developing engagement of the students in the research project; as students become familiar with the research literature, they write an Introduction; upon performing experiments, they write Materials and Methods; as they gather and interpret data, they write a Results section; and as they interpret and contextualize the results, they write the Discussion. Upon completion of each stage of the project and writing the corresponding section of the report, students receive input and recommendations from instructors on each section (Writing Deliverables in Tables 1 and 2). A grading rubric used in other laboratory courses in our school is used as a formative assessment and evaluation tool (see Appendix 11 and Appendix 12 for examples of student data).

In addition to being used as a means for student evaluation (Table 3), writing assignments and deliverables fulfill the critical objective of improving students’ ability to effectively communicate the results of research to peers, the scientific community, and the public. A second way of assessing student learning is through final oral presentations that are presented to instructors, peers and other researchers on campus. During one of the semesters that the course was offered, a local coral reef symposium was held near our campus. All students enrolled in the course attended the symposium and also submitted an abstract, which was required for evaluation as a writing deliverable during that semester. A group of three students gave an oral presentation to describe the project to all symposium participants (Appendix 13). To ensure that students were supported in the development of their scientific presentation skills and were prepared for the presentation, we provided opportunities to practice in class and offered
extended comments on presentations (Appendix 14). Other coral reef researchers and members of conservation groups were in attendance for this presentation. Although this was a unique scenario, it clearly evidenced that students fulfilled the learning objective of effectively communicating the results of research to peers and members of the scientific community.

SAMPLE DATA

CREARE participants engage in different aspects of the experimental workflow including tissue extraction, estimation of protein concentrations and enzyme assays. Participants also analyze environmental data, as well as use statistical analysis to interpret the results from biochemical experiments (Fig. 2). Fig. 3 and Appendices 12 and 13 show sample data generated by students and used in written deliverables and presentations.

SAFETY CONCERNS

Students received instruction on lab safety on the first day of the course (Appendix 1) and were required to complete two online courses prior to beginning work in the laboratory; a six-part “Online Ethics Research Course” from the Office of Research Integrity and a laboratory safety course from the campus Office for Environmental Protection and Occupational Safety. Furthermore, students are instructed about the proper use of laboratory equipment and handling of chemical reagents used for experiments. None of the reagents used for the experiments pose any serious health and safety concerns.

DISCUSSION

Field testing

CREARE was offered as an elective course for three consecutive semesters, fall 2016, spring 2017, and fall 2017. Three cohorts of six to nine students participated in CREARE and successfully performed biochemical experiments in the laboratory, analyzed environmental data using the R software environment, and disseminated the results of this research project that addresses the effect of climate change on a critically endangered reef-building coral. Once the course content and practices were optimized within its first iteration (cohort 1), we gathered assessment and perception data from cohorts 2 and 3. We are only presenting results for cohort 2 since the third semester that the course was offered was disrupted by two successive category 4 hurricanes (Hurricanes Irma and María), which resulted in major disruptions to academic activities and research efforts.

Evidence of student learning

The gathered data and figures generated by students in CREARE serve as evidence of student learning (Table 2).
These products demonstrate fluency in recording, analyzing and processing data from large data sets of biochemical and environmental data (Fig. 3, Appendices 12 and 13). Furthermore, these student-generated data have contributed to larger data sets that will be included in a research manuscript currently in preparation.

The impact of CREARE on student learning gains and attitudes toward science was measured using the Classroom
Undergraduate Research Experience Survey (6). Students reported that participation in CREARE led to significant gains in readiness for demanding research, understanding that scientific assertions require supporting evidence and understanding how knowledge is constructed. Furthermore, students reported improved attitudes toward science and significant perceived increases in their abilities to contribute to a research project and to collect, analyze, and present data (data not shown). Student self-reporting often holds little correlation to measurable learning gains or the mechanisms describing what makes CUREs effective at influencing learning gains or career choice behaviors (2, 9, 29). To verify that claims of learning correlate to acquisition of content knowledge, students who participated in CREARE during spring 2017 (N = 9) were given a 14-item exam to assess their knowledge about the underlying concepts related to the research project before and after participation (Appendix 15). Collectively, scores were higher after CREARE [mean, 7.78; standard deviation (SD), 1.86] compared to scores at the beginning of the experience (mean, 6.89; SD, 2.02) (Fig. 4). This improvement in student performance with regard to content is statistically significant (t = 2.28, P = 0.02) and validates student claims of learning gains after participation in CREARE.

Several lines of evidence suggest that the benefits of CUREs, beyond content acquisition and technical mastery, are the increase of self-efficacy, clarification of career goals, and development of a scientific identity (14–16, 30). To peer deeper into the mechanisms that mediate the changes in student perception regarding their abilities to perform scientific research and improve their view as scientists, a focus group interview was carried out with the same spring 2017 student cohort to survey which elements of the CURE experience are considered by participants to be the most important for their development. Audio from interviews was recorded and transcribed to identify phrases within the text that represented statements or reflections by students regarding how the experience impacted their personal and professional development (Appendix 16). Phrases within the text that made mention of what students perceived as opportunities for development were classified and grouped into four
emergent categories that mostly related to the valuation of the experience as an opportunity to develop professionally and to define a career trajectory (Table 4). As one student put it succinctly: “You do not know, really what career you want to go to until you start practicing it.” Within the four categories, codes that reflected improvements in self-efficacy and skill development were abundant, as were those that highlighted the value that students place on the development of interpersonal relationships as a factor of high impact. Within the latter category, there were multiple mentions of mentoring, teamwork, and a shift in their perception of positions within a scientific hierarchy (Table 4 and Appendix 16).

The categories that emerged from our analysis of focus group interviews mirror those described in the TIMSI model of social integration (15). Hence, we searched for phrases associated with the three modes of orientation described in the TIMSI framework for integrating research projects into courses. Only one of four learning objectives in CREARE is specific to the research project, and it can alternatively be replaced with the specific aim of other research projects that survey the impact of the environment on an organism, from behavior to molecular biology. Likewise, modules such as the Data Analysis Module can be used as an individual exercise to develop students’ fluency in analyzing environmental data within a course or a CURE. This can include instances where open source data sets accessible via the Internet are used for data analysis as well as carrying out the exercises using other statistical programs (see notes about instruction in Appendix 8). Similarly, the Science Writing module (Appendix 9) can be used in any other course where scientific results must be communicated through writing.

It merits mentioning that the main struggle for students in CREARE was using R, especially when applying it to real world circumstances and large data sets. Spending more time (two modules rather than one) to introduce students to R and starting with a small data set for them to work with before handling the real data were very helpful. Furthermore, keeping students engaged after setbacks in experiments and/or data analysis is always a challenge. On occasion, we had students switch tasks to avoid excessive frustration, specifically with data analysis in R.

**TABLE 4**

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<tr>
<th>Emergent category</th>
<th>Representative quote</th>
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<tbody>
<tr>
<td>Definition of career trajectory</td>
<td>“...you do not know, really what career you want to go to until you start practicing it”</td>
</tr>
<tr>
<td>Self-efficacy</td>
<td>“I thought that I could not do what I am doing, so the next thing I want to do, probably I can also do it if I put effort as well, as it happened now.”</td>
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<tr>
<td>Opportunities for development of research skills</td>
<td>“At the beginning of the investigation, you know very little about what you are investigating, and then, in the end, you go, you know so much, and then you continue branching out, and you want to know more about something that has to do with it.”</td>
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<tr>
<td>Opportunities for development of interpersonal relationships</td>
<td>“It changes your perspective a bit too, because sometimes you think that professors are like, like, someone like beyond human, they do not eat or anything...we spend a lot of time, uh, with them and you realize that, well, they also have their doubts and that they also learn from us some things as well, but they also learn things with us and that, and that they, in reality, they are basically there for us.”</td>
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Possible modifications

The semester-long CURE design presented here, with topical modules as a main mode of instruction and writing deliverables for assessment and evaluation, can be adapted as a framework for integrating research projects into courses.
work in developing and implementing CREARE offers further support of the effectiveness of CUREs in integrating cutting-edge research into courses thereby broadening the positive benefits of research experiences to a more diverse population of students.

**SUPPLEMENTAL MATERIAL**

- Appendix 1: Laboratory safety guidelines
- Appendix 2: Coral sampling protocol
- Appendix 3: Coral tissue extraction protocol
- Appendix 4: Biochemical examination of FP protocol
- Appendix 5: Biochemical examination of PO enzymatic activity protocol
- Appendix 6: Environmental measurement protocol
- Appendix 7: Biochemistry module
- Appendix 8: Data analysis module
- Appendix 9: Scientific writing module
- Appendix 10: Ecology module
- Appendix 11: Rubric to evaluate written reports
- Appendix 12: Examples of student-generated data and reports
- Appendix 13: Student presentation at local coral reef symposium
- Appendix 14: Example of feedback on student presentation
- Appendix 15: 14-item content exam
- Appendix 16: Transcript of focus group interview

**ACKNOWLEDGMENTS**

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