I. Abstract - The information that you provide in this section may be used in general publications as promotional material. This summary should be original and not an excerpt taken directly from the Project Proposal.

Name(s): Jenna Whitmore

Name of Faculty Collaborator/Mentor: Jerald S. Bricker

Department and Division: Biology Department, Natural and Health Sciences

Title of Project: A PCR-RFLP based genotyping system for *Acropora cervicornis* (staghorn coral).

Date: September 14, 2018

Abstract (maximum of 300 words)

Acropora cervicornis (staghorn coral) is one of many Endangered coral species in the Mesoamerican reef ecosystem. Beginning in the late 1980s, *Acropora* spp. populations have declined by 97% throughout the Caribbean. This has produced major changes in the ecosystem as major reef building species are replaced by "weedy" algal growth. Conservation efforts are underway to save or rehabilitate coral communities. A lack of knowledge, however, about the genetic diversity of staghorn coral hampers selection of candidates most suitable for culturing and relocation. The goal of this research project is to use genetic markers to genotype staghorn coral. This knowledge will allow the Roatan Institute of Marine Sciences (RIMS) to identify different "strains" of staghorn growing in protected communities near Roatan, Honduras. Coral genotypes best able survive the harsh environmental changes will be identified and used to establish transplant populations thus favoring growth and local recovery of the species.

II. Project Proposal

Name: Jenna Whitmore
E-mail address: jwhitmor@nebrwesleyan.edu
Student Mailbox (UC applicants - mailing address): 7012
Major: Biology
Year: Sophomore
(Please provide information for all students involved.)

Name of Faculty Collaborator/Mentor: Jerald S. Bricker
Department and Division: Biology Department, Natural and Health Sciences
E-mail address: jbricker@nebrwesleyan.edu

Title of Project: A PCR-RFLP based genotyping system for *Acropora cervicornis* (staghorn coral).
Amount Requested: $3684.00
Date: September 14, 2018

Period of performance: (Start date) October 1, 2018 **(End date)** May 1, 2019

Have you previously received a grant from the Student-Faculty Collaborative Research Fund? No

What other funding sources may be available to you? None

Does the research involve the use of human subjects? No

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Amount Requested: $3684.00

1. Describe the purpose of your scholarship (research or creative endeavor).

Coral reefs cover less than 1% of Earth's surface and provide approximately $375 billion worth of good and services. A key factor of the coral reefs, biodiversity, could lead to the discovery and development of drugs to cure cancer, arthritis, and many other diseases (NOAA, 2018). Unfortunately, coral reefs are in decline due to pressing environmental factors such as global warming and pollution. *Acropora cervicornis* ("staghorn coral") was once a dominant species of reefs throughout the Mesoamerican reef system. Since the 1980s, the population has declined 97% due to white band disease and other environmental stressors (NOAA, 2018). Population crashes combined with unsuccessful reproduction and recovery attempts has led to staghorn coral being classified as Endangered throughout its range (https://goo.gl/2EsL3D). The Coral Restoration Foundation (CRF) has worked to initiate and coordinate conservation efforts for coral restoration. CRF's primary tool for coral community restoration is a program where coral species are grown in nurseries on coral trees (PVC pipe tethered by buoys to the ocean floor and suspending coral fragments) that are later outplanted to the reef. The Roatan Institute of Marine Sciences (RIMS) is an active participant in the CRF program. The largest staghorn coral population remaining in the Caribbean is located near Coxen Hole and French Harbor, Roatan, Honduras (https://goo.gl/6yRU48). From this location, coral fragments are collected and relocated to the RIMS coral nursery located off shore of Sandy Bay. Thus far, fragment collection has been based on attempting to obtain a diverse population of transplants using trial and error (i.e., apparent morphological diversity). RIMS has limited funding, but would like to identify the coral being grown in the nursery to see if they belong to different "strains." That information would be used to guide outplant placement from the nursery onto the reef. In essence, RIMS staff needs to ensure established populations show high genetic diversity while ensuring the best coral strains are selected as transplants.

This project will utilize a set of nuclear and mitochondrial DNA genetic markers to genotype staghorn coral. A DNA genotyping system will be developed that utilizes the polymerase chain reaction (PCR) followed by restriction endonuclease digestion (RFLP analysis) and gel electrophoresis. Our plan is to perform PCR using sets of primers previously developed for invertebrates followed by DNA sequencing to map the restriction sites. We will then decide on the primer and enzyme combination that provides the best genetic "fingerprint" for guiding coral transplantation efforts.

Our approach recognizes that the marine station in Honduras (RIMS) running the conservation project has limited funding and expertise in DNA analysis. Any genotyping effort should be quick, reliable, and involve minimal analysis. Thus, we envision a PCR and RFLP-based approach being preferable to that of micro-satellite analysis or another bioinformatics intensive method. After perfecting genotyping protocols, RIMS will collect coral samples from their nursery and do the initial DNA extraction. These would be either be shipped to Dr. Bricker for analysis or held until he visits the station to teach BIO 3540: Applied Marine Biology.

2. Describe your preparation for pursuing this project. Include both formal and informal training and relevant experiences.

This past summer, I traveled to Roatan, Honduras, on a 2-week faculty study abroad program (BIO 3540: Applied Marine Biology) with Dr. Bricker. During those weeks, I studied the Mesoamerican reef system and its marine life. While in Roatan, I experienced the "Jewel of the Caribbean" firsthand during a SCUBA dive in the staghorn coral forest at Cordelia Bank. Prior to the Honduras trip, I took an oceanography and marine biology class (BIO 3530: Principles of Oceanography and Marine Biology) which introduced me to the geology and biology of coral reefs. I have also completed a bioinformatics class that enabled me to analyze and understand DNA sequences. This past summer, I was also involved in a 4-week research program that allowed me to become familiar with prep. work and how to set up and run PCRs to amplify DNA.

3. Describe how you intend to accomplish your project, the project steps and timeline, the methods) or processes chosen and how they are appropriate for the discipline. Explain the feasibility of your activity. (Consider time and funding restraints as well as other factors.) If more than one student is involved, please describe exactly what each student will do.

Location

Recognizing that *Acropora cervicornis* is an Endangered species, samples of staghorn coral utilized in this study must come from captive populations. Mitch Carl, Curator of Aquatics at the Henry Doorly Zoo, Omaha, has agreed to provide coral samples from the zoo's living collection. After PCR and DNA analysis techniques are perfected, coral samples previously collected (under CITES and Honduran government permits held by RIMS) by Dr. Bricker in Roatan, Honduras, will be analyzed to confirm our methodology.

Sample Analysis

The necessary lab work will be performed on the UNL East Campus in the laboratory of Dr. Josh Herr (Dr. Bricker's research collaborator during his sabbatical leave).

DNA extraction

DNA will be extracted from living coral samples obtained from the Henry Doorly Zoo. We will use a DNA extraction kit purchased from IBI Scientific and will follow the manufacturer's recommendations when developing our own protocol.

PCR

A PCR-based method for assessing variation among genes in *Acropora cervicornis* will be developed. During an exhaustive search of the primary literature, have identified several

molecular markers/PCR primer sets that show strong potential for success. Experimentation with primer combinations will be necessary to design the optimal approach to analysis.

DNA Sequencing and Restriction (Endonuclease) Enzyme Digestion
Positive PCR products will be identified by agarose gel electrophoresis and sent to the Genomics Core Facility at the University of Nebraska Medical Center (UNMC) for sequencing. Sequence data will be analysed using standard bioinformatics tools to identify restriction sites suitable for genotyping coral samples. Once the appropriate restriction enzymes are identified they will be used to digest to produce gel banding patterns (= Restriction Fragment Length Polymorphism analysis, RFLPs) that distinguish coral cultivars (i.e., produce a suitable "fingerprint").

4. What is your expected graduation date? May, 2021

How many credit hours are needed to complete your degree? 78 credit hours

5. **Describe the role of your faculty mentor or faculty collaborator. How will s/he be involved in your project?**

Dr. Jerald Bricker, Associate Professor of Biology, is my faculty mentor for this project. He will assist me in designing and troubleshooting the PCR and RFLP-based techniques that we'll develop to analyze coral tissue samples we receive from the Omaha Zoo.

5. **Describe your plan for a discipline appropriate dissemination of your scholarship beyond the Nebraska Wesleyan Student Symposium at the end of the Spring semester (e.g., peer-reviewed journal publication, oral presentation, poster presentation, performance, juried competition and/or exhibition).**

I will present the data collected from this research at the Nebraska Wesleyan Research Symposium next April. I also plan to present my results at the Nebraska Academy of Sciences (NAS) Annual Meeting which will be held on April 12, 2019 on the Nebraska Wesleyan University campus. If funds are available, I would also like to present this research at the West Coast Biological Sciences Undergraduate Research Conference in San Diego, California, in May 2019.

III. Project Budget

Name(s) of Student(s): Jenna Whitmore

Name of Faculty Collaborator/Mentor: Jerald S. Bricker

Title of Project: A PCR-RFLP based genotyping system for *Acropora cervicornis* (staghorn coral).

A. ITEMIZED BUDGET
Your itemized budget proposal must be submitted on the provided Excel spreadsheet. **All expenses should have documentation supporting the cost** (supply a copy of a price list or website order form, etc).

1. Equipment

Provided by the laboratory of Dr. Josh Herr, UNL Plant Pathology Department. Dr. Bricker is on sabbatical leave for the the 2018-2019 academic year and is working in Dr. Herr's lab on other genomic analysis projects. I will join Dr. Herr's lab and have access to the necessary equipment needed to complete my project.

2. Supplies

See the provided Excel spreadsheet (website ordering URLs are provided for each item). **All items are priority supplies** since the DNA analysis depends on the completion of each step in the process (i.e., obtaining samples from the Omaha Zoo → DNA extraction → PCR → gel electrophoresis → DNA sequencing → Restriction enzyme digestion and analysis).

B. BUDGET JUSTIFICATION

A. Equipment (Please indicate the department's contribution toward the purchase of permanent equipment or software). No equipment will be purchased for this project.

B. Supplies - research budget is directed towards doing the DNA/genotype analysis of staghorn coral. We expect that initial attempts at DNA extraction and PCR will be unsuccessful as we perfect our methodology. Thus, it is expected that over 150 DNA extractions will be attempted with 100 PCR products being sequenced at UNMC and subjected to RFLP analysis.

1) Laboratory supplies (microcentrifuge tubes, pipette tips, buffers, agarose, general chemicals, etc.)
2) DNA Extraction Kit – 100 Preps (IBI Scientific)
3) PCR primers (Eurofins Genomics)
4) ReadyMix™ Taq PCR Reaction Mix (Sigma-Aldrich #P4600)
5) DirectLoad™ 1 kb DNA Ladder (Sigma-Aldrich #D3937)
6) Restriction endonucleases (New England Bio. Labs)
7) DNA sequencing (UNMC DNA Sequencing Core Facility)

C. Travel

1) Round trip from the NWU campus to Omaha Zoo (110 miles x $0.55/mile) to receive coral samples (these must be obtained fresh and transported to the lab immediately). Travel reimbursement: $242.00 to allow travel at least four times over the course of seven months.

D. Other