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Development and Testing of an Enviromental DNA Assay to Detect and Monitor the Federally Endangered Alabama Cavefish

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Project Description

Conserving biodiverse habitats has become an increasingly difficult task as many threats, such as habitat destruction and degradation and pollution, continually impact biodiversity. Severely hampering our abilities to effectively manage and conserve biodiversity is a fundamental lack of knowledge on distributions and abundances of species, particularly those that are small and that live in habitats extremely difficult to survey and study. One such group are obligate subterranean species in which our knowledge of subterranean biodiversity is extremely limited, as subterranean ecosystems are particularly challenging to access and study. The analysis of environmental DNA (eDNA) from water samples offers an exciting and potentially effective way to detect and monitor subterranean biodiversity that might

otherwise be difficult or impossible to survey using traditional approaches. However, the use of eDNA as a tool for subterranean biodiversity monitoring is still new and its efficacy has not been thoroughly tested.

This project will develop and test the efficacy of eDNA to detect and monitor an endangered groundwater fish – the Alabama Cavefish (*Speoplatyrhinus poulsoni*). The Alabama Cavefish is known only from a single cave system along the Tennessee River near Florence in Lauderdale County, Alabama. Aquatic surveys of other nearby cave systems for this federally endangered cavefish have failed to uncover new locations. Environmental DNA offers potential to expand survey efforts for this species into groundwater habitats that cannot be accessed using traditional survey approaches. During this project, we will (1) develop a reference DNA sequence database from existing DNA sequence databases, such as GenBank and the Barcode of Life Database (BoLD), and through generation of novel sequences of select cavefish specimens and tissues from Key Cave and other nearby cave systems; (2) develop and test species-specific primer-probe assays for quantitative polymerase chain reaction (qPCR); and (3) screen water samples collected from caves and springs in northwestern Alabama for the Alabama Cavefish. This project will assist in addressing current knowledge gaps impeding conservation and management of the Alabama Cavefish by providing a better understanding of its occupancy, distribution, and ecology.

Student Duties, Contributions, and Outcomes

The RCEU student will be responsible for (1) field collection of eDNA water samples; (2) generation of a reference sequence database using traditional PCR and Sanger sequencing from previously collected specimens and tissues; (3) *in silico* design and testing and *in vivo* testing of a species-specific assay

involving primers and a probe; and (4) extraction and screening of eDNA samples using qPCR. The RCEU student will learn the basics of caving and field research safety. The student also will learn to extract DNA from tissue and environmental samples, setup and run traditional PCR and qPCR reactions, and other molecular laboratory techniques. The student will learn how to pipette, use sterile technique, and other essential molecular laboratory skills that are highly marketable. The RCEU student also will learn how to use software to design and test PCR primer and probe assays and align and analyze sequencing data. The student will participate in data analyses and report/manuscript preparation. A manuscript(s) will be submitted to a refereed journal based on the results of this project, with the RCEU

student as first author or coauthor.

Student Selection Criteria

The RCEU student should be a rising Junior or Senior and is required to have completed the Principles of Biology and Organismal Biology sequence (BYS 119 and 120), Genetics and Evolution (BYS 219), and the Chemistry sequence (CH 121/125 and 123/126). Previous molecular lab experience is preferred but not required.

Project Mentorship

The RCEU student will be taught the field and laboratory techniques by Dr. Niemiller and graduate students in the Niemiller Lab, who will work with the student daily as they learn the procedures. The student will be assigned scientific papers to read regarding eDNA techniques and will review these papers each week with Dr. Niemiller. The RCEU student will meet with Dr. Niemiller at least twice times a week while learning techniques then transitioning to a less frequent meeting schedule as the project progresses, but no less than at least once per week. The student is expected to attend lab meetings. Dr. Niemiller will also work with the student to improve scientific writing and data analysis skillsets. The student will produce a report and presentation of the results of his/her project at the end of the summer that will be evaluated.

Safety and Contingency Plan

The RCEU student will be trained and taught both field and molecular laboratory safety, including a laboratory safety training courses offered through OEHS, including Biological Safety, Building Evacuation Procedures, Fire Extinguisher Use, Lab Safety Orientation, Personal Protective Equipment

for the Lab, and Universal Waste Training. The student will be trained in caving safety as well as White nose Syndrome decontamination protocols to minimize the spread of the pathogenic fungus that impacts cave-roosting bats.

If COVID-19 concerns continue into Summer 2021 and require limiting or preventing in-person contact or on-campus research activities during the RCEU performance period, contingency plans will be implemented. First, existing DNA sequence data can be leveraged to design and test the specificity of primer-probe qPCR assays in silico for the Alabama Cavefish. Second, once initially trained, the RCEU

student can work independently in the laboratory as long as on-campus research activities are permitted. Regular meetings with the student can be coordinated via Zoom. Third, the RCEU student can use and analyze existing data already collected for an associated project on using eDNA for detecting and monitoring the Alabama Cave Shrimp (*Palaemonias alabamae*) in northern Alabama.