

Title:	Developing and Assessing eDNA Survey Methodology for Protected Mussel Species in Texas
The Problem:	The evaluation and permitting of impacts from TxDOT activities on federally and state protected mussels is a financially demanding and time-consuming effort that can delay project development and progress. Currently, Texas hosts two species of freshwater mussels that are listed as endangered under the Endangered Species Act (ESA), and 10 species proposed to be listed as threatened or endangered under the ESA (protected mussels). There are an additional five species listed as threatened by the state of Texas. These protected mussels and their potential impacts to TxDOT project delivery range across 124 Texas counties, the majority of which are in the more heavily populated central and eastern portions of the state. Current protocols defined by the U.S. Fish and Wildlife Service (USFWS) and Texas Parks and Wildlife Department (TPWD) include rigorous surveys at sites with proposed construction with in-water impacts.
	To comply with the survey protocols TxDOT has funded efforts ranging from \$30,000 to \$194,000 for survey alone, which excludes the required mussel relocation. Relocation efforts often add tens of thousands of dollars to the final total. Beyond cost, these surveys include scheduling constraints, require extensive logistical planning, and can be limited by the minimal availability of qualified professionals and are subject to variable river conditions. The surveys include risks to the safety of these professionals with many efforts requiring they dive in deep waters that have limited or no visibility that may contain entrapment obstacles, potentially dangerous wildlife, and contaminated waters. These surveys are not 100 percent effective at locating all protected mussels within an area which can lead to unexpected delays in project delivery. Multiple studies indicate survey methodologies using environmental DNA (eDNA) can provide more reliable results with less cost, time, and risk.
Technical Objectives:	 TxDOT is seeking to develop a method of survey for protected mussels using eDNA that minimizes cost and risk to project delivery and human health but matches or increases mussel detection efficiency rates when compared to the current mussel survey protocols. The objectives of this project are: Evaluate eDNA survey methodologies for the 12 federally listed and proposed mussel species in Texas, including comparisons of multi-species vs single species eDNA analyses. Identify or develop the necessary species-specific DNA tests (primers) for all 12 federally listed and proposed species and validate the primer accuracy and detection efficiency in a lab setting. Develop field tests to assess efficacy of eDNA surveys for each species including evaluation of impacts on persistence and degradation of eDNA from environmental variables such as flow rates, seasonality, and type of river habitat. Evaluate detection rate as a factor of sampling protocols including distance from known mussel individuals, sample location and depth, and collection method. Conduct field tests, to include both experimental settings with caged individuals and direct survey comparisons of detection rates between the USFWS current protocols and eDNA sampling. Provide cost comparisons of USFWS protocol and eDNA surveys at three sites with different conditions in the state. Provide a guidance document outlining the eDNA sampling protocols for future use shall be provided, pending successful findings of survey efficacy.
	The expected technology readiness level (TRL) for this project is 8.

Anticipated Deliverables:	 Technical memorandum for each task completed. Monthly progress reports. Guidance document outlining eDNA sampling protocols for freshwater mussels in Texas. Project Summary Report Research report documenting the findings of this research, including: Detailed eDNA survey plan including the generation of the appropriate species or community primers and lab validation, Detailed field-testing plan, Potential uses and limitations of eDNA surveys to assess protected freshwater mussel occupancy in
	 Texas as related to TxDOT project types including a cost comparison with USFWS mussel survey protocols. Value of Research (VoR) that includes both qualitative and economic benefits.
Proposal Requirements:	 The Research Staff shall include a member with a minimum of 5 years experience of eDNA research. The project duration shall not exceed 36 months. RFP#1 Q&A Deadline: 12:00 p.m. Central Time, Friday, May 10, 2024. Proposal Deadline: 12:00 p.m. Central Time, Tuesday, May 21, 2024. Use the current "ProjAgre" and "PA Forms" templates located at the <u>RTI Forms webpage</u>. Proposals will be considered non-responsive and will not be accepted for technical evaluation if they are not received by the deadline or do not meet the requirements stated in RTI's <u>University Handbook</u>. Proposals should be submitted by the University Liaison in PDF format; (1) PDF file per proposal. File name should include project name and university abbreviation. This project will be tracked during the life of the project using the Technology Readiness Level (<u>TRL</u>) scale. The 2021 Texas Legislative Session requires that universities be in compliance with Senate Bill 475 by submitting a completed and signed TxDOT Security Questionnaire (TSQ) to <u>RTIMAIN@txdot.gov</u>. Universities that have not submitted a completed and signed TSQ one week after award will be considered non-compliant and unable to participate in the Program.