Environment and Natural Resources Trust Fund

2026 Request for Proposal

General Information

Proposal ID: 2026-379

Proposal Title: Detecting Native Fishes and Mussels Using Molecular Tools

Project Manager Information

Name: Lynn Waterhouse

Organization: U of MN - College of Food, Agricultural and Natural Resource Sciences

Office Telephone: (801) 550-4065

Email: lwater@umn.edu

Project Basic Information

Project Summary: This project aims to develop a cost-effective, color-based method for detecting native fish and mussels, using genome sequencing to enhance biodiversity monitoring and support sustainable conservation of Minnesota's aquatic ecosystems.

ENRTF Funds Requested: $468,000

Proposed Project Completion: June 30, 2029

LCCMR Funding Category: Fish and Wildlife (D)

Project Location

What is the best scale for describing where your work will take place?

Statewide

What is the best scale to describe the area impacted by your work?

Statewide

When will the work impact occur?

During the Project and In the Future

Narrative

Describe the opportunity or problem your proposal seeks to address. Include any relevant background information.

Minnesota's lakes, rivers, and wetlands are essential to the state's ecological health and provide over one billion dollars in economic value, with native rough fish species playing a critical role in sustaining these ecosystems. Species like the longnose gar, shortnose gar, bowfin, mooneye, and goldeye are essential to freshwater habitats, acting as both predators and prey within food webs and supporting aquatic biodiversity. Some native rough fish are hosts for freshwater mussels during their parasitic life cycle phase, when larval mussels attach to fish gills or fins. Much remains unknown about native rough fish within Minnesota due to their historical labeling as "rough fish" alongside invasive species (e.g., Common Carp), and perceived lesser value relative to game fish. These populations face numerous threats, including habitat degradation, invasive species, overharvest, and climate change. Monitoring native rough fish with traditional methods (e.g., electrofishing) is often difficult due to their low population densities. These methods are often time-consuming, costly, and logistically difficult, resulting in many species going undetected. Some rough fish species look-alike, making accurate monitoring challenging. By developing an affordable, reliable, and efficient method to locate these species, agencies can better support their sustainable management and conservation.

What is your proposed solution to the problem or opportunity discussed above? Introduce us to the work you are seeking funding to do. You will be asked to expand on this proposed solution in Activities & Milestones.

The proposed solution is to develop a colorimetric (color-based) method to monitor native rough fish and freshwater mussel populations in Minnesota's lakes, rivers, and wetlands. This method enables real-time, on-site monitoring by detecting species-specific genetic markers from environmental DNA (eDNA) samples. The approach uses whole genome sequencing and skim-sequencing to identify unique genetic markers for 20 species including longnose gar, shortnose gar, bowfin, mooneye, goldeye, and spectaclecase mussels. These markers will be used to design DNA probes that specifically target these species in environmental DNA (eDNA) samples. The colorimetric assay will allow field technicians to test samples and identify target species by producing a visible color change when the DNA sequences match. This method is rapid, delivering results in less than 30 minutes, and is cost-effective (final product will cost between $2 and $10 to test for 20 species), making it accessible to researchers, conservationists, and state agencies like the Minnesota Department of Natural Resources (MNDNR). By enabling early detection and continuous monitoring of native rough fish populations, this tool will support better decision-making for their conservation and management. It provides an efficient, user-friendly solution for tracking these species and ensuring the health and biodiversity of Minnesota's aquatic ecosystems.

What are the specific project outcomes as they relate to the public purpose of protection, conservation, preservation, and enhancement of the state's natural resources?

The project will directly contribute to the protection and conservation of Minnesota's natural resources by providing a cost-effective, accurate tool for monitoring native rough fish populations, including species like longnose gar, shortnose gar, and bowfin. It will enable early detection of population shifts due to environmental stressors or ecological disturbances. The project will support resource managers, such as MNDNR and Tribal Nations , in making informed decisions regarding the protection and restoration of native habitats. Additionally, it will create a genetic library for species-specific monitoring, contributing to the long-term sustainability and health of Minnesota's aquatic ecosystems.

Activities and Milestones

Activity 1: Collection, whole genome sequencing, skim-sequencing, and DNA markers development for native rough fish and freshwater mussels in Minnesota

Activity Budget: $130,000

Activity Description:
This activity will involve the collection of native rough fish and freshwater mussel tissue samples from freshwater ecosystems across Minnesota. The species selected will represent a diverse range of aquatic habitats, including lakes, rivers, and wetlands throughout the state. These species will include the longnose gar (Lepisosteus osseus), shortnose gar (Lepisosteus platostomus), bowfin (Amia ocellicauda), mooneye (Hiodon tergisus), and goldeye (Hiodon alosoides), among other native rough fish and freshwater mussel species. DNA will be extracted from the samples using Qiagen's DNeasy® Blood & Tissue Kit. Depending on species genome availability, either whole genome sequencing will be done on the PacBio Revio platform or skim-sequencing on the Illumina NovaseqX platform. The sequence data will then be analyzed to identify conserved regions and species-specific genetic markers for each species. Species specific tailored primers will be developed according to their distinct genetic regions. The primers will be validated through PCR amplification and sequencing using the Nanopore minION™ platform. This validation process will ensure that the primers are specific to the target species, enabling accurate species identification, which will aid in the monitoring and conservation of rough fish species in Minnesota's aquatic ecosystems.

Activity Milestones:

Description	Approximate Completion Date
Collection of rough fish fin tissue and freshwater mussel tissue samples	September 30, 2026
DNA extraction and Whole genome sequencing	December 31, 2026
Bioinformatic analysis for identification of distinct DNA markers	October 31, 2027
Designing of metabarcoding primers	December 31, 2027
Validation of markers using PCR and amplicon sequencing	March 31, 2028
Development of rough fish metabarcoding database and making it publicly available on NCBI	December 31, 2028

Activity 2: Development of calorimetric method for detection of native rough fish and freshwater mussels from environmental DNA

Activity Budget: $120,000

Activity Description:
The main goal of this activity is to develop a rapid, cost-effective colorimetric method that can accurately detect native rough fish and freshwater mussel species from eDNA samples. The DNA markers identified in the previous activity will be used to design specific DNA probes that will hybridize with target genetic sequences unique to each rough fish species. The probes will be carefully designed to ensure high specificity and sensitivity for each target species, minimizing the risk of cross-reactivity with non-target species. The hybridization conditions will be optimized to ensure efficient binding of the probes to the target sequences, generating a visible color change in less than 30 minutes, including sample preparation. The colorimetric nature of the assay will allow field technicians to perform on-site detection without the need for complex laboratory equipment. Once the assay is developed, it will undergo validation using controlled rough fish samples and eDNA collected from natural habitats. Field trials will test the assay's effectiveness across various freshwater environments in Minnesota, ensuring its utility for monitoring rough fish populations in diverse ecosystems.

Activity Milestones:

Description	Approximate Completion Date
Design and laboratory validation of DNA probes	June 30, 2028
Testing of colorimetric assays in different environments	October 31, 2028

Activity 3: Deployment of colorimetric assays for monitoring data collection

Activity Budget: $120,000

Activity Description:
In this phase, the colorimetric method will be deployed across Minnesota's aquatic ecosystems to monitor native rough fish and freshwater mussel species in real time. Assay plates pre-loaded with DNA probes will be provided to field teams. Field teams will collect eDNA samples from a variety of lakes, rivers, and wetlands. These eDNA samples will be processed to purify DNA and then applied to colorimetric assay plates for detection. When the DNA of a target species hybridizes with the DNA probe, a visible color change will occur, indicating the presence of the species. This colorimetric result will allow for rapid, on-site identification of species in less than 30 minutes, facilitating quick responses and more efficient monitoring. The effectiveness of the assay will be assessed by evaluating its sensitivity, specificity, and reliability across environmental conditions. This phase will also help identify any adjustments needed to optimize the tool's performance in real-world scenarios, ensuring that it remains a reliable method for monitoring across Minnesota's freshwater ecosystems. Additionally, the monitoring data collected across Minnesota will be used for predictive modeling research on rough fish detection and conservation. This data will be compared with other monitoring databases, providing valuable insights for fish conservation efforts.

Activity Milestones:

Description	Approximate Completion Date
Native rough fish monitoring using colorimetric assays across Minnesota	April 30, 2029
Model analysis to estimate sensitivity, specificity and reliability	May 31, 2029

Activity 4: Sharing method via outreach and publication

Activity Budget: $98,000

Activity Description:
In this phase, the colorimetric method will be shared with potential future users through a variety of activities. We will develop a manual on the standard operating procedures for using the colorimetric methods to monitor native rough fish and freshwater mussel populations. We will also complete a cost-analysis and detailed supply list. These will be written assuming potential end users could be high school classes, undergraduate classes, citizen science groups, recreational angling programs, university and Tribal extension programs, and state, federal, and Tribal management agencies. We will share the colorimetric method, cost-analysis, and stand operating procedures through a workshop. The workshop will be digitally archived, enabling future access. We will also develop at least one manuscript for submission to an open-access peer-reviewed scientific journal. The results will be publicly shared and made accessible to state and federal agencies, ensuring transparency and aiding broader conservation efforts.

Activity Milestones:

Description	Approximate Completion Date
Development of Standard Operating Procedures and Cost-Analysis for using colorimetric assays to conduct Native	June 30, 2029

Development of manuscript for submission to an open-access peer-reviewed journal based on project findings	June 30, 2029
Build and present outreach activity based on results and submit other outreach	June 30, 2029

Project Partners and Collaborators

Name	Organization	Role	Receiving Funds
Dr. Solomon David	University of Minnesota	Dr. Solomon David is an assistant professor at the University of Minnesota, and an aquatic ecologist with training in conservation and restoration of native fish biodiversity, science communication, and over 20 years of experience studying gars and bowfins. He will oversee the project and co-supervise the students and research scientist.	No
Dr. Todd Michael	Salk Institute	Subcontract recipient. Dr. Todd Michael is a Research Professor at at Salk Institute. He has extensive experience in using sequencing technology and computational biology. He will lead the sequencing work and plate development. For the subcontract he will lead data collection, analysis, and report writing.	Yes

Long-Term Implementation and Funding

Describe how the results will be implemented and how any ongoing effort will be funded. If not already addressed as part of the project, how will findings, results, and products developed be implemented after project completion? If additional work is needed, how will this work be funded?

Results from the project would be shared through a variety of outreach activities. The genetic sequences will be uploaded to NCBI. The project data and results will be archived online through the USGS data portal along with being published in peer-reviewed open-access (free) journals. The colorimetric tool will be shared through a workshop archived online. The general results will be shared with a broader audience through short articles in regional newsletters, presentations, and outreach activities with interested stakeholders, including citizen science groups, recreational angling groups, and extension programs.

Other ENRTF Appropriations Awarded in the Last Six Years

Name	Appropriation	Amount Awarded
Predicting the Future by Understanding the Past	M.L. 2023, , Chp. 60, Art. 2, Sec. 2, Subd. 03g	$170,000

Project Manager and Organization Qualifications

Project Manager Name: Lynn Waterhouse

Job Title: Assistant Unit Leader, USGS Minnesota Cooperative Fish and Wildlife Unit and Assistant Professor at University of Minnesota

Provide description of the project manager's qualifications to manage the proposed project.

Lynn Waterhouse is the Assistant Unit Leader in Fisheries at the US Geological Survey (USGS) Minnesota Cooperative Fish and Wildlife Research Unit (MNCFWRU) which is a USGS Cooperative Research Unit at the University of Minnesota (UMN). L. Waterhouse is also an Assistant Professor in the Department of Fisheries, Wildlife, and Conservation Biology. L. Waterhouse has a PhD in Biological Oceanography and MS degrees in Fisheries Science and Statistics. L. Waterhouse joined MNCFWRU in June 2021 and is working on growing her lab, and she currently has 2 MS students, 1 PhD student, and 2 postdoctoral research fellows in her lab, and is recruiting 1 new MS student. L. Waterhouse has experience working with and leading collaborative research projects. She has ongoing projects utilizing environmental DNA (eDNA) to detect aquatic invasive plants, modeling invasion prone and resilient waterbodies through machine learning, and informing stock assessment of valuable Minnesota fishes through quantitative modeling. L. Waterhouse just concluded working on a large collaborative project assessing the growth of bigeye tuna in the Atlantic ocean with researchers from Europe, Africa, South America, and North America. As part of UMN, funds awarded to L. Waterhouse will go to UMN and have oversight from accounting people there (Kelsey Grachek, grach013@umn.edu). L. Waterhouse is able to bring in

additional students and postdoctoral fellows through UMN Department of Fisheries, Wildlife, and Conservation Biology which is a strong program. L. Waterhouse has training in science outreach. As part of her job with MNCFWRU, L. Waterhouse works closely with other scientists from Minnesota Department of Natural Resources (MNDNR), USGS, and the US Fish and Wildlife Services.

Organization: U of MN - College of Food, Agricultural and Natural Resource Sciences

Organization Description:
In the College of Food, Agricultural and Natural Resources Sciences (CFANS) we look at the bigger picture. We use science to find answers to the world's grand challenges and solve tomorrow's problems. The goal of CFANS is to advance Minnesota as a global leader in food, agriculture, and natural resources through extraordinary education, science-based solutions, and dynamic public engagement that nourishes people and enhances the environment in which we live. Few other public universities come close to the breadth of our expertise, allowing us to tackle challenges in novel ways. We develop leaders that see more possibilities and produce solutions that work for real people. This creates a powerful force for change. The university also hosts a cutting edge Minnesota Supercomputing Center which researchers use to tackle cutting edge problems. Twelve academic departments and 10 research and outreach centers make up our college, along with the Minnesota Landscape Arboretum, the Bell Museum, and dozens of interdisciplinary centers. The Department of Fisheries, Wildlife, and Conservation Biology has about 20 faculty, 40 staff, 60 graduate students, 200 undergraduates, 1200 alumni, and many friends….all working together to advance our knowledge of fisheries, wildlife, and conservation biology.

Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	$ Amount
Personnel								
2 Undergraduate research assistants		Two undergraduate student research assistants. They will assist with field work, lab work, all aspects of the project. 25% during 1 academic year and 50% time for two summers for 2 persons.			0%	0.28		$19,856
Research scientist, UMN		Research Scientist - FWCB at UMN -50% time, 3 years, $67,898/year base salary. 3% increase each year. Collect representative samples, perform extractions, assist in probe development and testing, assist in plate development and testing, and co-lead outreach activities with local stakeholders to share molecular test and explain how to use it for sampling for roughfish and mussels. Co-lead data collection and organization and dissemination of results through report and manuscript writing.			32.3%	1.5		$138,826
Graduate Student, UMN		Graduate Student, for Fall 2026 to summer 2028, involved with all aspects of project including publications. Full time all summers, 50% time during academic years. Collect representative samples, perform extractions, assist in probe development and testing, assist in plate development and testing, and co-lead outreach activities with local stakeholders to share molecular test and explain how to use it for sampling for roughfish and mussels. Co-lead data collection and organization and dissemination of results through report writiing and manuscript writing. Tuition for 4 semesters at UMN ($9,990 a semester).			23.2%	1.54		$139,192
Bioinformatics (Researcher 5)		20% for 2 months per PacBio Genome Assembly. 6 assemblies. 20% for 12 months. $120,000 base salary Assemble whole genome from sequencing done at UMN. Load genomes to NCBI database. Lead data organization and storage from genome sequencing work. ASsista with dissemination of results through report and manuscript writing.			36.6%	0.2		$32,784

							Sub Total	**$330,658**
Contracts and Services								
University of Minnesota Genomics Center (UMGC)	Service Contract	Perform whole genome sequencing of 6 species whose genome has not yet been sequenced on PacBio.				-		$24,000
Dr. Todd Michael, Salk Institute	Service Contract	Perform skim-sequencing, probe development, and plate development at Salk Institute utilizing multi-million dollar laboratory and expertise in molecular methods and bioinformatics.		X		-		$49,999
							Sub Total	**$73,999**
Equipment, Tools, and Supplies								
	Tools and Supplies	Supplies for field work (ethanol ($100), eppendorf tubes ($200), scissors ($200), scalpels ($66), gloves ($100), parafilm ($50), ziploc bags ($50), bleach ($100), life jackets ($200)).	Supplies for field work and collection of DNA materials from native rough fish specimens and freshwater mussels.					$1,066
	Tools and Supplies	Purification kits, extraction kits, PPE, bleach, vials, bags, and shipment fees.	Lab supplies for extracting, preparing, and shipping samples for whole genome sequencing.					$10,000
	Tools and Supplies	DNA extraction, PCR reagents, and nanopore sequencing flow cells.	Lab supplies and kits for validating DNA markers at UMN.					$15,000
	Tools and Supplies	Field supplies and consumables for testing colorimetric plate assays. eDNA sample collection supplies (filters, tubes, dessicant, bags, PPE, bleach) and lab supplies (gels, stains).	Field supplies and consumables for testing colorimetric plate assays.					$10,600
	Tools and Supplies	Coffe/tea	Coffee/Tea for outreach meetings with stakeholders to train them on the molecular test for native roughfish and native mussel detection from eDNA water sampling.	X				$400
	Tools and Supplies	Computer setup (laptop, dual monitors, mouse/keyboard)	Computer setup (laptop, dual monitors, mouse/keyboard) for project use. Necessary for data management, bioinformatics work, and anlyses.	X				$2,000

							Sub Total	**$39,066**
Capital Expenditures								
		Gel documentation system (ex. Analytik Jena NO.:849-97-0944-03)	The Analytik Jena UVP Solo Elite is a compact system for the rapid processing of gel analysis and documentation in labs.	X				$16,000
							Sub Total	**$16,000**
Acquisitions and Stewardship								
							Sub Total	**-**
Travel In Minnesota								
	Conference Registration Miles/ Meals/ Lodging	Prices from 2024 Conference in St. Cloud with 5% increase per year. Student rate $75, Professional rate $200. Using Duluth, MN for location placeholder. 3 nights hotel ($264/night - $220 lodging max plus tax)= $792, per diem $64.50/day x 2 days travel + $86/day x 2 days = $301. hotel and per diem is $1,093 per person x 2 person =$2,186. mileage (300 miles x $0.70/mile)= $210. $2,396 for 2 people in 2024 x 5% increase 2025 x 5% increase 2026 = $2,642 plus $275 in registration fees.	Graduate student and research scientist attend MN American Fisheries Society Meeting in 2028, location TBD in MN. Share results from project with scientific audience at MN American Fisheries Society Meeting.					$2,917
	Miles/ Meals/ Lodging	Mileage to sample sites local to twin-cities. Using 2025 rate of $0.70/mile x 80mi/trip x 60 trips	Travel to field sites to collect samples for DNA extraction, collect water for eDNA analysis for probe validation, and water sampling for testing of colorimetric plates and monitoring of native rough fish and freshwater mussel populations.					$3,360
							Sub Total	**$6,277**
Travel Outside Minnesota								
							Sub Total	**-**
Printing and Publication								

	Printing	Printed materials for outreach activities (stickers, protocols, infographics)	Printed materials for outreach activities to raise awareness of native roughfish and native mussels. Materials will be used when giving workshops and demonstrations of how the plate works to key stakeholders.					$2,000
							Sub Total	**$2,000**
Other Expenses								
							Sub Total	**-**
							Grand Total	**$468,000**

Classified Staff or Generally Ineligible Expenses

Category/Name	Subcategory or Type	Description	Justification Ineligible Expense or Classified Staff Request
Contracts and Services - Dr. Todd Michael, Salk Institute	Service Contract	Perform skim-sequencing, probe development, and plate development at Salk Institute utilizing multi-million dollar laboratory and expertise in molecular methods and bioinformatics.	Salk Institute (La Jolla, California) has a multi-million dollar laboratory and expertise in molecular methods and bioinformatics. They have the expertise in skim-sequencing and probe development that is necessary to accomplish the project.
Capital Expenditures		Gel documentation system (ex. Analytik Jena NO.:849-97-0944-03)	The gel documentation system is essential for accurately visualizing and documenting the results of DNA gel electrophoresis. This equipment allows for the analysis of PCR products, confirming the presence of species-specific genetic markers in environmental DNA (eDNA) samples. It enhances data quality by providing high-resolution images, ensuring precise and reproducible results. The system is crucial for validating the genetic markers identified from whole genome seqeuncing which will be key components of monitoring native fish and mussel populations. This equipment will help and support our project for regular genetic monitoring. The purchase of this system ensures that the project can operate efficiently and maintain high standards of scientific accuracy throughout set timeline. **Additional Explanation :** The gel documentation system will be used during the project for the analysis of PCR products, confirming the presence of species-specific genetic markers in environmental DNA (eDNA) samples. This instrument will play a key role in the validation of the probes and colorimetric plate. In the future, the whole genomes and skim-sequencing data created by this project, could be leveraged into another molecular tool for these native rough fish and freshwater mussel species and the the gel documentation system would continue to be useful for verifying the species-specific genetic markers presence in the eDNA in the tool development and validation phase. The gel documentation system will continue to be useful in studying native rough fish and freshwater mussel species via molecular methods.
Equipment, Tools, and Supplies		Coffe/tea	Meeting to share colorimetric method with those interested in learning the molecular method we used. This is one of the important ways we share the results of this project. We believe these meetings will be better attended if we are able to offer coffee, tea, and light snacks. Also the caffeine will help everyone with better focus on our exciting and important results and molecular methods.
Equipment, Tools, and Supplies		Computer setup (laptop, dual monitors, mouse/keyboard)	A large component of this project is computer based. A computer is necessary in order to complete the data collection and statistical analyses to validate the probes. It will be more efficient to use that computer with a proper keyboard, mouse, and dual monitors given that the project will often involve having multiple datasets open and coding chunks. To ensure the graduate student has a computer able to complete the project it is best to

			purchase them a new one.

Non ENRTF Funds

Category	Specific Source	Use	Status	Amount
State				
			State Sub Total	**-**
Non-State				
			Non State Sub Total	**-**
			Funds Total	**-**

Total Project Cost: $468,000

This amount accurately reflects total project cost?

Yes

Attachments

Required Attachments

Visual Component

File: 66b80d5e-a5f.pdf

Alternate Text for Visual Component

This technique is quick and requires minimal effort, providing results within 30 minutes after sample collection by generating a color-based detection. The figure shows 4 main steps in using the color-based method: sample collection, DNA extraction, loading eDNA onto plates, and color change and final detection of fish and mussels....

Supplemental Attachments

Capital Project Questionnaire, Budget Supplements, Support Letter, Photos, Media, Other

Title	File
Letter of approval from UMN Regents / SPA	11cadfe1-e76.pdf

Administrative Use

Does your project include restoration or acquisition of land rights?

No

Do you understand that travel expenses are only approved if they follow the "Commissioner's Plan" promulgated by the Commissioner of Management of Budget or, for University of Minnesota projects, the University of Minnesota plan?

Yes, I understand the UMN Policy on travel applies.

Does your project have potential for royalties, copyrights, patents, sale of products and assets, or revenue generation?

No

Do you understand and acknowledge IP and revenue-return and sharing requirements in 116P.10?

N/A

Do you wish to request reinvestment of any revenues into your project instead of returning revenue to the ENRTF?

N/A

Does your project include original, hypothesis-driven research?

Yes

Does the organization have a fiscal agent for this project?

Yes, Sponsored Projects Administration

Does your project include the pre-design, design, construction, or renovation of a building, trail, campground, or other fixed capital asset costing $10,000 or more or large-scale stream or wetland restoration?

No

Do you propose using an appropriation from the Environment and Natural Resources Trust Fund to conduct a project that provides children's services (as defined in Minnesota Statutes section 299C.61 Subd.7 as "the provision of care, treatment, education, training, instruction, or recreation to children")?

No

Provide the name(s) and organization(s) of additional individuals assisting in the completion of this proposal:

Dr. Solomon David, Dr. Danny Gotarkar, Patrick McDonald (UMN), UMN SPA

Do you understand that a named service contract does not constitute a funder-designated subrecipient or approval of a sole-source contract? In other words, a service contract entity is only approved if it has been selected according to the contracting rules identified in state law and policy for organizations that receive ENRTF funds through direct appropriations, or in the DNR's reimbursement manual for non-state organizations. These rules may include competitive bidding and prevailing wage requirements

Yes, I understand