

# **Environment and Natural Resources Trust Fund**

# 2026 Request for Proposal

## **General Information**

Proposal ID: 2026-084

Proposal Title: Enhancing the Integrity of Minnesota's Waterway Bioassessment

# **Project Manager Information**

Name: Matthew Petersen Organization: U of MN - College of Food, Agricultural and Natural Resource Sciences Office Telephone: (515) 441-0882 Email: pet03207@umn.edu

# **Project Basic Information**

**Project Summary:** Water quality bioassessment using aquatic insects can be improved using DNA-based methods. This approach increase taxonomic resolution and will better detect temporal and spatial variation of Minnesota's water quality

**ENRTF Funds Requested:** \$299,000

Proposed Project Completion: September 30, 2028

LCCMR Funding Category: Small Projects (G) Secondary Category: Water (B)

#### **Project Location**

What is the best scale for describing where your work will take place? Region(s): Central, SE, NE,

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.

Aquatic insects, largely in the immature stage, are widely used to assess the integrity of Minnesota's streams and rivers based on their ecological tolerances to stressors or pollution. Most immature aquatic insects can not be identified to the species-level using morphology, meaning only genus or higher identifications are used in bioassessment programs. This means valuable data is lost and information detailing the health of aquatic communities and whether a stream is polluted, are reduced. For example, the most diverse group of aquatic stream insects are the family Chironomidae (non-biting midges), which are also the most difficult to identify to species based on morphology. Over 100 species of Chironomidae can be found in a single Minnesota stream, but in bioassessment studies this group would instead have species' tolerances averaged across 12 genera. Accurate estimates of water quality, and an ability to detect change due to natural or anthropogenic disturbance, depend on our ability to precisely identify insects. Emerging DNA-based identification tools provide an opportunity to overcome many of these biomonitoring challenges by providing consistent species-level identification. Additionally, the use of DNA-based identification tools can also determine the presence of aquatic species of concern, which currently can only be identified as adults.

# 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.

We aim to safeguard Minnesota's rivers and streams by developing a DNA-based aquatic monitoring tool that will reduce processing time and costs, identify species that are otherwise missed using traditional assessment methods, and return greater resolution in freshwater bioassessments. We will build off of existing protocols used by the Minnesota Pollution Control Agency (MPCA) to assess surface water quality using aquatic insects. Critical to our research will be the development of DNA mitogenomic libraries that allow accurate species-level identifications to be made. By extracting DNA from adult life-stages, where determination is possible, we can match DNA sequence data to immature stages, thus making immature insect identification possible. This procedure will open the ability to utilize species-level data in biomonitoring for groups that are difficult to identify morphologically as larvae. We will carry out a study that compares traditional methods of freshwater assessment based on morphological identification with a modern DNA-based approach (i.e., metabarcoding). We will test the hypothesis that increased taxonomic resolution will allow greater accuracy in biotic assessment and more readily detect temporal and spatial variation of Minnesota's water quality. This work will also assign tolerance values to aquatic insect species, increasing resolution over generic tolerance values.

# 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 outcomes of this work will support decision-making efforts related to Minnesota waterways. Accurate, consistent, and timely assessment of water quality will be enhanced. Our created DNA library will document, catalog, and sequence aquatic insects in Minnesota, including species of conservation concern, thereby creating the capability to implement a DNA-based assessment approach. We will determine species-level tolerance values to pollution that can be used in biological tools. Our research will build off of existing monitoring capabilities to provide local decision-makers, landowners, and the public an efficient, cost-effective, and consistent approach to assess and protect water resources.

# Activities and Milestones

# Activity 1: Building capacity for a modern DNA-based assessment approach

Activity Budget: \$85,748

#### **Activity Description:**

DNA metabarcoding has become a standard approach for identifying species using short sections of DNA, often the COX1 gene. This technique offers cost-effective solutions, enhances detection capabilities, and can reduce the need for lethal specimen collection. Additionally, it can be applied to identify insect life-stages, such as pupae and larvae, where identification to species level is not possible based on morphology. This approach relies on reference databases of sequences associated with known species identity. Although existing databases for some aquatic insect groups are robust (e.g., mayflies, caddisflies), they are poor for others. We will focus on the fly family Chironomidae (non-biting midges), the most diverse group of aquatic insects where the capacity to identify species as immatures is impossible. Modern barcoding approaches utilize multiple genes across the mitochondrial genome ("mitogenome"), greatly increasing detection probability. The hired Technician will be in charge of generating resources for DNA-based identifications, where they will create mitogenomes for MN midges. We will sample adult midges from target sites (Activity 2), identify them to species, extract DNA, then develop mitogenomes for each species. These mitogenome libraries will allow for current DNA-based assessment (Activity 2) and for testing of individual tolerances for aquatic midges (Activity 3).

#### **Activity Milestones:**

Description	Approximate
	Completion Date
Collection and identification of adult insects	September 30, 2026
DNA extraction, amplification, and sequencing	July 31, 2027
Publishing of DNA databases	July 31, 2028

## Activity 2: Assessing a modern DNA-based approach to biotic assessment

#### Activity Budget: \$106,626

#### **Activity Description:**

Biotic assessment of streams and rivers based on aquatic benthic macroinvertebrates are recommended to determine the integrity of aquatic ecosystems. This approach, which is widely adopted by the Minnesota Pollution Control Agency (MPCA) and many other states, can provide long-term assessment data that detects the impacts of human activities, or natural disturbances, by assessing faunal turnover and loss of sensitive taxa. Our project will build off of this existing framework by developing and testing a modern DNA metabarcoding approach. Metabarcoding extracts, often nondestructively, DNA from bulk insect samples before amplifying, sequencing, and determining the identity of included species. This approach can provide finer-detail taxon identification over a traditional morphology-based approach, thus increasing the resolution possible by biotic assessment. We will sample aquatic macroinvertebrates using a standardized MPCA approach for bioassessment across a continuum of sites within target watersheds (e.g., Cannon, Snake River). At each site we will determine community composition using i) standard morphological identifications and ii) metabarcoding. The goals of this activity are to then assess the degree to which metabarcoding increases species identification potential and to determine if sampled insect communities between the two approaches are equivalent.

#### **Activity Milestones:**

•	Approximate Completion Date
Assess MPCA data and locate sampling sites	August 31, 2026

Year 1 macroinvertebrate collections	September 30, 2026			
Specimen identification (DNA, Morphology) January 31, 2				
Year 2 macroinvertebrate collections	August 31, 2027			
Specimen identification (DNA, Morphology)	January 31, 2028			
Complete Data Analyses	June 30, 2028			
Submit Publication	July 31, 2028			

#### Activity 3: Assessing species vs. genus level tolerance levels for Minnesota midges

#### Activity Budget: \$106,626

#### **Activity Description:**

Aquatic insects are used to determine indices of biotic integrity (IBI) that examine the impact of nonpoint source pollution (sediment, nutrients, other pollutants) on stream health. Examples of metrics involve the number of macroinvertebrate taxa, number of sensitive taxa (i.e., caddisflies, mayflies), or number of insensitive taxa present at a site. We will test the hypothesis that increased taxonomic resolution will increase accuracy in biotic assessment and more readily detect temporal and spatial variation of water quality. We anticipate a higher degree of granularity in specimen identification will lead to greater potential to assess water quality. This work will focus on midges and utilize the genomic resources developed in Activity 1 and be conducted as sites from Activity 2. MIdges are an ideal group as they are highly diverse, often environmentally sensitive, but currently underutilized because they can not be identified to species. We will use a threshold indicator taxa analysis (TITAN) to identify species that are sensitive to biotic change and determine threshold levels at which water quality decrease causes insect assemblages to change. We will contrast the highly sensitive barcoding approach to better capture environmental change against the traditionally used morphological-based approach.

#### **Activity Milestones:**

Description	Approximate Completion Date
Year 1 water collections	September 30, 2026
Year 2 water collections	August 31, 2027
Specimen identification (DNA)	January 31, 2028
Complete Data Analyses	June 30, 2028
Submit Publication	July 31, 2028

# **Project Partners and Collaborators**

Name	Organization	Role	Receiving Funds
Dr. Will Bouchard	Minnesota Pollution Control Agency	Dr. Bouchard will advise on location choice for aquatic insect sampling. He will lend support in his role as a taxonomic expert on midges (Chironomidae) which are an emphasis of this project.	No
Dr. Cristian	University of	Dr. Beza-Beza will coordinate the molecular work associate with the construction	No
Beza-Beza	Minnesota	of the DNA database.	

# 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?

This study will provide critical information needed to modernize water quality bioassessment in Minnesota. Modern DNA-based approaches like those proposed here can save time and money while increasing assessment resolution. All study results will be shared with the MN DNR, Minnesota Pollution Control Agency(MPCA), and Minnesota tribes as a way of highlighting the approaches and findings of this study. By basing our study methods on MPCA protocols, outcomes of our study will be directly comparably to currently used protocols. We will additionally use the findings of this research as a proof of concept for further research funding proposals.

# Other ENRTF Appropriations Awarded in the Last Six Years

Name	Appropriation	Amount Awarded
Monitoring Minnesota's Insects: Connecting Habitat to	M.L. 2024, , Chp. 83, Art. , Sec. 2, Subd. 03f	\$199,000
Insect Prey		

# Project Manager and Organization Qualifications

Project Manager Name: Matthew Petersen

Job Title: Associate Teaching Professor

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

Dr. Matt Petersen is an insect researcher focused on determining the underlying mechanisms responsible for insect presence and abundance. As an ecologist and taxonomist, he has been involved in large-scale initiatives to survey and document insect diversity. He has led river and stream biomonitoring initiatives based on aquatic insect community composition and has published on the application of DNA-based methods for aquatic insect monitoring. The goal of his program is to highlight how understanding the ecology of individual insect species can lead to better informed habitat management practices, sustained populations of beneficial insects, and assessment of environmental habitat quality.

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

#### **Organization Description:**

College of Food, Agricultural and Natural Resources Sciences (CFANS) is comprised of Twelve academic departments and 10 research and outreach centers, along with the Minnesota Landscape Arboretum, the Bell Museum, and dozens of interdisciplinary centers. As part of a major urban university located in the heart of the Twin Cities, we also provide immersive study opportunities across the state. Our living laboratories allow students, faculty, and staff to study throughout Minnesota's diverse ecosystems.

# Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineli gible	% Bene fits	# FTE	Class ified Staff?	\$ Amount
Personnel								
Principle Investigator		Principle Investigator			37%	0.12		\$20,773
Lab Technician		to conduct molecular analyses			37%	1.5		\$107,632
Graduate Research		to lead field research and analyze data			37%	2		\$106,311
Undergraduate Researcher		to assist the graduate student with field research			0%	0.5		\$18,099
							Sub Total	\$252,815
Contracts and Services								
							Sub Total	-
Equipment, Tools, and Supplies								
	Tools and Supplies	Field supplies are needed for specimen collection [Slides and Cover slips (\$2,290), Euparal (\$200/100ml), 100% ethanol (\$2,000; \$518.50/4L*4); KOH (\$186.80/500), Glacial acetic acid (\$30), 2-dram vials (\$1,423; 142.34/144*10), Forceps (\$100), Malaise traps (\$150*3), Sweep nets (\$300), Dip nets (\$343)]. Lab materials are needed for DNA extraction and amplification [centrifuge tubes: (\$416/500), pipetted tips and vials (\$556)]. Reagents for DNA extraction (\$880), DNA amplification (\$2,150/1000 reactions).	to conduct field research, curate collected material, and conduct molecular analysis					\$11,325
							Sub Total	\$11,325
Capital Expenditures								
							Sub Total	-
Acquisitions and Stewardship								

				Sub Total	-
Travel In Minnesota					
	Miles/ Meals/ Lodging	Funds will offset travel to field sites for insect collections in Minnesota. Vehicle rental: 1 vehicle for 3 months each during project duration @ \$1,100/month/vehicle = \$6,600. Mileage for 2 years of fieldwork requiring 12,000 miles (200 miles/event*30 events*2 yrs) of travel for collecting adult and larval samples @ \$0.23 per mile = \$2,760).	for field collection of research samples		\$9,360
				Sub Total	\$9,360
Travel Outside Minnesota					
	Conference Registration Miles/ Meals/ Lodging	Two trips to the annual meeting of the entomological society of America (\$1,000 * 2 yr).	Travel support for Graduate Research Assistant and Project Assistant to attend one professional meeting each year for 2 years. Presentations are needed to disseminate project methods, results, and implications at the annual meeting of the entomological society of America		\$2,000
				Sub Total	\$2,000
Printing and Publication					
				Sub Total	-
Other Expenses					
		DNA sequencing at the UMN Genomics Center for next generation (estimate ~40 samples + library) and sanger sequencing (\$7 sample @ 100 samples) (\$8,000); mitogenomes (\$15,000),	to sequence DNA from collected material		\$23,500
				Sub Total	\$23,500
				Grand Total	\$299,000

# Classified Staff or Generally Ineligible Expenses

Category/Name	Subcategory or Type	Description	Justification Ineligible Expense or Classified Staff Request
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## Non ENRTF Funds

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

Total Project Cost: \$299,000

This amount accurately reflects total project cost?

Yes

# Attachments

#### **Required Attachments**

*Visual Component* File: <u>fae45e9b-7d5.pdf</u>

#### Alternate Text for Visual Component

Title: Enhancing the Integrity of Minnesota's Waterway Bioassessment. Image comparing morphology-based vs. DNAbased methods for identifying aquatic insects that are used in bioassessment. Minnesota's water quality bioassessment will be enhanced by developing a high-resolution DNA-based protocol based on DNA libraries and enhanced tolerance information for aquatic species....

#### Supplemental Attachments

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

Title	File
LCCMR Approval Letter	<u>13b6afc0-9b7.pdf</u>

#### 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?

No

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?

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:

Andrea Little - University of Minnesota

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

N/A