

# **Environment and Natural Resources Trust Fund**

2024 Request for Proposal

# **General Information**

Proposal ID: 2024-260

Proposal Title: Biogeography of Cyanobacteria and Their Toxins Across Minnesota

# **Project Manager Information**

Name: Beatriz Baselga Cervera

Organization: U of MN - College of Biological Sciences

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# **Project Basic Information**

**Project Summary:** Knowledge regarding the occurrence and spread of cyanobacteria and their cyanotoxins across time and space is only in its infancy. We propose a systematic phylogenetic survey of Minnesota freshwater bodies.

Funds Requested: \$285,000

Proposed Project Completion: December 31, 2026

**LCCMR Funding Category:** Water Resources (B)

# **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?

In the Future

# **Narrative**

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

With the current development of molecular techniques, microbial biogeography has been widely studied. Recent studies have challenged the "cosmopolitan microbial rule" (everything is everywhere), identifying geographical isolation and dispersal barriers in some microbial groups. However, this field has moved at a relativity slower pace in some bacterial phyla, like cyanobacteria.

Cyanobacteria is a diverse phylum of photosynthetic microorganisms that inhabits a wide range of aquatic ecological niches. Despite their ecological role as atmospheric carbon and nitrogen fixer, cyanobacteria can pose serious risks to aquatic bodies and their users when they undergo rapid growth (blooms). Harmful algae blooms (HABs) can reduce water quality by generating anoxic conditions and releasing odors, off-flavors, and cyanotoxins. Cyanotoxins are produced by a variety of cyanobacteria genera and present toxic effects to both aquatic and terrestrial fauna as well as humans.

Within Minnesota, there is an increasing concern regarding the increased incidence, persistence, and toxicity of blooms. There is an increased focus on understanding and predicting cyanobacterial blooms. But there is no comprehensive survey of the occurrence of cyanobacteria and their toxins across Minnesota. Here, we propose a systematic phylogenetic survey of Minnesota water bodies to identify patterns across space and time.

# 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 propose the study of distribution patterns of cyanobacteria and their cyanotoxins across the estate of Minnesota. Knowledge of cyanobacteria and cyanotoxins biogeography across Minnesota will shed light on population distributions, diversity, and species mobility (invasion and dispersal).

Employing molecular techniques, we propose to use several genetic markers of Cyanobacteria and cyanotoxin genes (multi-gene analyses) to determine the genetic diversity and population structure at multiple locations across Minnesota. We propose a sampling design of thirty water bodies across the four ecological regions of the state across two years. This design will include multiple water bodies, ranging from lakes to shallow water bodies. We propose sampling twice each year, one at the beginning and the other in the middle of the warm months, to be able to make predictions regarding blooms.

This study will contribute to the taxonomic, environmental, and geographical diversity knowledge of cyanobacteria and their cyanotoxins in Minnesota. As a result, this research will help the State to achieve the UN Sustainable Development Goals 3 (good health and well-being), 6 (clean water), and 14 (life below water).

# 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 biogeographical survey of Minnesota's cyanobacteria and their cyanotoxins will result in an extensive database (outcomes (O) O1 & O2) of geographical and temporal data. This database will be widely available and accessible to researchers, state holders, and the public. Phylogenetic and taxonomic analyses will result in at least two publications (O3 to O5) addressing questions regarding population structure, ecological segregation, and species dispersal. Generated information can lead to better monitoring, management, regulation, and advice to neighborhood associations, local stakeholders, and government agencies.

#### **Activities and Milestones**

#### Activity 1: Sampling Design and sampling campaigns.

**Activity Budget: \$50,000** 

#### **Activity Description:**

We will create a sampling design (milestone 1 (M1)) across the four ecological regions of the state of Minnesota that will include thirty water bodies. Within each ecological region, seven or eight water bodies that span diverse water body types, from shallow waters to lakes, and land usage. We will purposely include some of the Sentinel Lakes' long-term lake ecosystem monitoring program locations.

Over two years, we will sample twice each year (2025 (M2) and 2026 (M3)), once at the beginning and the other at the hottest weeks of the warm months, to be able to make predictions regarding blooms. Samples at the beginning of the warm seasons will be taken both from the sediment and at different water depths if thermal stratification is present (between April and May). The second annual sampling will take place in mid-August, taking samples of the water column. During the sampling, water profile measurements (e.g., pH, oxygen dissolved, etc.) will be collected. Samples will be preserved and stored refrigerated in the field and frozen in the laboratory.

The proposed budget for this activity will cover lodging and mileage to carry out the samplings.

#### **Activity Milestones:**

Description	Approximate	
	Completion Date	
Set a sampling design: identify the number of the locations of the sampling sites (M1)	December 31, 2024	
Sampling campaign year 1: carry out an effective sampling (M2)	September 30, 2025	
Sampling campaign year 2: carry out an effective sampling (M2)	September 30, 2026	

# Activity 2: Sequencing and data collection

Activity Budget: \$60,000

#### **Activity Description:**

Employing molecular techniques, we propose to use several genetic markers to detect, identify, quantify, make a profiling, and determine the genetic diversity of cyanobacteria and cyanotoxins across Minnesota water bodies. To identify cyanobacteria, specifically; 16S-23S ITS, phycocyanin intergenic spacer region (PC-IGS), nifH, nifD, rpoC1, rbcLX, rbcS, rbcS, hetR, and ftsZ. In the case of cyanotoxins, we will target the detection of microcystin (mcy gene cluster), nodularin (nda genes), cylindrospermopsin (cyr genes), and saxitoxin (sxt cluster).

Samples will be stored in cold temperatures until analyses. DNA will be extracted, isolated, and submitted for sequencing to the University of Minnesota Genomic Center (UMGC). With the support of the UMGC, a library preparation will be created for Illumina high-throughput sequencing (output 1 (O1)).

The proposed budget for this activity will cover sequencing costs.

#### **Activity Milestones:**

Description	Approximate Completion Date
DNA extraction isolation and sequencing (year 1) (M4).	December 31, 2025
Library preparation (M5).	March 31, 2026
DNA extraction isolation and sequencing (year 2) (M6).	December 31, 2026

#### Activity 3: Data analyses

Activity Budget: \$175,000

#### **Activity Description:**

Post-sequence processing will be conducted to merge reads, screen, trim, align and classify the sequences as operational taxonomic units (OTUs). Resulting of these analyses we expect to create a database focusing on the biogeography of cyanobacteria and cyanotoxins in Minnesota (O1).

Community diversity analyses (richness and diversity indexes) and toxic profiles of the obtained sequences will be crossed with geographical, land-use, type of water body, sample type, and temporal data. Resulting of these analyses we expect to generate two to three scientific publications and several conference presentations (O2-O4).

The proposed budget for this activity will cover the salary and fringe costs of a two-year postdoctoral researcher that will work in the sampling, processing, and analysis of the generated data.

# **Activity Milestones:**

Description	Approximate Completion Date
Biogeography database of cyanobacteria and cyanotoxins in Minnesota (O1)	June 30, 2026
Publications and scientific communications (O3-O5)	December 31, 2026

# **Project Partners and Collaborators**

Name	Organization	Role	Receiving Funds
Michael	Univeristy of	Co-manager	Yes
Travisano	Minnesota		

# 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?

The proposed project includes a long-term plan set for two years, extendible for another two into the future, to address not only the cyanobacteria and their toxin patterns across space (the state of Minnesota) but also across time (four years). During this time, additional partners (e.g., St. Croix Watershed Research Station) and funding (e.g., USGS/NIWR Competitive Grants) will be obtained to add other survey data to the existing database. Additional partners and funding will strengthen the data collection, resulting in better resolution, and the generation of new testable hypotheses to inform future policies.

# **Project Manager and Organization Qualifications**

Project Manager Name: Beatriz Baselga Cervera

Job Title: Postdoctoral researcher

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

Experimental Microbiologist with interdisciplinary laboratory-field background and practice. Specialized in linking ecological, evolutionary, and applied scientific methods within the fields of adaptive microbial diversity and freshwater microbiology. 10+ years of research experience in the field of freshwater microbiology and limnology. Eighteen scientific publications in the field of applied limnology, freshwater microbial diversity, and experimental microbiology (orcid.org/0000-0003-3423-4780). Two patents (ES2531010B2 / WO2014087030A1 and ES2529362B1 Spain) using freshwater microorganisms to degrade cyanotoxins and selectively uptake heavy metals. Currently, co-Pi in an MNDrive Environment grant addressing: Cyanotoxin Bioremediation with Microbes. Advanced skill set in data analysis, statistics, science communication, wet laboratory safety & management, and microbial techniques (culturing and molecular methods for yeast, microalgae, and cyanobacteria). Five years of experience running independent research projects and coordinating undergraduate and graduate students.

Organization: U of MN - College of Biological Sciences

#### **Organization Description:**

The UMN Twin Cities is among the nation's top public research universities. The School of Biological Sciences at the UMN supports a diverse research profile, from molecules to ecosystems from investigating the mechanisms driving disease to new approaches to monitoring changes in biodiversity. CBS has an annual operating budget of \$103 million and has a robust research portfolio including sponsored research expenditures of \$33 million for the fiscal year 2022. CBS is on the leading edge in research, defining the future of the biological sciences. Concretely, The UMN was ranked #2 in the world for ecology and environment in 2022 due to their researchers' achievements.

# **Budget Summary**

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineli gible	% Bene fits	# FTE	Class ified Staff?	\$ Amount
Personnel								
Postdoctoral researcher		Researcher's salary to support with sampling, processing, and analyses of the samples. UMN job code: 9546. Ph. D. qualification. Salary and fringe cost for two years. First year salary: \$55,000 + fringe (21%) Second year salary: \$57,000 + fringe (21%) Additional costs (moving costs, increased salary based on experience, etc.): \$14,000			21%	54		\$150,000
							Sub Total	\$150,000
Contracts and Services								
							Sub Total	-
Equipment, Tools, and Supplies								
	Tools and Supplies	Ambar glass vials, microalgae nets, core tube samples, PCR regalements (dyes, primers, agarose, rulers, Tag polymerase, etc.)	Field and laboratory supplies to obtain and process the water and soil samples from Minnesota lakes					\$5,000
	Equipment	Ultra-Low freezer (x1)	Preserve the samples and the microbes within. It will ensure the long-term storage of the samples for future studies.					\$20,000
	Tools and Supplies	Illumina sequencing and library preparation costs.	Illumina NextSeq 2000 Next Generation Sequencing and library preparation costs of 180 samples using multiple markers. Estimated cost based on the UMN Genome Center.					\$60,000
	Equipment	Water quality Multiprobe (x1)	Multiparameter sonde for monitoring water quality in freshwater.					\$5,000
			. ,				Sub Total	\$90,000
Capital Expenditures								
							Sub Total	-

Acquisitions and Stewardship					
				Sub Total	-
Travel In Minnesota					
	Miles/ Meals/ Lodging	Sampling across Minnesota costs for 5 trips of 3 or 4 days. The estimated cost of identifying and sampling 30 locations across the state. The budget will cover mileage, meals, and lodging.	Initial trips will be carried out to identify the water bodies and create the sampling design. Afterward, we propose 4 sampling campaigns of the 30 locations over two years.		\$20,000
				Sub Total	\$20,000
Travel Outside Minnesota					
	Conference Registration Miles/ Meals/ Lodging	Attendance to two conferences (domestic or international (e.g., ASM, ISME, Gordon conferences)) one each year for three people (both managers and hired postdoctoral researcher).  Approximate cost per person and conference of \$2,500. Including conference registrations, lodging costs, transportation (mileage/airfare), and per diem.	Domestic and international conferences are perfect examples to disseminate cutting-edge scientific knowledge to the scientific community. It is also the perfect venue to find partners and talk with funding agents.		\$15,000
		(		Sub Total	\$15,000
Printing and Publication					
	Publication	Open access articles processing charger. Two to three high-impact publications in scientific journals such as Phycology, Plos One, Frontiers in Microbiology	Open access to scientific content offers unrestricted access to the papers and the science.		\$10,000
				Sub Total	\$10,000
Other Expenses					
				Sub Total	-
				Grand Total	\$285,000

# Classified Staff or Generally Ineligible Expenses

Category/Name	Subcategory or	Description	Justification Ineligible Expense or Classified Staff Request
	Туре		

# Non ENRTF Funds

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

# **Attachments**

#### **Required Attachments**

Visual Component

File: 5c15004c-42b.pdf

Alternate Text for Visual Component

Figure 1. A) Neon-blue bloom on Burnt Lake in the Boundary Waters in Sept. 2022 (photo source: Lienne Sethna). B) Map of the Ecological Regions across Minnesota from where thirty water bodies locations will be selected....

# **Optional Attachments**

Support Letter, Photos, Media, Other

Title	File
Board Resolution ro be requested	<u>b5d5086c-8c5.pdf</u>

#### Administrative Use

Does your project include restoration or acquisition of land rights?

No

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

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 design, construction, or renovation of a building, trail, campground, or other capital asset costing \$10,000 or more?

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?

No