Environment and Natural Resources Trust Fund

2023 Request for Proposal

General Information

Proposal ID: 2023-233

Proposal Title: Reducing Beach Closures through Improved Microbiological Monitoring

Project Manager Information

Name: Raymond Hozalski

Organization: U of MN - College of Science and Engineering

Office Telephone: (612) 626-9650

Email: hozal001@umn.edu

Project Basic Information

Project Summary: The goal of this research will be to develop better, faster, and more reliable methods for determining whether Minnesota's lakes are unsafe for swimming, hopefully limiting unnecessary beach closures.

Funds Requested: $726,000

Proposed Project Completion: June 30, 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?

During the Project

Narrative

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

Microbes that make people sick, called pathogens, can enter Minnesota lakes from domestic sources like old or poorly functioning septic systems and from animal waste released directly into lakes (e.g., by geese, beavers) or onto the surrounding land (i.e., watershed) where it can be washed into the lake via runoff. Exposure to many pathogens that may be present in lakes requires that the water be ingested, either intentionally or accidentally, via the so-called fecal-oral route. Lake water quality is currently monitored by measuring total coliforms and/or E. coli in the water via traditional culture-based methods; if levels of total coliforms or E. coli become excessive, then swimming beaches can be closed. These traditional culture-based testing methods, however, merely indicate the *potential* for pathogenic organisms in our lake water, not the presence of *genuine* pathogenic organisms. This study will test and validate quantitative PCR methods for directly quantifying pathogenic microbes in lake water. The goal of this research will be to develop better, faster, and more reliable methods for determining whether Minnesota's lakes are unsafe for swimming, hopefully limiting unnecessary beach closures while simultaneously ensuring that swimming in potentially unsafe waters is avoided.

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 to monitor 50-60 lakes throughout Minnesota over a 2-year period to better understand the spatial and temporal variability of harmful microbes in Minnesota lakes. We will assess spatial variability both statewide, by sampling lakes from the far south to the Canadian border, and within lake by sampling at several locations in each lake, including designated beaches and swimming areas. The standard approach for assessing fecal contamination is based on cultivating coliform bacteria which takes at least 36 hours of incubation not including sampling and sample preparation. New molecular biology techniques can provide results much faster, within hours of sampling. These new techniques can also be more reliable and more specific. They do not simply quantify all coliform bacteria but can target the pathogenic strains with high specificity, which can aid in reducing the frequency and duration of beach closures significantly. We will develop and evaluate new molecular assays to quantify harmful microbes in lake water samples and in lake sediment samples based on quantitative PCR (qPCR) and flow cytometric fingerprinting (FCM). We will compare these new qPCR and FCM techniques with the current standard approaches for microbial water quality assessment to improve Minnesota's future water quality monitoring capabilities.

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?

Research project findings will provide the state with the newest techniques to monitor biological water quality in our recreational waters. These new techniques will minimize unnecessary beach closures and enable the state to be proactive and efficient in prioritizing lakes for future monitoring of harmful microbes to conserve precious state resources. The research also expects to identify watershed management strategies for reducing harmful microbe levels, such as buffer strips or other stormwater treatment approaches used to reduce nutrient or bacterial inputs to surface waters via stormwater runoff.

Activities and Milestones

Activity 1: Quantify pathogens in water and sediment from Minnesota Lakes

Activity Budget: $450,000

Activity Description:
Water and sediment samples will be collected from five distinct clusters of lakes within the State of Minnesota; each cluster will include 10-12 lakes. These clusters will be located in southern Minnesota (vicinity of Albert Lea), the Twin Cities Metro area, central Minnesota (the Brainerd Lakes area), north-central Minnesota (near and within Itasca State Park), and northeastern Minnesota (Boundary Waters area). Each sampling event will include collecting water samples (~ 1.0 liter) from three near-shore areas and one open water area. We also plan to collect sediment samples from at least one near-shore location as some harmful microorganisms can be associated with sediments. Each lake will be sampled at least 3 times per year during the swimming season for 2 consecutive years for totals of 300-360 sediment samples, and 1200-1440 water samples. We will perform standard culture-based coliform testing on all water samples using the Colilert method. We will extract the DNA from the microorganisms in the water and sediment samples and then use quantitative PCR (qPCR) to quantify pathogens of interest. Flow cytometric fingerprinting will be used on selected water samples to calibrate the technique based on the cultivation and DNA-based results.

Activity Milestones:

Description	Completion Date
Sample collection and Genomic DNA extractions	October 31, 2025
Quantification of pathogens via qPCR and FCM	December 31, 2025
Data analysis	May 31, 2026

Activity 2: Assess lakes sampled in Activity 1 for conventional water quality indicators and physical characteristics and correlate with pathogen occurrence

Activity Budget: $150,000

Activity Description:
We will use water quality sensors to analyze for temperature, dissolved oxygen, light, and pH as a function of depth at the time of sampling. We will also collect water samples and return them to the laboratory for analysis of chloride (an indicator of stormwater runoff from roads, parking lots, and sidewalks), nitrogen and phosphorus (nutrients that can stimulate algal growth), chlorophyl (an indicator of algal abundance and eutrophication), total suspended solids (which affects water clarity), and total organic carbon/color which are associated with inputs from forests and wetlands. Color is of interest because it provides protection for microorganisms in the water column by absorbing solar irradiation including UV light that can damage DNA but it also blocks light from reaching algae, inhibiting their growth. We will evaluate the water quality data for statistical correlations with pathogen concentrations. We will also obtain data on maximum and average lake depth, lake area, watershed area, land use, and other parameters. Identification of water quality or other parameters that correlate strongly with pathogen concentrations will potentially provide state or county agencies with simple low-cost screening tools for identifying lakes that may be at risk of pathogen contamination to prioritize for follow-up testing.

Activity Milestones:

Description	Completion Date
Water sample collection and field measurements	August 31, 2025
Water quality analyses	December 31, 2025
Data Analysis	May 31, 2026

Activity 3: Quantify coliforms and fecal coliforms in lake water samples

Activity Budget: $126,000

Activity Description:

We will employ commercial test kits (i.e., Colilert from IDEXX) to quantify total coliforms and E. coli in the lake water samples as an indicator of harmful microorganisms in the lakes. Coliform testing is a standard water quality assessment approach for lakes to ensure their safety for swimming. We will evaluate whether such a simple, standardized, and widely available test provides any correlation with results from our highly specific DNA-based testing of harmful microorganisms including known pathogenic strains of E. coli, other pathogenic bacteria, cyanotoxin-producing algae, and pathogenic amoeba. Quantified pathogen levels (Activity 1 and 2) will be statistically analyzed to quantify their signatures (mean, standard deviation, probability distribution) and correlation with physiochemical water quality parameters and precipitation pattern at time of sampling. The data will be augmented in two categories, including the days with dry weather and wet weather (i.e., during and up to 48 hrs after a storm). The analyzed data will be crucial for the development and verification of new, faster, and more specific monitoring strategies to detect pathogens in Minnesota's lakes.

Activity Milestones:

Description	Completion Date
Water sample collection	August 31, 2025
Colilert testing for coliforms and E. coli	September 30, 2025
Data Analysis	May 31, 2026

Project Partners and Collaborators

Name	Organization	Role	Receiving Funds
Timothy M. LaPara	University of Minnesota	Co-project manager	Yes
Sebastian Behrens	University of Minnesota	Co-project manager	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?

We will share results with the Minnesota Pollution Control Agency, Minnesota Department of Health, as well as regional Park and Recreation boards. We will work with the responsible states agencies to develop press releases to more broadly disseminate our findings. State and county officials will be informed about the use and implementation of the new monitoring techniques for pathogens and about their time and cost advantages in comparison to business-as-usual approaches. They can then use our recommendations to future-proof their routine monitoring for pathogens to ensure that Minnesota lakes remain safe for swimming and other recreational uses.

Other ENRTF Appropriations Awarded in the Last Six Years

Name	Appropriation	Amount Awarded
Bacterial Assessment of Groundwater Supplies Used for Drinking Water	M.L. 2016, Chp. 186, Sec. 2, Subd. 04f	$299,000
Assessment of Surface Water Quality With Satellite Sensors	M.L. 2016, Chp. 186, Sec. 2, Subd. 04i	$345,000
Evaluate Emerging Pathogens in Lakes, Rivers, and Tap Water to Keep Drinking Water Safe	M.L. 2018, Chp. 214, Art. 4, Sec. 2, Subd. 04f	$325,000
Improving Drinking Water for Minnesotans through Pollution Prevention	M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 04f	$345,000

Project Manager and Organization Qualifications

Project Manager Name: Raymond Hozalski

Job Title: Professor

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

Dr. Hozalski has been a professor at the University of Minnesota since 1997 in the Department of Civil, Environmental, and Geo- Engineering. His research primarily focuses on surface water quality, drinking water treatment, and water distribution and he has worked with water utilities throughout Minnesota, around the USA, and in Europe. He has authored or co-authored 87 manuscripts published in the peer-reviewed technical literature. According to the Web of Science, his research has been cited more than 2,700 times by other peer-reviewed research publications. He has served as a member of the U.S. Environmental Protection Agency's Science Advisory Board Drinking Water Committee. He also has served as project manager or co-project manager on 5 LCCMR-sponsored research projects.

Organization: U of MN - College of Science and Engineering

Organization Description:

The University of Minnesota is one of the largest, most comprehensive, and most prestigious public universities in the

United States (http://www1.umn.edu/twincities/01_about.php). The laboratories directed by the project managers contain the majority of the equipment needed to perform the proposed project, including centrifuges, pumps, water meters, analytical balances, and a real-time PCR machine. The University of Minnesota also has "core facilities" that offer additional equipment, which can be used by University researchers "at cost." For this project, the core facility that is most germane is the University of Minnesota Genomics Center (UMGC; http://genomics.umn.edu). UMGC offers state-of-the-art DNA sequencing capabilities, numerous real-time PCR machines, droplet digital PCR machines, and experts available for consultation on an as-needed basis.

Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	$ Amount
Personnel								
Project manager		Manage project, supervise staff, prepare reports			33.5%	0.3		$76,042
Co-project manager		Manage project, supervise staff, prepare reports			33.5%	0.3		$68,954
Post-doctoral researcher		Coordinate sample collection and analysis, data analysis, supervise graduate and undergraduate students			20.9%	3		$205,530
Undergraduate research assistant		Assist in water and sediment sample collection and analysis			0%	3		$87,163
Sebastian Behrens		Co-project manager			33.5%	0.3		$66,334
Graduate student research assistant		Collect and analyze water and sediment samples, supervise undergraduate researchers			23.6%	1		$106,348
							Sub Total	**$610,371**
Contracts and Services								
Lab user fees	Internal services or fees (uncommon)	Use of genomics center facility for digital droplet PCR to quantify pathogens in water samples				0		$20,000
							Sub Total	**$20,000**
Equipment, Tools, and Supplies								
	Equipment	Sealer for IDEXX Colilert quantitrays	Will be used to perform standard culture-based assay for coliforms and E. coli bacteria					$5,000
	Tools and Supplies	Laboratory supplies including sampling bottles, filters, DNA extraction kits, qPCR supplies, Colilert supplies	Laboratory supplies needed for sample collection and analysis					$60,000

							Sub Total	**$65,000**
Capital Expenditures								
							Sub Total	**-**
Acquisitions and Stewardship								
							Sub Total	**-**
Travel In Minnesota								
	Miles/ Meals/ Lodging	48 trips, 12000 miles total, 2-3 people per trip, 72 nights in hotels, meals	Travel to MN lakes for water and sediment sample collection					$20,629
							Sub Total	**$20,629**
Travel Outside Minnesota								
							Sub Total	**-**
Printing and Publication								
	Publication	Journal publishing charges	Journal publishing charges for open access publications					$5,000
							Sub Total	**$5,000**
Other Expenses								
		Lab equipment repair and maintenance	Funds needed to maintain equipment used for this project					$5,000
							Sub Total	**$5,000**
							Grand Total	**$726,000**

Classified Staff or Generally Ineligible Expenses

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

Non ENRTF Funds

Category	Specific Source	Use	Status	Amount
State				
			State Sub Total	**-**
Non-State				
In-Kind	University of Minnesota	Indirect costs not charged to the project	Secured	$377,955
			Non State Sub Total	**$377,955**
			Funds Total	**$377,955**

Attachments

Required Attachments

Visual Component

File: 4d2838c2-788.pdf

Alternate Text for Visual Component

Map of the Minnesota showing major watersheds. Sign showing temporary beach closure. Comparison of methods for biological water quality testing: slow and non-specific culture-based testing versus two faster and highly specific cutting-edge molecular methods called quantitative PCR and flow cytometry....

Optional Attachments

Support Letter or Other

Title	File
University of Minnesota endorsement	d9b6c220-765.doc

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?

Yes, Sponsored Projects Administration

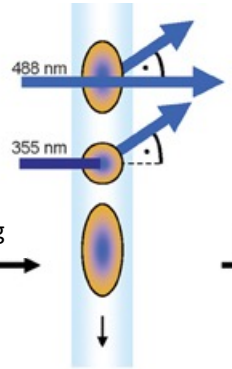
Environment and Natural Resources Trust Fund (ENRTF)
2022 Main Proposal VISUAL
Project Title: Reducing Beach Closures through Improved Microbiological Monitoring

Advanced molecular methods

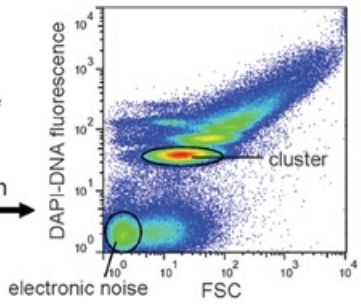
1) qPCR

2) Flow cytometry

Cell staining

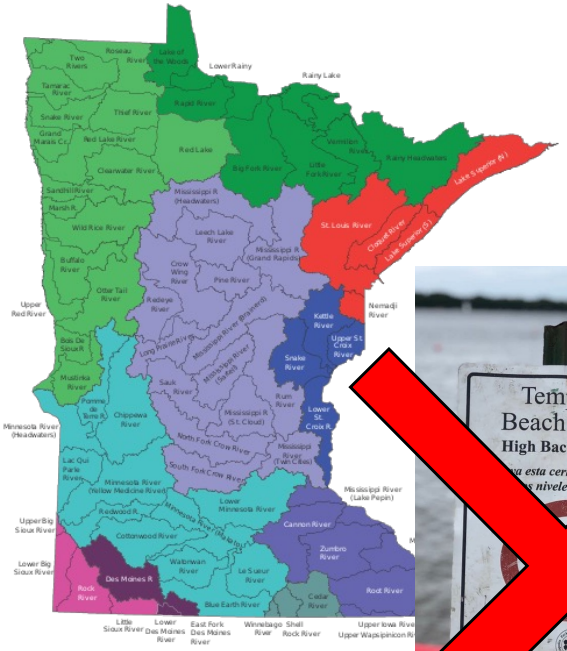


Visualization



Cytometric fingerprints

Basins and Major Watersheds in Minnesota



Standard culture-based tests

The Colilert test detects & quantifies total coliforms and *Escherichia coli*

https://www.mprnews.org/story/2019/07/10/beach-closed-in-twin-cities-blame-it-on-the-rain
Koch et al. (2014) Cytometric fingerprints: evaluation of new tools for analyzing microbial community dynamics. *Front. Microbiol*. 5:273
Fernández-Baca et al. (2021) Rapid qPCR-Based Water Quality Monitoring in New York State Recreational Waters. *Front. Water* 3:711477