



Environment and Natural Resources Trust Fund

2022 Request for Proposal

General Information

Proposal ID: 2022-098

Proposal Title: Assessing Prevalence of Brain-Eating Amoeba in Minnesota Lakes

Project Manager Information

Name: Raymond Hozalski

Organization: U of MN - College of Science and Engineering

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Project Basic Information

Project Summary: This research project will determine the presence and concentration of the brain-eating amoeba (*Naegleria fowleri*) in Minnesota Lakes and identify lake characteristics that correlate with occurrence of the amoeba.

Funds Requested: \$487,000

Proposed Project Completion: June 30 2025

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.

Naegleria fowleri is a waterborne pathogen that can infect humans after having contaminated water enter their nose. Once the organism enters nasal or olfactory nerve tissue, it migrates to the brain where it consumes brain tissue. Symptoms of this rare but serious infection, termed primary amoebic meningoencephalitis (PAM), appear within a few days of exposure and initially include headache, nausea, and fever; the disease then progresses to confusion, loss of balance, and seizures, and ultimately ends in death of nearly all patients (>95%). The organism occurs naturally in warmer surface waters, such as those in southern states in the USA. Nevertheless, the organism appears to be “moving north” as *Naegleria fowleri* was detected in Lily Lake in Washington County, now closed to swimming, following two deaths from PAM of children who swam in the lake. PAM from *Naegleria fowleri* exposure is also the suspected cause of death of two other children after swimming in Minnesota lakes (Fawn Lake in Stacy and Island Lake near Bemidji), but was not confirmed by autopsies. Several other lakes in Washington County have tested positive for the amoeba, but not every year they were sampled. The reason for the variability over time is unclear.

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

We propose to monitor numerous lakes in Minnesota over a 2-year period to better understand the natural life cycle of *Naegleria fowleri* in Minnesota lakes. We will assess spatial variability in *Naegleria fowleri* levels both statewide, by sampling lakes from the far south to the Canadian border, and within lake by sampling at several locations in selected lakes. We will quantify *Naegleria fowleri* in lake water samples by extracting DNA from the microorganisms in the water and analyzing it using a technology called quantitative PCR (qPCR). Water samples will also be analyzed for coliform bacteria as an indicator of fecal contamination, as well as conventional water quality parameters including temperature, pH, chlorophyll, total suspended solids, chloride, and nutrients (nitrogen and phosphorous). Physical lake characteristics, including maximum and average depth, will also be compiled. We will provide state and county officials with guidance for how, when, and where to monitor for *Naegleria fowleri* in Minnesota lakes to better define occurrence and risk to Minnesotans and out-of-state visitors. With improved monitoring programs and occurrence data, those officials can make informed decisions and take appropriate actions, if necessary, to limit *Naegleria fowleri* exposure such as posting warning signs and/or imposing beach closures.

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?

It is essential that Minnesota's lakes remain safe for recreational uses, including swimming. The goal of the proposed research is to prevent exposure to brain-eating amoeba via swimming and other water activities, loss of life resulting from exposure, negative publicity, and a potential decline in visitors and associated tourism revenue.

Research project findings will allow the state to be proactive and efficient in prioritizing lakes for monitoring of *Naegleria fowleri* to conserve precious state resources. The research also potentially could point to watershed management strategies for reducing *Naegleria fowleri* levels, such as reducing nutrient or bacterial inputs from stormwater runoff.

Activities and Milestones

Activity 1: Quantify *Naegleria fowleri* in water and sediment from Minnesota Lakes

Activity Budget: \$248,608

Activity Description:

Water and sediment samples will be collected from four distinct clusters of lakes within the State of Minnesota; each cluster of lakes will include 4-6 different lakes. These clusters will be located in southern Minnesota (vicinity of Albert Lea), the east Twin Cities Metro area (i.e., Lily Lake and other nearby lakes), central Minnesota (the Brainerd Lakes area), and northern Minnesota (near and within Itasca State Park). Water and sediment samples will be collected from four locations within each lake. Each sampling event will include collecting small volume water samples (0.1 to 1.0 liters) from three shallow areas and one large volume water sample (10-40 liters) from an open water area; sediment samples will also be collected from the same locations. Each lake will be sampled 6 times per year (i.e., once per winter through the ice, once per spring, once per fall, and three per summer) for 2 consecutive years for a total of 960 sediment samples, 720 small volume water samples, and 240 large volume samples. We will extract the DNA from the microorganisms in the water and sediment samples and then use quantitative PCR (qPCR) to quantify the presence of *Naegleria fowleri*.

Activity Milestones:

Description	Completion Date
Sample collection and Genomic DNA extractions	October 31 2024
Quantification of <i>Naegleria fowleri</i>	December 31 2024
Data analysis	March 31 2025

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

Activity Budget: \$194,714

Activity Description:

We will use in situ 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), nitrate/ammonia/total nitrogen/phosphate/total phosphorus (nutrients that can stimulate algal growth), chlorophyll (an indicator of algal growth and eutrophication), total suspended solids (which affects water clarity), and total organic carbon/color which are associated with inputs from forests and wetlands. Water color is of interest because it provides protection for microorganisms in the water column by absorbing solar irradiation including UV light that can kill or damage microorganisms. We will evaluate the water quality data for statistical correlations with *Naegleria fowleri* concentrations. We will also obtain data on maximum and average lake depth, lake area, watershed area, and other lake parameters. Identification of water quality or other parameters that correlate strongly with *Naegleria fowleri* concentrations will potentially provide state or county agencies with simple low-cost screening tools for identifying lakes that may be at risk of *Naegleria fowleri* contamination to prioritize for follow-up testing.

Activity Milestones:

Description	Completion Date
Water sample collection	August 31 2024
Water quality analyses	December 31 2024
Data Analysis	March 31 2025

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

Activity Budget: \$43,678

Activity Description:

We will employ commercial test kits (i.e., Colilert from IDEXX) to quantify total and fecal coliforms in the lake water samples as an indicator of waste inputs to the lakes. Coliform testing is a standard water quality assessment for lakes to ensure their safety for swimming. We will evaluate whether such a simple, standardized, and widely available test provides any correlation with *Naegleria fowleri* occurrence. These data will also be useful in their own right as an assessment of lakes for impairment and their suitability for swimming.

Activity Milestones:

Description	Completion Date
Water sample collection	August 31 2024
Testing for coliforms	September 30 2024
Data Analysis	March 31 2025

Project Partners and Collaborators

Name	Organization	Role	Receiving Funds
Timothy M. LaPara	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 be funded?

We will share results with the Minnesota Pollution Control Agency and Minnesota Department of Health and work with those agencies to develop press releases to more broadly disseminate our findings. State and county officials can then use our recommendations to prioritize lakes for routine monitoring for the brain-eating amoeba to ensure that those lakes remain safe for swimming. The research potentially could also point to watershed management strategies that may be beneficial in limiting *Naegleria fowleri* occurrence, such as nutrient abatement. If our findings suggest generally negligible risk, then that could be promoted for the benefit of Minnesota's tourism industry.

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. He has authored or co-authored more than 80 manuscripts published in the peer-reviewed technical literature. According to the Web of Science, his research has been cited more than 2,300 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			36.5%	0.24		\$61,262
Co-project manager		Manage project, supervise staff, prepare reports			36.5%	0.24		\$55,553
Post-doctoral researcher		Collect and analyze samples, supervise undergraduate students			25.4%	3		\$199,129
Undergraduate research assistant		Assist in sample collection and analysis			0%	2.25		\$60,840
							Sub Total	\$376,784
Contracts and Services								
Lab user fees	Internal services or fees (uncommon)	Use of genomics center facility for qPCR and DNA sequencing.				-		\$30,000
							Sub Total	\$30,000
Equipment, Tools, and Supplies								
	Equipment	Two Hydrolab water quality measurement devices	Will be used to measure a suite of water quality parameters versus depth in lakes					\$15,000
	Tools and Supplies	Laboratory supplies including sampling bottles, DNA extraction kits, qPCR supplies	Laboratory supplies needed for sample collection and analysis					\$30,216
							Sub Total	\$45,216
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					\$25,000
							Sub Total	\$25,000
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	\$487,000

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				
In-Kind	University of Minnesota	Indirect costs not charged to the project	Secured	\$259,482
			Non State Sub Total	\$259,482
			Funds Total	\$259,482

Attachments

Required Attachments

Visual Component

File: [abf17217-dad.pdf](#)

Alternate Text for Visual Component

Map of the USA showing Naegleria fowleri infection cases by state. Image of a swimming advisory sign at Lily Lake. Image of water samples collected from MN lakes. Image of lake sediment sampling from canoes. Image of the brain-eating amoeba, Naegleria fowleri....

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

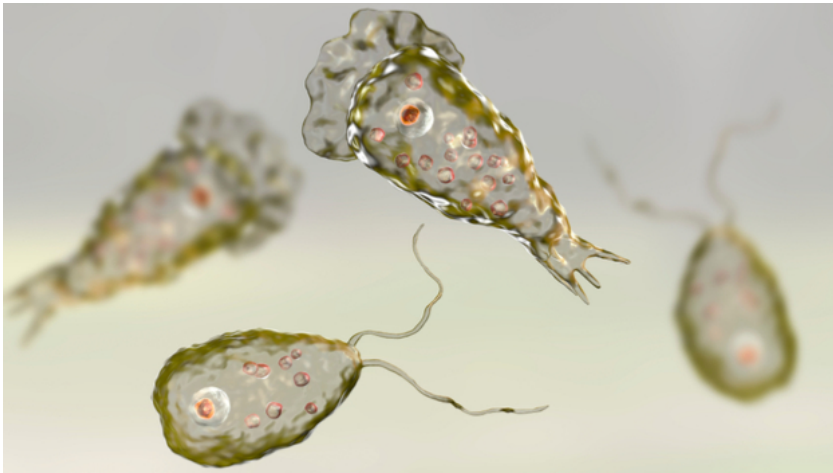
Does your project include original, hypothesis-driven research?

Yes

Does the organization have a fiscal agent for this project?

Yes, Sponsored Projects Administration

Concern is *Naegleria fowleri* in lakes, deaths, and tourism impacts



<https://www.biospace.com>



<https://healthworldnet.com>

Our approach is to sample water and sediment to test for the brain-eating amoeba

