



# Environment and Natural Resources Trust Fund

2021 Request for Proposal

## General Information

**Proposal ID:** 2021-190

**Proposal Title:** Quantitative Risk Assessment of Pathogens in Urban Waters

## Project Manager Information

**Name:** Miki Hondzo

**Organization:** U of MN - St. Anthony Falls Laboratory

**Office Telephone:** (612) 644-1850

**Email:** mhondzo@umn.edu

## Project Basic Information

**Project Summary:** In the interest of public health and safety, this project aims to quantify risks associated with the presence of viral and bacterial pathogens in urban waters in the Twin Cities.

**Funds Requested:** \$499,000

**Proposed Project Completion:** 2024-06-30

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

## Project Location

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

Region(s): Central, Metro,

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

Region(s): Metro

**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.**

The past two decades have seen significant advances in environmental microbiology pertinent to the better management of pathogen risks to bathers/recreators in natural waters. Microbiologists/engineers have utilized hydrodynamic modelling to provide insights into microbial contaminant movement. Further, improved knowledge has become available on the causative agents of water-associated illness, particularly enteric viruses and fecal indicator bacteria (FIB). The City of Minneapolis conducted a bacterial source identification study in Minnehaha Creek and concluded that the majority of FIB originate from birds, dogs, and “naturalized” E.coli. From a microbial ecology context, there has been a revolution in our understanding of human and animal gut microbioms that has resulted from various genomic approaches, which improved host-specific target identification. There has also been increasing application of risk assessment to inform management actions. Nonetheless, we do not generally see these new approaches reflected in how the microbiological safety of recreational waters is managed, nor is it clear how to deal with locations that have high temporal and spatial variability due to nutrient input and water flow. Secondly, we need to devote more effort to “finding a better indicator,” more formally viral and microbial pathogen source tracking and tracing via specific gene quantification.

### **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.**

Quantitative Microbial Risk Assessment (QMRA) has been shown to be a valuable tool in many aspects of assessing water safety including system assessment, monitoring, and management. A requirement for the practical implementation of QMRA for urban water systems is the quantification of pathogen concentration (viruses and bacteria) in untreated source water. Virus monitoring is seldom routinely done. The vast majority of aquatic systems in Minnesota (and nationally) do not have any virus data. While historical sets of fecal indicator bacteria data are available, only a fraction of potential pathogenic bacteria are routinely monitored. Consequently, estimated pathogen concentrations may be biased because only a fraction of the diversity and temporal variability is currently quantified. New approaches are needed to integrate knowledge about hydrologic events and the large diversity of scenarios for pathogen contamination (viruses, bacteria, protozoa), associated with or derived from birds, domestic animals, pets, cyanobacteria blooms, resuspended sediments and from humans themselves. We proposed to use a dual coupled modeling and a novel pathogen detection system to predict the potential of pathogen presence at the public beaches. Molecular biology technologies enable the detection and quantification of pathogen in the urban water cycle with new speed, sensitivity, and simplicity of use.

### **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?**

This project addresses major issues in managing the safety of urban waters for Minnesotans. The project is designed to ‘fill-in- the-gaps’ of knowledge about numerous environmental scenarios that may lead to microbial pollution at recreational sites. The predictive ‘modeling’ approach we propose to aid in our understanding of pathogens in urban waters, is microbial risk assessment based on data generated by fast and cost-effective quantification of host-specific target gene quantification. Outcomes from this project will provide local authorities with timely and quantitative information to minimize public health risk of pathogen exposure at public beaches and during water recreational activities.

## Activities and Milestones

### Activity 1: Seasonal monitoring of pathogens (viruses & bacteria) in ponds, lakes, and rivers

**Activity Budget:** \$200,000

**Activity Description:**

The seasonal monitoring of pathogens (human viruses, protozoa, cyanobacteria), and hydraulic-hydrologic data will be conducted at the southwest corner of Bde Maka Ska in three-cell wetland pond designed to assimilate stormwater runoff from approximately 3.6 km<sup>2</sup> of urban watershed. The data will be collected concurrently at five sampling locations including 1) inlet to wetland pond, 2) outlet, and 3) nearby stormwater outlets. Automatic water samples, water depth, precipitation, and turbidity loggers will be positioned at the proposed sampling sites. The collection of microbiological data will be based on massively parallel DNA sequencing as a comprehensive screening tool to overcome limitation of conventional diagnostic methods for the detection and identification of pathogenic agents. Pathogen screening based on metagenomic sequence data will comprise but not be limited to human norovirus, adenoviruses, rotavirus, human and animal (avian/pig) influenza viruses, coronaviruses (MERS, SARS), Campylobacter, Salmonella, Giardia, Cryptosporidium, E. coli (O157:H7), and other human-associated fecal marker genes (such as BacHum-UCD, HumM2, and HF183). A high-resolution vertically profiling buoy will be deployed at the southwest corner of Bde Maka Ska to collect local meteorological and lake temperature data, vertical profiles of water samples, and water quality parameters data every 2 hours with 1m resolution.

**Activity Milestones:**

Description	Completion Date
Seasonal monitoring and data logging	2023-10-31
Setup of field monitoring equipment	2023-10-31
DNA/RNA extraction from collected water samples and metagenomic sequencing	2023-12-31

### Activity 2: Development of molecular assays to detect and quantify viruses and bacteria of potential human health risk

**Activity Budget:** \$150,000

**Activity Description:**

The state of Minnesota is in critical need to provide improved viral and bacterial water quality monitoring tools that have the potential to safeguard human and environmental health. The improved resolution of pathogen detection and quantification enabled by microbiome research provides a significant opportunity for improved water quality management tools to address this need. The DNA sequence information of water samples collected in Activity 1 will enable comparative metagenomic analysis and provide genome assembly of viral, bacterial, and protozoan pathogens. The assembled sequences can be scanned for potential PCR primers that meet quality assurance and control guidelines for PCR analyses on environmental samples according to U.S. EPA guidelines. The developed PCR assays will then be adapted to quantitative PCR or digital PCR chemistry to enable target quantification in the collected water samples. The newly developed PCR assays will be applied to quantify the seasonal and spatial distribution of viral, bacterial, and protozoan pathogens in Bde Maka Ska and its stormwater inflow ponds. The amplification-based PCR data will be verified with amplification-independent analyses using fluorescence in situ hybridization and flow cytometry where possible. Data from Activity 2 will inform Quantitative Microbial Risk Assessment (QMRA) modeling as described in Activity 3.

**Activity Milestones:**

Description	Completion Date
Comparative metagenomic sequence analysis of pathogens in collected urban water samples	2022-06-30
Development and quality assurance of PCR methods for the detection of pathogens in environmental samples	2023-07-31
Quantification of seasonal and spatial distribution of pathogens in water samples from lake Bde Maka Ska	2023-12-31

### Activity 3: Integrated data analysis and model development for risk assessment

**Activity Budget:** \$149,000

**Activity Description:**

The QMRA framework will consist of four steps, including 1) hazard identification, 2) dose–response, 3) exposure assessment and 4) risk characterization. Quantified pathogen and marker gene levels (Activity 1 and 2) will be statistically analyzed to quantify their statistical signatures (mean, standard deviation, probability distribution). The data will be augmented in two categories, including the days with dry weather and wet weather (during and 48 hrs after a storm). The analyzed data will be crucial for the development and verification of the mass balance model of marker and pathogen concentrations in the receiving wetland and lake water. The dose of each reference pathogen and marker will be determined by multiplying the volume of swimmer-ingested water by the ambient concentration of pathogen. The dose will be used to determine the probabilities of infection and illness. The estimated risk (step 4) will be evaluated with the health benchmark of 36/1000 (36 illnesses per 1000 swimmers, EPA standard). Monte Carlo simulations will provide a range of uncertainty in infectious illness risks to human health from lake water. A series of pathogen exposure risk maps of the entire lake will be generated for 2, 10, 100-year storm events.

**Activity Milestones:**

Description	Completion Date
Statistical data analysis of marker and pathogen concentrations	2022-11-30
Mass balance model verification of marker and pathogen concentrations	2023-12-31
Pathogen risk assessment and development of guidelines to manage recreation safety in urban aquatic environments.	2024-06-30

## Project Partners and Collaborators

Name	Organization	Role	Receiving Funds
Assoc. Prof. Dr. Sebastian Behrens	Department of CEGE, University of Minnesota	Environmental microbiologist. Expert in detection, identification, and quantification of diverse microbial target sequences in environmental samples based on massively parallel sequencing technologies, quantitative PCR, and flow cytometry. Integration of molecular tools for water quality assessment with human and environmental health effects.	Yes
Ms. Rachael Crabb	Minneapolis Park and Recreation Board (MPRB)	Ms. Rachael Crabb, Water Resources Supervisor, will be the contact person of MPRB regarding the sampling dates of pathogen sampling in urban waters and will contribute for the development of the microbial risk assessment model.	No
Dr. Shahram Missaghi	Minneapolis Public Works - Surface Water & Sewers Division	Dr. Missaghi will facilitate the implementation of QMRA modeling and communicate the results of molecular assays to the Minneapolis Park and Recreation Board.	No

## 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?**

Outcomes from this project will provide local authorities (City of Minneapolis Public Works Department, MPWD, and Minneapolis Park and Recreation Board, MPRB) with quantitative information to manage human health and safety in Minnesota's aquatic environments by developing quick pathogen detection system and formulating Quantitative Microbial Risk Assessment platform. The short-term success of the project will be measured by our ability to detect new pathogens and quantify the health risk from exposure to contaminated water. Long-term success of the project will be measured by the extent to which state stakeholders (MPWD and MPRB) adopt our findings to manage recreational waters.

## Other ENRTF Appropriations Awarded in the Last Six Years

Name	Appropriation	Amount Awarded
Assessing the Increasing Harmful Algal Blooms in Minnesota Lakes	M.L. 2016, Chp. 186, Sec. 2, Subd. 04b	\$270,000

## Project Manager and Organization Qualifications

**Project Manager Name:** Miki Hondzo

**Job Title:** James L. Record Professor

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

Miki Hondzo (PI), Professor

Department of Civil, Environmental, and Geo- Engineering, St. Anthony Falls Laboratory, University of Minnesota  
 Dr. Hondzo will be responsible for the development and guidance of the field monitoring and the development of Quantitative Microbial Risk Assessment (QMRA) modeling. He will guide the development of modeling efforts in the prediction of pathogen concentrations in the Bde Maka Ska and stormwater detention ponds. Hondzo has 20 years of experience in physical limnology and water quality monitoring and modeling in lakes, rivers, and ponds . Furthermore,

he will be responsible for communicating the research reports to the LCCMR and coordinating sampling schedules with Minneapolis Park and Recreation Board. Dr. Hondzo is an Associate Editor of the Environmental Fluid Mechanics journal.

Dr. Sebastian Behrens (Co-PI), Associate Professor, is an environmental microbiologist and an expert in detection, identification, and quantification of diverse microbial target sequenced in environmental samples based on massively parallel sequencing technologies, quantitative PCR, and flow cytometry. Dr. Behrens follows an interdisciplinary approach that combines the disciplines environmental engineering, and molecular biology to understand the basic ecological principles driving the bioremediation of metals, the biodegradation of organic contaminants, and the transport and fate of nutrient in the environment. Dr. Behrens will be responsible for the detection of pathogens in water samples and he will guide the development of molecular assays to detect and quantify viruses and bacteria of potential human health risk development of new studies natural and engineered ecosystems.

Dr. Shahram Missaghi (Co-PI), Minneapolis Public Works - Surface Water & Sewers Division, Water Resources Regulatory Coordinator, will facilitate the implementation of QMRA modeling and communicate the results of molecular assays to the Minneapolis Park and Recreation Board. Dr. Missaghi will be responsible for the public outreach and dissemination of project outcomes to the regulatory agencies and authorities on water quality.

**Organization:** U of MN - St. Anthony Falls Laboratory

**Organization Description:**

The proposed research is a collaborative effort between the Department of Civil, Environmental and Geo-Engineering (CEGE) and the St. Anthony Falls Laboratory (SAFL), University of Minnesota. The University of Minnesota is the State's main research and graduate teaching institution. The University partners with communities and governmental agencies across Minnesota to engage students, faculty, and staff in addressing society's most pressing issues. The CEGE focuses on collaborative and interdisciplinary research within critical areas such as managing and sustaining water and land-use infrastructure, mitigating disaster of the natural and built environments, engineering and developing earth resources, and designing renewable energy systems. The SAFL comprises 4460 m<sup>2</sup> of flumes, basin, tanks and offices and houses several smaller labs, including wet chemistry, sediment analysis, and a biological laboratory with phytoplankton-growth chambers, and incubators. The EcoFluids Laboratory, developed by PI Hondzo, allows SAFL researchers to study the interactions among fundamental fluid mechanics, microbiological processes, and chemical reactions that are mediated by biological organisms. In situ micro-profiling sensors with a robotic computer-controlled traversing system for dissolved oxygen, nitrate, pH, temperature, conductivity, fluid flow velocity, and fluorescence have been developed and tested under laboratory and field conditions.

## Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	\$ Amount
<b>Personnel</b>								
Miki Hondzo		PI			27%	0.12		\$34,930
Sebastian Behrens		Co PI			27%	0.12		\$28,113
Graduate Student		Graduate Student			43%	1.5		\$158,692
Graduate Student		Graduate Student			43%	1.5		\$158,692
Chris Ellis		Engineer			7%	0.04		\$5,262
Ben Erickson		Research Scientist			24%	0.08		\$6,680
Undergraduate student		assist with research			0%	0.45		\$11,869
Undergraduate student		assist with research			0%	0.45		\$11,869
							<b>Sub Total</b>	<b>\$416,107</b>
<b>Contracts and Services</b>								
							<b>Sub Total</b>	-
<b>Equipment, Tools, and Supplies</b>								
	Tools and Supplies	Consumable supplies	DNR/RNA extraction, cDNA synthesis, qPCR reagents, primers, plastic ware, general lab supplies					\$30,000
	Tools and Supplies	Field supplies, monitoring sensors replacement	monitoring station supplies, chemicals for water sample preservation, water chemistry analysis (DOC, nitrate phosphate), particle size					\$22,893
	Tools and Supplies	Flow cytometry supplies	dyes, standard beads, buffer, filters					\$7,500
							<b>Sub Total</b>	<b>\$60,393</b>
<b>Capital Expenditures</b>								

5/18/2020

							<b>Sub Total</b>	-
<b>Acquisitions and Stewardship</b>								
							<b>Sub Total</b>	-
<b>Travel In Minnesota</b>								
	Other	Travel to the designated field sites	Data collection in the field					\$500
							<b>Sub Total</b>	<b>\$500</b>
<b>Travel Outside Minnesota</b>								
							<b>Sub Total</b>	-
<b>Printing and Publication</b>								
	Publication	Publication fees	Dissemination the results of new testing protocols					\$1,500
							<b>Sub Total</b>	<b>\$1,500</b>
<b>Other Expenses</b>								
		Wireless data transfer fees	Online data transfer and analysis over the Internet					\$500
		Lab services	UMGC DNA sequencing					\$20,000
							<b>Sub Total</b>	<b>\$20,500</b>
							<b>Grand Total</b>	<b>\$499,000</b>



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
<b>State</b>				
			<b>State Sub Total</b>	-
<b>Non-State</b>				
In-Kind	Unrecovered F&A	Support of SAFL facilities where research will be conducted.	Secured	\$218,599
			<b>Non State Sub Total</b>	<b>\$218,599</b>
			<b>Funds Total</b>	<b>\$218,599</b>

## Attachments

### Required Attachments

#### *Visual Component*

File: [9db03b37-174.pdf](#)

#### *Alternate Text for Visual Component*

The visual of there project activities include

Activity 1: The experimental fieldsite Bde Maka Ska (Thomas Beach), stormwater Bde Maka Ska ponds, stormwater, and pathogen sampling by the Minneapolis Park and Recreation Board (12 public beaches in the metropolitan area);

Activity 2: Pathogen source detection and the development of new molecular assays for viruses and bacteria of potential human health risk; and

Activity 3: The development of Quantitative Microbial Risk Assessment alerting platform to manage the safety of the public in urban aquatic environments.

### Optional Attachments

#### *Support Letter or Other*

Title	File
MPRB Letter of support	<a href="#">d9b03cf1-b36.pdf</a>

## Administrative Use

**Does your project include restoration or acquisition of land rights?**

No

**Does your project have patent, royalties, or revenue potential?**

No

**Does your project include research?**

Yes

**Does the organization have a fiscal agent for this project?**

Yes, Sponsored Projects Administration

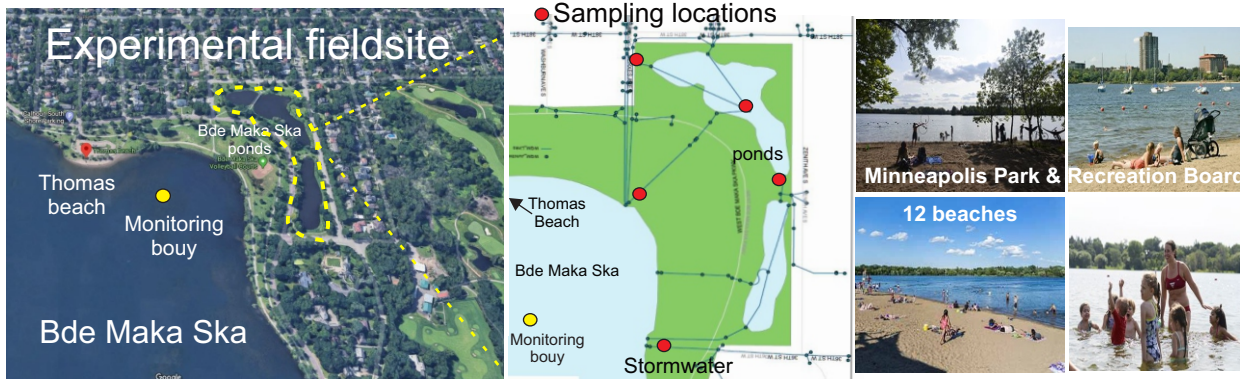
# Quantitative Risk Assessment of Pathogens in Urban Waters

Hondzo M., Behrens S., Missaghi S.

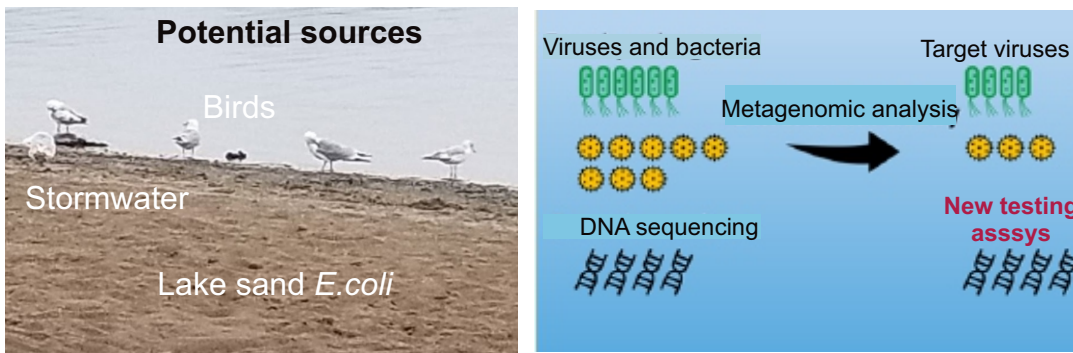
University of Minnesota and City of Minneapolis

## Can we swim yet?

Activity 1: Seasonal monitoring of pathogens (viruses and bacteria) in lakes, ponds, stormwater



Activity 2: Development of molecular assays to detect and quantify viruses and bacteria of potential human health risk



Activity 3: Integrated data analysis and model development for quantitative microbial risk assesment

