Environment and Natural Resources Trust Fund
2018 Request for Proposals (RFP)

Project Title: **ENRTF ID: 042-B**

Rapid Detection of Algal Toxins in Minnesota Lakes

Category: B. Water Resources

Total Project Budget: $ 686,013

Proposed Project Time Period for the Funding Requested: 3 years, July 2018 to June 2021

Summary:

We will use novel genetic and toxin characterization techniques to develop DNA based indicators of toxin risk. Citizen scientist sampling will be used to evaluate risk model application statewide.

Name: Andrew Bramburger

Sponsoring Organization: U of MN - Duluth NRRI

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Location

Region: Statewide

County Name: Statewide

City / Township: Multiple

Alternate Text for Visual:

The top panel gives the project title and states: Harmful Algal Blooms: Rapidly intensifying in MN inland lakes. Panels down the left side illustrate approach to Q1 – Characterization techniques for eDNA and cyanotoxins. Right side panels describe citizen science approaches. Bottom panels show stat e risk map and potential applications (i.e. mobile HAB lab and cell phone app).

_____ Funding Priorities	_____ Multiple Benefits	_____ Outcomes	_____ Knowledge Base
_____ Extent of Impact	_____ Innovation	_____ Scientific/Tech Basis	_____ Urgency
_____ Capacity Readiness	_____ Leverage	_____ TOTAL	_____%

PROJECT TITLE: Rapid Detection of Algal Toxins in Minnesota Lakes

I. PROJECT STATEMENT

The proposed research will characterize the statewide distribution of harmful algae species and algal toxin exposure risk within Minnesota inland lakes**.** We will also identify lake conditions that are favorable to harmful algae bloom (HAB) formation in order to determine priority lakes for future monitoring. The primary goal of the project is as follows:

- **To develop a cost-effective, rapid system for monitoring statewide algal toxin exposure risk in Minnesota.**

In order to achieve our project objectives, we will:

1. **Use cutting edge genetics and toxin characterization techniques in 200 focal lakes statewide in order to identify genomic indicators of high algal toxin concentrations and produce a gene-based model of toxin exposure risk.**
2. **Train a network of citizen science volunteers to collect genetic samples from algae occurrences in a large number (~2000) of Minnesota lakes in order to apply risk model at a statewide scale.**

While Cyanobacteria (blue-green algae) are integral primary producers in aquatic food-webs, Cyanobacteria produce toxins that can threaten the ecosystem and human health. Reports of Cyanobacteria-based HABs have been increasing in frequency in freshwater systems both within Minnesota and worldwide. Recent advances in environmental genomics technology, coupled with the abundance and diversity of inland lakes in Minnesota provide a unique opportunity to produce a cost-effective and widely applicable long-term monitoring tool that will be effective for protecting Minnesotans from risks associated with HABs at a statewide scale. Additionally, partnerships with agencies that support ongoing outreach and public engagement will allow us to leverage citizen science participation in order to apply these cutting-edge monitoring tools to a large number of lakes in all regions and counties within the state.

II. PROJECT ACTIVITIES AND OUTCOMES

Activity 1: Training and mobilization of citizen scientist volunteers for algal sampling in 2000 lakes. **Budget: $54,150**

Citizen scientist volunteers will be recruited through social media, public outreach events, and through partner organizations. Volunteers will be equipped with sampling kits and trained in sampling technique.

Outcome	Completion Date
1. Prepare 2000 Citizen Scientist sampling kits for deployment in lakes statewide. Kits will include basic genetics sampling equipment, preservative, and a self-addressed padded mailer for returning samples to the project team.	July 15, 2018
2. Develop 2 online awareness/ sampling training videos (YouTube).	July 15, 2018
3. Host 3 regional training events (proposed in southern MN, Brainerd, and Ely) to discuss HABs, distribute sampling kits (500 for year 1), and train volunteers.	July 31, 2018
4. Design and production of outreach materials in order to bolster citizen scientist recruitment for year 2.	Apr. 30, 2019
5. Host 3 regional follow up/outreach and training events (proposed in southern MN, Brainerd, Ely) to discuss HABs and their health risks with the public, disseminate findings from 2017 sampling season, distribute additional sampling kits (1500 for year 2), and to train volunteers on proper sampling techniques.	May 31, 2019

Activity 2: Comprehensive sampling of 200 focal lakes and determination of genomic indicators of high toxin concentration. **Budget: $534,465**

We will characterize water quality conditions and sample water and environmental DNA annually in 200 Minnesota lakes. Metagenomics and toxin signatures of focal lake samples will be fully characterized using cutting-edge techniques. Citizen scientist samples will be scanned for presence of indicator genes. Gene-based risk models will be constructed and calibrated using focal lake data, and applied to citizen science data.

Outcome	Completion Date
1. Focal Lake genetic and toxin sampling and analysis (Comprehensive analysis; Round 1; 200 samples)	Nov 30, 2018
2. Analysis of citizen scientist sample kits (Indicator gene scan; Round 1; 500 samples)	Jan. 31, 2019
3. Focal Lake genetic and toxin sampling and analysis (Comprehensive analysis; Round 2; 200 samples)	Nov. 30, 2019
4. Analysis of citizen scientist sample kits (Indicator gene scan; Round 2; 1500 samples)	Jan 31, 2020
5. Quality control of genetic, toxin, and water quality data, construction/calibration of toxin risk model for application to full suite of citizen science data.	June 30, 2020

Activity 3: Development and application of statewide toxin risk model in 2000+ lakes **Budget: $97,398**
We will work with UMN Extension and MN Sea Grant to disseminate materials to stakeholders. Targeted avenues for outreach include publication of an article in the MN Sea Grant publication, The Seiche; a podcast episode on The Sea Grant Files; and participation in MN Sea Grant and UMN Extension outreach activities including the state fair booth, as well as specific products and outcomes described below.

Outcome	Completion Date
1. Produce outreach materials including visual guide to potentially harmful algae, instructions for reporting suspicious blooms, initial feedback to citizen scientists and snapshot of algal toxin risk in Minnesota lakes.	Sept. 30, 2020
2. Produce statewide algal toxin risk map, disseminate algal toxin risk model to stakeholders, distribute results via conferences and research articles.	Apr. 30, 2021
3. Provide long-term monitoring outreach and guidance materials including user guide and training manual for sampling kit use, report on feasibility of using citizen science monitoring for HAB toxins.	June 30, 2021

III. PROJECT STRATEGY

A. Project Team/Partners

Lead PI, **Andrew Bramburger** (Research Associate, UMD-NRRI) will receive funds and coordinate all project activities. Co-PIs **Cody Sheik** (Assistant Professor, UMD-LLO) **Christopher Filstrup** (Research Associate, UMD-LLO) **Kathryn Schreiner** (Assistant Professor, UMD-LLO), **and John Downing** (Professor, UMD-LLO, Director MNSG) will receive funds to fulfil analytical and outreach objectives. **Marte Kitson** (MNSG) will receive funds serve as outreach coordinator for the project. Project partners including the White Iron Chain of Lakes Association, The Minnesota Coalition of Lake Associations, and UMN Extension will not receive funds, but will facilitate outreach initiatives.

B. Project Impact and Long-Term Strategy

Outcomes of this project will lead directly to the establishment of a long-term genomics-based HAB monitoring system, leveraging novel techniques and citizen scientist volunteer network developed through the project. This approach will embody significant improvements in spatial and temporal coverage, response time, and cost effectiveness relative to current monitoring techniques. Potential future applications of this project include a publicly-accessible statewide toxin risk map that could be updated monthly based on citizen science sampling. This map could be accessed via a searchable, geo-linked cellular app.

C. Timeline Requirements

Three years, from July 2018 through June 2021.

2018 Detailed Project Budget

Project Title: Rapid Detection of Algal Toxins in Minnesota Lakes

IV. TOTAL ENRTF REQUEST BUDGET 3 years

BUDGET ITEM	AMOUNT
Personnel:	$ 444,296
Andrew Bramburger, Principal Investigator: $62,242 (66.5% salary, 33.5% benefits); 20% each year for 3 yrs	
Cody Sheik, Co-Investigator: $46,033 (66.5% salary, 33.5% benefits); 70% in 2 summer mos. each year for 3 yrs	
John Downing, Co-Investigator: $588 (66.5% salary, 33.5% benefits); 0.1% each year for Y1 & Y2	
Christopher Filstrup, Co-Investigator: $32,778 (66.5% salary, 33.5% benefits); 15% each year for 3 yrs	
Kathryn Schreiner, Co-Investigator: $47,508 (66.5% salary, 33.5% benefits); 70% in 2 summer mos. each year for 3 yrs	
Julia Halbur, Lab Tech: Genomics Sample Prep: $90,327 (72.8% salary, 27.2% benefits); 75% each year for 3 years	
Marte Kitson, Outreach Coordinator: $17,161 (66.5% salary, 33.5% benefits); 10% each year for 3 years	
Elizabeth Alexson, Lab Tech: $17,522 (72.8% salary, 27.2% benefits); 10% effort each year for 3 years	
Graduate Student: $130,137 (85% salary, 15% fringe); 50% effort each year for 3 years	
Equipment/Tools/Supplies:.	$ 86,005
YSI EX02 Water Quality Sonde used to quantify water quality. Quote from YSI. Will follow University policy for selection of vendor. Item purchased in Y1. State-of-the-art water quality sonde for measurement of multiple parameters. Equipment will be retained for use in future HAB research and monitoring. Total: $19,560	
Sample Processing and Analysis Supplies ($32,615): Phytoplankton sample processing ($7,050): beakers (case of 100 = $400), centrifuge bottles (case of 1000 X 2 = $1,500), slides (case of 1000 X 2 = $800), coverslips (case of 1,000 X 2 =$450), reagents and preservatives (HNO3 X 8L = $1,200, H2O2 X 16L = $700, K2Cr2O7 X 8L = $700, mounting media X 100 mL = $300, formalin X 3L = $300, acetic acid X 2L = $200, iodine X 500 mL = $300, KI crystals = $200). Purchase half Year 1 and Year 2. Molecular analysis supplies ($14,765): primers ($400), PCR MasterMix solution ($10,200 Agarose ($1150, TBE Bugger ($200), PCR plates ($895), PCR film ($420), Multi-channel pipettes ($1500) Purchase half year 1 and half year 2 Toxin characterization supplies ($10,800): Liquid Chromatograph columns (2 sets/year $2600), cyanotoxin standards (1 set / 6 months $8200) Purchase half year 1 and half year 2	
Lab and field supplies ($4,360): Sample bottles and vials, preservatives, Rite in the Rain notebooks, sunscreen, insect repellent, gloves, test vials, labortory glassware, bags, general storage supplies $2,130 each	
Data storage device ($450): 3x 3TB hard drives for genomics data storage @ $150	
Citizen science sampling kits ($26,000): $13/kit x 2,000 kits. Purchased in Y1.	
Li-Cor LI192 submersible light quantum sensor, logger, and cable for measuring underwater light levels in lakes ($3,020) - Equipment will be retained for use in future HAB research and monitoring	
Travel:	$ 30,021
Field Travel ($21,948): 8 trips per year to sample 200 inland lakes in MN (8 trips x 4 nights @ $120 + per diem for 2 travelers @ $64 + mileage + 32 days truck and boat use = $10,974/yr)	
Outreach ($8,073): distribution of citizen science kits (4 trips per year to attend public events and stakeholder meetings x 1 night @ $120 + partial perdiem for 2 travelers @ $48 + 24 days truck use =$2691/ yr)	
Additional Budget Items:	$ 125,691
Scientific services ($121,191): Water quality analysis ($18,000): $30/sample x 200 samples for total nitrogen and phosphorus each year for 3 years; Genomics analysis ($54,000): DNA extraction (2400 samples x $6.25/sample), genome sequencing and toxin gene database screening ($16.25/sample x 2400 samples); Toxin characterization ($49,191): Extraction, prep, sample cleanup, extraction supplies, solvents ($8391), LC-MS analysis time (200 samples x $80/sample), LS-MS standards & Calibration (50 hours x $80/hour)	
Mailing/shipping to cover cost for citizen scientists to mail back sampling kits: $2.25/kit x 2,000 kits. Total: $4,500	
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$ 686,013

V. OTHER FUNDS

SOURCE OF FUNDS	AMOUNT	Status
Other Non-State $ To Be Applied To Project During Project Period:	N/A	
Other State $ To Be Applied To Project During Project Period:	N/A	
In-kind Services To Be Applied To Project During Project Period: Unrecovered indirect: 54% MTDC, $606,177 base (TDC less graduate student fringe and equipment costs)	$ 327,335	Secured
Past and Current ENRTF Appropriation:	N/A	
Other Funding History:	N/A	

Rapid Detection of Algal Toxins in Minnesota Lakes

Harmful Algae Blooms
Rapidly intensifying across Minnesota

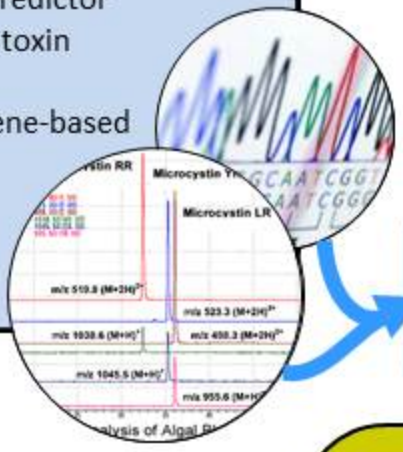
Variable Toxicity | Multiple Species | Multi-Gene Systems

Q1: What genes predict toxin exposure risk?
Q2: What are the distribution and frequency of these genes in MN inland lakes?

Q1

Comprehensive Genetics and Toxin Survey of Minnesota Lakes

- 200 lakes.
- Establish relationships between predictor genes and toxin levels
- Produce gene-based toxin risk model.



Q2

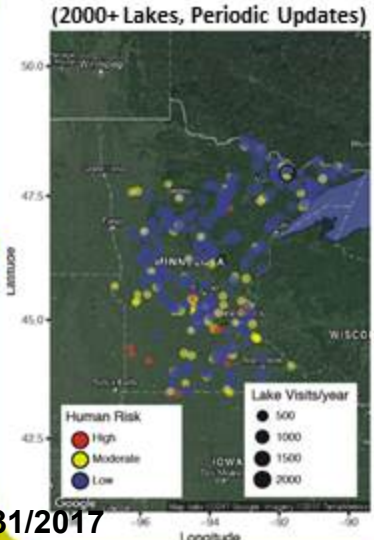
Citizen Science Sampling of Minnesota Lakes

- Establish rapid-detection sampling network.
- 2000 lakes.
- Open data access

Cutting-edge Statewide HAB Monitoring Program

Extended Impact / Future Applications

Statewide Toxin Risk Map
(2000+ Lakes, Periodic Updates)



PROJECT TITLE: Rapid Detection of Algal Toxins in Minnesota Lakes

Project Manager's Qualifications and Organization Description

Dr. Andrew Bramburger is a Research Associate at the Natural Resources Research Institute (NRRI) at the University of Minnesota Duluth. His research interests and expertise lie within the field of phycology (the study of algae), and he has been conducting research on freshwater algae for over 15 years. Bramburger has published 15 peer-reviewed articles on algal communities and presented over 50 conference presentations. Since 2010, Bramburger has served as PI or Co-PI on research programs totaling over $3.5 million in total funding, including ongoing EPA Great Lakes phytoplankton monitoring programs and several projects related to harmful algae blooms and the use of citizen science in both Canada and the U.S.

The NRRI is a U.S. based research institute established by the Minnesota state legislature within the University of Minnesota Duluth. NRRI is a non-profit applied research organization that works to develop and deliver the understanding and tools needed to utilize our mineral, forest, energy and water resources in a balanced and environmentally responsible manner. The NRRI facility in Duluth MN is a 110,000 square foot facility dedicated to providing research-based solutions for empowering sustainable development. NRRI is equipped with the facilities for GIS, water quality, and algal analyses, including a wide variety of sampling equipment, boats and field vehicles, as well as sample processing, inverted microscopy and image analysis capabilities. NRRI is a well-established laboratory and research facility and can provide ~$500,000 in analytical equipment, computers, and microscope facilities at no cost to the project. NRRI works in close collaboration with other departments at UMD including the Large Lakes Observatory (LLO) and Minnesota Sea Grant.

The Phycology / Paleolimnology Lab (Bramburger) at the Natural Resources Research Institute (NRRI) is fully equipped for microscopic analysis of phytoplankton. The laboratory has several microscopes including Olympus BH-2 and BX-60 compound microscopes equipped with DIC, RIC, and phase contrast optics, as well as Olympus CX-40 inverted microscopes equipped with phase contrast optics and epifluorescence accessories. Auxiliary equipment includes a freeze-dryer, centrifuges, hot-plates, and slide warmers, as well as a dedicated radioisotope preparation facility featuring a Hitachi Aloka Accu-Flex 8000 liquid scintillation counter. Shared facilities within NRRI also consist of a LaChat multi-channel flow-injection nutrient autoanalyzer and a Hitachi TM3030 Plus scanning electron microscope.

The Sheik Geomicrobiology lab housed at the Large Lakes Observatory (LLO) and associated with the Biology Department is equipped as a modern microbiology laboratory with emphasis on culturing and processing samples from the environment. The lab is outfitted with common area bench space with power and gas outlets, a laminar flow hood, fume hood, centrifuges, PCR machine, Qubit DNA quantification platform, agarose gel electrophoresis systems, transilluminator with gel capture camera system, diH2O, Milli-Q water system, incubator, lighted and refrigerated growth chambers, an autoclave, and -20 and -80 °C storage.

The Organic Geochemistry Laboratory (Schreiner) has two Agilent 6890 GCs, one interfaced to an Agilent 5973 quadropole MS and one interfaced to a flame ionization detector. Additionally, this laboratory contains various extraction and other equipment, including Soxhlet extractors, an Accelerated Solvent Extractor, and glassware, hoods, and other equipment necessary for organic geochemical analyses.

The aquatic chemistry laboratory houses an HPLC, Total Organic Carbon analyzer, and FTIR spectrometer, in addition to multiple ovens and furnaces, hoods, microscopes, and chemical glassware and other equipment. The LLO also houses a dedicated LC-MS laboratory, which contains an Agilent LC triple quadrupole MS, along with a variety of peripherals including fraction collectors.