Environment and Natural Resources Trust Fund
2020 Request for Proposals (RFP)

Project Title: **ENRTF ID: 105-B**

Cold Temperature Ammonia Consuming Bacteria during Wastewater Treatment

Category: B. Water Resources

Sub-Category:

Total Project Budget: $ 462,351

Proposed Project Time Period for the Funding Requested: June 30, 2023 (3 yrs)

Summary:

This project will investigate ammonia consuming microorganisms during municipal wastewater treatment in the winter. These bacteria protect Minnesota's environment by preventing the release of ammonia and estrogenic hormones.

Name: Timothy LaPara

Sponsoring Organization: U of MN

Job Title: Professor

Department: Department of Civil, Environmental, and Geo- Engineering

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Minneapolis MN 55455

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Web Address:

Location:

Region: Statewide

County Name: Statewide

City / Township:

Alternate Text for Visual:

This project will benefit the State of Minnesota and its environment by better understanding the bacteria that consume ammonia during wastewater treatment. It will lead to reduced fish toxicity and reduced feminization of fish populations by leading to better year-round ammonia removal.

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

PROJECT TITLE: *Cold Temperature Ammonia Consuming Bacteria during Wastewater Treatment*

I. PROJECT STATEMENT

This project will investigate the presence and activity of ammonia-consuming bacteria during the winter months in Minnesota's wastewater treatment facilities. These organisms are critically important for protecting Minnesota's surface water quality, by preventing the release of ammonia (which is especially toxic to fish) and estrogenic hormones. Ammonia-consuming bacteria, however, are exceptionally sensitive to cold temperatures, such that ammonia discharges from wastewater treatment facilities are typically not regulated from November through April. Because these organisms are slow-growing in the laboratory and ammonia is not regulated in the winter, our knowledge and understanding of these critically important organisms during the cold weather months is sorely lacking. Research is needed to better understand these organisms to better protect Minnesota's surface water quality, to optimize energy consumption for wastewater treatment, and to better remove hormones and other contaminants of emerging concern from Minnesota's wastewaters.

Ammonia is a critically important environmental pollutant because of its toxicity to fish, its contribution to eutrophication (i.e., excessive growth of weeds and algae in lakes and streams), and its cost of treatment. Ammonia is not typically a primary pollutant (i.e., directly released to the environment), but instead forms as proteins decompose. Because municipal wastewater contains a substantial quantity of protein, ammonia is a particularly important pollutant to wastewater treatment plant operators. Ammonia is one of the most difficult pollutants to remove from wastewater because it requires a lot of aeration to fully treat (which is expensive) and the organisms that consume ammonia are very slow-growing.

Ammonia-consuming organisms are historically thought to be inactive at low temperatures, which is why most wastewater treatment facilities in Minnesota are not regulated for ammonia discharges in the winter. Our recent research, however, has demonstrated that these organisms are retained during the winter months at similar quantities as the summer months. This suggests that these organisms remain active during the winter, even if they are not responsible for measurable ammonia removal. If ammonia-consuming bacteria remain active during the winter, this has several beneficial impacts on the State of Minnesota. First, wastewater treatment facilities need to be less concerned about the resumption of ammonia removal each Spring. This would allow wastewater treatment operators to better control aeration rates, potentially saving Minnesota taxpayers an unnecessary expense. Second, ammonia-consuming bacteria have been linked to the degradation of numerous contaminants of emerging concern, such as the hormone estrone. If ammonia-consuming organisms remain active in the winter months, Minnesota's wastewater treatment facilities can discharge lower quantities of estrogenic substances, reducing the feminization of fish and help sustain Minnesota's fish populations.

II. PROJECT ACTIVITIES AND OUTCOMES

Activity 1 Title: *Identify and characterize the ammonia consuming organisms in Minnesota's wastewater treatment facilities*

Description: Samples will be collected from up to 10 municipal wastewater treatment facilities in Minnesota in February (cold) and in August (warm). We will then characterize the ammonia-consuming bacteria by using DNA sequencing technology on a gene common to all bacteria (16S rRNA gene) and a gene that is specifically involved in ammonia consumption (*amoA*). We will then sequence all of the DNA in these samples and then use this data to assemble the majority of the genome of the ammonia-consuming bacteria in Minnesota's wastewater treatment facilities (this technique is known as "shotgun metagenomics"). This work will allow us to determine if the ammonia-consuming bacteria vary from summer to winter and from treatment plant to treatment plant. The assembled genome sequences will then provide us the fundamental information to allow us to track the activity of ammonia consuming organisms in Activity 2.

ENRTF BUDGET: $227,426

Outcome	Completion Date
1. Sample collection and Genomic DNA extractions	March 31, 2021
2. DNA sequence analysis	August 31, 2021
3. Analysis of DNA sequence data	March 31, 2022

Activity 2 Title: *Quantify the activity of ammonia consuming bacteria during wastewater treatment in the winter*
Description: This activity will determine the activity of ammonia consuming bacteria during the winter months. We will quantify the expression of different genes during the winter months by targeting their RNA (a direct measure of activity) rather than their DNA (linked to the potential to do an activity). Because ammonia consuming bacteria appear to be active in the winter but yet do not consume ammonia to a great extent, we will also track their ability to remove estrone, the most prominent estrogenic compound in municipal wastewater. This research is important because ammonia consuming bacteria have been previously linked to the removal of estrone from wastewater, such that the reduction in ammonia consumption could also be linked to the removal of estrogenicity in the winter months.
ENRTF BUDGET: $227,425

Outcome	Completion Date
1. Sample collection, RNA extraction, and cDNA Synthesis	May 31, 2022
2. DNA sequence analysis	December 31, 2021
3. Analysis of DNA sequence data	December 31, 2022
4. Quantification of estrone degradation activity	December 31, 2022

Activity 3 Title: *Disseminate research results to stakeholders.*
Description: The first two activities will significantly improve our knowledge of the ammonia consuming organisms in the Minnesota. The final activity will be to disseminate these results at local conferences (e.g., the Annual Innovative Conference by the Central States Water Environment Association). In addition, we will publish our research results in open-access, peer-reviewed journals, which will allow us and LCCMR staff to disseminate our results to the fullest extent possible.
ENRTF BUDGET: $7,500

Outcome	Completion Date
1. Presentations at in-state scientific conferences (on-going/continuous)	June 30, 2023
2. Publication in open access scientific journals	June 30, 2023

III. PROJECT PARTNERS AND COLLABORATORS:
The project team will be led by Drs. Timothy LaPara and Sebastian Behrens (University of Minnesota, Department of Civil, Environmental, & Geo-Engineering) who are experts in wastewater treatment and in microbiology. The team also will include one post-doctoral research associate, a graduate student, and numerous participating wastewater treatment facilities.

IV. LONG-TERM IMPLEMENTATION AND FUNDING:
The long-term goal of the proposed research is to protect the Minnesota's surface water through better wastewater treatment. This research will help optimize removal of ammonia from Minnesota's municipal wastewater throughout the year and hopefully reduce the estrogenicity of Minnesota's wastewater discharges.

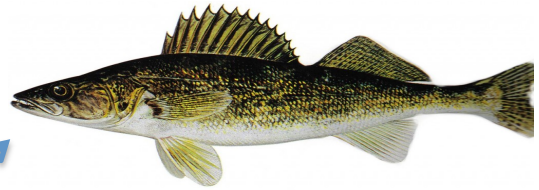
Attachment A: Project Budget Spreadsheet
Environment and Natural Resources Trust Fund
M.L. 2020 Budget Spreadsheet
Legal Citation:
Project Manager: Timothy LaPara
Project Title: Cold Temperature Ammonia Consuming Bacteria during Wastewater Treatment
Organization: University of Minnesota
Project Budget: $461,315
Project Length and Completion Date: 3 years; June 30, 2023
Today's Date: March 15th, 2019

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Budget	Amount Spent	Balance
BUDGET ITEM			
Personnel (Wages and Benefits)	$ 362,351	$ -	$ 362,351
Timothy LaPara, Professor (74% salary, 26% benefits); 8% FTE for three years; project supervision, supervision of a post-doctoral researcher and a graduate research assistant, project reporting.			
Sebastian Behrens, Associate Professor (74% salary, 26% benefits); 8% FTE for three years; co-supervision of a post-doctoral researcher and a graduate research assistant. ($62,892)			
Postdoctoral research associate (80% salary, 20% benefits); 100% FTE for 24 months; metagenomic DNA sequencing and analysis ($133,523)			
Graduate Research Assistant (57% salary, 43% benefits); 50% FTE for 24 months; community analysis, estrone degrading activity experiments ($99,312)			
Professional/Technical/Service Contracts			
University of Minnesota Genomics Center: High-throughput DNA Sequencing and other tasks	$ 37,500	$ -	$ 37,500
Equipment/Tools/Supplies			
Lab supplies (DNA/RNA extraction kits; Reagents for PCR and DNA sequencing; Cell sorting)	$ 50,000	$ -	$ 50,000
Travel expenses in Minnesota			
In-state travel to collect samples: (Approximately 10,000 miles at .58/mile)	$ 5,000	$ -	$ 5,000
Other			
Publication Fees for Open Access Publication	$ 7,500	$ -	$ 7,500
COLUMN TOTAL	$ 462,351	$ -	$ 462,351

SOURCE AND USE OF OTHER FUNDS CONTRIBUTED TO THE PROJECT	Status (secured or pending)	Budget	Spent	Balance
Non-State:		N/A	N/A	N/A
State:		N/A	N/A	N/A
In kind:				
The University of Minnesota does not charge the State of Minnesota its typical overhead rate of 54% of the total modified direct costs.	Secured	$ 232,141	$ -	$ 232,141
Other ENRTF APPROPRIATIONS AWARDED IN THE LAST SIX YEARS	**Amount legally obligated but not yet spent**	**Budget**	**Spent**	**Balance**

Cold Temperature Ammonia Consuming Bacteria during Wastewater Treatment

Ammonia Consuming Bacteria
Wastewater Treatment



Protect Fish Against Ammonia Toxicity

$$$$$

A major cost of wastewater treatment due to aeration costs

Prevent the Release of Hormones and Other Emerging Contaminants

This project will benefit the State of Minnesota and its environment by better understanding the bacteria that consume ammonia during wastewater treatment. It will lead to reduced fish toxicity and reduced feminization of fish populations by leading to better year-round ammonia removal.

Project Manager Qualifications and Organization Description

Timothy M. LaPara

Professor, Department of Civil, Environmental, and Geo- Engineering, University of Minnesota

B.S.C.E., Civil Engineering, 1995, University of Notre Dame, Notre Dame, IN
Ph.D., Civil Engineering, 1999, Purdue University, West Lafayette, IN

Dr. Timothy LaPara will be responsible for overall management of the proposed project. Dr. LaPara's research is focused on the role of municipal and industrial wastewater treatment plants in preserving environmental quality and in protecting public health. His research has a strong interdisciplinary nature, stemming from his unique background in both environmental engineering and microbiology.

Sebastian F. Behrens

Associate Professor, Department of Civil, Environmental, and Geo- Engineering, University of Minnesota

B.S., Biology 1997, University of Bremen, Germany
Diploma, 2000, Microbiology, University of Bremen, Germany
Ph.D., Microbial Ecology, 2003, Max Planck Institute for Marine Microbiology, Germany

Dr. Behrens' research focuses on linking environmental processes to the spatial-temporal distribution and metabolic activity of key functional groups of microorganisms. He follows an interdisciplinary approach that combines the disciplines biogeochemistry, microbiology, and molecular biology to understand the basic microbial ecology principles driving the biogeochemical cycling of metals and metalloids, the biodegradation of organic contaminants, and the emission of greenhouse gases from the molecular to the ecosystem scale. The gained knowledge on microbial transformation processes in natural and engineered ecosystems is then implemented in order to optimize microbial remediation approaches, resource recovery, and the biological treatment of water (drinking water, surface water, groundwater, or waste water), thereby spanning the gap between basic and applied research aspects of bioremediation.

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/about/index.html). The laboratories and offices of the PIs contain the necessary fixed and moveable equipment and facilities needed for the proposed studies.