Environment and Natural Resources Trust Fund
2019 Request for Proposals (RFP)

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

New Technology for Removing Mercury from Minnesota Waters

Category: H. Proposals seeking $200,000 or less in funding

Sub-Category: B. Water Resources

Total Project Budget: $ 199,000

Proposed Project Time Period for the Funding Requested: June 30, 2021 (2 yrs)

Summary:

We will demonstrate that minnows equipped with two genes from environmental bacteria will be able to detoxify mercury in our aquatic ecosystems, making game-fish safer to eat.

Name: Michael Smanski

Sponsoring Organization: U of MN

Title: Asst. Professor

Department: Biological Sciences/Biochemistry/Biotechnology Institute

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St. Paul MN 55108

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Web Address

Location

Region: Statewide

County Name: Statewide

City / Township:

Alternate Text for Visual:

By equipping minnows with genes for mercury detoxification, the whole food chain will be made safer.

_____ Funding Priorities	_____ Multiple Benefits	_____ Outcomes	_____ Knowledge Base
_____ Extent of Impact	_____ Innovation	_____ Scientific/Tech Basis	_____ Urgency
_____ Capacity Readiness	_____ Leverage	_____ TOTAL	_____%
____ If under $200,000, waive presentation?			

PROJECT TITLE: New technology for removing mercury from Minnesota waters: Phase I

I. PROJECT STATEMENT

Mercury is a major global pollutant and public health hazard. Mercury levels in the environment are increasing, primarily due to human activity including coal-powered energy generation, cement kilning, industrial production of chlorine-containing materials, and gold mining. Mercury is a potent neurotoxin in animals and is particularly hazardous during prenatal and postnatal neurological development. **We plan to equip fish with genes from bacteria that will allow them to detoxify the mercury present in aquatic ecosystems.**

Mercury does not degrade in the environment, but it cycles between several forms that differ in their mobility and toxicity. Elemental mercury, Hg(0) is the least dangerous form and is a gas that will escape to the upper atmosphere. The oxidized form, Hg(II), is slightly more dangerous and can be modified by several species of bacteria to form methylmercury, MeHg. Of these most-abundant mercury species, methylmercury (MeHg) is the most toxic and tends to accumulate in living organisms that are higher in the food chain. The global burden resulting from ingesting methylmercury was estimated at $8B (USD), compared to a global burden of $2.9M (USD) for inhalation of mercury as Hg(0). Thus, converting methylmercury present in aquatic environments to the less toxic Hg(0) elemental form will have a substantial positive impact on human health.

Here, we **propose** to modify fish by adding genes to make them capable of detoxifying methylmercury. This is Phase I of a multi-phase research plan. In Phase I, we will add two bacterial genes to a commonly studied laboratory fish (*D. rario*, or 'zebrafish') that should allow the fish to degrade methylmercury and remove it from the water. We will test that our modified fish can remove methylmercury from water in laboratory aquarium studies. If we are successful with this 'proof of principle', we will apply for Phase II funding at a later date. Phase II will entail modifying a small native fish, such as a minnow, with the same genes and testing its ability to remove methylmercury from an outdoor pond in controlled field trials. All modified fish will be sterilized to prevent their spread in native ecosystems. If Phase II is successful, Phase III will involve seeking regulatory approval to test our methylmercury remediation technique in Minnesota lakes to reduce methylmercury in the environment and in seafood. Phase III will also confirm that there are no non-target effects. Because our methylmercury detoxifying fish will be low on the food chain, they will reduce mercury levels in all gamefish, making them safer to eat.

II. PROJECT ACTIVITIES AND OUTCOMES

Activity 1: Use modern precision genetics to engineer fish that can remove mercury from the environment.

ENRTF BUDGET: $ 100,000

In our first task, we will use modern tools for DNA synthesis and precise genome editing to make engineered fish. We will engineer several different varieties that will have to be tested to determine which are best at removing methylmercury from the food chain.

Outcome	Completion Date
1. Design genes that will allow fish to remove methylmercury from aquatic ecosystems	8/1/2019
2. Engineer laboratory aquarium fish by introducing these genes to their genome	6/1/2020

Activity 2: Test the ability of laboratory aquarium fish to detoxify methylmercury by converting it to the less dangerous gas, Hg(0).

ENRTF BUDGET: $ 99,000

Activity 2 is divided into two subtasks. The first seeks quantify different mercury species in the engineered fish that are fed a high-mercury diet. The second quantifies the mercury that gases off and is present in the headspace above the tank. Since the bioconversion of methylmercury and elemental mercury occurs within the tissue of the fish, the two subtasks essentially quantify how well Hg(0) is eliminated from the fish. Published literature on Hg(II) elimination from tilapia suggests this will occur 10-fold faster than for methylmercury. Even if Hg(0) fails to eliminate, the presence of Hg(0) in fish will be a meaningful result, as it is less toxic than methylmercury.

Outcome	Completion Date
1. Confirm that toxic methylmercury is broken down to less toxic Hg(0) in engineered fish	1/1/2021
2. Confirm that Hg(0) produced in our fish is released as a gas, thus reducing mercury levels in the water.	6/30/2021

III. PROJECT PARTNERS:
None.

IV. LONG-TERM- IMPLEMENTATION AND FUNDING: The work described in this proposal is Phase I of a multi-phase project to remove mercury from Minnesota waters. At the end of this Phase, we will have proven that our approach – leveraging genetically engineered fish to remove methyl mercury from lakes and rivers – works in controlled laboratory environments. Phase II of this project will require us to transition our system to a native minnow species for trail experiments in outdoor ponds. Prior to and during Phase II we will work with regulatory directors at the Environmental Protection Agency to determine a path towards regulatory approval. Pending success of this Phase I project, we will seek Phase II funding from the UMN MnDrive program and the LCCMR.

V. TIME LINE REQUIREMENTS:
The experiments described here for this project are realistic and will be completed entirely in controlled lab environments and so will not require field work. We will demonstrate our new technology in fast-growing, easy-to-handle laboratory fish species during the first year, and we will demonstrate the feasibility in laboratory experiments during the second year.

VI. SEE ADDITIONAL PROPOSAL COMPONENTS:

A. Proposal Budget Spreadsheet
B. Visual Component or Map

2019 Proposal Budget Spreadsheet

Project Title: New technology for removing mercury from Minnesota waters

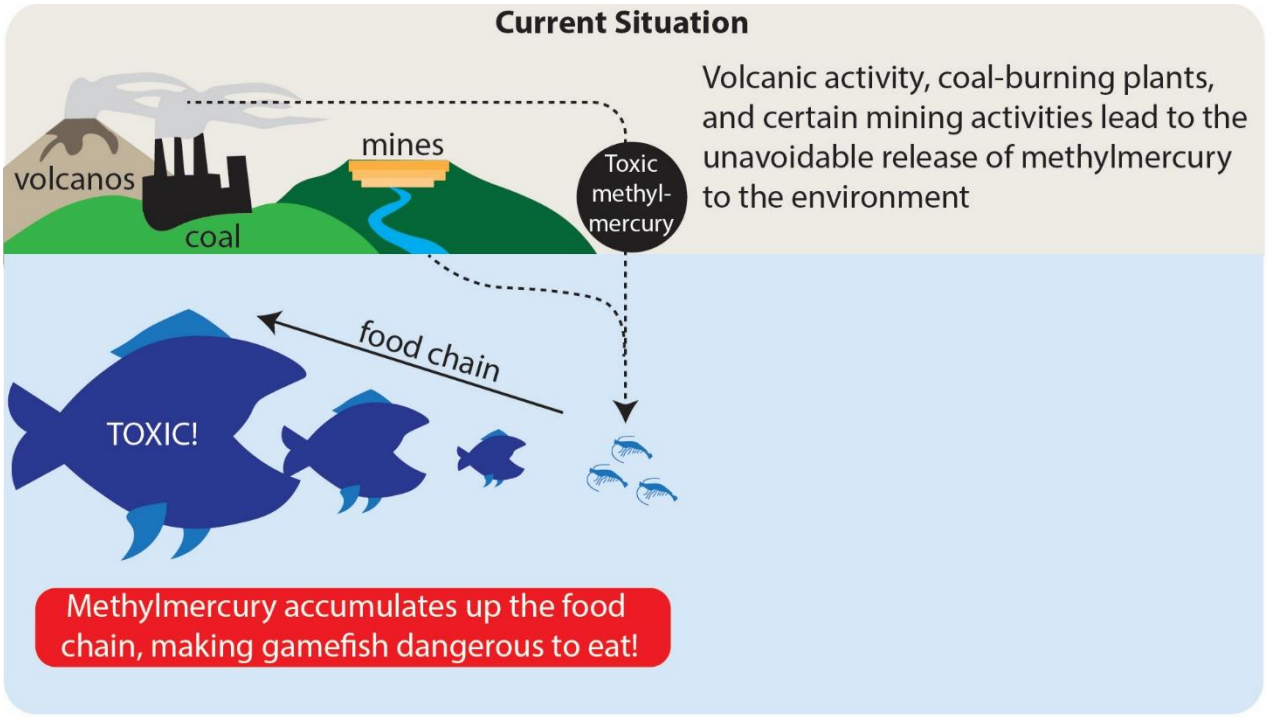
IV. TOTAL ENRTF REQUEST BUDGET *2* **years**

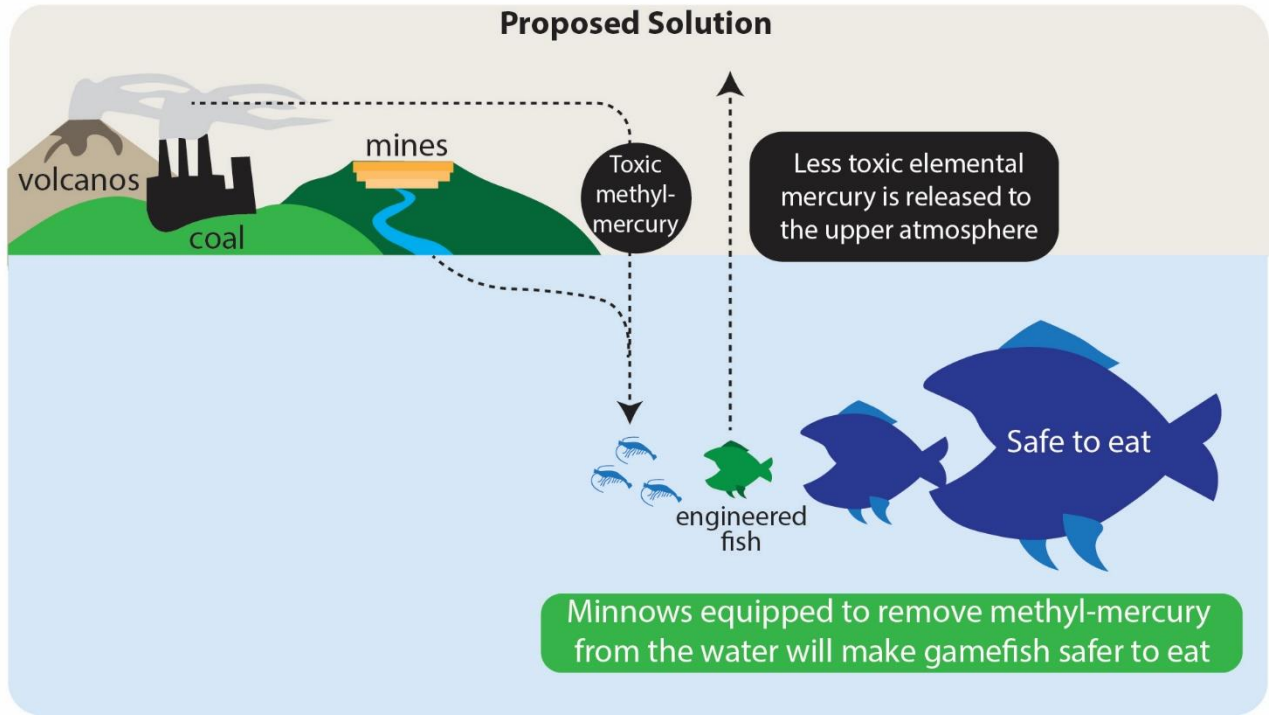
BUDGET ITEM (See "Guidance on Allowable Expenses")	**AMOUNT**
Personnel:	**$154,000**
Project Manager (75% salary, 25% benefits) (5% summer salary for 2 years) Prof. Smanski: $18,000	
Postdoctoral Researcher (82% salary, 18% benefits)(100% FTE for 2 years) Dr. Siba Das: $136,000	
Equipment/Tools/Supplies:	**$45,000**
Controlled laboratory fish rearing and handling facilities for initial laboratory fish studies, includes costs for space rental, food, tank and handling equipment, tools for egg harvesting, and cleaning equipment (for 2 years): $10,000	
Molecular biology reagents (includes $8,000 per year to purchase enzymes and chemicals to perform modern precision genetics tests on fish, testing of engineered fish, and also includes an additional $2,000 to purchase DNA elements that are required for tests performed in this study): $18,000	
Core facility costs (DNA sequencing): will cover costs associated with 'reading' the DNA of the fish that are used in experiments to determine success of experiments: $7,000	
Lab consumables (including disposable plasticware, for example test tubes and petri plates, as well as media needed for production of molecular biology reagents made in the lab; $5,000 per year for two years): $10,000	
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	**$ 199,000**

V. OTHER FUNDS *(This entire section must be filled out. Do not delete rows. Indicate "N/A" if row is not applicable.)*

SOURCE OF FUNDS	**AMOUNT**	**Status**
Other Non-State $ To Be Applied To Project During Project Period:	$ -	
Other State $ To Be Applied To Project During Project Period:	$ -	
In-kind Services To Be Applied To Project During Project Period: In kind services will be provided by the University of Minnesota to cover indirect costs associated with managing the research project and providing administrative support to researchers as indirect costs are not allowed on state awards. (54% of total costs).	$ 107,460	
Past and Current ENRTF Appropriation:	$ -	
Other Funding History:	$ -	

B. Visual Component





PROJECT MANAGER QUALIFICATIONS: *Michael J. Smanski (PI)*

PROFESSIONAL PREPARATION

University of California, San Diego	Biochemistry	B.S., 2006
University of Wisconsin, Madison	Microbiology	Ph.D., 2011
Massachusetts Institute of Technology	Biological Engineering	2011-2014

APPOINTMENTS

Since 2014 Assistant Professor, Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota, Twin Cities

QUALIFICATION STATEMENT

Dr. Smanski's research group leverages the latest technologies in precision genetics for diverse applications, including the discovery of new antibiotics and the control of invasive species. He is well-trained in Biochemistry, Microbiology, Molecular Biology, and Engineering. His research focuses on applying state-of-the-art genetic engineering tools to address grand challenges in the environment, agriculture, public health, and medicine.

HONORS AND AWARDS

HHMI Fellow of the Damon Runyon Cancer Research Foundation	2012-2014
Dale F. Frey Award for Breakthrough Scientists	2014
DARPA Young Faculty Award	2017

MOST CLOSELY RELATED PUBLICATIONS

1. Maselko M, Heinsch SC, Chacon J, Harcombe W, Smanski MJ (2017) Engineering species-like barriers to sexual reproduction. Nat. Comm. 8:883 doi:10.1038/s41467-017-01007-3.
2. Bajer P, Lechelt J, Hansen G, Kornis M, Maselko M, Smanski MJ (2018) Biological control of invasive fish and aquatic invertebrates: a brief review with case studies. Chapter in AFS book on Integrated Pest Management. Accepted.
3. Hsu S, Smanski MJ (2017) Designing and implementing algorithmic DNA assembly pipelines for multigene systems. In Synthetic Metabolic Pathways, eds Jensen MK, Keasling JD. Methods Mol. Biol. ISBN 978-1-4939-7295-1.
4. Smanski MJ, Bhatia S, Zhao D, Park YJ, Woodruff L, Giannoukos G, Ciulla D, Busby M, Calderon J, Nicol R, Gordon DB, Densmore D, Voigt CA. (2014) Functional optimization of gene clusters by combinatorial design and assembly. Nat. Biotechnol. 32:1241-1249.
5. Smanski MJ, Zhou H, Claesen J, Shen B, Fischbach MA, Voigt CA (2016) Synthetic biology to access and expand nature's chemical diversity. *Nat. Rev. Microbiol.* **14**:135-142.

ORGANIZATION

The University of Minnesota is a world-class research university. Its official mission statement can be found at http://regents.umn.edu/sites/regents.umn.edu/files/policies/Mission_Statement.pdf