Environment and Natural Resources Trust Fund
2015 Request for Proposals (RFP)

Project Title: **ENRTF ID:** **089-D**

Biocontrol of Eurasian Water Milfoil Based on DNA Sequencing

Category: D. Aquatic and Terrestrial Invasive Species

Total Project Budget: $ 574,000

Proposed Project Time Period for the Funding Requested: 3 years, July 2015 - June 2018

Summary:

Invasive plant species pose a threat to aquatic ecosystems. In this proposal we will develop microbiological control agents for invasive Eurasian Water Milfoil by using DNA sequencing and cultures-based approaches.

Name: Michael Sadowsky

Sponsoring Organization: U of MN

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St. Paul MN 55108

Telephone Number: (612) 624-2706

Email sadowsky@umn.edu

Web Address

Location

Region: Metro

County Name: Carver, Hennepin

City / Township:

Alternate Text for Visual:

Project Flow

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

PROJECT TITLE: Biocontrol of Eurasian Water Milfoil Based on DNA Sequencing Analysis

I. PROJECT STATEMENT

Invasive plant species pose a common threat to public health, and the structure, and function of aquatic ecosystems. Eurasian Water Milfoil (*Myriophyllum spicatumas*) is an aquatic invasive plant found in Minnesota waterways (~160 lakes, rivers, and streams). This species disrupts native biodiversity, lowers water quality, and causes nutrient loading and eutrophication. The negative ecological impacts of Eurasian Water Milfoil (EWM) on freshwater aquatic systems also affect human resource use and ecosystem functioning. Moreover, interbreeding of EWM with the native northern water milfoil (*M. sibiricum* Komarov) has been reported in Minnesota lakes and this may lead to even faster growth rates over the parental species.

These aquatic plants are capable of supporting a variety of micro- and macro-organisms, including enteric bacteria and human pathogens. Moreover, these microorganisms can detach and be released to surrounding waterways, influencing water quality and recreational activity. Such is the case for interaction of the aquatic alga *Cladophora* and bacteria in Lake Michigan. While we propose here that EWM also supports large populations of bacteria and some harmful pathogens, our knowledge of the microbial community associated with EWM in Minnesota is limited. This project aims to evaluate the distribution, spread, and ecological effects of EWM in Minnesota waterways across time and space using metagenomic, PCR, and microbiological approaches. We will characterize the total microbial community structure of EWM using a 16S rDNA-based, metagenomic DNA sequencing approach and examine microbial biodiversity and ecology within benthic, epiphytic, and endophytic regions of EWM in various states of decay. This will provide a distribution map of microbes associated with EWM that can be extremely distinctive, as well as potential health hazards associated with microbes from EWM. More importantly, this characterization may allow us to eventually develop effective, specific and cost effective biological control agents for EWM, which may have advantages over the conventional mechanical and chemical management methods. That is, we can identify bacteria that are unique and pathogenic to EWM. This same approach is often taken with other bacterial pathogens of terrestrial plants.

Currently, large infestations of EWM are managed most often by mechanical and chemical means. However, chemical control has many disadvantages, including possible toxicity to humans and aquatic organisms, nutrient release by decaying material, anoxic conditions, and destruction of native biota. Development of effective biological control agents to reduce EWM biomass without use of herbicides or dredging may minimize damage to native aquatic wildlife. This can help protect native species, increase aquatic biodiversity, improve ecosystem health, and manage natural resources for recreational use. This approach may ultimately be of use for conservation practices to protect native aquatic wildlife.

These studies will put Minnesota at the forefront of this important area of aquatic invasive species research. Project outcomes will provide more insight into conservation practices of native aquatic wildlife and ecological effects of EWM on water quality. We also believe that one of the best approaches to protect and restore native species in Minnesota is to engage the public through outreach program in collaboration with the University of Minnesota Aquatic Invasive Species Research Center (MAISRC) and the MN Department of Natural Resource (DNR), and through exhibits at the Science Museum of Minnesota and the Bell Museum of Natural History.

II. PROJECT ACTIVITIES AND OUTCOMES

Activity 1: Metagenomic characterization of microbial community of EWM **Budget: $286,000**

We will sample EWM from Lake Minnetonka and Cedar Lake, which was previously studied to manage EWM and considered as a case study of EWM invasion. We will sample from multiple sites, chosen for their anthropogenic influence, water quality status and milfoil abundance. Since the growth of EWM is limited to the littoral zone of most Minnesotan lakes from April to September, we will sample benthic, epiphytic and endophytic regions of EWM, in various state of decay, monthly at each sampling location for two years. Along with EWM, we will also obtain water and sediment samples near the EWM sampled. We will examine total microbial communities associated with EWM and assess the distribution of rare and abundant microbes in relation EWM growth stage and various states of decay. We will also obtain information on other indicators of water quality (temperature, turbidity, O_2 levels, nutrient status, pH, land use, and *E. coli* counts). Each sample will be

sequenced to a depth of 200,000 to obtain taxonomic data on the microbes present. This activity will contribute to the overall understanding of microbial biodiversity and ecology of EWM.

Outcome	Completion Date
1. Sample collection and water quality monitoring.	October 31, 2017
2. Identify microbial community associated with EWM using metagenomic approach.	March 31, 2017
3. Correlations of EWM microbial community to biological characteristics of EWM and water quality.	April 30, 2018

Activity 2:Development of microbiological control agent for EWM **Budget: $233,000**

Based on the characterization of EWM microbiota in Activity 1, we will select potential candidate microbes which are naturally-occurring and non-infectious for humans, but may be specifically associated with EWM, in a symbiotic or pathogenic manner. Based on this information, we will isolate and extensive screen about 500 potential candidate microbes that are potentially lethal to native, invasive, and hybrid water milfoil. Once we narrow down the bacteria, we will test their potential pathogenicity to EWM in terms of effectiveness at various growth stages and environmental conditions (water temperature, pH, dissolved oxygen, etc.). Furthermore, treatment concentration/duration and specificity will be examined.

Outcome	Completion Date
1. Selection of potential pathogenic microbes to EWM.	April 30, 2017
2. Laboratory screening of microbes that may be lethal to native, invasive, and hybrid water milfoil.	December 31, 2017
3. Determine effectiveness and specificity of biological control microbes.	April 30, 2018

Activity 3: Project data dissemination **Budget: $55,000**

In this result, we will disseminate our results through personal presentations, fact sheets, and papers published in professional, peer reviewed journals. We will utilize existing national web resources, such as the Mothur database to facilitate metagenome data and Genbank for archiving, retrieval, and analysis. We will also develop public displays at the Bell Museums and MN state parks to reach large number of adults and children for aquatic invasive species and water quality.

Outcome	Completion Date
1. Production of public exhibits.	December 31, 2017
2. Dissemination of project data and results via seminars, and workshops.	June 30, 2018

III. PROJECT STRATEGY

A. Project Team/Partners

The project will be carried out under the direction of Drs. Michael Sadowsky (PI) and Chanlan Chun. This work will be done in consultation with Peter Sorensen and Raymond Newman of the MAISRC at the UMN. Funded project partners will include Pat Hamilton of the Science Museum of Minnesota, Susan Weller, Bell Museum Director and Mike Berndt and Megan Kelly at MNDNR for dissemination activities.

B. Project Impact and Long-Term Strategy

This request seeks funding for the first 3 years of this program. This will provide key information on the protection of native species and aquatic biodiversity and management of natural resources for recreation in Minnesota water resource for a long-term. Additional funding for long term and more extensive operation will be obtained from the MNDNR, National Science Foundation, and other agencies.

C. Timeline Requirements

The project will be completed in 3 years, but the impact will last for many more. Multiple years of sampling and analysis are required to obtain adequate, reliable data and for effective incorporation into public education venues.

2015 Detailed Project Budget

Project Title : Biocontrol of Eurasian Water Milfoil Based on DNA Sequencing Analysis

IV. TOTAL ENRTF REQUEST BUDGET *3* years

BUDGET ITEM	AMOUNT
Personnel:	
Chanlan Chun:Research Associate ($44,290, 16% time, 34% fringe, 3 years, 1 person)	$ 30,000
Postdoctoral Associate ($41,000, 100% time, 20.75% fringe, 3 years, 1 person)	$ 152,000
Graduate Student (50% time, 37% tuition, 9 % fringe, 3 years, 1 person)	$ 116,000
Technician ($51,355, 24% time, 36% fringe, 3 years, 1 person)	$ 53,000
Contracts:	$ -
Publication and Exhibit at MNDNR (staff time and materials)	$ 30,000
Exhibit at Science Museum of Minnesota and Bell Museum (staff time and materials)	$ 25,000
Equipment/Tools/Supplies:	$ -
Laboratory supplies: $17,000/year for year 1 and $22,000/year for year 2 and 3	$ 61,000
Publication	$ 5,000
Acquisition (Fee Title or Permanent Easements): *In this column, indicate proposed number of acres and and name of organization or entity who will hold title.*	$ -
Travel:.	$ -
In-state Travel for 12 samplings per year × 2 years @6000 mi × $0.50/mi	$ 3,000
Conferences	$ 2,000
Additional Budget Items: Sample analysis: 50 water and 80 water milfoil samples: Genome preparation @$200/sample =$18,000, genome anlayisis= @$600/sample=$54,000, physiochemical analysis=$3,000). Genome preparation and all genomic analyses are done most cost effectively in	$ 97,000
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$ 574,000

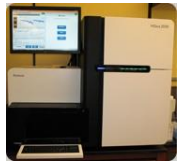
V. OTHER FUNDS

SOURCE OF FUNDS	AMOUNT	Status
Other Non-State $ To Be Applied To Project During Project Period: N/A	$ -	*Indicate: Secured or*
Other State $ To Be Applied To Project During Project Period: N/A	$ -	*Pending*
In-kind Services To Be Applied To Project During Project Period: *N/A*	$ -	*Indicate: Secured or*
Funding History: N/A	$ -	
Remaining $ From Current ENRTF Appropriation:N/A	$ -	*Indicate: Unspent? Legally Obligated? Other?*

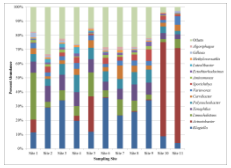
Sampling of native and invasive water milfoil

DNA extraction

DNA Sequence analysis

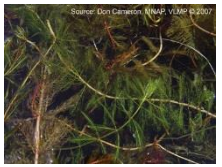


Microbial community analysis



Isolation of bacteria as potential biocontrol agents

Development of microbiological control agents for water milfoil



Project Manager Qualifications and Organization Description:

Project Manager: Dr. J. Michael Sadowsky

Title: McKnight University Professor and Director BioTechnology Institute

Affiliation: University of Minnesota, Department of Soil, Water and Climate, and BioTechnology Institute

The UMN Biotechnology Institute (BTI) provides advanced research, training, and university-industry interactions in biological process technology, and other areas of biotechnology research. Faculty in the BTI have broad expertise in: Biocatalysis, Metabolic engineering/microbial physiology, Population dynamics, Molecular biology, Proteomics and focused expertise in defined areas such as bioremediation, biomaterials, biosensors, and bioinformatics.

Education:
Ph.D., 1983. University of Hawaii, Honolulu, Hawaii. Major: Microbiology
M.S., 1979. University of Wisconsin-Oshkosh, Wisconsin. Major: Microbiology
B.S, 1977. University of Wisconsin-Madison, Wisconsin. Major: Bacteriology

Professional Experience:
Director BioTechnology Institute, University of Minnesota, St. Paul, Minnesota, 2009 - present.
Co-Director, Microbial and Plant Genomics Institute, University. of Minnesota, 2006-2009.
Distinguished McKnight University Professor: Department of Soil, Water, & Climate, and BioTechnology Institute, University of Minnesota, St. Paul, Minnesota, 04/04 - present.
Professor: Department of Soil, Water, and Climate and Department of Microbiology
University of Minnesota, St. Paul, Minnesota, 07/96 – 04/04.
Associate Professor: Departments of Soil Science and Microbiology
University of Minnesota, St. Paul, Minnesota, 07/93 - 6/96.
Assistant Professor: Departments of Soil Science and Microbiology
University of Minnesota, St. Paul, Minnesota, 06/89 - 6/93.
Microbiologist: U.S. Department of Agriculture-ARS; Beltsville, Maryland, 01/86 - 05/89.

Dr. Sadowsky will have chief management responsibilities for overseeing the proposed project. He will be responsible for working with the Research Associate (Dr. Chun), project partners and cooperators to ensure that project goals, results and timelines are met. He will also be responsible for working with the graduate graduates, and postdoctoral associate at UMN, staff at museums and partners at MN DNR. Dr. Sadowsky is an environmental microbiologist with 30 years research experience in the analysis and use of microorganisms in environmental settings. Dr. Sadowsky's laboratory studies the distribution and diversity of microorganisms in aquatic and soil environments and uses genetic, genomic, and biotechnology tools to examine how microorganism become established in new environments. He is currently Director of the BioTechnology Institute, and is currently involved in three large metagenome projects; the soil metagenome to define novel microbial genes for agriculture, biofuels and bioenergy; the Mississippi River metagenome project (M3P) that examines the impact of human activity on the diversity and function of microbes in the Mississippi River; and the Human Intestinal metegenome project that defines changes in human intestinal tract microbiota due to *Clostridium difficile* diarrheal disease and fecal transplantation.