Environment and Natural Resources Trust Fund
2011-2012 Request for Proposals (RFP)

Subd: 06a

Project Title: Improved Detection of Harmful Microbes in Ballast Water

Category: E. Aquatic and Terrestrial Invasive Species

Total Project Budget: $ $250,000

Proposed Project Time Period for the Funding Requested: 3 yrs, July 2011 - June 2014

Other Non-State Funds (secured): $ 0

Summary:

This project will identify the potentially harmful bacteria transported to Lake Superior in ships ballast water that can cause ecological and economic damage and threaten human and aquatic animal health.

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Sponsoring Organization: U of MN

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Location:

Region: NE

Ecological Section: Northern Superior Uplands (212L)

County Name: Statewide

City / Township:

PROJECT TITLE: Improved Detection of Harmful Microbes in Ballast Water

I. PROJECT STATEMENT

While the Great Lakes face many threats, the presence of invasive species threatens not only Lake Superior but also Minnesota's people and coastal economies. The transport of organisms in the ballast water of ships is of global concern. Over 182 species of non-indigenous algae, invertebrates, fish, and plants have been identified in the Great Lakes, and it has been estimated that 65% of those species were introduced by the discharge of ballast water from ships. The appearance of the fish virus VHS in the Great Lakes and the recent discovery of its DNA in parts of Lake Superior have led many to recognize that some microbes transported in the ballast water of commercial ships can be viewed as harmful invasive species, just like invasive species of plants and animals that threaten our natural resources.

In 2005, more than 5 billion gallons of ballast water was discharged into the Duluth-Superior Harbor, the largest volume discharged in any harbor within the Great Lakes. This fact makes early detection of ballast-water derived invasive microbes an extremely important goal. Ship-mediated transport of bacteria is of particular concern due to their abundance, potential pathogenicity, and the ability of some bacterial species to form resting stages. Some of the bacteria being released into Lake Superior may cause ecological damage, impact local coastal economies, and even threaten human and aquatic animal health in other inland lakes in Minnesota. Yet, very little is known about the diversity of bacteria that are being transported by ships into Lake Superior, and their potential for causing irreparable harm. The potential transport of water-borne pathogenic bacteria from warmer climate zones into Lake Superior is of increasing concern as this lake's water temperature is rising due to global climate change.

Our team will sample freshwater ('Lakers') and ocean-going ('Salties') commercial ships to identify harmful bacteria that are being transported in ballast water and discharged into Lake Superior. Our studies will focus on bacteria present in the ballast water of ships but not already common in the Duluth-Superior harbor. We will use state-of-the-art DNA sequencing techniques to identify the harmful bacteria we should be most concerned about. The methods employed have the potential to detect rare microbes before they become common inhabitants in Lake Superior. Identifying harmful microbes of concern is the first step on the path to develop sensitive monitoring techniques that provide early detection of harmful microbes in the ballast water of ships an to devise effective remediation strategies to limit their spread.

II. DESCRIPTION OF PROJECT ACTIVITIES

Activity 1: Collect Ballast Water from Commercial Ships and Extract DNA **Budget: $39,208**

Large volumes of ballast water will be sampled from up to ten commercial ships. We have already collected ten ballast and matching harbor water samples from Lake Superior. Ballast water samples from freshwater and ocean-going ships will be filtered to concentrate microbial communities and their DNA will be extracted. Microbes in harbor water sampled adjacent to areas where ships discharge ballast water have already been collected to compare ballast and harbor water microbial communities.

Outcome	**Completion Date**
1. Establish a ballast water collection characteristic of ships entering Lake Superior	September 2012
2. Develop and archive a repository of purified microbial DNA from ballast water	September 2012

Activity 2: Sequence Bacterial Genes Found in Ship Ballast Water **Budget: $110,560**

Taxonomically important bacterial genes (16S rDNA) will be sequenced from bacterial community DNAs from 'Laker', 'Salty', and harbor microbial communities. This will allow us to evaluate differences in the bacterial assemblages and identify potentially harmful bacteria present in ballast water. Partial sequences (V5 region) of the bacterial 16S rRNA gene will be collected for 20 ballast water and 10 harbor water bacterial assemblages using Illumina next generation sequencing technology to identify potentially harmful members

of ballast water bacterial communities. Fosmid libraries will be constructed from ten microbial DNA ('metagenomes') samples from 'Laker' and 'Salty' ballast water bacterial assemblages to identify functional genes of concern like those involved in pathogenesis, and antibiotic and heavy metal resistance.

Outcome	Completion Date
1. Create a 16S rDNA sequence database of ballast bacteria from commercial ships	May 2012 and 2013
2. Construct functional fosmid libraries to detect harmful genes and processes	May 2012 and 2013

Activity 3: Analyze Gene Sequences of Bacteria found in Ships' Ballast Water **Budget: $100,232**

Our goal is to describe the composition of bacterial assemblages in the ballast water of ships and resolve differences of bacterial diversity found between ballast water assemblages and microbial communities from the Duluth-Superior harbor, the receiving water where large volumes of ballast water are discharged. Two approaches will be used to reach this goal. First, 16S rRNA gene sequences (V5 region) obtained using a massively parallel sequencing approach will be cross-compared to a reference database of taxonomically important bacteria. This next generation sequencing approach will yield extremely large numbers (millions) of sequence data, so that even rare bacterial species within ballast water samples can be detected and quantified. In addition, the fosmid libraries of microbial genomes in ten ballast water samples will be screened to identify any harmful microbial genes present, like those that code for pathogenic functions and resistance to antibiotics.

Outcome	Completion Date
1. Construct phylogenetic trees and develop comparisons between bacterial assemblages and functional genes from different sources	June 2012 and 2013
2. Identify and rank most potentially harmful bacteria transported to the Duluth-Superior Harbor in the ballast water of commercial ships	July 2013

III. PROJECT STRATEGY

A. Project Team/Partners

Randall Hicks and Michaeal Sadowsky (co-project managers), University of Minnesota, will coordinate the project and oversee the work of a postdoctoral associate and graduate student who will help collect ballast water samples, extract DNA, and sequence and analyze the bacterial community DNA to identify potentially harmful microbes. We will collaborate with personnel from the MPCA in Duluth, MN to obtain ballast water samples from freshwater and ocean-going ships. In 2009, we worked with John Thomas and Jeff Stollenwerk (MPCA) to successfully collect ballast water samples from ten ships that visited the Duluth-Superior Harbor. They have agreed to help us collect new ballast water samples as part of this project to augment our existing samples. ENRTF funds will be used to support University of Minnesota personnel. MPCA personnel who collaborate with us do not require funds from the ENRTF.

B. Timeline Requirements

We are proposing a two-year project period. Ballast water samples will be collected each year from commercial ships to provide a sufficient sample set to determine the inter-annual variability of harmful bacteria in the ballast water of these ships.

C. Long-Term Strategy and Future Funding Needs

This project will identify potentially harmful bacteria of concern in ballast water and can be completed within two years. A future project that might develop a suite of sensitive, early warning, monitoring methods would naturally follow this project after the most harmful bacteria being discharged by commercial ships into our waterways are identified. However, we are not seeking funds for such a project until the types of harmful bacteria found in ballast water are established, the most potentially harmful microbes are ranked, and the potential risks to Lake Superior's ecosystem, animals including fish and humans, and Minnesota's coastal economies are estimated.

2011-2012 Detailed Project Budget

IV. TOTAL TRUST FUND REQUEST BUDGET [2 years]

BUDGET ITEM	AMOUNT
Personnel:	
Randall E. Hicks (2 years; 1 mo @ 100% time + 33.3% Fringe Benefits)	$ 27,227
Postdooctoral Associate (2 years; 12 mo @ 100% time + 20.22% Fringe Benefits)	$ 124,464
Graduate Research Assistant (2 years; 7.9 mo @ 50% time + 24.2% Fringe Benefits + Tuition Benefit)	$ 48,754
Contracts: *N/A*	$ -
Equipment/Tools/Supplies:	
Ballast water sampling supplies	$ 805
Portable temperature/DO/salinity meter (YSI 2030)	$ 1,600
DNA extraction and PCR Reagents	$ 5,500
Fosmid library costs (10 libraries @ $1,500 ea)	$ 15,000
Illumina sequencing costs (30 samples @ $5,000 per 10 samples)	$ 15,000
Chemicals and expendable lab supplies	$ 9,150
Publication costs	$ 1,000
Acquisition (Fee Title or Permanent Easements): *N/A*	$ -
Travel: Ballast water sampling: 8 trips/year; Travel between UM campuses: 3 trips/year.	$ 1,500
Additional Budget Items: N/A	$ -
TOTAL ENVIRONMENT & NATURAL RESOURCES TRUST FUND $ REQUEST	**$ 250,000**

V. OTHER FUNDS

SOURCE OF FUNDS	AMOUNT	Status
Other Non-State $ Being Applied to Project During Project Period: *N/A*	$ -	
Other State $ Being Applied to Project During Project Period: *N/A*	$ -	
In-kind Services During Project Period: *Hicks Salary Match (0.5 mo/year+33.3% FB)*	$ 13,613	*Secured*
Remaining $ from Current ENRTF Appropriation (if applicable): *N/A*	$ -	
Funding History: Great Lakes Protection Fund grant (through Northeast-Midwest Institute) 2007-2010	$ 148,274	

CO-PROJECT MANAGER QUALIFICATIONS

Randall Hicks is a Professor of Biology and Director of the Center for Freshwater Research and Policy at the University of Minnesota Duluth (UMD). He completed a Ph.D. in Ecology at the University of Georgia and did postdoctoral work at Woods Hole Oceanographic Institution and the Illinois Natural History Survey before joining the faculty at the University of Minnesota Duluth. Dr. Hicks is an environmental microbiologist who studies the diversity and productivity of aquatic microbial communities, their role in the degradation and transformation of organic compounds, and the survival and virulence of pathogenic microbes in these communities. This work has taken him to the bottom of different great lakes using a manned submersible, to Russia and various oceans, but his current research efforts are focused on the North American Great Lakes and watersheds in northern Minnesota. He is the author or coauthor of over 30 scientific journal articles and book chapters. Dr. Hicks brings several decades of organizational experience and expertise ranging from heading a large academic department, organizing a national scientific meeting, to directing a university center. For eight years, he was head of a department of 50-60 employees that serves over 800 undergraduate and graduate students working on biology-related degrees.

Professor Michael Sadowsky, a fellow in the prestigious American Academy of Microbiology, is internationally known and respected for his research work in the area of environmental microbiology. He is a McKnight Distinguished Professor in the Department of Soil, Water, and Climate and currently is the Director of the Biotechnology Institute at the University of Minnesota. He has published more than 100 original articles, and his work is widely cited by researchers in several scientific disciplines.

The collective research, organizational, and administrative experiences of the project team members and the resources available to this project from the University of Minnesota should ensure the successful completion of the proposed project goals.

ORGANIZATIONAL DESCRIPTION

The University of Minnesota is a non-profit, state-funded educational institution of the State of Minnesota.

Dr. Hicks’s research laboratory is located in the research wing of the new Swenson Science Building (SSB 171) on the University of Minnesota Duluth campus. In addition to research laboratories, this wing has special rooms for culturing, epifluorescence microscopy, tissue culture, work with radioisotopes, equipment rooms, cold rooms, and variable temperature rooms. There is a support room on each floor that has an autoclave, dishwasher, and pyrogen-free Milli-Q water system. Dr. Hicks's laboratory is equipped for research in the areas of microbial ecology, organic geochemistry, and molecular biology. His laboratory includes approximately 1,200 square feet for bench research and includes computers and special software (e.g., BioNumerics, ARB) for genetic and phylogenetic analyses. The Department of Biology is well equipped for microbiological, limnological, and molecular biology research.

Dr. Sadowsky’s research laboratories are located in the Borlaug Hall on the St. Paul campus of the University of Minnesota. His labs are well equipped for microbiological, molecular biology, and biotechnology research.