Environment and Natural Resources Trust Fund
2016 Request for Proposals (RFP)

Project Title: **ENRTF ID: 123-D**

Advancing Microbial Invasive Species Monitoring from Ballast Discharge

Category: D. Aquatic and Terrestrial Invasive Species

Total Project Budget: $ 368,995

Proposed Project Time Period for the Funding Requested: 2.5 years, July 2016 to December 2018

Summary:

We will identify bacteria in ship ballast water and St. Louis River Estuary sediments, assess the risk of introducing invasive bacteria, and evaluate techniques for their removal from ballast water.

Name: Randall Hicks

Sponsoring Organization: U of MN

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Duluth MN 55812

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Web Address

Location

Region: NE

County Name: St. Louis

City / Township:

Alternate Text for Visual:

Map of the St. Louis River Estuary and the Duluth-Superior Harbor. Dots indicate the sites where sediment and water samples will be collected for the “Advancing Microbial Invasive Species Monitoring from Ballast Discharge” project.

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

PROJECT TITLE: Advancing Microbial Invasive Species Monitoring from Ballast Discharge

I. PROJECT STATEMENT

The movement of aquatic invasive species via ship ballast water associated with maritime commerce is a long-standing concern. Ballast water can be a primary source of invasive species and a vector for microbial introductions and movements. In fact, the Duluth-Superior Harbor (DSH) receives twice the ship ballast water discharge volume and events of any other Great Lakes port and is one of four invasion "hotspots" within the Great Lakes based on initial discoveries of non-indigenous species. Some of the microbes being released into the DSH may threaten human and aquatic animal health, cause ecological damage, and impact local coastal economies. This makes early detection of potentially harmful microbes, assessing the risk posed, and development of mitigation strategies extremely important goals.

It is well known that ballast water can transport a wide array of invasive species including potentially harmful bacteria. The introduction and rapid spread of the VHS virus in fish throughout the Great Lakes led many to recognize that some microbes can be viewed as harmful invasive species, just like their invasive animal and plant counterparts. The potential for this threat to cause real human or environmental hazards in Lake Superior waters has not been extensively evaluated. A recent conceptual analysis underscored the importance of hazards from fish and wildlife pathogens. Recently completed research funded by the Minnesota ENRTF confirmed our concern about the potential for microbial invasions in the DSH because DNA sequences from 33 and 14 bacterial genera containing human, and fish and wildlife pathogens, respectively, were found in ship ballast waters. These bacterial DNA sequences were often more common than those of indicator bacteria recommended by the International Maritime Organization for monitoring microbiological safety of ballast and recreational waters.

The federally required ballast water treatment systems in ocean-going vessels, and best management practices in Great Lakes vessels can be evaluated prior to their installation for their capacity to significantly reduce the importation and redistribution of larger invasive organisms. However, little is known about the effectiveness of these treatment systems to remove or inactivate many bacterial pathogens. Thus, the University of Minnesota and Northeast-Midwest Institute (NEMWI) will address these issues in cooperation with the MPCA. The overall goals are to build an empirical database for assessing the actual hazard, if any, and evaluate ballast water treatment technology effectiveness for attenuating such a hazard.

II. DESCRIPTION OF PROJECT ACTIVITIES

Activity 1: Collect Ballast Water from Commercial Ships **Budget: $90,994**

Ballast water will be sampled from at least five commercial freshwater and ocean-going ships to identify potentially harmful microbes and test ballast water treatment technologies (see Activity 2). This information will expand a database of bacterial diversity and potentially harmful microbes in commercial ship ballast water discharged in Minnesota.

Outcomes	Completion Date
1. Collect and expand repository of microbial DNA from commercial ship ballast water	*November 2017*
2. Expand a database of microbial diversity and bacterial pathogens in a variety of ship ballast waters	*November 2018*

Activity 2: Determine Current Ballast Water Treatment Technology Effectiveness for Neutralizing Potentially Harmful Bacteria **Budget: $92,404**

Our goal is to determine if current ballast water treatment technologies are effective for neutralizing potentially harmful bacteria in ballast water and whether this removal can be assessed effectively using standard microbial assessment tools for fecal indicator bacteria. This objective will test how current ballast

water treatment technologies alter the composition and relative abundance of potentially harmful microbes and determine whether these changes could be more readily and cheaply indicated by monitoring removal of indicator bacteria or larger invasive organisms. The results will be applicability to in-line, in-tank, and on-shore treatment systems.

Outcomes	Completion Date
1. Conduct bench-scale experiments to evaluate indicator and potentially harmful bacteria removal using common ballast water treatment technologies	*November 2017*
2. Correlate removal and inactivation of culturable bacterial indicators with DNA-based molecular methods to detect removal of bacterial indicators and potential pathogens	*February 2018*

Activity 3: *Taxonomically Identify the Common and Rare Bacteria in the SLRE* **Budget: $161,348**

Sediments and water will be collected from multiple sites across the SLRE during the ice-free season (see attached map). 16S rDNA gene amplicons will be sequenced to identify common and rare bacterial genera. This dataset will be compared with new and existing data on the bacterial compositions of ship ballast water and treated wastewater. This activity will focus on forecasting the risk that different bacteria pose on the sustainability of recreational fisheries, human health, and a functional SLRE ecosystem and help develop management strategies that prevent the unintended introduction of potentially harmful microbial species.

Outcomes	Completion Date
1. Collect sediments and water from the SLRE and extract microbial DNA	*July 2018*
2. Create a 16S rDNA sequence database of bacteria in SLRE sediments and water	*September 2018*
3. Identify potentially harmful bacteria in SLRE sediments and sediments to compare with new and existing datasets to identify potential sources of pathogens and estimate risk	*December 2018*

Activity 4: Public Outreach and Educational Enrichment **Budget: $24,249**

Outcomes	Completion Date
1. Provide an annual internship for a science teacher to engage K-12 students	*August 2018*
2. Provide presentations at the Great Lakes Aquarium and other outreach efforts	*December 2018*

III. PROJECT STRATEGY

A. Project Team/Partners

Randall Hicks (project manager), will coordinate the project and oversee the work of research staff at the University of Minnesota. The NEMWI will design and conduct the bench scale lab experiments at the Great Ships Initiative's facilities. We will cooperate with the MPCA and Wisconsin DNR to obtain additional ballast water samples, and the Lake Superior National Estuarine Research Reserve and UMD Large Lakes Observatory to obtain sediment and water samples. A letter of support for this project from the MCPA and Minnesota DNR is being sent to the LCCMR.

B. Timeline Requirements

We are proposing a 2.5-year project period. Ballast, sediment and SLRE water samples will be collected and tested over two years to provide sufficient samples to determine the inter-annual variability of microbial communities.

C. Long-Term Strategy and Future Funding Needs

This project will identify the prevalence of potentially pathogenic bacteria within the SLRE and compare these findings with new and existing data about the sources of these microbes. This information can be used to forecast the potential risks of introducing harmful microbes on the ecological and economic sustainability of the SLRE and the ballast water treatment activity will provide management strategies to mitigate the risk of introducing new bacterial invasive species.

2016 Detailed Project Budget

Project Title: *Advancing Microbial Invasive Species Monitoring from Ballast Discharge*

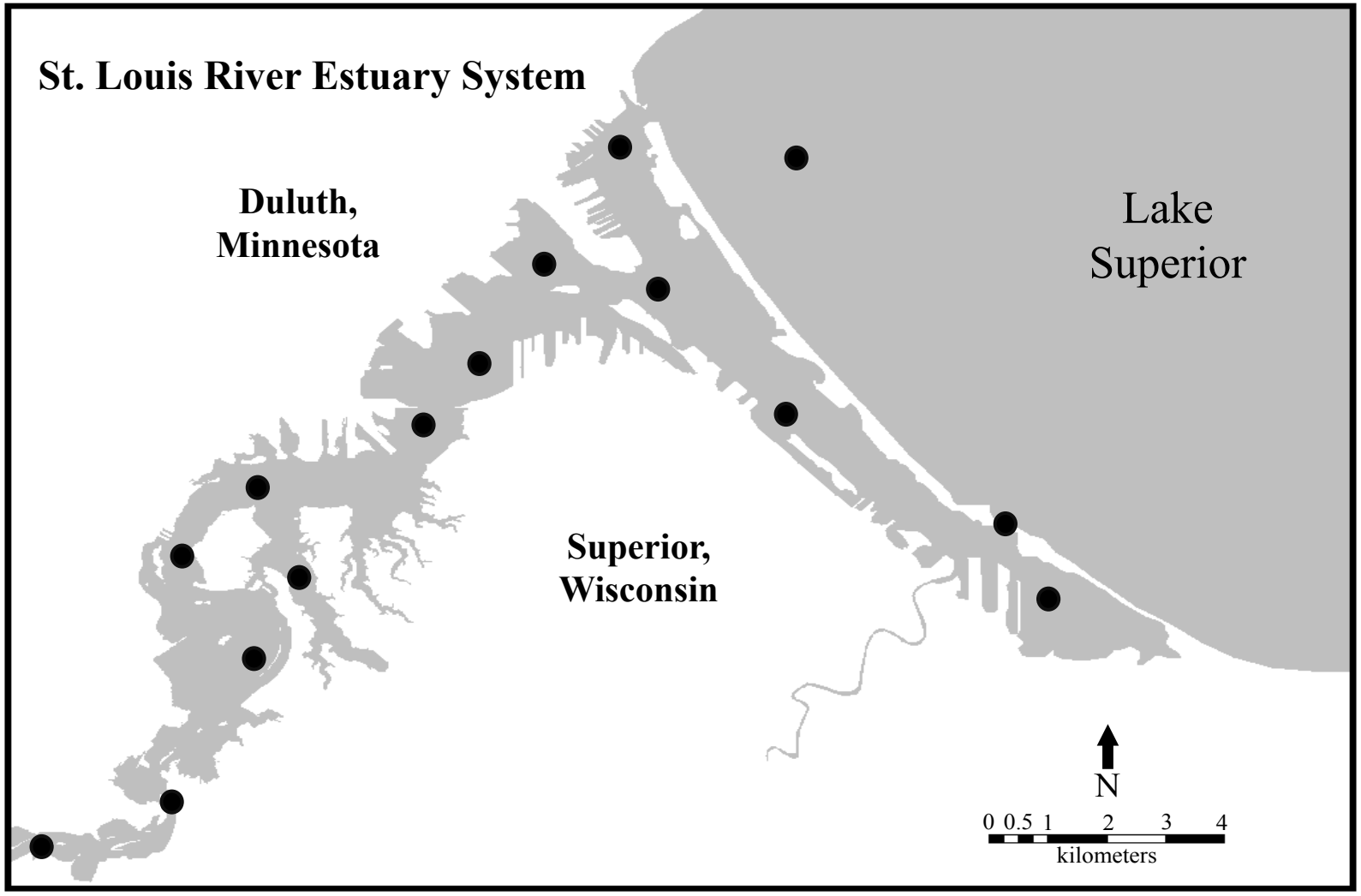
IV. TOTAL ENRTF REQUEST BUDGET *[2.5 years; July 1, 2016 - December 31, 2018]*

BUDGET ITEM	AMOUNT
Personnel:	
Randall E. Hicks (74.8% salary+25.2% fringe; 8.3% FTE) (summer)	**$ 30,838**
Postdoctoral Associate (81.7% salary+18.3% fringe: 100% FTE) (12 mo/yr; July 1, 2016-Aug 31, 2018)	**$ 137,547**
Graduate Research Assistant (55.0% salary+45.0% fringe: 50% FTE) (12 mo/yr; July 1, 2016-Aug 31, 2018)	**$ 86,550**
Professional/Technical/Service Contracts:	
Northeast-Midwest Institute Subcontract	**$ 44,500**
K-12 Teacher Stipend	**$ 1,000**
Equipment/Tools/Supplies:	
Sediment and water sampling supplies	**$ 1,515**
DNA extraction and PCR reagents	**$ 12,150**
Illumina sequencing and data storage costs	**$ 19,270**
Chemicals and expendable lab supplies	**$ 11,200**
Acquisition (Fee Title or Permanent Easements): N/A	$ -
Travel: Sampling trips: 3 trips; Present results at 2 conferences	**$ 3,108**
Additional Budget Items:	
Publication costs	**$ 2,045**
R/V Blue Heron ship time (2 days @ $8,500/day)	**$ 17,000**
Small boat rental (8 days @ $250/day)	**$ 2,272**
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	**$ 368,995**

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:		
Other State $ Being Applied to Project During Project Period: UM and NEMWI F&A Match	$ 191,849	*Secured*
In-kind Services During Project Period: Randall Hicks PI effort	$ -	*Secured*
Funding History:	$ -	
ENRTF Funding (Project ML2011-188-E) 2011-2013 (Ballast Water Bacteria)	$ 250,000	
Remaining $ from Current ENRTF Appropriation (if applicable): N/A	$ -	

Map of the St. Louis River Estuary and the Duluth-Superior Harbor. Dots indicate the sites where sediment and water samples will be collected for the "Advancing Microbial Invasive Species Monitoring from Ballast Discharge" project.



Project Manager: Randall E. Hicks

PROJECT MANAGER QUALIFICATIONS AND ORGANIZATION DESCRIPTION

Randall E. Hicks is a Professor of Biology 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 UMD. Dr. Hicks is an environmental microbiologist who studies the diversity and productivity of aquatic microbial communities, 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, Africa and various oceans, but his current research is focused on the North American Great Lakes. He has published over 40 scientific journal articles and book chapters. Dr. Hicks brings several decades of organizational experience and expertise ranging from heading a large academic department (UMD Biology; 1998-2006), organizing an international scientific conference (IAGLR 2011), to directing a university center (UMD Center for Freshwater Research and Policy; 2007-2011).

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 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 (~1,200 ft^2) is equipped for research in the areas of microbial ecology, organic geochemistry, and molecular biology and includes computers and special software for genetic and phylogenetic analyses. The Department of Biology is well equipped for microbiological, limnological, and molecular biology research. In addition, his laboratory and this project have access to DNA sequencing facilities at the University of Minnesota Biomedical Genomics Center and the Minnesota Supercomputing Institute for analysis of DNA sequence data generated by this project.

The Northeast-Midwest Institute oversees the operation of the Great Ships Initiative ballast water testing facility in Superior, WI. These facilities include laboratories to test new ballast water treatment technologies at the bench-scale and mesocoms, a full-scale, on-land testing facility, and scientific labs to process samples.