

**Environment and Natural Resources Trust Fund
2014 Request for Proposals (RFP)**

Project Title:

ENRTF ID: 102-D

Bacterial Biodiversity for Sustainable St. Louis River Estuary

Category: D. Aquatic and Terrestrial Invasive Species

Total Project Budget: \$ 359,849

Proposed Project Time Period for the Funding Requested: 2.5 Years, July 2014 - December 2016

Summary:

We will identify the common and rare bacteria that currently inhabit the St. Louis River Estuary to evaluate the potential ecological and economic damage caused by new invasive bacterial species.

Name: Randall Hicks

Sponsoring Organization: U of MN - Duluth

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Location

Region: Northeast

County Name: St. Louis

City / Township:

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



PROJECT TITLE: Bacterial Biodiversity for a Sustainable St. Louis River Estuary

I. PROJECT STATEMENT

Healthy and functional bacterial communities in urban harbors and ports are critical for sustaining aquatic ecosystems and coastal economies. Many stressors affect the ecological and economic sustainability of the St. Louis River Estuary (SLRE), such as invasive bacterial species and rapid steel corrosion of port infrastructure. While the Great Lakes face many threats, the presence of invasive species also threatens Minnesota’s people and coastal economies. The ballast water of ships can be a vector for the global transport of aquatic microorganisms. Some of the bacteria being released into the Duluth-Superior Harbor (DSH) may threaten human and aquatic animal health, cause ecological damage, and impact local coastal economies. The 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. These facts make early detection of potentially harmful microbes an extremely important goal.

With recent ENRTC funding, we found DNA sequences from 33 bacterial genera containing human pathogens and 14 bacterial genera containing fish and wildlife pathogens in ship ballast water that were discharged into the DSH in 2011 and 2012. The sequences from these bacterial genera were often more common than sequences of indicator bacteria (e.g., *Escherichia*, *Enterococcus*, *Vibrio*) recommended by the International Maritime Organization for monitoring the microbiological safety of ballast water. Unlike aquatic plants and animals, a taxonomic evaluation of bacterial biodiversity has never been completed in the SLRE. Yet, such data is the missing link for understanding risks to the ecological and economic sustainability of the SLRE. Current concern about introducing harmful and potentially invasive bacterial species into waterways and regional concern about microbiologically influenced corrosion in the SLRE justify the need for a comprehensive evaluation of bacterial biodiversity in the SLRE and DSH. We will identify the common and rare bacteria that currently inhabit the SLRE and compare the composition of bacteria within the SLRE with those bacteria discharged in ship ballast water and found on corroding steel structures in the SLRE. Our overarching goal is to identify harmful bacteria that are introduced or exist in the SLRE so future efforts can focus on bacteria that pose the greatest risk to the sustainability of recreational fisheries, human health, port infrastructure, and a functional ecosystem.

II. DESCRIPTION OF PROJECT ACTIVITIES

Activity 1: Collect Water and Sediments from the SLRE and Extract DNA **Budget: \$64,255**

Water and sediment samples will be collected three times from at least 15 sites across the SLRE and nearshore Lake Superior during the ice-free season (see attached map). Water samples will be filtered and bacterial community DNA on these filters and in sediment will be extracted for next generation sequencing.

Outcomes	Completion Date
1. Build water and sediment collections from the SLRE during three ice-free seasons	October 2015
2. Develop a repository of purified microbial DNA from SLRE water and sediment	November 2015

Activity 2: Taxonomically Identify the Common and Rare Bacteria in the SLRE **Budget: \$149,615**

The 16S rDNA gene of DNA samples will be amplified and sequenced using Illumina next generation sequencing technology to identify common and rare bacterial genera in water and sediments. This dataset will be compared with existing data on the bacterial compositions of ship ballast water and steel corrosion microbial communities.

Outcomes	Completion Date
1. Create a 16S rDNA sequence database of bacteria in SLRE water and sediments	March 2016



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2. Create lists of bacterial genera found in SLRE water and sediments	<i>July 2016</i>
3. Evaluate season and geographic changes in SLRE bacteria communities	<i>September 2016</i>

Activity 3: Analyze Bacterial Compositions of the SLRE, Ballast Water, and Corroding Steel Structures **Budget: \$106,522**

We will compare results from this project with data from ongoing investigations on the bacterial biodiversity of ballast water of commercial ships and corroding steel structures supported by the LCCMR and Minnesota Sea Grant program, respectively. These activities will focus on bacteria that pose the greatest risk to the sustainability of recreational fisheries, human health, port infrastructure, and a functional ecosystem. Results will be useful for creating best management plans to prevent the unintended transport of potentially harmful microbial species.

Outcomes	Completion Date
1. Develop taxonomic comparisons of bacterial communities from different sources	<i>October 2016</i>
2. Identify the most potentially harmful bacteria introduced in the ballast water ships	<i>December 2016</i>
3. Identify bacteria that may influence the corrosion of steel structures in the DSH	<i>December 2016</i>

Activity 4: Public Outreach and Educational Enrichment **Budget: \$39,457**

The graduate student will present our findings for the public at the Great Lakes Aquarium in Duluth, MN through their teacher workshops and adult seminar presentation series. We will provide an intern opportunity for a K-12 teacher, who will translate her/his experience into lesson plans to engage K-12 students.

Outcomes	Completion Date
1. Presentations at the Great Lakes Aquarium and other public venues	<i>December 2016</i>
2. Hire a student team member from a group that is underrepresented in the STEM sciences	<i>August 2014</i>
3. Provide a science enrichment opportunity for a K-12 teacher	<i>June 2015</i>

III. PROJECT STRATEGY

A. Project Team/Partners

Randall Hicks and Michael Sadowsky (co-project managers), University of Minnesota, will coordinate the project and oversee the work of the postdoctoral associate, graduate and undergraduate students, and a K-12 teacher who will collect samples, extract DNA, and sequence and analyze the bacterial community DNA to identify potentially harmful microbes. We will collaborate with personnel from the UM Large Lakes Observatory to obtain water and sediment samples.

B. Timeline Requirements

We are proposing a 2.5 year project period. Water and sediment samples will be collected each year from the St. Louis River Estuary to provide a sufficient sample set to determine the inter-annual variability of bacterial communities to compare with potentially harmful bacteria released from the ballast water of commercial ships.

C. Long-Term Strategy and Future Funding Needs

This project will identify potentially harmful bacteria within the St. Louis River Estuary and compare them with bacteria introduced by discharge of ship ballast water and bacteria found on severely corroding port structures to identify the risk of introducing bacteria on the ecological and economic sustainability of the SLRE. In a future project, we will develop a suite of sensitive, early warning, monitoring methods to identify these harmful bacteria. However, we are not seeking funds for such a project until the types of bacteria already present in the SLRE are well established, the most potentially harmful new microbes entering this estuary have been evaluated and ranked using the information obtained by this project, and the potential risks to Lake Superior’s ecosystem and Minnesota’s coastal economies are estimated.

2014 Detailed Project Budget

Project Title: *Bacterial Biodiversity for a Sustainable St. Louis River Estuary*

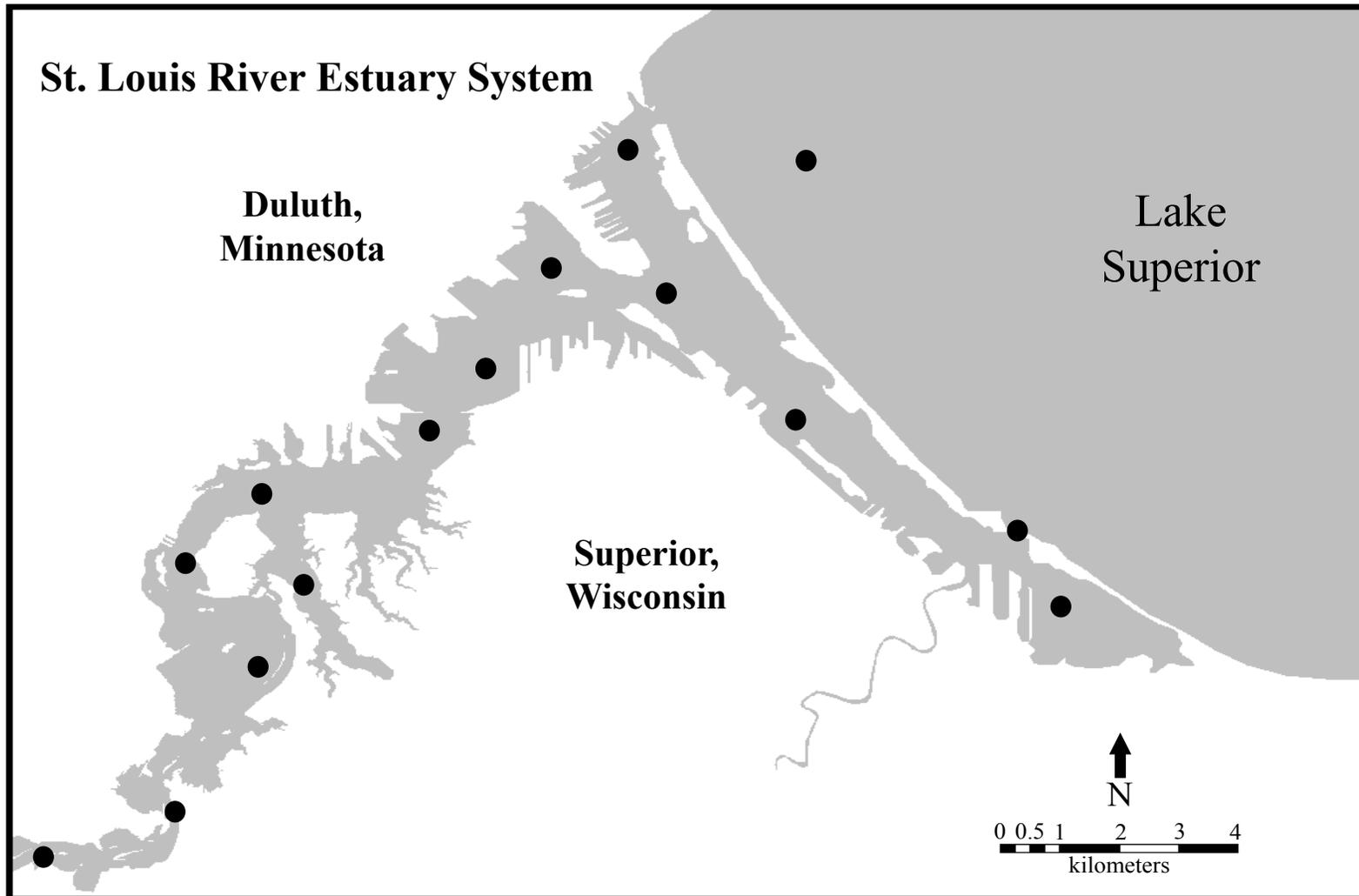
IV. TOTAL ENRTF REQUEST BUDGET [2.5 years]

<u>BUDGET ITEM</u>	<u>AMOUNT</u>
Personnel:	
Randall E. Hicks (2 mo @ 100% time + 33.6% Fringe Benefits; 74.9% salary+25.1% FB; 22% FTE)	\$ 28,094
Michael J. Sadowsky (1 mo @ 100% time + 33.6% Fringe Benefits; 74.9% salary+25.1% FB; 11% FTE)	\$ 20,909
Postdoctoral Associate (26 mo @ 100% time + 20.75% Fringe Benefits; 82.8% salary+17.2% FB; 50% FTE)	\$ 127,213
Graduate Research Assistant (26 mo @ 50% time + 23.1% Fringe Benefits + Tuition Benefit; 82.8% salary+17.2% FB; 25% FTE)	\$ 82,119
Undergraduate Research Assistant (6.82 mo @ 100% time + 7.34% Fringe Benefits; 93.2% salary+6.8% FB; 57% FTE)	\$ 13,714
Contracts:	
K-12 Teacher Stipend (1 teacher)	\$ 1,000
Equipment/Tools/Supplies:	
Water and sediment sampling supplies	\$ 1,000
DNA extraction and PCR reagents	\$ 12,500
Illumina sequencing and data storage costs	\$ 31,500
Chemicals and expendable lab supplies	\$ 10,200
Publication costs	\$ 1,000
Acquisition (Fee Title or Permanent Easements): N/A	\$ -
Travel: Sampling trips: 3 trips; Travel between UM campuses: 3 trips/year	\$ 3,600
Additional Budget Items:	
R/V Blue Heron ship time (3 days @ \$8,500/day)	\$ 25,500
Small boat rental (6 days @ \$250/day)	\$ 1,500
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND \$ REQUEST =	\$ 359,849

V. OTHER FUNDS

<u>SOURCE OF FUNDS</u>	<u>AMOUNT</u>	<u>Status</u>
Other Non-State \$ Being Applied to Project During Project Period: N/A	\$ -	
Other State \$ Being Applied to Project During Project Period:	\$ -	
In-kind Services During Project Period: Hicks Salary Match (0.5 mo/yr + 33.6% FB)	\$ 14,047	<i>Secured</i>
Remaining \$ from Current ENRTF Appropriation (if applicable): N/A	\$ -	
Funding History:		
ENRTF Funding (Project ML2011-188-E) 2011-2013 (Ballast Water Bacteria)	\$ 250,000	
Minnesota Sea Grant Program grant 2012-2014 (Steel Corrosion in Duluth-Superior Harbor)	\$ 211,096	

Map of the St. Louis River Estuary and the Duluth-Superior Harbor. Dots indicate the sites where water and sediment samples will be collected for the “Bacterial Biodiversity for a Sustainable St. Louis River Estuary” 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 in 1986. 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, Africa 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 35 scientific journal articles and book chapters. Dr. Hicks brings several decades of organizational experience and expertise ranging from heading a large academic department, organizing an international scientific meeting, to directing a university center. For eight years (1998-2006), he was the Head of UMD Biology, a department of 50-60 employees that serves over 800 undergraduate and graduate students working on biology-related degrees. He was Director of the UMD Center for Freshwater Research and Policy for four years (2007-2011).

Michael J. Sadowsky 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 completed a Ph.D. in Microbiology at the University of Hawaii after finishing M.S (Microbiology) and B.S. (Bacteriology) degrees at the University of Wisconsin campuses in Oshkosh and Madison, respectively. Dr. Sadowsky is an environmental microbiologist with 30 years research experience in the analysis and use of microorganisms in environmental settings. He is a fellow in the prestigious American Academy of Microbiology and his research is internationally known and respected. Dr. Sadowsky has published more than 100 original articles, and his work is widely cited by researchers in several scientific disciplines. His 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. Dr. Sadowsky is currently involved in three large metagenome projects; the soil metagenome to define novel microbial genes for biofuel and bioenergy, the Mississippi River metagenome 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.

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 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.