

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

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**Project Title:**

**ENRTF ID: 088-D**

Forecasting Microbial Invasions: Muskie Pox and VHS Virus

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**Category:** D. Aquatic and Terrestrial Invasive Species

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**Total Project Budget:** \$ 467,589

**Proposed Project Time Period for the Funding Requested:** 2.5 years, July 2015 - December 2017

**Summary:**

We will determine the prevalence of "muskie pox" bacteria, VHS virus, and other invasive microbes in St. Louis River Estuary muskellunge, water, and sediment to forecast risk to recreational fisheries.

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**Location**

**Region:** NE

**County Name:** St. Louis

**City / Township:**

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**Alternate Text for Visual:**

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 "Forecasting Microbial Invasions: Muskie Pox and VHS Virus" project.

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



**PROJECT TITLE: Forecasting Microbial Invasions: Muskie Pox and VHS Virus**

**I. PROJECT STATEMENT**

Healthy and functional microbial communities in urban harbors and ports are critical for sustaining aquatic ecosystems and coastal economies. In the St. Louis River Estuary (SLRE), however, stressors such as invasive microbes are affecting ecological and economic sustainability. While the Great Lakes face many threats, the presence of large and small invasive species threatens Minnesota’s natural resources, people, and coastal economies. The introduction and rapid spread of the VHS virus in fish throughout the Great Lakes, including Lake Superior and likely the Duluth-Superior Harbor (DSH), have led many to recognize that some microbes can be viewed as harmful invasive species, just like their invasive animal and plant counterparts.

The ballast water of ships can be a primary source of invasive species and a vector for microbial introductions and movements. In fact, 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. 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 an extremely important goal.

Recent research funded by the Minnesota ENRTF confirmed our concern about microbial invasions in the SLRE. We found DNA sequences from 33 and 14 bacterial genera containing human, and fish and wildlife pathogens, respectively, in ship ballast waters that were discharged in 2011 and 2012. These bacterial DNA sequences were often more common than those of fecal indicator bacteria recommended by the International Maritime Organization for monitoring microbiological water safety. The bacterium *Piscirickettsia* was detected that causes “muskie pox” disease in muskellunge (*Esox masquinongy*). DNA from this bacterium was found in 25% of the ships sampled, including ships transporting ballast water from Lake St. Clair where *Piscirickettsia* was found in dead muskellunge during a 2006 fish kill. Fishermen in the SLRE have observed lesions similar to those caused by “muskie pox” and VHS on muskellunge. Thus, we will determine the abundance of *Piscirickettsia* and VHS virus in muskellunge, water and sediment and also identify other potentially harmful bacteria and viruses in these samples. Our overarching goal is to forecast which bacteria and viruses pose the greatest risk to the sustainability of recreational fisheries, human health, and a functional ecosystem.

**II. DESCRIPTION OF PROJECT ACTIVITIES**

**Activity 1:** Collect Water, Sediments, and Muskellunge from the SLRE **Budget: \$115,087**

Water and sediment samples will be collected from at least 15 sites across the SLRE and nearshore Lake Superior during the ice-free season (see attached map). Bacterial community DNA in water and sediment will be collected and extracted for next generation sequencing. Skin and internal organs of the SLRE muskellunge population will be sampled to extract DNA to identify microbial pathogens and estimate infection rates.

Outcomes	Completion Date
1. Build water and sediment collections from the SLRE	August 2016
2. Collect muskellunge from the SLRE	August 2016
3. Develop a repository of purified microbial DNA from SLRE muskies, water and sediment	November 2016

**Activity 2:** Measure Abundance of *Piscirickettsia* Bacteria and VHS Virus in Muskellunge and Identify Potentially Harmful Bacteria in the SLRE **Budget: \$285,736**

DNA from *Piscirickettsia* bacteria and VHS virus will be quantitatively amplified to identify the extent of existing “muskie pox” and VHS infections in the SLRE muskellunge population. Microbial DNA and RNA (bacterial and viral, respectively) will be amplified and sequenced using next generation Illumina sequencing technology to



**Environment and Natural Resources Trust Fund (ENRTF)**

**2015 Main Proposal**

**Project Title:** *Forecasting Microbial Invasions: Muskie Pox and VHS Virus*

identify other potentially harmful microbes in SLRE water and sediments and evaluate potential sources (ship ballast, tributary water, stormwater, treated wastewater) of these microbes. This activity will focus on forecasting the risk that different microbes pose on the sustainability of recreational fisheries, human health, and a functional ecosystem and help develop management strategies that prevent the unintended introduction of potentially harmful microbial species.

<b>Outcomes</b>	<b>Completion Date</b>
1. Measure <i>Piscirickettsia</i> bacteria and VHS virus abundances in muskellunge, water, sediment, and potential sources, and determine “muskie pox” and VHS virus infection rates in SLRE muskellunge population	<i>October 2017</i>
2. Compare lesion diaries from Muskies, Inc. with <i>Piscirickettsia</i> and VHS abundance data to determine if lesion diaries can be used for future SLRE muskellunge monitoring	<i>December 2017</i>
3. Create databases to identify other potentially harmful bacteria and viruses in SLRE water and sediments, and then compare with new and existing datasets to identify potential sources of pathogens	<i>December 2017</i>

**Activity 3: Public Outreach and Educational Enrichment**

**Budget: \$66,766**

Partnering with the Lake Superior Chapter of Muskies, Inc. will provide an opportunity for citizen involvement that may lead to a sustained monitoring program. We will provide an intern opportunity for a STEM teacher, who will translate her/his experience into lesson plans to engage K-12 students. The graduate students and postdoctoral associate will present our findings for the public at the Great Lakes Aquarium in Duluth, MN through their teacher workshops and adult seminar presentation series.

<b>Outcomes</b>	<b>Completion Date</b>
1. Partner with Lake Superior Chapter of Muskies, Inc. to develop SLRE muskellunge population and lesion diaries	<i>September 2017</i>
2. Provide graduate education and postdoctoral training and a science enrichment opportunity for a K-12 science teacher	<i>August 2016</i>
3. Presentations at the Great Lakes Aquarium and other public venues	<i>December 2017</i>

**III. PROJECT STRATEGY**

**A. Project Team/Partners**

Randall Hicks (project manager), Nicholas Phelps, and Michael Sadowsky from the University of Minnesota will coordinate the project and oversee the work of the postdoctoral associate, graduate students, and a K-12 teacher who will collect samples, extract DNA, and sequence and analyze the microbial DNA to identify potentially harmful microbes. We will collaborate with Lake Superior Chapter of Muskies, Inc. to acquire diaries of muskie lesions and the UMD Large Lakes Observatory to obtain water and sediment samples.

**B. Timeline Requirements**

We are proposing a 2.5-year project period. Water, sediment, and muskellunge samples will be collected 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 “muskie pox” bacteria and VHS virus in SLRE muskellunge. We will also identify the prevalence of these and other potentially harmful microbes within the water and sediments of the St. Louis River Estuary 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 inform management strategies to mitigate the risk of potential dispersal to inland lakes of Minnesota.

## 2015 Detailed Project Budget

Project Title: *Forecasting Microbial Invasions: Muskie Pox and VHS Virus*

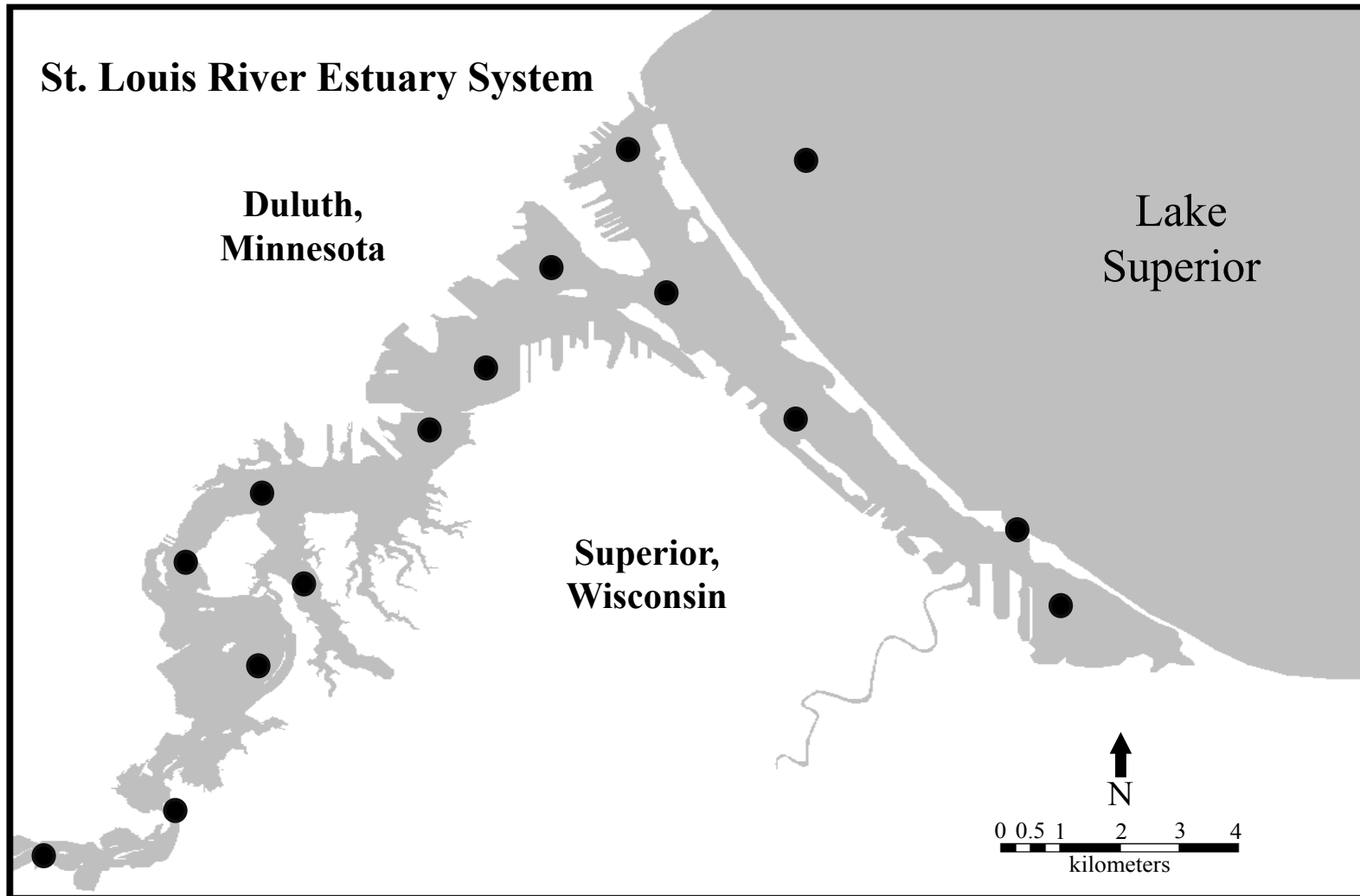
### IV. TOTAL ENRTF REQUEST BUDGET [2.5 years; July 1, 2015 - December 31, 2017]

<u>BUDGET ITEM</u>	<u>AMOUNT</u>
<b>Personnel:</b>	
Randall E. Hicks (2 mo @ 100% time + 33.6% Fringe Benefits; 74.9% salary+25.1% FB; 8.3% FTE)	\$ 29,527
Nicholas Phelps (1 mo @ 100% time + 33.6 Fringe Benefits; 74.9% salary+25.1%FB; 4.2% FTE)	\$ 11,479
Michael J. Sadowsky (1 mo @ 100% time + 33.6% Fringe Benefits; 74.9% salary+25.1% FB; 4.2% FTE)	\$ 22,015
Postdoctoral Associate (26 mo @ 100% time + 20.75% Fringe Benefits; 82.8% salary+17.2% FB: 100% FTE)	\$ 135,693
Graduate Research Assistant-Hicks (1 student; 26 mo @ 50% time + 15.7% Fringe Benefits + Tuition Benefit; 82.8% salary+17.2% FB; 50% FTE)	\$ 83,947
Graduate Research Assistant-Phelps (1 student; 26 mo @ 50% time + 15.7% Fringe Benefits + Tuition Benefit; 82.8% salary+17.2% FB; 50% FTE)	\$ 90,995
<b>Contracts:</b>	
K-12 Teacher Stipend	\$ 1,000
<b>Equipment/Tools/Supplies:</b>	
Water and sediment sampling supplies	\$ 1,000
Muskellunge sampling supplies and effort	\$ 5,075
DNA extraction and PCR reagents	\$ 15,428
Illumina sequencing and data storage costs	\$ 31,972
Chemicals and expendable lab supplies	\$ 15,240
<b>Acquisition (Fee Title or Permanent Easements):</b> N/A	\$ -
<b>Travel:</b> Sampling trips: 3 trips; Travel between UM campuses: 3 trips/year	\$ 3,651
<b>Additional Budget Items:</b>	
Publication costs	\$ 2,045
R/V Blue Heron ship time (2 days @ \$8,500/day)	\$ 17,000
Small boat rental (6 days @ \$250/day)	\$ 1,522
<b>TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND \$ REQUEST =</b>	<b>\$ 467,589</b>

### V. OTHER FUNDS

<u>SOURCE OF FUNDS</u>	<u>AMOUNT</u>	<u>Status</u>
<b>Other Non-State \$ Being Applied to Project During Project Period:</b> N/A	\$ -	
<b>Other State \$ Being Applied to Project During Project Period:</b> UM F&A Match	\$ 206,927	<i>Secured</i>
<b>In-kind Services During Project Period:</b> Hicks Salary Match (0.5 mo/yr + 33.6% FB)	\$ 14,764	<i>Secured</i>
<b>Remaining \$ from Current ENRTF Appropriation (if applicable):</b> N/A	\$ -	
<b>Funding History:</b>		
ENRTF Funding (Project ML2011-188-E) 2011-2013 (Ballast Water Bacteria)	\$ 250,000	

**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 “Forecasting Microbial Invasions: Muskie Pox and VHS Virus” 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. Dr. Hicks is an environmental microbiologist who studies the diversity and productivity of aquatic microbial communities, and the survival 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 37 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).

**Nicholas Phelps** is an Assistant Professor in the Department of Veterinary Population Medicine at the University of Minnesota College of Veterinary Medicine. He earned a Ph.D. in Veterinary Medicine (University of Minnesota), an M.S. in Aquaculture/Fisheries with an emphasis on fish health (University of Arkansas–Pine Bluff), and a B.S. in Aquatic Biology (Bemidji State University). His research focuses on emerging threats to fisheries sustainability, which lie at the intersection of animals, humans, and environmental health. Dr. Phelps has ongoing research in emerging virus discovery, aquatic invasive species control, risk management, diagnostic development, and significant efforts focused on detection, control, and management of the viral hemorrhagic septicemia (VHS) virus of fish.

**Michael J. Sadowsky** is a McKnight Distinguished Professor in the Department of Soil, Water, and Climate and currently 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. Dr. Sadowsky has published more than 100 original articles and his research is internationally known and respected. 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 metagenome 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. Dr. Hicks's laboratory (~1,200 ft<sup>2</sup>) 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.

Dr. Phelps' and Sadowsky's research laboratories are located on the St. Paul campus of the University of Minnesota. Their labs are well equipped for microbiological, viral, molecular biology, fish pathogen, and biotechnology research.