



Environment and Natural Resources Trust Fund (ENRTF) M.L. 2016 Work Plan

Date of Report: May 29, 2016

Date of Next Status Update Report: January 31, 2017

Date of Work Plan Approval: June 7, 2016

Project Completion Date: December 31, 2018

Does this submission include an amendment request? No

PROJECT TITLE: Advancing Microbial Invasive Species Monitoring from Ballast Discharge

Project Manager: Randall E. Hicks

Organization: University of Minnesota Duluth

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Location: St. Louis County

Total ENRTF Project Budget:

ENRTF Appropriation: \$368,000

Amount Spent: \$0

Balance: \$368,000

Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 06c

Appropriation Language:

\$368,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota to identify bacteria in ship ballast water and St. Louis River estuary sediments, assess the risks posed by invasive bacteria, and evaluate treatment techniques for effectiveness at removing the bacteria from ballast water. This appropriation is available until June 30, 2019, by which time the project must be completed and final products delivered.

I. PROJECT TITLE: Advancing Microbial Invasive Species Monitoring from Ballast Discharge

II. 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. In this project, we will expand a database of bacterial diversity and potentially harmful microbes collected from commercial ship ballast water discharged in the Duluth-Superior Harbor. Common and rare bacterial taxa will also be identified in water and sediments of the St. Louis River estuary and compared with bacteria discharged with ballast water to predict the risk different bacterial groups pose on sustainable fisheries, human health, and a functional St. Louis River Estuary ecosystem.

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 Northeast-Midwest Institute (NEMWI) and the University of Minnesota will conduct bench scale experiments with mock ballast water treatment processes to contrast changes to the composition and relative abundance of fecal bacteria and indicator organisms using standard methods, versus non-conventional potentially harmful bacteria concentrations using genetic detection probes.

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. Addressing these issues will inform management strategies that mitigate the risk of inadvertently introducing potentially harmful bacteria.

III. OVERALL PROJECT STATUS UPDATES:

Project Status as of January 31, 2017:

Project Status as of July 31, 2017:

Project Status as of January 31, 2018:

Project Status as of July 31, 2018:

Overall Project Outcomes and Results:

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Collect Ballast Water from Commercial Ships

Description:

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). We will work closely with the Minnesota Pollution Control Agency and the Wisconsin Department of Natural Resources to collect ballast water from commercial ships arriving in the Duluth-Superior Harbor, and with the NEMWI to collect ballast samples from other ships. DNA will be extracted from ballast water samples and bacterial community DNA sequenced to expand a database of bacterial diversity and potentially harmful microbes in commercial ship ballast water discharged in Minnesota.

Summary Budget Information for Activity 1:

ENRTF Budget: \$ 103,983
Amount Spent: \$ 0
Balance: \$ 103,983

Outcome	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 Status as of January 31, 2017:

Activity Status as of July 31, 2017:

Activity Status as of January 31, 2018:

Activity Status as of July 31, 2018:

Final Report Summary:

ACTIVITY 2: Comparison of Standard Versus Non-Standard Approaches for Assessing Current Ballast Water Treatment Technology Effectiveness for Neutralizing Potentially Harmful Bacteria

Description:

Our goal is to determine if neutralizing potentially harmful bacteria in ballast water can be most effectively assessed using standard microbial assessment tools for fecal indicator bacteria (standard approach), or genetic detection alternative methods. This objective will test whether BWMS induced changes in microbial composition of ballast water could be more readily and cheaply indicated by monitoring fecal indicator organisms, or genetic signals for potential microbial invaders. These bench-scale tests will be conducted on two ballast water samples at the Great Ships Initiative (GSI) facility in Superior, WI. In addition to UV radiation, other conventional and treatment methods in development are also being considered for evaluation. GSI will conduct standard fecal indicator bacteria analyses while the University of Minnesota will complete genetic detection analyses. The results will be applicability to in-line, in-tank, and on-shore treatment systems.

Summary Budget Information for Activity 2:**ENRTF Budget: \$ 124,276****Amount Spent: \$ 0****Balance: \$ 124,276**

Outcome	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 Status as of January 31, 2017:**Activity Status as of July 31, 2017:****Activity Status as of January 31, 2018:****Activity Status as of July 31, 2018:****Final Report Summary:****ACTIVITY 3: Taxonomically Identify the Common and Rare Bacteria in the SLRE****Description:**

Sediments and water will be collected from multiple sites across the SLRE during the ice-free season (see attached map). Final sites selected for water and sediment samples will be determined after consultation with personnel from the the Lake Superior National Estuarine Research Reserve, the U.S. EPA, and universities who have previously characterized the distributions of larger invasive species and chemical concentrations in this harbor. Water and sediment samples will be collected from research vessels using standard limnological and microbiological techniques. Microbial DNA will be extracted from the samples and bacterial 16S rDNA gene amplicons will be sequenced to identify common and rare bacterial taxa. 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 ballast water 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

Summary Budget Information for Activity 3:**ENRTF Budget: \$ 139,741****Amount Spent: \$ 0****Balance: \$ 139,741**

Outcome	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 water to compare with new and existing datasets to identify potential sources of pathogens and estimate risk	December 2018

Activity Status as of January 31, 2017:**Activity Status as of July 31, 2017:****Activity Status as of January 31, 2018:****Activity Status as of July 31, 2018:**

Final Report Summary:

V. DISSEMINATION:

Description:

Our research results will be disseminated to several target audiences. First, we will periodically present and discuss the results of our investigation with our collaborators at the Minnesota Pollution Control Agency. We also intend to present our research results to our scientific peers at national and international scientific meetings, and develop manuscripts for scientific publications. We will upload the genetic data into national databases for searching and retrieval by researchers, regulatory agencies, and the public to better understand the diversity of microbes in ballast water. In addition, there are other target audiences we wish to reach; ship owners and agents, port authorities and other organizations such as the Great Lakes Maritime Task Force and the Great Lakes Maritime Research Institute. Data and results from our testing will be discussed with our collaborators at the Minnesota Pollution Control Agency, other interested Minnesota and Wisconsin agencies, dock owners, the Duluth Seaway Port Authority, and the Great Lakes Maritime Research Institute. We expect these existing networks will in turn disseminate information about this issue and our activities to other areas of the great lakes. In addition, we intend to provide updates through public presentations at venues such as the Great Lakes Aquarium.

Status as of January 31, 2017:

Status as of July 31, 2017:

Status as of January 31, 2018:

Status as of July 31, 2018:

Final Report Summary:

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget Overview:

Budget Category	\$ Amount	Overview Explanation
Personnel:	\$ 252,805	Salary support for the project manager (24 months @ 8.3% time), a postdoctoral investigator (27 months @ 100% time), and a graduate research assistant (26 months @ 50%-time)
Professional/Technical/Service Contracts:	\$ 44,500	The Northeast-Midwest Institute will provide professional services to test ballast water treatment process effectiveness using conventional microbial assessment methods. It will also supply ship ballast water samples for DNA analysis. The funds include Ms. Cangelosi @ 55 hours and UWS-LSRI staff at 480 hours (collectively) for laboratory analyses, and ship sample collection.
Equipment/Tools/Supplies:	\$ 52,042	Supplies for water and sediment sampling (\$3,650), DNA extraction and PCR reagents for assays (\$13,922), DNA sequencing and data storage and analysis costs for DNA analyses (\$20,270), chemical and expendable lab

		supplies to support field sample analyses and laboratory experiments (\$14,200)
Travel Expenses in MN:	\$ 3,108	Sampling trips to the St. Louis River estuary and GSI facility (30 trips, \$600) and travel support for project personnel to disseminate project results at public venues, scientific conferences, and outreach events with target organizations (\$2,508). Matching conference travel funding will be sought from the University of Minnesota.
Other:	\$ 15,545	Publication costs for journal articles (\$2,045), R/V Blue Heron ship time (1 day @ \$8,500/day), small boat rental (10 days @ \$500/day)
TOTAL ENRTF BUDGET:		\$368,000

Explanation of Use of Classified Staff: N/A

Explanation of Capital Expenditures Greater Than \$5,000: N/A

Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: 3.58

Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation: 0.03 (NEMWI) + 0.23 (UWS)

B. Other Funds:

Source of Funds	\$ Amount Proposed	\$ Amount Spent	Use of Other Funds
Non-state			
NEMWI F&A Match	\$10,112	\$	Indirect Cost Match
State			
UM F&A Match	\$163,939	\$	Indirect Cost Match
TOTAL OTHER FUNDS:	\$174,051	\$	

VII. PROJECT STRATEGY:

A. Project Partners:

Great Ships Initiative, Northeast-Midwest Institute

Ms. Allegra Cangelosi will lead the GSI-based contribution to the project, namely, the collection of ballast water samples and the bench-scale experiments (Activity 2). She will also provide review of all project objectives and their outcomes. Dr. Matthew Teneyk, University of Wisconsin-Superior will conduct the GSI-based bench-scale experiments under supervision of Ms. Cangelosi, using standard indicator bacteria assays for comparison with genetic detection approaches implemented by Dr. Randall Hicks. Research activities will take place in Duluth-Superior Harbor, at the Great Ships Initiative facility in Superior, WI, and at the University of Wisconsin Superior's Lake Superior Research Institute. The NEMWI will receive ENRTF funds in a subcontract from the University of Minnesota, and will subcontract funds to the UWS-LSRI.

Jeff Stollenwerk, Minnesota Pollution Control Agency. This partner will provide guidance and assistance in collecting ballast water samples from commercial ships entering the Duluth-Superior Harbor. He will not receive ENRTF funds from this appropriation.

B. Project Impact and Long-term Strategy:

This project will identify the prevalence of potentially harmful 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 St. Louis River Estuary and the ballast water treatment activity will provide management strategies to mitigate the risk of unintentionally introducing new bacterial invasive species.

C. Funding History:

Funding Source and Use of Funds	Funding Timeframe	\$ Amount
Environmental and Natural Resources Trust Fund: M.L. 2011, First Special Session, Chp. 2, Art.3, Sec. 2, Subd. 06a; M.L. 2014, Chapter 26, Section 2, Subdivision 19	7/1/2011 to 6/30/2015	\$250,000

VIII. FEE TITLE ACQUISITION/CONSERVATION EASEMENT/RESTORATION REQUIREMENTS:

A. Parcel List: N/A

B. Acquisition/Restoration Information: N/A

IX. VISUAL COMPONENT or MAP(S): See attached map

X. RESEARCH ADDENDUM: A research addendum was peer reviewed for the parent project earlier in 2011 - M.L. 2011, First Special Session, Chp. 2, Art.3, Sec. 2, Subd. 06a. A research addendum was not requested for this continuing project.

XI. REPORTING REQUIREMENTS:

Periodic work plan status update reports will be submitted no later than January 2017, July 2017, January 2018, and July 2018. A final report and associated products will be submitted between June 30 and August 15, 2019.

Environment and Natural Resources Trust Fund

M.L. 2016 Project Budget



Project Title: Advancing Microbial Invasive Species Monitoring from Ballast Discharge

Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 06c

Project Manager: Randall E. Hicks

Organization: University of Minnesota Duluth

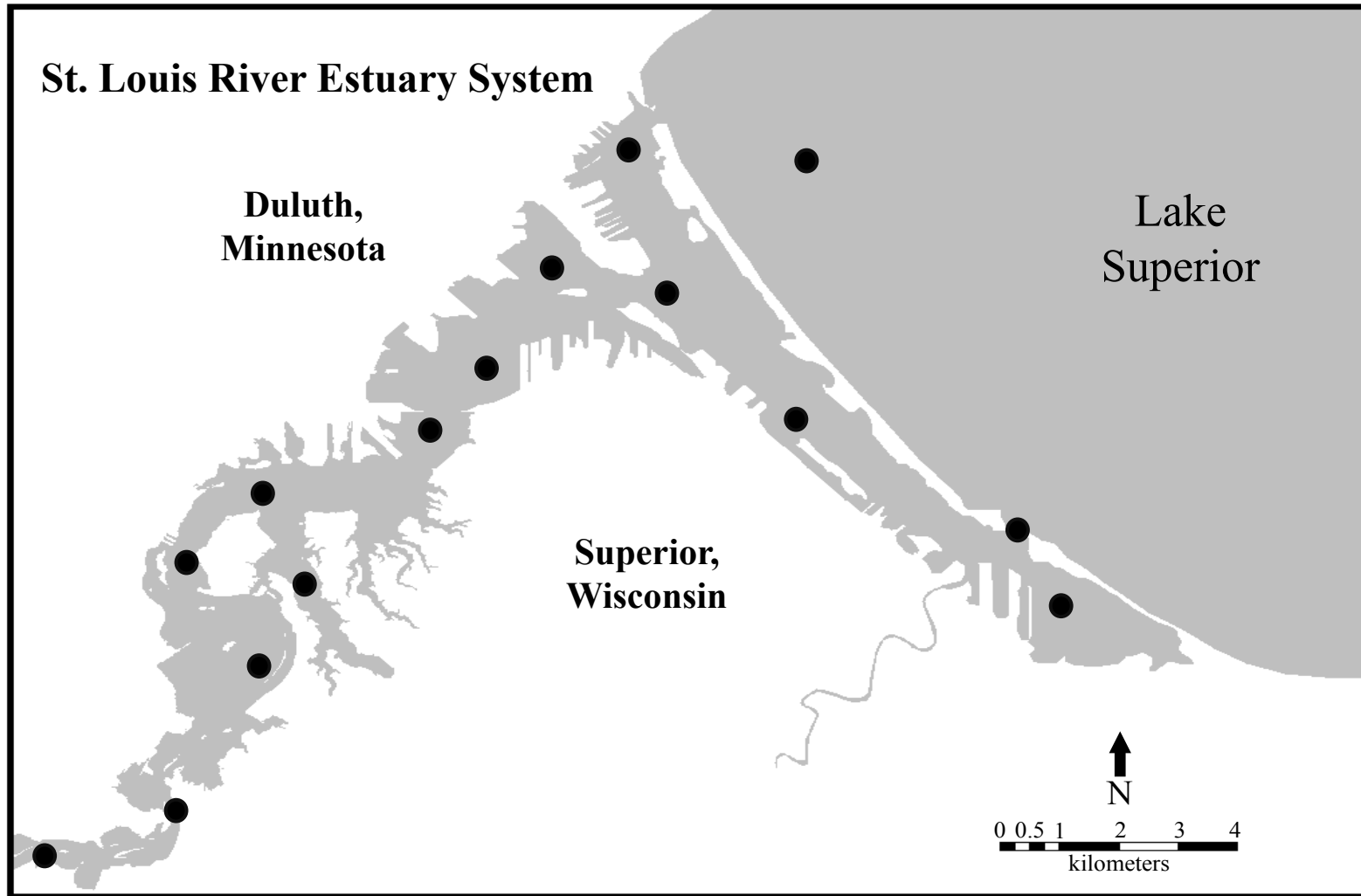
M.L. 2016 ENRTF Appropriation: \$368,000

Project Length and Completion Date: 2.5 years, December 31, 2018

Date of Report: May 29, 2016

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Activity 1 Budget	Amount Spent	Activity 1 Balance	Activity 2 Budget	Amount Spent	Activity 2 Balance	Activity 3 Budget	Amount Spent	Activity 3 Balance	TOTAL BUDGET	TOTAL BALANCE
BUDGET ITEM	<i>Collect Ballast Water from Commercial</i>			<i>Comparison of Standard Versus Non-</i>			<i>Taxonomically Identify the Common and</i>				
Personnel (Wages and Benefits)	\$86,530		\$86,530	\$65,850		\$65,850	\$100,420		\$100,420	\$252,800	\$252,800
<i>Project Manager (Randall Hicks; \$29,939; 74.8% salary+25.2% fringe; July 1, 2016-June 30, 2018; 8.4% FTE)</i>											
<i>Postdoctoral Associate (1 person to be announced; \$138,892; 81.7% salary+18.3% fringe; July 1, 2016-Sept 20, 2018; 100% FTE)</i>											
<i>Graduate Research Assistant (1 person to be announced; \$83,974; 55.0% salary+45.0% fringe; July 1, 2016-Aug 31, 2018; 50% FTE)</i>											
Professional/Technical/Service Contracts											
<i>Northeast-Midwest Institute subcontract to design, set up, run bench-scale experiments and evaluate standard microbial assessment tools for fecal indicator bacteria.</i>				\$44,500		\$44,500				\$44,500	\$44,500
Equipment/Tools/Supplies	\$15,550		\$15,550	\$12,568		\$12,568	\$23,920		\$23,920	\$52,040	\$52,040
<i>Water and sediment sampling supplies (\$3,650)</i>											
<i>DNA extraction and PCR reagents for assays(\$13,922)</i>											
<i>Illumina sequencing and data storage costs for DNA analyses(\$20,270)</i>											
<i>Chemicals and expendable lab supplies(\$14,200)</i>											
Travel expenses in Minnesota											
<i>Sampling trips to the St. Louis River estuary (20 trips) and GS facility (10 trips) - \$600; Travel support to disseminate project results at public venues, scientific conferences, and outreach events with target organizations - \$2,508. Matching conference travel funding will be sought from the University of Minnesota.</i>	\$1,200		\$1,200	\$708		\$708	\$1,200		\$1,200	\$3,108	\$3,108
Other											
<i>Publication costs for journal articles</i>	\$700		\$700	\$645		\$645	\$700		\$700	\$2,045	\$2,045
<i>R/V Blue Heron ship time</i>			\$0			\$0	\$8,500		\$8,500	\$8,500	\$8,500
<i>Small boat rental (10 days @ \$500/day)</i>			\$0			\$0	\$5,000		\$5,000	\$5,000	\$5,000
COLUMN TOTAL	\$103,980		\$103,980	\$124,276		\$124,276	\$139,740		\$139,740	\$368,000	\$368,000

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



Project Manager: Randall E. Hicks

