



## Environment and Natural Resources Trust Fund (ENRTF) M.L. 2011 Work Plan

---

**Date of Status Update:**

**Date of Next Status Update:** 1/1/2012

**Date of Work Plan Approval:** 6/23/2011

**Project Completion Date:** 6/30/2014

**Is this an amendment request?** \_\_\_\_\_

---

**Project Title:** Improved Detection of Harmful Microbes in Ballast Water

**Project Manager:** Randall Hicks

**Affiliation:** U of MN - Duluth

**Address:** 1035 Kirby Dr, SSB 207

**City:** Duluth **State:** MN **Zipcode:** 55812

**Telephone Number:** (218) 726-8438

**Email Address:** rhicks@d.umn.edu

**Web Address:**

---

**Location:**

**Counties Impacted:** St. Louis

**Ecological Section Impacted:** Northern Superior Uplands (212L)

---

<b>Total ENRTF Project Budget:</b>	<b>ENRTF Appropriation \$:</b>	250,000
	<b>Amount Spent \$:</b>	<u>0</u>
	<b>Balance \$:</b>	250,000

---

**Legal Citation:** M.L. 2011, First Special Session, Chp. 2, Art.3, Sec. 2, Subd. 06a

**Appropriation Language:**

\$125,000 the first year and \$125,000 the second year are from the trust fund to the Board of Regents of the University of Minnesota for the University of Minnesota Duluth to identify and analyze potentially harmful bacteria transported into Lake Superior through ship ballast water discharge. This appropriation is available until June 30, 2014, by which time the project must be completed and final products delivered.

## I. PROJECT TITLE: Improved Detection of Harmful Microbes in Ballast Water

## II. PROJECT SUMMARY:

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. 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 may be harmful invasive species, just like invasive species of plants and animals that threaten our natural resources. Our team will examine freshwater ('Lakers') and ocean-going ('Salties') commercial ships to identify harmful bacteria that are being transported in ballast water and discharged into Lake Superior. We will use state-of-the-art DNA sequencing techniques to identify 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 and nearby watersheds. Lastly, we will rank the most potentially harmful bacteria transported to the Duluth-Superior Harbor in the ballast water of commercial ships, which should be useful for developing guidelines for the microbiological safety of ballast water in the future.

## III. PROJECT STATUS UPDATES:

**Project Status as of January 2012:**

**Project Status as of July 2012:**

**Project Status as of January 2013:**

**Project Status as of July 2013:**

## IV. PROJECT ACTIVITIES AND OUTCOMES:

### ACTIVITY 1: Collect Ballast Water from Commercial Ships and Extract DNA

#### Description:

Large volumes of ballast water will be collected from up to 10 commercial vessels in the Duluth-Superior harbor throughout the summers 2011 and 2012 as sampling opportunities arise with the Minnesota Pollution Control Agency (MPCA). Typically, ballast water samples will be collected by siphoning water using polyethylene tubing from a ballast tank, through a sounding tube off the side of the ship to the dock where it is captured. Ballasting history of the ballast water tanks will also be obtained through personnel interviews and access to ballast log records as determined by the ship officer present at the time of sampling. Each ballast water sample will be filtered onto a large Durapore membrane filter to concentrate bacterial cells. A portion of each membrane filter will be used to prepare DNA for sequence analysis of 16S rDNA and for the development of bacterial fosmid libraries. Total DNA will be extracted using MoBio PowerSoil® DNA extraction kits, eluted in nuclease-free water and then frozen (-80°C) until used for sequencing and constructing fosmid libraries.

#### Summary Budget Information for Activity 1:

**ENRTF Budget: \$ 37,874**  
**Amount Spent: \$ 0**  
**Balance: \$ 37,874**

**Activity Completion Date: November 2012**

Outcome	Completion Date	Budget
1. <i>Collect ballast water from up to 10 commercial ships and establish ballast water collection</i>	October 2012	\$ 18,790
2. <i>Extract microbial community DNA from water samples and develop a repository of purified microbial DNA from ballast water</i>	November 2012	\$ 19,084

**ACTIVITY 2: Sequence Bacterial Genes Found in Ship Ballast Water****Description:**

Once extracted, DNA corresponding to the V5 and V6 hypervariable regions of the full-length 16S rDNA gene will be amplified by PCR using primers. The amplicons from multiple samples will be pooled together and the multiplexed amplicons will be sequenced on an Illumina/Solexa Sequencer at the National Center for Genomic Research (NCGR) in Santa Fe, New Mexico. In addition to phylogenetic information from 16S rDNA, our metagenomic analyses will also examine the functionality of microbial communities (i.e., genes conferring resistance to antibiotics and heavy metals) in up to ten samples of ship ballast water. To do this, we will send a portion of the extracted DNA samples (as described above) to the Clemson University Genome Institute for the construction of functional gene libraries. Library clones will be picked into 384 well microplates using a Qbot colony-picking robot. Functionally active fosmid clones will be sequenced at the Biomedical Genomics Center at the University of Minnesota and screened by the graduate student for functionally active genes involved in resistance to antibiotics and heavy metals.

**Summary Budget Information for Activity 2:**

**ENRTF Budget: \$ 109,103**  
**Amount Spent: \$ 0**  
**Balance: \$ 109,103**

**Activity Completion Date: April 2013**

Outcome	Completion Date	Budget
1. <i>Sequence bacterial 16S rRNA gene using the Illumina system and create a sequence database for ballast water bacteria</i>	April 2013	\$ 68,869
2. <i>Construct fosmid libraries of function genes to detect harmful genes and processes</i>	April 2013	\$ 40,234

**ACTIVITY 3: Analyze Gene Sequences of Bacteria Found in Ships' Ballast Water****Description:**

The 16S rDNA sequence data obtained will be compared to V5 and V6 region reference databases and the taxonomic classification of 16S rDNA PCR products will be assigned using reference databases and taxonomic classification tools and software. The taxonomic signature of microorganisms in each sample will be compared within and across samples and statistically analyzed. The resulting relationships that are identified will be tested by constructing bootstrapped phylogenetic trees. Comparisons of bacterial constituents in the ballast waters will be determined by examining the numbers and types of phyla (or operational taxonomic units) in each sample. When completed, these analyses will give us a comprehensive picture of the bacterial structure of ballast water. Additional analysis will be done to examine the fosmid libraries for functionally active genes that are involved in the resistance to antibiotics and heavy metals. Sequence data from functionally active fosmid clones will be assembled into contigs, and analyzed by Blast and IMG-ACT software and websites. The DNA sequences in these fosmid clones will be annotated (identified) by the graduate student and postdoctoral associate and submitted to the IMG-ACT database.

**Summary Budget Information for Activity 3:**

ENRTF Budget: \$ 103,023  
 Amount Spent: \$ 0  
 Balance: \$ 103,023

**Activity Completion Date: June 2013**

<b>Outcome</b>	<b>Completion Date</b>	<b>Budget</b>
1. Analyze 16S rDNA sequences and construct phylogenetic trees to identify and rank the most common and the potentially harmful bacteria in ballast water	June 2013	\$ 70,439
2. Annotate genes in fosmid libraries to identify harmful genes and processes	June 2013	\$ 32,584

**Activity Status as of January 2012:****Activity Status as of July 2012:****Activity Status as of January 2013****Activity Status as of July 2013:****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 metagenomic data into national databases (e.g., Genbank and IMG-ACT) 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 distributed (by email or personal visits) to 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. At the discretion of Minnesota Pollution Control Agency, we will help disseminate information about this project to legislators and citizens.

**Status as of January 2012:****Status as of July 2012:****Status as of January 2013:****Status as of July 2013:****Final Report Summary:**

**VI. PROJECT BUDGET SUMMARY:**

**A. ENRTF Budget:**

<b>Budget Category</b>	<b>\$ Amount</b>	<b>Explanation</b>
Personnel:	\$ 200,445	<p>One month of summer salary is requested each year for Dr. Hicks (8% FTE), who will participate in all aspects of this project and oversee the activities of the postdoctoral associate and graduate research assistant. No salary is requested for Dr Sadowsky.</p> <p>Salary is requested for Dr. Andrew Reed (100% FTE), a postdoctoral associate, who will be responsible for collecting ballast water samples, extracting DNA and preparing samples for gene sequence determinations, analysis of the 16S rDNA and fosmid library DNA sequence results, and help writing reports and publications.</p> <p>Salary is also requested for a graduate research assistant (33% FTE), who will work with the postdoctoral associate on all aspects of the project and develop a thesis on functional genes that are identified in the fosmid libraries.</p>
Professional/Technical Contracts:	\$	
Service Contracts	\$	
Equipment/Tools/Supplies:	\$48,055	Supply funds are requested for collecting and extracting DNA from ballast and harbor water samples, expendable laboratory materials, and purchasing a meter to measure water DO and conductivity. The majority of supply funds are required for Illumina sequencing of DNA from ballast water samples, and constructing and analysis of the fosmid libraries. Other funds are requested to disseminate the project results in scientific journals and other publications.
Capital Equipment over \$3,500:	\$	
Fee Title Acquisition:	\$	
Easement Acquisition:	\$	
Professional Services for Acq:	\$	
Printing:	\$	
Travel Expenses in MN:	\$1,500	Travel funds are requested to collect ballast water samples and for travel (including lodging and meals) between Dr. Hicks' and Dr. Sadowsky's laboratories in Duluth and St. Paul, respectively.
Other:	\$	
<b>TOTAL ENRTF BUDGET:</b>	<b>\$250,000</b>	

**Explanation of Use of Classified Staff:** N/A

**Explanation of Capital Expenditures Greater Than \$3,500:** N/A

**Number of Full-time Equivalent (FTE) funded with this ENRTF appropriation:** 1.41

**B. Other Funds:**

Source of Funds	\$ Amount Proposed	\$ Amount Spent	Use of Other Funds
<b>Non-state</b>			
	\$	\$	
<b>State</b>			
UMD Biology Department	\$13,613	\$	Dr. Hicks salary
<b>TOTAL OTHER FUNDS:</b>	<b>\$</b>	<b>\$</b>	

**VII. PROJECT STRATEGY:****A. Project Partners:**

John Thomas and Jeff Stollenwerk, Minnesota Pollution Control Agency. These partners will not receive ENRTF funds from this appropriation.

**B. Project Impact and Long-term Strategy:**

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 may be harmful invasive species, just like invasive species of plants and animals that threaten our natural resources. The ballast water of ships can be a vector for the global transport of aquatic microorganisms. 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. 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. 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 types of bacteria that are being transported by ships into Lake Superior, and their potential for causing irreparable harm.

**C. Spending History:**

Funding Source	FY 2010	FY 2011
Great Lakes Protection Fund grant (through Northeast-Midwest Institute)	\$58,952	\$89,322

**VIII. ACQUISITION/RESTORATION LIST: N/A****IX. MAP(S): N/A****X. RESEARCH ADDENDUM: See Research Addendum****XI. REPORTING REQUIREMENTS:**

Periodic work plan status update reports will be submitted not later than January 2012, July 2012, January 2013, and July 2013. A final report and associated products will be submitted between June 30 and August 1, 2013 as requested by the LCCMR.

Attachment A: Budget Detail for M.L. 2011 (FY 2012-13) Environment and Natural Resources Trust Fund Projects											
Project Title: <i>Improved Detection of Harmful Microbes in Ballast Water</i>											
Legal Citation: <i>Fill in your project's legal citation from the appropriation language</i>											
Project Manager: <i>Randall E. Hicks</i>											
M.L. 2011 (FY 2012-13) ENRTF Appropriation: <i>\$ 250,000</i>											
Project Length and Completion Date: <i>July 2013</i>											
Date of Update: <i>May 13, 2011</i>											
ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Activity 1 Budget	Amount Spent	Balance	Activity 2 Budget	Amount Spent	Balance	Activity 3 Budget	Amount Spent	Balance	TOTAL BUDGET	TOTAL BALANCE
BUDGET ITEM	<i>Collect Ballast Water and Extract DNA</i>			<i>Sequence Bacterial Genes found in Ballast</i>			<i>Analyze Gene Sequences of Bacteria</i>				
<b>Personnel (Wages and Benefits)</b>	32,169		32,169	68,053		68,053	100,223		100,223	200,445	200,445
Randall Hicks, Project Manager: \$27,227 ( 2 mo @ 100% time + 33.3% fringe benefits)											
Postdoctoral Associate: \$124,464 (24 mo @ 100% time + 20.22% fringe benefits)											
Graduate Research Assistant: \$48,754 (7.9 mo@ 50%-time + 24.2% fringe benefits + tuition benefit)											
<b>Professional/Technical Contracts</b>											
<b>Service contracts</b>											
<b>Equipment/Tools/Supplies</b>											
Ballast water sampling supplies	805		805							805	805
Portable temperature/DO/salinity meter	1,600		1,600							1,600	1,600
DNA extraction and PCR reagents	2,000		2,000	3,500		3,500				5,500	5,500
Fosmid library costs (10 libraries@ \$1,550 ea)				15,000		15,000				15,000	15,000
Illumina sequencing costs (30 samples @ \$5,000 per 10 samples)				15,000		15,000				15,000	15,000
Chemicals and expendable lab supplies	1,000		1,000	7,150		7,150	1,000		1,000	9,150	9,150
Publication costs							1,000		1,000	1,000	1,000
<b>Capital equipment over \$3,500</b>											
<b>Fee Title Acquisition</b>											
<b>Easement Acquisition</b>											
<b>Professional Services for Acquisition</b>											
<b>Printing</b>											
<b>Travel expenses in Minnesota</b> <i>(Specify types of travel expenses, e.g., mileage, lodging, meals. Per diems are not allowed.)</i>	300		300	400		400	800		800	1,500	1,500
<b>Other</b> <i>(Describe the activity and cost - be specific)</i>											
<b>COLUMN TOTAL</b>	<b>\$37,874</b>	<b>\$0</b>	<b>\$37,874</b>	<b>\$109,103</b>	<b>\$0</b>	<b>\$109,103</b>	<b>\$103,023</b>	<b>\$0</b>	<b>\$103,023</b>	<b>\$250,000</b>	<b>\$250,000</b>