Research Addendum for Peer Review

Project Manager Name: Randall E. Hicks
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Project Title: Improved Detection of Harmful Microbes in Ballast Water
Project Number: 118-E

Total Dollars Requested: $250,000

1. Abstract

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 sample 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. 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.

2. Background

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. There is an estimated 3 to 10 metric tons of ballast water transported around the world each year carrying as many as 7,000 different species per day (GBWMP 2002). 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 (Riciarrdi 2006). The zebra mussel (*Dreissena polymorpha*), a non-indigenous bivalve first detected in 1988, is a well-known example of an invasive species in the Great Lakes (Hebert et al. 1989). Other examples of invasive animals and plants in the Great Lakes include the sea lamprey (*Petromyzon marinus*; Smith and Tibbles 1980), the spiny water flea (*Bythotrephes longimanus*; Johannsson et al. 1991), and numerous diatom and algal species (Ricciardi 2006). The appearance of the fish virus VHS in the Great Lakes and the recent discovery of its DNA in parts of Lake Superior (Bain et al. 2010) 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.

A long-held view in microbiology is that free-living microorganisms can be found essentially everywhere, because of their small size, great abundance, and the ability of some species to form

physiologically inactive stages, spores, and cysts. This concept has been summed up in the saying, 'Everything is everywhere, and the environment selects', which has been attributed to Bass-Becking in 1934 (Staley and Gosink 1999). There is substantial evidence to support this view. Increasingly, however, there is evidence that microbial communities contain both indigenous and widespread species (Staley and Gosink 1999, Neufeld and Mohn 2005).

Water and air currents, birds during migration, and other biological vectors may contribute to the global distribution of microorganisms (Staley and Gosink 1999, Griffin 2002). The ballast water of ships can also 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 (Finlay 2002, Drake et al. 2007). McCarthy and Khambaty (1994) reported the detection of toxigenic *Vibrio cholera* O1, serotype Inaba in 1991 and 1992 from ballast, bilge, and sewage water collected from four cargo ships docked in ports of the U.S. Gulf of Mexico that had carried ballast taken from cholera-infected waters off the coast of Latin America or Mexico. This same strain of bacterium, significantly different from the endemic strains found in the Gulf Coast, was also isolated from oysters harvested off the coast of Mobile Bay, Alabama in 1990 and may have been introduced by ballast water discharge (McCarthy and Khambaty 1994). Ballast water discharged from commercial vessels into the Chesapeake Bay has been estimated to contain 8.3×10^8 bacteria cells liter^{-1} (Ruiz 2000). Drake et al. (2007) estimated that as many as 10^{18} bacterial cells and 10^{19} viruses are discharged annually into the lower Chesapeake Bay region and that 56% could survive in the Bay after discharge.

In 2005, more than 5 billion gallons of ballast water was discharged into the Duluth-Superior harbor (MPCA 2008), 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. The potential transport of water-borne pathogenic bacteria from warmer climate zones into Lake Superior is of increasing concern as this lake's water temperature is rising due to global climate change (Drake et al. 2007). Consequently, it is important to determine whether ballast water transports harmful microbes and whether bacterial assemblages exhibit biogeographic patterns within the Laurentian Great Lakes (Dobbs and Rogerson 2005).

3. Hypothesis

Shipping through the Duluth-Superior harbor, the largest port by total cargo volume in the Great Lakes, has a $200 million annual impact on Minnesota's economy. The Duluth-Superior harbor also receives the largest volume of ballast water discharged from commercial ships of any harbor within the Great Lakes (MPCA 2008). The hypothesis guiding this research is that harmful bacteria are discharged with ballast water, some of these bacteria may not already be present in this harbor, and if they do survive and flourish then they may become harmful invasive species similar to well known examples of invasive animal and plant species. Our goal is to identify potentially harmful bacteria in the ballast water of commercial ships by extracting DNA from these cells and then comparing DNA sequences in these bacterial genomes to those found in the harbor. This will allow us to identify

potentially harmful bacteria that may be discharged with ballast water and determine if they may become invasive species of concern.

4. Methodology

Our team will sample freshwater ('Lakers') and ocean-going ('Salties') commercial ships to identify harmful bacteria that are being transported in ballast water and discharged into Lake Superior. Our studies will focus on bacteria present in the ballast water of ships but not already common in the Duluth-Superior harbor. We will use state-of-the-art DNA sequencing and bioinformatic techniques to identify the harmful bacteria of most concern to human and ecological health. The methods employed have the potential to detect rare microbes before they become common inhabitants in Lake Superior. Identifying harmful microbes of concern is the first step on the path to develop sensitive monitoring techniques that provide early detection of harmful microbes in the ballast water of ships and to devise effective remediation strategies to limit their spread in this important environment.

Activity 1: *Collect Ballast Water from Commercial Ships and Extract DNA*

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). We have already collected 10 ballast and matching harbor water samples from the Duluth-Superior harbor. In 2009, we worked with John Thomas and Jeff Stollenwerk from the MPCA and collected ballast water samples from 7 freshwater and 3 ocean-going ships. They have agreed to help us collect new ballast water samples as part of this project to augment our existing samples (see attached letter of support).

Fig. 1. Collection of a ballast water sample from a sounding tube of a commercial ship in the Duluth-Superior harbor.

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 (Fig. 1). The polyethylene tubing will be pushed through the ship's sounding tube into the ballast tank to

ensure that water retained in the sounding tube is not inadvertently sampled instead of water in the ballast tank. Approximately 45 to 60 liters of ballast water will be collected from each ship in three 20-liter carboys to be used as replicate subsamples. Ballast water temperature, pH, and conductivity will be recorded. Ballasting history of the ballast water tanks will also be obtained through personnel interviews and access to ballast log records (e.g., USCG Ballast Water Reporting forms) as determined by the ship officer present at the time of sampling. In addition, prior to ballast water sampling, harbor water samples will be collected off the dock using a 5-liter Niskin bottle. Three replicate samples of harbor water (~15 liters each) will also be collected and water temperature, pH, and conductivity will be recorded.

All ballast water, from freshwater and ocean-going ships, and harbor water samples will be quickly returned to the laboratory, placed in a cold room (at 5°C), and immediately filtered (typically within 1 hour of sampling) to capture bacteria. Each ballast and harbor water sample will be filtered onto a large (142 mm diameter, 0.22 μm pore size, Millipore Corp.) Durapore membrane filter to concentrate bacterial cells as previously described (Hicks and Pascoe 2001, Pascoe and Hicks 2004). All filters will be frozen (-80°) until DNA is extracted.

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. The large 142 mm filters will be divided into one-eighth portions by weight and one portion will be used per extraction tube. Total DNA will be extracted using MoBio PowerSoil® DNA extraction kits (MoBio Laboratories, Solana Beach, CA) as previously described (Ishii et al. 2006). We have found that these kits give maximal DNA extraction, with minimal PCR inhibitors (Kish 2010). Extracted DNA will be eluted in nuclease-free water and then frozen (-80°C) until used for sequencing and constructing fosmid libraries.

Activity 2: *Sequence Bacterial Genes Found in Ship Ballast Water*

Once extracted, DNA corresponding to the V6 hypervariable region of the full-length 16S rDNA gene will be amplified by PCR using primers as described by Wang et al 2007 and Lazarevic et al. 2009. The taxonomy of microorganisms assigned to the V6 hypervariable regions has been shown to be very similar to the taxonomy assigned to microorganisms obtained via analysis of full-length SSU rRNA (Huse et al. 2008), making this a cost effective approach (Lazarevic et al. 2009) to obtain near complete taxonomic information on ballast water bacteria. The PCR primers will contain a unique sequence tag (Binladen et al. 2007) such that amplicons from each sample will contain a unique identifier sequence. The amplicons from each of 10 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. Since each amplicon is initiated via a different primer tag, it will allow us to deconvolute sequence data arising from a single sequence run. Thus, sequences from individual samples in these mixtures can be separated using these unique sequence tags so that sequences from individual samples are identified. This sequencing technique is very cost effective because 10 individual samples can be sequenced together. Sequence data will be obtained by the paired-end read method. Using this approach, about 20 million reads can be obtained from each sequence run, and will result in the collection of approximately 1 to 2 million reads of taxonomically-useful 16S rDNA from each sample.

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 ten samples of ship ballast water. These samples will be selected based on preliminary information we obtain from the 16S rDNA phylogenetic analysis of these samples. To do this, we will send a portion of the extracted DNA samples (as described above) to the Clemson University Genome Institute (http://www.genome.clemson.edu/) for the construction of functional gene libraries. The libraries, consisting of randomly sheared metagenomic DNAs, will be constructed in fosmid vector pEPIFOS-5 and transformed into *E. coli* DH10 as the host. Each fosmid will have an average insert size of ~40 kb, enough to encode about 20-40 bacterial genes. We will obtain ~10,000 clones (containing about 400,000 kb of DNA) from each library. Library clones will be picked into 384 well microplates using a Qbot colony-picking robot and screened by the graduate student for functionally active genes involved in resistance to antibiotics and heavy metals.

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

The 16S rDNA sequence data obtained will be compared to V6 region reference databases as described by Dethlefsen et al. 2008 and Lazarevic et al. 2009. The taxonomic classification of 16S rDNA PCR products will be assigned using the GAST (Global Alignment for Sequence Taxonomy) taxonomic classification tool as described by Sogin et al. (2006), and by analyses done using a reference database of V6 rDNA sequences (RefHVR_V6) from SILVA (Pruesse et al. 2007), the taxonomy from known cultured isolates, the Entrez Genome and the Ribosomal Database Project (Cole et al., 2005), Greengenes (DeSantis et al. 2006) and ARB (Ludwig et al. 2004). The taxonomic signature of microorganisms in each sample will be compared within and across samples and statistically analyzed as described by Dethlefsen et al. (2008) and by using tools available from ARB. Sequences occurring < 3 times will be filtered to reduce the number of unique sequences. Phylotype clusters and diversity estimates will also be obtained by using the MOTHUR software program (Schloss and Handelsman, 2005). Local alignments and graphical representation of data will be facilitated by MOTHUR and phylogenetic relationships of the sequences will be examined by maximum-parsimony into the ARB dataset. The resulting phylogenetic relationships that are identified will be tested by maximum-likelihood bootstrap trees (with 1000 iterations) using distance-based subsampling and a minimum distance of 3% between sequences.

Operational taxonomic units (OTUs) will be determined and compared by using the sortx subroutine of XplorSeq (Frank 2008). Comparisons of bacterial constituents in the ballast and harbor waters will be determined by examining the numbers and types of phyla (or operational taxonomic units) in each sample. We will also determine species diversity, species richness, and evenness using rarefaction analysis as described (Robertson et al. 2009). When completed, these analyses will give us a comprehensive picture of the bacterial structure of ballast water and the receiving water in the Duluth-Superior harbor, where ballast water is discharged. The phylogenetic and comparative analyses obtained from these studies will be used to develop a relational database so that information can be readily downloaded and utilized by researchers, regulatory agencies and stakeholders to examine the types of bacteria present in 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. Functionally active fosmid clones will be sequenced at the Biomedical Genomics Center at the University of Minnesota, sequence data will

be assembled into contigs, and analyzed by Blast and IMG-ACT software and websites (http://img.jgi.doe.gov/cgi-bin/pub/main.cgi). The IMG-ACT is a database of microbial genomes and metagenomic data that is maintained by the Joint Genome Institute of the U.S. Department of Energy. 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.

5. Results and Deliverables

We recently completed a community-level comparison of bacteria found in the ballast water of commercial ships and receiving water in the Duluth-Superior harbor collected in 2009. Terminal restriction fragment length polymorphism (T-RFLP) analysis of the 16S rDNA gene in these bacterial communities indicated that ballast water from ocean-going and freshwater ships have significantly different bacterial communities than water in the Duluth-Superior harbor (Fig. 2). The broad scope of this analysis did not determine how these bacterial communities were different or what bacterial species were present in the different samples. However, our analysis did indicate that there is reason to suspect that new or different species of bacteria are introduced to this harbor when ballast water is discharged.

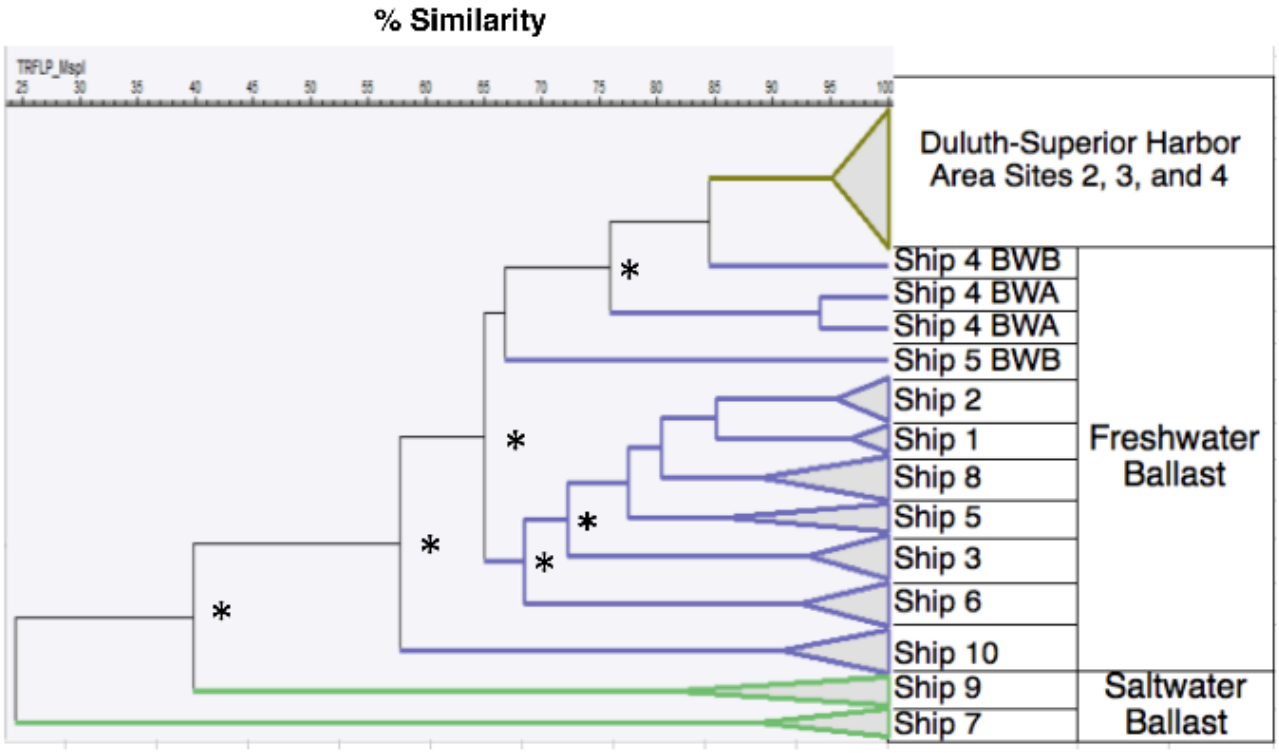

Fig. 2. Comparison of the overall similarity of bacterial communities in the ballast water of commercial ships and sites in the Duluth-Superior harbor where ballast water is routinely discharged. Asterisks indicate clusters of bacterial communities whose DNA similarity was significantly different. All samples were collected in 2009. [Welch. J. and R. E. Hicks, unpublished data]

We also conducted a preliminary analysis of a few ballast water samples collected during 2009 to determine what bacterial species might be present using established cloning and sequencing methods. Although this analysis is still incomplete, one relevant finding has emerged. DNA from a bacterium most similar to *Tenacibaculum* species was identified in the ballast water of an ocean-going ship that discharged its ballast into the Duluth-Superior harbor. This bacterial genus contains several species that are fish pathogens, which can cause diseases (e.g., fin and mouth rot, skin lesions) in bottom water fish like sole and turbot. These pathogenic bacteria have been isolated and identified in marine fish from farming operations in Spain (Piñeiro-Vidal et al. 2008a, b). Interestingly, the ocean-going ship containing this bacterial DNA had picked up ballast water off the coast of Spain two weeks prior to arriving in Minnesota, and discharged some ballast water into the Duluth-Superior harbor. This example demonstrates that potentially harmful bacteria can be transported long distances in ballast tanks of ships. It also points out the great need to identify the full range of potentially harmful bacteria which may be introduced into Minnesota's coastal waters. We believe these studies warrant a more in-depth investigation with improved detection methods, which can identify a larger portion of the bacteria that are present in ballast water being discharged into Minnesota's coastal waters.

There are several products and deliverables that are outcomes of this project. These outcomes include the:

1. Establishment a ballast water collection characteristic of ships entering Lake Superior
2. Development and archiving a repository of purified microbial DNA from ballast water
3. Creation of a 16S rDNA sequence database of ballast bacteria from commercial ships
4. Construction of functional fosmid libraries to detect harmful genes and processes
5. Construction of phylogenetic trees and development of correlations between bacterial assemblages and functional genes from different ballast and harbor water samples
6. Identification and ranking of the most common and the potentially harmful bacteria transported to the Duluth-Superior harbor in the ballast water from commercial ships

6. Timetable

The project will take place over a two-year period, beginning on July 1, 2011. We have already obtained ballast water samples from 10 freshwater or ocean-going ships during summer 2009. The diversity of bacteria in these samples can be analyzed immediately and will be followed with additional ballast water samples collected in 2011 and 2012. Our analyses should reliably identify bacterial species and microbial genes that may be of concern to scientists, policymakers and the public, which are present in the ballast water of commercial ships entering the Duluth-Superior harbor.

Activity or Task	**2011**	**2012**	**2013**
	JASOND	JFMAMJJASOND	JFMAMJ
Collect ballast water samples with MPCA	xxxx	xxxxx	
Extract microbial community DNA	xxxxx	xxxxxx	
Illumina sequencing of bacterial 16S rDNA	xxxxxx	xxxxx xxxx	xxxx
Analysis of 16S rDNA sequences and construction of phylogenetic trees	xxxxx	xxxxxxxxxxxxxxx	xxxx
Construct fosmid libraries of functional genes		x xx	xxxxx

Annotate gene identities in fosmid libraries		xxxxx	xxxxxxx
Presentations to MPCA and other organizations	x	x	x
Presentations and publication in scientific literature		x	x x
Final report to LCCMR			x

7. Budget

2011-2012 Detailed Project Budget		
IV. TOTAL TRUST FUND REQUEST BUDGET (2 years)		
BUDGET ITEM	**AMOUNT**	
Personnel:		
[illegible]	$	27,227
[illegible]	$	124,464
[illegible] Benefit)	$	48,754
Contracts: *N/A*	$	-
[illegible]		
[illegible]	$	565
[illegible]	$	1,600
[illegible]	$	5,500
[illegible]	$	15,300
[illegible]	$	15,000
[illegible]	$	9,150
[illegible]	$	1,000
[illegible]	$	-
[illegible]	$	1,500
[illegible]	$	-
TOTAL ENVIRONMENT & NATURAL RESOURCES TRUST FUND $ REQUEST	**$**	**230,499**
V. OTHER FUNDS		
SOURCE OF FUNDS	**AMOUNT**	**Status**
[illegible]	$ -	
[illegible]	$ -	
[illegible]	$ 13,613	*Secured*
[illegible]	$ -	
[illegible]	$ 166,274	

Budget Narrative and Justification

One month of summer salary is requested each year for Dr. Hicks, who is on a 9-month appointment at the University of Minnesota Duluth. He will donate 0.5 months of his time each year to this project as a matching contribution. Dr. Hicks 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, who holds a 12-month appointment at the University of Minnesota. Dr. Sadowsky will provide guidance on next generation sequencing, consult on DNA sequence results and fosmid library screening, and participate in the outreach and dissemination of the project results.

Salary is requested for a full-time Postdoctoral Associate, Dr. Andrew Reed, who will take primary responsibility for collecting ballast and harbor water samples with MPCA personnel. He will also be responsible for extracting DNA and preparing samples for gene sequence determinations, spend a large portion of his time analyzing the 16S rDNA and fosmid library DNA sequence results using web-based and online software analysis packages, and help write reports and publications. Dr. Reed, completed his Ph.D. degree by investigating the diversity of phylogeny of microbes in oceans using molecular biology and bioinformatic techniques. Thus, he is well versed in these techniques but will work with other postdoctoral investigators and technicians in Dr. Sadowsky's lab who are practiced in doing large-scale (>25 million data points) taxonomic analyses.

A partial salary is requested for a graduate research assistant (to be named), 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, like those involved in antibiotic and heavy metal resistance.

Supply funds are requested for collecting and extracting DNA from 20 ballast and 10 harbor water samples, and for expendable laboratory materials. Additional funds are required for Illumina sequencing for 30 samples and constructing and analysis of 10 fosmid libraries. The costs for these services have dropped significantly since our project was originally proposed. These reduced costs are reflected in this budget. Funds are also requested to collect samples and travel between Dr. Hicks and Dr. Sadowsky's laboratories in Duluth and St. Paul, respectively. Other funds are requested to disseminate the project results in scientific journals and other publications. Prior funding from the Great Lakes Protection Fund supported the collection of some ballast and harbor water samples that will be further evaluated in this project.

8. Credentials

NAME:	**RANDALL E. HICKS**
TITLE:	Professor & Director, Ctr. for Freshwater Research & Policy
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CAMPUS ADDRESS:	1035 Kirby Drive, SSB 207, University of Minnesota Duluth
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EDUCATION:

B.S. (Honors in Zoology) 1977, University of Oklahoma, Norman, Oklahoma.
Ph.D. (Ecology) 1983, University of Georgia, Athens, Georgia.

POSITIONS HELD:

2006 to present	Director, UMD Center for Freshwater Research and Policy
1998 to 2006	Department Head, Department of Biology, University of Minnesota Duluth
2004 to present	Professor, Department of Biology, University of Minnesota Duluth
1994 to 1995	Adjunct Associate Professor, Center for Microbial Ecology, Michigan State University
1993 to 2004	Associate Professor, Department of Biology, University of Minnesota Duluth
1986 to 1993	Assistant Professor, Department of Biology, University of Minnesota Duluth
1985 to 1986	Assistant Professional Scientist, Illinois Natural History Survey
1983 to 1985	Postdoctoral Scholar, Woods Hole Oceanographic Institution

RESEARCH INTERESTS:

Aquatic microbial ecology, bacterial growth and survival, organic geochemistry, detrital dynamics

HONORS & AWARDS:

1973 TRW, Inc. National Exploration Award
1976 Undergraduate Research Grant (Oklahoma Academy of Science)
1977 Arthur N. Bragg Award in Natural History (University of Oklahoma)
1977 Sigma Xi Grant-in-Aid of Research
1978 NSF Graduate Fellowship - Honorable Mention
1979 Marine Sciences Summer Student Fellowship (University of Georgia)
1980 University-Wide Assistantship (University of Georgia)
1981 University-Wide Assistantship (University of Georgia)
1983 Woods Hole Oceanographic Institution Postdoctoral Scholar
1989 University of Minnesota Single Quarter Leave
1994 Bush Foundation Sabbatical Research Award

ACTIVE GRANTS AND CONTRACTS:

Developing a Risk Assessment Tool to Predict the Accelerated Corrosive Loss of Port Transportation Infrastructure. 2009-2010. Great Lakes Maritime Research Institute. $50,000. (PI – R. E. Hicks)

Collaborative Research-Microscopic Islands: Modeling the Theory of Island Biogeography for Aquatic Pathogens Colonizing Marine Aggregates. National Science Foundation (EID). 2009-2013. $2,322,000. (Co-PI with F. C. Dobbs, J. E. Ward, and J. M. Drake; $369,316 to R. E. Hicks)

Temporal Pathogenicity Potential of Bacteria on Lake Superior Beaches and in Waterways. Minnesota Sea Grant Program. 2009-2010. $32,500. (Co-PI with M. Sadowsky).

MRI Proposal for Acquisition of a Flow Cytometer for Aquatic Ecosystem Research. National Science Foundation (MRI). 2008-2010. $193,827. (Co-PI with S. Guildford and J. Etterson)

Ship-Mediated Harmful Microbes: Protecting the Great Lakes Ecosystem. Great Lakes Protection Fund. 2007-2010. $1,028,299 ($148,273 to R. E. Hicks). (Co-PI with A. Cangelosi, M. Bein, J. W. Casey, P. R. Bowser, J. Winton, F. Dobbs, T. Elder, and N. Mays).

Cooperative Training Partnership in Aquatic Toxicology and Ecosystem Research: A Training Partnership for Students and Postdoctoral Scientists with the University of Minnesota. U.S. Environmental Protection Agency. 2007-2010. $554,856. (PI with D. L. Swackhamer, M. T. Andrews, and R. M. Newman).

RECENT PUBLICATIONS (past three years):

Ishii, S., D. L. Hansen, R. E. Hicks, and M. J. Sadowsky. 2007. Beach sand and sediments are temporal sinks and sources of *Escherichia coli* in Lake Superior. Environ. Sci. Technol. 41:2203-2209.

Ksoll, W. B., S. Ishii, M. J. Sadowsky, and R. E. Hicks. 2007. Presence and sources of fecal coliform bacteria in epilithic periphyton communities of Lake Superior. Appl. Environ. Microbiol. 73(12):3771-3778.

Hansen, D. L., J. J. Clark, S. Ishii, M. J. Sadowsky, and R. E. Hicks. 2008. Sources and sinks of *Escherichia coli* in benthic and pelagic fish. J. Great Lakes Res. 34(2):228-234.

Hansen, D. L., S. Ishii, M. J. Sadowsky, and R. E. Hicks. 2009. *Escherichia coli* populations in Great Lakes waterfowl exhibit spatial stability and temporal shifting. Appl. Environ. Microbiol. 75(6):1546–1551.

Lyons, M. M., J. E. Ward, H. Gaff, R. E. Hicks, J. M. Drake, and F. C. Dobbs. 2010. Theory of island biogeography on a microscopic scale: organic aggregates as islands for aquatic pathogens. Aquat. Microb. Ecol. 60(1):1-13.

Ishii, S. T. Yan, H. Vu, D. L. Hansen, R. E. Hicks, and M. J. Sadowsky. 2010. Factors controlling long-term survival and growth of naturalized *Escherichia coli* populations in temperate field soils. Microbes and Environ. 28:8-14.

STUDENT ADVISING AND MENTORING:

Graduated Advisees: 14 M.S.

Current Graduate Advisees: 4 M.S., 2 Ph.D.

Service on Graduate Student Examining Committees: 26 M.S., 5 Ph.D.

Graduate Faculty Appointments: Water Resources Science, Integrated Biosciences, Microbial Ecology

Undergraduate Research Students (UROP, REU, etc): 38

NAME:	**MICHAEL J. SADOWSKY**
TITLE:	McKnight Distinguished University Professor, Director BioTechnology Institute, Director Graduate Studies Microbial Ecology Program
DEPARTMENT:	Department of Soil, Water, and Climate, and Biotechnology Institute, University of Minnesota
CAMPUS ADDRESS:	1991 Upper Buford Crl, 439 BorH
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EDUCATION:

1979 - 1983 Ph.D. University of Hawaii, Honolulu, Hawaii - Microbiology
1977 - 1979 M.S. University of Wisconsin-Oshkosh, Wisconsin - Biology/Microbiology
1973 - 1977 B.S. University of Wisconsin-Madison, Wisconsin – Bacteriology

POSITIONS HELD:

2009 - present Director, BioTechnology Institute
2006 - 2009 Co-Director, Microbial and Plant Genomic Institute
2004 - present Distinguished McKnight University Professor
1996 - 2004 Professor, Department of Soil, Water, and Climate, Biological Process Technology Institute, and Department of Microbiology, University of Minnesota
1993 - 1996 Associate Professor, Department of Soil, Water, and Climate and Department of Microbiology, University of Minnesota
1989 - 1993 Assistant Professor, Department of Soil, Water, and Climate and Department of Microbiology, University of Minnesota
1986 - 1989 Microbiologist, U.S. Department of Agriculture-ARS; Beltsville, Maryland,
1985 Molecular Biologist, Allied Corporation; Plant Sci. Lab., Syracuse, New York.

HONORS AND AWARDS:

Fellow, American Association for the Advancement of Science, 2008, Fellow - American Academy of Microbiology, 1999, Editor, Molecular Plant-Microbe Interactions 2009-present, Editor, Applied and Environmental Microbiology, 1999-2004; Young Investigator Award, American Society for Microbiology, 1990; Editorial Board, Symbiosis, 1997-present; Editorial Board, Microbe and Environment 2000-present; Associate Editor, Applied Environmental Microbiology 1989-1999, 2007-present. CFANS Distinguished Graduate Teaching Award, 2009, CFANS Distinguished Diversity and Inclusion Award, 2008, Time Magazine Innovator Article, 2006.

SELECTED PUBLICATIONS (16 out of 162)**:**

1. **Badgley, B. D., J. Ferguson, A.Vanden Heuvel, G. T. Kleinheinz, C. M. McDermott, T. R. Sandrin, J. Kinzelman, E. Junion, M. N. Byappanahalli, R. L. Whitman, and M. J. Sadowsky.** 2010. Multi-scale temporal and spatial variation in genotypic composition of *Cladophora*-borne *E. coli* populations in Lake Michigan. Water Res.: **In Press.**

2. **Hamilton, M.H., A. Z. Hadi,, J. F. Griffith, Satoshi Ishii, and M. J. Sadowsky.** 2010. Large scale analysis of virulence genes in *Escherichia coli* strains isolated from Avalon Bay, CA. Water Res.: **In Press.**
3. **Unno, T., J. Jang, D. Han, J. Ha Kim, M. J. Sadowsky, O.-S. Kim, J. Chun and H.-G. Hur.** 2010. Use of barcoded pyrosequencing and shared OTUs to determine sources of fecal bacteria in watersheds. Environ. Sci. Tech. : **In Press**
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9. Dissemination and Use

Shipping through the Duluth-Superior harbor, the largest port by total cargo volume in the Great Lakes, has a $200 million annual impact on Minnesota's economy. Development of an improved approach to determine whether potentially harmful bacteria, which may become invasive species in the future, are imported into Minnesota's coastal waters will be valuable to Minnesota as well as other cities and states that border the Great Lakes. Our goal is to generate reliable information about potentially harmful microbes that may be imported into the Duluth-Superior harbor by discharge of water from the ballast tanks of commercial ships. If we do identify potentially harmful bacteria that are discharged in the Duluth-Superior harbor, then this information will be useful for risk analyses that may help policymakers if they decide to set new standards for the microbiological safety of ballast water discharged into the Great Lakes.

Our research results will be disseminated to several target audiences. First, we will 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 also 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.

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Minnesota Pollution Control Agency
Duluth Office

September 22, 2010

Dr. Randall E. Hicks
Director, UMD Center for Freshwater Research and Policy
Department of Biology
1035 Kirby Drive, SSB 207
University of Minnesota Duluth
Duluth, Minnesota 55812

Re: LCCMR Proposal

Dear Dr. Hicks,

The purpose of this letter is to document Minnesota Pollution Control Agency (MPCA) staff availability to provide assistance with your project entitled "Improved Detection of Harmful Microbes in Ballast Water". It is our understanding that you are currently requesting funding for this project by the Legislative-Citizen Commission on Minnesota Resources (LCCMR).

MPCA staff anticipates providing assistance to the project during the summers of 2011 and 2012. MPCA staff can assist the project with obtaining access to vessels docking within the Port of Duluth/Superior as well as with assisting University of Minnesota – Duluth personnel with obtaining samples of ballast water from approximately 10 vessels. As in the past, our facilitation of vessel access will be on a volunteer-only basis.

We look forward to working with you and your project partners, as we had during our joint ballast sampling efforts conducted during the 2009 shipping season.

Sincerely,

John Thomas
Pollution Control Specialist Senior
Compliance and Enforcement Section
Industrial Division

JT:kmk

cc: Jeff Stollenwerk, MPCA

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