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

Project Title: **ENRTF ID:**   041-B

Sources of Microbial Pollution in the Upper Mississippi River

Category: B. Water Resources

Total Project Budget: $ 690,972

Proposed Project Time Period for the Funding Requested: 3 Years, July 2014 - June 2017

Summary:

In this project we will develop a DNA-sequence-based method to determine sources of fecal bacteria in the Mississippi River to assess water quality, mitigate health risks, and develop effective control strategies.

Name: Michael   Sadowsky

Sponsoring Organization: U of MN

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Web Address http://www.cbs.umn.edu/m3p

Location

Region: Statewide

County Name: Statewide

City / Township:

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

PROJECT TITLE: Sources of Microbial Pollution in the Upper Mississippi River

I. PROJECT STATEMENT

This project aims to develop a next generation, high throughput, sequence based system to determine the sources of the fecal pollutants in the Mississippi River. We have called this system SeqMiST, for sequence-based microbial source tracking. We will comprehensively characterize the total microbial community and assess relative abundance of taxa associated with fecal pollution using 16S rDNA-based, metagenomic DNA sequencing approaches. This new method will increase the predictive power of environmental monitoring assays for fecal pollution and the information gained will be essential for health advisories, to formulate restoration strategies, to develop total maximum daily loads (TMDL), and to detect other potential unsuspected sources of fecal contaminants in waterways.

Contamination of water by fecal material results from deposition by wildlife, agricultural runoff, inadequate sewage treatment, or faulty septic systems. The Mississippi River is often contaminated with human fecal materials, and this is generally regarded as creating a greater risk to human health than other fecal sources, as they most likely contain human-specific viruses and enteric pathogens. To address some of these problems, the microbiological quality of the upper Mississippi River, with respect to fecal indicator bacteria is now subject to a TMDL protection plan, which aims to restore water quality. While this is necessary as the river is an important habitat for fish and wildlife and provides for recreational activity and transport, we often do not know the sources of fecal bacteria in the river - SeqMiST can solve this problem.

Fecal pollution levels in waterways are typically monitored by measuring the presence of the fecal indicator bacteria (FIB), chiefly *E. coli.* Unfortunately, current efforts to monitor water quality in Mississippi River using FIB are overshadowed by our lack of knowledge of > 99% of the other microorganisms in the water, including pathogens, and our inability to determine the sources of fecal pollution. This research project fills this knowledge gap concerning the Mississippi River within Minnesota using a 16S rDNA, metagenomics-based approach. This technology has the power to comprehensively determine nearly all the bacteria and fecal contaminants in the water. This is done by comparing microbial taxa in waterways to what is present in the feces from 16 animal species and humans. This will lead to more confidence in the validity of our regulations and policies, and lead to more targeted remediation efforts by both state and local agencies. This data will be available for use in large scale (*e.g.*, Minnesota or national) studies to address human impacts on the Mississippi River. This river is more than a transportation corridor, it is also a watershed and drinking water source for over 50 cities and 18 million people and a habitat providing for fish and wildlife and recreational activities for millions of people in over 31 states.

These studies will put Minnesota at the forefront of this important area of environmental research. Project outcomes will provide more insight into selection of proper remediation efforts and the future environmental needs of the watershed to improve water quality. We also believe that one of the best approaches to remediate the Mississippi is to engage the public through exhibits at the Science Museum of Minnesota, the Bell Museum of Natural History, and the Lake Itasca visitor's center. These efforts will engage the state's citizens in this novel exploration of the Mississippi.

II. DESCRIPTION OF PROJECT ACTIVITIES

Activity 1: SeqMiST – Fecal Taxon Libraries (FTL)   Budget: $241,250

We will develop microbial fecal taxon libraries (FTL) comprised of nearly all bacteria from feces of 16 animal species and humans (Beaver, Cat, Chicken, Dairy Cow, Beef Cow, Deer, Dog, Duck, Goat, Goose, Gull, Horse, Human, Pig, Sheep, Tern, and Turkey). Once created, the FTL will be compared to all bacteria at each sampling site using high throughput bioinformatics tools. Ten individuals from each animal species will be pooled together and this will be done from 5 farms from across the state. Each fecal sample will be sampled to a depth of 20,000 OTUs. Fecal taxa will be compared to microbes in the waterway as well as the metadata from each site.

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Outcome	Completion Date
1. Establish fecal taxon libraries (FTL) for Mississippi River Watershed	October 31, 2016
2. Correlations of sources of fecal pollution OTU data to distribution and persistence of genetic markers of fecal pollution and eco-chemical-physical factors and land uses.	December 31, 2016

Activity 2: SeqMiST - Microbial Source Tracking **Budget: $378,426**

This proposal will fund three years of sampling and metagenome and molecular analysis of water samples from the Mississippi River at 10 critical junctures in the Minnesota, from Lake Itasca to La Crescent (see attached map), focusing on the headwaters and confluences with other major Rivers. We are currently obtaining data from these 10 sites as part of our current LCCMR-sponsored project, and are proposing here to request funding for in depth studies of these sites, at multiple depths and seasons, for three additional years. At each sampling location and time point (3 times per year), we will take replicate water samples at three depths (surface, 1 m, and 3m) as well as sediment samples. When possible, we will choose sampling locations that correspond to the TMDL site of the MPCA and USGS monitoring stations. We will also obtain information on other indicators of water quality (temp., turbidity, pH, land use, *E. coli* and *Enterococcus* counts, and fecal marker genes) and watershed management that impact fecal contamination. Each water sample will be sequenced to a depth of 200,000 operational taxonomic units (OTUs) and compared to the FTL developed in Activity 1 to determine sources.

Outcome	Completion Date
1. Sampling of the Mississippi River and analysis of samples for determination of fecal pollution sources at each sampling location using the FTL	December 31, 2016
2. Correlations of FTL data to OTU data from the river to determine sources of fecal pollution at each sampling location.	June 30, 2017
3. Correlations of OTU data to distribution and persistence of genetic markers of fecal pollution and eco-chemical-physical factors and land uses at each sampling location.	June 30, 2017

Activity 3: Project data dissemination **Budget: $ 71,296**

In this result we will develop a user-friendly web interface for SeqMiST data that significantly reduces the analysis time required to determine potential source of fecal contamination in the environment. This website will be accessible to everyone. We will utilize existing national web resources, such as the Mothur database to facilitate archiving, retrieval, and analysis of metagenome data. We will also develop public displays at Museums and Lake Itasca.

Outcome	Completion Date
1. Production of a web interface and database of 16S rDNA sequencing-based microbial source tracking for the Upper Mississippi River.	December 31, 2016
2. Production of public exhibits.	December 31, 2016
3. Dissemination of project data and results via seminars, and workshops	June 30, 2017

III. PROJECT STRATEGY
A. Project Team/Partners. The project will be carried out under the direction of Drs. Michael Sadowsky (PI) and Chanlan Chun (Research Associate). Funded project partners will include Pat Hamilton of the Science Museum of Minnesota, Susan Weller, Bell Museum Director, and Itasca State Park. We will also collaborate with the National Park Service at the SMM and Barb Peichel at MPCA for dissemination activities.
B. Timeline Requirements. The project will be completed in 3 years, but the impact will last for many more. Multiple years of sampling and analysis are required for adequate, reliable data and multiple years are required for effective incorporation into public education venues.
C. Long-Term Strategy and Future Funding Needs. This request seeks funding for the first 3 years of this program. This will provide the basis for a long-term, continuing study of the health of the River. Additional funding for more long term and more extensive analyses (of the upper and lower Mississippi River) will be obtained from the National Science Foundation, other states, and other foundations.
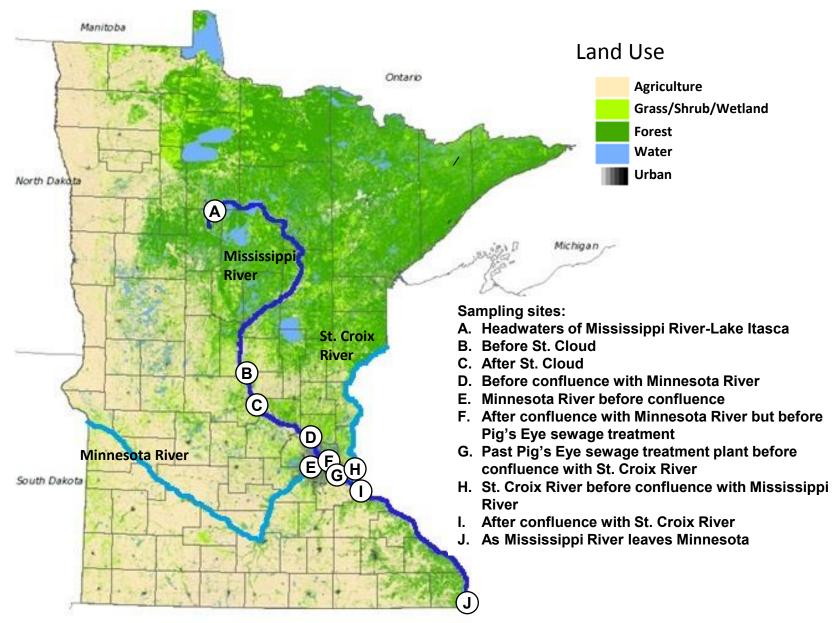
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2014 Detailed Project Budget

Project Title: Sources of Microbial Pollution in the Upper Mississippi River

IV. TOTAL ENRTF REQUEST BUDGET 3 years

BUDGET ITEM	AMOUNT	
Personnel:		
Research Associate (30% time, 34% fringe, 3 years, 1 person)	$	60,300
Postdoctoral Associate (100% time, 22% fringe, 3 years, 1 person)	$	146,400
Graduate Student (50% time, GRA tution and benefits, 3 years, 1 person)	$	115,500
Technician (100% time, 36% fringe, 3 years, 1 person)	$	142,800
Website staff (100% time, 36% fringe, 1 years, 1 person)	$	1,496
Contracts:		
Exhibit at Science Museum of Minnesota (staff time and materials)	$	54,800
Exhibits at Bell Museum and Lake Itasca	$	10,000
Equipment/Tools/Supplies:		
Laboratory supplies: $12,000/year × 3 years	$	36,000
Acquisition (Fee Title or Permanent Easements):	$	-
Travel:		
In-state Travel for 10 samplings per year × 3 years @3000 mi × $0.50/mi	$	1,500
Room & boad for 4 people × 3 days/year × 3 years for sampling: $3,496 for lodging and $1,680 for	$	5,176
Additional Budget Items:		
Sample analysis: 50 animal fecal samples and 90 water samples: Genome preparation @$200/sample =$28,000, genome anlayisis= @$600/sample=$84,000, physiochemical analysis=$5,000). Genome preparation and all genomic analyses are done most cost effectively in specialty labs that charge by the sample	$	117,000
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$	690,972

V. OTHER FUNDS

SOURCE OF FUNDS	AMOUNT	Status
Other Non-State $ Being Applied to Project During Project Period: Fecal Taxon Library (FTL) generated by MN Sea Grant will be used to leverage this project	$ 114,608	*Pending*
Other State $ Being Applied to Project During Project Period:	0	
In-kind Services During Project Period:	$ -	
Remaining $ from Current ENRTF Appropriation (if applicable):	$ -	
Funding History: LCCMR project, *Mississippi Water Quality – Deeper Look, Broader Impacts will be leverage to this project (July 2011-December 2014).*	$557,000	

Land Use

Agriculture
Grass/Shrub/Wetland
Forest
Water
Urban

Manitoba

Ontario

North Dakota

Michigan

Mississippi River

St. Croix River

Minnesota River

South Dakota

Sampling sites:
A. **Headwaters of Mississippi River-Lake Itasca**
B. **Before St. Cloud**
C. **After St. Cloud**
D. **Before confluence with Minnesota River**
E. **Minnesota River before confluence**
F. **After confluence with Minnesota River but before Pig's Eye sewage treatment**
G. **Past Pig's Eye sewage treatment plant before confluence with St. Croix River**
H. **St. Croix River before confluence with Mississippi River**
I. **After confluence with St. Croix River**
J. **As Mississippi River leaves Minnesota**

Project Manager Qualifications and Organization Description:

Project Manager: Dr. J. Michael Sadowsky

Title: McKnight University Professor and Director BioTechnology Institute

Affiliation: University of Minnesota, Department of Soil, Water and Climate, and BioTechnology Institute

The UMN Biotechnology Institute (BTI) provides advanced research, training, and university-industry interactions in biological process technology, and other areas of biotechnology research. Faculty in the BTI have broad expertise in: Biocatalysis, Metabolic engineering/microbial physiology, Population dynamics, Molecular biology, Proteomics and focused expertise in defined areas such as bioremediation, biomaterials, biosensors, and bioinformatics.

Education:
Ph.D., 1983. University of Hawaii, Honolulu, Hawaii. Major: Microbiology
M.S., 1979. University of Wisconsin-Oshkosh, Wisconsin. Major: Microbiology
B.S, 1977. University of Wisconsin-Madison, Wisconsin. Major: Bacteriology

Professional Experience:
Director BioTechnology Institute, University of Minnesota, St. Paul, Minnesota, 2009 - present.
Co-Director, Microbial and Plant Genomics Institute, University. of Minnesota, 2006-2009.
Distinguished McKnight University Professor: Department of Soil, Water, & Climate, and BioTechnology Institute, University of Minnesota, St. Paul, Minnesota, 04/04 - present.
Professor: Department of Soil, Water, and Climate and Department of Microbiology
University of Minnesota, St. Paul, Minnesota, 07/96 – 04/04.
Associate Professor: Departments of Soil Science and Microbiology
University of Minnesota, St. Paul, Minnesota, 07/93 - 6/96.
Assistant Professor: Departments of Soil Science and Microbiology
University of Minnesota, St. Paul, Minnesota, 06/89 - 6/93.
Microbiologist: U.S. Department of Agriculture-ARS; Beltsville, Maryland, 01/86 - 05/89.

Dr. Sadowsky will have chief management responsibilities for overseeing the proposed project. He will be responsible for working with the Research Associate (Dr. Chun), project partners and cooperators to ensure that project goals, results and timelines are met. He will also be responsible for working with the undergraduates, and postdoctoral associate at UMN and staff at the SMM and Lake Itasca. Dr. Sadowsky is an environmental microbiologist with 30 years research experience in the analysis and use of microorganisms in environmental settings. Dr. Sadowsky's 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. He is currently Director of the BioTechnology Institute, and is involved in teaching metagenomic courses at the University and Lake Itasca. 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.