

**Environment and Natural Resources Trust Fund
2017 Request for Proposals (RFP)**

Project Title:

ENRTF ID: 031-A

Minnesotas Freshwater Sponges: Mapping Taxonomy and Environmental Toxicology

Category: A. Foundational Natural Resource Data and Information

Total Project Budget: \$ 258,000

Proposed Project Time Period for the Funding Requested: 3 years, July 2017 - June 2020

Summary:

Data on sponges in Minnesota are scarce despite their vital role in aquatic ecosystems. This project will determine sponge distribution, identify and quantify accumulated pollutants, strengthen undergraduate research and education.

Name: Venugopal Mukku

Sponsoring Organization: U of MN

Address: 2900 University Ave
Crookston MN 56716

Telephone Number: (218) 280-2342

Email mukku002@umn.edu

Web Address _____

Location

Region: Statewide

County Name: Statewide

City / Township:

Alternate Text for Visual:

The visual shows ten watersheds in MN, graphically portrays the distribution of sponges, and the potential transfer of accumulated pollutants in sponges to humans via the food chain

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



Environment and Natural Resources Trust Fund (ENRTF)
2017 Main Proposal
Project Title: Minnesota’s Freshwater Sponges: Mapping Taxonomy and Environmental Toxicology

I. PROJECT STATEMENT

Sponges are the simplest forms of animal life and play a vital role in the aquatic ecosystem by providing habitat and nutrients for other aquatic life. Most of the sponges that have been studied are found in the marine environment and are known to accumulate pollutants similar to those found in MN waters (e.g., polyaromatic hydrocarbons or PAHs). Due to their similar structure, freshwater sponges are likely accumulating these toxic substances found in MN waters and may be transferring them through the food chain to game fish and other economically important aquatic and terrestrial organisms. Despite their importance in aquatic ecosystems, information on distribution of freshwater sponges in MN lakes and rivers is very limited. The primary goals of this project are to **(1) determine the diversity and distribution of freshwater sponges in Minnesota’s water basins and watersheds and to (2) determine if these freshwater sponges are accumulating toxic pollutants.** The proposed study will generate novel and critically important information regarding distribution of freshwater sponges and bioaccumulation, aiding in the fight to protect Minnesota’s aquatic ecosystems.

We will collect and identify sponges from two lakes and/or rivers in each of the ten watersheds in MN and map their geographic distribution. Taxonomic identification will be performed using morphological and molecular analyses. Subsequent to the identification of sponges, chemical analyses will be performed to identify and quantify accumulating pollutants. Chemical analyses will be carried out by extraction of organic compounds from the sponges and screening for the presence of pollutants using sophisticated chromatographic methods such as gas and liquid chromatography coupled with mass spectrometry (GC-MS and LC-MS).

Another goal of this project is to **(3) further strengthen interdisciplinary research among faculty at University of Minnesota Crookston (UMC) and provide its students with practical skills that could be translated into their careers.** UMC is uniquely poised to tackle this project because of its faculty and student profile. UMC faculty are recognized for their expertise in natural resources and in the natural sciences. The associated faculty have established productive interdisciplinary collaborations involving Minnesota’s undergraduate students with varied academic backgrounds.

II. PROJECT ACTIVITIES AND OUTCOMES

Activity 1: Collection and taxonomic identification of freshwater sponges sampled from the ten major basins and watersheds in Minnesota Budget: \$119,000

Collection and taxonomic identification of freshwater sponges will be carried out from May through September in the first two years. Two lakes/ivers will be selected based on primary literature and available water quality data. Using the water quality data, sponges will be collected from one “pristine” lake/river and one contaminated lake/river within each watershed. Multiple sites will be sampled within each lake/river. A small portion of each sponge will be collected and transported to UMC and identified by morphological and molecular analysis. Morphological analysis will be performed by preparation of sponge spicules. Molecular analysis will be performed after morphological analysis to further classify the collected sponges. DNA will be extracted from each collected sponge and sequenced for identification. GIS data will be collected from each sampling site and used to construct a preliminary geographic distribution map of freshwater sponges in Minnesota.

Outcome	Completion Date
1. Collection of sponges from two lakes/ivers within each major basin/watershed in MN	September 2018
2. Morphological and molecular identification of collected sponges	January 2019
3. Geographic mapping of the distribution of freshwater sponges in MN	June 2020

Activity 2: Detection of contaminants present in collected freshwater sponges Budget: \$89,000

Collected sponges will be freeze-dried and organic material will be extracted with suitable solvents. Contaminants of emerging concern in each sponge extract, if any, will be identified using EPA protocols where available such as GC-MS. Chemical standards will be used for the identification and quantification of contaminants. The presence of PCBs, PAHs, and other emerging contaminants such as pharmaceuticals,



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pesticides, and estrogenic compounds in the sponge will be determined. A similar analysis will be performed for inorganic contaminants.

Outcome	Completion Date
1. Extraction of contaminants from collected freshwater sponges	January 2019
2. Identify and quantify contaminants present in sponge extracts using established analytical methods (e.g. EPA protocols)	June 2020

Activity 3: Training undergraduate students in interdisciplinary research and dissemination of research findings to the appropriate stakeholders **Budget: \$50,000**

During year 1, 4-6 students will learn how to collect and identify sponge specimens using accepted techniques (Activity 1). During year 2, 4-6 students will learn how to perform chemical analyses on these specimens using appropriate laboratory techniques and instrumentation (Activity 2). By the end of year 3, 6-8 students and 2-3 faculty will have disseminated much of their findings to interested state agencies (e.g., MPCA) and at professional conferences by way of poster and/or oral presentations or white papers. Based on the results of this project, certain aspects of this work will be incorporated into laboratory courses from freshman-level in biology, chemistry. Students engaged in this research will be well prepared for required internships and future employment.

Outcome	Completion Date
1. 10-12 Students will be trained in proper field collection and laboratory procedures	June 2019
2. 6-8 Students and 2-3 faculty will disseminate research findings to potentially interested state (MPCA, MN DNR) agencies and scientific conferences.	June 2020
3. Integration of sponge research into undergraduate curricula (e.g., biology, chemistry)	June 2020

III. PROJECT STRATEGY

A. Project Team/Partners: Venugopal Mukku, U. MN Crookston. Will extract and quantify contaminants from the collected sponges (Activity 2; Outcome 1 and 2). Will coordinate projects and assist with results dissemination. Anthony Schroeder, U. MN Crookston. Responsible for the morphological and molecular identification of collected of all freshwater sponges (Activity 1; Outcome 2). Also responsible for the geographic mapping of the distribution of freshwater sponges in Minnesota (Activity 1; Outcome 3). Timothy Dudley, U. MN Crookston. Responsible for organizing training of students and integrating this research into the undergraduate programs at UMC (Activity 3; Outcome 1 and 3). Responsible for coordinating the dissemination of research to state agencies and science community (Activity 3; Outcome 2). Also, responsible for making sure all reports are filed on time. All will supervise students and all will receive ENRTF funds.

B. Project Impact and Long-Term Strategy: The long-term goal of the proposed study is to provide an understanding of the distribution of the freshwater sponges found in Minnesota lakes/ivers and to determine if these sponges are accumulating pollutants. This research is especially important because there is no current data regarding these essential aquatic organisms and how they may be involved in bioaccumulation of pollutants which would be transferred to gamefish and ultimately humans. Another impact of this project is to provide Minnesota students training in relevant field and laboratory protocols, ensuring a well-prepared scientific workforce. Results will be disseminated through scientific presentations, peer-reviewed publications, and presented to interested state agencies. Ultimately, this project’s growing database and understanding will be incorporated into various UMC science programs, providing long-term enrichment opportunities for Minnesota students.

C. Timeline Requirements: This project is expected to conclude within 36 months, by June 2020. It will require two field seasons to sample two lakes/ivers within each watershed for collecting sponges. Laboratory work and collection of sponges in year two will be performed in parallel throughout the rest of the project, with findings from each activity informing the work of the other.

2017 Detailed Project Budget

Project Title: Minnesota's Freshwater Sponges: Mapping Taxonomy and Environmental Toxicology

IV. TOTAL ENRTF REQUEST BUDGET 3 years

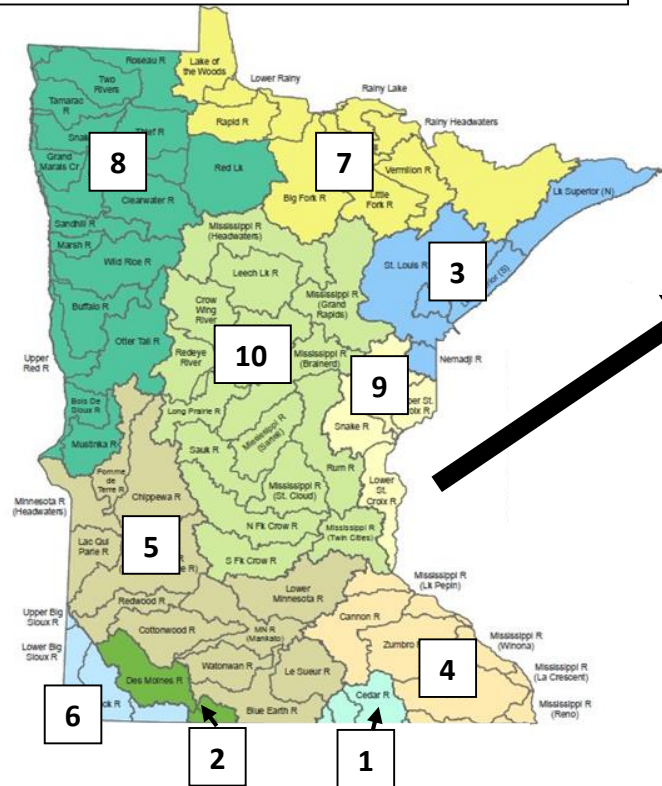
BUDGET ITEM	AMOUNT
Personnel:	
Project Manager. Venugopal Mukku (66% salary, 34% benefits); 11% FTE each year. Supervise students and coordinate projects.	\$ 29,000
Co-Project Manager. Anthony Schroeder (66% salary, 34% benefits): 11% FTE first 2 years, 5.5% FTE third year. Supervise students, assist with coordinating project, disseminating results.	\$ 23,000
Co-Investigator. Timothy Dudley (66% salary, 34% benefits): 5.5% FTE first year, 11% FTE for last 2 years. Supervise students, develop curricula, compile and file reports, and organize the dissemination of results.	\$ 26,000
Undergraduate students. 4 students during each academic year (two at 5 hours per week for 32 weeks at \$11.50 per hour (\$3,996), two at 10 hours per week for 32 weeks at \$11.50 per hour (\$7992)), 3 students each summer full time (30 hours per week for 12 weeks at \$11.50 per hour (\$13,485)). 92.1% salary, 7.9% fringe benefits.	\$ 74,000
Professional/Technical/Service Contracts:	
Sponge collection from 10 watersheds and basins during years 1 and 2 will be contracted out. This will involve 1-2 technicians assisting with collections within each watershed during the summer months. The costs include the salaries/wages of the technicians, scuba and other equipment rentals, and supplies.	\$ 50,000
Equipment/Tools/Supplies:	
DNA extraction kits (~100 samples/\$20 per sample)	\$ 2,000
Reagents for DNA sequencing (primers, Big Dye Reagent, tubes) (~100 samples/\$40 per sample)	\$ 4,000
DNA sequencing for sponge identification (~100 samples/\$10 per sample)	\$ 10,000
Lyophilizer (freeze-drying instrument) for sampling organic material from each sponge. This will be used to remove water from the sponge samples.	\$ 10,000
General chromatographic and spectrophotometric supplies (e.g. 5 HPLC columns, HPLC solvents and buffers, analytical standards for PAHs and PCBs, 4 UV-visible quartz tubes, reagents for spectrophotometric analyses)	\$ 12,000
LC/MS and GC/MS instrument access. These analyses will be performed off-site (e.g., Univ. of Minnesota Twin Cities) and will be used to identify and quantify contaminants in the sponge samples (~120 samples/\$100 per sample)	\$ 12,000
Travel: Mileage costs (59 cents per mile, 6000 miles total) for PIs to travel to sampling sites to oversee collection of sponges and perform preliminary chemical analysis of sample sites. 2 trips to each watershed and basin (20 round trips total) originating from UMC (Crookston).	\$ 4,000
Additional Budget Items: Shipping costs to send samples for sequencing and chemical analysis.	\$ 2,000
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND \$ REQUEST =	\$ 258,000

V. OTHER FUNDS

SOURCE OF FUNDS	AMOUNT	Status
Other Non-State \$ To Be Applied To Project During Project Period:	N/A	
Other State \$ To Be Applied To Project During Project Period:	N/A	
In-kind Services To Be Applied To Project During Project Period: Indirect costs (waived)	134,160	
Funding History: Anthony Schroeder has a service contract for a project recommended for funding through ENRTF unrelated to the current proposal (053-B Eliminating Contaminants to Protect Endangered Native Fish/Mussels).	N/A	Project will begin 7/2016
Remaining \$ From Current ENRTF Appropriation:	N/A	

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Basins and Major Watersheds in Minnesota

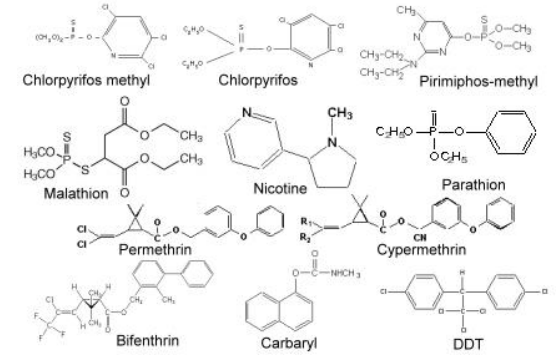


- 1: Cedar River
- 2: Des Moines River
- 3: Lake Superior
- 4: Lower Mississippi River
- 5: Minnesota River
- 6: Missouri River
- 7: Rainy River
- 8: Red River of the North
- 9: St. Croix River
- 10: Upper Mississippi River

Where are freshwater sponges found in MN?



Are sponges accumulating pollutants?



Why do we care?



**Freshwater Sponge
Accumulating Pollutants
and Bio-Magnification in
the Food Chain**

Minnesotans
eat fish,
resulting in
potentially
serious
illnesses



Project Title: Minnesota's Freshwater Sponges: Mapping Taxonomy and Environmental Toxicology

2017 LCCMR Project Manager and Co-Manager Qualifications and Organization Description

Project Manager: Dr. Venugopal Mukku, University of Minnesota Crookston

Project Co-Manager: Anthony Schroeder, University of Minnesota Crookston

Key Qualifications

Dr. Venugopal Mukku is a tenured Associate Professor of Organic Chemistry at the University of Minnesota Crookston (UMC). He has considerable experience in characterizing organic compounds from marine organisms, including sponges, soft corals, star fishes, and other invertebrates.

Dr. Anthony Schroeder is an assistant professor of Biology at UMC. He was previously a postdoctoral associate with the University of Minnesota Twin Cities in cooperative agreement with U.S. EPA. He has considerable research experience in developing and utilizing approaches to better and more rapidly assess the biological effects of complex environmental mixtures on aquatic ecosystems. He uses a variety of molecular biology techniques to assess biological effects on various aquatic species.

Education

Dr. Mukku: Andhra University, Organic Chemistry, Ph.D. 1995; Sri Sathya Sai Institute of Higher Learning (SSSIHL), Chemistry, M.Sc. 1990, SSSIHL, Chemistry, B. Sc., 1988.

Dr. Schroeder: University of North Dakota, Biology, Ph.D. 2013; University of North Dakota, Biology, B.S. 2006;

Selected Publications

Venugopal J. Mukku, Shawn Friedland, Nicole E. Sorlie, Heather S. Donati-Lewis, Brian J. Dingmann. Antimicrobial activity of selected Native American seeds. *Journal of Medicinal Plants Research*, **2013**, 7, 2928-2932.

George R. Pettit, **Venugopal J. Mukku**, Gordon Cragg, Delbert L. Herald, John C. Knight, Cherry L. Herald and Jean-Charles Chapuis. Antineoplastic Agents. 558. *Ampelocissus* sp. Cancer Cell Growth Inhibitory Constituents. *Journal of Natural Products*, **2008**, 71, 130-133.

Venugopal J. Mukku, Speitling M, Laatsch H, Helmke E. New butenolides from two marine Streptomyces. *Journal of Natural Products*, **2000**, 63, 1570-1572.

Anjaneyulu, A.S.R., **Venugopal J. Mukku**, Prakash C.V.S. Two new furanosesquiterpenoids and a phenolic dibromo compound from a marine sponge of *Axinella* genus of Andaman and Nicobar Islands. *Indian Journal of Chemistry B*, **1994**, 33, 148-151.

Schroeder, A.L., Ankley, G.T., Houck, K., Villeneuve, D. **2016**. Environmental Surveillance and Monitoring – The Next Frontiers for High-throughput Toxicology. *Environmental Toxicology Chemistry* 35:513-525.

Nelson, K.R., **Schroeder, A.L.**, Ankley, G.T., *et al.*, **2016**. Inhibition of thyroid peroxidase leading to impaired anterior swim bladder inflation – Part I: Fathead Minnow. *Aquatic Toxicology* 173:192-203.

Organization Description: University of Minnesota Crookston is part of the University of Minnesota System and predominantly serves rural Northwestern MN and Eastern ND. UMC has made impressive strides in the last ten years in hiring tenure track faculty with research agendas. In addition to local facilities, the PIs have the resources (instrumental and library) of the Main campus for their use.