

**Environment and Natural Resources Trust Fund  
2020 Request for Proposals (RFP)**

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**Project Title:**

**ENRTF ID: 040-A**

Freshwater Sponges and AIS: Engaging Citizen Scientists

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**Category:** A. Foundational Natural Resource Data and Information

**Sub-Category:**

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**Total Project Budget: \$** 460,000

**Proposed Project Time Period for the Funding Requested:** June 30, 2023 (3 yrs)

**Summary:**

The project will study the geographical spread, taxonomic diversity and anti-fouling potential of freshwater sponges against aquatic invasive species by involving faculty, students and citizen scientists.

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**Name:** Venugopal Mukku

**Sponsoring Organization:** U of MN

**Job Title:** \_\_\_\_\_

**Department:** \_\_\_\_\_

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Crookston MN 56716

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**Location:**

**Region:** Statewide

**County Name:** Statewide

**City / Township:**

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**Alternate Text for Visual:**

Freshwater sponges; Collection, Identification and Chemistry; Citizen Scientists; Aquatic Invasive Species

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



**I. PROJECT STATEMENT**

**This project addresses the LCCMR funding priorities by 1) identifying compounds from freshwater sponges that will be used to combat the spread of aquatic invasive species (AIS) such as zebra mussels 2) Incorporating Minnesota’s citizen involvement in the collection and identification of freshwater sponges and 3) stimulating STEM education for students in Minnesota.**

**Need:** As a result of 2017 LCCMR funding, two previously undocumented species of freshwater sponges were identified from the lakes and rivers of Minnesota in the ten county region around Crookston. Gas Chromatography - Mass Spectrometry (GC-MS) analysis of some sponge extracts revealed that some sponges produce a compound (oleamide) that was reported to have antifouling properties. Antifouling chemicals inhibit the attachment and growth of barnacles and other marine organisms on a ship’s hull. We hypothesize that one or more of the naturally occurring antifouling compounds from sponges may inhibit the spread of zebra mussels. Natural antifouling compounds have an added advantage in that they are biodegradable. The utilization of antifouling compounds found in Minnesota freshwater sponges contributes to addressing AIS as noted in Minnesota Statute 84D, specifically addressing 84D.02: aiding in preventing and curbing the spread of invasive species such as zebra mussels.

The current project was highlighted in the Minnesota DNR’s Conservation Magazine in July 2017, which sparked scientific curiosity among Minnesotans. Over 50 citizens and schools reported locations of sponges and/or requested more information about the project. The proposed project will integrate the clearly expressed public interest with one of the funding priorities of LCCMR and will provide Minnesotans with opportunities to be directly involved in learning about the habitat and collection of freshwater sponges. Incorporating citizen scientists will enable the project team to expand the geographical focus of the project to the entire state. The project team will leverage the experience of the new Center for Citizen Science at the University of Minnesota Twin Cities. Additionally, the team will focus on stimulating STEM education for students in Minnesota.

**II. PROJECT ACTIVITIES AND OUTCOMES**

**Activity 1: Expand the taxonomy mapping of freshwater sponges by the inclusion of Minnesota Citizen Scientists** **Budget: \$248,000**

Numerous rivers and lakes remain to be explored for freshwater sponges. Collections will continue with the additional focus of citizen scientist involvement. Town hall meetings will be held at strategic locations across the state to explain the freshwater sponge project, and encourage citizen scientist participation. Using developed specimen collection packets, citizens will be able to collect sponge samples and mail them to UMC for analyses described in activity 3.

Faculty/researchers will travel to schools in Northwest Minnesota to engage students and teachers in STEM activities. Freshwater sponge-focused activities will be designed to get students interested in scientific inquiry and stimulate participation in local and regional science fairs.

<b>Outcome</b>	<b>Completion Date</b>
1. Continuation of freshwater sponge collections in MN rivers/lakes	October 2022
2. Develop information/collection packets to send to public for a broader search for sponges throughout the state	March 2021
3. Initiate/perform outreach activities using town halls, schools, etc. to teach about sponges and encourage citizen engagement in the project.	June 2023



**Environment and Natural Resources Trust Fund (ENRTF)**

**2020 Main Proposal**

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**Activity 2: Determine the chemical ecology of freshwater sponges and identify antifouling compounds**

**Budget: \$148,000**

Freshwater sponges, as well as water and sediment samples, will be collected. Sponges will be freeze-dried prior to chemical extractions. Organic components of these samples will be analyzed using chromatographic techniques (e.g., GC-MS with NIST library, LC-MS). Inorganic components will be analyzed using chromatographic (e.g., IC) and spectroscopic (e.g., ICP-MS) techniques.

All sponge extracts will be tested for their potential antifouling activity using zebra mussel attachment and *in vitro* assays. Compounds of interest will be identified using GC-MS and LC-MS and isolated in order to fully test their efficacy.

Outcome	Completion Date
1. Examine the chemical ecology of freshwater sponges	June 2023
2. Isolate antifouling compounds and perform biological assays to assess the antifouling ability of sponge extracts	June 2023

**Activity 3: Perform morphological and genetic analyses to identify sponges collected by the investigators and citizen scientists**

**Budget: \$64,000**

Collected freshwater sponges will be identified by morphological and molecular analysis. A sub-sample of the sponge will be used for morphological analysis through spicule preparation. DNA will also be extracted from each sponge. The common barcoding gene, cytochrome oxidase, will be amplified and sequenced for identification. Morphological and molecular results from sponges collected from citizens (Activity 1) will be disseminated through a list-serv, providing the citizens with information about their collected sponges and how they are contributing to the project and understanding of animal diversity in Minnesota. All collected freshwater sponges will be catalogued in the University of Minnesota’s Bell Museum.

Outcome	Completion Date
1. Catalogue collected sponge species by morphological and genetic analyses	June 2023
2. Develop and disseminate sponge results to each citizen scientist who assisted with sponge collections	June 2023

**III. PROJECT STRATEGY**

**A. Project Team/Partners: Venugopal Mukku, UMN Crookston, Ph.D., Chemistry.** Project Manager.

Responsible for isolating and characterizing antifouling compounds from freshwater sponges (Activity 2), supervising a postdoctoral associate and coordinating the project activities. **Anthony Schroeder, UMN Crookston, Ph.D., Biology.** Responsible for biological work associated with Activity 3. **Timothy Dudley, UMN Crookston, Ph.D., Chemistry.** Responsible for analysis of the chemical ecology of freshwater sponges (Activity 2) and outreach (Activity 1). **Postdoctoral associate.** Primarily freshwater sponge collections (Activity 1), working with outreach program (Activity 1), assisting in analyses (Activities 2, 3), and manuscript preparations. 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 expand on our understanding of the species of the freshwater sponges found in Minnesota, while also providing an opportunity for citizen scientist involvement and STEM promotion. This project will begin to identify connections between freshwater sponges and their environment via chemical analyses. The project also has great potential to find and develop a natural biocide to help combat the spread of zebra mussels. Results will be disseminated through scientific presentations by faculty and students, peer-reviewed publications, and presented to interested state agencies.

**C. Timeline Requirements:** This project is expected to conclude within 36 months, by June 2023. It will require three field seasons for the investigators to collect sponges, perform outreach, chemical examination and *in vitro* assays for antifouling activity.

**Attachment A: Project Budget Spreadsheet**  
**Environment and Natural Resources Trust Fund**  
**M.L. 2020 Budget Spreadsheet**



**Legal Citation:**  
**Project Manager:** Venugopal Mukku  
**Project Title:** Freshwater Sponges and AIS: Engaging Citizen Scientists  
**Organization:** University of Minnesota Crookston  
**Project Budget:** \$460,000  
**Project Length and Completion Date:** 3 years; June 30, 2023  
**Today's Date:** April 15, 2019

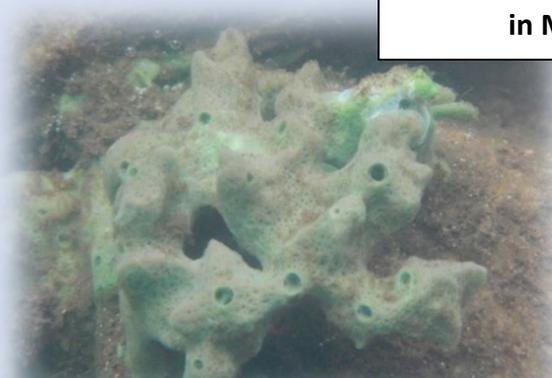
<b>ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET</b>	<b>Budget</b>	<b>Amount Spent</b>	<b>Balance</b>
<b>BUDGET ITEM</b>			
<b>Personnel (Wages and Benefits)</b>	\$ 377,700	\$ -	\$ 377,700
Project Manager. Venu Mukku: \$43,032 (74% salary, 26% fringe); 11% FTE each year. 1 month salary in year 1 and 2 and 2 months salary in year 3. Supervise students, coordinate the project, compile and file reports, disseminate results.			
Co-Principal Investigator. Timothy Dudley: \$46,300; (74% salary, 26% fringe). 11% FTE each year. 1 month of salary for first two years and 2 months for third year. Supervise students, develop curricula, compile and file reports, and organize the dissemination of results.			
Co-Principal Investigator. Anthony Schroeder: \$38,973; (74% salary, 26% fringe); 11% FTE each year. 2 months salary in year 1 and 1 month salary in years 2 and 3. Responsible for activity 3.			
Undergraduate students. \$62,945; 2 students during each academic year (two at 10 hours per week at \$12.50 per hour for 32 weeks (\$8000/year*3=\$24,000) (100% salary, no fringe) 2 students each summer full time (40 hours per week at \$12.50 per hour for 12 weeks @8.2% fringe (\$12,984/year*3=\$38,945). 92% salary, 8% fringe)			
Postdoctoral Associate: \$186,450; (salary 80%, fringe 20%). 100% FTE for 3 years. The University of Minnesota post-doc will have recently completed Ph.D. in Environmental Ecology.			
<b>Equipment/Tools/Supplies</b>			
Supplies for freshwater sponge and water collections by investigators (tubes, bags, supplies to fix and store samples) (~100 sponge samples and ~100 water samples/\$45 per sample)	\$ 9,000	\$ -	\$ 9,000
Supplies to develop sponge collection kits for sending to citizen scientists (Tubes, mailing boxes, reagents for fixing sponges) (~200 samples/\$45 per sample)	\$ 9,000	\$ -	\$ 9,000
General chromatography (analytical chemistry) supplies (e.g. columns, standards, quartz tubes, reagents)	\$ 9,000	\$ -	\$ 9,000
Maintenance contract for GC-MS (\$6600/year for the GC that will be used at UMC to run samples)	\$ 19,800	\$ -	\$ 19,800
Supplies for zebra mussel bioassays (Culturing reagents and other consumables) (~300 assays at \$25 per assay)	\$ 7,500	\$ -	\$ 7,500
Reagents for DNA sequencing (Primers, Big Dye Reagent, tubes) and cost to perform DNA sequencing (~300 sponge samples between citizen collections and investigator collections at	\$ 4,500	\$ -	\$ 4,500
Chemicals for sponge morphology and chemical extractions (~300 samples at \$15 per sample)	\$ 4,500	\$ -	\$ 4,500
Instrument use and access for water and sponge analyses (LC-MS/MS; ICP-MS) (~300 samples at \$20 a sample)	\$ 6,000	\$ -	\$ 6,000
<b>Printing</b>			
Cost for printing infographs and other documents for citizens	\$ 2,500	\$ -	\$ 2,500
<b>Travel expenses in Minnesota</b>			
Mileage costs (58 cents per mile; ~14,000 miles total) for traveling to sampling sites for sponge collections and for outreach events	\$ 8,000	\$ -	\$ 8,000
<b>Other</b>			
Shipping costs for sending prepaid collection kits to citizens, shipping samples for chemistry analysis and DNA sequencing	\$ 2,500	\$ -	\$ 2,500
<b>COLUMN TOTAL</b>	\$ 460,000	\$ -	\$ 460,000
<b>SOURCE AND USE OF OTHER FUNDS CONTRIBUTED TO THE PROJECT</b>	<b>Budget</b>	<b>Spent</b>	<b>Balance</b>
<b>Non-State:</b>	\$ -	\$ -	\$ -
<b>State:</b>	\$ -	\$ -	\$ -
<b>In kind: Indirect costs (waived)</b>	\$ 248,400	\$ -	\$ 248,400
<b>Other ENRTF APPROPRIATIONS AWARDED IN THE LAST SIX YEARS</b>	<b>Budget</b>	<b>Spent</b>	<b>Balance</b>
Anthony Schroeder has a service contract for proposal (M.L. 2016, Chp. 186, Sec. 2, Subd. 04d).	\$ 33,000	\$ 28,311	\$ 4,689
Current ENRTF Appropriation: M.L. 2017, Chp. 96, Sec. 2, Subd. 03m	\$ 258,000	\$ 125,781	\$ 132,219



# Freshwater Sponges and AIS: Engaging Citizen Scientists



**Citizen Scientists Provided Information to Assist in Identifying and Locating Freshwater Sponges in Minnesota's Lakes and Rivers**



**Collected Freshwater Sponges Identified and Analyzed in the Laboratory by UMC Students and Faculty**



**UMN Center for Citizen Science Assists in Sharing Project Findings with Citizen Scientists**



**Students and Faculty Analyze Samples for Natural Biofouling Compounds Capable of Mitigating AIS such as Zebra Mussels**

## **Project Title: Freshwater Sponges and AIS: Engaging Citizen Scientists**

### **Project Manager Qualifications**

#### **Venugopal Mukku, Math, Science, and Technology Department, University of Minnesota Crookston**

**Education:** **B.Sc.**, 1988, Chemistry, Sri Sathya Sai Institute of Higher Learning (SSSIHL); **M.Sc.**, 1990, Chemistry, SSSIHL; **Ph.D.**, 1995, Marine Natural Products, Andhra University, Visakhapatnam, India.

**Employment:** **Associate Professor**, 2014-present, Math, Science, and Technology Department, University of Minnesota Crookston; **Assistant Professor**, 2008-2013, Math, Science, and Technology Department, University of Minnesota Crookston; **Visiting Assistant Professor**, 2006-2008, Austin College, Sherman, Texas.

**Research:** Dr. Mukku's doctoral and postdoctoral research involved identifying organic compounds from marine organisms such as sponges, corals, as well as marine and terrestrial Streptomyces. His research is currently focused on terrestrial species and freshwater sponges.

#### **Timothy J. Dudley, Math, Science and Technology Department, University of Minnesota Crookston**

**Education:** **B.S.**, 1997, Chemistry, University of North Dakota; **Ph.D.**, 2002, Physical Chemistry, University of North Dakota.

**Employment:** **Associate Professor**, 2017-present, Math, Science, and Technology Department, University of Minnesota Crookston; **Assistant Professor**, 2012-2017, Math, Science and Technology Department, University of Minnesota Crookston; **Associate Professor**, 2011-2012, Department of Chemistry, Villanova University, **Assistant Professor**, 2005-2011, Department of Chemistry, Villanova University, **Post-doctoral Fellow**, 2002-2005, Ames Laboratory, Iowa State University.

**Research:** Dr. Dudley's research involves the synthesis, characterization, and computational analysis of benzimidazole compounds, as well as computational studies of complexes of transition metals with nitrogen-containing ligands.

#### **Anthony Schroeder, Math, Science, and Technology Department, University of Minnesota Crookston**

**Education:** **B.S.**, 2006, Biology, University of North Dakota; **Ph.D.**, 2012, Biology, University of North Dakota.

**Employment:** **Assistant Professor**, 2015-present, Math, Science, and Technology Department, University of Minnesota-Crookston; **Post-Doctoral Research Associate**, 2013-2015, University of Minnesota in Cooperative Agreement with U.S. Environmental Protection Agency.

**Research:** Dr. Schroeder's research has focused on 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.

### **Organization Descriptions**

The University of Minnesota Crookston (UMC) is an institution of higher education that focuses primarily on educating undergraduate students. UMC is part of the University of Minnesota System and predominantly serves rural northern MN and eastern ND. UMC has made impressive strides in the last ten years in hiring tenure track faculty with research agendas. As part of the University system, faculty have the resources (instrumental and library) of the Twin Cities campus for their use.

UMC has provided over 3,000 square feet of laboratory space to the Principal Investigators (PI). The PIs applied for and received funding (> \$750,000) over several years, from multiple funding sources, including LCCMR. In 2018, UMC has opened its first Center for Collaborative Research. The proposed research is especially suitable to be conducted at UMC due to the existing programs and inter-departmental collaborations. In addition, UMC is known for its programs in Natural Resources, and there is a high number of those students interested in the proposed research. The University investigators will manage pieces of the project while supervising undergraduate students.