**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**

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| **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.

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| **Outcome** | | **Completion Date** |
| 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 |
| **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.

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| **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.

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| **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 bedisseminated 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.