



Environment and Natural Resources Trust Fund (ENRTF) M.L. 2017 LCCMR Work Plan

Date of Submission: September 14, 2016

Date of Next Status Update Report: February 1, 2018

Date of Work Plan Approval:

Project Completion Date: June 30, 2020

Does this submission include an amendment request? No

PROJECT TITLE: 031-A: Minnesota's Freshwater Sponges: Mapping Taxonomy and Environmental Toxicology

Project Manager: Venugopal Mukku

Organization: University of Minnesota Crookston

Mailing Address: 2900 University Ave

City/State/Zip Code: Crookston, MN, 56716

Telephone Number: (281) 281-2342

Email Address: mukku002@crk.umn.edu

Web Address: www.crk.umn.edu

Location: Statewide

Total ENRTF Project Budget:

ENRTF Appropriation: \$258,000

Amount Spent: \$0

Balance: \$258,000

Legal Citation: M.L. 2017, Chp. xx, Sec. xx, Subd. xx

Appropriation Language:

I. PROJECT TITLE: Minnesota’s Freshwater Sponges: Mapping Taxonomy and Environmental Toxicology

II. 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 (Cedar River, Des Moines River, Lake Superior, Lower Mississippi River, Minnesota River, Missouri River, Rainy River, Red River of the North, St. Croix River, Upper Mississippi River Basins) 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 undergraduate students with varying academic backgrounds.

III. OVERALL PROJECT STATUS UPDATES:

Project Status as of February 1, 2018:

Project Status as of August 1, 2018:

Project Status as of February 1, 2019:

Project Status as of August 1, 2019:

Project Status as of February 1, 2020:

Overall Project Outcomes and Results:

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Collection and taxonomic identification of freshwater sponges sampled from the ten major basins and watersheds in Minnesota

Description:

Freshwater sponges will be collected from two lakes and or rivers from each of the ten watersheds during the summer months (June to October) of 2017 and 2018. To provide information about freshwater habitat selection in Minnesota waters, sponges will be collected at depths ranging from 0.15 to 12 meters by two methods: 1) wading into shallow water and examining appropriate substrate (e.g., rocks, sunken trees); 2) SCUBA diving into deeper areas and examining appropriate substrate. For each sampling location the GIS coordinates, data pertaining to the body of water, the physical, chemical, and biological conditions will be obtained. ArcGIS will be used to map the physical location with various conditions where the sponges were found. These data will be used to provide the first map of where freshwater sponges are located in Minnesota.

Collected sponges from each location will be separated for genetic and morphological analyses and for chemical analysis. A sub-sample of sponge will be placed in 70% ethanol, and stored at -20°C until used for genetic and morphological analyses (Activity 1). The remaining wet sponge material will be freeze-dried, dry weight of each sponge specimen recorded and used for chemical analysis (Activity 2).

A sub-sample of sponge (less than 1 cm³) will be digested in boiling concentrated nitric acid (2 mL) for 10-15 minutes in order to retain only spicules and gemmules. The digested samples will be rinsed and prepared for initial light microscopy analysis as well as subsequent scanning electron microscope analysis. Species will be distinguished by identifying morphotraits which include examination of megascleres, microscleres, and gemmular theca.

Another sub-sample of sponge will be used for molecular identification. Nuclear and mitochondrial DNA will be extracted using commercially available extraction kits. Polymerase chain reaction (PCR) will be used with primers previously designed to amplify 18s rDNA and cytochrome oxidase I (COI) using cycling protocols previously published for each primer set. Amplified nuclear and mitochondrial DNA for each sponge collected will be sequenced using BigDye™ Terminator v3.1 and an ABI PRISM™ 3100 Genetic Analyzer to obtain nucleotide sequences. Nucleotide sequences will be subjected to a BLASTN search using the National Center for Biotechnology Information (NCBI) database. BLASTN results will be used to identify the species of the collected sponges.

The data obtained from sequencing the collected sponges will be used for determining phylogenetic relationships to identify potentially new freshwater sponge species. Other DNA sequences for COI and 18s rDNA from other freshwater sponge species that have been deposited in NCBI's GenBank will be extracted. Sequences for each gene will be aligned using BioEdit v7.2 software. Aligned sequences for each gene will be used for phylogenetic analysis using maximum parsimony implemented in PAUP* v4.0 and Bayesian inference implemented in MrBayes v3.2. For the Bayesian inference, ModelTest v3.7 will be used to determine the best of fit of the data for phylogenetic analyses. When these analyses are complete we will be able to further document which species of sponges are present in Minnesota's lakes and rivers and potentially identify new species of freshwater sponges.

Summary Budget Information for Activity 1:

ENRTF Budget: \$ 119,000
Amount Spent: \$ 0
Balance: \$ 119,000

Outcome	Completion Date
1. Collection of sponges from two lakes/ivers within each major basin/watershed in MN	October 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 1 Status as of February 1, 2018:

Activity 1 Status as of August 1, 2018:

Activity 1 Status as of February 1, 2019:

Activity 1 Status as of August 1, 2019:

Activity 1 Status as of August 1, 2019:

Activity 1 Status as of February 1, 2020:

Final Report Summary:

ACTIVITY 2: Detection of contaminants present in collected freshwater sponges

Description:

Collected sponges will be freeze-dried and organic material will be extracted with suitable solvents such as hexane, dichloromethane and methanol. Chemical standards will be used for the identification and quantification of contaminants. The presence of PAHs, PCBs, and other emerging contaminants such as pharmaceuticals, pesticides, and estrogenic compounds in the sponge will be determined using EPA protocols when available. For example, the Environmental Protection Agency (EPA) has a number of protocols for qualitative and quantitative determination of PAHs. We will adapt EPA method 550.1 which is used to determine PAHs in drinking water using HPLC and EPA method 525.2 (Revision 2.0) using GC-MS. In preliminary studies, 14 of the 16 compound mixture of PAHs bought from Sigma-Aldrich were clearly separated using a reverse phase Zorbax C₁₈ column (250 X 4.6 mm) with an acetonitrile-water gradient at a flow rate of 2.0 mL/min. The compounds were detected at 254 nm using a diode-array detector. A similar analysis will be performed for inorganic contaminants.

Summary Budget Information for Activity 2:

ENRTF Budget: \$ 89,000
 Amount Spent: \$ 0
 Balance: \$ 89,000

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

Activity 2 Status as of February 1, 2018:

Activity 2 Status as of August 1, 2018:

Activity 2 Status as of February 1, 2019:

Activity 2 Status as of August 1, 2019:

Activity 2 Status as of February 1, 2020:

Final Report Summary:

ACTIVITY 3: Training undergraduate students in interdisciplinary research and dissemination of research findings to the appropriate stakeholders

Description:

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.

Summary Budget Information for Activity 3:

ENRTF Budget: \$50,000
 Amount Spent: \$ 0
 Balance: \$ 50,000

Outcome	Completion Date
1. <i>10-12 Students will be trained in proper field collection and laboratory procedures</i>	June 2019
2. <i>6-8 Students and 2-3 faculty will disseminate research findings to potentially interested state (MPCA, MN DNR) agencies and scientific conferences</i>	June 2020

Activity 3 Status as of February 1, 2018:

Activity 3 Status as of August 1, 2018:

Activity 3 Status as of February 1, 2019:

Activity 3 Status as of August 1, 2019:

Activity 3 Status as of February 1, 2020:

Final Report Summary:

V. DISSEMINATION:

Description: The results of the work outlined in this proposal will be disseminated in a number of formats and venues. Since undergraduate students will be heavily involved in this work, they will be presenting their results via poster and oral presentations at any number of local, regional, and national conferences and symposia. UMC hosts an undergraduate research day each year in which students discuss the research projects they work on to their peers and other faculty. Recently, UMC students have presented their research findings at both regional and national meetings (e.g., American Chemical Society, National Council of Undergraduate Research). Similarly, UMC faculty have been presenting at many of these and other conferences (e.g., Society of Environmental Toxicology and Chemistry). We plan to continue our attendance at these conferences in order to disseminate our findings for this project. One new set of venues we anticipate disseminating our result to are various state agencies (e.g., DNR, MPCA) that may be interested in our work.

Besides dissemination of results, we also anticipate informing the public of our work on this project through various media outlets. The Minnesota Conservation Volunteer has already expressed interest in covering our preliminary work on this project. UMC Public Relations often does stories on students and faculty doing research and those stories appear in local newspapers and the hometown newspapers of the students involved. As a land-grant institution UMC is obligated to engage in public outreach and education, thus we expect that this project will be utilized by our institution to achieve its land-grant mission.

While the scientific merit of this project has been thoroughly outlined, the educational impact of this work is just as important to UMC and its students. Having students engaged in meaningful research is known to help in student retention at both the academic institution and in STEM fields. Thus, we plan on incorporating this project into a number of our biology and chemistry courses to give as many of our students as possible the opportunity to engage in original research. We have already started this process by incorporating some of the methods outlined in this project into our first-year experience (called Nature of Life) designed to get students introduced to research-based methods in the biological sciences. Students during this experience go to the University of Minnesota's Biological station for a weekend field-trip composed of laboratory and field experiences. This academic year, we chose to involve the students in some simple analyses of sponges that were collected in a region near the park. We intend to expand on the work these students performed in the Nature of Life course in a number of other courses: Zoology, Cell Biology, Genetics, Organic Chemistry, and Analytical Chemistry. The

goal is to expose all of our students majoring in biological sciences to some original research with the hopes that a few of them will engage in more involved research projects.

Status as of February 1, 2018:

Status as of August 1, 2018:

Status as of February 1, 2019:

Status as of August 1, 2019:

Status as of February 1, 2020:

Final Report Summary:

VI. PROJECT BUDGET SUMMARY:

A. Preliminary ENRTF Budget Overview:

Budget Category	\$ Amount	Overview Explanation
Personnel:	\$152,000	Project manager 1 month salary each year (\$29,000); project partner 1 month salary first two years, 0.5 month salary third year (\$23,000); project partner 0.5 month salary the first year, 1 month salary the last two years (\$26,000); three undergraduate students full time in each summer (\$40,455); 4 undergraduate students averaging 7.5 hours per week during each academic year (\$33,120)
Professional/Technical/Service Contracts:	\$50,000	1 technical contract, selected through competitive bidding, for sponge collections and preliminary storage from the 10 watersheds and basins during years 1 and 2. Costs include salaries/wages of the technician collecting the samples, SCUBA equipment rental for the technician performing the collections, and supplies for sponge storage.
Equipment/Tools/Supplies:	\$40,000	Supplies for extraction, amplification, and sequencing of DNA from ~100 sponge samples (Per sponge sample - \$20 for DNA extraction, \$40 for amplification, and \$100 for sequencing reactions = \$16,000 total); Supplies for chemical analysis (columns, solvents and buffers, analytical standards, UV-visible quartz tubes; \$12,000 total); LC/MS and GC/MS Instrument access at the Center for Mass Spectrometry and

		Proteomics at the UMNTC to perform chemical analyses (~120 samples at \$100 = \$12,000 total)
Capital Expenditures over \$5,000:	\$10,000	Lyophilizer (Freeze-dryer from Fisher Scientific - Labconco™ 7752040) - needed to remove water from sponge samples in order to perform extraction of organic substances.
Travel Expenses in MN:	\$4,000	Mileage for PIs to travel to sampling sites to oversee collection of sponges and perform preliminary chemical analysis of sample sites.
Other: Shipping	\$2,000	Shipping costs to send samples to UMNTC for DNA sequencing and chemical analysis.
TOTAL ENRTF BUDGET:	\$258,000	

Explanation of Use of Classified Staff: N/A

Explanation of Capital Expenditures Greater Than \$5,000: One lyophilizer (freeze-dryer) will be purchased (\$10,000; Labconco™ model no. 7752040) for removal of water from sponge samples in order to extract organic substances. Upon the completion of this project, the instrument will continue to be used by the University of Minnesota Crookston for similar projects and purposes. Currently, there is no such instrument available on campus nor at surrounding colleges or universities. Other groups who need such instrumentation will be able to utilize the equipment without charge. If the instrument is sold prior to the end of its useful life, proceeds from the sale will be paid back to the Environment and Natural Resources Trust Fund.

Total Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: 6.0 FTE

Total Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation: 2 FTE

B. Other Funds:

Source of Funds	\$ Amount Proposed	\$ Amount Spent	Use of Other Funds
Non-state			
In-Kind Support	\$ 134,160	\$	Indirect costs (waived)
State			
	\$	\$	
TOTAL OTHER FUNDS:	\$ 134,160	\$	

VII. PROJECT STRATEGY:

A. Project Partners:

Partners receiving ENRTF funding

- Venugopal Mukku, (Associate Professor), Anthony Schroeder (Assistant Professor), Timothy Dudley (Assistant Professor), University of Minnesota Crookston: \$208,000 to extract and quantify contaminants from the collected sponges, perform morphological and molecular identification of collected of all freshwater sponges and integrate this research into the undergraduate programs at UMC. All University of Minnesota Crookston faculty receiving funding will also be supervising students.

- **Technical support to be contracted (Competitive bid)**, \$50,000 to technical staff for sponge collections and preliminary storage from the 10 watersheds and basins during years 1 and 2. Costs include salaries/wages of the technician collecting the samples, SCUBA equipment rental for the technician performing the collections, and supplies for sponge storage. (Activity 1; Outcome 1). A qualified SCUBA diver needs to be contracted because the students and investigators are not allowed to perform this due to safety concerns of SCUBA diving and liability issues associated with the University of Minnesota.

Partners NOT receiving ENRTF funding: N/A

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. This research is especially important because there is no current data regarding these essential aquatic organisms. Consequently, the potential of sponges to uptake pollutants results in bioaccumulation of pollutants which would be transferred to gamefish and ultimately humans. Results will be disseminated through scientific presentations, peer-reviewed publications, and presented to interested state agencies. This project will provide opportunities for Minnesota students to receive training in relevant field and laboratory protocols, thereby ensuring a well-prepared science workforce.

C. Funding History:

Funding Source and Use of Funds	Funding Timeframe	\$ Amount
Service contract to Anthony Schroeder funded through the ENRTF unrelated to the current proposal (053-B Eliminating Contaminants to Protect Endangered Native Fish/Mussels). Funds used to develop molecular and physiological endpoints in fish and mussels	7/01/2016 - 6/30/2019	\$ 33,000
Vice Chancellor of Academic Affairs, University of Minnesota Crookston – Interdisciplinary grant to Anthony Schroeder and Timothy Dudley. Funds were used to perform initial collections and obtain preliminary data related to freshwater sponges in Minnesota	7/1/2016 – 6/30/2017	\$ 20,000

VIII. REPORTING REQUIREMENTS:

- The project is for 3 years, will begin on 07/01/17, and end on 06/30/20.
- Periodic project status update reports will be submitted February 1 and August 1 of each year.
- A final report and associated products will be submitted between June 30 and August 15, 2020.

IX. VISUAL COMPONENT or MAP(S): See attached graphic.

X. FEE TITLE ACQUISITION/CONSERVATION EASEMENT/RESTORATION REQUIREMENTS:

A. Parcel List: N/A

B. Acquisition/Restoration Information: N/A

**Environment and Natural Resources Trust Fund
M.L. 2017 Project Budget**



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

Legal Citation:

Project Manager: Venugopal Mukku

Organization: University of Minnesota Crookston

M.L. 2017 ENRTF Appropriation: \$258,000

Project Length and Completion Date: 3 Years, June 30, 2020

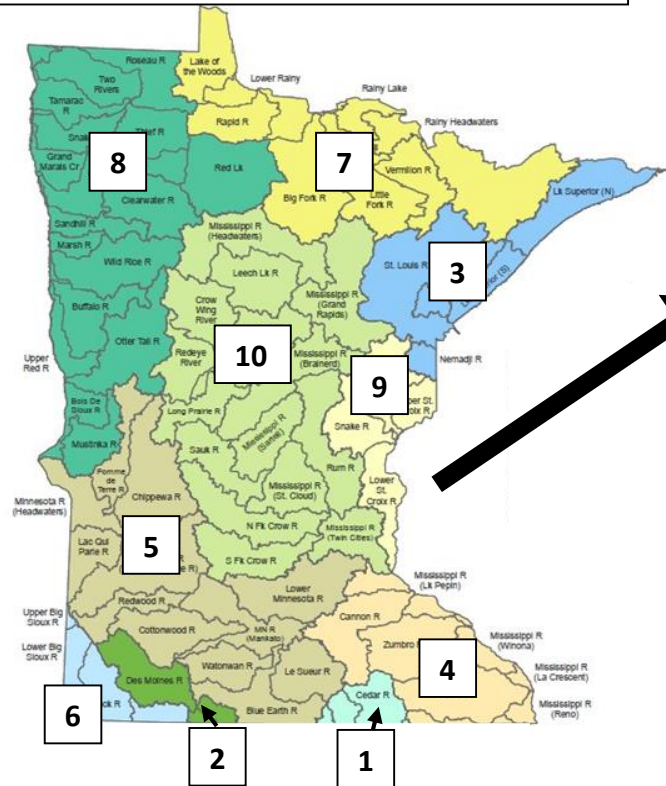
Date of Report: September 14, 2016

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Activity 1 Budget	Amount Spent	Activity 1 Balance	Activity 2 Budget	Amount Spent	Activity 2 Balance	Activity 3 Budget	Amount Spent	Activity 3 Balance	TOTAL BUDGET	TOTAL BALANCE
BUDGET ITEM	<i>Collection and taxonomic identification of freshwater sponges sampled from the ten major basins and watersheds in Minnesota</i>			<i>Detection of contaminants present in collected freshwater sponges</i>			<i>Training undergraduate students in interdisciplinary research and dissemination of research findings to the appropriate stakeholders</i>				
Personnel (Wages and Benefits)	\$48,000	\$0	\$48,000	\$54,000	\$0	\$54,000	\$50,000	\$0	\$50,000	\$152,000	\$152,000
Venugopal Mukku, Project Manager: \$29,000 (66% salary, 34% benefits); 11% FTE each year.											
Anthony Schroeder, Senior Personnel: \$23,000 (66% salary, 34% benefits); 11% FTE first 2 years, 5.5% FTE third year.											
Timothy Dudley, Senior Personnel: \$26,000 (66% salary, 34% benefits); 5.5% FTE first year, 11% FTE for last 2 years.											
4 Undergraduate Research Assistants during academic year: \$ (92.1% salary, 7.9% benefits). 2 students at 25 FTE (10 hours/week) each year; 2 students at 12.5 FTE (5 hours each week) each year											
3 Undergraduate Research Assistants during each summer: \$ (92.1% salary, 7.9% benefits). 3 at 100% FTE (3 months of summer) each year											
Professional/Technical/Service Contracts	\$50,000	\$0	\$50,000							\$50,000	\$50,000
TBD (competitive bid): Contract in years 1 and 2 to perform sponge collections by scuba diving in 2 lakes or rivers in the 10 watersheds or basins in Minnesota											
Equipment/Tools/Supplies											
DNA extraction kits to isolate DNA from collected sponges (~100 samples/\$20 per sample)	\$2,000	\$0	\$2,000							\$2,000	\$2,000
Reagents and supplies to perform DNA sequencing reactions (primers, Big Dye reagent, tubes) (~100 samples/\$40 per sample)	\$4,000	\$0	\$4,000							\$4,000	\$4,000

DNA sequencing performed by the Genomics Center, UMNTC) for sponge identification (~100 sponge samples/4 sequencing reactions per sample at \$25 per reaction)	\$10,000	\$0	\$10,000						\$10,000	\$10,000
General chromatographic and spectrophotometric supplies (columns, solvents and buffers, analytical standards, UV-visible quartz tubes)				\$12,000	\$0	\$12,000			\$12,000	\$12,000
LC/MS and GC/MS instrument access. These analyses will be performed off-site (Center for Mass Spectrometry and Proteomics, UMNTC) and will be used to identify and quantify contaminants in the sponge samples (~120 samples/\$100 per sample)				\$12,000	\$0	\$12,000			\$12,000	\$12,000
Capital Expenditures Over \$5,000										
Lyophilizer (Freeze-dryer from Fisher Scientific -Labconco™ 7752040) - needed to remove water from sponge samples in order to perform extraction of organic substances				\$10,000	\$0	\$10,000			\$10,000	\$10,000
Travel expenses in Minnesota										
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 of the 10 watersheds and basins (10 round trips year 1 at a cost of \$2000, 10 round trips year 2 at a cost of \$2000) originating from UMC (Crookston).	\$4,000	\$0	\$4,000						\$4,000	\$4,000
Other										
Shipping costs to send samples for DNA sequencing (400 sequencing reactions) and chemical analyses at UMNTC (120 samples)	\$1,000	\$0	\$1,000	\$1,000	\$0	\$1,000			\$2,000	\$2,000
COLUMN TOTAL	\$119,000	\$0	\$119,000	\$89,000	\$0	\$89,000	\$50,000	\$0	\$50,000	\$258,000

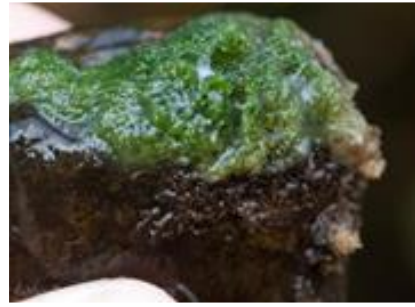
Project Title: Minnesota's Freshwater Sponges: Mapping Taxonomy and Environmental Toxicology

Basins and Major Watersheds in Minnesota

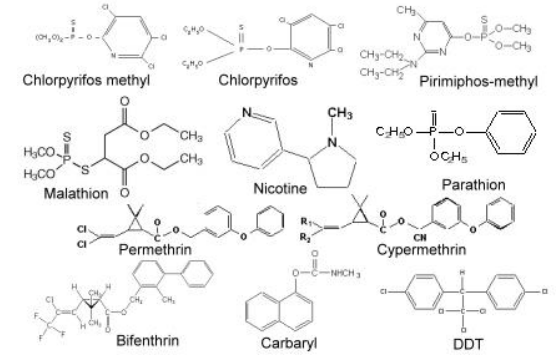


- 1: Cedar River
- 2: Des Moines River
- 3: Lake Superior
- 4: Lower Mississippi River
- 5: Minnesota River
- 6: Missouri River
- 7: Rainy River
- 8: Red River of the North
- 9: St. Croix River
- 10: Upper Mississippi River

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

