Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2015 Work Plan

Date of Report: October 10, 2014

Date of Next Status Update Report: January 1, 2016

Date of Work Plan Approval:

Project Completion Date: June 30, 2018

Does this submission include an amendment request? _No_

PROJECT TITLE: Biological Consequences of Septic Pollution in Minnesota Lakes

Project Manager: Heiko L. Schoenfuss

Organization: St. Cloud State University

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Location: Statewide

Total ENRTF Project Budget:	**ENRTF Appropriation:**	**$364,000**
	Amount Spent:	**$0**
	Balance:	**$364,000**

Legal Citation: M.L. 2015, Chp. 76, Sec. 2, Subd. 04c

Appropriation Language:
$364,000 the first year is from the trust fund to the Board of Trustees of the Minnesota State Colleges and Universities System for Saint Cloud State University to assess the presence and possible sources of contaminants of emerging concern in Minnesota lakes in order to determine their effects on fish health, understand the potential contribution from septic systems, and inform options for remediation and prevention to protect Minnesota lakes from these contaminants in the future. This appropriation is available until June 30, 2018, by which time the project must be completed and final products delivered.

I. PROJECT TITLE: Biological Consequences of Septic Pollution in Minnesota Lakes

II. PROJECT STATEMENT:

Contaminants of Emerging Concerns (CECs), which include hormones, pharmaceuticals and compounds found in personal care products, have the potential to harm lake fish populations. We will link the established presence of CECs in Minnesota Lakes to observed biological effects in lake fish and provide the foundation needed for subsequent remediation efforts. The ENRTF has been instrumental in establishing that CECs are present and cause biological effects in Minnesota rivers and lakes. Riverine research has progressed to identifying sources and assessing the feasibility of remediation. However, for Minnesota lakes, only the presence has been established: the actual **sources of CECs** to the lakes have yet to be conclusively elucidated. **Understanding the sources of CECs is a crucial and necessary step to remedying the problem of CECs in Minnesota lakes.** Lake environments respond to pollution very differently than rivers as the sources of pollution are usually more diffuse (non-point sources) and as the long residence time of lake water (months to years) prevents the dilution of incoming pollution often observed in riverine environment. As a consequence, biological effects already observed in riverine fish exposed to CECs are likely more pronounced in lake fish and are more difficult to assess. In many lakes, obvious point sources of pollution are usually lacking.

Among potential CEC sources to Minnesota Lakes, onsite septic systems stand out for three reasons: they are commonly used around Minnesota lakes; they are not designed to remove CECs from household waste water; and CEC composition and concentrations measured in Minnesota Lakes in previous studies had distinct human "signatures" with many compounds present that are usually only associated with human household consumption (for example mood altering drugs, fragrances). The goals of this project are, therefore, to (i) validate the presence and biological effects of CECs in representative lake-types in Minnesota; (ii) identify a broad suite of approximately 50 CECs and their currently unknown metabolites that are contributing to the observed biological effects, and (iii) and conduct detailed analysis of potential sources with particular focus on onsite septic systems. The outcome of the proposed study is the identification of specific sources of CECs to Minnesota lakes and the documentation of the hydrologic pathways that result in discharge to lakes. As part of this approach, we will examine traditional and advanced (aerated) onsite septic system to assess their potential to treat CECs more effectively than is the case with traditional septic systems. This knowledge will aid natural resource managers in water conservation districts, watershed associations, and county zoning offices in identifying sources of CECs in their aquatic resources and will provide the information needed for water treatment specialists to assess potential remediation and/or preventative actions.

III. OVERALL PROJECT STATUS UPDATES:

Project Status as of January 1, 2016:

Project Status as of July 1, 2016:

Project Status as of January 1, 2017:

Project Status as of July 1, 2017:

Project Status as of January 1, 2018:

Overall Project Outcomes and Results:

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Integrated Surface and Ground Water Sampling for CECs

Description: To identify sources of CECs in Minnesota Lakes, both surface and ground water needs to be sampled continuously and across seasons in lakes with varying infrastructure characteristics. The USGS has developed a passive sampling technology that allows for continuous sampling of surface and ground water for a month at a time. This technology will be used to explore the continuous input of CECs through ground water and surface water runoff in four Minnesota lakes (with known concentrations of CECs). Lakes and lake sites will be chosen to encompass different discharge pathways (surface water runoff; sewered vs. onsite septic systems) and septic technologies (traditional systems, advanced aeration). In addition, a preliminary sample of lakes will be assessed in summer 2016 for logistical feasibility of water and fish sampling.

- We will assess four lakes and examine in detail two septic-impacted and two reference sites in each lake (total of 16 sampling sites for the study).
- Passive sampling devices (allowing for the continuous sampling for four weeks at a time) will be installed at two sites in each lake.
- Approximately 128 environmental samples (plus quality assurance/quality control samples) will be screened using inexpensive high-throughput assay technology and augmented with detailed analytical chemistry (Activity 2) when warranted by assay results.
- Due to the potential high temporal variability in water quality of some potential CEC sources (i.e., septic tank leachate), detailed temporal grab samples will also be collected to verify the validity of the passive sampling approach (Activity 2).

These results will inform the biological effects testing (Activity 3).

Summary Budget Information for Activity 1:

ENRTF Budget: $ 125,000
Amount Spent: $ 0
Balance: $ 125,000

Outcome	Completion Date
1. Identify 4 lakes and appropriate study sites and establish sampling devices in lakes	June 30, 2016
2. Sample ground and surface water for eight week periods	December 31, 2016
3. Quantify CEC concentrations in seasonal ground water from 4 lakes using screening methods for model CECs such as estrogens, detergents and pharmaceuticals	June 30, 2017
4. Quantify CEC concentrations in seasonal surface water from 4 lakes using screening methods for model CECs such as estrogens, detergents and pharmaceuticals	December 31, 2017

Activity Status as of January 1, 2016:

Activity Status as of July 1, 2016

Activity Status as of January 1, 2017

Activity Status as of July 1, 2017

Activity Status as of January 1, 2018

Final Report Summary:

ACTIVITY 2: Analysis of samples for CECs

Description: Identifying the sources of CECs to Minnesota lakes will require looking for both CECs that are expected to be released to surface waters (i.e., from storm water runoff) and CECs that might leach from onsite septic systems (i.e., pharmaceuticals and personal care products). State of the art liquid chromatography tandem mass spectrometry will be employed for known CEC analysis of selected samples (Activity 1). CECs to be quantified will include approximately 50 known or suspected CECs from all compound classes. These include pharmaceuticals (for example carbamazepine, diazepam, acetaminophen, sulfamethoxazole, etc.), antimicrobial agents (triclosan, triclocarban, etc.), ingredients in personal care products (DEET), detergents (nonylphenol, octylphenol, etc.), flame retardants (TCEP, TCPP) and steroid hormones (estradiol, testosterone, progesterone, etc.). In addition, these samples will be screened for non-target compounds using high throughput LC-time-of-flight MS, which will enable the identification of a broad range of contaminants. This non-target screening is essential, as many known CECs can be biologically or chemically transformed in the environment to often unknown transformation products that retain substantial biological activity, and may confound the biological assays (Activity 3).

Summary Budget Information for Activity 2:

ENRTF Budget: $ 142,125
Amount Spent: $ 0
Balance: $ 142,125

Outcome	Completion Date
1. Develop and validate sampling and extraction protocols	June 30, 2016
2. Accurate quantitation of approximately 50 CECs in passive sampler extracts and grab samples	June 30, 2017
3. Screening of grab samples and extracts for non-target analytes such as metabolites of pharmaceuticals and personal care products	June 30, 2018

Activity Status as of January 1, 2016:

Activity Status as of July 1, 2016

Activity Status as of January 1, 2017

Activity Status as of July 1, 2017

Activity Status as of January 1, 2018

Final Report Summary:

ACTIVITY 3: Analysis of Fish for Effects and Causal Linkage to CEC Exposure

Description: CECs are diverse in their presence, concentrations and chemical nature. To establish a causal relationship between CECs measured through chemical analysis and biological effects observed in resident fish, two assumptions have to be tested: (i) fish in lakes with CEC occurrence present pathologies consistent with CEC exposure and (ii) laboratory reared fish exposed to the mixture of CECs measured in surface and ground water will develop similar pathologies. To test these assumption we will:

- We will collect fish from 16 sampling sites in the four study lakes and assess for a comprehensive range of pathological indications consistent with exposure to CECs.

- We will collect approximately 100 egg clutches from the 16 lake study sites to quantify fecundity and health of embryos and juveniles and use the information in population models established as part of previous ENRTF funded research.
- We will expose eggs in the laboratory to ground water from all 16 field sites and expose larvae and adults of both species to four mixtures of CECs based on analytical findings from Activities 1 & 2 and assess similar endpoints.

Taken together, these field and laboratory studies will provide the information needed to establish a causal link between septic system discharge and endocrine disruption in lake fish if such a linkage exists.

Summary Budget Information for Activity 3:

ENRTF Budget: $ 96,875
Amount Spent: $ 0
Balance: $ 96,875

Outcome	Completion Date
1. Identify four spawning habitats (two septic influenced, two reference) in each of the four study lakes	June 30, 2016
2. Collect and analyze larvae and adults of fathead minnows and sunfish from four sites in each of the four study lakes (Activity 1) for CEC exposure effects	June 30, 2017
3. Expose egg clutches and adult fathead minnows and sunfish to CEC mixtures representing lake exposure conditions (Activity 2)	December 31, 2017
4. Establish causality between lake CEC exposure and biological effects through laboratory fish exposures based on measured environmental concentrations (Activity 2)	June 30, 2018

Activity Status as of January 1, 2016:

Activity Status as of July 1, 2016

Activity Status as of January 1, 2017

Activity Status as of July 1, 2017

Activity Status as of January 1, 2018

Final Report Summary:

V. DISSEMINATION:

Description: The target audience for results from this research will be professionals in the areas of wastewater treatment and natural resource management. Specific targets will be environmental engineers and scientists in academia, industry, state agencies such as the DNR and MPCA, and environmental consultants. Results will be disseminated through scholarly publications in peer-reviewed journals such as *Environmental Science and Technology*. Results from the research project will also be presented at regional conferences such as the annual meeting of the *Midwest Chapter of the Society for Environmental Toxicology & Chemistry (SETAC)* and the *Minnesota Water* conference and if possible, at targeted seminars at the DNR and MPCA. Results will be used to determine which whether advanced aeration septic systems provide additional ecological protection.

Status as of January 1, 2016:

Status as of July 1, 2016

Status as of January 1, 2017

Status as of July 1, 2017

Status as of January 1, 2018

Final Report Summary:

VI. PROJECT BUDGET SUMMARY:
A. ENRTF Budget Overview:

Budget Category	$ Amount	Overview Explanation
Personnel:	$ 76,047	**Heiko Schoenfuss, PI (SCSU)** ($20,180 salary, $7239 fringe, 26% fringe rate; $27,419 total for 3 years; 10% effort per year). **One Graduate Research Assistant (SCSU)** ($27,300 salary, $21,328 fringe including tuition; $48,628 total for two years; 50% effort each year)
Professional/Technical/Service Contracts:	$267,125	**Subcontract USGS (Mounds View, MN)** The subcontract amount will include characterization of groundwater flow into lakes; acquisition and deployment of groundwater and surface water passive sampling devices ($89,800), and bi-weekly sample collection and analysis of water samples for common contaminants of emerging concern using ELISA kits ($35,200). The subcontract costs include salary and benefits for a Hydrologist for 3 years at 12% effort ($53,800) and salary and benefits for Co-PI Kiesling for three years at 6% effort ($34,700). **Subcontract Colorado School of Mines** The subcontract amount will include salary and benefits for 50% of a postdoctoral fellow (annual average of $23,412 salary and $9,839 benefits per year for 3 years) and one day of summer salary for Co-PI Higgins ($524 annual average in salary and $237 in benefits per year for three years). In addition, supplies for experiments and measurements (chemicals, analysis time, etc.) will be $13,397 (on average) per year for three years. No travel funds are requested.
Equipment/Tools/Supplies:	$18,600	**Equipment/Tools/Supplies (SCSU)** Fish acquisition and maintenance ($1,000/year for 3 years), gene expression assays ($2,500/year for

		3 years), histopathology ($1,200/year for 3 years), exposure experiment setup and execution ($1,500/year for 3 years).
Travel Expenses in MN:	$2,228	Travel to field sites located on four Minnesota lakes for 3 consecutive summers (1,500 miles/year @ $0.495/mile for three years).
TOTAL ENRTF BUDGET:	**$364,000**	

Explanation of Use of Classified Staff: N/A

Explanation of Capital Expenditures Greater Than $5,000: N/A

Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: 1.3 FTEs

Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation: 2.04

B. Other Funds:

Source of Funds	$ Amount Proposed	$ Amount Spent	Use of Other Funds
Non-state			
USGS Cooperative Water Program Match Funding	$54,000	$54,000	Support during project period
State			
	$0	$0	
TOTAL OTHER FUNDS:	**$54,000**	**$54,000**	

VII. PROJECT STRATEGY:

A. Project Partners:

The project team consists of the Principal Investigator (PI) Heiko Schoenfuss (St. Cloud State University) and co-PIs Richard W. Kiesling (USGS, Mounds View, MN) and Christopher Higgins (Colorado School of Mines). Dr. Heiko L. Schoenfuss will guide the biological impact research and organize the entire project effort. Dr. Richard Kiesling is a USGS Hydrologist and Limnologist who will guide the lake sampling and characterization effort.

Dr. Chris Higgins will lead the analytical characterization of selected samples. The proposed study requires a specific blend of advanced instrumentation and expertise. It is the unique blend of analytical chemical instrumentation and experience with onsite wastewater treatment systems that can only be brought to this study by CSM and Dr. Higgins. The CSM lead, Associate Professor Christopher P. Higgins, has a significant track record of publications in which his laboratory has measured wastewater-derived organic contaminants such as CECs that may result in endocrine disruption activity. Moreover, several of these publications have specifically examined the occurrence and removal of CECs in onsite wastewater systems. Dr. Higgins' environmental chemistry analytical laboratory is one of the best equipped in the nation. Dr. Higgins has two liquid chromatography tandem mass spectrometry (LC-MS/MS) systems (ABSCIEX 3200 and ABSCIEX 3200 QTrap) for use in the targeted analysis of compounds. Moreover, Dr. Higgins will also use an ABSCIEX 5600+ quadropole time of flight (TripleTOF) MS system for the analysis of "known unknowns" and "unknown unknowns." Dr. Higgins' substantial expertise in targeted analysis of CECs will be supplemented by an on-going collaboration with ABSCIEX to further the use of non-targeted screening of chemicals (i.e., the "unknown unknowns). Further justification for Dr. Higgins collaboration on this project has been provided in a separate attachment.

B. Project Impact and Long-term Strategy:

The proposed research fits into a larger research agenda centered at St. Cloud State University and the USGS focused on contaminants of emerging concern and protection of lake ecosystems. We have previously determined that fish exposed to estrogens (a known class of potent CECs) in small pond-like settings will delay spawning which may have detrimental effects on fish populations (ML 2009, Chp. 142, Sec. 2, Subd. 5b). These effects were found to be of environmental relevance when we assessed in the context of estrogen concentrations in point-source (municipal treatment plants and industrial discharge) (M.L. 2010, Chp. 362, Sec. 2, Subd. 5c). Furthermore, in addition to point-source discharge, our recent studies also determined that estrogenic compounds are found in lake habitats near onsite septic systems (M.L. 2010, Chp. 362, Sec. 2, Subd. 5e). These findings, mostly related to the potent estrogens associated with human and animal excretions lead to a recently funded proposal to assess how already scheduled changes in wastewater treatment technology to reduce effluent nitrogen loads may further benefit the environment through reduction in estrogens (M.L. 2014, Chp. 226, Sec. 2, Subd. 03d). The current proposal builds on these findings and other information in the published literature to examine how non-point sources such as septic systems affect fish reproducing in the areas of lakes most impacted by septic seepage. The addition of Dr. Higgins at the Colorado School of Mines to this project will vastly expand the analytical component of this study to encompass all major groups of known and suspected CECs and to include their metabolites. This capability is critical as it is becoming increasingly apparent that the overall adverse biological effect observed in lake fish (for example intersex) cannot be the result solely of estrogenic exposure. The proposed research, therefore, builds upon and complements current and prior research in this area. When taken together, this research will provide a more complete picture of how to assess the environmental impact of onsite septic system, improve treatment, and safeguard our fish populations.

C. Funding History:

Funding Source and Use of Funds	Funding Timeframe	$ Amount
ML 2009, Chp. 142, Sec. 2, Subd. 5b "Vulnerability of Lakes to Endocrine Disruption"	July 1, 2009 - June 30, 2013	$297,000
M.L. 2010, Chp. 362, Sec. 2, Subd. 5c "Ecological Impacts of Effluent in Surface Waters and Fish"	July 1, 2010 – June 30, 2014	$340,000
M.L. 2010, Chp. 362, Sec. 2, Subd. 5e "Assessing Septic System Discharge to Lakes"	July 1, 2010 – June 30, 2014	$594,500
M.L. 2014, Chp. 226, Sec. 2, Subd. 03d "Cost-benefit Analysis of Wastewater Treatment and Fish Abundance" (Novak, PI)	July 1, 2014 – June 30, 2017	$500,000

VIII. FEE TITLE ACQUISITION/CONSERVATION EASEMENT/RESTORATION REQUIREMENTS: N/A

IX. VISUAL COMPONENT or MAP(S): attached

X. RESEARCH ADDENDUM: See attached Research Addendum subject to peer review by the U.S. Geological Survey

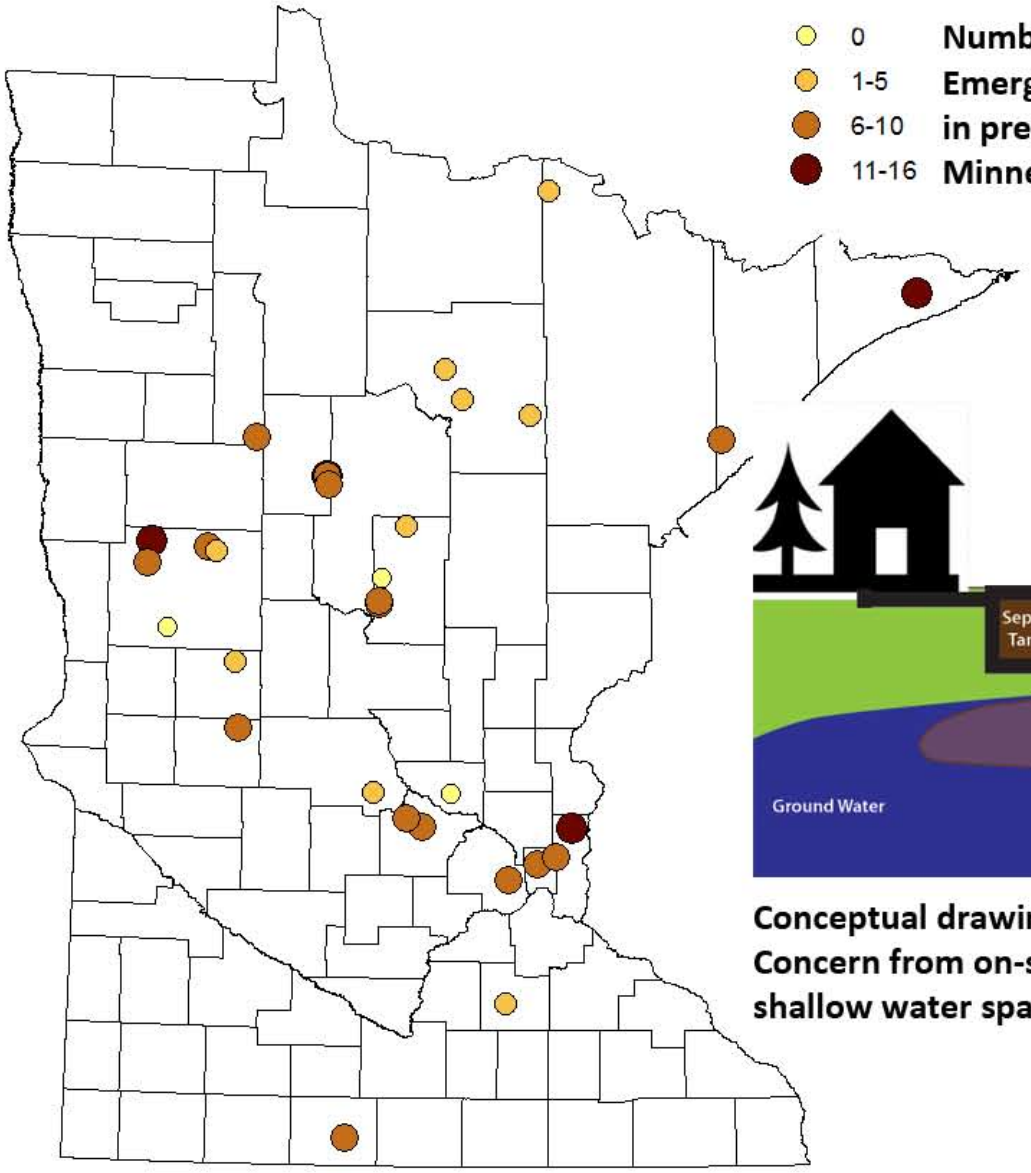
XI. REPORTING REQUIREMENTS:

Periodic work plan status update reports will be submitted no later than January 1, 2016; July 1, 2016; January 1, 2017; July 1, 2017; January 1, 2018. A final report and associated products will be submitted between June 30 and August 15, 2018.

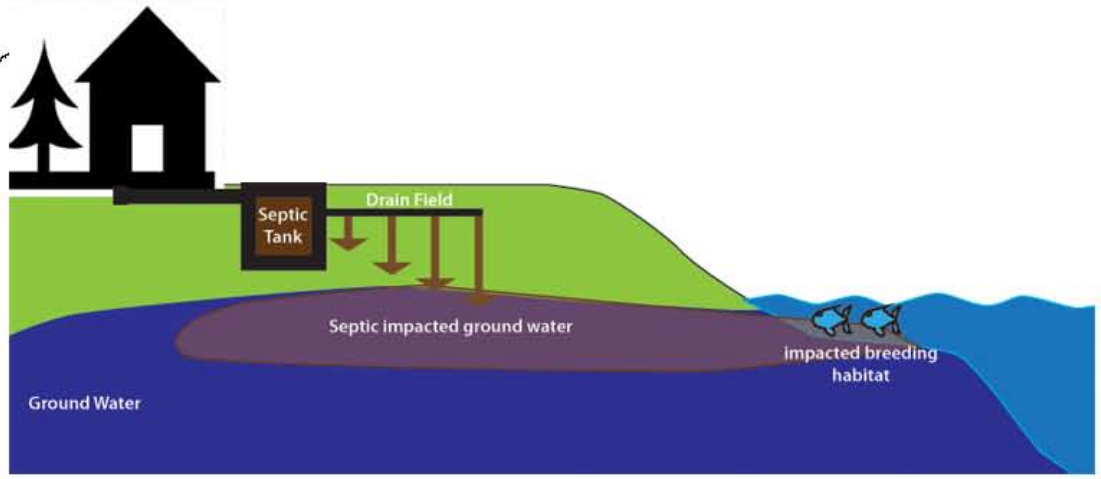
Environment and Natural Resources Trust Fund
M.L. 2015 Project Budget

Project Title: Biological Consequences of Septic Pollution in Minnesota Lakes
Legal Citation:
Project Manager: *Heiko L. Schoenfuss*
Organization: St. Cloud State University
M.L. 2015 ENRTF Appropriation: $364,000
Project Length and Completion Date: 3 years, June 30, 2018
Date of Report: 10/10/2014

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	**Integrate surface and groundwater sampling for CECs**			**Analysis of samples for known and unknown CECs**			**Determining the causal linkage between CEC exposure and biological effects observed in lake fishes.**				
Overall Personnel (Wages and Benefits)							$76,047	$0	$76,047	$76,047	$76,047
Heiko Schoenfuss, PI (SCSU) ($20,180 salary, $7239 fringe, 26% fringe rate; $27,419 total for 3 years; 10% effort per year)											
One Graduate Research Assistant (SCSU) ($27,300 salary, $21,328 fringe including tuition; $ 48,628 total for two years; 50% effort each year)											
Professional/Technical/Service Contracts											
Subcontract USGS (Mounds View, MN) The subcontract amount will include characterization of groundwater flow into lakes; acquisition and deployment of groundwater and surface water passive sampling devices ($89,800), and bi-weekly sample collection and analysis of water samples for common contaminants of emerging concern using ELISA kits ($35,200). The subcontract costs include salary and benefits for a Hydrologist for 3 years at 12% effort ($53,800) and salary and benefits for Co-PI Kiesling for three years at 6% effort ($34,700).	$125,000	$0	$125,000								
Subcontract Colorado School of Mines The subcontract amount will include salary and benefits for 50% of a postdoctoral fellow (annual average of $23,412 salary and $9,839 benefits per year for 3 years) and one day of summer salary for Co-PI Higgins ($524 annual average in salary and $237 in benefits per year for three years). In addition, supplies for experiments and measurements (chemicals, analysis time, etc.) will be $13,397 (on average) per year for three years. No travel funds are requested.				$142,125	$0	$142,125				$142,125	$142,125
Equipment/Tools/Supplies											
Fish acquisition and maintenance ($1,000/year for 3 years), gene expression assays ($2,500/year for 3 years), histopathology ($1,200/year for 3 years), exposure experiment setup and execution ($1,500/year for 3 years).							$18,600	$0	$18,600	$18,600	$18,600
Travel expenses in Minnesota											
Travel to field sites located on four Minnesota lakes for 3 consecutive summers (2,500 miles/year @ $0.495/mile for three years).							$2,228	$0	$2,228	$2,228	$2,228
COLUMN TOTAL	**$125,000**	**$0**	**$125,000**	**$142,125**	**$0**	**$142,125**	**$96,875**	**$0**	**$96,875**	**$364,000**	**$364,000**



0 Number of Contaminants of
1-5 Emerging Concerns detected
6-10 in previously sampled
11-16 Minnesota Lakes



Conceptual drawing on the movement of Contaminants of Emerging Concern from on-site septic systems into Minnesota Lakes. Note that shallow water spawning habitats are most impacted by the septic flow.