Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2016 Work Plan

Date of Report: May 29, 2016

Date of Next Status Update Report: February 1, 2017

Date of Work Plan Approval: June 7, 2016

Project Completion Date: June 30, 2019

Does this submission include an amendment request? No

PROJECT TITLE: Assessing Techniques for Eliminating Contaminants to Protect Native Fish and Mussels

Project Manager: Kristine Wammer

Organization: University of St. Thomas

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Location: Statewide

Total ENRTF Project Budget:		
	ENRTF Appropriation:	**$287,000**
	Amount Spent:	**$0**
	Balance:	**$287,000**

Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 04d

Appropriation Language:

$287,000 the second year is from the trust fund to the commissioner of natural resources for an agreement with the University of St. Thomas to evaluate the use of ultraviolet treatment of wastewater to remove certain commonly detected wastewater contaminants, in order to reduce the contaminants' toxicity to native fish and mussels. This appropriation is available until June 30, 2019, by which time the project must be completed and final products delivered.

I. PROJECT TITLE: Assessing Technique for Eliminating Contaminants to Protect Native Fish and Mussels

II. PROJECT STATEMENT:

In 2009 the MPCA was directed by the legislature to monitor surface waters for endocrine disrupting compounds (EDCs) in the vicinity of at least 20 wastewater treatment plants (WWTPs); in the resultant study tonalide and galaxolide were detected in 84% and 96% of effluent samples respectively, as well as in many sediments downstream from WWTPs (30-60%). This project will determine whether (a) UV disinfection would effectively remove these contaminants prior to discharge into surface waters and (b) whether the products formed when the contaminants break down would still be of concern.

Although tonalide and galaxolide are among the most commonly detected contaminants of emerging concern (CECs) in Minnesota WWTP effluents, the effects of these high production volume chemicals and their byproducts on the quality of Minnesota drinking water and aquatic life remain largely unknown. Municipalities in various locations are considering costly modifications of existing wastewater treatment processes to enhance removal of such CECs to protect surface waters, many of which serve as sources of drinking water, without adequate understanding of whether such treatments are effective and/or necessary. UV treatment is commonly considered because it can be used simultaneously to improve chemical removal and disinfect wastewater.

Both contaminants to be studied have worldwide production volumes of over 6,000 tons per year and account for 90% of the US market for polycyclic musks, which are used as synthetic fragrances in a wide range of products. Musks can impair transporters involved in the first line of defense against toxicants, known as MXR/PXR defenses. These transporters are involved in substrate translocation across membranes and mediate cellular efflux of a variety of organic chemicals. If detoxification ability is impaired, organisms cannot effectively eliminate other toxic chemicals found in MN waters - this has been demonstrated in mussels. This is of great concern as 25 of Minnesota's 48 native mussel species are listed as endangered, threatened, or of special concern. Tonalide and galaxolide are also known to induce other types of toxicity (e.g. liver damage, DNA/genetic damage) and are suspected EDCs, meaning they can disrupt hormones and impair growth and reproduction, and are thus a potential threat to mussel and fish populations.

This project will assess whether UV treatment of wastewater will effectively remove toxicity attributable to these common wastewater contaminants, including assessing toxicity of products formed during UV treatment. UV treatment can be effective at reducing tonalide concentrations in effluent, but galaxolide is tougher to break down. It is of particular concern that most galaxolide degradation products have been classified as very persistent and/or toxic. Therefore, there is an urgent need to further our understanding of what is formed when these contaminants are broken down by UV light as it is very possible these UV products could also be an important unknown source of toxicity for endangered native mussels and fish in MN waters. This work will provide valuable insight into the ability of UV treatment to mitigate contribution of these contaminants to toxicity of wastewaters, in addition to identifying contaminants and products of particular interest for monitoring and further study and enabling municipalities to make better informed decisions about the need for treatment upgrades.

III. OVERALL PROJECT STATUS UPDATES:

Project Status as of February 1, 2017:

Project Status as of August 1, 2017:

Project Status as of February 1, 2018:

Project Status as of August 1, 2018:

Project Status as of February 1, 2019:

Overall Project Outcomes and Results:

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Quantify removal of contaminants by UV treatment and measure toxicity and endocrine disrupting activity

Description:
Tonalide and galaxolide photolysis rates, quantum yields and extent of removal with UV treatment will be quantified in the laboratory to enable estimation of transformation efficiency during wastewater treatment. Mixtures of UV degradation products will be generated for toxicity testing, with a focus on adverse effects on fish and mussels. In situations where chemicals and their photoproducts are present in complex mixtures, biological analyses that can quantify total toxicological activity without knowledge of specific chemical composition can be used to streamline identification of chemicals/fractions responsible for the observed biological activity. The following battery of *in vitro* assays will be used to determine whether UV exposure can reduce/eliminate toxicity of galaxolide and tonalide by screening parent compounds and photoproduct mixtures/fractions:

- *Detoxification assays – test for impairment of organism's ability to eliminate contaminants*
    - If MXR toxicity is detected, we will conduct native fish and mussel tests to determine whether exposure to musks/their UV products can increase toxicity of common contaminants that normally co-occur in WWTP effluents with musks.
- *Endocrine toxicity assays - test for disruption of reproductive hormones (e.g., testosterone, estrogen)*
    - If endocrine cell toxicity is detected, we will conduct 48 h exposure of adult fathead minnows (an excellent model for MN natives) to the musks/photoproducts of interest and evaluate effects on expression of genes involved in endocrine function
- *General toxicity assays* – a series of widely recognized tests indicative of toxicity used for human and ecological hazard evaluation will be measured. Parent compounds and their photoproducts will be analyzed for approximately 90 different toxicity endpoints (including carcinogenesis, DNA damage, endocrine disruption, neurotoxicity etc.) using commercially available, cutting-edge techniques where living cells/proteins are exposed to water samples and screened for changes in biological activity that are indicative of toxic effects. If resources allow, other toxic pathways of interest (especially those that are initiated at environmentally relevant concentrations) indicated by general toxicity assays will be evaluated.

If biological assays suggest that some of these UV degradation products are toxic they will be further characterized primarily via liquid chromatography coupled with Time-of-Flight mass spectrometry; see Activity 2 for a more detailed description of the analytical methods. If possible, active products will be isolated or, if available, purchased for individual compound toxicity testing.

Summary Budget Information for Activity 1:

ENRTF Budget: $ 200,000
Amount Spent: $ 0
Balance: $ 200,000

Outcome	Completion Date
1. Measure photolysis rates and quantum yields of tonalide and galaxolide under UV light.	December 2017
2. Perform biological screening tests and, where appropriate, follow-up fish and mussel studies to determine if UV treatment can minimize toxicity to native fish and mussels.	January 2019
3. Identify toxic products formed during UV treatment	June 2019

Activity Status as of February 1, 2017:

Activity Status as of August 1, 2017:

Activity Status as of February 1, 2018:

Activity Status as of August 1, 2018:

Activity Status as of February 1, 2019:

Final Report Summary:

ACTIVITY 2: Quantify contaminants and their UV products in municipal wastewater

Description:
Effluents from wastewater treatment plants with and without UV disinfection will be analyzed for the presence of the two target contaminants and the products formed when they undergo degradation by UV light. Professor Stoll's laboratory at Gustavus is equipped with state-of-the-art equipment that enables: 1) separation of a complex sample such as WWTP effluent and 2) identification of unknown compounds and quantitation of compounds of interest. This instrumentation will be especially useful to this project because it enables the identification of most products formed after UV treatment, and the measurement of very low concentrations of target compounds and products (akin to finding the 'needle in the haystack'). The Stoll group has extensive experience developing analyses for complex matrices.

In the first phase aimed at detection of the target musks and their known UV photoproducts, we will use online Solid-Phase Extraction (SPE) coupled with two-dimensional high performance liquid chromatography (2D-LC) with mass spectrometric detection. The high resolving power of 2D-LC will be especially valuable to the quantitation of photoproducts for which stable isotope labeled internal standards are not available, because of the mitigation of matrix effects that results from higher resolution of the sample constituents. This approach will also be used in the final phase of the work aimed at quantitation of photoproducts in WWTP effluent that have demonstrated endocrine activity or toxicity in the course of this project.

For the identification of additional UV photoproducts in WWTP effluent we will use comprehensive 2D-LC coupled with Time-of-Flight mass spectrometry to establish putative identities of the photoproducts using accurate mass measurements. These identities will be verified through retention time matching between authentic standards and the peaks observed in the mixture of transformation products.

Summary Budget Information for Activity 2:

ENRTF Budget: $ 87,000
Amount Spent: $ 0
Balance: $ 87,000

Outcome	Completion Date
1. Determine concentrations of target compounds and known UV products in at least six WWTP effluents and WWTP-impacted sites at least twice per year. Sites will be chosen to overlap significantly with those being studied in existing LCCMR-funded mussel-related work (e.g. Minnesota River basin, St. Croix River); the PI of the other study (Kozarek) will share sampling plans and field site locations.	June 2017
2. Identify additional UV degradation products in WWTP effluents based on major products observed during laboratory UV treatment studies.	June 2018
3. Measure concentrations of products determined to have endocrine activity or toxicity based on work carried out during this project.	June 2019

Activity Status as of February 1, 2017:

Activity Status as of August 1, 2017:

Activity Status as of February 1, 2018:

Activity Status as of August 1, 2018:

Activity Status as of February 1, 2019:

Final Report Summary:

V. DISSEMINATION:
Description: The results of this study will be disseminated through oral and poster presentations by the students and faculty involved in the project, briefings to the LCCMR as requested, and peer-reviewed publication. We also intend to present progress on this project periodically to relevant personnel working on related ENRTF projects who have been made aware of this project and may be interested in the results.

Status as of February 1, 2017:

Status as of August 1, 2017:

Status as of February 1, 2018:

Status as of August 1, 2018:

Status as of February 1, 2019:

Final Report Summary:

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

Budget Category	$ Amount	Overview Explanation
Personnel:	$ 92,986	Project manager 1 month of salary first two years, 0.5 month third year ($20,866); project partner 1 month of salary each year ($28,003); two undergraduate students full time in summer and an average of 7.5 hours per week during the academic year ($44,117).
Professional/Technical/Service Contracts:	$ 120,000	1 contract with Gustavus Adolphus College for assistance with identification of products and measurements of concentrations in WWTP effluents ($87,000); 1 contract with University of Minnesota Crookston for method development related to molecular/physiological endpoints for fish and mussel studies ($33,000).
Equipment/Tools/Supplies:	$ 61,429	Sample prep supplies ($3,500), cells and supplies ($4,400), general toxicity tests ($27,000), supplies for in vivo assays ($6,500), supplies for mussel assays ($4,600), general supplies for biological studies ($2,800), fish and mussels and supplies ($2,500), photolysis and

		chromatography supplies ($9,769), shipping costs ($360).
Capital Expenditures over $5,000:	$ 11,085	1 LuzChem UV photoreactor instrument.
Travel Expenses in MN:	$ 1,500	Mileage for obtaining WWTP effluent samples.
TOTAL ENRTF BUDGET:	**$ 287,000**	

Explanation of Use of Classified Staff: N/A

Explanation of Capital Expenditures Greater Than $5,000: One LuzChem UV photoreactor is being purchased and will continue to be used by the University of St. Thomas for the life of the instrument for similar projects and purposes. 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.

Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: 2.5

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

B. Other Funds:

Source of Funds	$ Amount Proposed	$ Amount Spent	Use of Other Funds
Non-state			
In-Kind Support	$ 71,200	$	Indirect costs (waived)
TOTAL OTHER FUNDS:	**$ 71,200**	**$**	

VII. PROJECT STRATEGY:

A. Project Partners:

Project partners receiving funds:

- Kristine Wammer and Dalma Martinovic-Weigelt, University of St. Thomas: $167,000 to measure UV photolysis rates, generate product mixtures for activity assay testing, isolate suspected active products (Wammer) and to lead work on toxicity assays and fish and mussel studies (Martinovic-Weigelt) (Activity 1).
- Anthony Schroeder, University of Minnesota – Crookston: $33,000 to develop molecular/physiological endpoints for fish and mussel studies (Activity 1).
- Dwight Stoll, Gustavus Adolphus College: $87,000 to assist with identification of products (Activity 1) and measure concentrations in WWTP effluents (Activity 2).

All project partners will supervise students.

B. Project Impact and Long-term Strategy: In addition to disseminating our work through peer-reviewed scientific publications and presentations, we will communicate and work with the PI of an existing ENRTF mussel study and MPCA personnel involved in WWTP effluent survey work as appropriate during the project. If warranted by our findings, we will collaborate with WWTPs statewide to introduce appropriate UV technologies that will facilitate removal of the toxic contaminants and be protective of fish and mussel health.

C. Funding History: N/A

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

A. Parcel List: N/A

B. Acquisition/Restoration Information: N/A

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

X. RESEARCH ADDENDUM: See attached Research Addendum.

XI. REPORTING REQUIREMENTS:
Periodic work plan status update reports will be submitted no later than February 1, 2017; August 1, 2017; February 1, 2018; August 1, 2018; and February 1, 2019. A final report and associated products will be submitted between June 30 and August 15, 2019.

Environment and Natural Resources Trust Fund
M.L. 2016 Project Budget

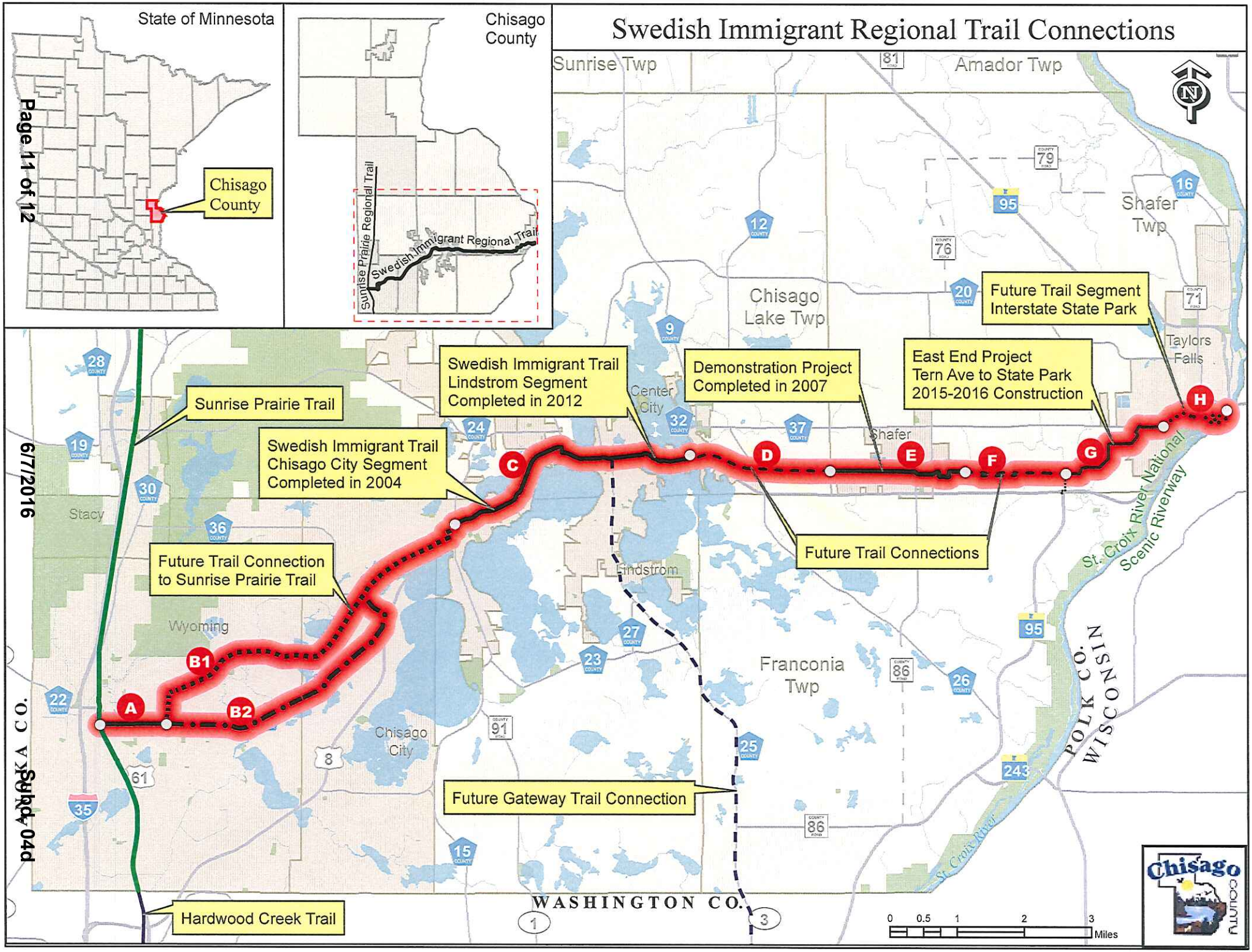
Project Title: Assessing Techniques for Eliminating Contaminants to Protect Native Fish and Mussels
Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Sub. 04d
Project Manager: Kristine H. Wammer
Organization: University of St. Thomas
M.L. 2016 ENRTF Appropriation: $287,000
Project Length and Completion Date: 3 Years, June 30, 2019
Date of Report: May 29, 2016

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Activity 1 Budget	Amount Spent	Activity 1 Balance	Activity 2 Budget	Amount Spent	Activity 2 Balance	TOTAL BUDGET	TOTAL BALANCE
BUDGET ITEM	**Quantify removal of contaminants by UV treatment and measure toxicity and endocrine disrupting activity**			**Quantify contaminants and their UV products in municipal wastewater**				
Personnel (Wages and Benefits)	$92,986	$0	$92,986				$92,986	$92,986
Kris Wammer, Project Manager: 1 month of salary first 2 years, 0.5 month third year (432 hours total, .2 FTE total). Supervise students, coordinate project, compile and file reports, disseminate results. 93% salary, 7% fringe ($20,866).								
Dalma Martinovc-Weigelt, Project Partner: 1 month of salary each year (516 hours total, 0.25 FTE total). Supervise students, assist with coordinating project, disseminating results. 93% salary, 7% fringe ($28,003).								
Undergraduate students. 2 students during the academic year (one 5 hours per week for 32 weeks, one 10 hours per week for 32 weeks), 2 students each summer 40 hours per week for 11 weeks (4080 hours total, 2.0 FTE total). 95% salary, 5% fringe benefits ($44,117).								
Professional/Technical/Service Contracts								
Gustavus Adolphus College				$87,000	$0	$87,000	$87,000	$87,000

Dwight Stoll, Project Partner. 0.5 month of salary per year for first two years, 0.33 month salary for third year (229 hours total, 0.1 FTE total). Supervise Gustavus undergraduate students and reseach technician. 86% salary, 14% fringe benefits ($10,084).								
Research technician 10 hours per week in years 1 and 2, 7 hours per week in year 3 (1404 hours total, 0.7 FTE total). 75% salary, 25% fringe benefits ($42,100).								
Undergraduate students. 1 student during the first two summers (40 hours per week for 10 weeks) (80% salary, 12% housing, 8% fringe benefits). 1 student during the academic year, 8 hours per week for 15 weeks per semester, (100% salary) (1520 hours total, 0.7 FTE total). ($15,283)								
General lab supplies, e.g. solvents, vials, analytical standards ($7,483).								
LC/MS instrument access ($11,000).								
Travel (meetings with other groups, some sampling) ($1,050).								
University of Minnesota – Crookston	$33,000	$0	$33,000				$33,000	$33,000
Anthony Schroeder, Project Partner. 0.5 month of salary per year for first two years, 1 month of salary for third year (344 hours total, 0.2 FTE total). Supervise undergraduate student. 100% salary ($13,333).								
Undergraduate students. One student during the first and second academic years, 8 hours per week for 16 weeks per semester. One student during the first summer, 40 hours per week for 10 weeks. (912 hours total, 0.4 student FTE total). ($9476)								
Lab supplies, e.g. disposable plastics, primers, Sybr green mastermix, RNA extraction kits, enzyme assays, hormone assays ($10,091).								
Equipment/Tools/Supplies	$61,429	$0	$61,429				$61,429	$61,429
Sample filtration, extraction and preparation for all analyses - 20 samples @ $175/sample ($3,500).								
Cells/supplies, media, standards for endocrine in vitro asessments - 20 samples @ $220/sample ($4,400).								

90 general toxicity tests - parent/UV degradation compounds - 10 samples/ $30/sample/test ($27,000)								
Reagent supplies for in vivo molecular/physiological assessment (enzyme/hormone/ gene assays) - 45 ($6,500).								
Reagents and disposables for mussel MXR defense assays and nutrient chemistry ($4,600).								
Miscellaneous lab supplies (pipette tips, culture plates, tubing, sterile syringes/containers, assay plates ($2,800).								
Fish and mussels, holding supplies, and food ($2,500).								
General photolysis and chromatography supplies (e.g. columns, quartz tubes, reagents, solvents) ($9,769).								
Shipping costs to send samples between institutions for analysis ($360).								
Capital Expenditures Over $5,000	$11,085	$0	$11,085				$11,085	$11,085
LuzChem UV photoreactor instrument								
Travel expenses in Minnesota	$1,500	$0	$1,500				$1,500	$1,500
Mileage for obtaining WWTP effluent samples (6 treatment plants, at least twice per year; most sites in MN River Basin or St. Croix River)								
COLUMN TOTAL	**$200,000**	**$0**	**$200,000**	**$87,000**	**$0**	**$87,000**	**$287,000**	**$287,000**

Swedish Immigrant Regional Trail Connections



6/7/2016

Subd. 04d