

**Environment and Natural Resources Trust Fund  
2014 Request for Proposals (RFP)**

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**Project Title:**

**ENRTF ID: 039-B**

Pharmaceuticals and Nitrogen Interact to Harm MN Fish/Ecosystems?

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**Category:** B. Water Resources

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**Total Project Budget:** \$ 269,000

**Proposed Project Time Period for the Funding Requested:** 3 Years, July 2014 - June 2017

**Summary:**

Determine whether pharmaceuticals (alone/combined with environmental stressor nitrogen-nitrate) widespread in Minnesotas waters impact fish health and microbial processes that regulate nutrient cycling and water quality in rivers and lakes.

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**Name:** Dalma Martinovic

**Sponsoring Organization:** St. Thomas University

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**Location**

**Region:** Statewide

**County Name:** McLeod, St. Louis, Stearns

**City / Township:**

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



**PROJECT TITLE: Pharmaceuticals and nitrogen interact to harm MN fish/ecosystems?**

**I. PROJECT STATEMENT**

Current understanding of environmental pharmaceuticals in MN indicates that they are widespread in our state’s lakes and rivers, and they could disrupt fish health. Unfortunately, much of existing pharmaceutical toxicity research is irrelevant to MN risk assessors because it uses much higher levels of pharmaceuticals than are found in MN waters; thus it remains unknown whether levels of pharmaceuticals found in MN pose threat to wild fish. Of further concern is that pharmaceuticals often co-occur with other environmental stressors (especially nitrates) that could exacerbate their negative effects. Exposure to certain pharmaceuticals or nitrates alone can impair fish reproduction, and their combination could have even more harmful effects. In addition to this direct potential interaction between pharmaceuticals and nitrates on fish health, there is also the possibility of an indirect interaction, if exposure to pharmaceuticals alters the microbial processes of nitrogen-fixation and denitrification, thereby altering nitrogen levels in the aquatic ecosystem. Our study aims to fill these knowledge gaps in order to facilitate development and implementation of standards for nitrates and chemicals of emerging concern by MN agencies.

**Our goals are** to (1) measure the direct interaction between four common pharmaceutical compounds and nitrate on fish health (physiology, behavior, and reproduction), and (2) measure effects of these pharmaceutical compounds on microbial processes that alter nitrate levels in rivers and lakes.

This study will focus on four pharmaceutical compounds that are commonly found in MN surface waters at very high concentrations, in combination with nitrates (Bupropion, Carbamazepine, Venlafaxine & Caffeine; see 2011 report to MN Legislature for concentrations; lrp-ei-1sy11). We will combine field measurements and laboratory analyses, focusing on sites located above and below four wastewater treatment plants (important source of nitrates and pharmaceuticals) in different eco-regions of MN. Targeted field sites are: St. Louis River/Lake Superior, Duluth; Lake Shagawa, Ely; Sauk River, Sauk Centre; Crow River, Hutchinson). Previous studies at these sites show that these rivers contain high levels of above mentioned pharmaceuticals and nitrates, and that fish reproduction may be adversely impacted by pharmaceuticals (reported in lrp-ei-1sy11).

Nitrate and pharmaceutical pollution are connected issues of great importance to multiple MN agencies (MPCA, MN DNR, MDH) that are interested in advancing development or implementation of surface and drinking water quality standards and/or identification of the causes of changes in fish health. Understanding relative importance of nitrate vs. pharmaceutical-driven effects, and their interactions could make standard development more germane to aquatic environments where chemicals occur in mixtures with other stressors, and would help prioritize regulatory actions for these chemicals.

**II. DESCRIPTION OF PROJECT ACTIVITIES**

**Activity 1: Field survey of pharmaceuticals and nutrients**

**Budget: \$95,551**

Investigate seasonal patterns (Fall/Winter, Spring, Summer) of occurrence of pharmaceuticals (Bupropion, Carbamazepine, Venlafaxine, Caffeine), nitrogen forms (nitrate, ammonia, nitrite), and baseline rates of nitrogen fixation at five sites above and five below WWTPs at St. Louis River/Lake Superior, Lake Shagawa, Sauk River, Crow River. Measure biological, neuroendocrine activities of the water samples to determine what portion of total biological effect can be ascribed to the pharmaceuticals we investigated.

Outcomes	Completion Date
1. Complete analyses and disseminate data on spatial and seasonal patterns of occurrence of pharmaceuticals and nitrogen stressors for four watersheds representative of MN habitats.	June 30 2015
2. Provide entities involved in water quality standard development and risk assessment (e.g., MPCA, MDH) with exposure data for pharmaceuticals and nitrates.	June 30 2015



**Activity 2: Lab study of biological effects of pharmaceuticals, nutrients and their mixtures Budget: \$119,047**

- Determine whether/how Bupropion, Carbamazepine, Venlafaxine, Caffeine, and Nitrates impact fish health (physiology, behavior & reproduction) using fathead minnow, a regulatory toxicology fish model.
- Determine whether addition of nitrate to pharmaceutical mixtures exacerbates effects of the pharmaceuticals on fish health.
- Determine concentrations of pharmaceuticals that affect the addition of nitrogen (through nitrogen fixation) and the removal of nitrogen (through denitrification) by microbes.

Outcomes	Completion Date
1. Determine effects of environmentally relevant concentrations of pharmaceuticals on fish health and microbial processes alone and in context of co-occurring environmental stressor nitrate.	Jun 30 2016
2. Provide entities involved in water quality standard development, risk assessment, and fish and nutrient management (e.g., MPCA, MDH, DNR) with biological effects data (low effect and no effect concentrations) for pharmaceuticals, nitrates and their mixtures.	Jun 30 2016

**Activity 3: Field study of biological effects of pharmaceuticals and nutrients Budget: \$54,715**

Evaluate significance of laboratory findings by integrating those with the field exposure and effects data. Return to the field to determine whether the comparable effects on fish and microbes are observed there, and whether these effects can be ascribed to these pharmaceuticals.

Outcomes	Completion Date
1. Compare thresholds of pharmaceutical effects on fish and microbes from lab experiments with measured concentrations in Minnesota waters.	Jun 30 2017
2. Evaluate biological significance of measured effects from pharmaceuticals, by comparing biological effects from laboratory experiments with spatial and temporal variation in these traits and processes measured at these sites.	Jun 30 2017

**III. PROJECT STRATEGY**

**A. Project Team/Partners**

This project will be supervised by Drs. Dalma Martinovic – ecotoxicologist/fisheries scientist (U St. Thomas), with collaborators Dr. Gaston Small ecosystem ecologist (U St. Thomas), and Dr. Heiko Schoenfuss (St. Cloud State U).

**B. Timeline Requirements**

Field sampling will begin the summer of 2014 and will be completed in the spring of 2017. Final reports will be issued July 2017 and project scientists will begin disseminating results.

**C. Long-Term Strategy and Future Funding Needs**

This project fulfills need to “Increase understanding of effects of contaminants on natural resources and develop strategies for reducing contamination” identified in LCCMR’s 2009 Six-Year Strategic Plan. Our findings will be integrated with data for other pollutants found in MN (e.g., estrogens) and used as basis for seeking additional funds from other entities to expand this research to additional stressors (e.g., hypoxia) and pharmaceuticals.

## 2014 Detailed Project Budget

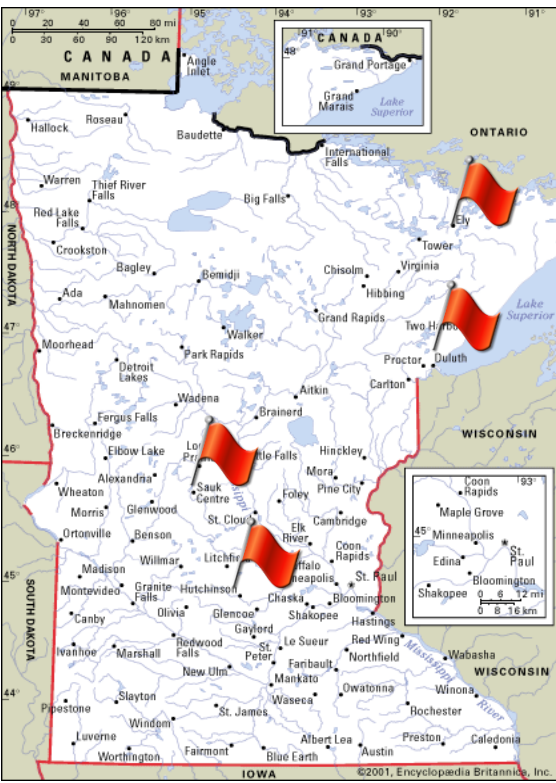
**Project Title: Pharmaceuticals and nitrogen interact to harm MN fish/ecosystems?**

### IV. TOTAL ENRTF REQUEST BUDGET [3] years

<u>BUDGET ITEM</u>	<u>AMOUNT</u>
<b>Personnel:</b> University of St. Thomas <span style="float: right;"><i>Dalma</i></span> <i>Martinovic, Principal Investigator (PI)</i> , Duties: Coordinate project, generate chemical, molecular, physiological measurements, compile reports and disseminate results. 1 month salary (8.33% effort) per yr (totaling \$22,650 for 3 yrs) plus 7.65% fringe (\$1,733/3 years). <i>Gaston Small, Co-PI</i> , Duties: Generate lab and field microbial data and reports. 1 month salary (8.33% effort) per yr, (\$20,949 for 3 yrs), 7.65% fringe (\$1,603 for 3 yrs). <i>Undergraduate student research assistants</i> , Duties: Assist with field sampling and lab analyses. 1 student during the academic year, 5 hrs for 32 weeks each year \$10/hr, totaling \$4,800 for three years, no fringe (0%). 1 student each summer, 40 hrs/week for 10 weeks @ \$10/hour totaling \$12,000 plus 7.65% fringe (\$918).	\$ 64,653
<b>Contracts:</b> St. Cloud State University (SCSU) - Co-PI Heiko Schoenfuss Fish lab/field exposures, reproduction assays will be conducted or supervised by <i>SCSU Co-PI Heiko Schoenfuss</i> . The subcontract will include salary for Schoenfuss (\$17,566, \$4,567 fringe, 26% fringe rate; total for three years; 5.8% effort), one graduate student for two years (\$33,800, \$2,586 fringe, 7.65% fringe rate; 12,532 tuition; total effort for two years 100%), one undergraduate student for three summers (\$12,000, \$918 fringe, 7.65% fringe rate; total effort 25% for three years), supplies for experiments (fish, chemicals, pumps, aquaria maintenance, etc., \$31,825 for three years), and travel to field sites in Minnesota (\$3,706 for three years).	\$ 119,500
<b>Equipment/Tools/Supplies:</b> Sample collection containers, filtration and extraction supplies for microbial/endocrine analyses \$10,500; Cells/supplies, media, standards for neuroendocrine in vitro assessments - \$5,100; Custom fathead minnow 15K microarrays - 7 treatments x 7 fish x \$500 = \$24,500; Supplies for molecular/physiological endpoint assessment (primers, probes, sybr mastermix, enzyme assays, hormone assays) - \$8,200; Reagents, plates, tubes for microbial assays and nutrient chemistry - \$8,000	\$ 56,300
<b>Acquisition (Fee Title or Permanent Easements):</b>	N/A
<b>Travel:</b> All travel funds for field site travel in MN. YR 1 - Lodging/Food -Sample 4 field MN sites 3 times/yr x 2 days per sampling trip x \$110 x 2 persons= \$5280; Mileage - 3 round trips to 4 MN locations (miles x \$0.56)= \$1752; YR 3 - Lodging and food - Sample 4 field MN sites 1 time/yr x 6 nights per sampling trip = 24 days x \$110 x 1 persons= \$2640; Mileage for 1 round trip to each of 4 locations @\$0.56/mile = \$584.	\$ 10,560
<b>Additional Budget Items:</b> Chemistry analyses - Analytical lab of Dr. Duane Hugget at the University of North Texas. The contract amount of \$15,800 will cover cost of 160 chemical analyses for five chemicals. Specialized software for microarray analyses (JmpGenomics academic 1 yr license)- \$1800 Courier/Mailing - Sample shipping overnight on dry ice for analyses - \$700	\$ 18,300
<b>TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND \$ REQUEST =</b>	\$ 269,313

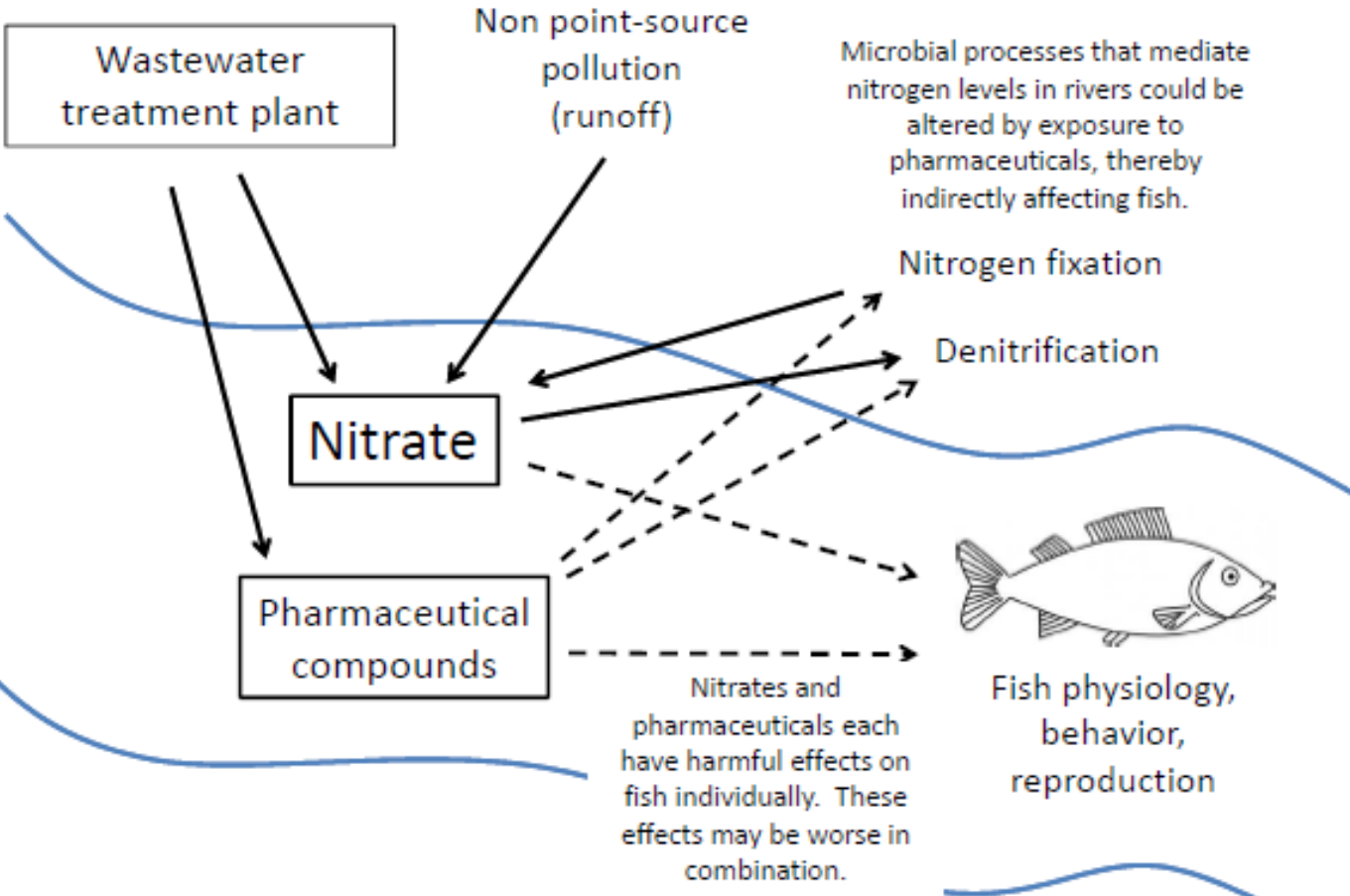
### V. OTHER FUNDS

<u>SOURCE OF FUNDS</u>	<u>AMOUNT</u>	<u>Status</u>
<b>Other Non-State \$ Being Applied to Project During Project Period:</b> Academic year salary and fringe benefits for two undergraduate students will be provided by University of Saint Thomas Each Year (10 hrs/week for 32 weeks @ \$10/hour totaling \$19,200 plus 0% fringe).	\$ 19,200	<i>Indicate: Pending</i>
<b>Other State \$ Being Applied to Project During Project Period:</b>	N/A	N/A
<b>In-kind Services During Project Period:</b>	N/A	N/A
<b>Remaining \$ from Current ENRTF Appropriation (if applicable):</b>	N/A	N/A
<b>Funding History:</b>	N/A	N/A



**Field Sites:**

1. St. Louis River/Lake Superior, Duluth
2. Lake Shagawa, Ely
3. Sauk River, Sauk Centre
4. Crow River, Hutchinson



*Solid arrows denote inputs/outputs of nitrates and pharmaceuticals. Dashed arrows denote potential effects.*

07/25/2013

## **Project Manager Qualifications and Organization Description**

**The University of St. Thomas (UST) - Dalma Martinovic, PhD** - completed Ph.D. in Fisheries Science and Water Resources at the U of MN (2005), and served as National Academies Research Associate at the U.S. Environmental Protection Agency (US EPA) from 2006-09. Dr. Martinovic is an assistant professor at UST, St. Paul, MN (2009-present). Dr. Martinovic has co-authored two reports to MN Legislature (Endocrine Disrupting Compounds, lrp-ei-1sy08; Wastewater Treatment Plant Endocrine Disrupting Chemical Monitoring Study, lrp-ei-1sy11) and circa 30 research manuscripts that assess occurrence and the effects of chemicals of emerging concern and other stressors on fish and aquatic ecosystems. Dr. Martinovic is a recipient of three *US EPA's Scientific and Technological Achievement Awards*. She has served on *The Contaminant Screening Criteria and Prioritization Development Task Group for MN Dept. of Health*, and the *Editorial Board of the Environmental Toxicology and Chemistry Journal*. Dr. Martinovic has managed several externally-funded projects at UST (ca \$300,000). Drs. Martinovic and Schoenfuss have collaborated for 10 years and have successfully completed several large-scale research projects.

**The University of St. Thomas (UST) - Gaston Small, PhD** – is an assistant professor at UST (2012-present). He is an aquatic ecologist who completed his Ph.D. at the University of Georgia in 2010, and served as a postdoctoral research associate at the U of MN studying nitrogen in the Great Lakes from 2010-2012. Dr. Small has published 13 peer-reviewed manuscripts describing the effects of nutrients on aquatic ecosystems. He currently studies nutrient transformations in the Saint Louis River Estuary, and in 2010 led a scientific working group studying carbon transformations in tropical rivers.

UST was founded in 1885 and emphasizes values-based education and career preparation, it helps solve community problems through education and service-learning programs. 56% of UST students receive need-based *scholarship or grant* aid. The largest private university in Minnesota (11,000 students, 461 full-time faculty), it offers bachelor's degrees in 85 major fields of study and 45 graduate degree programs, and is ranked as a National University. UST's Biology Department views -faculty research as essential - over the past 10 years, the faculty in Biology Department have received research grants from the NSF, NIH, USDA, USEPA, USGS, and multiple MN agencies (DNR, MPCA). Currently, the UST's Science Division has ca \$5.7 million of capital equipment, nearly half of which is owned/ maintained by Biology.

**St. Cloud State University (SCSU) - Heiko L. Schoenfuss, PhD** - Dr. Schoenfuss is a *Professor at the St. Cloud State University (SCSU)*. He received PhD in Evolutionary Biology from Louisiana State University in 1997. After three years of post-doctoral training at the U of MN, he joined the faculty at SCSU in 2001. Dr. Schoenfuss has published over 50 manuscripts detailing the effects of environmental changes on anatomical structure. He has focused for the past 15 years on the effects of endocrine active compounds (EAC) on aquatic ecosystems. He has served on the *EPA Science Advisory Board* (2008), an EAC review committee for the *National Institute for Environmental Health* (2009), and on the *MN Health Advisory Board* (2010).

SCSU is the largest of the Minnesota State Colleges and Universities system and the second largest university in Minnesota (18,300 students, 600 faculty). SCSU offers more than 200 majors, minors and pre-professional, and circa 60 graduate programs. The elements of SCSU education include community engagement, active learning, sustainability, and globalization. SCSU supports active and applied learning through the university-wide Student Research Colloquium, funding research and projects with Student Research Funds, and supporting faculty research to engage undergraduate and graduate students.