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

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Project Title:

Predicting Smallmouth Bass Exposure to Endocrine Active Compounds

Category: F. Methods to Protect, Restore, and Enhance Land, Water, and Habitat

Total Project Budget: $ 241,073

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

Other Non-State Funds: $ 0

Summary:

We will develop a model that will predict the exposure of native fish to endocrine active compounds and identify lakes and rivers where these compounds are likely to be found.

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Sponsoring Organization: U of MN - NRRI

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Location

Region: Northeast

County Name: Cook, Lake, St. Louis

City / Township:

MP: 0613-2-153-proposa
Budget: 0613-2-153-bud
Qual: 0613-2-153-qualifi
Map: 0613-2-153-map-4
Resolution:
List:

_____ Funding Priorities _____ Multiple Benefits _____ Outcomes _____ Knowledge Base

_____ Extent of Impact _____ Innovation _____ Scientific/Tech Basis _____ Urgency

Capacity Readiness Leverage Employment TOTAL

PROJECT TITLE: Predicting smallmouth bass exposure to endocrine active compounds

I. PROJECT STATEMENT

Endocrine active compounds (EACs) are manmade and natural chemicals that mimic hormones and thus can interfere with normal physiological processes. Male fish exposed to low concentrations of estrogen mimics can develop abnormal testes containing egg cells (testicular oocytes). Wild fish from many Minnesota waterways have been found with such abnormalities, which in severe cases may reduce reproductive capabilities. Although we know that major waterways receive EAC inputs from municipal wastewater effluents and numerous industrial and unregulated sources, the potential risks to organisms in most Minnesota lakes are virtually unknown because we lack sufficient tools to predict nonpoint EAC inputs. The ultimate goal of the project is to develop a model that can be used to identify lakes in which aquatic organisms are likely to be exposed to estrogenic EACs. We intend to combine computer modeling (geographic information systems, GIS), chemical analysis for selected EACs and other anthropogenic indicators, and biological analysis of EAC activity in cell cultures and in native fish to build a testable model. The specific goals of this project are to: 1) use GIS analysis of landscape factors to develop a comprehensive map showing potential EAC inputs in northeast MN; 2) measure the concentrations of selected estrogenic EACs and other indicators of anthropogenic activity and determine the total estrogenicity of water samples from selected lakes; and 3) use the results from mapping, chemical, and biological analysis to develop a predictive model of EAC activity and test it by assessing testicular oocyte biomarkers in smallmouth bass from our target lakes.

II. DESCRIPTION OF PROJECT ACTIVITIES

Activity 1: Develop cumulative map of factors that contribute EACs to lakes **Budget: $ 52,419**

Outcome	Completion Date
1. Literature review on landscape characteristics and EACs; quantify watershed factors and likely EAC sources	Dec 2014
2. Develop a map of potential EAC inputs for lakes across NE MN	April 2015

We will identify, quantify, and develop maps of watershed factors that could contribute EACs to lakes in NE MN. GIS will be used to create a comprehensive map of a gradient of inputs for lakes across NE Minnesota that categorizes lakes based on the intensity of potential EAC impact (see attached graphic).

Activity 2: Evaluate potential EAC inputs, quantify selected EACs and anthropogenic indicators, and assess total estrogenicity of water from selected NE MN lakes **Budget: $ 113,765**

Outcome	Completion Date
1. Gather and review existing data on endocrine active compounds concentrations and total estrogenicity	Jan 2015
2. Collect water from 10 lakes, ranging from likely impacted to likely unimpacted; measure anthropogenic compounds and total estrogenicity	May 2016
3. Evaluate the quantitative range of anthropogenic inputs and total estrogenicity, and determine their relationship with landscape factors	May 2016

The relationship between landscape factors and the EAC contamination will be evaluated using data from public databases (e.g. MPCA, USGS, US FWS, US EPA) and sampling. In addition, we will determine the concentrations of estrogenic EACs (via contractor) and total estrogenicity in three water samples collected from

ten NE MN lakes, which will be selected to approximate the range of anthropogenic impacts. Chemicals will include

commonly detected estrogenic EACs, such as bisphenol A, and alkylphenols, and anthropogenic tracers, such as caffeine and triclosan. Nutrients (phosphate and nitrogen) will be analyzed by the NRRI Analytical Lab (UMD). Total estrogenicity will be determined in our lab using a cell culture-based assay employing the estrogen sensitive human breast cancer T47D cell line modified to contain a luciferase reporter gene. This assay has been used to measure total estrogenicity in wastewater treatment plant effluents and in other environmental samples, and can be used on water taken directly from lakes or after concentration by C-18 solid phase extraction (SPE).

Activity 3: Predict EAC exposure and test biomarkers in fish from northeast MN lakes **Budget: $ 74,889**

1. Collect male smallmouth bass from 10 target lakes and evaluate TO prevalence	Oct 2016
2. Correlate TO prevalence with measured EAC and indicator concentrations, total estrogenicity, and landscape factors	Dec 2016
3. Use cumulative results from Activities 1 and 2 to predict likelihood of EACs causing endocrine disruption in native fish within the study region	June 2017

Testicular oocytes are used as indicators of estrogenic EAC exposure in many vertebrate species, including smallmouth bass (SMB). We are currently conducting studies on normal and abnormal gonadal development in lab-exposed and wild SMB from NE MN field sites. We propose to expand that dataset with additional collections from selected lakes (see Activity 1), which will be analyzed for TOs using standard histological procedures. This biomarker will be used to test our model's response to measured EAC and anthropogenic input indicators, total estrogenicity, and landscape factors. The final product is intended to be a predictive map of lakes in which SMB and other fish populations could be at risk.

III. PROJECT STRATEGY

A. Project Team/Partners

Team: Dr. Patrick Schoff (PI), Jennifer Olker (Co PI), Sarah Hoheisel (PhD student).
Partners: MN DNR (contact - John Lindgren), Fond du Lac Natural Resources Management (contact - Brian Borkholder), will participate in project planning, site selection, field collection strategies, and general logistical planning. Both partners have contributed similarly to our current Sea Grant project on smallmouth bass (see below). These partners are not requesting ENTRF funding.

B. Timeline Requirements

The project requires two full field seasons; water chemistry will be evaluated in Year 1 (2014-2015) to determine locations for targeted smallmouth bass collections in Year 2 (2015-2016), along with evaluations of TO prevalence. Models will be constructed based on literature and landscape data in year 1 (2014-2015) and combined chemistry, landscape, and biomarker data in year 3 (2016-2017).

C. Long-Term Strategy and Future Funding Needs

This proposal builds on a currently active project funded by Minnesota Sea Grant investigating gonad development patterns and the occurrence of testicular oocytes as an indicator of endocrine disruption in smallmouth bass. The funding requested for this project will extend the scope and relevance of our SMB developmental research and enhance both projects. Results will be disseminated via academic and professional presentations and publications, along with formal and informal communications with our partners and collaborators. No further investment will be required to complete the goals stated in this proposal.

2014 Detailed Project Budget

Project Title: Predicting smallmouth bass exposure to endocrine active compounds

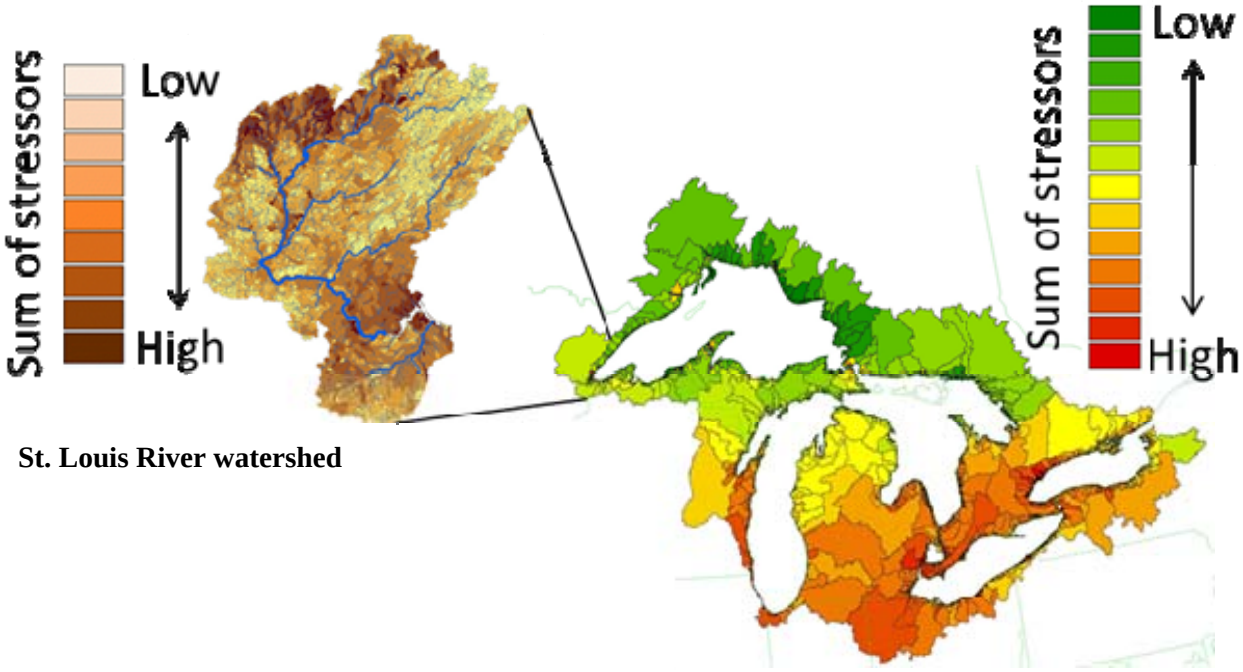
IV. TOTAL ENRTF REQUEST BUDGET: 3 years

BUDGET ITEM	AMOUNT
Personnel:	
Schoff, PI: oversee the entire project and directly manage the tissue culture portions; 15% fte; salary 75%/ fringe 25%, 36 mos. ($51,872)	$ 185,819
Olker, ResFellow: direct field & GIS portion; 25% fte; 75% salary/25% fringe, 36 mos ($50,128)	
Hoheisel, GRA: field collections, cell culture experiments & histology & histochemistry; 50% fte-9 mo AY, 50% fte-6 mo SUM; 61% salary/39% fringe ($48,959)	
TBD, GRA: participate in GIS modeling; 50% fte SUM; 81% salary/19% fringe, 6 mos. ($13,428)	
TBD, Seasonal field workers: 20 % fte; salary 92%/fringe 8%; 2 ppl, 24 mos ($14,614)	
TBD, Under workstudy; participate in field collections; 30% fte; 100% salary; 2 ppl, 9 mos ($6,818)	
Contracts:	$ 30,000
Chemical analysis (Contractor TBD), $30,000 [30 water samples @ $1,000 each]	
Equipment/Tools/Supplies:	
Water quality meter (YSI) is necessary for field water quality assessments, $1,500, Lab and field supplies, $6,400, includes solid phase extraction columns for concentrating estrogens, plasticware, culture media, antibiotics, histochemicals, staining kits, CO_2, cell cultures, euthanizing chemicals, water and tissue transport materials, dissection tools, fixatives, safety gear	$ 7,900
Travel:	$ -
Field trips to lakes in NE MN for water and specimen collection, $5594 (30 days of travel over two field seasons, most travel within 100 mi of Duluth, no overnight stays; 9900 mi @ $0.565/mi), $750 vehicle and boat rental (NRRI, 30 days @ $25/d)	$ 6,344
Additional Budget Items:	$ 11,010
Nutrient and water quality sample analysis, NRRI (UMD) analytical lab, $6,000 [30 samples @ $200 each]	
GIS lab service (NRRI, UMD), $3,690 [300 hr/yr @ $4.10/h]; field cell phone/emergency contact, $1,320 for yr 1 & 2 only @ $55/mo	
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$ 241,073

V. OTHER FUNDS

SOURCE OF FUNDS	AMOUNT	Status
Other Non-State $ Being Applied to Project During Project Period:	$ -	
Other State $ Being Applied to Project During Project Period: Graduate student support from the UMD Integrated Biosciences Program	$ 60,790	*Pending*
In-kind Services During Project Period:	$ -	
Remaining $ from Current ENRTF Appropriation (if applicable):	$ -	
Funding History: "Gonadal deformities in smallmouth bass as indicators of endocrine disruption in St Louis River Estuary" funded by MN Seat Grant, PI P. Schoff, 2012-2014. This proposal builds on a currently active project funded by Minnesota Sea Grant. The funding requested for this project will extend the scope and relevance of this SMB developmental research and enhance both projects.	$ 41,065	

Predicting smallmouth bass exposure to endocrine active compounds



St. Louis River watershed

Map estimating combined effects of stressors in the Great Lakes (right) and St. Louis River (left) watersheds. Stressor evaluations were a product of GIS interpretation and empirical measurements. We propose to perform a similar analysis focusing on endocrine active compound inputs for lakes and watersheds in NE MN. (Maps courtesy of Lucinda Johnson).

Predicting smallmouth bass exposure to endocrine active compounds

2014 - 2017 LCCMR Project Manager Qualifications and Organization Description

Patrick K. Schoff, PhD, holds a research appointment at the Natural Resources Research Institute at the University of Minnesota Duluth. Schoff is trained in developmental biology and ecotoxicology, and directs a research program focused on investigating the effects of anthropogenic and natural stressors on developing fish and amphibians. Current and past research programs have been funded by NIH, EPA, and MN Sea Grant, and include lab and field studies of skeletal and gonadal abnormalities in native frogs and fish and development of cell culture-based detection systems for retinoic acid mimics in surface water and effluents. A current project investigating gonadal abnormalities in smallmouth bass in northeast MN acts as background for this proposal.

The Natural Resources Research Institute (NRRI) is a part of the University of Minnesota Duluth. NRRI contains research facilities and staff appropriate to support lab and field projects of the scale proposed here, and the Institute has a proven record of both logistical and administrative support for research projects of this nature. NRRI's mission is to promote private sector employment based on natural resources in an environmentally sensitive manner. NRRI scientists have extensive experience in applied ecological research on terrestrial and aquatic systems.