### Environment and Natural Resources Trust Fund 2012-2013 Request for Proposals (RFP)

Project Title: ENRTF ID: 007-A	
Endocrine-Active Contaminants in Minnesota's Shallow Lakes	
<b>Fopic Area:</b> A. Fisheries & Wildlife Research	
otal Project Budget: \$ _293,000	
roposed Project Time Period for the Funding Requested: 2 yrs, July 2013 - June 2015	
other Non-State Funds: \$ 18,000	
ummary:	
lsing tissue gathered from turtles and minnows from shallow Minnesota lakes, we will determine if nvironmental estrogens affect aquatic wildlife and make recommendations about lake and wildlife nanagement.	
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ocation	
egion: Statewide	
county Name: Statewide	
ity / Township:	
Funding Priorities Multiple Benefits Outcomes Knowledge Base	
Extent of Impact Innovation Scientific/Tech Basis Urgency	
Capacity Readiness Leverage Employment TOTAL%	

### Project Title: Endocrine-active contaminants in Minnesota's shallow lakes

### **Project summary**

Using tissue gathered from turtles and minnows from shallow Minnesota lakes, we will determine if environmental estrogens affect aquatic wildlife and make recommendations about lake and wildlife management.

### **Project Proposal**

Shallow lakes provide ecosystem services and benefit citizens of MN by providing clean water, groundwater recharge, sequestration of chemicals, and a multitude of recreational opportunities (e.g., fishing, hunting). Past studies have established that endocrine disrupting contaminants (environmental estrogens or EEs) are present in Minnesota's streams and larger lakes at concentrations which have adverse impacts on fish. Conversely, very little is known about the occurrence, sources, and effects of EEs in small, shallow lakes. Furthermore, it is unknown how the use of surrounding land, and the associated lake management practices, may interact with the EEs in these systems. Our preliminary data strongly suggest that EEs are ubiquitous in Minnesota's shallow lakes and that they cause morphological structural changes in the brains of exposed turtles.

The goals of this project are to: 1) Determine whether exposure to EEs is common in shallow lake fish and wildlife, 2) Determine whether EEs correlate with lake states (clear versus turbid), land-use practices (urban, agriculture- and forest-dominated ecosystems), lake water quality conditions, community characteristics, and surface water connectivity. These analyses will allow us to identify which land-use and shallow lake management practices may be beneficial to minimizing EEs, and which shallow lakes are most vulnerable. Ultimately, the outcomes of this project fulfill the need to "Increase understanding of effects of contaminants on natural resources and develop strategies for reducing contamination" as identified in the LCCMR's 2009 Six-Year Strategic Plan for the Environment and Natural Resources Trust Fund.

We will achieve the above by 1) measuring vitellogenin (a quantifiable biological indicator of EEs) in fish and turtles we already collected from ca 50 shallow lakes representing five geographic regions across MN, 2) conducting additional fish and turtle collections in approximately 70 Minnesota lakes dispersed across the geographic regions our preliminary studies indicated as areas of interest (including Lake Christina and Heron Lake), and 3) test whether exposure to EEs is related to land use in watersheds by using our EE data and watershed data previously funded through an LCCMR project.

The work proposed herein is an important contribution to our understanding of EE contamination in shallow lakes, and is financially conservative as the preliminary sample collection and analyses are completed, and were supported by UST's substantial commitment and in-kind UST's services and heavily leveraged off the existing efforts of MN DNR and LCCMR. This is a unique opportunity to investigate whether current shallow-lake management practices can be used to minimize EEs and it time sensitive as the data needed for analysis, especially land use and water quality, collected in a previously funded LCCMR study need to be used before they are too dated for inclusion in our work.

### **DESCRIPTION OF PROJECT ACTIVITIES**

Activity 1 Assess the level of EE exposure in fish and turtles inhabiting shallow lakes in five ecoregions in Minnesota.

- Analyze fish samples collected in summer of 2011.
  - We will complete analyses of fish (fathead minnow) blood VTG analyses -VTG is an excellent biological indicator of EE exposure. Fathead minnows are selected because they are abundant in the shallow lakes, are an important food source for other commercially important species, and VTG measurement methods are readily available.
- Develop biochemical assay to measure EE exposure in painted turtles.
  - Fathead minnows are short-lived, and in some cases may not survive winter if lakes become very low in oxygen. Because of this we will also examine VTG in painted turtles, which are longer-lived, widely found, less likely to be impacted by low oxygen levels, and are of interest to nature lovers (we have letters of support from Friends of the Mississippi, the National Parks Service, and Ramsey County Parks), and are commercially harvested turtle species in MN.
- Complete the analyses of turtle samples collected in summer of 2011.
  - This includes the brain feminization assessment (sex-specific changes in brain structure as an indicator of adverse developmental effects) and VTG assessment (EE exposure indicator). Turtle DNA analysis of the samples will allow metapopulation description for ecoregion isolation.
- Test for relationships between EEs and land use patterns across Minnesota.
  - We will use a previously collected GIS data set (LCCMR funded) to test whether land use in watersheds (e.g. agriculture versus native grasslands) is related to EE levels in fish and turtles, and we will also assess whether these relationships vary across the state. We will also test whether EEs are related to other lake characteristics, such as whether lakes are turbid versus clear and overall water quality characteristics of lakes (e.g. algal abundance and nutrient levels).

### **Outcomes: YR 1**

- 1. Identify geographic areas/shallow lakes in MN that are impacted by EEs.
- 2. Develop and disseminate the painted-turtle specific VTG assay.
- **3.** Integrate EE-exposure data we generated with the existing ecological data to guide experimental design and site selection for YR2.

Activity 2 Conduct sampling of additional lakes to enrich sample sizes for the areas of interest and to extend the fish and turtle data set where it can be used to examine correlations with the management strategies, lake quality etc.

Outcomes: YR 2

- 1. Conduct fish and turtle sampling
- 2. Measure VTG and developmental effects in fish and turtles using assays developed over year 1.
- 3. Analyze DNA samples to determine metapopulation isolation across ecoregions
- 4. Sample abundance of submerged aquatic plants, algae in the water column, phosphorus and nitrogen, and water clarity. From these data we will be able to classify each lake as existing in a clear-water versus turbid-water state at the time of fish and turtle sampling for EEs.

# Activity 3. Integrate EE exposure data for turtles and fish with the existing ecological and management data to facilitate identification of practices beneficial for reducing contamination. Outcomes: YR 3

- 1. Identify land and lake management practices associated with EE occurrence and exposure.
- 2. Recommend and disseminate information to managers (DNR, MPCA) and citizens about the best management practices.

The outcomes of this project fulfill need to "Increase understanding of effects of contaminants on natural resources, including ground water, and develop strategies for reducing contamination" as identified in the LCCMR's 2009 Six-Year Strategic Plan for the Environment and Natural Resources Trust Fund. The outcomes are measureable, valuable, and have clear natural resource management implications.

## 2012-2013 Detailed Project Budget

INSTRUCTIONS AND TEMPLATE (1 PAGE LIMIT)

Attach budget, in MS-EXCEL format, to your "2012-2013 LCCMR Proposal Submit Form".

(1-page limit, single-sided, 10 pt. font minimum. Retain bold text and DELETE all instructions typed in italics. ADD OR DELETE ROWS AS NECESSARY. If a category is not applicable write "N/A", leave it blank, or delete the row.)

### IV. TOTAL ENRTF REQUEST BUDGET 3 years

BUDGET ITEM (See list of Eligible and Non-Eligible Costs, p. 11)	AMOUNT
Personnel: Timothy Lewis, Collection of painted turtles, project management, data analysis	\$ 44,000
and writing, five months work over three years, 14% employment over the three year period,	
99.235 % towrds salray, 7.65% FICA	
Personnel: Kyle Zimmer, Collection of fathead minnows, data analysis and writing, three	\$ 26,000
months work over three years, 8% employment over the three year study, 99.235 % towards	
salary, 7.65% FICA	
Personnel: Dalma Martinovic, VTG analysis in fish and turtles, data analysis and writing,	\$ 14,000
two months work over three years, 6% employment over the three year study, 99.235 %	
towards salary, 7.65% FICA	
Personnel: Kurt Illig, brain morphology work, data analysis and writing, three months work	\$ 23,000
over three years, 8% employment over the three year study, 99.235 % towards salary,	
7.65% FICA	
Personnel: 20 undergraduate researchers providing 60 person-months work over three	\$ 87,000
years 99.235 % towards salary, 7.65% FICA	
Contracts: Stehpan Bartell, Normandale Community College, VTG assay development	\$ 14,000
Contracts: Brad Swanson, ATCG Lab, Central Michigan University, DNA Population	\$ 12,000
Analysis	
Equipment/Tools/Supplies: VTG development supplies and consumbles	\$ 36,000
Equipment/Tools/Supplies: traps, glassware, reagents, field suplies	\$ 22,000
Acquisition (Fee Title or Permanent Easements):NA	\$ -
Travel: In state milage to lakes, housing and per diem at study sites	\$ 15,000
Additional Budget Items: In this column, list any additional budget items that do not fit	\$ -
above categories. List by item(s) or item type(s) and explain how number was reached. One	
row per type/category.	
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND \$ REQUEST =	\$ 293,000

### **V. OTHER FUNDS**

SOURCE OF FUNDS	AMOUNT		<u>Status</u>
Other Non-State \$ Being Applied to Project During Project Period: U of St Thomas	\$	18,000	Secured
funding for student reserachers			
Other State \$ Being Applied to Project During Project Period: NA	\$	-	
In-kind Services During Project Period: Vehicles, canoes, labs, extensive equipment list	\$	22,000	Secured
Remaining \$ from Current ENRTF Appropriation (if applicable): NA	\$	-	
Funding History: UST supplied funding for faculty research time	\$	22,000	Secured
Funding History: UST supplied funding for undergraduate researchers	\$	24,000	Secured
Funding History: UST supplied funding for field equipment, vehicles, consumables	\$	4,000	Secured
Funding History: UST supplied funding for lab equipment, vehicles, consumables	\$	12,000	Secured

# Sampling Regions (Shallow lakes with painted turtles, fathead minnows)



3 Study Years: 2013-2015

The Project Director, Dr. Timothy L. Lewis, has 30 years of wildlife research experience including 25 years of turtle work. He has a PhD in Wildlife Ecology from the University of Wisconsin-Madison, and has published results of his work in the scientific peerreviewed literature as well as at numerous national and international conferences. He also brings 15 years administrative experience managing deadlines, personnel, and budgets as a department chair for two separate universities Wittenberg University, Springfield, Ohio and the University of St. Thomas). He is currently a Professor of Biology and the Biology Department Chair at the University of St. Thomas, St. Paul.

Lewis' turtle research includes long term population and habitat use studies of spotted turtles (*Clemmys guttata*), wood turtles (*Glyptemys insculpta*), and painted turtles (*Chrysemys picta*). Techniques employed for these projects include basking, box, and hoop traps, radio telemetry, blood and tissue sampling, behavior analysis, GIS analysis, and habitat management. Most of this research has been funded by either the Ohio Department of Natural Resources or the U.S. Forest Service because of the clear management implications of his work. The Minnesota research has been done in collaboration with MN DNR. This research over the years has involved various collaborators including over 200 undergraduate researchers. Most relevant to this application, for the last three years he has been trapping painted turtles for a project in 40 state-wide shallow lakes. This has included the acquisition of nearly a hundred blood samples that this funding will allow to be analyzed for estrogenic compounds and brain samples used in our preliminary structural analysis.

Management of an extensive collaborative projects such as the one proposed also requires administrative skills. Lewis has overseen annual department budgets of several million dollars per year including detailed accounting and specific purchasing requirements. His department has 20 faculty and staff including three of the collaborators on this project. He has worked closely with this team for a year as they gathered initial samples and data. Further, his extensive experience with undergraduate researchers ensures not only an educational benefit to them but the clear confidence that comes from experience that they with the faculty researchers can accomplish the outcomes of this proposal.

In short, he has the research experience including trapping turtles on the very lakes of this proposal needed to get the samples for this research, a team of collaborators form his department with the skills to do the lab analysis, access to a labor pool of students to cover the necessary lakes and ensure data integrity, and the administrative and grants experience to comply with the money and personnel management aspects of this proposal.

Founded in 1885, the University of St. Thomas is a Catholic, diocesan university in the Midwest based in the Twin Cities of St. Paul and Minneapolis. The largest private university in Minnesota, St. Thomas offers bachelor's degrees in over 85 major fields of study and more than 45 graduate degree programs including master's, education specialist, juris doctor and doctorates. Within it, the Department of Biology offers undergraduate BA and BS degrees as well as degrees in Neuroscience and Biochemistry.