Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2014 Work Plan

Date of Report: 10 February, 2014

Date of Next Status Update Report: 15 January, 2015

Date of Work Plan Approval:

Project Completion Date: 30 June 2017

Does this submission include an amendment request? No

PROJECT TITLE: Impacts of Estrogen Exposure on Minnesota's Shallow Lake Wildlife

Project Manager: Kurt R. Illig, PhD

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Location:

Statewide

Total ENRTF Project Budget:		
	ENRTF Appropriation:	**$136,000**
	Amount Spent:	**$0**
	Balance:	**$136,000**

Legal Citation: M.L. 2014, Chp. 226, Sec. 2, Subd. 03f

Appropriation Language:
$136,000 the second year is from the trust fund to the commissioner of natural resources for an agreement with the University of St. Thomas to use biological samples already gathered from shallow lakes across Minnesota to determine the environmental estrogen exposure impacts on aquatic wildlife in shallow lakes for enhanced land and lake management. This appropriation is available until June 30, 2017, by which time the project must be completed and final products delivered.

I. PROJECT TITLE: Impacts of Estrogen Exposure on Minnesota's Shallow Lake Wildlife

II. PROJECT STATEMENT:

Minnesota's smaller lakes play an important role in the ecosystem by providing clean water, recharging groundwater stores, and sequestering chemical and soil runoff. These lakes also benefit citizens, both by providing opportunities for recreation (e.g., fishing, swimming) and by providing economic value as a site for various commercial ventures (e.g., summer camps, commercial fisheries). Endocrine-disrupting contaminants, including environmental estrogens (EEs) are present in Minnesota's larger lakes and streams at concentrations which have adverse impacts on wildlife. However, very little is known about the sources and effects of EEs in small, shallow lakes. Importantly, the use of surrounding land and associated lake management practices may exacerbate the effects of contaminants in these systems. Our preliminary data strongly suggest that EEs are present in Minnesota's shallow lakes, and that wildlife exposed to these contaminants exhibit changes in the nervous system that may impact survival and reproduction.

There are three goals of this project, as follows:

- Determine whether EE exposure is common for aquatic wildlife in shallow lakes
- Determine land-use practices that correlate with EE exposure (e.g., urban, agriculture- and forest-dominated ecosystems)
- Identify the effects of EE exposure on the nervous system of aquatic species.

These analyses will allow us to identify which land-use and shallow lake management practices are most beneficial to minimizing EE exposure, and associate EE exposure with impacts on wildlife. The outcomes of this project directly address three 2014 LCCMR funding priorities:

- to protect or restore water quality by…improving water and land use practices;
- to evaluate and identify the causes of observed changes in the health of fish and wildlife that may pertain to contaminants of emerging concern;
- to protect the health of humans and aquatic and terrestrial species by advancing the development of standards for contaminants.

We will achieve these goals by measuring blood vitellogenin levels (a quantifiable indicator of EE exposure) in turtles from approximately 50 shallow lakes across five geographic regions of MN, testing whether exposure to EEs is related to land use by combining vitellogenin data with watershed data previously obtained, and analyzing brain structures associated with foraging and reproductive behavior in the brains of turtles for which blood vitellogenin levels are available.

This work is an important contribution to our understanding of the effects of contamination in Minnesota's shallow lakes by estrogen and estrogen-activating compounds. Moreover, the project leverages over $100,000 of funding and in-kind services, in addition to work performed by faculty and students at the University of St. Thomas (including sample collection, preliminary specimen preparation and analyses). Further, this study takes advantage of recently-obtained, up-to-date GIS data on land use and water quality. Thus, the study provides a high impact for relatively low cost, to deliver an important investigation of how land- and lake-management practices correlate with exposure and effects of EEs.

III. PROJECT STATUS UPDATES:

Project Status as of 15 January 2015:

Project Status as of 15 July 2015:

Project Status as of 15 January 2016:

Project Status as of 15 July 2016:

Project Status as of 15 January 2017:

Overall Project Outcomes and Results:

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Assess EE exposure levels in wildlife

Description: To assess EE exposure, we will develop an assay to examine vitellogenin (VTG) levels in painted turtles (Chrysemys picta), which have been shown to vary with EE exposure in this species (Irwin et al, 2001). The VTG assay will be developed using methods similar to previously published reports (e.g., Bartell and Schoenfuss, 2012; Irwin et al., 2001). Plasma vitellogenin will be measured by antibody-capture competitive ELISA incorporating a species-validated anti-vitellogenin antibody and purified vitellogenin as standard. Polyclonal antisera will be produced by ProSci Inc. (San Diego, CA) from purified painted turtle plasma vitellogenin. Microtiter plate wells will be coated with 600 ng species-validated vitellogenin in carbonate coating buffer (pH 9.6). A pre-competition step will be performed with the antibody (1:20,000 final dilution) and either standard vitellogenin, sample plasma or control plasma in 1% BSA/PBS (pH 7.5). After incubation this mixture will be loaded into the wells and incubated at room temperature for 1 h, followed by secondary antibody (anti-rabbit IgG-HRP, Sigma-Aldrich, St. Louis, MO) at a concentration of 1:10,000. The substrate tetramethylbenzidine (TMB) will be added and incubated for 20 min at room temperature and color development measured at 620 nm on a Thermo Multiscan plate reader (Waltham, MA). Each plate will contain a set of standards for standard curve generation, and will be read precisely at 20 min post-TMB addition. Average VTG levels for males and females will be calculated for each lake, ecological region, and statewide. Comparisons will be made using analysis of variance, and significant main effects (sex, lake, ecological region) and interactions will be explored using appropriate post-hoc analyses (e.g., Tukey's HSD).

Summary Budget Information for Activity 1:

ENRTF Budget: $60,000
Amount Spent: $0
Balance: $60,000

Activity Completion Date: 30 June, 2017

Outcome	Completion Date
1. Develop an assay to determine vitellogenin levels in painted turtles (*Chrysemys picta*). This assay will be used as an initial measure to examine environmental estrogen exposure in these animals.	April, 2015
2. Correlate vitellogenin levels with lakes and watersheds impacted by environmental estrogens. This correlation will involve integrating data about vitellogenin levels with GIS maps of Minnesota.	February, 2016
3. Evaluate and refine methods and data related to turtle vitellogenin assay (ongoing). Although an initial assay should be available within the first year of the project, we will be interested in perfecting the assay to ensure that the assay is sensitive and robust enough for widespread use.	June, 2017

Activity Status as of 15 January 2015:

Activity Status as of 15 July 2015:

Activity Status as of 15 January 2016:

Activity Status as of 15 July 2016:

Activity Status as of 15 January 2017:

Final Report Summary:

Activity 2: Correlate land use and EE exposure
We will integrate EE exposure data collected in Activity 1 with previously-collected GIS data sets containing information about ecological and land-management to test whether watershed land use is related to EE levels in turtles, leveraging the wide collection range to assess whether these relationships vary across the state. In particular, we will combine the geospatial information collected during the collection of the turtle blood and brains with land use data available from the Minnesota Geospatial Information Office (for example, data on land use practices is available http://www.mngeo.state.mn.us/chouse/land_use.html).

This process will involve merging land-use data with the vitellogenin level data collected in Activity 1. Additionally, because some of the turtles in our study are relatively long-lived, we will explore the degree to which vitellogenin levels may correlate with historical land use, to the extent that this data is available. To facilitate this, we will explore correlations between vitellogenin levels collected in Activity 1 and data available on land cover and land use from the data clearinghouse of the Minnesota Geospatial Information Office (e.g., http://www.mngeo.state.mn.us/chouse/land_use_historic.html). For both recent and historical data sets, these correlations will help us identify land use patterns that are most favorable for reducing EE exposure in shallow lake wildlife.

Summary Budget Information for Activity 2:

ENRTF Budget: $28,000
Amount Spent: $0
Balance: $28,000

Activity Completion Date: 30 June, 2017

Outcome	Completion Date
1. Identify land- and water-management practices associated with EE exposure. This will involve working with GIS mapping software to determine what land-use practices are most closely associated with elevated vitellogenin levels.	April, 2017
2. Draft recommendations to share with managers (DNR, MPCA) and citizens (ongoing). As results become available, we will work with leaders of appropriate local and state agencies to explore ways of managing land use in vulnerable shallow lakes and watersheds to reduce environmental estrogens.	June, 2017

Activity Status as of 15 January 2015:

Activity Status as of 15 July 2015:

Activity Status as of 15 January 2016:

Activity Status as of 15 July 2016:

Activity Status as of 15 January 2017:

Final Report Summary:

Activity 3: Analyze EE exposure effects on nervous system structures

We will analyze brain regions associated with foraging and reproductive behavior in turtles for which blood vitellogenin levels are available from Activity 1. This will include examining the nucleus paraventricularis (NPV), a structure that is sexually dimorphic and may be related to reproductive behavior in the turtle. Brains were fixed in the skull with 4% paraformaldehyde for >92 hours, then removed and placed in a cryoprotectant solution (25% sucrose) for 48 hours. Serial sections were cut at 40 microns using a cryostat (Leica Instruments) and transferred to well plates containing phosphate buffered saline (PBS) and 0.1% sodium azide until further processing. Sections were stained with Cresyl violet, dehydrated, mounted on gelatin-coated slides and coverslipped. Preliminary data on the size of the NPV was collected in the same manner as the proposed work, as follows. Sections of turtle brain that contain the NPV will be marked and prepared for counting. All procedures from this point on are carried out with the experimenter blind to the identity and sex of the specimen. Using a microscope connected to a computer running Neurolucida software (MicroBrightField), experimenters will circumscribe the NPV for every section from each animal. Each section will be subject to circumscription by at least two experimenters, and the average area for each section will be computed. These areas will be combined into a 3-D volumetric measurement by the software. Preliminary results have shown that the difference in size of the NPV between males and females varies widely throughout the state. Therefore, average volumes for males and females will be computed for each lake and ecoregion where turtles were collected. Comparisons will be made using analysis of variance, and significant main effects (sex, lake, ecoregion) and interactions will be explored using appropriate post-hoc analyses (e.g., Tukey's HSD). These results, taken together with EE exposure data, will suggest whether exposure to EEs might affect behavior related to survival and reproduction in this species.

Summary Budget Information for Activity 3:

ENRTF Budget: $48,000
Amount Spent: $0
Balance: $48,000

Activity Completion Date: 30 June, 2017

Outcome	Completion Date
1. Identify how brain structures are impacted by EE exposure. This will take the shape of at least one scientific research paper published in a peer-reviewed journal in the appropriate field.	June, 2017

Activity Status as of 15 January 2015:

Activity Status as of 15 July 2015:

Activity Status as of 15 January 2016:

Activity Status as of 15 July 2016:

Activity Status as of 15 January 2017:

Final Report Summary:

V. DISSEMINATION:

Description: We will disseminate our findings to managers, citizens and scientists about the impact of EE exposure to facilitate development of exposure standards. As results become available, we will work with leaders of appropriate local and state agencies to explore ways of managing land use in vulnerable shallow lakes and watersheds to reduce environmental estrogens. Further, we will present our findings at regional, national and international scientific conferences. This will benefit the scientific community as we explore the relationships between organisms and the quality of their environment, and it will benefit the project as we gain valuable insight and input from recognized experts in the field.

Status as of 15 January 2015:

Status as of 15 July 2015:

Status as of 15 January 2016:

Status as of 15 July 2016:

Status as of 15 January 2017:

Final Report Summary:

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget Overview:

Budget Category	$ Amount	Explanation
Personnel:	$ 68,000	Funding for Kurt Illig: Project director; brain morphology; personnel training and management; data analysis; project outcome dissemination. Salary and fringe benefit support for four months over three years. Funding for undergraduate research assistants: Brain morphology and data analysis; hourly wage support for eight students for three years (24 person-months).
Professional/Technical/Service Contracts:	$ 14,000	Contract for Steven Bartell, Normandale Community College for vitellogenin assay development.
Equipment/Tools/Supplies: These items are expected to be needed with estimated dollar amounts. Actual equipment and supplies will be determined as the project progresses.	$ 54,000	Total funding for vitellogenin assay and brain structure equipment, supplies, services and consumables as detailed below.
Microplate Reader	$ 4900	Equipment for vitellogenin assay development
Microplate Washer	$ 4975	Equipment for vitellogenin assay development
Pipettors P2-P1000 (2 sets)	$ 1520	Equipment for vitellogenin assay development
Repeat Pipettors (2)	$ 2460	Equipment for vitellogenin assay development
Multichannel Pipettors (2)	$ 1800	Equipment for vitellogenin assay development
DEAE agarose/sepharose column matrix	$ 180	Supplies for vitellogenin assay development

Chromatography columns (3)	$ 300	Supplies for vitellogenin assay development
Rubber Tubing (1/4" ID)	$ 20	Supplies for vitellogenin assay development
Pre-cast PAGE gels, ladder, buffers	$ 440	Supplies for vitellogenin assay development
50ml Conical Tubes	$ 200	Supplies for vitellogenin assay development
15 ml Conical Tubes	$ 180	Supplies for vitellogenin assay development
2.0 ml microtubes (no-cap)	$ 100	Supplies for vitellogenin assay development
0.5 ml microtubes (no-cap)	$ 100	Supplies for vitellogenin assay development
Microtube caps	$ 60	Supplies for vitellogenin assay development
Bovine Serum Albumin	$ 500	Supplies for vitellogenin assay development
96-well microplates	$ 500	Supplies for vitellogenin assay development
Tips for pipettors	$ 500	Supplies for vitellogenin assay development
Buffer reservoirs	$ 100	Supplies for vitellogenin assay development
Antiserum Services	$ 4500	Services for vitellogenin assay development
Microscope slides	$ 2270	Supplies for brain structure analyses
Microscope coverslips	$ 700	Supplies for brain structure analyses
Microscope slide storage bins	$ 400	Supplies for brain structure analyses
Slide mounting media	$ 600	Supplies for brain structure analyses
Ethanol reagent (absolute)	$ 620	Supplies for brain structure analyses
Ethanol reagent (70%)	$ 650	Supplies for brain structure analyses
Histoclear reagent	$ 780	Supplies for brain structure analyses
Cresyl Violet stain	$ 1055	Supplies for brain structure analyses
Virtual Slice Module	$ 4450	Software for brain structure analyses
Solid Modeling Module	$ 4500	Software for brain structure analyses
Image Stack Module	$ 4950	Software for brain structure analyses
Digital Image File Storage	$ 400	Equipment for brain structure analyses
Nanopure water system	$ 4690	Equipment for brain structure analyses
Nanopure cartridges	$ 1800	Equipment for brain structure analyses
pH meter and microelectrodes	$ 2800	Equipment for brain structure analyses
TOTAL ENRTF BUDGET:	**$ 136,000**	

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: Approximately 2.11 FTE will be directly funded with this ENRTF appropriation (undergraduate students: 2 FTE; Kurt Illig, 0.11 FTE)

Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation: Approximately 0.25 FTE will be funded through contracts with this ENRTF appropriation (Steven Bartells)

B. Other Funds:

Source of Funds	$ Amount Proposed	$ Amount Spent	Use of Other Funds
Non-state			
University of St. Thomas (cash support)	$ 18,000	$ 0	Funding for additional student research assistants
University of St. Thomas (in-kind support)	$ 24,000	$ 0	Equipment use and supplies

State			
	$ 0	$	
TOTAL OTHER FUNDS:	**$ 42,000**	**$**	

VII. PROJECT STRATEGY:

A. Project Partners: The project team will be led by Dr. Kurt R. Illig, Assistant Professor of Biology and Director of Neuroscience at the University of St. Thomas. He will be in charge of data collection and analysis, and will direct a team of undergraduate students who will assist in these efforts. In addition, Dr. Illig and the University of St. Thomas will contribute over $100,000 worth of equipment use, supplies and undergraduate student labor in support of this project. Dr. Stephen E. Bartell of Normandale College, who has developed vitellogenin assays for multiple non-model aquatic species in Minnesota, will be contracted to develop such an assay for the painted turtle. Dr. Timothy Lewis and Dr. Kyle Zimmer at the University of St. Thomas will assist Dr. Illig with merging brain morphology and EE exposure results with existing GIS data.

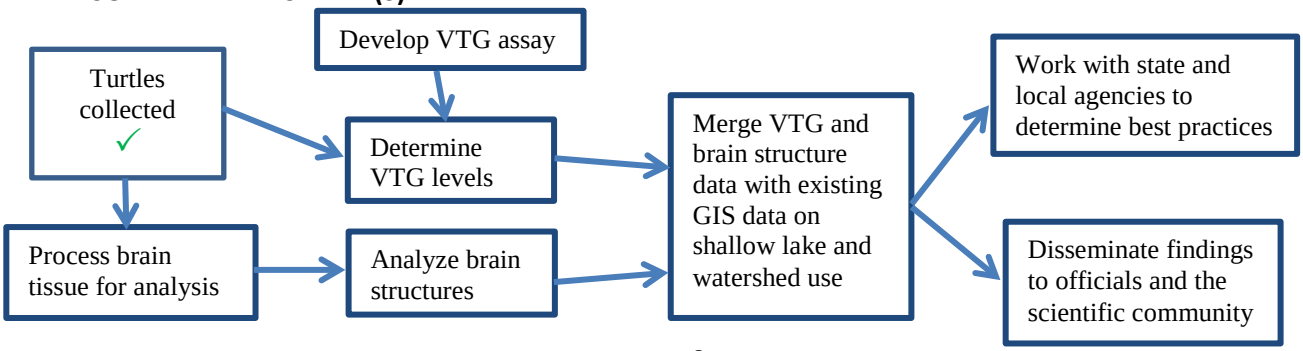
B. Project Impact and Long-term Strategy: The project has the potential of providing a significant impact into our understanding of how land use management affects Minnesota's shallow lake wildlife. Although the focused, directed studies in the proposed project are not likely to require more than three years, we expect that the results will lead to many further questions of interest regarding shallow lake wildlife and habitat. For example, if preliminary results hold and it is confirmed that EE exposure leads to differences in brain structures related to feeding and reproduction (see visual element, below), follow-up studies will be required to examine questions such as: 1) What levels of EE exposure cause behavioral effects? 2) Are such effects seen in other aquatic species? 3) Does EE exposure threaten the long-term health of aquatic organisms that are important for the health of Minnesota's lakes and economy? In the future, we also may wish to expand the study by collecting different organisms, or more painted turtles from more lakes. Future projects addressing such questions will likely be of interest to LCCMR and to governmental and non-governmental agencies both within and outside of Minnesota (e.g., the US Environmental Protection Agency, National Science Foundation, the National Institutes of Health); funding for such projects will be sought from these sources.

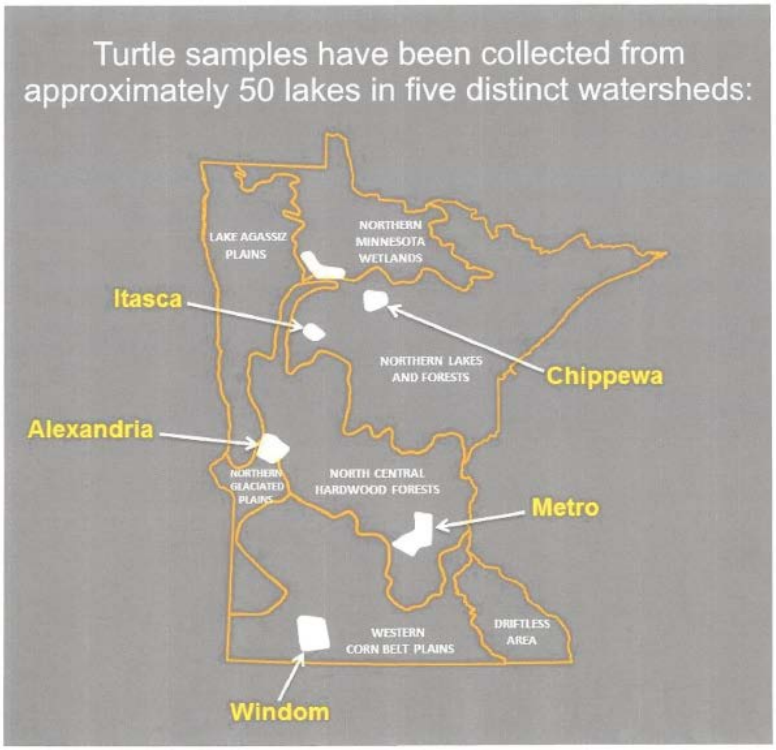
C. Spending History: A large amount of work already has been completed for this project, made possible by funding from the University of St. Thomas for student research, equipment and supplies, as outlined below:

Funding Source	**M.L. 2008 or FY09**	**M.L. 2009 or FY10**	**M.L. 2010 or FY11**	**M.L. 2011 or FY12-13**	**M.L. 2013 or FY14**
University of St. Thomas			$54,000	$8,000	

VIII. ACQUISITION/RESTORATION LIST: N/A

IX. VISUAL ELEMENT or MAP(S):





X. ACQUISITION/RESTORATION REQUIREMENTS WORKSHEET: N/A

XI. RESEARCH ADDENDUM: See attached research addendum

XII. REPORTING REQUIREMENTS:
Periodic work plan status update reports will be submitted no later than 15 January 2015, 15 July 2015, 15 January 2016, 15 July 2016 and 15 January 2017. A final report and associated products will be submitted between June 30 and August 15, 2017.

Environment and Natural Resources Trust Fund											
M.L. 2014 Project Budget											
Project Title: Impacts of Estrogen Exposure on Minnesota's Shallow Lake Wildlife											
Legal Citation: M.L. 2014, Chp. 226, Sec. 2, Subd. 03f											
Project Manager: Kurt R. Illig											
Organization: University of St. Thomas											
M.L. 2014 ENRTF Appropriation: $ 136,000											
Project Length and Completion Date: 3 Years; June 30, 2017											
Date of Report: 10 February, 2104											
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	***Assess EE exposure levels in wildlife***			***Correlate Land Use and EE exposure***			***Analyze EE exposure effects on nervous system structures***				
Personnel (Wages and Benefits)											
Kurt Illig, Project Manager: $32,000 (92.35% salary, 7.65% FICA); 0.11 FTE				$6,000	$0	$6,000	$26,000	$0	$26,000	$32,000	$32,000
Undergraduate Students: Eight undergraduate researchers for 24 person-months work over three years (92.35 % towards salary, 7.65% FICA); 2 FTE				$8,000	$0	$8,000	$28,000	$0	$28,000	$36,000	$36,000
Professional/Technical/Service Contracts											
Stephen Bartell, Normandale Community College, VTG assay development contract; 0.25 FTE	$14,000	$0	$14,000							$14,000	$14,000
Equipment/Tools/Supplies: Equipment such as: Microplate Reader, microplate washer, pipettors, etc.; Tools such as: Chromotography columns, software tools, etc.; Supplies such as: Bovine serum albumin, 96-well microplates, pipette tips, microscope slides, etc.	$23,085	$0	$23,085				$30,915	$0	$30,915	$54,000	$54,000
COLUMN TOTAL	**$37,085**	**$0**	**$37,085**	**$14,000**	**$0**	**$14,000**	**$84,915**	**$0**	**$84,915**	**$136,000**	**$136,000**