2014 Project Abstract
For the Period Ending June 30, 2017

PROJECT TITLE: Impacts of Estrogen Exposure on Minnesota's Shallow Lake Wildlife
PROJECT MANAGER: Kurt R. Illig, PhD
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2014, Chp. 226, Sec. 2, Subd. 03f

APPROPRIATION AMOUNT: $ 136,000
AMOUNT SPENT: $ 112,917
AMOUNT REMAINING: $ 23,083

Overall Project Outcomes and Results

Minnesota's shallow 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, 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 influence or exacerbate the effects of contaminants in these systems. By developing an assay that allows us to look at levels of EE exposure in the painted turtle, we have found that lakes across Minnesota have widely different chemical makeup, and that turtles in these lakes show different levels of exposure. By examining the brains of these animals, we have discovered that the size of certain structures related to reproduction is highly correlated with EE exposure. We are now examining the relationship between land-use practices in lake watersheds and the chemical makeup of these lakes.

Project Results Use and Dissemination

We presented work resulting from our efforts to develop a species-specific measure for VTG in painted turtles at the Midwest Regional Chapter meeting of the Society for Environmental Toxicology and Chemistry in April, 2017. We presented work that describes the relationship between VTG levels and brain structure at the North American Society for Environmental Toxicology and Chemistry annual meeting in November, 2017.

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

Date of Report: 15 December, 2017

Final Report

Date of Work Plan Approval: 4 June, 2014

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

Organization: University of St. Thomas

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Location:

Statewide

Total ENRTF Project Budget:		
	ENRTF Appropriation:	**$136,000.00**
	Amount Spent:	**$112,917.00**
	Balance:	**$23,083.00**

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 January 2015: Work on the project began in July 2014 with the project manager working to recruit students, gather materials, prepare analyses and set up collaborative research structures. Although work was done on all three activities, the bulk of activity was done to prepare for Activity 3; the project manager recruited students, configured equipment, and initiated measurements. Training and some preliminary analyses

were completed in September 2014. Collaborations for Activity 2 involving the management and addition of data to existing GIS databases have been established, and collaborative work for Activity 1 was finalized, including materials and equipment and a timeline required for the work.

Project Status as of July 2015: We are progressing very steadily on all aspects of this project. We are over halfway complete with Activity 1, the vitellogenin antibody development. Although this project was off to a somewhat slow start, progress on development of the antibody has been rapid. Vitellogenin isolated from painted turtles has been purified and initial results show good cross-reactivity with antibodies from other species, which suggests a good prognosis for the remainder of the antibody development. The final steps in the assay development should be complete by December, 2015. No further activity has occurred on Activity 2, because the beginning of this Activity awaits the use of the antibody being developed in Activity 1. On Activity 3, good progress has been made in processing neural tissue and preparing it for analysis. Students are now engaged in analyzing this material, but because of the double-blind nature of these experiments, we have no results to report yet. Additionally, we have been fortunate to recover extraneural organ tissue from these animals, allowing us to complete measurements that will serve as a biological correlate of sex, improving and extending our findings in Activity 3.

Project Status as of January 2016: Vitellogenin assay development (Activity 1) is now complete, and we are gathering data from our samples (see details below). Correlating vitellogenin levels with land use data in GIS (Activity2) will not be possible until Activity 1 is complete. Analyzing brain structures (Activity 3) is now largely complete for one brain region, and data analysis is proceeding well (see details below).

Project Status as of July 2016: Blood levels of vitellogenin have now been obtained using the assay developed in Activity 1. Correlating vitellogenin levels with land use data (Activity 2) is approximately 20% complete, with lakes and land use data being merged with the turtle data sets. Analyzing brain structures (Activity 3) is now largely complete for one brain region, and data analysis is proceeding well (see details below). The final year of this project will be devoted to completing Activities 2 and 3, which have taken longer to complete than anticipated. To expedite our process and ensure completion of the project on time, I am proposing to hire an additional consultant on the project (see amendment request below).

Amendment Request (07/31/2016): With the completion of the vitellogenin assay and data gathering in full swing, I would like to recruit an additional person to the project to help us analyze the effects of EE exposure levels on nervous system structures (Activity 3). I propose to hire a second Professional Consultant on this project whose background includes comparative neuroanatomy, particularly in examining male/female differences in a variety of species and identifying neural correlates of these differences. The consultant will provide expertise and will assist by working with the project director and students to analyze the neural data detailed in Activity 3, particularly by looking at additional brain regions. I will post an advertisement and will evaluate candidatescontract with someone who has experience with examining hormonal influence on behavior in vertebrate organisms. Experience analyzing the changes that occur after hormonal activation at multiple levels (cellular, neural and behavioral) will be preferred. To pay for this work, I would like to move $16,005 from the equipment/tools/supplies budget for Activity 3 to Personnel. This budgetary shift is made possible because the University of St. Thomas continues to provide generous support, by purchasing some of the equipment and supplies that were included in the original budget. As a consequence, this results in no change in the total project budget. Approved by LCCMR 7-28-2016.

Project Status as of January 2017: After a competitive search, a second Professional Consultant has been hired for this project. The consultant is now analyzing the neural data detailed in Activity 3, and performing literature reviews to determine the appropriate additional brain regions to study.

Overall Project Outcomes and Results: Minnesota's shallow 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, 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 influence or exacerbate the effects of contaminants in these systems. By developing an assay that allows us to look at levels of EE exposure in the painted turtle, we have found that lakes across Minnesota have widely different chemical makeup, and that turtles in these lakes show different levels of exposure. By examining the brains of these animals, we have discovered that the size of certain structures related to reproduction is highly correlated with EE exposure. We are now examining the relationship between land-use practices in lake watersheds and the chemical makeup of these lakes.

More specifically, this project has yielded three important and significant results to date. First, we have been able to develop a species-specific measure for VTG levels in painted turtles. This is a major asset to the field, as it will allow scientists to study, for the first time, exposure to EEs in a long-lived species found in Minnesota's shallow lakes. This will enable future projects to explore more directly the impact of EEs and changes in land-use practices in this turtle species.

Second, we have discovered that VTG levels in turtles from Minnesota's shallow lakes vary widely. This finding suggests that the shallow lakes in Minnesota cannot be considered as a single entity; each one is unique and provides a different environment to the wildlife that lives there. The extent to which the chemical environment in these lakes is due to watershed use is the subject of ongoing work.

Finally, we have discovered a significant relationship between VTG levels in turtles and the size of a sexually-dimorphic nucleus (the paraventricular nucleus, or NPV) in their brains. In lakes where overall VTG levels were low, the size of the NPV in males was significantly larger than in females, as found previously. However, in high-VTG lakes, the average NPV was significantly smaller in males than in females. These results suggest that environmental estrogen exposure impacts brain structure in painted turtles in a sex-dependent manner.

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: $37,085
Amount Spent: $30,175
Balance: $6,910

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 January 2015: A list of materials and supplies was finalized with Dr. Steven Bartell, and the timeline for assay development was negotiated and finalized. Supplies are on order to be delivered to Dr. Bartell in the coming weeks.

Activity Status as of July 2015: We are progressing steadily with Vitellogenin assay development. This took somewhat longer than we initially expected, in part because it was impossible to obtain enough turtle blood early in the season (turtles are difficult to find under the ice!). Through collaborative efforts with members of the Biology Department at the University of St. Thomas, we obtained sufficient samples of turtle blood to isolate the vitellogenin and have performed the necessary purification steps for antibody development. Stephen Bartell is now completing antibody services, including development of the antibody, final testing and validation. The vitellogenin is showing good purity, and shows some cross-reactivity to an antibody for red-eared slider turtle vitellogenin, which bodes well for the rest of the antibody development. The final steps in the assay development should be complete by December, 2015.

Activity Status as of January 2016: Assay development is complete. Stephen Bartell has completed development of the antibody, final testing, and validation, including standard calibrations. We have begun running sample turtle blood that was collected for this purpose, and we anticipate recovering the data on these samples by early February, 2016. We will then proceed to correlate vitellogenin levels with external and internal markers of sex of the turtles, and to correlate vitellogenin levels in males and females with the lakes where turtles were collected. This work should be well underway by July 2016, with preliminary findings available in January 2017. We are on track to complete this Activity by July 2017.

Activity Status as of July 2016: We have completed running all of our sample turtle blood through the assay, and we are in the middle of analyzing this data now. Preliminary results suggest that overall, vitellogenin levels are higher in females than in males, which is in agreement with work in other species. However, we have found that there are many individual turtles in which this relationship is reversed. This is important because some of the individual turtles have ambiguous external sex characteristics, or exhibit a mismatch between external and

internal sex characteristics. Our ongoing task is to correlate vitellogenin levels in males and females with the lakes where turtles were collected (Activity 2), and levels in individuals with brain structure size (Activity 3).

Activity Status as of January 2017: Activity is complete; no additional update.

Final Report Summary: We successfully developed a species-specific measure for VTG levels in painted turtles (Outcome 1). This is a major asset to the field, as it will allow scientists to study, for the first time, exposure to EEs in a long-lived species found in Minnesota's shallow lakes. This will further enable scientists and future studies to explore more directly the impact of EEs and changes in land-use practices in this turtle species. Outcomes 2 and 3 are ongoing, as we prepare and refine the assay and historic GIS maps that correspond with land use in the watersheds where turtles were collected (See Activities 2 and 3 below). We disseminated some of this work at the Midwest Regional Chapter meeting of the Society for Environmental Toxicology and Chemistry in April, 2017.

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: $ 14,000
Amount Spent: $ 14,000
Balance: $ 0

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 January 2015: Collaborations have been established with clear expectations for GIS and scientific data. This was an important step to ensure that data collected in the other phases of the project could be used with the existing GIS software and databases.

Activity Status as of July 2015: Further progress on this activity is awaiting data from EE exposure (Activity 1).

Activity Status as of January 2016: Progress on this activity is awaiting data from EE exposure (Activity 1).

Activity Status as of July 2016: As our data analysis from EE exposure (Activity 1) progresses, we are preparing to integrate this data with GIS databases. We have begun coding our data to make this transition efficient.

Activity Status as of January 2017: Data analysis and integration is ongoing; this work is painstaking and time-consuming, but the addition of the second professional consultant to the grant is getting us back on track for this activity. We estimate that this activity is approximately 20% complete, and we should be able to complete this task by late April or early May, 2017.

Final Report Summary: We have discovered that VTG levels in painted turtles from Minnesota's shallow lakes vary widely. This finding suggests that the shallow lakes in Minnesota cannot be treated as a single, homogenous entity; each one is unique and provides a different environment to the wildlife that lives there. The extent to which the chemical environment in these lakes is due to watershed use is the crux of Activity 2, and is the subject of ongoing work. Specifically, we need to determine the age of the turtles collected in the lakes, collect historical land-use data in the watershed of various lakes, and correlate these factors. Differential availability of this data for different watersheds will undoubtedly be problematic for these analyses, but we hope to have enough data for enough of the watersheds to discover some general principles that correlate land use with VTG levels in shallow-lake wildlife.

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: $84,915**
Amount Spent: $70,201

Balance: $14,734

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 January 2015: The project manager trained two students how to take prepare materials, configure equipment, initiate measurements, and record data. Training and some preliminary analyses were completed in September, when students began taking classes. In addition, biological material from 64 turtles (approximately 85% of the total) has been sectioned, mounted and prepared for analysis. This is a major portion of the work necessary to prepare for the work that will take place over the next two years.

Activity Status as of July 2015: Two students have been preparing materials, taking measurements, and recording data. Biological material from all turtles has now been sectioned, mounted and prepared for analysis. The data collection and analysis of this material is ongoing, and is currently being examined with the computer-microscope setup under a double-blind paradigm. Because it is essential that all data is collected before analyses begin, statistical analyses and group identification cannot occur until all sections from all animals have been completed; thus, we have no results to report at this time.
Additionally, we have been able to recover extraneural tissue from the turtles involved in this study to examine how the morphology of other organs in the body may correlate with our findings in brain tissue. Measurements including carapace and plastron length, and follicle, penis, and testis size and weight have now been added to our database to provide a biological determinant for each animal's sex (rather than relying solely on appearance). This effort will improve the interpretation of the results from Activity 3.

Activity Status as of January 2016: Students have finished collecting data for one brain structure (the NPV) from over 400 sections from over 70 turtles. This data is now being prepared for analysis, including coding for the additional, extraneural variables collected. Because of the large number of sections, there is quite a bit of variability in the appearance of the NPV at similar locations, and the bulk of work currently is devoted to making sure that sections are "in register" with each other, so that analysis and interpretation of our data will be sound. This is slow but essential work for ensuring that the conclusions of our study are sound. In addition, literature and additional background searches currently being performed will enable us to choose other areas of the brain that we can examine for effects of exposure to environmental estrogens.

Activity Status as of July 2016: Data for the NPV has been prepared for analysis, including much coding for the additional, extraneural variables and merging data sets for integration with lake data. We have begun to look at ways to solve the problem of variability in the appearance of the NPV, which is turning out to be a difficult task. Again, this is slow but essential work, as our effect sizes may be small in relation to our measurements. The addition of a consultant on this task will reduce the time it takes to complete this activity. We may also need to collect additional turtles to carry out this work, depending on how the effect size compares with the variability in the data.

Activity Status as of January 2017: The professional consultant hired to carry out this activity has been extremely helpful. She is organizing the data, collecting and analyzing new data, and performing literature reviews to determine appropriate additional brain regions to study. We are now in the process of combining

these results into a comprehensive analysis to determine the relationship between the likelihood of EE exposure and brain structure.

Final Report Summary: We have discovered a significant relationship between VTG levels in turtles and the size of a sexually-dimorphic nucleus (the paraventricular nucleus, or NPV) in the brains of painted turtles. In lakes where overall VTG levels were low, the size of the NPV in males was significantly larger than in females, as has been previously reported in the literature. However, in lakes where the overall VTG levels measured in painted turtles was high, the average NPV was significantly smaller in males than in females. These results suggest that environmental estrogen exposure impacts brain structure in painted turtles, and does so in a sex-dependent manner. We presented this work at the North American Society for Environmental Toxicology and Chemistry annual meeting in November, 2017.

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 January 2015: No change from previous report.

Status as of July 2015: No change from previous report.

Status as of January 2016: No change from previous report.

Status as of July 2016: No change from previous report.

Status as of January 2017: No change from previous report.

Final Report Summary: So far, we have presented our findings to two regional and national conferences of the Society for Environmental Toxicology and Chemistry (SETAC). These meetings have been a good venue for dissemination of these findings, and have fostered communication with local, regional, national, and international contacts involved in similar work.

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget Overview:

Budget Category	$ Amount	Explanation
Personnel:	$ 51,447	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:	$ 13,250	Contract for Steven Bartell, Normandale Community College for vitellogenin assay development.
	$ 17,105	Contract for professional services work on brain correlates of EE exposure
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.	$ 31,115	Total funding for vitellogenin assay and brain structure equipment, supplies, services and consumables as detailed below.
	$ 5,845	Equipment for vitellogenin assay development
	$ 6,500	Supplies for vitellogenin assay development
	$ 4,500	Antiserum services for vitellogenin assay development
	$ 2,270	Supplies for brain structure analyses
	$ 12,000	Equipment for brain structure analyses
TOTAL ENRTF BUDGET:	**$ 112,917**	

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 (Stephen Bartell)

B. Other Funds:

Source of Funds	$ Amount Proposed	$ Amount Spent	Use of Other Funds
Non-state			
University of St. Thomas (cash support)	$ 18,000	$ 29,574	Funding for additional student research assistants
University of St. Thomas (in-kind support)	$ 24,000	$ 35,509	Equipment use and supplies
State	$ 0	$ 0	
TOTAL OTHER FUNDS:	**$ 42,000**	**$65,083**	

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. A professional contract will be issued to a qualified individual, identified through a competetive process, paid on an hourly basis, to provide assistance with analysis of neural correlates of environmental estrogen exposure.

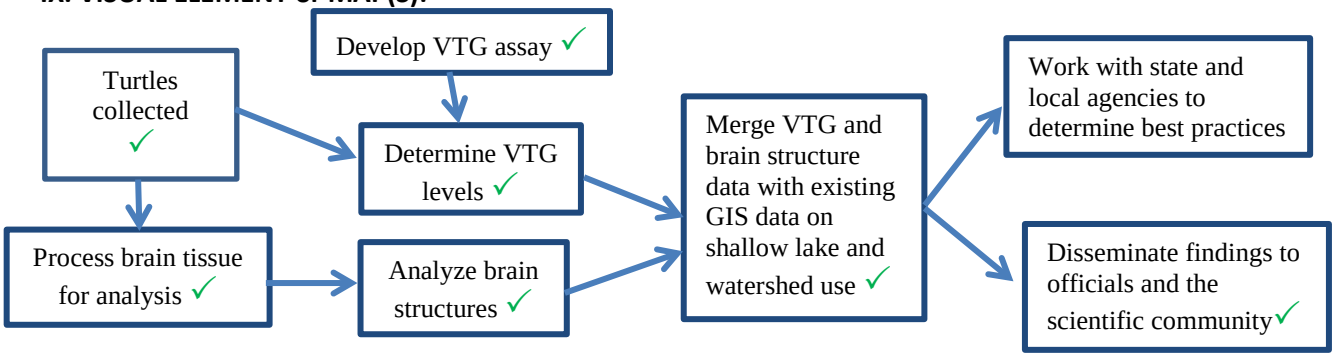
B. Project Impact and Long-term Strategy: The project has the potential of providing a significant impact into our understanding of how land use management affects Minnesota's shallow lake wildlife. Although the focused, directed studies in the proposed project are not likely to require more than three 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.

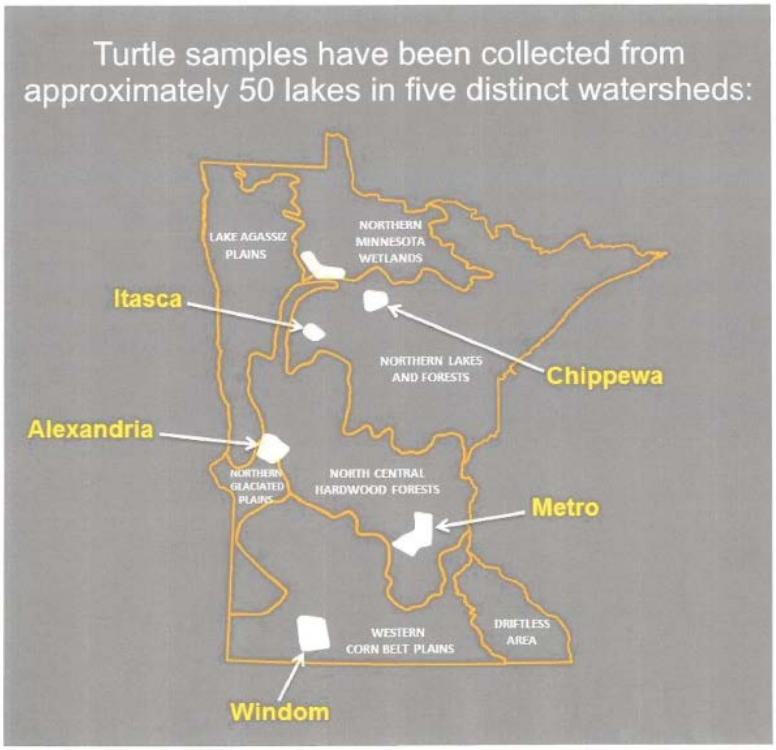
C. Spending History: A large amount of work already has been completed for this project, made possible by funding from the University of St. Thomas for student research, equipment and supplies, as outlined below:

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 January 2015, July 2015, January 2016, July 2016 and January 2017. A final report and associated products will be submitted between June 30 and August 30, 2017.

Environment and Natural Resources Trust Fund

FINAL 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: August 30, 2017

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Activity 1 Budget	Amount Spent	Activity 1 Balance	Activity 2 Budget	Amount Spent	Activity 2 Balance	Revised Activity 3 Budget 07/31/2016	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	$6,000	$0	$26,000	$26,107	-$107	$32,000	-$107
Undergraduate Students: Eight undergraduate researchers for 24 person-months work over three years (92.35 % towards salary, 7.65% FICA); 2 FTE				$8,000	$8,000	$0	$28,000	$11,340	$16,660	$36,000	$16,660
Professional/Technical/Service Contracts											
Stephen Bartell, Normandale Community College, VTG assay development contract; 0.25 FTE	$14,000	$13,250	$750							$14,000	$750
TO BE NAMED: Consultant (7/31/2016) Nervous system analyses; 0.25 FTE							$16,005	$17,105	-$1,100	$16,005	-$1,100
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	$16,925	$6,160				$14,910	$15,649	-$719	$37,995	$6,880
COLUMN TOTAL	**$37,085**	**$30,175**	**$6,910**	**$14,000**	**$14,000**	**$0**	**$84,915**	**$70,201**	**$14,734**	**$136,000**	**$23,083**