M.L. 2014 Project Abstract
For the Period Ending June 30, 2018

PROJECT TITLE: Evaluation of Wastewater Nitrogen and Estrogen Treatment Options
PROJECT MANAGER: Paige Novak
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2014, Chp. 226, Sec. 2, Subd. 03d as extended M.L. 2017, Chapter 96, Section 2, Subdivision 18

APPROPRIATION AMOUNT: $ 500,000
AMOUNT SPENT: $454,288
AMOUNT REMAINING: $45,712

Overall Project Outcome and Results
Wastewater treatment plants (WWTPs) discharge effluent that contains contaminants of emerging concern (CECs), including estrogens. These estrogens have caused ecological damage, such as fish feminization, with unknown long-term consequences. The most important estrogen exiting WWTPs is a chemical called estrone. In this project we studied how different treatment systems performed with respect to estrone degradation and how temperature effected degradation. We also studied how fish vulnerability changed seasonally. Finally, we combined these laboratory efforts with models of fish population dynamics to extrapolate the results.

We determined that the technology used by a treatment plant is likely to have an impact on the estrogenicity of WWTP effluent, with some technologies performing very well and others failing to remove estrone. In addition, natural seasonal fluctuations in temperature and expected fluctuations in estrone concentration can cause negative changes in exposed fish. Mathematical models were used to expand this research to whole river systems and showed that the impacts of estrone on fish populations varied depending on the characteristics of the environment. Impacts were expected to be low in systems in which fish were limited by food and high in systems in which fish were limited by predators. Therefore, fish populations in Minnesota rivers are likely to vary in their response to wastewater estrone. The cost of various wastewater improvements were calculated, which could be compared to the value associated with recreational fishing.

Overall, this research showed that low energy treatment systems do exist that are capable of excellent estrone removal, which should be considered so that multiple ecological benefits can be reaped as treatment plants upgrade. Nevertheless, modeling results suggest that the impacts of estrone vary at the population scale based on river characteristics. Therefore, the impact of estrogens at the fishery scale should be evaluated for a given river of interest.

Project Results Use and Dissemination
Information from this project has been shared broadly and multiple peer-reviewed manuscripts have been published from this work and submitted to the LCCMR.

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

Date of Status Update Report: September 4, 2018

Final Report

Date of Work Plan Approval: June 4, 2014

Project Completion Date: June 30, 2018

PROJECT TITLE: Evaluation of Wastewater Nitrogen and Estrogen Treatment Options

Project Manager: Paige J. Novak

Organization: University of Minnesota

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Location: Statewide

Total ENRTF Project Budget:		
	ENRTF Appropriation:	**$500,000**
	Amount Spent:	**$454,288**
	Balance:	**$45,712**

Legal Citation: M.L. 2014, Chp. 226, Sec. 2, Subd. 03d
M.L. 2017, Chapter 96, Section 2, Subdivision 18

Appropriation Language:
$500,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota to examine the performance of new wastewater contaminant treatment options under Minnesota weather conditions in order to understand how to improve wastewater treatment of nitrogen and estrogenic compounds, decrease costs and energy use, and safeguard aquatic species. This appropriation is available until June 30, 2017, by which time the project must be completed and final products delivered.

Carryforward (a) The availability of the appropriations for the following projects are extended to June 30, 2018: (1) Laws 2014, chapter 226, section 2, subdivision 3, paragraph (d), Evaluation of Wastewater Nitrogen and Estrogen Treatment Options.

I. PROJECT TITLE: Wastewater estrogen: removal options, fish abundance, and cost

II. PROJECT STATEMENT:
Wastewater treatment plants discharge effluent that contains contaminants of emerging concern (CECs), including estrogens. These estrogens have caused dramatic ecological effects such as fish feminization and fish population collapses, with unknown long-term consequences. The most important estrogen exiting wastewater treatment plants, in terms of contributing to the feminization potential of effluent, is a chemical called estrone, which is an estrogen that is released naturally from women via the waste stream. Although this and other estrogens are present in Minnesota lakes and rivers and can be ecologically harmful, their treatment and discharge are not regulated.

Interestingly, the discharge of estrone and other estrogens is a function of how (and how well) a treatment plant removes nitrogen. Nitrogen discharge is regulated to some extent in Minnesota and will be more heavily regulated in the future, requiring additional wastewater treatment plant upgrades. With this research we will determine how different nitrogen removal processes perform over the range of temperatures experienced in Minnesota with respect to both CEC (and in particular, estrone) and nitrogen removal so that the very best processes for the protection of Minnesota's natural resources can be put into place. In addition, we will determine how fish vulnerability changes seasonally so that treatment to extremely low levels of CECs is only required during critical periods (e.g., during egg maturation or spawning) to save energy and costs from excessive (and unnecessarily rigorous) treatment. Finally, we will combine laboratory efforts with predictive mathematical models so that we can extrapolate to cost and whole population behavior.

III. PROJECT STATUS UPDATES:

Project Status as of January 31, 2015:
We have completed design and construction for the five reactor systems to be used in this project. Initial "base case" experiments with synthetic wastewater and an influent estrone concentration of 10 µg/L have been completed and show 90% removal of estrone in the effluent. A control experiment to quantify estrone sorption within the reactor has also been completed. Three additional experiments are currently in operation: (1) conventional treatment using actual wastewater from the Metropolitan Wastewater Treatment Plant in St. Paul; (2) conventional treatment using a lower influent concentration of estrone (100 ng/L); and (3) granular aerobic sludge. These experiments will allow us to determine whether experiments performed with synthetic wastewater and slightly higher estrone feed concentrations (for ease of analysis) behave similarly to those performed with real wastewater and environmentally relevant estrone concentrations. Experiments with granular aerobic sludge represent experiments with a cutting edge lower-energy nutrient removal technology. Results for this experiment are expected by July, 2015.

We have also nearly completed the fathead minnow larval exposures (Activity 2). We collected eggs from minnow breeding groups and exposed each clutch to one of 20 treatments for 21 days (5 concentrations of estrone at 4 temperatures). We have collected data on hatching success, survival, predator avoidance behavior, and feeding efficiency sufficient for assessing the effects of temperature and exposure concentration on larvae. In addition, exposed fathead minnow larvae have been preserved appropriately for future vitellogenin analysis and assessment of growth and developmental abnormalities.

Amendment Request (07/16/2015):
The addendum is to formally request a re-budgeting of funds for this project.

As part of the project, we would like to establish one additional personnel category: undergraduate researcher. The undergraduate will assist the graduate student researchers with routine activities (running reactors) to enable the graduate student to spend more time on higher-level functions such as data analysis and estrone analysis. All of the required rebudgeting will remain within the "**Personnel**" category and will simply move from sub-category to sub-category.

The movement of money between sub-categories will not affect project objectives or timelines.

Amendment Approved: July 20, 2015

Project Status as of July 31, 2015:
We have completed the following experiments regarding estrone degradation during biological nitrogen treatment: (1) conventional nitrification with synthetic and actual wastewater at 10 µg/L influent estrone, (2) conventional treatment using a lower influent concentration of estrone (100 ng/L) and synthetic wastewater, (3) Modified Ludzack-Ettinger (MLE) process fed actual wastewater and (4) sequencing batch reactor systems fed high (1000 mg/L) and standard (200 mg/L) wastewater carbon to study estrone removal in a granular aerobic sludge system. We found excellent removal of estrone in the conventional, MLE, and low carbon sequencing batch reactor systems (>90% removal), but no estrone removal for the high carbon sequencing batch system (the granular aerobic sludge system). Results to date suggest that aeration benefits estrone degradation and that the repeated exposure to high carbon concentrations and/or granulation is detrimental to estrone removal. We are currently operating conventional reactors at low temperatures (15°C), as well as several additional novel reactor systems for total nitrogen removal. These additional experiments will show whether low oxygen systems can be effective for estrone removal and will also demonstrate the effect of cold temperatures on estrone degradation. Results for all these experiments are expected by January 2016.

All fathead minnow experiments have been completed. Both adult flow-through exposures and larval batch exposures were conducted at estrone concentrations of 0, 25, 125 or 625 ng/L and at temperatures of 15, 18, 21, or 24°C. A total of 2,292 fish were studied. Data is currently being analyzed to determine the statistical significance of the results and to verify the estrone concentrations used during the exposures.

Population modeling and economic modeling studies will be starting shortly.

Project Status as of January 31, 2016:
We have completed all of the wastewater experiments in which estrone degradation during biological nitrogen treatment was monitored. Final analysis of the estrone concentrations in the SHARON and anammox reactors is being completed. Anaerobic estrone controls have been performed to assess sorption, but an additional abiotic control experiment may be completed in the next several months to clarify sorption of estrone to the membrane used to filter the bacteria from the effluent. This is not expected to alter our conclusions, but will provide the necessary quality assurance for a strong publication. New observations indicated that during low temperature (15°C) operation nitrification slows, but after a very short lag period, estrone degradation is unaffected. Estrone appears to be effectively degraded at the low oxygen addition required for only partial ammonia oxidation in the SHARON system, but this is currently being verified through additional estrone analysis. Effluent estrone concentrations have not yet been analyzed in the anammox experiment; nevertheless, estrone degradation is not anticipated because anammox operates under complete anaerobic conditions. Two manuscripts should be completed in the next six months: one that incorporates the temperature data from the wastewater experiments with the minnow data, and a second that focuses on estrone degradation in novel nitrogen treatment systems.

Larval and adult behavioral data have now been quantified from video tapes and the statistical data analyses assessing the effects of E1 on hatching success, early survival, fecundity, fertility, behavioral impairment (4 assays), and biochemical endpoints are largely completed. Additional analysis of the adult fathead minnow tissues is ongoing. Our initial data provide some evidence that temperature modulates the effects of estrogen exposure in both adult and larval fathead minnows, but that its influence is limited and unpredictable. A preliminary draft of a manuscript reporting the results of the behavioral assays has been written.

The mathematical model for predicting minnow and bass abundance is in development. We are focusing on the most important pathway in this model, which links environmental estrogen concentration to population-level effects via the estrone concentration in individual fish and the effect that this concentration has on the feeding, maintenance, growth, and/or reproduction of individual fish. We are using a well-tested ecotoxicological model to convert environmental estrogen concentrations to internal estrogen concentrations, and a bioenergetics approach to convert these internal concentrations to individual-level effects. We have developed and parameterized this part of the model for fathead minnows, and are now testing it to make sure that model output is consistent with results from Activity 2.

Economic modeling studies will be starting shortly.

Amendment Request (01/22/2016):
Our findings from Activity 2 (Outcome 1) indicate that temperature does not strongly or consistently modulate the effects of E1 on reproduction, development, and egg survival. However, our results suggest that temperature may interact with E1 to alter larval predator avoidance performance. In light of these findings, we propose to amend the work plan for Activity 2. Specifically, we propose to conduct a new, large-scale (40 treatment), factorial predation experiment in lieu of collecting basic data on the reproduction and survival of smallmouth bass exposed to wastewater contaminants. The proposed changes to the work plan will allow a more thorough understanding of the effects of estrogen exposure on trophic interactions under different thermal regimes. This will not alter the budget.

A second amendment request involves changing the budget such that Jay Coggins, Professor, Department of Applied Economics, can be paid from the project as well. Dr. Frances Homans has accepted a position as Department Head of the Department of Applied Economics at the University of Minnesota and has therefore brought Dr. Coggins into the project to assist with the economic/costing tasks (Activities 1 and 3). This will not result in budget changes, only the addition of Jay Coggins' name to the project so that he and Dr. Homans can allocate the currently-budgeted salary as they see appropriate.

Amendments Approved: January 27, 2016

Retroactive Amendment Request (02/5/2016):
After submitting our project status report and amendment request 1/22/16 we realized that our travel budget was overspent by $774. In addition, travel funds have been needed to pick up wastewater from the Metropolitan Wastewater Treatment (Metro) Plant in St. Paul and transport it to the laboratory for use in experiments, rather than only for project meetings. When we began the research we thought that we would be able to use synthetic wastewater created in the laboratory. We verified this assumption by performing replicate estrone and nitrogen degradation experiments with both synthetic wastewater and real wastewater from the Metro Plant. Although estrone behaved identically in the two experiments, nitrogen did not; therefore, we felt that it was important to perform experiments with real wastewater rather than synthetic so that we could be sure that our results were transferrable to actual wastewater systems. This change necessitates the collection of 100 L of wastewater per week and therefore requires the student performing the experiments to collect the wastewater using a vehicle from fleet services at the University of Minnesota. We therefore request that $1000 be moved from the "Personnel" line item to the "Travel expenses in MN" line item. We also request that the following language be added to the budget descriptor within the "Travel expenses in MN" line item: "*Travel funds are ~~extremely~~ minimal ($1,400) and are included for travel to meetings at either St. Cloud State University or the University of Minnesota for project coordination or to the Metropolitan Wastewater Treatment Plant for sample collection for use in experiments.*" The proposed changes to the work plan will allow a more accurate and realistic assessment of estrone and nitrogen removal during wastewater treatment. This will not alter the overall budget or timing of the project.

Amendment Approved: February 9, 2016

Project Status as of July 31, 2016:
The experiments for Activity 1 on the degradation of estrone in different nitrogen removal systems have been completed. Estrone removal was excellent when fed to the nitrification (room temperature and cool temperature), MLE, and sequencing semi-batch systems, at 96%, 96%, and 97% mean estrone loss, respectively. The aerobic granular sludge system failed to remove estrone (14% mean estrone loss), which was perhaps not unexpected given the very high COD loading that is required for granulation to occur. Surprisingly, the anammox system also resulted in excellent estrone removal (99.8% mean estrone loss), despite the anaerobic nature of anammox. The anammox results are particularly exciting, as this is a low-energy alternative for total nitrogen removal. The estrone removal results from the SHARON reactor system were variable and unreliable, despite repeating this experiment three times. Two manuscripts (one that includes data from the minnow experiments) are in preparation and should be submitted by the next project update.

We have completed our analysis of larval and adult fathead minnow exposures at four temperatures (15, 18, 21, 24°C) and four concentrations of estrone (0 ng/L, 25 ng/L, 125 ng/L, 625 ng/L nominal concentrations). Results from the behavioral assessment of these exposed fish have been summarized in a manuscript draft that is currently being circulated among the co-authors for comment. Results from physiological and anatomical assessments of adult fathead minnows exposed in the above experiments are currently being prepared as a draft manuscript that will be circulated among co-authors shortly. Larval fathead minnows exposed in these experiments are currently processed for gene expression. RNA has been extracted from all 140 larvae and gene expression assays will be conducted shortly.

We have made some progress on the mathematical model for predicting minnow and bass abundance. The post-doc who was a part of this activity left at the end of February 2016, but has continued to work on the model in his spare time and taken steps to ensure that his replacement, who starts December 1 2016, can pick up where he left off.

The cost information on biological nutrient removal processes has been collected and summarized from recent literature. The capital cost includes the impact of design capacity of wastewater treatment facilities, but the positive relationship between the cost and corresponding treatment capacity does not seem significant for all treatment technologies. A remaining challenge is to isolate cost performance and efficiency as they depend upon treatment capacity, suitable treatment technology and spatial characteristics. Work is underway to develop and refine a set of mathematical models of the costs of alternative treatment technologies.

Retroactive Amendment Request (07/25/2016):
The addendum is to formally request a re-budgeting of funds for this project.

We would like to move funds ($4,393) from the personnel category to the laboratory supplies category to cover an over-expenditure of $4,393, which was required to complete the wastewater experiments. The cost of estrone analysis was higher than anticipated as a result of these analyses being performed in a core facility (charge-per-analysis) and resulted in overruns in the laboratory supplies category. It is difficult to estimate in advance (at the proposal stage) exactly how many samples will need to be analyzed to generate high quality data. In addition, sometimes samples need to be re-analyzed as a result of the need for dilution for better quantification of analytes. We have now completed the experimental work in Activity 1 and should not have further laboratory supply expenditures. The graduate student working on the wastewater experiments in Activity 1 graduated at the beginning of June, 2016, rather than August, 2016 as planned, and the funds that would have paid her over the summer are therefore available for this transfer.

The movement of funds between these categories will not affect project objectives or timelines.

Approved by LCCMR 7-28-2016

Project Status as of January 31, 2017:
The experiments for Activity 1 on the degradation of estrone in different nitrogen removal systems have been completed. One additional experiment was performed that showed that estradiol was not a product of estrone degradation under anammox conditions. One manuscript has been resubmitted and a second (that includes data from the minnow experiments) is in preparation.

The large-scale, factorial predation experiment with the predator, bluegill sunfish (*Lepomis macrochirus*), and prey, larvae of the fathead minnow (*Pimephales promelas*), has been completed and the data have undergone a preliminary analysis. The results of this analysis indicate the following main results: (i) sunfish exposed to 625 ng/L estrone exhibited pronounced physiological responses to estrone exposure at all four temperatures, (ii) sunfish exposed at 125 ng/L show a less pronounced and consistent response, (iii) sunfish exposed at the lowest temperature (15°C) fed less than sunfish at the three higher temperatures, (iv) independent of temperature, estrone exposed larval fathead minnows were eaten at a greater frequency then ethanol control larvae as indicated by the approximately 12% greater survival of control fish across trials, (v) estrone concentration (125 ng/L and 625 ng/L) did not affect the greater rate at which exposed larvae were eaten suggesting that a detrimental threshold of exposure exists below 125ng/L. The results of these experiments are being incorporated into the population models under development.

We are making up lost ground on the mathematical model for predicting fish abundance and the impacts of estrone. The new post-doc for this activity started December 1 2016. He brought himself up to speed by reviewing existing material and the literature, and working with the former post-doc and project leaders. We are now coding the heart of the model, which are two sub-routines that govern how individual fish allocate food energy to metabolism, growth, reproduction, etc. (and how this is impacted by estrone concentrations). We will present this work at the Minnesota Chapter of the American Fisheries Society annual meeting in February.

We are currently developing tables to be used for a cost-benefit analysis of different wastewater treatment processes, including the MLE process, nitritation-denitritation and anammox, which show both the treatment costs and the additional benefits of different nitrogen removal processes. The treatment cost is categorized into two parts: the capital cost and the operation and management cost. The additional benefit category considers the benefits of a change in fish population (in monetary units based on recreational and economic value) as a result of changes in estrone removal. The efficiency of nitrogen removal can be easily computed from the table, to assist with a comparison among different wastewater treatment processes.

<u>Amendment Request (01/31/2017):</u>
The addendum is to formally request a 6-month extension for this project. The project is scheduled to end 30 June 2017, but we are requesting that this be changed to 31 December 2017. The extension is necessary because of an unexpected change in personnel. The 2-year post-doc who we hired for Activity 3 left the project after 8 months (28 February 2016). We found a highly-qualified replacement in just three months, but he could not begin work until 1 December 2016. Because we lost 9 months of productivity, a 6-month extension shortens Activity 3 from 24 to 21 months; this is, however, a necessary compromise given the length of the vacancy and remaining funds. These funds are sufficient to pay this post-doc until 31 December 2017. Outcomes will be met by the new deadline.

Approved by LCCMR 5-30-2017

Project Status as of July 31, 2017:
The experiments for Activity 1 on the degradation of estrone in different nitrogen removal systems have been completed. One manuscript has been accepted for publication; a second (containing data from both Activity 1 and Activity 2) has been submitted for publication.

The experiments for Activity 2 on the effects of temperature, life stage, and estrone concentration on fathead minnows are also completed. One manuscript has been published, one submitted (including Activity 1 data, see above), and a third is in preparation.

The fish population model is now coded. The sub-routines that govern how individual fish allocate food energy to metabolism, growth, reproduction, and activity are parametrized, and we are calibrating the model to observed fish densities in Minnesota rivers. We are making good progress on incorporating estrone effects into this model. We will present our results at the Society of Environmental Toxicology and Chemistry North America 38th Annual Meeting at Minneapolis in mid-November.

Project Status as of January 31, 2018:
The experiments for Activity 1 and Activity 2 on the degradation of estrone in different nitrogen removal systems and their effects on fish at different temperatures have been completed. Three manuscripts have been published or accepted for publication on this work; 2 additional manuscripts (containing data from Activity 2) are being prepared for publication.

We calibrated the population model to reproduce realistic fish densities in different river systems. We ran simulations based on the results of Activity 2 and are also running additional simulation scenarios. A first manuscript is in preparation, one conference presentation was made in mid-November at a National meeting, and an additional conference presentation will be made in late January, 2018.

The economic analysis of alternative nutrient removal processes is well along and will be completed in the current semester. The goal of this work is to compare the cost of nutrient removal, in terms of plant upgrade and operations cost, to the value of estrone removal, in terms of fish population value.

The cost information on biological nutrient removal processes has been collected and summarized from recent literature. Depending on available technologies for wastewater treatment, total capital cost on upgrading facilities has been generalized, with a focus on comparing the cost of anammox and granular aerobic sludge facilities. Remaining steps are first, to estimate to the nitrogen removal achieved by the treatment facilities under study and the benefits of such removal and second, to link costs/benefits of nitrogen removal to the value of estrone removal. Because the valuation of fish populations is highly variable, we will compute the dollar value per fish that would be minimally necessary to justify the expense associated with an improved treatment technology.

Overall Project Outcomes and Results:
Wastewater treatment plants (WWTPs) discharge effluent that contains contaminants of emerging concern (CECs), including estrogens. These estrogens have caused ecological damage, such as fish feminization, with unknown long-term consequences. The most important estrogen exiting WWTPs is a chemical called estrone. In this project we studied how different treatment systems performed with respect to estrone degradation and how temperature effected degradation. We also studied how fish vulnerability changed seasonally. Finally, we combined these laboratory efforts with models of fish population dynamics to extrapolate the results.

We determined that the technology used by a treatment plant is likely to have an impact on the estrogenicity of WWTP effluent, with some technologies performing very well and others failing to remove estrone. In addition, natural seasonal fluctuations in temperature and expected fluctuations in estrone concentration can cause negative changes in exposed fish. Mathematical models were used to expand this research to whole river systems and showed that the impacts of estrone on fish populations varied depending on the characteristics of the environment. Impacts were expected to be low in systems in which fish were limited by food and high in systems in which fish were limited by predators. Therefore, fish populations in Minnesota rivers are likely to vary in their response to wastewater estrone. The cost of various wastewater improvements were calculated, which could be compared to the value associated with recreational fishing.

Overall, this research showed that low energy treatment systems do exist that are capable of excellent estrone removal, which should be considered so that multiple ecological benefits can be reaped as treatment plants upgrade. Nevertheless, modeling results suggest that the impacts of estrone vary at the population scale based on river characteristics. Therefore, the impact of estrogens at the fishery scale should be evaluated for a given river of interest.

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Determine the performance of different wastewater treatment processes with respect to nitrogen removal, CEC and estrone removal, energy use, and cost

Description: Five laboratory-scale reactor systems will be set-up to mimic different wastewater treatment systems, including conventional (NH_3 removal-only) treatment (*CONV*) and four treatment systems designed for total nitrogen removal. Reactors will be constructed from glass and will be designed to mimic the most basic total nitrogen removal process (the Modified Ludzack-Ettinger (MLE) process), a nitritation-denitritation system (*N-D*), an *ANAMMOX* system, and a low-oxygen granular sludge process (*GRAN*). All of the reactors, except for the *GRAN* reactor, will be fed continuously and operated with solids residence times of 10-25 days depending on the reactor. A membrane separation system will be used with each reactor set-up to retain the biomass for recirculation. Experiments will initially be performed at approximately 72°F followed by a second set of experiments performed at approximately 59°F. Two exceptions are the experiments performed with the *N-D* and *ANAMMOX* reactors, which require heating of the aerobic (both, 90°F) and second anaerobic (*ANAMMOX* only, 97°F) reactors to function optimally. In these cases the feed to the reactors will be heated and the energy used for heating will be incorporated into the cost calculations. When appropriate, reactors will be aerated. The flow rate of oxygen required to meet the dissolved oxygen set-point will be monitored daily and will be incorporated into energy utilization and cost calculations. Reactors will be fed synthetic wastewater amended with estrone. Reactor effluents will be monitored for soluble COD, estrone, NH_3, NO_3^-, NO_2^-, and dissolved oxygen. Two of the experiments will be repeated in triplicate to verify reproducibility. Finally, two to three experiments will be repeated with influent secondary wastewater from the Metropolitan Plant in St. Paul, MN to which estrone has been amended. This will allow verification of the trends observed with synthetic wastewater amended with estrone with a more complex feed. The effluent will also be collected, and the CECs present will be extracted and amended to aquaria in fish exposure experiments (described under Activity 2). We have experience operating similar systems.

Summary Budget Information for Activity 1:	**ENRTF Budget:**	**$ 140,450**
	Amount Spent:	**$ 140,311**
	Balance:	**$ 139**

Activity Completion Date: February 28, 2017

Outcome	Completion Date	Budget
1. Nitrogen removal efficiency in five different wastewater treatment plant configurations	10/31/2016	$62,125
2. Estrone/CEC removal efficiency in five different wastewater treatment plant configurations	10/31/2016	$62,125
3. An estimate of the energy use for the various treatment options	02/28/2017	$5,000
4. An estimate for the cost of the various treatment options	02/28/2017	$10,000

Activity Status as of January 31, 2015:
Reactor design, configuration, and construction for the five reactor systems (conventional, Modified Ludzack-Ettinger, nitrification-denitrification, anammox, and granular aerobic sludge) have been completed. Analytical methods for quantification of estrone, dissolved organic carbon, ammonia, nitrate and nitrite have been

developed and tested. A method for total nitrogen analysis is still undergoing development due to digestion issues. Sludge samples for seeding reactors have been obtained from the Metropolitan Wastewater Treatment Plant in St. Paul and preserved for use in all experiments.

The base case study, conventional treatment with synthetic wastewater with an influent concentration of 10 µg/L of estrone, has been completed. Results show 90% removal of estrone in the effluent. An anaerobic control to account for estrone removal through sorption to sludge or membrane module has also been completed, with results pending sample analysis.

Three additional experiments are currently in operation: (1) conventional treatment using actual wastewater from the Metropolitan Wastewater Treatment Plant in St. Paul; (2) conventional treatment using lower influent concentrations of estrone (100 ng/L); and (3) granular aerobic sludge. The first two configurations will run until 2/2 and 2/9 respectively. Once (1) and (2) are complete, verifying that the base case results are applicable to real wastewater and environmentally relevant estrone concentrations, a second base case configuration and a Modified Ludzack-Ettinger configuration will be operated. The granular aerobic sludge reactor has been operating for a month and a half, and granule formation has begun, though full granulation is still not complete. We expect to operate this reactor for at least another month, and possibly longer, depending on the granulation timeline and changes in reactor performance.

Activity Status as of July 31, 2015:
Several quality assurance experiments were performed with the conventional reactor configuration to determine whether (1) synthetic wastewater could be reliably used in experiments, as opposed to real wastewater, and (2) degradation of estrone differed between reactors fed 10 µg/L versus 0.1 µg/L estrone. Results showed that although estrone degradation was identical in experiments performed with real wastewater and synthetic wastewater, the nitrogen removal differed. Therefore, all experiments are being conducted with real wastewater so that clear linkages between the different technologies designed for nitrogen removal can be made with estrone degradation performance. Results also showed that the degradation of estrone was very similar (approximately 90% degraded) in experiments fed 10 µg/L versus 0.1 µg/L estrone. Therefore, experiments are being performed with a 10 µg/L estrone feed to simplify the analytical chemistry required.

The following experiments have been completed with real wastewater feed at an influent of 10 µg/L estrone: (1) conventional nitrification, (2) Modified Ludzack-Ettinger (MLE) (3) sequencing batch reactor operation fed high concentrations of wastewater carbon (1000 mg/L) to induce sludge granulation, and (4) sequencing batch reactor operation fed low concentrations of wastewater carbon (200 mg/L) with no sludge granulation. Excellent estrone degradation was observed in the conventional, MLE, and low carbon sequencing batch systems (>90% removal), but no estrone removal occurred in the high carbon sequencing batch system containing granular sludge. These results suggest that repeated exposure to high carbon concentrations and/or granulation is detrimental to estrone removal. This is as expected based on our previous published research. We are currently operating conventional reactors at low temperatures (15°C), as well as a reactor operating with partial nitrification, called nitritation (SHARON), which is fed only very low concentrations of oxygen and is operated at a high temperature to control the microbial populations present. Preliminary results for SHARON show good estrone removal (>90%), though we have not yet been able to achieve consistent partial nitritation at this time. Two additional sets of reactors will be evaluated for estrone removal in the coming months: high-rate anaerobic treatment followed by partial nitritation by SHARON, and anammox. Results for all these experiments are expected by January 2016.

Activity Status as of January 31, 2016:
The following additional experiments have been completed since July 31, 2015: (1) cool temperature (15°C) conventional nitrification (2) cool temperature (15°C) conventional nitrification duplicate, (3) SHARON and (4) anammox. Experiments 1, 2, and 3 were conducted with 10 ug/L estrone added to real wastewater from the local Metropolitan Wastewater Treatment Plant. Experiment 4 used a synthetic wastewater feed that delivered

the required nitrite to ammonia ratio for successful anammox activity; 10 ug/L estrone was also added in this experiment.

Estrone degradation (>90% removal) was observed in both 15°C conventional nitrification experiments at the end of the 50 to 60 day experiments. The cool temperature (15°C) experiments exhibited a slight delay in the initiation of estrone degradation after experiment start up as compared to the room temperature conventional nitrification experiments performed in fall 2014. Preliminary data indicated that the room temperature experiments exhibited >90% removal prior to Day 3 of the experiment, whereas the 15°C experiments did not exhibit >90% estrone removal until after Day 3.

The SHARON and anammox experiments were successfully completed. The method of estrone analysis is currently being assessed for quality assurance and quality control prior to the analysis of the SHARON and anammox estrone samples. Preliminary results suggest that estrone removal did occur in the SHARON reactor. Final results from estrone analysis for all experiments are expected by May 2016, completing Activity 1.

Activity Status as of July 31, 2016:
All experiments for Activity 1 have been completed. Unexpectedly, estrone was effectively degraded in the anammox reactor, with 99.8% mean estrone removal in this reactor. These results are very exciting and were not anticipated, as anammox operates under anaerobic conditions, which are not expected to be conducive to estrone degradation.

Upon further analysis of the SHARON results, the estrone removal was determined to be quite variable. Because SHARON and anammox are operated in series at full-scale installations, the degradation of estrone in the anammox reactor will enable the use of this coupled system for effective estrone treatment, despite the variable performance of the SHARON system.

We are collaborating on a manuscript on the influence of temperature on estrone degradation and on the impact of estrone on fish. This manuscript is in preparation and should be completed by late summer/early fall 2016. We have also drafted a second manuscript focusing on the degradation of estrone in different nitrogen removal treatment systems. This manuscript is being reviewed by one of the coauthors and should be submitted by the end of summer.

Activity Status as of January 31, 2017:
All experiments for Activity 1 have been completed. One additional experiment was performed, as requested by reviewers of our manuscript, to determine whether estrone transformation to estradiol occurs during anammox treatment. This experiment showed that estrone was again degraded under anammox conditions but without the concomitant formation of estradiol. This is important because it shows that estrone degradation follows an unknown pathway to unidentified products.

We have resubmitted the manuscript describing this research to the top disciplinary journal in Environmental Engineering and expect a decision within two months. In addition, Kira Peterson, the Master’s student who completed the research on Activity 1 and wrote her Master’s thesis on this work, was awarded the Cale Anger Master’s Thesis Award and the University of Minnesota Distinguished Master's Thesis Award in Mathematics, Physical Sciences and Engineering for her research. The collaborative manuscript on the influence of temperature on estrone degradation and on the impact of estrone on fish is in progress.

Activity Status as of July 31, 2017:
All experiments for Activity 1 have been completed and results accepted or submitted for publication. In this research estrone removal was compared in a laboratory-scale system that modeled current conventional wastewater treatment with removal in laboratory-scale systems designed to remove total nitrogen from wastewater: the Modified Ludzack-Ettinger (MLE) system (a two-stage anaerobic-aerobic system with recycle), a

granular activated sludge system (cycled anaerobic-aerobic), a sequencing batch reactor (cycled anaerobic-aerobic), and an anaerobic ammonia oxidation (anammox) system. Estrone removal was excellent when fed to the nitrification, MLE, and sequencing batch reactors, at >96% mean estrone loss. Excellent estrone removal occurred in a nitrification system operated at 15°C as well, though there was a slight lag in degradation. The granular activated sludge system operated in our laboratory failed to remove estrone, which was perhaps not unexpected given the high carbon loading under which our system was operated. Despite the anaerobic nature of anammox, it also resulted in excellent estrone removal (95% mean estrone loss) without concomitant 17β-estradiol production. This work demonstrates that the choice of nitrogen removal technology used by a treatment plant could have an impact on the estrogenicity of WWTP effluent, but low energy total nitrogen removal systems do exist that are capable of excellent estrone removal.

Activity Status as of January 31, 2018:
All experiments have been completed and there are no additional updates.

Final Report Summary:
In this study, the degradation of a common and persistent human estrogen, estrone (E1) was studied in several different laboratory-scale reactor configurations and in one case, at two different temperatures. E1 removal in a laboratory-scale system that modeled current conventional wastewater treatment (designed only to transform, but not completely remove total nitrogen) was compared with that in laboratory-scale systems designed to remove total nitrogen from wastewater: the Modified Ludzack-Ettinger (MLE) system (a two-stage anaerobic-aerobic system with recycle), a granular activated sludge system (cycled anaerobic-aerobic), a sequencing batch reactor (cycled anaerobic-aerobic), and an anaerobic ammonia oxidation (anammox) system. E1 removal was excellent when fed to the conventional (nitrification) system, as well as the MLE system and the sequencing batch reactor, with >96% mean E1 loss in all cases. Excellent E1 removal occurred in a nitrification system operated at 15°C as well, though there was a slight lag before degradation began, demonstrating that E1 degradation slows when the temperature drops. Although this was not a problem at 15°C, with eventual excellent E1 degradation, it is possible that at lower temperatures (5-10°C), E1 could persist. E1 did not degrade in the granular activated sludge system operated in our laboratory, which was perhaps not unexpected given the high carbon loading under which our system was operated. Interestingly, despite the anaerobic nature of anammox, E1 degraded well in this reactor system (95% mean E1 loss) without concomitant 17β-estradiol production. This work demonstrates that the choice of nitrogen removal technology used by a treatment plant could have an impact on the estrogenicity of WWTP effluent, but low energy total nitrogen removal systems do exist that are capable of excellent E1 removal. As treatment plants upgrade to implement total nitrogen removal, anammox would be a system worth considering for this reason.

ACTIVITY 2: Determine how temperature and life stage alter the reproduction and survival of fathead minnows after exposure to a common wastewater contaminant

Description: First, we will employ a staggered blocked design using a model fish species (fathead minnow, *Pimephales promelas*), at two life stages, two temperatures and five exposure treatments to determine windows of vulnerability for fish to effluent exposure. The life history of non-migrating North American fishes usually contains two life stages during which the fish are assumed to be particularly vulnerable to the effects of environmental estrogens: (i) the embryonic/early larval stage during which organogenesis occurs and (ii) the period during which adult fish produce gametes and reproduce. We will expose fathead minnow to estrone-amended water during both stages. This species was chosen because it is native to North America, widespread and abundant in many aquatic environments, readily available from controlled culture facilities, and has been used as model species for laboratory and field studies of CECs in the past. Both life stages will be exposed at four temperatures (15°C; 18°C; 21°C; 24°C) to mimic conditions across the life history stages of these fishes. Following exposure, larvae will be assessed in their ability to perform innate predator avoidance behaviors. Adult fishes (males and females) will be assessed for changes in their reproductive behavior. Fish will also be analyzed for vitellogenin concentrations (a precursor protein involved in egg production and a sign of feminization of male fish) and their livers and reproductive organs will be evaluated for changes. Second, we will conduct a large-scale predation experiment to determine how variation in the thermal regime modulates the effects of contaminants present in wastewater effluent on natural predator-prey relationships. Juvenile (45 dph) fathead minnows (prey) and adult piscivorous bluegill sunfish (*Lepomis macrochirus*) (predators) will be exposed to E1 (i.e., 25, 125, or 625 ng L^{-1}) or maintained under control conditions (0 ng L^{-1}) at four temperatures (15°C; 18°C; 21°C; 24°C) for 45 days. Trials will be conducted in a factorial manner (i.e., exposed predator/non-exposed prey; non-exposed predator/exposed prey; exposed predator/exposed prey; non-exposed predator/non-exposed prey) (40 total treatments). The resultant data will be used to develop the fish biomass model described in Activity 3.

Summary Budget Information for Activity 2: **ENRTF Budget: $ 186,800**
Amount Spent: $ 185,130
Balance: $ 1,670

Activity Completion Date: March 31, 2017

Outcome	Completion Date	Budget
1. Reproduction and survival data for larval and adult fathead minnows exposed to estrone (E1) at seasonally-appropriate temperatures	03/31/2016	$93,500
2. Predation experiment assessing the effect of exposure to wastewater contaminants on predator-prey relationships (fathead minnows and bluegill sunfish)	03/31/2017	$93,500

Activity Status as of January 31, 2015:

Exposures, hatching success and early survival

Thus far, we have collected fathead minnow eggs from 45 different breeding groups. We randomly exposed these eggs to one of 20 different treatments (i.e., a concentration of 0, 5, 25, 125 or 625 ng L^{-1} estrone at a temperature of 15, 18, 21, or 24°C). To date, a total of 229 clutches have successfully hatched under exposed conditions, or are currently undergoing exposure. Current sample sizes for each treatment range from 10-12, or 83-100% of our minimum goal sample size of 12 replicates in each treatment. For each clutch hatched to date, we recorded the proportion of fertilized eggs that hatched (hatching success) and the proportion of hatched eggs that survived to Day 21 (larval survival). We also recorded the latency to hatching and the duration of the hatching period (in days). Preliminary analyses indicate that the data generated to date are sufficient for assessing hatching success and early survival.

Behavioral endpoints – predator avoidance and feeding efficiency

To date, we have conducted a total of 372 behavioral trials (i.e., between one and three trials per treatment replicate, per behavioral assay). Current treatment sample sizes for the predator avoidance assay (C-start assay) range from 7-12 (total 183 trials). Replicate sample sizes for the larval feeding assay range from 6-12 (total 189 trials). The treatment replicates tested to date are between 60% and 120% of our minimum goal sample size of 10 replicates in each behavioral assay. For the predator avoidance trials conducted thus far, we used a high-speed camera to generate a video of the response of a single larva to a simulated predator. These videos were stored off-line and are currently awaiting quantification by eye for several measures of escape behavior, including response latency, velocity of escape, and turning angle. To quantify feeding efficiency, we recorded the number of brine shrimp consumed by two larvae over a 1 min period. Each trial conducted to date was filmed using a digital video camera. Preliminary analyses indicate that the data that we have generated are sufficient for assessing the selected behavioral endpoints of exposure.

Vitellogenin analysis and developmental abnormalities

All of the larvae used to date in the C-start and predator avoidance assays (i.e., 1620 individual fish) were placed in RNAlater immediately following the completion of each trial. These samples were subsequently frozen at -20°C and are currently awaiting vitellogenin gene expression analysis. The remaining fish from each treatment replicate were euthanized on Day 21 of exposure via an overdose of MS-222, and stored in formalin. These fish will be used to assess the effects of exposure on larval growth and development.

Activity Status as of July 31, 2015:

Adult fathead minnow flow-through exposures

We have completed the adult fathead minnow exposures using the flow-through system. We exposed a total of 672 fish (two females and one male per tank) to each of 16 different estrone treatments (i.e., a concentration of 0, 25, 125 or 625 ng L^{-1} estrone at a temperature of 15, 18, 21, or 24°C) for 30 days. Between 8 and 14 breeding groups were established in each treatment. We monitored the fish daily throughout the exposure period and recorded the number of eggs laid and the proportion of eggs that were fertilized for each breeding group. Beginning on Exposure Day 10 and continuing through Exposure Day 20, we collected one clutch from each breeding group and placed it in a commercial fry basket in the exposure tank containing the parental fish. We monitored the clutch and recorded the proportion of fertilized eggs that hatched (hatching success) and the proportion of hatched eggs that survived to Day 21 (larval survival). We also recorded the latency to hatching and the duration of the hatching period (in days). Behavioral assays (feeding rate, male-male aggression) were conducted for all adult minnows on Day 30; the fish were then immediately sacrificed and dissected (see *behavioral endpoints* and *vitellogenin analysis and histology* below). Exposures continued to Day 21 for the larvae in the fry basket. On the morning of Day 22, the larvae were tested in the same behavioral assays described above for static-exposed larvae (i.e., predation evasion and feeding rate). These data will allow for comparisons between our flow-through and static exposure set-ups.

Adult behavioral endpoints – male-male aggression and feeding rate

At the end of the exposure period (Day 30) we examined whether estrone exposure altered the aggressive intensity with which males defended their nests from intruding males, and whether exposure affected the ability of fish to capture live prey (*Daphnia pulex*). A total of 224 resident, exposed males (10-14 males per treatment) were assessed for aggressive intensity by quantifying the number of aggressive acts, and the latency to first response, performed towards a (non-exposed) intruder male over a 5-min period. A total of 384 fish were assessed for feeding rate (192 males and 192 females, 12-28 fish per treatment). Individual fish were introduced to a feeding arena and provided with 30 mature *D. pulex.* The number of daphnia consumed over a 10-min period was recorded. These behavior data are currently undergoing statistical analysis.

Adult behavioral endpoints – predator evasion and feeding rate

All of the larvae from the static exposures have now been analyzed. Several measures of predator evasion were scored from high-speed videos and feeding rate was quantified. These data are currently undergoing statistical analysis. All of the larvae from the flow-through exposures have been tested and are currently awaiting quantification for several measures of escape behavior, including response latency, velocity of escape, and turning angle.

Vitellogenin analysis and histology

Larvae: all of the larvae used in the C-start and predator avoidance assays were placed in RNAlater immediately following the completion of each trial. These samples were subsequently frozen at -20°C and are currently awaiting vitellogenin gene expression analysis. The remaining fish from each treatment replicate were euthanized on Day 21 of exposure via an overdose of MS-222, and stored in formalin. These fish will be used to assess the effects of exposure on larval growth and development.

Adults: the minnows were dissected on exposure Day 30, following behavioral testing. Each minnow was weighed and measured for standard length. The liver and gonads were dissected from each fish and weighed. Blood was collected and the presence and quantity of vitellogenin was measured from each fish using commercial ELISA kits. These data are currently undergoing statistical analysis.

Activity Status as of January 31, 2016:

Larval and adult behavioral data have now been quantified from video tapes, and the statistical data analyses assessing the effects of E1 on hatching success, early survival, fecundity, fertility, behavioral impairment (4 assays), and biochemical endpoints are largely completed. Our initial data provide some evidence that temperature modulates the effects of estrogen exposure in both adult and larval fathead minnows, but that its influence is limited and unpredictable. A preliminary draft of a manuscript reporting the results of the behavioral assays has been written.

In addition, the preparation of adult fathead tissues for histological analysis is approximately 80% complete. Guided by the results of the behavioral analyses, we have also chosen several genes for qPCR analysis, and have begun to extract RNA from preserved larvae.

Based on our initial finding that temperature has only a weak or unpredictable modulating effect on the fecundity, fertility and development of fish exposed to E1, but that temperature significantly interacts with E1 to alter larval predator avoidance performance, we have proposed to amend Activity 2 to conduct a large, factorial predation experiment to clarify how temperature interacts with wastewater contaminants to alter natural predator-prey relationships, in lieu of collecting basic survival and reproduction data for smallmouth bass.

Activity Status as of July 31, 2016:

Adult and larval fathead minnow exposures

Exposure experiments with adult and larval fathead minnows have been completed and are fully analyzed. A manuscript detailing the behavioral impact of estrone exposure at various temperatures is currently being circulated among co-authors in preparation of journal submission in the near future. A second manuscript, detailing the effects of estrone exposures at various temperatures on the physiology and anatomy of fathead minnows is currently being prepared, will be circulated among co-authors shortly and will be readied for journal submission in the coming months.

Larvae preserved in RNAlater for subsequent analysis of gene expression patterns have been processed to extract RNA. Extracts will soon be used in the gene expression analysis.

Predation trials with adult sunfish and larval fathead minnows

We developed the experimental design and standard operating procedure to examine the effect of estrone exposure at various temperatures on the predator-prey interactions of adult sunfish and larval fathead minnows. These experiments have been completed for the 15°C and 21°C treatments at all estrone concentrations. The 18°C and 24°C treatments are currently in the exposure phase and predation trials will resume in late July.

Activity Status as of January 31, 2017:

Predation trials with adult sunfish and larval fathead minnows

We executed the complete large-scale, factorial predation experiment described in our approved amendment (1/22/2016). This experiment provides the information for a more thorough understanding of the effects of estrogen exposure on trophic interactions under different thermal regimes. Our predator species, the bluegill sunfish (*Lepomis macrochirus*), was exposed at four experimental temperatures (15, 18, 21, 24°C – matching the previous fathead minnow exposure experiments) for 30 days to one of three treatments: ethanol carrier control, estrone low (125 ng/L), and estrone high (625 ng/L). Concurrently, our prey species, larvae of the fathead minnow (*Pimephales promelas*) were exposed for 30 days at the four temperatures and three concentrations of estrone. A subset of larval minnows were assessed for their feeding and predator avoidance performance after 21 days exposure to match previously established experimental protocols. Following the 30 day exposures, individual predators were placed into 800L circular tanks acclimated to the same water temperature as the exposure temperature of the specific fish treatments. Tanks contained foliage to provide a more natural habitat and cover for the prey fish. After a 90-minute acclimation period, the prey fish (larval fathead minnows) were introduced in groups of five control and five estrone exposed (either estrone low or high treatment) fish for a total of 10 prey fish in the tank. After a 60-minute forage period, predators were removed and processed for all anatomical, physiological and histological endpoints that had previously been collected for all other adult fish analyzed in the larger project. Surviving prey were then recovered from the tank and identified (by a previously applied stain only visible at a wave-length outside the visual spectrum of fishes) by treatment. Great care was taken to randomize treatments for predator and prey and for the identifying stain.

We have completed these exposure experiments at all temperatures, all treatments and in all randomized trials. These combinations resulted in almost 300 individual (=one tank) trials. All sunfish have been processed for all endpoints while all feeding efficiency and predator avoidance performance data for larval fathead minnows have been gathered. Data for survival of prey fish in the mixed predation experiments have been compiled by treatment and have undergone a preliminary analysis. The results of this analysis indicate the following main results: (i) sunfish exposed to 625 ng/L estrone exhibited pronounced physiological responses to estrone exposure at all four temperatures, (ii) sunfish exposed at 125 ng/L show a less pronounced and consistent response, (iii) sunfish exposed at the lowest temperature (15°C) fed less than sunfish at the three higher temperatures, (iv) independent of temperature, estrone exposed larval fathead minnows were eaten at a greater frequency then ethanol control larvae as indicated by the approximately 12% greater survival of control fish across trials, (v) estrone concentration (125 ng/L and 625 ng/L) did not affect the greater rate at which exposed larvae were eaten suggesting that a detrimental threshold of exposure exists below 125ng/L. The results of these experiments have been discussed with our collaborators on this study and are being incorporated into the population models under development (Activity 3).

Adult and larval fathead minnow exposures
Larvae preserved in RNA*later* for analysis of gene expression patterns have been processed to extract RNA. Quality control of the extract indicates excellent extraction efficiencies. Gene expression analysis has begun with the evaluation of putative primers. We expect to complete gene expression analysis in spring 2017.

Activity Status as of July 31, 2017:
Adult and larval fathead minnow exposures
Gene expression analysis for larval fathead minnows has been completed. All data have been quality checked and analyzed. Results of the analysis, which indicated changes in gene expression that are consistent with observed changes in predator avoidance performance in larval fathead minnows, have been completed. The results of these investigations are currently being prepared as manuscript for submission during the next reporting period.

Activity Status as of January 31, 2018:
All exposure experiments have been completed and all data have been analyzed. Results are described above. Data not already published are currently readied for publication.

Final Report Summary:
In this study, we exposed reproductively mature and larval fathead minnows (*Pimephales promelas*) to three environmentally relevant concentrations of a common environmental estrogen, estrone (E1), at four water temperatures reflecting natural spring and summer variations. We then conducted a series of experiments to assess the independent and interactive effects of temperature and E1 exposure on individual fish and on interactions between the prey fish (fathead minnows) and their natural predators (bluegill sunfish, *Lepomis macrochirus*). Our results document important consequences of E1 exposure to the fitness, reproduction, foraging, and predator evasion of fathead minnows. Our data demonstrated significant independent effects of temperature and/or E1 exposure on the physiology, survival, and behavior of adult fish. Some endpoints (body size, growth, organ size) were more susceptible to temperature-modulating effects in female fish while others (biosynthesis of proteins) were more strongly affected in males. Conversely, larval fathead minnows were smaller and impaired in their ability to avoid predators when exposed to E1 at all temperatures. Most notably, the concentration-dependent predation survival rates declined by almost 25% in E1-exposed larval fathead

minnows when compared to control fish. The prey catching abilities of the sunfish were also impaired, although not as severely as predator avoidance behaviors in minnows, potentially mitigating the predation effects on the minnows.

Collectively, our data demonstrate that natural seasonal fluctuations in temperature and E1 concentrations are sufficient to induce sex-dependent physiological and anatomical changes in exposed fish and alter population-level dynamics. These findings improve our understanding of the outcomes of interactions between anthropogenic stressors and natural abiotic environmental factors, and suggest that such interactions can have ecological and evolutionary implications for freshwater populations and communities.

ACTIVITY 3: Conduct a cost-benefit analysis that links the cost of different wastewater treatment options to mathematical predictions of fathead minnow and smallmouth bass abundance

Description: During Activity 3 we will conduct an empirical analysis of alternative water quality trading systems, incorporating information about costs of upgrading wastewater treatment facilities and ongoing operating costs from Activity 1 and from the literature, and solve for the cost of attaining a set of water quality levels. Mathematical modeling will link these water quality levels to the equilibrium biomass of fathead minnows and smallmouth bass in a river that receives treated wastewater. To link treatment options to fish biomass, we will develop a mathematical simulation model that uses environmental cues (e.g., seasonal wastewater effluent temperature) and fish biology to predict minnow and bass biomass under various scenarios of exposure to treated effluent. This information will allow us to express the cost of treating effluent in terms of benefits related to the biomass of different fish species

Summary Budget Information for Activity 3: **ENRTF Budget: $ 172,750**
Amount Spent: $ 128,847
Balance: $ 43,903

Activity Completion Date: June 30, 2018

Outcome	**Completion Date**	**Budget**
1. A predictive mathematical model that simulates minnow and bass abundance in a pristine, Minnesota river during different seasons	12/30/2015	$52,325
2. A predictive mathematical model that simulates minnow and bass abundance under exposure to treated wastewater effluent during different seasons	12/31/2017	$52,325
3. A cost-benefit analysis of treatment options and fish abundance	5/30/2018	$69,100

Activity Status as of January 31, 2015:
No work has been completed on this activity to date because results from Activity 2 will be used for Activity 3.

Activity Status as of July 31, 2015:
No work has been completed on this activity to date because results from Activity 2 will be used for Activity 3. Work will begin this summer.

Activity Status as of January 31, 2016:
The mathematical model for predicting minnow and bass abundance is in development. We began work on this model in July 2015 with a conceptual framework that describes the different parts of the model and how they fit together. Because the details of the model depend on how we incorporate estrone effects, we have decided to develop the complete model rather than a pristine version followed by an impacted one. We are focusing on the most important pathway in this model, which links environmental estrogen concentration to population-level effects via the estrone concentration in individual fish and the effect that this concentration has on the feeding, maintenance, growth, and/or reproduction of individual fish. We are using a well-tested ecotoxicological model to convert environmental estrogen concentrations to internal estrogen concentrations, and a bioenergetics

approach to convert these internal concentrations to individual-level effects. We have developed and parameterized this part of the model for fathead minnows, and are now testing it to make sure that model output is consistent with results from Activity 2. We have focused solely on fathead minnows so far because they are well studied, and because the results from Activity 2 are from fathead minnows. Late in 2015, the post-doc who is in charge of this work informed us that he will be leaving at the end of February 2016 to start his dream job. Since that time, our focus has been on hiring a replacement, and on documenting the model and related work so far so that the transition is as smooth as possible. Once we are back up and running, we can finish testing and then duplicate this part of the model for smallmouth bass and/or bluegill sunfish. We will then scale the model up to multiple individuals: first in a homogeneous environment, then in a pristine river, and then in a river that is receiving wastewater effluent as per results from Activity 1.

Activity Status as of July 31, 2016:
We have made some progress on the mathematical model for predicting minnow and bass abundance. The post-doc who was a part of this activity left for a permanent position at the end of February 2016. He has documented the model and related work to date so that his replacement, who starts December 1 2016, can begin working on the model in an effective way immediately. The former post-doc is also continuing to work on the model in his spare time. He recently replaced the energetics part of the model with a version that the replacement post-doc can easily implement, and is currently improving how fish feeding and energetics scale with temperature and updating the documentation accordingly. Last week, we shared the most recent data from Activity 2 with the former post-doc so that he can ensure that model fish respond to estrone the same way that laboratory fish do.

The cost information on biological nutrient removal processes has been collected and summarized from recent literature. Depending on available technologies for wastewater treatment, total capital cost on upgrading facilities has been generalized for Bardenpho, A^2/O, Step Feed, Biolac, Methanol, MLE, Oxidation Ditch and Sequencing Batch Reactor. The capital cost includes the impact of design capacity of wastewater treatment facilities, but the positive relationship between the cost and corresponding treatment capacity does not seem significant for all treatment technologies. A remaining challenge is to isolate cost performance and efficiency as they depend upon treatment capacity, suitable treatment technology and spatial characteristics. Assuming a 20-year project lifetime and a 6% discount rate, the total capital cost of building a new but small wastewater treatment facility has also been summarized for MLE, Sequencing Batch Reactor, Submerged Biofilter, Rotating Biological Contactor and Activated Sludge. Work is underway to develop and refine a set of mathematical models of the costs of alternative treatment technologies.

Activity Status as of January 31, 2017:
We are once again making good progress on the mathematical model for predicting minnow and bass abundance. The new post-doc for this activity began work December 1 2016. He has quickly brought himself up to speed by reviewing the files that were left by the previous post-doc. The two post-docs (former and current) have also been in communication via phone and email. We have met with Co-PI Heiko Schoenfuss to discuss results from Activity 2. A follow-up meeting is scheduled for mid-February. We are also back to coding the model, with an emphasis on using individual-scale data (growth, reproduction, and other life history traits) from both Activity 2 and the literature to parameterize the Dynamic Energy Budget models for the two fish species. These components govern how an individual fish allocates food energy to different processes (e.g., metabolism, growth, reproduction) along a gradient of estrone contamination.

Our previous work summarized the cost of different nitrogen removal technologies both in a new facility and in an upgraded facility. Based on this work, we have developed tables for a cost-benefit analysis of different wastewater treatment processes, including the MLE process, nitritation-denitritation and anammox. This table can be adjusted based on cost assumptions from the literature or from specific information obtained from experiments. The table lists both the treatment cost and the additional benefit of different nitrogen removal processes. The treatment cost is categorized into two parts: (1) the capital cost, which includes the construction cost and the indirect cost, and (2) the operation and management cost, which includes maintenance, labor, electricity, chemicals, taxes and insurance, and miscellaneous. All the costs are converted to an annual basis using a parameter that relies on the discount rate and the life of the equipment. The additional benefit category considers the benefits of a change in fish population (in monetary units based on recreational and economic value) as a result of changes in estrone removal. In this case the change in the fish population is linked to the mathematical model developed in this project. By combining the efficiency of nitrogen removal for different wastewater treatment processes, the average cost to remove nitrogen can be computed, which can in turn be used to assist with comparisons among different wastewater treatment processes.

Activity Status as of July 31, 2017:
The population model is in the final stages of development. We have parameterized the Dynamic Energy Budget sub-routine, which describe how food is allocated to different processes within an individual of a given species. We have also obtained bass and minnow abundance data in Minnesota Rivers from the Minnesota Pollution Control Agency. We are using these data to calibrate model fish abundance in the absence of estrone. We are also beginning to integrate results from Activity 2 (metabolism, growth, and reproduction) into the model. When this step is complete, we will be able to link wastewater treatment and individual-level responses to estrone to population-level impacts on fish.

Activity Status as of January 31, 2018:
The population model was calibrated to reproduce realistic population densities and reproduction patterns based on both literature and in situ data obtained from the Minnesota Pollution Control Agency. Temperature data from a pristine river in Minnesota (Kawishiwi river) was used for model input; this was obtained from the USGS Water Data for the Nation website. Our model takes into account three kinds of mortalities: Aging, which is computed from the internal metabolism of an individual, starvation, which happens when food availability is too low for too long to enable individuals to survive, and predation, which is based on a daily probability. Inclusion of multiple mortality processes allows us to represent different types of river ecosystems, one in which fish population density is more controlled by food availability than by predation pressure (bottom-up controlled system) and a system in which fish population density is more controlled by predation (top-down controlled system).

Predator-prey scenarios:
Results from Activity 2 showed that exposure to estrone significantly alters larval predator avoidance. Consequently, we ran simulations in which prey survival probability in response to predation decreased according to these experimental results. In these simulations, estrone is homogeneously distributed in the river. Our results suggest that the outcomes at the population level are dependent on the river system (top-down or bottom-up) and that fish population density can be significantly reduced in the presence of estrone. We are now developing simulations in which estrone concentrations within the river can vary spatially.

Nest-defense scenarios:
Results from Activity 2 showed that male fathead minnows are less aggressive when exposed to estrone, which could lead to a decreased efficiency in nest defense. Although a proper link between loss of aggressiveness and reduction of nest defense efficiency does not exist at this time, we ran simulations with reduced egg survival due to increased predation (a logical consequence of reduced efficiency in nest defense). In these simulations, estrone is homogeneously distributed in the river. Consistent with our predator-prey scenarios above, impacts on fish population density appear to depend on the river system; further simulations are needed.

Reduced sperm scenarios:
We ran simulations in which sperm quality is reduced, leading to fewer reproductive events per male. In these simulations, estrone is homogeneously distributed in the river. Estrone effects on sperm quality appear to have limited impacts on population density, but further analyses are needed.

The economic analysis of alternative nutrient removal processes is well along and will be completed in the current semester. The goal of this work is to compare the cost of nutrient removal, in terms of plant upgrade and operations cost to the value of estrone removal, in terms of fish population value.

The cost information on biological nutrient removal processes has been collected and summarized from recent literature. Depending on available technologies for wastewater treatment, total capital cost on upgrading facilities has been generalized, with a focus on comparing the cost of anammox and granular aerobic sludge facilities. The cost performance and efficiency as a function of treatment capacity, treatment technology, and spatial characteristics are currently being determined. Two additional steps remain. First, we are currently estimating the nitrogen removal achieved by the treatment facilities under study in terms of economic benefit. This is a challenge, but is currently being estimated based on literature values relating home costs to nitrogen pollution. Second, we are linking costs/benefits of nitrogen removal to the value of estrone removal. Because the valuation of fish populations is highly variable, we will compute the dollar value per fish that would be minimally necessary to justify the expense associated with an improved treatment technology. This will then be carefully analyzed to determine whether these values are realistic, and under what scenarios estrone removal makes economic sense.

Final Report Summary:
We estimated the population-level effects of wastewater estrone by first developing individual-based, mathematical models that represent the full life cycle of fathead minnows and walleye in pristine Minnesota rivers (Outcome 3.1). Individual-based models simulate populations and communities by following individuals and their properties in response to environmental cues (e.g., temperature, food availability, predation rate). Each individual has a set of attributes and behaviors. Simulated rivers, all in Minnesota and based on data obtained from the US Geological Survey, differed in terms of food availability (high vs. low) and predation pressure (high vs. low). We parametrized two population models: one for the fathead minnow and one for the walleye, both culturally and economically important fish in Minnesota. We calibrated the fathead minnow model to reproduce population densities and reproduction patterns that have been reported in the literature and observed in data obtained from the Minnesota Pollution Control Agency. We calibrated the walleye model to reproduce realistic population densities and reproduction patterns based on literature data only. This process of calibration allowed us to simulate population dynamics on four rivers; one for each combinations of food availability and predation pressure. It was important for us to model these combinations because fish population densities in some rivers can be largely controlled by food availability (bottom-up controlled system) or predation (top-down controlled system). The technical document that is attached to this report (“DEB_parameterization.pdf”) describes both models, details how they were parameterized and calibrated, and compares individual processes such as growth and reproduction to observed data.

We incorporated the observed effects of estrone on individual fish (Activity 2) into our population model (Outcome 3.2). Lab results indicated that temperature does not strongly or consistently modulate the effects of estrone on reproduction, development, and egg survival. However, results do suggest that estrone alters larval predator avoidance performance. We incorporated this result into our model by reducing larval survival by 10% (fatheads only) or 25% (fatheads and walleye) when estrone was present. Experiments also showed a decline in aggressive behaviors in male fathead minnows that were exposed to estrone. Because male fathead minnows must defend their nests from predation, reduced aggressiveness in male fathead minnow likely translates into reduced egg survival. We therefore included simulations in which fathead egg survival was reduced by 25% when estrone was present.

We ran our fathead and walleye models on all four simulated rivers and with the effects of estrone exposure on egg and larval survival separately and in combination. We ran each simulation for 20 years – the last 5 years of which included exposure to estrone (if present) – and then compared population sizes between the exposed and non-exposed scenarios. The attached technical document “IBMs_Methods_and_Results.pdf” describes each model, the simulations that we considered, and results.

Model results suggest that the impact of exposure to wastewater estrone on fish abundance in Minnesota rivers is context-dependent. Individuals in food-limited systems do not have enough to eat, and are therefore smaller and less reproductive. When estrone exposure increases predator-induced egg or larval mortality in these systems, the initial decrease in population size results in a decrease in competition (i.e., an increase in per capita food availability), and therefore a corresponding increase growth, size, and reproduction. In some cases, these changes are enough to fully compensate for the increased mortality due to estrone exposure - the result being that estrone is not predicted to impact fish populations. Conversely, individuals are less likely to compete for food in systems in which population density is largely controlled by predation. Individuals in such systems are therefore more likely to grow quickly and large, and have high reproductive output. An estrone-induced increase in egg or larvae mortality in this system is predicted to cause a significant decline in fish abundance because individuals in the population are already growing and reproducing at their maximum rate. These individuals are already well fed, so they are not released from competition and therefore unable to compensate for the additional mortality that is imposed by estrone. Results are intermediate to these extremes in systems in which fish are limited by some combination of food and predation.

Our overall conclusion is two-fold. First, measuring exposure effects on individuals alone is insufficient to determine whether a stressor (or the removal of a stressor through wastewater treatment) will cause population-level impacts; food web dynamics play a large role in determining this response. Second, it is insufficient to assume that population-level responses to wastewater estrone (treatment) will be the same in all rivers. Variation in river food webs (e.g., in terms of the degree of limitation by food or predators) means that fish populations in these rivers will also vary in their respond to wastewater estrone. Taken together, these results suggest that impacts of estrone should be evaluated at the population scale, and for the conditions that are appropriate for river of interest.

Our research summarized the cost information for different nitrogen removal technologies either in a new facility or in an upgraded facility. Tables were developed to be used in a cost-benefit analysis for different wastewater treatment processes. In terms of cost, the treatment cost of different nitrogen removal processes was categorized into two parts: capital cost, which included construction and indirect costs, and the operation and management cost, which included maintenance, labor, electricity, chemicals, taxes and insurance, and miscellaneous costs. All costs were converted to an annual basis. These costs can be compared to the estimated benefit to fish from the population models, based on the literature value of estimated walleye value to recreational anglers of $13.52 to $28.38 per fish (after conversion to 2017 dollars).

V. DISSEMINATION:

Description: The target audience for results from this research will be professionals in the areas of wastewater treatment and natural resource management. Specific targets will be environmental engineers and scientists in academia, industry, state agencies such as the DNR and MPCA, and environmental consultants. Results will be disseminated through scholarly publications in peer-reviewed journals such as *Environmental Science and Technology*. Results from the research project will also be presented at regional conferences such as the *Minnesota Water* conference and if possible, at targeted seminars at the DNR and MPCA. Results will be used to determine which wastewater treatment upgrades offer the most ecological protection while incorporating the value of fisheries and energy use.

Status as of January 31, 2015:
No dissemination efforts have been made, as the project is not advanced enough at this point.

Status as of July 31, 2015:
No dissemination efforts have been made, as the project is not advanced enough at this point.

Status as of January 31, 2016:
Data from this project were presented at the SETAC meeting in Duluth in March 2015 and the Minnesota Water Resources Conference in St. Paul in October 2015. In addition, one manuscript has been drafted for submission to a peer-reviewed journal.

Status as of July 31, 2016:
Three manuscripts (one collaborative between Activity 1 and Activity 2, one focused on Activity 1, and a third focused on Activity 2) are in internal review among the collaborators and should be submitted by the end of the summer/early fall.

Status as of January 31, 2017:
A manuscript detailing the behavioral impact of estrone exposure at various temperatures is currently in peer review. A second manuscript, detailing the effects of estrone exposures at various temperatures on the physiology and anatomy of fathead minnows is being circulated among co-authors and will be readied for journal submission in the coming month. The preparation of a third manuscript draft detailing the linkage between gene expression and behavioral effects is planned for spring 2017. A fourth manuscript describing the majority of the Activity 1 results has been resubmitted for peer review. We have submitted an abstract for a poster that will be presented at the annual meeting of the Minnesota Chapter of the American Fisheries Society at St. Cloud State in February. This poster will describe the project, and focus on our simulation model as an up-scaling tool that infers the population-level effects of estrone on individual patterns of energy allocation and survival.

Kira Peterson, the Master's student who completed the research on Activity 1 and wrote her Master's thesis on this work, was awarded the Cale Anger Master's Thesis Award and the University of Minnesota Distinguished Master's Thesis Award in Mathematics, Physical Sciences and Engineering for her research.

Status as of July 31, 2017:
Results from our estrone biodegradation experiments are accepted for publication in *Environmental Science: Water Research and Technology* (Peterson KN, Tan DT, Bezares-Cruz JC, Novak PJ. Estrone Biodegradation in Laboratory-Scale Systems Designed for Total Nitrogen Removal from Wastewater. *Environmental Science: Water Research and Technology*). Results from our adult fathead minnow exposure experiments have recently been published in the journal *Hormones and Behavior* (Ward JL, Cox MK, Schoenfuss HL. 2017. Thermal modulation of anthropogenic estrogen exposure on a freshwater fish, *Pimephales promelas*, at two life stages. *Hormones and Behavior* 94:21-32.). A second manuscript, detailing the effects of estrone exposures at various temperatures on the physiology and anatomy of fathead minnows is in journal review. A third manuscript detailing the linkage between gene expression and behavioral effects in larval fathead minnows is being readied for journal submission. We presented a poster at the annual meeting of the Minnesota Chapter of the American Fisheries Society at St. Cloud State in February. We also presented a poster at the 5th International symposium on Dynamic Energy Budget Theory in Tromsø, Norway. Both posters described the project, and focused on our modelling approach as an up-scaling tool that infers the population-level impacts of estrone effects on individual energy allocation and survival. We will also present a poster at the Society of Environmental Toxicology and Chemistry North America 38th Annual Meeting at Minneapolis in mid-November.

Status as of January 31, 2018:
Three manuscripts have been published on this work (1 from Activity 1, 1 from Activity 2, and a third that combines results from both Activity 1 and 2 *(Cox MK, Peterson KN, Tan D, Novak PJ, Schoenfuss HL, Ward JL. 2017. Temperature modulates estrone degradation and biological effects of exposure in fathead minnows. Science of the Total Environment. https://doi.org/10.1016/j.scitotenv.2017.10.069)*). A fourth manuscript detailing the linkage between gene expression and behavioral effects in larval fathead minnows is being readied for journal submission. Similarly, a fifth manuscript detailing the effects of estrone at various temperatures on

the predator prey interactions is currently readied for publication. A sixth publication is being written on the fish population modeling efforts.

We presented the results of some specific scenarios (Predator-prey and Nest-defense scenarios) at the Society of Environmental Toxicology and Chemistry North America 38th Annual Meeting at Minneapolis in mid-November. We will have an oral presentation about the impacts of behavioral effects at population scale at the 78th Midwest Fish & Wildlife Conference at Milwaukee, Wisconsin in late January.

Final Report Summary:
The research was widely disseminated at conferences, including the national/international conferences (the 5th International symposium on Dynamic Energy Budget Theory in Tromsø, Norway and the Society of Environmental Toxicology and Chemistry North America 38th Annual Meeting) as well as multiple local/regional meetings. Three manuscripts have been published on this research and have been sent to the LCCMR with the final project report. X manuscripts have been submitted for publication on this research and 3 manuscripts are in preparation for submittal (*"Population level impacts of measured individual behavioral impact after exposure to estrone in fathead minnow," by Vaugeois, Forbes, and Venturelli*; *"Estrone Exposure Interacts with Temperature to Alter Predator Escape Performance and Gene Expression," by Cox, Ward, Matsuura, Aing, Schoenfuss, Kohno*; *"Does Temperature Modulate the Effects of Estrogenic Exposure in a Piscivore Freshwater Fish?" by Korn, Ward, Edmiston, Schoenfuss*). Once accepted, they will be forwarded to the LCCMR.

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget Overview:

Budget Category	$ Amount	Explanation
Personnel:	$ 277,407	Over the course of the 3-year project, two years of support for two graduate students (Activity 1 and the economic aspects of Activity 3), two years of support for a postdoctoral researcher (the population modeling aspects of Activity 3), and two years of support for a research associate (Activity 2), are budgeted. Funds for the research associate will be covered under a subcontract to St. Cloud State University (see below). Funds will also be used to pay an undergraduate researcher for approximately 13 weeks. The PI (Novak) will each receive 2 weeks of salary a year for the first 2 years of the project. The Co-PIs Venturelli and Homans/Coggins will each receive 1 week of salary a year for the first 2 years of the project. No salary is requested for Schoenfuss who will be granted one semester 100% re-assign time by St. Cloud State University to focus on the analysis of biological data. The PIs will be responsible for project oversight, guidance of the graduate students and postdoctoral researchers, data interpretation and analysis, and report preparation and submission. Two graduate

		student research assistants will each devote 100% of their research time to the project over a 2-year period. Fringe benefits for graduate students include tuition, health insurance, and summer FICA. All fringe benefit rates are set by the University of Minnesota and St. Cloud State University.
Equipment/Tools/Supplies:	$ 34,393	Funds ($34,393) are requested for materials, supplies, consumables, analytical costs and upkeep associated with the LC-MS, computers (to be used only on this project), and software. Required materials include, but are not limited to: pipette tips, glassware, solid phase extraction cartridges for extractions, chemicals for standards and experiments, pumps, analytical consumables, analytical fees, solvents, reagents, gloves, digital data storage media, and laboratory notebooks. A portion of the Materials & supplies are budgeted for support of the fish exposure experiments (fish, chemicals, pumps, aquaria maintenance, etc., $54,000/3 years) and will be part of the subcontract to St. Cloud State University (see below).
Travel Expenses in MN:	$1,400	Travel funds are minimal ($1,400) and are included for travel to meetings at either St. Cloud State University or the University of Minnesota for project coordination or to the Metropolitan Wastewater Treatment Plant for sample collection for use in experiments.
Other: Subcontract to St. Cloud State University	$186,800	The subcontract amount ($186,800) will include salary for a research associate ($48,825 salary, $17,575 fringe (36% fringe rate) per year for 2 years) and supplies for experiments (fish, chemicals, pumps, aquaria maintenance, etc., $54,000 for 3 years) to complete Activity 2.
TOTAL ENRTF BUDGET:	**$500,000**	The total proposed project amount is $500,000. No indirect costs for the University of Minnesota or St. Cloud State University are included in the budget.

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: 4.15

Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation: 2

B. Other Funds: N/A

VII. PROJECT STRATEGY:

A. Project Partners: The project team consists of the Principal Investigator (PI) Paige Novak (University of Minnesota) and co-PIs Dr. Heiko Schoenfuss (St. Cloud State University), Paul Venturelli (UMN), and Frances Homans (UMN). Dr. Jay Coggins will also assist Homans. Novak will direct Activity 1; Schoenfuss will direct Activity 2; Venturelli will direct Activity 3; Homans and Coggins will direct the cost analysis and economic modeling efforts. MCES has agreed to provide access to wastewater.

B. Project Impact and Long-term Strategy: The proposed work fits into a larger research agenda centered at UMN and St. Cloud State focused on environmental estrogens and improved wastewater treatment. The proposed research complements current and prior research in this area. This project builds on what we have learned and takes it further, factoring in cost, how CEC removal is impacted by changes in treatment (focused on nitrogen removal), and how temperature impacts both removal efficiency and fish vulnerability. It also expands the impact of the research by incorporating fish population modeling to scale the findings to a whole-state level. When taken together, this research will provide a more complete picture of how to improve treatment, decrease costs and energy use, and safeguard our fish populations.

C. Spending History: N/A

VIII. ACQUISITION/RESTORATION LIST: N/A

IX. VISUAL ELEMENT or MAP(S): See attached graphic.

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 31, 2015, July 31, 2015, January 31, 2016, July 31, 2016, January 31, 2017, July 31, 2017, and January 31, 2018. A final report and associated products will be submitted between June 30 and August 15, 2018.

Environment and Natural Resources Trust Fund
M.L. 2014 Project Budget

Project Title: Evaluation of Wastewater Nitrogen and Estrogen Treatment Options
Legal Citation: M.L. 2014, Chp. 226, Sec. 2, Subd. 03d
Project Manager: Paige J. Novak
Organization: University of Minnesota
M.L. 2014 ENRTF Appropriation: $ 500,000
Project Length and Completion Date: 4 Years, June 30, 2018
Date of Report: September 4, 2018

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	*Determine the performance of different wastewater treatment processes with respect to nitrogen removal, CEC and estrone removal, energy use, and cost*			*Determine how temperature and life stage alter the reproduction and survival of fathead minnows and smallmouth bass after exposure to treated synthetic or real*			*Conduct a cost-benefit analysis that links the cost of different wastewater treatment options to mathematical predictions of fathead minnow and smallmouth bass*				
Personnel (Wages and Benefits)	$104,657		$0	$0		$0	$172,750		$43,903	$277,407	$43,903
Paige Novak, PI ($12,700 salary, $4,300 fringe, 33.6% fringe rate; total for 2 years; 3.8% effort),		$4,828			$0			$0			
Paul Venturelli, Co-PI ($4,300 salary, $850 fringe, 19.8% fringe rate; total for 2 years; 1.9% effort)		$5,667			$0			$5,749			
Frances Homans, Co-PI and Jay Coggins ($5,650 salary, $1,400 fringe, 24.7% fringe rate; total for 2 years; 1.9% effort)		$0			$0			$0			
One Postdoctoral Researcher ($82,400 salary, $17,100 fringe (includes healthcare); total for 2 years; performing the mathematical modeling of fish populations)		$24,487			$0			$67,834			
Two Graduate Research Assistants ($79,293 salary, $65,414 fringe (includes healthcare and tuition); total for 2 years for each student; one student will perform the research on the removal of nitrogen and CECs during wastewater treatment and the other will perform research on the cost and value of wastewater treatment upgrades with respect to the preservation of fish populations))		$65,693			$0			$55,264			
An Undergraduate Research Assistant ($5,000 salary plus fringe) to assist with the wastewater treatment reactor experiments.		$3,981			$0			$0			
Equipment/Tools/Supplies Laboratory supplies and analytical costs (includes, but is not limited to, chemicals for all analyses, supplies to maintain analytical equipment, supplies for reactor construction, and pumps ($34,393/3 years))	$34,393	$34,393	$0	$0	$0	$0	$0	$0	$0	$34,393	$0
Travel expenses in Minnesota Travel between St. Cloud and Minneapolis for research progress meetings (in state) and to the Metropolitan Wastewater Treatment Plant for sample collection for use in experiments	$1,400	$1,261	$139	$0	$0	$0	$0	$0	$0	$1,400	$139
Other: Subcontract Some of the work will be conducted at St. Cloud State University (Activity 2). The subcontract amount will include salary for a research technician ($55,000 salary, $11,400 fringe (20.75% fringe rate) per year for 2 years) and supplies for experiments (fish, chemicals, pumps, aquaria maintenance, etc., $54,000/3 years)	$0	$0	$0	$186,800	$185,130	$1,670	$0	$0	$0	$186,800	$1,670
COLUMN TOTAL	**$140,450**	**$140,311**	**$139**	**$186,800**	**$185,130**	**$1,670**	**$172,750**	**$128,847**	**$43,903**	**$500,000**	**$45,712**