Environment and Natural Resources Trust Fund

2022 Request for Proposal

General Information

Proposal ID: 2022-271

Proposal Title: Perfluorinated Pollutants And Raising Temperature Exterminate Turtles

Project Manager Information

Name: Satomi Kohno

Organization: Minnesota State Colleges and Universities - St. Cloud State University

Office Telephone: (320) 200-9173

Email: skohno@stcloudstate.edu

Project Basic Information

Project Summary: Many Minnesota turtle species are considered threatened or of special concern. The proposed study elucidates how known feminizing factors, Perfluorinated pollutants, and rising temperatures, impact the sexes of turtle offspring.

Funds Requested: $348,000

Proposed Project Completion: June 30 2025

LCCMR Funding Category: Methods to Protect, Restore, and Enhance Land, Water, and Habitat (F)

Project Location

What is the best scale for describing where your work will take place?

Statewide

What is the best scale to describe the area impacted by your work?

Statewide

When will the work impact occur?

In the Future

Narrative

Describe the opportunity or problem your proposal seeks to address. Include any relevant background information.

Nearly half of species of turtles in Minnesota are considered threatened or of special concern. The proposed study will determine how two known male-eliminating (feminizing) factors, the presence of Perfluorinated pollutants and rising summer-time water temperatures, interact to eliminate male turtle offspring. Since turtles are long-lived species (> 50 years), relatively high in the food web, and faithful to a location, they are essential sentinels for long-term aquatic ecosystem health. Especially, freshwater turtles could be great indicator species for the local aquatic ecosystem since they do not migrate to other locations and spend their whole lives in the local environment. The presence of estrogenic contaminants (including hormones, industrial chemicals, and plasticizers such as Bisphenol-A, BPA) and the surface water temperature in Minnesota lakes and streams both have dramatically increased in recent decades (Figure 1), whereas Minnesota turtle populations have concurrently declined. In addition to these feminization factors, Perfluorinated chemicals known to enhance the activity of estrogenic pollutants are contaminated in some water bodies of Minnesota. It is essential to maintain the appropriate sex ratio of turtle species for their conservation and ecosystem sustainability, which leads to our human health.

What is your proposed solution to the problem or opportunity discussed above? i.e. What are you seeking funding to do? You will be asked to expand on this in Activities and Milestones.

The goal of this project is, therefore, to validate the presence and biological effects of Perfluorinated chemicals in the common snapping turtles from Minnesota lakes. We chose the common snapping turtle as a study species since it is relatively abundant and its sex is determined by ambient temperature, similar to other Minnesota turtle species. We will measure perfluorinated and estrogenic contaminants in turtle eggs at a reference site (expected relatively pristine, Diamond Lake, Kandiyohi County) and three contaminated lakes (Lake Elmo, Washington County; Lake Harriet, Hennepin County; Lake Johanna, Ramsey County), (Activity-1). We will also monitor the temperature of turtle nests at reference and contaminated lakes during egg incubations to elucidate the effects of Perfluorinated pollutions and increasing temperature (Activity-2). Firstly, we will transfer turtle eggs to our laboratory at the early egg incubation and incubate eggs under an ideal condition at the laboratory to elucidate contaminants effects without increasing temperature in the wild (Activity-2A). We will also collect eggs at the later egg incubation after monitoring nest temperature in the wild to reveal the combined effects of all feminizing factors (Activity-2b). Identified contaminants that reduce male production will be experimentally validated through egg exposure at the laboratory (Activity-3).

What are the specific project outcomes as they relate to the public purpose of protection, conservation, preservation, and enhancement of the state's natural resources?

The results of this study will be extrapolated to other turtle species and lakes in Minnesota to assess the threat to species diversity in the ecosystem. This information will help identify sensitive nesting areas that require habitat improvement, as described above. The results will provide novel information for water resource managers and aquatic reptilian conservation in Minnesota's 10,000 lakes and stream ecosystem. It is essential to maintain the appropriate sex ratio of turtle species for their conservation and ecosystem sustainability, which leads to our human health.

Activities and Milestones

Activity 1: Perfluorinated and estrogenic contaminants in turtle eggs

Activity Budget: $122,000

Activity Description:
We will collect eggs of the common snapping turtles and measure perfluorinated and estrogenic contaminants in turtle eggs. We will compare the contaminant concentrations in the egg collected at four sites: One reference site, which is expected relatively pristine, Diamond Lake (Kandiyohi County) and three testing lakes (Lake Elmo, Washington County; Lake Harriet, Hennepin County; Lake Johanna, Ramsey County). Three eggs from each nest will be collected. The eggs will be collected from six nests at each lake for 2 years. Total 144 eggs will be analyzed (3 eggs x 6 nests x 4 lakes x 2 years).

- Analyze perfluorinated contaminants composites of 144 eggs.
- Quantify estrogenic contaminants of the contaminants in composites from 144 eggs.

Outcome Completion Date
1. Perfluorinated contaminants in 144 eggs June 2024
2. Estrogenic potential in 144 eggs June 2024

Activity Milestones:

Description	Completion Date
Perfluorinated contaminants in 144 eggs	April 30 2024
Estrogenic potential in 144 eggs	April 30 2024

Activity 2: EFFECTS OF PERFLUORINATED POLLUTIONS AND INCREASING TEMPERATURE ON SEX RATIO

Activity Budget: $141,000

Activity Description:
We will monitor the temperature of turtle nests at reference and contaminated lakes during egg incubations to elucidate the effects of Perfluorinated pollutions and increasing temperature (Activity-2). We will collect eggs two-timing. We will transfer turtle eggs to our laboratory at the early egg incubation and incubate eggs under an ideal condition at the laboratory to elucidate contaminants effects without increasing temperature in the wild (Activity-2A). We expect to obtain a 50:50 sex ratio in the reference group. We will also collect eggs at the later egg incubation after monitoring nest temperature in the wild to reveal the combined effects of all feminizing factors (Activity-2b). Eggs will be collected at one reference lake and two testing lakes. Up to 30 eggs will be collected from each nest. Eggs will be collected from 3 nests at 2 timing before and after the sex determination period. incubated until hatch-out. Total 1080 hatchlings will be analyzed (30 eggs x 3 nests x 2 timing x 3 lakes x 2 years). At one week of age, babies will be sacrificed. The sex will be asses by morphological, histological, and molecular analyses of the gonad.

Activity Milestones:

Description	Completion Date
Effects of contaminants on sex ratio with Lab incubation	April 30 2024
Effects of contaminants on sex ratio with incubation in wild nest	April 30 2024

Activity 3: Effects of identified contaminants on sex ratio in the laboratory experiments

Activity Budget: $85,000

Activity Description:

Eggs will be collected at the reference lake, and before the sex determination period, eggs will be exposed to contaminants identified in Activity-1. Total 120 eggs will be utilized (30 eggs x 4 exposures (1 control & 3 contaminant mixture identified in 3 lakes)). Eggs will be incubated at intermediate temperature producing a 50:50 sex ratio, and we will let them hatch out. At one week of age, babies will be sacrificed. The sex will be asses by morphological, histological, and molecular analyses of the gonad as same as activity-2.

Activity Milestones:

Description	Completion Date
Preparation of contaminant mixture identified in Activity-1	May 31 2024
Exposure to contaminants in the Lab	August 31 2024
Analyses of gonad in Histology	June 30 2025
Analysis of gonad in Gene Expression	June 30 2025

Long-Term Implementation and Funding

Describe how the results will be implemented and how any ongoing effort will be funded. If not already addressed as part of the project, how will findings, results, and products developed be implemented after project completion? If additional work is needed, how will this be funded?

The proposed research supports a state-wide research agenda centered at SCSU and focused on estrogenic contaminants of emerging concern and the protection of lake ecosystems. The proposed research complements current and prior research that to date did not address the declining turtle populations in Minnesota lakes. This research will assess the environmental impact of estrogenic contaminants of emerging concern, identify especially vulnerable lake ecosystems, and help safeguard turtle populations in Minnesota. This study will provide guidance in identifying and remediating some of the adverse impacts of estrogenic contaminants and rising ambient temperature on Minnesota turtle populations.

Project Manager and Organization Qualifications

Project Manager Name: Satomi Kohno

Job Title: Assistant Professor

Provide description of the project manager's qualifications to manage the proposed project.

Dr. Kohno has been involved in developing the research field of endocrine disruption with molecular biological techniques in wildlife, which is a major issue of global concern for both environmental and human health since 2003. A major part of my research involves experimentally understanding the molecular mechanisms of endogenous and/or exogenous endocrine signals on the steroid hormone signaling systems during development in a variety of vertebrates as sentinel species. Dr. Kohno has published 65 peer-reviewed research papers and 2 book chapters. Dr. Kohno is highly qualified to manage the proposed project. based on his adequate and unique experiences in ecotoxicology field.

Organization: Minnesota State Colleges and Universities - St. Cloud State University

Organization Description:

St. Cloud State University makes a positive, long-term impact on the lives of our students. We provide rigorous and relevant academic experiences with engaged, active learning opportunities in an intellectually vibrant, inclusive, and diverse campus community. Our graduates are well-prepared to act as responsible global citizens and professionals who remain actively connected with our university.

The Department of Biological Sciences offers undergraduate and graduate degree programs in biological disciplines that prepare students for a variety of careers and professional degree programs (e.g., medicine, dentistry, physical therapy, ecology, evolution, wildlife conservation, natural resources). Talented faculty and staff, dedicated to teaching and learning by doing, paired with well-equipped labs and research centers provide an active learning environment for students. Faculty mentors guide laboratory, fieldwork, and independent research projects to enhance the academic curriculum and provide hands-on learning experiences. Our facilities include laboratories that promote research and hands-on learning in microbiology, cell and tissue culture, phytoplankton, physiology, aquatic toxicology, genomics, systematics, evolutionary biology, taxonomy, ecology, and histology. We maintain specimen-based collections for teaching and research including zoological collections and a herbarium. Our greenhouse and vivarium support a variety of educational and research experiences.

Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	$ Amount
Personnel								
Mannager		PI			40%	150		$138,000
Graduate Student		Research Assistant			20%	200		$43,000
							Sub Total	**$181,000**
Contracts and Services								
							Sub Total	**-**
Equipment, Tools, and Supplies								
	Equipment	Environmental chamber	egg incubation					$13,000
	Tools and Supplies	Chemical Analysis at SGS AXYS ANALYTICAL SERVICES LTD. , $395 x 144 samples = $56,880	Perfluorinated Pollutants analysis					$57,000
	Tools and Supplies	Analysing biological activity of contaminants, $120 x 144 samples = $17,280	Analysing biological activity					$17,000
	Tools and Supplies	Histology, $10 x (1080 + 120 samples) =$12,000	Histological analysis					$12,000
	Tools and Supplies	Quantitative gene expression analysis, $50 x (1080 + 120 samples) =$60,000	Quantitative gene expression analysis					$61,000
							Sub Total	**$160,000**
Capital Expenditures								
							Sub Total	**-**
Acquisitions and Stewardship								
							Sub Total	**-**
Travel In Minnesota								

	Miles/ Meals/ Lodging	Visiting 4 different lakes 4 times for 3 years, $100 lake x 4 lakes x 4 times x 3 years=$4,800	Egg collection on Field					$5,000
							Sub Total	**$5,000**
Travel Outside Minnesota								
	Conference Registration Miles/ Meals/ Lodging	$2000 x 1	Scientific conference registration & accomodation					$2,000
							Sub Total	**$2,000**
Printing and Publication								
							Sub Total	**-**
Other Expenses								
							Sub Total	**-**
							Grand Total	**$348,000**

Classified Staff or Generally Ineligible Expenses

Category/Name	Subcategory or Type	Description	Justification Ineligible Expense or Classified Staff Request

Non ENRTF Funds

Category	Specific Source	Use	Status	Amount
State				
			State Sub Total	**-**
Non-State				
			Non State Sub Total	**-**
			Funds Total	**-**

Attachments

Required Attachments

Visual Component

File: 89767f95-223.pdf

Alternate Text for Visual Component

Figure 1. Contaminant burden in Lakes and increased temperature in Minnesota....

Administrative Use

Does your project include restoration or acquisition of land rights?

No

Does your project have potential for royalties, copyrights, patents, or sale of products and assets?

No

Do you understand and acknowledge IP and revenue-return and sharing requirements in 116P.10?

N/A

Do you wish to request reinvestment of any revenues into your project instead of returning revenue to the ENRTF?

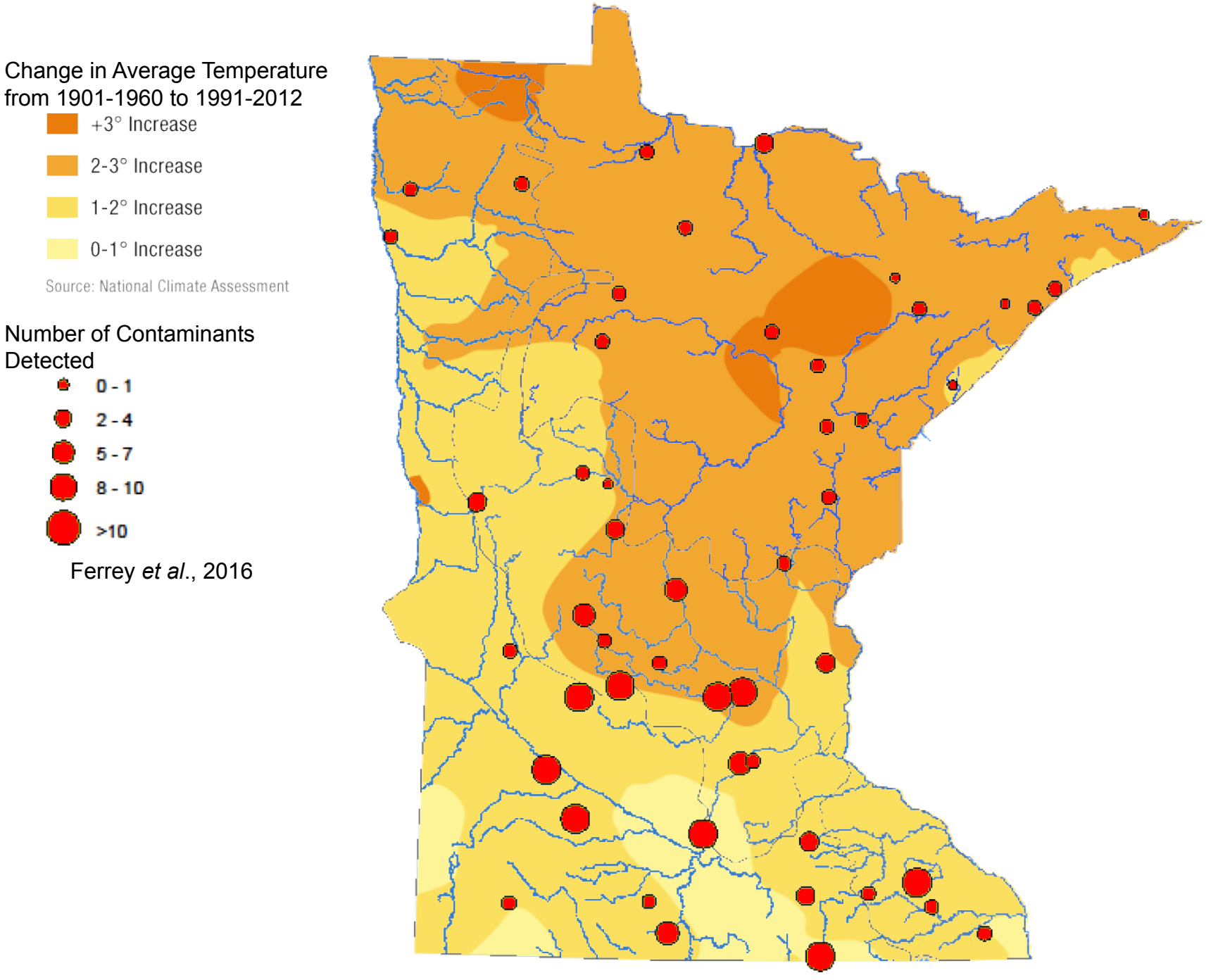
N/A

Does your project include original, hypothesis-driven research?

Yes

Does the organization have a fiscal agent for this project?

No



Ferrey *et al*., 2016