Environment and Natural Resources Trust Fund
2018 Request for Proposals (RFP)

Project Title: **ENRTF ID: 072-B**

Increasing Contaminants and Temperature Eliminate Minnesota Turtles

Category: B. Water Resources

Total Project Budget: $ 248,632

Proposed Project Time Period for the Funding Requested: 3 years, July 2018 to June 2021

Summary:

Quantifying factors, including estrogenic contaminates and rising temperature, responsible for turtle decline in Minnesota Lakes to provide natural resource managers opportunities to remediate sensitive habitats and stabilize populations.

Name: Satomi Kohno

Sponsoring Organization: St. Cloud State University

Address: 720 4th Avenue South, ISELF-217

St Cloud MN 56301

Telephone Number: (320) 200-9173

Email skohno@stcloudstate.edu

Web Address http://web.stcloudstate.edu/skohno/

Location

Region: Statewide

County Name: Statewide

City / Township:

Alternate Text for Visual:

Six selected research locations (3 low and 3 high contaminated lakes at Polk, Beltrami, Cow Wing, Meeker and McLeod County) on the States heat-map with rising ambient temperature. Listed Minnesota turtle species (6 and 3 species determining gender by ambient temperature and gene, respectively; 2 species in each gender-determining mechanism are threatened and special concern).

_____ Funding Priorities	_____ Multiple Benefits	_____ Outcomes	_____ Knowledge Base
_____ Extent of Impact	_____ Innovation	_____ Scientific/Tech Basis	_____ Urgency
_____ Capacity Readiness	_____ Leverage	_____ TOTAL	_____%

PROJECT TITLE: Increasing Contaminants and Temperature Eliminate Minnesota Turtles

I. PROJECT STATEMENT

Nearly half species of turtles in Minnesota are considered threatened or of special concern. **The proposed study will determine how two known male-eliminating factors, the presence of estrogenic contaminants of emerging concern 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 important sentinels for the long-term aquatic ecosystem health. The presence of estrogenic contaminants (including hormones, industrial chemicals and plasticizer such as Bisphenol-A, BPA) and the surface water temperature in Minnesota lakes and streams both have dramatically increased in recent decades (see map), whereas Minnesota turtle populations have concurrently declined. In six of nine Minnesota turtle species, a gender is determined by the ambient temperature during egg-incubation with higher temperatures resulting in less male offspring. Painted turtles will exclusively become male offspring if the nest cavity is 80˚F; while 86˚F will result in all females. Minnesota ambient temperature has increased by up to 3˚F in past 40 years (see map). At the same time, estrogenic contaminants, whose presence has been repeatedly confirmed by the Minnesota Pollution Control Agency (MN PCA) in Minnesota waters, will reduce male offspring production in both temperature- and gene-determining gender turtle species (i.e., with greater estrogenic contaminant load, more females are produced at the same temperature than it without estrogens present). As the adverse effects of estrogenic contaminants and rising temperature are individually concerned, their combined impacts on turtle population sustainability is poorly understood despite strong evidences that synergistic interactions between these stressors are likely. Indeed, increased temperature experimentally enhanced the male-eliminating ability of estrogenic contaminants in other reptiles. According to recent MN PCA reports (Ferrey *et al., 2013*) some highly contaminated lakes are located in areas that have experiences of a 2-3˚F temperature increase in recent decades (see map). Turtles in these lakes face the combined impact of estrogenic contaminants and rising temperature. In a worst-case scenario, male turtle will disappear and the turtle population will be extinct in Minnesota. If these effects are confirmed, conservation actions, such as shade planting to reduce ambient temperature at nest sites or estrogenic source remediation in especially vulnerable lakes, are needed to counter the detrimental effects on turtle populations.

The goal of this project is, therefore, to validate the presence and biological effects of estrogenic contaminants in Minnesota turtles by utilizing the painted turtles from Minnesota lakes. We chose the painted turtle as a study species, since it is relatively abundant and its gender is determined by ambient temperature similar to other Minnesota turtle species. By measuring nest temperature and contaminant levels in maternal blood and turtle eggs in three pairs of reference and contaminated lakes, we will investigate the synergistic effects of estrogenic contaminants and increasing temperature (Activity-1). Identified contaminants which reduce male productions will be validated through laboratory exposure experiments (Activity-2). The results of this study will be extrapolated to other turtle species and Minnesota lakes to assess threats to species diversity in the ecosystem (Activity-3). This information will help to identify sensitive nesting areas that require habitat improvement as described above. The results will provide novel information for water resource managers as well as aquatic reptilian conservation in Minnesota lake and stream ecosystem.

II. PROJECT ACTIVITIES AND OUTCOMES

Activity 1: *For 60 nests, measure nest temperature, quantifying contaminants in eggs & maternal blood, and count male offspring.* **Budget: $86,254**

- Collect 120 eggs from three pairs of exposed and reference lakes (see attached map) immediately after egg deposition for contaminant analyses.
- Measure nest temperature in 60 nests using continuous temperature loggers for duration of egg incubation.
- Analyze estrogenic contaminants composites of 120 eggs and maternal plasma.
- Quantifying biological activity of the contaminants in composites from 120 eggs.

- Determine offspring gender and health one week after hatching (approximately 300 animals).

Outcome	Completion Date
1. Contaminants and total biological potential in 120 egg and maternal blood	*December 2019*
2. Nest temperature profiles and predicted male-production in 60 nests	*December 2019*
3. Number of male-offspring in 60 nests	*June 2020*

Activity 2: *Validate results from Activity 1 through laboratory exposure of 120 turtle eggs from reference lake.* **Budget: $83,134**

- Eggs will be exposed at 1x or 10x environmental relevant concentration or positive/negative control. Eggs will be incubated at the pivotal temperature (80˚F) and male-producing temperature (83˚F) in the laboratory (4 x 2 factorial design), and allowed to hatch out.
- Determine offspring gender and health one week after hatching using histological and molecular techniques.

Outcome	Completion Date
1. Identify three reference lakes	*December 2019*
2. Expose 120 turtle eggs in 4 x2 factorial design to validate Activity 1 results	*December 2020*
3. Quantifications of gonadal gene expressions in 120 turtle eggs	*June 2021*

Activity 3: *Expanding results to other Minnesota turtles and lakes* **Budget: $79,245**

- Quantify contaminants in 3 additional turtle species with temperature-dependent sex determination in 5 additional Minnesota lakes (in collaboration with MN DNR and US FWS).
- Molecular cloning and characterization of estrogen receptor from additional 3 turtle species.
- Biological assay using culture cells to estimate total biological activity.

Outcome	Completion Date
1. Estrogenic contaminants in blood of 45 turtles (3 species x 5 lakes x 3 specimens)	*June 2021*
2. Molecular cloning and characterization of hormone receptor from 3 turtle species	*December 2020*
3. Quantification of total biological activities in blood of all 45 turtles	*June 2021*

III. PROJECT STRATEGY

A. Project Team/Partners

The project team has considerable experience in all aspects of the proposed study. The principal investigator Dr. Satomi Kohno (St Cloud State University, SCSU - Biology) is an expert in reptilian sex-determination and endocrine toxicology and will oversee the entire study. Dr. Heiko Schoenfuss (SCSU - Biology) has studied the biological impacts of estrogenic compounds in Minnesota water for the past 20 years and will supervise exposure experiments. Dr. Nathan Bruender (SCSU - Chemistry) is an analytical biochemist and a new faculty at SCSU. He will lead the analytical characterization of egg and blood specimens using existing infrastructure.

B. Project Impact and Long-Term Strategy

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.

C. Timeline Requirements

Activity 1 will be completed in the first two years of the study. Activity 2 will begin in year 2 and will be completed with Activity 3 in Year 3. The proposed project will be completed in the allotted three-year period.

2018 Detailed Project Budget

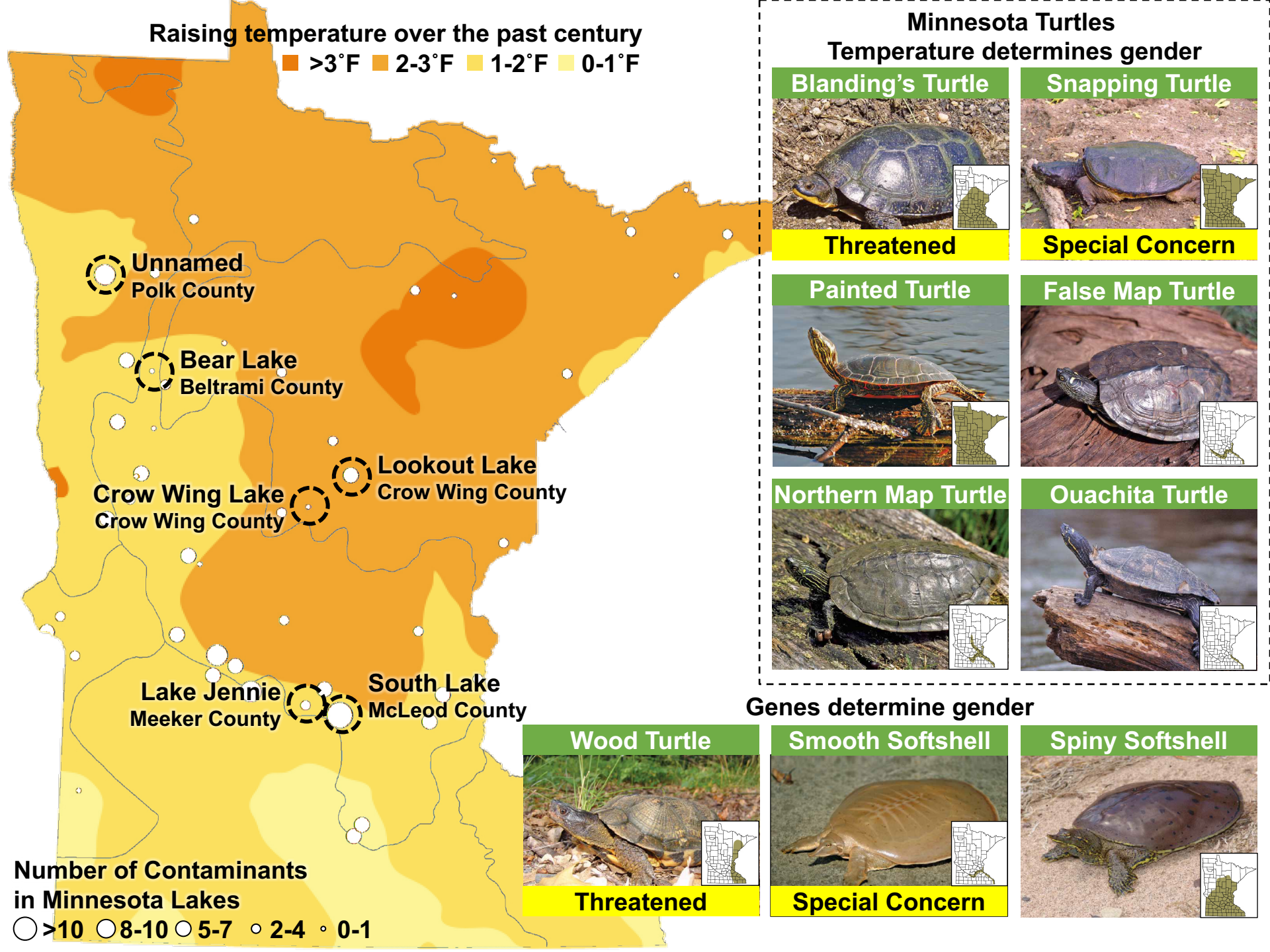
Project Title: Increasing Contaminants and Temperature Eliminate Minnesota Turtles

IV. TOTAL ENRTF REQUEST BUDGET *3* **years**

BUDGET ITEM	AMOUNT
Personnel: Kohno - six months salary (50%/year) and fringe (38%) = $124,575; Schoenfuss - one month of salary (8.3%/year) and fringe (38%) per year = $30,000	$ 154,575
Professional/Technical/Service Contracts: Transcriptome analyses for 4 species at Uof M, $1,200 each species x 4 = $4,800 (Activity 3)	$ 4,800
Equipment/Tools/Supplies: Environmental chamber for egg incubation, $13,417; Developing chemical analyses and contaminant quatification, $9,000 for each compound x 5 = $45,000; Analysing biological activity of contaminants, $120 x 117 samples = $14,040; Histological analysis, $10 x 420 samples =$4,200; Quantitative gene expression analysis, $50 x 120 samples =$10,000	$ 85,657
Acquisition (Fee Title or Permanent Easements): *N/A*	$ -
Travel: For sampling, visiting 6 different lakes twice for 3 years, $200 lake x 6 lakes x 3 years=$3,600	$ 3,600
Additional Budget Items: *N/A*	$ -
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$ 248,632

V. OTHER FUNDS

SOURCE OF FUNDS	AMOUNT	Status
Other Non-State $ To Be Applied To Project During Project Period: *N/A*	$ -	*N/A*
Other State $ To Be Applied To Project During Project Period: *N/A*	$ -	*N/A*
In-kind Services To Be Applied To Project During Project Period: *N/A*	$ -	*N/A*
Past and Current ENRTF Appropriation: *N/A*	$ -	*N/A*
Other Funding History: *N/A*	$ -	*N/A*


Raising temperature over the past century
>3˚F
2-3˚F
1-2˚F
0-1˚F
Unnamed
Polk County
Bear Lake
Beltrami County
Lookout Lake
Crow Wing County
Crow Wing Lake
Crow Wing County
Lake Jennie
Meeker County
South Lake
McLeod County
Number of Contaminants
in Minnesota Lakes
>10
8-10
5-7
2-4
0-1
Minnesota Turtles
Temperature determines gender
Blanding's Turtle
Threatened
Snapping Turtle
Special Concern
Painted Turtle
False Map Turtle
Northern Map Turtle
Ouachita Turtle
Genes determine gender
Wood Turtle
Threatened
Smooth Softshell
Special Concern
Spiny Softshell

Project Manager Qualifications & Organization Description

Project Manager Qualifications

Satomi Kohno will serve as the project manager for this project. Kohno is an assistant professor at St Cloud State University (SCSU, Biology), and an expert in reptilian sex-determination and endocrine toxicology and will oversee the entire study. Kohno is an environmental scientist and comparative reproductive endocrinologist with 15 years' experience in research. Kohno has played a key role in developing the research field of endocrine disruption with molecular biological technique in wildlife, which is a major issue of global concern for both environmental and human health.

Kohno has started an Assistant Professor at Department of Biology in SCSU since October 2016. Kohno has been using the American alligator as sentinel species with a concept 'Bad Environments for Wildlife Won't Be Good for Humans'. Since joining the Aquatic Toxicology Laboratory at SCSU, Kohno has been trying to extend the question to the Minnesota wildlife.

At the previous position in Medical University of South Carolina (MUSC), Kohno has established strong collaboration with analytical chemists at U.S. National Institute of Standards and Technology (NIST) in Hollings Marine Laboratory (HML). This collaboration makes it possible to address the contamination / endocrine disruptor issues with an analytical chemical approach in both wildlife and human. Kohno has also collaborated with Obstetrics clinicians, enabling us to collect samples from pregnant women to address the questions based on Baker hypothesis 'Fetal Origins of Adult Disease'.

Kohno is highly motivated with excellent skills in collaboration and with a passion for biology research and education. No other laboratory in Minnesota has not analyzed the impacts of environmental contaminants to a reptile reproductive health using both field and laboratory experiments. Therefore, Kohno is highly qualified to conduct the proposed project.

Organization Description

SCSU runs 200 academic programs, 60 graduate degrees and education abroad choices on six continents emphasize hands-on learning through community engagement and close interaction with professors. Our 100-acre campus is between downtown St. Cloud and the Beaver Islands, a group of more than 30 islands that form a natural maze for a two-mile stretch of the Mississippi River. The St. Cloud metropolitan area is 24th on Forbes magazine's "Best Small Places for Businesses and Careers." St. Cloud State is ranked in Forbes magazine's "America's Top Colleges" and Money magazine's "Money's Best Colleges."

SCSU prepares our students for life, work and citizenship in the twenty-first century. In our rigorous academic programs, students apply their knowledge in real-world settings. Through active discovery, applied knowledge and creative interaction, SCSU positively transform our students and the communities where they live and work (www.stcloudstate.edu).