



# Environment and Natural Resources Trust Fund

M.L. 2023 Approved Work Plan

## General Information

**ID Number:** 2023-153

**Staff Lead:** Corrie Layfield

**Date this document submitted to LCCMR:** June 5, 2023

**Project Title:** Northward Expansion of Ecologically-Damaging Amphibians and Reptiles

**Project Budget:** \$163,000

## Project Manager Information

**Name:** Kenneth Kozak

**Organization:** U of MN - College of Food, Agricultural and Natural Resource Sciences

**Office Telephone:** (612) 624-3982

**Email:** kozak016@umn.edu

**Web Address:** <https://cfans.umn.edu/>

## Project Reporting

**Date Work Plan Approved by LCCMR:** June 22, 2023

**Reporting Schedule:** April 1 / October 1 of each year.

**Project Completion:** July 31, 2025

**Final Report Due Date:** September 14, 2025

## Legal Information

**Legal Citation:** M.L. 2023, Chp. 60, Art. 2, Sec. 2, Subd. 06a

**Appropriation Language:** \$163,000 the first year is from the trust fund to the Board of Regents of the University of Minnesota to assess the distribution and potential for expansion of key detrimental and nonnative amphibians and reptiles in Minnesota.

**Appropriation End Date:** June 30, 2026

## Narrative

**Project Summary:** American bullfrogs and Red-eared sliders are non-native predators and competitors in Minnesota's native fish communities. This research will assess the distribution and potential for expansion of these species in Minnesota.

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

Minnesota has experienced dramatically warmer temperatures in the past decades. Many species reach a northern limit to their distribution in Minnesota. To the extent that this pattern is influenced by the adaptive capacity of populations at the range edge (e.g. limited tolerance to cold), non-native species with limited ranges in Minnesota (or range limits to the south of the state), are predicted to expand their ranges northward as the climate warms.

American bullfrogs and Red-eared slider turtles are non-native species that have recently become established in Minnesota, and are two of the most pervasive and detrimental invasive species when they are introduced into waterways in which they are not native. Both species outcompete and feed on native fish species, restructuring the food webs and threatening native species with extinction. Given that fish populations contribute greatly to local community revenue and the health of the ecosystems, understanding the current and predicted future geographic distributions of these non-natives, and their impact on fish communities is critical. Although American bullfrogs and Red-eared sliders have limited ranges in Minnesota, they are expected to expand northward through the state as temperatures continue to increase with climate change.

**What is your proposed solution to the problem or opportunity discussed above? Introduce us to the work you are seeking funding to do. You will be asked to expand on this proposed solution in Activities & Milestones.**

The proposed research will use environmental DNA (eDNA) and thermal-tolerance data to assess the current distributions of American bullfrogs and Red-eared sliders in Minnesota and their potential for expansion across the state.

These species are likely expanding their ranges into locations in Minnesota where they have yet to be detected. eDNA is new a tool that can document species that are in low abundance and/or have limited activity windows (such as amphibians and reptiles) by detecting traces of species' DNA left in its habitat. By collecting water samples in drainage systems immediately to the north of the currently known distributions of the focal species, we will be able to determine whether these two species are expanding their ranges beyond their currently-known range limits.

We will measure the thermal tolerances of these species at the northern range limit and in more in southern localities. Broader thermal tolerance and/or increased tolerance to cold at the range edge would be evidence of adaptive evolution to climate and more rapid northward range expansion than expected based on species simply tracking favorable climate. Thermal-tolerance data will be used to generate species-specific models of the species' current and future distributions across Minnesota.

**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 proposed research will provide predictions of impact on Minnesota's aquatic communities by two non-native species that are notorious aquatic ecosystem invaders. The results will inform the management of aquatic ecosystems and will identify the potential threats of non-native predators and competitors to Minnesota's native fish communities. The modeling of the future distributions of American bullfrogs and Red-eared sliders will predict whether continued expansion across the state's waterways is expected in response to Minnesota's changing climate, providing a foundation for developing adaptive management strategies to protect the state's aquatic ecosystems.

## 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?**

During the Project and In the Future

## Activities and Milestones

### Activity 1: Graduate Assistant Recruitment, Species surveys and eDNA sample collection

**Activity Budget:** \$72,000

#### Activity Description:

A graduate student will be recruited and trained to carry the activities over the two-year duration of the project. Following recruitment, we will sample for eDNA of American bullfrogs and Red-eared sliders by collecting water samples from locations where these species are known to occur and also in adjacent drainages to the north where they have yet to be detected. Known locations will be queried in the Minnesota Biodiversity Atlas (<https://bellatlas.umn.edu>) and HerpMapper (<https://herpmapper.org>) databases. By sampling from locations where these species have been detected with traditional biological surveys (visual detection and trapping for turtles, visual detection and mating calls for frogs) we will be able to assess the efficacy of eDNA sampling at varying distances from known source populations, thereby guiding the distance of sampling intervals in locations where these species have not yet been documented. Sampling eDNA in drainage systems immediately to the north of the currently known distributions of the focal species, will determine whether these two species have expanded their ranges beyond their currently-known range limits. In total we expect to collect approximately 800 water samples to assess for eDNA using standardized published protocols for sampling eDNA.

#### Activity Milestones:

Description	Approximate Completion Date
Recruit Graduate Research Assistant	June 30, 2023
Documentation of the distributions of American bullfrogs and Red-eared sliders	July 31, 2024
Collection of eDNA water samples from survey locations	July 31, 2024
Filtration of eDNA water samples and extraction of eDNA for qPCR	July 31, 2024

### Activity 2: Thermal tolerance and role of climate in range limits and range expansion

**Activity Budget:** \$71,000

#### Activity Description:

To assess the role that climate and thermal tolerance play in setting the northern range limits of the focal species, we will use correlative and mechanistic niche models to map the range limits based on cold temperatures. For the correlative niche model, we will use known, georeferenced locations for each species and GIS-based maps of temperature at those locations to generate maps of climatically suitable locations across Minnesota (using the software package Maxent). The mechanistic niche model will estimate climatically suitable locations based on thermally-suitable activity temperature windows of the two species, and their critical thermal minima (CTMin). Activity temperatures will be measured by taking the body temperatures of animals observed in the field over the duration of their active season. CTMin will be assessed in the lab by measuring the temperature at which animals can no longer locomote. Mechanistic model construction will follow modifications of methods and software previously published by Kozak's lab group. Mapping the predicted geographic distributions of each species using correlative- and mechanistic niche models will provide an evaluation whether the current range limits of these species are determined by climate, or whether climatically suitable locations exist beyond where they currently occur in the state.

#### Activity Milestones:

Description	Approximate Completion Date
Determine the role of climate in restricting the northward expansion of species	July 31, 2025
Predict the current and future distributions of species based on thermal tolerance	July 31, 2025

### Activity 3: eDNA data collection and analysis

**Activity Budget:** \$20,000

**Activity Description:**

Water samples collected and filtrated during Activity 1 will be analyzed for American bullfrog and Red-eared slider eDNA. eDNA extraction from filtered water samples and gene primer evaluation will follow standardized protocols, and qPCR will be used to assay for and amplify eDNA. The collection and analysis of eDNA samples from drainages in which these species are already documented (Activity 1) will serve as a positive control for the efficacy of our eDNA methodology for detecting the focal species in other locations. Any amplification of the focal species eDNA from locations where they have yet to be documented (see sampling design in Activity 1) will be used to map the spread of theses two invasions . Following eDNA analysis from all sampled locations, we will generate maps of the current distributions of American bullfrogs and Red-eared sliders in Minnesota.

**Activity Milestones:**

Description	Approximate Completion Date
qPCR of eDNA extracted from water samples	July 31, 2025
eDNA sequence assembly and species identification of amplified eDNA	July 31, 2025
Map drainages in which the focal species were detected with eDNA	July 31, 2025

## Dissemination

**Describe your plans for dissemination, presentation, documentation, or sharing of data, results, samples, physical collections, and other products and how they will follow ENRTF Acknowledgement Requirements and Guidelines.**

We will communicate the results to the general public at the Minnesota State Fair and the University of Minnesota's Bell Museum. Research results will be disseminated to the scientific community through journal publications and research presentations at regional and international meetings. All public presentations (e.g. PowerPoint Presentations, Posters) will include the LCCMR logo and include in the acknowledgments a statement of the contribution of LCCMR funding to the project. All scientific publications will acknowledge LCCMR funding the "Acknowledgments" section of the publication. To disseminate our results to the greater management community, we will communicate our results with key personnel at the MN Biological Survey (Bruce Carlson, Carol Hall).

## 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 work be funded?**

We will communicate the results to the general public at the Minnesota State Fair and the University of Minnesota's Bell Museum. Research results will be disseminated to the scientific community through journal publications and research presentations at regional and international meetings. Future development of the proposed research will focus on broadening its scope to explore the changing distributions of rare, native species of conservation concern, and also common native species, thereby providing a means to address how aquatic communities in Minnesota may shift and become restructured with climate change.

## Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	\$ Amount
<b>Personnel</b>								
12-month Graduate Research Assistant		Data collection and analysis			25%	1		\$101,000
Associate Professor (2-months summer salary)		Project supervision, data analysis, dissemination and publication of results			36%	0.16		\$28,000
							<b>Sub Total</b>	<b>\$129,000</b>
<b>Contracts and Services</b>								
UMN Genomics Center	Internal services or fees (uncommon)	Lab services: qPCR @ University of Minnesota Genomics Center (800 samples @ \$25/sample)				0		\$20,000
							<b>Sub Total</b>	<b>\$20,000</b>
<b>Equipment, Tools, and Supplies</b>								
	Tools and Supplies	DNA extraction kits (15 kits @ \$200/kit)	Supplies required to extract eDNA from samples					\$3,000
	Tools and Supplies	Sterlitech water filters 810kits @ \$200/kit)	For filtration of eDNA from water samples					\$2,000
							<b>Sub Total</b>	<b>\$5,000</b>
<b>Capital Expenditures</b>								
							<b>Sub Total</b>	<b>-</b>
<b>Acquisitions and Stewardship</b>								

							<b>Sub Total</b>	-
<b>Travel In Minnesota</b>								
	Miles/ Meals/ Lodging	Mileage to field sampling sites (2000 mi @0.585/mile)	To collect eDNA samples from MN waterways					\$1,000
	Miles/ Meals/ Lodging	GSA per diem (lodging + M&IE @ MN standard rate, 30 days @ \$155/day),	Costs for meals and lodging associated with travel to field sites to collect samples.					\$5,000
							<b>Sub Total</b>	<b>\$6,000</b>
<b>Travel Outside Minnesota</b>								
							<b>Sub Total</b>	-
<b>Printing and Publication</b>								
	Publication	Page charges to publish in open-access scientific journal	Dissemination of research results to scientific community					\$3,000
							<b>Sub Total</b>	<b>\$3,000</b>
<b>Other Expenses</b>								
							<b>Sub Total</b>	-
							<b>Grand Total</b>	<b>\$163,000</b>



Classified Staff or Generally Ineligible Expenses

Category/Name	Subcategory or Type	Description	Justification Ineligible Expense or Classified Staff Request
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## Non ENRTF Funds

Category	Specific Source	Use	Status	\$ Amount
<b>State</b>				
			<b>State Sub Total</b>	-
<b>Non-State</b>				
			<b>Non State Sub Total</b>	-
			<b>Funds Total</b>	-

## Attachments

### Required Attachments

#### *Visual Component*

File: [212ae1a7-e9e.pdf](#)

#### *Alternate Text for Visual Component*

Known county-level distribution for American bullfrogs and Red-eared slider turtles in Minnesota....

### Optional Attachments

#### *Support Letter, Photos, Media, Other*

Title	File
Background Check Form-signed	<a href="#">774f54af-81b.pdf</a>
2023-153: Kozak_ReasearchAddendum	<a href="#">b7747632-9aa.pdf</a>

## Difference between Proposal and Work Plan

### *Describe changes from Proposal to Work Plan Stage*

All tracked changes from peer review were accepted. Costs for UMN Genomics Center were moved to "internal fee"

## Additional Acknowledgements and Conditions:

The following are acknowledgements and conditions beyond those already included in the above workplan:

**Do you understand and acknowledge the ENRTF repayment requirements if the use of capital equipment changes?**

N/A

**Do you agree travel expenses must follow the "Commissioner's Plan" promulgated by the Commissioner of Management of Budget or, for University of Minnesota projects, the University of Minnesota plan?**

Yes, I agree to the UMN Policy.

**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?**

Yes, Sponsored Projects Administration