

Environment and Natural Resources Trust Fund (ENRTF)

M.L. 2020 ENRTF Work Plan (Main Document)

Today's Date: 02/24/20 Date of Next Status Update Report: 12/30/2020 Date of Work Plan Approval: Project Completion Date: 06/30/2023 Does this submission include an amendment request? No

PROJECT TITLE:

Project Manager: Christine Salomon Organization: University of Minnesota College, Department, or Division: Center for Drug Design Mailing Address: 312 Church St. SE, 4-130 NHH City, State, Zip Code: Minneapolis, MN 55406 Project Manager Direct Telephone Number: 612-626-3698 Email Address: csalomon@umn.edu Web Address:

Location: Statewide

Total Project Budget: \$440,000 Amount Spent: \$0 Balance: \$440,000

Legal Citation: M.L. 2020, Chp. xx, Sec. xx, Subd. xx **Appropriation Language:**

PROJECT STATEMENT:

White nose syndrome (WNS) is a devastating fungal disease of hibernating bats which has killed at least 90% of little brown bats (*Myotis lucifugus*) at many sites in Minnesota. Our proposal is focused on developing a biocontrol strategy to treat substrates and to monitor the fungal pathogen, *Pseudogymnoascus destructans*, in Minnesota hibernacula. Our previous research supported by LCCMR and USFW has allowed us to build a library of potential biocontrol microbes (>2000 strains) collected from major hibernacula (Soudan Iron Mine, Mystery Cave, and several sandstone caves). We have screened many of these strains, identified the most potent inhibitors, and are ready to test these strains/extracts on natural substrates and in limited field settings. We have also optimized a sensitive DNA based detection method (qPCR) and used this approach to measure the occurrence and abundance of *P. destructans* along transects of Mystery Cave and in the Soudan Mine (the two largest hibernacula in Minnesota). This quantification work will be combined with a method to assess viable (live) versus dead cells and spores, which should allow for a more accurate assessment of treatments.

We propose to continue monitoring *P. destructans* to better understand where the fungus is most abundant and likely to re-infect surviving/returning bats. This approach will be used to monitor treatment experiments, and this data will also be available to park managers to identify specific locations in Soudan Mine and Mystery Cave State Parks to focus treatments, decontamination of equipment, or to help regulate visitors/staff in those areas. Additionally, we recently identified one hibernaculum that surprisingly has a healthy population of tricolor bats (another Minnesota bat species that has been decimated in other nearby locations). An initial, small-scale test of some of the surfaces of this cave were negative for the presence of *P. destructans*. We propose to determine how this cave has remained free of *P. destructans* and WNS by studying the physical environment, substrates, and microbial populations. This information may help to develop a treatment strategy for other hibernacula, and to support conservation efforts for surviving bats.

II. OVERALL PROJECT STATUS UPDATES:

First Update December 30, 2020 Second Update June 30, 2021 Third Update December 30, 2021 Fourth Update June 30, 2022 Fifth Update December 30, 2022 Final Report June 30, 2023

III. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1 Title: Testing of most active microbial biocontrol agents with relevant substrates and field studies

Description: We have identified >100 microbial strains that inhibit the growth of *P. destructans*. Among these strains, approximately 10 have demonstrated consistent production of antifungal extracts, and we have purified, characterized and identified most of the active compounds. We have also tested these compounds against cultured fibroblast (skin) cells from two species of bats (Northern long eared and Gray bats) to assess their toxicity. The strains that produce the most antifungal but least toxic compounds were prioritized, and these will be inoculated onto natural substrates from the three major hibernacula locations together with *P. destructans*. We will also test extracts and the active pure compounds from these strains on substrates with established *P. destructans*. Experimental substrate challenges will be treated with a viability reagent propidium monoazide (PMA) before DNA extraction to differentiate between DNA from live versus dead *P. destructans*. qPCR with a specific Taqman probe will be used to quantify DNA from live cells. Once these experiments are analyzed, successful trials will be translated into small-scale field experiments in collaboration with the DNR. These experiments will be focused on human-made hibernacula including mines and smaller historic sandstone brewery caves that serve as hibernacula. *P. destructans* and antagonist growth in these field studies will be assessed using the PMA viability reagent combined with qPCR DNA quantification.

ACTIVITY 1 ENRTF BUDGET: \$149,524

Outcome	Completion Date
1. Optimization and testing of top (3-5) biocontrol agents/extracts on natural	12/31/2021
substrates	
2. Field testing of best biocontrol agents, extracts and pure compounds	06/31/2023
3. Quantification of P. destructans on substrates in lab and field experiments	06/31/2023

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ACTIVITY 2 Title: Quantification of viable *P. destructans* in hibernacula: Seasonal and spatial dynamics

Description: Bats afflicted with WNS change their hibernation patterns within caves, and will often move towards the entrance of caves. However, little is known about the extent of the environmental reservoirs of viable *P. destructans* on substrates in these different locations over time. We will map the occurrence and quantity of *P. destructans* along transects of hibernacula at twice per year from substrate locations (walls, sediments, ceiling) to better understand the spatial and seasonal dynamics of *P. destructans* growth and potential spread. Samples will be analyzed by treating with PMA (as in activity 1) before isolation of DNA and subsequent qPCR quantification of *P. destructans*. This information will be especially helpful to cave managers for focusing treatments or interventions. For example, the top of one door at the entrance of Mystery Cave in Forestville was found to have 3000x more *P. destructans* DNA than areas much deeper in the cave. These "pinch points" for bat entry/exit might be an obvious place for reinfection when bats return in the fall, and could be specifically disinfected. Our regular sampling and quantification before and after any treatments will also provide empirical data to assess management strategies.

ACTIVITY 2 ENRTF BUDGET: \$123,954

Outcome	Completion Date
1. qPCR quantification of samples collected from hibernacula transects, 2x per	06/2023
year, 3 years	
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ACTIVITY 3 Title: Assessment of WNS-free cave

Description: We identified a cave (not named here to minimize potential disturbance) with a healthy population of tricolor bats (*Perimyotis subflavus*) and no signs of WNS (as of 02/2019) with animal numbers consistent with pre-WNS census data. A preliminary analysis of a small number of substrates were all negative, suggesting that *P. destructans* is not present or not abundant in sampled areas. This is surprising and unexpected due to the widespread occurrence of WNS in all other Minnesota hibernacula. We propose to conduct a more thorough mapping of *P. destructans* throughout this cave (see activity 2) and from bats over the next three years, and methodically test a number of different possible factors that might be responsible for the absence of WNS. Various substrates (rocks, water, sediment, etc.) will be tested for their ability to host (or inhibit) *P. destructans* growth, and volatile air samples may be collected and tested. Concurrently, bacterial and fungal samples will be collected and tested for *P. destructans* inhibition. If the substrate or volatile materials show significant activity, the microbial experiments will be minimized. The results of these experiments should provide information about a naturally disease suppressive environment which will inform management strategies.

ACTIVITY 3 ENRTF BUDGET: \$166,522

Outcome	Completion Date			
1. Substrate testing against P. destructans	06/2021			
2. Microbial sampling, isolation and testing (depending on results of substrate	06/2022			
testing)				
3. qPCR quantification of P. destructans on bat and substrate samples	06/2023			
throughout cave				

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IV. DISSEMINATION:

Description:

The Minnesota Environment and Natural Resources Trust Fund (ENRTF) will be acknowledged through use of the trust fund logo or attribution language on project print and electronic media, publications, signage, and other communications per the <u>ENRTF Acknowledgement Guidelines</u>.

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V. ADDITIONAL BUDGET INFORMATION:

A. Personnel and Capital Expenditures

Explanation of Capital Expenditures Greater Than \$5,000:

Explanation of Use of Classified Staff: N/A

Total Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation:

Enter Total Estimated Personnel Hours for entire	Divide total personnel hours by 2,080 hours in
duration of project: 12480	1 yr = TOTAL FTE: 6 (3 years)

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

Enter Total Estimated Contract Personnel Hours	Divide total contract hours by 2,080 hours in 1
for entire duration of project:	yr = TOTAL FTE:

VI. PROJECT PARTNERS:

- A. Partners outside of project manager's organization receiving ENRTF funding N/A
- B. Partners outside of project manager's organization NOT receiving ENRTF funding N/A

VII. LONG-TERM- IMPLEMENTATION AND FUNDING: : If we accomplish the goals in this proposal, we expect this to be the last request for the basic research components of this WNS project. Depending on the success of the field trials, we may request additional funds to expand treatments or interventions (such as targeted disinfection) to more hibernacula sites. We plan to continue to request funds from USFW to leverage effort towards WNS treatment, and will seek additional funds from the National Science Foundation to expand studies of the natural history and environmental reservoir of *P. destructans*.

VIII. REPORTING REQUIREMENTS:

- Project status update reports will be submitted December 30 and June 30 each year of the project
- A final report and associated products will be submitted between June 30 and August 15, 2023

IX. SEE ADDITIONAL WORK PLAN COMPONENTS:

- A. Budget Spreadsheet
- **B. Visual Component or Map**
- C. Parcel List Spreadsheet N/A
- D. Acquisition, Easements, and Restoration Requirements N/A

E. Research Addendum

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Attachment A: Project Budget Spreadsheet Environment and Natural Resources Trust Fund M.L. 2020 Budget Spreadsheet Legal Citation: Project Manager: Christine Salomon



Organization: University of Minnesota

Project Budget: 440,000

Project Length and Completion Date: 3 years, July 2020-July 2023

Today's Date: 02/24/20

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET		Budget		Amount Spent		Balance	
BUDGET ITEM							
Personnel (Wages and Benefits)			-	\$ -		\$	-
PI Salomon 5% FTE for 3 years. Project mananager and chemistry and bioactivity tes	ting lead.	\$	27,410	\$-		\$	27,410
Co-PI Blanchette 5% (in kind) for 3 years. Lead for qPCR analysis and fungal work.		\$	-	\$-		\$	-
Postdoctoral researcher (82% salary, 18% benefits): 100% FTE for each of 3 years. Co	ollecting and	\$	197,380	\$-		\$	197,380
testing substrates and samples, fractionation and identification of active component	nts. Microbial						
isolations and characterizations. Field experiments with test biocontrol strains.							
Technician (79% salary, 21% benefits): 50% FTE for each of 3 years, biological assay	testing, database	\$	40,923	\$-		\$	40,923
management for bioactivity, chemistry and microbiology samples, general lab suppo	ort						
Research Scientist (75% salary, 25% benefits): 25% FTE for each of 3 years. Sample c	ollection and	\$	82,462	\$-		\$	82,462
qPCR analysis of samples for P. destructans quantification in field and laboratory exp	periments. Fungal						
isolations and characterizations.							
Undergraduate student technicians (100% salary): 20% FTE for each of 3 years, med	lia and sample	\$	15,825	\$-		\$	15,825
prep, sample management, fungal cultivations, general lab support							
Professional/Technical/Service Contracts							
		\$	-	\$	-	\$	-
Equipment/Tools/Supplies							
Activity 1		\$	-	\$	-	\$	-
Microbiology supplies (media, reagents, petri dishes, tubes, gloves, field sampling materials)		\$	8,500		0	\$	8,500
Microscopy supplies and costs (microscope use, fixatives, sample prep instrumentation, sam	ple supplies)	\$	4,500				
					0	\$	4,500
						\$	-
Activity 2						\$	-
Microbiology supplies (media, reagents, petri dishes, tubes, gloves, field sampling materials)		\$	8,000		0	\$	8,000
Molecular biology/sequencing costs: (DNA isolation kits, PCR supplies, enzymes, reagents, s	equencing costs)	\$	12,000		0	Ś	12,000
Activity 3						\$	-
Chemical supplies (solvents, chromatography materials, reagents, tubes, glassware, pipettes	5)	\$	18,000		0	\$	18,000
Supplies for biological assays (pipettes, pipette tips, epi tubes, culture tubes, petri dishes, mo	edia, 96 well plates,	\$	14,000		0	\$	14,000
Travel: In-state round trip travel : room/board for 2-3 researchers for overnight trips, milea	ge, est. 5-6 trips/yr	\$	6,000		-	,	,
(1-3 days each trip) for 3 yrs		-				\$	6,000
Publication costs: ~2 total, \$1000 per publication-page/color fee charges and/or open acces	ss charges for	\$	2,000			\$	2,000
Euipment repair and calibration: Repair of equipment and instrumentation (e.g. vacuum pu	mps, water baths,	\$	3,000				
incubators, shakers, etc.) and calibration of instruments (pipettes, balances) estimated at \$1	.000 per year for 3						
years						\$	3,000
Other						\$	-
		\$	-	\$	-	\$	-
COLUMN TOTAL		\$	440,000	\$	-	\$	440,000
SOURCE AND USE OF OTHER FUNDS CONTRIBUTED TO THE PROJECT	Status (secured		Dudaat	Creant		Da	I amaa
	or pending)		Budget	Spent		ва	lance
Non-State:		\$	-	\$	-	\$	-
State:		\$	-	\$	-	\$	-
In kind: Salary support for co-PI (Blanchette) 5% FTE for 3 years		\$	34,515	\$	-	\$	34,515
Other ENRTF APPROPRIATIONS AWARDED IN THE LAST SIX YEARS Obligated but							
		Budget		Spent		Balance	
	not yet spent						
Biological Control of White Nose Syndrome in Bats: Phase 2 (balance as of 12/2018)		\$	452,000	426,704.6	0	\$	25,295
Harnessing Soudan Mine Microbes: Bioremediation, Bioenergy and Biocontrol, ML					T	\$	-
2013-03F (WNS research was one of several sub-aims in this multi-PI project)		\$	838,000	\$ 838,000	С		



02/26/2020



- Testing of best biocontrol strains on substrates from 3 diverse hibernacula
- Small scale field trials
- Mapping of *P. destructans* in hibernacula
- Assessment of physical and chemical factors in WNS-free cave