



Environment and Natural Resources Trust Fund

M.L. 2021 Draft Work Plan

General Information

ID Number: 2021-321

Staff Lead: Michael Varien

Date this document submitted to LCCMR: January 29, 2021

Project Title: Microbiome In Raptors: A New Tool For Conservation

Project Budget: \$129,000

Project Manager Information

Name: Julia Ponder

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Project Reporting

Date Work Plan Approved by LCCMR:

Reporting Schedule: December 1 / June 1 of each year.

Project Completion: June 30, 2023

Final Report Due Date: August 14, 2023

Legal Information

Legal Citation:

Appropriation Language:

Appropriation End Date: June 30, 2023

Narrative

Project Summary: We will evaluate the impact of microbial interventions during captivity on the raptor gut microbiome, both in terms of treatment efficacy during rehabilitation and subsequent environmental dissemination.

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

Antimicrobials and probiotics are both commonly-used therapies in raptor rehabilitation. However, these treatments can alter raptor microbial communities (i.e. microbiomes), which in turn may have unintended consequences for raptor health, as well as wider implications for natural ecosystems. Specifically, exposure to antimicrobials and the treatment of raptors with probiotics formulated for different species may cause long-term harmful perturbations to the gut microbiome, particularly in at-risk individuals. Further, antimicrobials may create selection pressure for antimicrobial resistant bacteria, which could then be introduced into the environment upon reintroduction of animals into their natural habitat. Although antimicrobial resistance (AMR) is one of the greatest public health challenges of the 21st century and wild birds, including raptors, are considered one of the primary mechanisms by which AMR is disseminated in the environment, the impact of AMR on ecosystem health is still largely understudied. As such, it is crucial to understand whether microbial interventions in captivity lead to the release of animals with impaired gut microbiomes that make them less fit for survival as well as create mechanisms for AMR dissemination.

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.

Our goal is to understand the impact raptor rehabilitation (treatment and captivity) has on the raptor gut microbiome and on the emergence and spread of AMR in the natural environment. With this information, we will be able to understand whether we are releasing raptors into the wild with impaired and/or altered microbiomes that make them less fit for survival and ultimately use this information to make improvements to raptor treatment and husbandry. In order to achieve this goal, we are seeking funding to characterize and quantify the gut microbiome and antimicrobial resistance in raptors. Specifically, we will be comparing the gut microbiome of healthy raptors captured at Hawk Ridge Bird Observatory (HRBO) in Duluth MN, during fall migration with injured raptors of the same species admitted to The Raptor Center (TRC) during the same timeframe. We will assess the health of sampled raptors using standard biometrics to link health to gut microbiome outcomes. In addition, we will longitudinally sample raptors admitted at TRC to evaluate the potential emergence and development of antimicrobial resistance before being released back into the wild.

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?

We will have an improved understanding of the short- and long-term impacts of microbial interventions during captivity on the raptor gut microbiome and an estimate of AMR risk, both in terms of treatment efficacy during rehabilitation and subsequent environmental dissemination. This project will also advance raptor welfare in rehabilitation settings by providing clinicians with actionable recommendations for treatments and husbandry during rehabilitation and better prognoses for survival after release. As we become more knowledgeable about healthy and altered microbiomes in wildlife species, we will be able to include the microbiome among the important tools for wildlife management and conservation plans.

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: Evaluation of the emergence of antimicrobial resistance in raptors

Activity Budget: \$63,547

Activity Description:

We will conduct a longitudinal study to evaluate the development of AMR in raptors admitted to TRC. We will include raptors that have a fair prognosis of survival upon admission (to allow for sampling over time) and fall into two groups: raptors that do not receive antibiotic treatment (control group) and raptors that receive at least seven days of continued antibiotic treatment. We will collect two cloacal swabs from all enrolled birds at three time points: 1) at admission prior to any treatment, 2) after treatment (if applicable) and before the bird is moved to a recovery flight enclosure, and 3) before release. We will record information on antimicrobial interventions as well as information about husbandry practices for each bird. We will then utilize the cloacal swabs to conduct antimicrobial susceptibility testing using standard laboratory disk diffusion techniques to characterize resistant bacteria, and we will use a microfluidic qPCR (MF-qPCR) to quantify antibiotic resistance gene differences over time and between groups.

Activity Milestones:

Description	Completion Date
Raptor sample collection at TRC for antimicrobial resistance analyses	December 31, 2021
Laboratory analysis of antibiotic resistant bacteria	September 30, 2022
Laboratory analyses to quantify antibiotic resistance genes (MF-qPCR)	January 31, 2023
Data analyses	April 30, 2023
Publication and presentation of results at a conference	June 30, 2023

Activity 2: Microbiome analysis and link to raptor health

Activity Budget: \$65,453

Activity Description:

We will collect cloacal samples from raptors at both HRBO and TRC during the fall migration of 2021. At HRBO, raptors will be trapped and sampled with the collaboration of HRBO researchers. The same collection procedures will be used to collect samples from raptors at TRC upon admission prior to any clinical treatment, and at several time points subsequently to evaluate changes in the microbiome over time. We will then extract bacterial DNA from all cloacal swabs and use next generation sequencing technology in combination with bioinformatic analyses to characterize raptor gut microbial communities. To assess the general health of each bird, we will collect measurements of weight, body condition score, and basic hematological parameters. We will take additional morphometric parameters to determine age and sex. We will analyze these data together with the microbiome results to identify associations between raptor health and characteristics of the raptor microbiome.

Activity Milestones:

Description	Completion Date
Raptor sample collection at HRBO (Duluth, MN) for microbiome analysis	November 30, 2021
Raptor sample collection at TRC for microbiome analysis	December 31, 2021
DNA extraction and sequencing	June 30, 2022
Bioinformatics and data analyses	October 31, 2022
Publication and presentation of results at a conference	May 31, 2023

Project Partners and Collaborators

Name	Organization	Role	Receiving Funds
Matthew Ettersen	Hawk Ridge Bird Observatory	Collaborator - will provide access to samples of wild-trapped birds during banding season and contribute to data analysis.	No

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.

Our findings will be presented at state, regional and national meetings as appropriate given the results. Publications will be produced for peer-reviewed journals, outreach newsletters and annual reports. Media outreach will also be pursued. 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 ENRTF Acknowledgement Guidelines.

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?

Understanding the links between raptor health and gut microbiome changes will be used as a new tool to tailor treatments of raptors undergoing rehabilitation, which will ultimately contribute to raptor conservation efforts. Results will also provide the first steps toward understanding how microbiome alterations may affect raptor fitness in the wild, as well as how they may contribute to the widespread dissemination of antimicrobial resistance in natural environments. Minnesota will be the pioneer on this front, and results can be later extrapolated to other rehabilitation facilities and ecosystems across the country. Future funding options to expand the work include Morris Animal Foundation, Association of Avian Veterinarians, and National Science Foundation.

Other ENRTF Appropriations Awarded in the Last Six Years

Name	Appropriation	Amount Awarded
Raptor Lab Integrating Online and Outdoor Learning Environments	M.L. 2014, Chp. 226, Sec. 2, Subd. 09h	\$186,000
Game and Nongame Bird Pesticide Exposure	M.L. 2016, Chp. 186, Sec. 2, Subd. 03m	\$349,000
Expanding Raptor Center Online Education	M.L. 2017, Chp. 96, Sec. 2, Subd. 05d	\$270,000
Spruce Grouse as Indicators for Boreal Forest Connectivity	M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 03e	\$350,000

Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Bene fits	# FTE	Class ified Staff?	\$ Amount
Personnel								
Laboratory technician		DNA extractions, bacterial culturing, susceptibility testing and sample preparation for genetic analysis			31.8%	0.3		\$15,324
Veterinary intern		Sample collection, preparation, handling and curation at The Raptor Center			22.45%	0.2		\$3,429
Researcher		Sampling, performs bioinformatics and microbiome analysis; oversees susceptibility testing; manuscript writing			36.5%	0.2		\$25,707
Post Doctoral Associate		Survey, sampling, genetic analysis, manuscript writing			25.4%	0.6		\$38,651
Project manager		Project oversight, coordination and reporting			36.5%	0.1		\$21,210
							Sub Total	\$104,321
Contracts and Services								
University of Minnesota Genomics Center	Professional or Technical Service Contract	Genetic sequencing of samples			0.05			\$6,968
							Sub Total	\$6,968
Equipment, Tools, and Supplies								
	Tools and Supplies	Laboratory supplies - consumables	Sample collection supplies, reagents, culture supplies and laboratory consumables including solvents, standards, vials and columns					\$7,925
							Sub Total	\$7,925
Capital Expenditures								
							Sub Total	-

Acquisitions and Stewardship								
							Sub Total	-
Travel In Minnesota								
	Miles/ Meals/ Lodging	Mileage	Trips to Hawk Ridge in Duluth MN for sample collection					\$1,574
							Sub Total	\$1,574
Travel Outside Minnesota								
	Conference Registration Miles/ Meals/ Lodging	Travel expenses, per diem and lodging	Travel to scientific meetings to present research results	X				\$2,212
							Sub Total	\$2,212
Printing and Publication								
	Publication	Open access fees for peer-reviewed journals	Scientific communication of research results					\$6,000
							Sub Total	\$6,000
Other Expenses								
							Sub Total	-
							Grand Total	\$129,000

Classified Staff or Generally Ineligible Expenses

Category/Name	Subcategory or Type	Description	Justification Ineligible Expense or Classified Staff Request
Travel Outside Minnesota	Conference Registration Miles/Meals/Lodging	Travel expenses, per diem and lodging	Scientific reporting of results at a national conference to be determined based on submission and acceptance of presentation proposal

Non ENRTF Funds

Category	Specific Source	Use	Status	Amount
State				
			State Sub Total	-
Non-State				
In-Kind	Waived facilities and administrative costs	The University of Minnesota is waiving the income normally generated from extramural research grants that contribute Facilities and Administrative (F&A). The current full rate is 55% of direct costs.	Pending	\$70,950
			Non State Sub Total	\$70,950
			Funds Total	\$70,950

Attachments

Required Attachments

Visual Component

File: [1c82958c-066.pdf](#)

Alternate Text for Visual Component

The graphic shows the state of Minnesota with marks noting the two locations (University of Minnesota, Hawk Ridge - Duluth) where sampling of birds from around the state will be done, with a picture of an eagle and a flowchart noting how microbiome analysis (Activity 1) and identification of antimicrobial resistance will lead to evidence-based recommendations to improve raptor conservation....

Optional Attachments

Support Letter or Other

Title	File
Partnership letter from Hawk Ridge Bird Observatory	f490d56d-856.pdf
Cover letter - University of Minnesota Sponsored Projects	66f65036-35f.pdf
Raptor Microbiome Research Addendum	311a32df-229.docx

Difference between Proposal and Work Plan

Describe changes from Proposal to Work Plan Stage

Corrected mailing address in section 2. Added dissemination information and reviewed all sections.

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

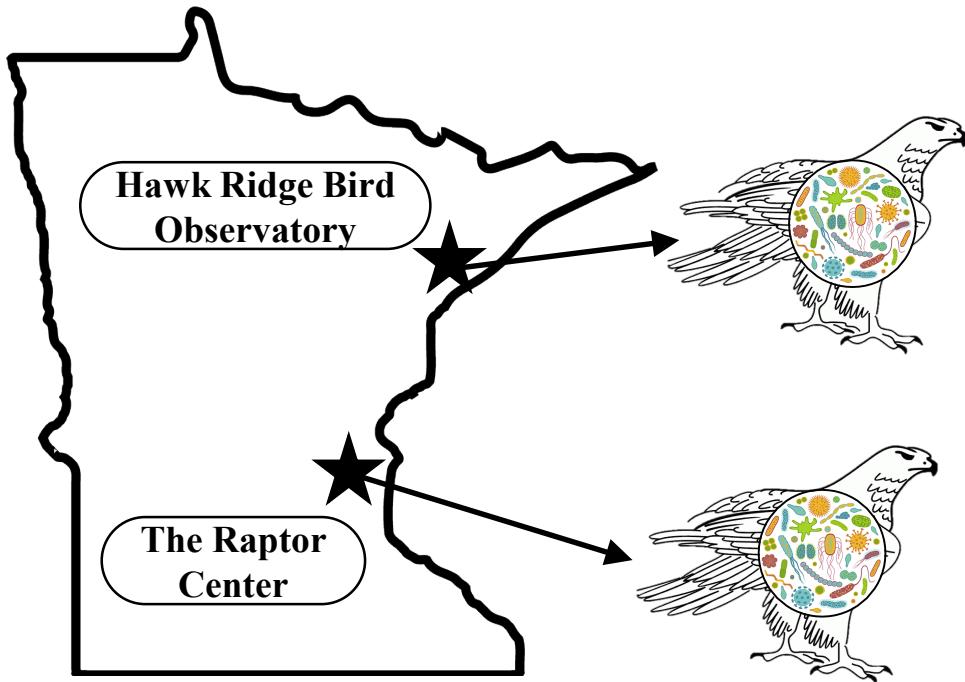
Does your project include original, hypothesis-driven research?

Yes

Does the organization have a fiscal agent for this project?

Yes, Sponsored Projects Administration

Microbiome in Raptors: A New Tool for Conservation



Activity 1
**Microbiome analysis and
link to raptor health**

Activity 2
**Emergence of antimicrobial
resistance in raptors**

**Evidence-based recommendations
to improve raptor conservation**