

**Environment and Natural Resources Trust Fund**

# M.L. 2021 Approved Work Plan

## **General Information**

**ID Number:** 2021-321

**Staff Lead:** Michael Varien

**Date this document submitted to LCCMR:** July 21, 2021

**Project Title:** Microbiome in Raptors: A New Tool for Conservation

**Project Budget:** $129,000

## **Project Manager Information**

**Name:** Julia Ponder

**Organization:** U of MN - Raptor Center

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## **Project Reporting**

**Date Work Plan Approved by LCCMR:** July 20, 2021

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

**Project Completion:** July 31, 2023

**Final Report Due Date:** September 14, 2023

## **Legal Information**

**Legal Citation:** M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 03m

**Appropriation Language:** $129,000 the first year is from the trust fund to the Board of Regents of the University of Minnesota for the Raptor Center to improve wildlife care and environmental stewardship by evaluating the impact of antibiotics administered during captivity on raptor gut microbiome, rehabilitation success, and the potential spread of antimicrobial resistance in the natural environment.

**Appropriation End Date:** June 30, 2024

## **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 Etterson | 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. Ineli gible** | **% 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](https://lccmrprojectmgmt.leg.mn/media/map/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](https://lccmrprojectmgmt.leg.mn/media/attachments/f490d56d-856.pdf) |
| Cover letter - University of Minnesota Sponsored Projects | [66f65036-35f.pdf](https://lccmrprojectmgmt.leg.mn/media/attachments/66f65036-35f.pdf) |
| Raptor Microbiome Research Addendum | [311a32df-229.docx](https://lccmrprojectmgmt.leg.mn/media/attachments/311a32df-229.docx) |
| Background check certification | [bcc72ace-2eb.pdf](https://lccmrprojectmgmt.leg.mn/media/attachments/bcc72ace-2eb.pdf) |

## **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.  
7/12/2021 - Adjusted capitalization in title as requested; background check form uploaded (no background check needed)  
7/13/2021 - Completion date changed in narrative as requested

## **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