

Final Abstract

Final Report Approved on January 6, 2025

M.L. 2021 Project Abstract

For the Period Ending June 30, 2024

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

Project Manager: Julia Ponder

Affiliation: U of MN - Raptor Center

Mailing Address: 1920 Fitch Avenue

City/State/Zip: St. Paul, MN 55108

Phone: (612) 624-3431

E-mail: ponde003@umn.edu

Website: <https://www.raptor.umn.edu/>

Funding Source:

Fiscal Year:

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

Appropriation Amount: \$129,000

Amount Spent: \$120,164

Amount Remaining: \$8,836

Sound bite of Project Outcomes and Results

This project (1) provided knowledge on the impacts to Minnesota's wildlife treated in rehabilitation facilities relative to antimicrobial resistance and gut microbiome changes, (2) found no increased risk of antibiotic resistance during wildlife rehabilitation treatment and (3) documented a need for standardized guidelines for antimicrobial use in wildlife rehabilitation.

Overall Project Outcome and Results

While antimicrobials and probiotics are commonly used therapies in wildlife/raptor rehabilitation, there is little known about potential unintended consequences for raptor health or implications for natural ecosystems. There are also questions about the potential of selecting for antimicrobial resistant bacteria, introduction of these bacteria into the natural environment, as well as impacts for public health. Our goal was to better understand the impact of medical treatment of injured/ill raptors relative to their gut microbiome make-up and possible emergence/spread of antimicrobial resistance bacteria into the natural environment. To accomplish this, we looked at the emergence of antimicrobial resistance in raptors admitted for rehabilitation and medical treatment as well as compared gut microbiome analyses between free-living raptors briefly trapped for banding studies and raptors in captivity for

rehabilitation and care.

Looking at red-tailed hawks and Northern saw-whet owls, we determined that the gut microbiome undergoes change during captivity and rehabilitation, although we were unable to determine if these changes impaired the birds' health or made them less fit for survival. We did not identify any change in risk for antimicrobial resistance in birds assessed over time while in captivity for rehabilitation. In addition, we conducted a novel national survey collecting data on the current level of knowledge and use of antimicrobials and probiotics in wildlife rehabilitation; the results documented the wide spread use of these therapies (used in all facilities at varying levels) and that 78% of surveyed wildlife rehabilitation facilities surveyed thought there would be benefits in standardized guidelines for the use of these therapies in wildlife (currently there are no guidelines).

This information will help wildlife rehabilitators in Minnesota by supporting best practice guidelines for the use of these therapeutic practices in wildlife.

Project Results Use and Dissemination

The results of our work have been published in the journal Plos One (see attachment) and presented as a poster and as a scientific presentation at conferences. We currently have another paper in preparation for publication. In addition, the results of this project are incorporated into the work done by the Partners for Wildlife program (UMN) does with Minnesota wildlife rehabilitators.



Environment and Natural Resources Trust Fund

M.L. 2021 Approved Final Report

General Information

Date: January 8, 2025

ID Number: 2021-321

Staff Lead: Mike Campana

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

Office Telephone: (612) 624-3431

Email: ponde003@umn.edu

Web Address: <https://www.raptor.umn.edu/>

Project Reporting

Final Report Approved: January 6, 2025

Reporting Status: Project Completed

Date of Last Action: January 6, 2025

Project Completion: June 30, 2024

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? Introduce us to the work you are seeking funding to do. You will be asked to expand on this proposed solution in Activities & 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	Approximate 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	Approximate 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 work 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	% Benefits	# FTE	Classified Staff?	\$ Amount	\$ Amount Spent	\$ Amount Remaining
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	\$104,014	\$307
Contracts and Services										
University of Minnesota Genomics Center	Professional or Technical Service Contract	Genetic sequencing of samples				0.05		\$8,057	\$5,425	\$2,632
							Sub Total	\$8,057	\$5,425	\$2,632
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					\$7,825	\$7,825	-
							Sub Total	\$7,825	\$7,825	-

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					\$800	\$800	-
							Sub Total	\$800	\$800	-
Travel Outside Minnesota										
	Conference Registration Miles/ Meals/ Lodging	Travel expenses, per diem and lodging	Travel to scientific meetings to present research results	X				\$1,997	\$1,997	-
							Sub Total	\$1,997	\$1,997	-
Printing and Publication										
	Publication	Open access fees for peer-reviewed journals	Scientific communication of research results					\$6,000	\$103	\$5,897
							Sub Total	\$6,000	\$103	\$5,897
Other Expenses										
							Sub Total	-	-	-
							Grand Total	\$129,000	\$120,164	\$8,836

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	\$ Amount Spent	\$ Amount Remaining
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.	Secured	\$70,950	\$66,090	\$4,860
			Non State Sub Total	\$70,950	\$66,090	\$4,860
			Funds Total	\$70,950	\$66,090	\$4,860

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....

Supplemental Attachments

Capital Project Questionnaire, Budget Supplements, Support Letter, Photos, Media, 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
Background check certification	bcc72ace-2eb.pdf
Wildlife Disease Association Conference Abstract	7532aebd-889.docx
Figures for June 2022 Status Update	74f9747f-b07.docx
Wildlife Disease Association Conference Poster (Dec. 2022 Update Attachment 1)	ff395130-e9c.pdf
Cover letter - University of MN SPA - amendment request	588380bc-bf7.pdf
Miller et al 2024_PLoS	57e95d86-330.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 understand that travel expenses are only approved if they 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 understand the UMN Policy on travel applies.

Does your project have potential for royalties, copyrights, patents, sale of products and assets, or revenue generation?

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

Work Plan Amendments

Amendment ID	Request Type	Changes made on the following pages	Explanation & justification for Amendment Request (word limit 75)	Date Submitted	Approved	Date of LCCMR Action
1	Amendment Request	<ul style="list-style-type: none"> • Budget • Narrative • Budget - Professional / Technical Contracts • Budget - Capital, Equipment, Tools, and Supplies • Budget - Travel and Conferences • Budget - Non-ENRTF Funds Contributed • Attachments 	An additional step is needed in microbiome sequencing, increasing the amount of funding for Univ of MN Genomics Center by \$1,089. We are requesting to redistribute unspent funds for Travel in MN (\$989) and \$100 of unspent supply funds to allow this extra sequencing step to be completed. The total redistribution of \$1,089 will not impact any other deliverables or activities of this project.	February 13, 2023	Yes	February 14, 2023
2	Completion Date	Previous Completion Date: 07/31/2023 New Completion Date: 06/30/2024	Sample analysis by UMGC (due June 2022) has been delayed until Mar 2023. Both lead researchers will be on maternity leave from Mar/Apr 2023 through July 2023, so unavailable to analysis sequencing data and finalize publications before July 2023 (original completion date). Extending the completion date would allow adequate time for completion of UMGC services and follow-up analysis work and publication.	February 13, 2023	Yes	February 14, 2023
3	Completion Date	Previous Completion Date: 06/30/2024 New Completion Date: 12/31/2024	LCCMR administrative workaround for final update.	August 5, 2024	Yes	August 5, 2024
4	Completion Date	Previous Completion Date: 12/31/2024 New Completion Date: 06/30/2024	LCCMR administrative workaround for final update.	August 5, 2024	Yes	August 5, 2024

Status Update Reporting

Final Status Update August 14, 2024

Date Submitted: August 17, 2024

Date Approved: November 22, 2024

Overall Update

Microbial profiling of red-tailed hawks and saw whet owls suggest that the raptor gut microbiome undergoes changes during captivity and rehabilitation. However, because we did not identify any correlation between raptor health status and gut microbial diversity or composition, we were unable to assess whether the changes that occur during captivity are potentially impairing the gut microbiomes and making the birds less fit for survival upon release.

Based on the longitudinal sampling of red-tailed hawks comparing admission to pre-release, we found that captivity and rehabilitation did not seem to have an effect on AMR risk.

The national scale survey we conducted, which was the first one of its kind, provided very useful information for clinicians and wildlife rehabilitators about optimizing antimicrobial and probiotic use in wildlife species as well as key gaps that need to be improved moving forward.

Activity 1

A total of 30 cloacal swabs from 15 red-tailed hawks were collected at two time points (admission and pre-release). These birds involved 5 adults, 6 hatch-years, and 4 second-years, and were 7 males, 7 females, and 1 of unknown sex. Seven birds received antibiotics during captivity, while 8 of them did not. Two types of bacteria were isolated: *Escherichia coli* and *Enterococcus* spp. The Sensititre method was used to identify antimicrobial resistance patterns. For *E.coli*, many of the samples showed resistance to fluoroquinolone and tetracycline antibiotics, which is common for this bacterium as they have intrinsic resistance mechanisms. However, we also found that 15% and 23% of the samples showed resistance to trimethoprim/sulfamethoxazole and streptomycin, respectively. For *Enterococcus* spp, 100% of samples were resistant to quinupristin/dalfopristin, 13% to nitrofurantoin and ciprofloxacin, and 7% to daptomycin. About 90% of the antibiotic resistance identified were in the admission samples. We also collected information on antimicrobial and probiotic use on wildlife rehabilitation centers across the U.S., and those results were published (attached as Miller et al 2024) and disseminated at international conferences (UoB_2023, WDA_2022, EWDA_2024). *(This activity marked as complete as of this status update)*

Activity 2

A total of 148 cloacal swabs were collected from both The Raptor Center (TRC) and Hawk Ridge Bird Observatory (HRBO) and underwent sequencing for gut microbiome profiling. There were 72 samples from red-tailed hawks and 76 samples from northern saw-whet owls, including 21 pairs of samples collected at two time points (TRC admission and pre-release). Primary microbiome analyses indicated that while the two raptor species are dominated by the same bacteria Phyla: Actinobacteriota, Firmicutes, Fusobacteriota, and Proteobacteria, their overall microbial communities are relatively distinct. Samples collected from TRC and HRBO had similar microbial community compositions, except for 11 northern saw whet owls from HRBO that had strikingly different microbiomes than all other samples. Between paired samples collected from the same TRC bird at different timepoints, there were noticeable shifts in the microbial communities of red-tailed hawks, but this was less pronounced in saw-whet owls. There was no indication that antibiotic treatment specifically had an effect on microbial community diversity or composition and there were no clear patterns between any raptor health metric (e.g. white blood cell count, body condition score, presence of blood parasites) and the gut microbiome. *(This activity marked as complete as of this status update)*

Dissemination

We submitted the work on antimicrobial and probiotic use in wildlife rehabilitation facilities to the journal Plos One and it is now published (<https://doi.org/10.1371/journal.pone.0308261>). We also submitted an abstract with results from the project to the European Wildlife Disease Association conference to be held in Germany in September 2024, and we will be presenting those results as an oral presentation (<https://ewda.org/upcoming-ewda-conference/>).

Throughout the project, we have published 1 paper, and we have another paper in preparation. We have presented results from the project at 3 international conferences (Wildlife Disease Association 2022, European Wildlife Disease Association 2024, and the Infection and Immunity Early Career Researchers' symposium at the University of Bristol, (UK) in 2023 for which we won a prize for one of the best poster presentations).

Additional Status Update Reporting

Additional Status Update August 14, 2024

Date Submitted: June 5, 2024

Date Approved: August 5, 2024

Overall Update

Per LCCMR staff guidance, due to system logic, this is place holder text for final update to be submitted in August 2024.

Activity 1

Per LCCMR staff guidance, due to system logic, this is place holder text for final update to be submitted in August 2024.

Activity 2

Per LCCMR staff guidance, due to system logic, this is place holder text for final update to be submitted in August 2024.

Dissemination

Per LCCMR staff guidance, due to system logic, this is place holder text for final update to be submitted in August 2024.

Status Update Reporting

Status Update June 1, 2024

Date Submitted: June 5, 2024

Date Approved: August 5, 2024

Overall Update

Since the last update, we have focused on a) disseminating results from the finished components of the project, and b) finalizing bioinformatic and statistical analyses from what remains to be done in the project. For a) we submitted a manuscript which summarizes the results from the survey we conducted on antimicrobial and probiotic use in wildlife rehabilitation, and it is currently 'under review'. We will be presenting these and other project results at an upcoming international conference. For b) we have been analyzing the final datasets from the project related to microbiome and antimicrobial resistance and writing up the results.

Activity 1

We have the results back from the laboratory on antibiotic resistant bacteria and antibiotic resistance genes from the raptor fecal samples. Two types of bacteria (a Gram negative and a Gram positive respectively) were isolated and tested for resistance against antibiotics using Sensititre: *Escherichia coli* (subjected to 13 different antibiotics and *Enterococcus* spp to 14 antibiotics). A total of 14 samples from seven birds were analyzed for 44 different antibiotic resistance genes using microfluidic qPCR (MF-qPCR). Additionally, data on antimicrobial treatments and other husbandry interventions were collected from the birds that were sampled. Data analyses on all these outputs are currently being finalized and are being incorporated into a manuscript.

Activity 2

Microbiome data (16S rRNA) files were obtained from the University of Minnesota Genomics Center. Data analyses for these data which include samples from two different bird species from both Hawk Ridge and The Raptor Center are currently being finalized and will be incorporated into the same manuscript as the results from Activity 1.

Dissemination

We submitted the work on antimicrobial and probiotic use in wildlife rehabilitation facilities (entitled 'Antimicrobial and probiotic use in wildlife rehabilitation in the United States') to the peer-reviewed journal Plos One and it is under review. We also submitted an abstract to the European Wildlife Disease Association conference to be held in Germany in September 2024, and we will be presenting the aforementioned paper plus results from Activity 1 as an oral presentation.

Status Update Reporting

Status Update December 1, 2023

Date Submitted: November 29, 2023

Date Approved: January 31, 2024

Overall Update

Progress since last update has been limited to focusing on writing of one publication (expected to be submitted early 2024) as both major contributors to this project have been on maternity leave. Overall, we have completed all data collection and laboratory sequencing of samples, the final analysis of which will provide insights into the impact of microbial interventions on raptor gut microbiome and potential dissemination of antimicrobial resistance in the environment. In addition to biological samples, we have collected information on use of antimicrobials and probiotics in wildlife rehabilitation facilities.

Activity 1

Completion of analysis of sample data on antibiotic resistant bacteria and antibiotic resistance genes is pending return of investigator from FMLA. Prior to this update, we reported isolating two types of bacteria from the samples and testing them for antimicrobial susceptibility to a range of antimicrobial drugs. We also submitted 14 samples from seven birds for evaluation of antimicrobial resistant genes - these assays have been run although results pending review by investigator.

Activity 2

Completion of microbiome analyses are pending return of investigator from FMLA. We previously reported collection of samples, DNA extraction results and sequencing.

Dissemination

We have drafted a publication on usage of antimicrobials and potential impacts by wildlife rehabilitation centers. Submission is pending review and final additions by investigator who is on FMLA.

Status Update Reporting

Status Update June 1, 2023

Date Submitted: June 5, 2023

Date Approved: June 13, 2023

Overall Update

We have completed all data collection and laboratory sequencing of samples and are on track to have analysis completed, which will provide insights into the impacts of microbial interventions on raptor gut microbiome community and the dissemination of antimicrobial resistance in the environment. As part of our process, we are also working with wildlife rehabilitation facilities to understand and document their needs for more information on antimicrobial use as well as probiotics.

Activity 1

All proposed laboratory processing and analyses have been completed:

- We isolated two types of bacteria from the samples: *Escherichia coli*, a Gram negative bacterium, and *Enterococcus* spp. a Gram positive bacterium. The bacterial isolates have been tested for antimicrobial susceptibility to a range of antimicrobial drugs by determining the minimum inhibitory concentration (MIC) via the Sensititre system. Irene Bueno and Elizabeth Miller are analyzing the resulting data.
- A subset of 14 fecal samples from seven birds (pre- and post- treatment samples) were selected for MF-qPCR targeting a range of antimicrobial resistance genes. This assay will allow us to identify resistance genes present in the raptor gut, not just those found in *E. coli* and/or *Enterococcus*. The DNA samples have been submitted for processing and we are currently waiting on the results.

Activity 2

Despite issues with lower than expected biomass, the 16S rRNA gene sequencing has finally been completed by the University of Minnesota Genomics Center and the sequences have been transferred to us for microbiome analyses. Analysis of the sequencing data has been started. Number of raw sequencing reads per sample range from 4,929 to 311,813, with a sample mean of 99,577.86 reads.

Dissemination

Irene Bueno presented a poster with the preliminary survey results from the antimicrobial and probiotic use survey at the 'Infection and Immunity Research Network' Early Career Symposium (University of Bristol, UK) on 1st February 2023 and she was awarded the 2nd place for best poster presentation. The title of the poster was: Antimicrobial resistance dynamics and antimicrobial use in wildlife rehabilitation.

We conducted an online survey of antimicrobial and probiotic use practices in U.S. wildlife rehabilitation facilities. Analyses of the survey data have been completed and a manuscript draft has been written. We are now in the process of reviewing the manuscript draft and readying it to send to collaborators. Overall, the study found that antimicrobials (specifically antibiotics and antifungals) were used in all rehabilitation facilities with more or less frequency and for a variety of reasons. 78% of respondents thought that their facility would benefit from standardized antimicrobial and antifungal use guidelines, which currently do not exist. A high proportion of facilities (66.67%) also reported use of a variety of probiotics, but none of the products used were developed for use in wild bird species. That said, 57% of respondents believed that probiotics improved their patient outcomes.

Status Update Reporting

Status Update December 1, 2022

Date Submitted: December 2, 2022

Date Approved: December 5, 2022

Overall Update

We have completed many of the milestones from Activities 1 and 2 and we are on track to achieve the goals we set for this project. For Activity 1 we have finished all of the laboratory work, and for Activity 2, despite some delay, we will be receiving the microbiome data in the upcoming weeks and begin analyzing it. In terms of dissemination, we presented the preliminary results from the antimicrobial use survey at an international conference during the Summer, and we are preparing a manuscript with the results that should be ready for submission at the beginning of 2023.

Activity 1

We have completed milestones 1 (“Raptor sample collection at TRC for antimicrobial resistance analyses”) and 2 (“Laboratory analysis of antibiotic resistant bacteria”) of Activity 1, as we collected raptor samples from The Raptor Center in Fall 2021, and have now completed the laboratory analyses for antibiotic resistant bacteria. We isolated two types of bacteria from the samples: *Escherichia coli*, a Gram negative bacterium, and *Enterococcus* spp. a Gram positive bacterium. The bacterial isolates have been tested for antimicrobial susceptibility to a range of antimicrobial drugs by determining the minimum inhibitory concentration (MIC) via the Sensititre system. We are currently analyzing the resulting data and preparing a subset of samples for MF-qPCR targeting a range of antimicrobial resistance genes.

Activity 2

For activity 2, we have completed most of the milestones. DNA was extracted from the cloacal swabs collected from raptors at The Raptor Center and Hawk Ridge Bird Observatory, and are currently being processed for sequencing. There was some delay in the laboratory work due to staff shortages and increases in the sequencing cost since the proposal was originally written, but this step should be finalized in a few weeks. While we wait to receive the sequencing data to conduct the bioinformatic analyses, the metadata collected from birds (blood samples, morphometric information, etc.) is being organized and prepared for inclusion in analyses.

Dissemination

We conducted an online survey of antimicrobial and probiotic use practices in U.S. wildlife rehabilitation facilities. Preliminary results from the survey were presented at the Wildlife Disease Association Conference in July 2022 in the form of scientific poster (Attachment 1). We are now finalizing the manuscript with the results from this survey to submit it for publication at the beginning of 2023. We will also be preparing a lay report to be shared with the participating wildlife rehabilitation facilities.

Status Update Reporting

Status Update June 1, 2022

Date Submitted: June 7, 2022

Date Approved: June 7, 2022

Overall Update

We have completed several milestones from Activities 1 and 2 and we are on track to achieve the goals we set for this project. We have collected the samples we needed and we are currently working on the analysis of samples in the laboratory for both antimicrobial resistance (Activity 1) and microbiome (Activity 2). We have also gathered data to inform both activities which we will use in the analytical phase along with the results from the samples. In terms of dissemination, we developed an online survey and delivered it to 106 wildlife rehabilitation facilities within the U.S. In this survey we asked about antimicrobial and probiotic use practices, as well as perceptions and knowledge of antimicrobial resistance and antimicrobial use in wildlife settings. We are working on a manuscript to report on the survey results and we have submitted a proposal to present it at an international conference in July 2022.

Activity 1

For activity 1, we completed the 1st milestone: “Raptor sample collection at TRC for antimicrobial resistance analyses”. We collected 41 fecal samples from red-tailed hawks at The Raptor Center (TRC) at two time points: at admission and before their final outcome (release or euthanasia). We also collected information from each bird included in the study consisting of general information (e.g., recovery location, age, sex), and treatments received during captivity, with a special focus on antimicrobial therapy. Samples are currently being processed in the laboratory for antimicrobial resistance analyses.

We had originally proposed to collect samples at three time points, but we were only able to collect samples at two time points. This change does not impact our research goals though, as the second sample represents any changes in the microbiome and antimicrobial resistance associated with captivity and/or treatment. The total number of birds enrolled was fewer than what we had estimated based on historical data for raptor admissions. Also, our IACUC approval took longer than expected, missing birds admitted during that time. Despite the slight deviations from the plan, we will be able to complete the outcomes we set out for this project without losing any scientific rigor.

Activity 2

For activity 2, we completed two milestones: “Raptor sample collection at HRBO (Duluth, MN) for microbiome analysis”, and “Raptor sample collection at TRC for microbiome analysis”. We collected a total of 105 cloacal swabs from northern saw-whet owls (Figs. 1, 2) and red-tailed hawks (Fig. 3) at Hawk Ridge Bird Observatory (HRBO) and 49 cloacal swabs from the same species at TRC (Fig. 4). In addition, we collected blood samples from each bird, weight, and body condition score to assess their general health status, as well as morphometric parameters, age, and sex. All cloacal swabs are currently being processed in the laboratory for DNA extraction and sequencing. Similarly to Activity 1, the number of samples collected (i.e., the number of birds enrolled) was smaller than originally anticipated. However, we will be able to complete our goals without losing any scientific rigor.

Dissemination

Our original outcomes included “to 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”. In order to accomplish this, aside from Activities 1 and 2, we developed an online survey and delivered it to 106 wildlife rehabilitation facilities within the U.S. In this survey we asked about antimicrobial and probiotic use practices, as well as perceptions and knowledge of antimicrobial resistance and antimicrobial use in wildlife settings. The results of this survey will be key to understanding antimicrobial prescribing practices in the treatment of captive wildlife. We are working on a manuscript to publish the results from this survey in a peer-reviewed journal, and we will also

provide a lay report for the wildlife rehabilitation community. The survey, in combination with the results from Activities 1 and 2 will be key to developing antimicrobial stewardship programs for wildlife rehabilitation as well as to provide an understanding of wildlife rehabilitation impact on the release of antimicrobial resistance into the environment. We submitted an abstract with a summary of the survey results to the Wildlife Disease Association conference in July 2022