

Bats are an important part of our ecosystems and the agriculture sector, as they provide essential ecosystem services in the form of pest control. The health of bats is indispensable, yet many bat species are threatened by the spread of white nose syndrome, pesticide use, windmill turbines, and habitat loss. Bat fecal samples can be used to profile the gut microbiome to gain a better understanding of the differences and similarities of their microbial composition. Learning more about the microbiome composition of bats may give us insight into why species vary in susceptibility to certain diseases. There is not much available information about bat gut microbiomes particularly among Myotis grisescens. The objective of this study is to sample and analyze the fecal microbiome of wild M. grisescens throughout the state of Missouri. We will extract the DNA from fecal samples using the ZymoBIOMICS DNA/RNA Miniprep kit and perform 16S rRNA sequencing using standard amplicon primer sets through the Genomics Technology Core (GTC) facility. Microbiome composition data received from the GTC will be processed using QIIME2 software. We plan to analyze the microbiome data of *M. grisescens* to gain a better understanding of the microbiome composition and variation of healthy gray bats sampled over the course of a few years from multiple sites throughout Missouri. These could reveal areas of further research to determine how differences in the composition of the gut microbiome translate to functional differences in diseased versus healthy states.

Background

M. grisescens is currently an endangered species, due to human disturbance of their colonies, pesticide use, and the spread of WNS. Gray bats are microchiropterans that inhabit caves in the Midwest and Southeast US that are close to areas of feeding, typically < 2 km away. Gray bats forage for insects like moths and flies over small, calm aquatic bodies such as streams and ponds, and they rely on echolocation to catch their prey. They play an important role in providing ecosystem services as one of the main regulators of night flying aquatic insects.

Comparative microbiome analysis of *Myotis grisescens*

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Methods

Fecal samples and health data were collected from wild gray bats that were humanely trapped at multiple known gray bat habitat locations in the state of Missouri from 2014 through 2022. To characterize the health of gray bats we collected data on age, sex, reproductive status, and wing condition and size. The gray bats were released after fecal samples and health data were collected. We also collected multiple bulk samples at maternity colonies.

The collected fecal samples were stored in DNA shield, and DNA was extracted from fecal samples using ZymoBIOMICs DNA/RNA Miniprep Kit. Then the extractions were quantified using a Qubit fluorometer. These samples were submitted to the Genomics Technology Core (GTC) at the University of Missouri at a concentration of 3.5 ng/uL in EB buffer for 16S sequencing.

The sequenced DNA will be processed utilizing QIIME2 software.



Figure 1. This figure shows the steps taken to collect and analyze samples. Figure 2. This figure shows the wing scoring system on a scale of 0 (no damage/healthy) to 3 (heavy damage/unhealthy).¹

Results

This project is ongoing. At this time, we have submitted our samples to the GTC and are waiting for these results to complete our analysis. We plan to analyze commonalities and differences in the microbiome of Myotis grisescens according to:

- •Geographic location
- •Age (juvenile vs adult)
- •Sex
- •Reproductive status
- •Change over time (2014 2022)

The bulk samples collected at maternity colonies will be analyzed for similarities and differences between maternity colonies.

Our goal is to characterize the microbiome of healthy gray bats, and to determine if there are any significant differences in the composition of their microbiomes between different groups.

We will utilize QIIME2 software to run our analysis. We plan to analyze beta diversity and dissimilarities between groups using an ordination technique such as principal coordinates analysis (PCoA).



Figure 3. This figure shows the counties in Missouri where samples were collected and the number of bats sampled.

Future Work

The ongoing study of the gut microbiome in bats is important in understanding the overall health of bats. Characterizing what a normal healthy gut microbiome in different species of bats will allow us to look at how different health conditions may disrupt the microbiome and contribute to disease processes.

Figure 4. These images show a gray bat being examined to collect health data before fecal samples are collected.

Acknowledgements

References

1. Jonathan D. Reichard Center for Ecology and Conservation Biology Department of Biology Boston University Boston, MA.Wing-Damage Index Used for Characterizing Wing Condition of Bats Affected by White-nose Syndrome. https://sbdn.org/wpcontent/uploads/2018/08/Reichard Scarring-indexbat-wings.pdf



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