Exit Report to the Western National Park Association

October 2018

Are caves a source of bat microbiota? A comparison of microbial communities in caves and cave roosting bats in El Malpais National Monument

Investigators: Dr. Diana E. Northup and Jennifer Hathaway

Park: El Malpais National Monument, New Mexico

1. Abstract:

The most critical threat to bats today is the rapid spread of white-nose syndrome (WNS) and the causative fungus, *Pseudogymnoascus destructans*, across the United States. There is an urgent need to study bats in the ever-shrinking WNS-free areas, like El Malpais National Monument (ELMA). Knowledge of the natural defenses a bat's external microbiota (bacteria and other microbes that live on the bats' skin and fur) can provide is slowly coming to light, but little is known as to where bats acquire their external bacteria. Which bacteria we find living on a bat's skin or fur depends on whether we caught the bat in a cave or on the surface (Winter et al., 2017). The cave-caught bats have a larger proportion of Actinobacteria in their external microbiota, a phylum of bacteria known to produce secondary metabolites including antifungals and antibiotics. Actinobacteria, including rare and novel species, are abundant in caves. We hypothesized that the microbial mats that adorn the walls and ceiling of caves may be the source of the external microbiota on bats. To test this, we compared the bacteria sequences obtained from swabbing the fur and wing surfaces of roosting bats in caves in ELMA (WNS-free at the time of this study) to microbial mats from the cave ceiling and walls found near the bats' roost using DNA sequencing technology. We chose ELMA to conduct this study as we have multi-year microclimate data showing that caves at ELMA have the conditions necessary to foster the growth of *P. destructans*, the WNS causative fungus. Furthermore, we have previously worked with ELMA in studying the external microbiota of bats and a previous pilot study indicated that there was a large overlap in the bacteria shared between the bats and the caves in which they roosted This comparison would allow us to determine if the caves are the source of the external microbiota on bats. By characterizing this link between caves and bats we would be better able to educate the public about cave and bat protection at ELMA. We are recommending educating ELMA visitors about the value of the cave microbial mats as a source of natural defenses for ELMA bats and are suggesting measures to protect these microbial mats.

2. Relevance to the Park:

Our research at El Malpais National Monument (ELMA) lays the foundation for continued investigation into the microbiota on western bats. Understanding the role external bacteria play in the health of an organism begins with knowing what bacteria are living on that organism and where the organism may be acquiring those bacteria. Understanding the connection between the ecosystem (caves) and the organisms (bats) is crucial for the protection of both. Caves are a fragile ecosystem and a crucial habitat for bats. Our

findings demonstrate a strong link between the microbial mats that are found on walls and ceilings of many ELMA caves and the bats that day roost or hibernate in these caves. This finding suggests several key management recommendations, including the following. We are recommending that ELMA staff educate park visitors about the nature and value of the microbial mats in caves for bats. Many visitors are not aware that they are looking at mega cities of microorganisms when they look at the white, tan, and yellow microbial mats on the walls of many of the caves. We have seen visitors "write" their names in the mats in caves worldwide, build fires in areas near the cave microbial mats, or other actions that may harm the microbial mats. Further, we encourage ELMA staff to remind visitors to not eat in the caves, or to eat over a bag, to prevent enriching the cave in human-sourced organic matter. Continuing to stress to park visitors the importance of not urinating, defecating, or spitting in the caves, as this brings unwanted, foreign microorganisms and organic matter. Emphasizing the importance of having clean boots, caves, and gear when entering caves on the Monument, something being done to prevent the spread of WNS, also will help protect the microbial mats from contamination with microorganism or organic matter from other caves or the surface. Our results reinforce ELMA staff's efforts to protect bat hibernacula and important day roost caves from human disturbance at critical times.

3. Process:

We decided to focus on two caves Classic Cave and West Cave to increase the statistical power of our data set. We worked with Eric Weaver and Laura Bauman of ELMA to select these caves. We swabbed 16 bats from four species found roosting in these two caves. While an experienced bat biologist (D. Buecher) held the bats, Dr. Northup swabbed each bat thoroughly on all fur surfaces, wings and uropatagium. Swabs were preserved in sucrose lysis buffer (SLB) and stored on dry ice in the field. Microbial mat samples from as close to the roosting site as possible were collected aseptically, and covered in SLB to preserve DNA. A total of 12 bats and 11 microbial mats were collected from the two caves. Swabs of the ambient air were also collected from each location with bats for a total of four air controls, an addition to our original proposed methods.

The microbial mats were preprocessed in the lab to ensure ample DNA extraction. All samples were sent to Mr. DNA, in Shallowater, TX, for DNA extraction and sequencing on March 27, 2017. Samples will be sequenced for the V4-V6 region of the 16S rDNA gene, the gene used to classify bacteria into species.

Analysis of the resulting sequences was performed using QIIME and statistical packages for R such as vegan, deseq and ggplot. We found that the microbial mats in caves appear to be contributing a great deal to the external microbiota of bats, especially of *C. townsendii*. Many of the shared bacteria between bats and microbial mats are from the phylum Actinobacteria. Further analysis is still being conducted to determine if any of these Actinobacteria could be providing health benefits to the bats. These methods are as we proposed, with the exception of the added air sample controls.

4. Bookkeeping:

Funds were spent as follows:

- Personnel: $4,040.50

- Travel: $148.20
- Consultant Fees: $322 (Bat Biologist)
- Technical Services fees: $2,700 (Sequencing)
- Supplies: $173.59

Funds from another source were secured for some of the travel expenses, allowing for some of those funds to be used to sequence additional samples.

Other/In-Kind Contributions included:
- Northup's time mentoring, doing fieldwork, and analyzing data @ $230/day X 5 days = $1150 unpaid labor donated by Northup.

5. Products Resulting From This WNPA-Funded Project

Manuscripts
1. Master's thesis by Nicole Caimi on these data is expected by April 2019.
2. A manuscript describing these results is expected in May 2019.

Interpretative Materials
1. Information poster made with input from the ELMA staff highlighting the important connection between bats and their roosts. Poster is included below.
2. CD of photographs taken by Kenneth Ingham of bats and caves at ELMA for the Park Staff to use in promotional and interpretive materials was delivered in August 2018.

Presentations to the ELMA Staff:
Northup, D. 20 August 2018. Testing for the Presence of *Pseudogymnoascus destructans* and Bat-Cave Wall Microbiota in ELMA Caves. Presentation given to the Interpretive Ranger Staff of El Malpais National Monument. Grants, NM.

Scientific Presentations:
Caimi, Nicole A., Hathaway, Jennifer JM., Buecher, Debbie C., and Northup, Diana E. Are caves a source of bat microbiota? A comparison of bacterial and fungal communities in caves and cave roosting bats in El Malpais National Monument, New Mexico. Presented at the Intermountain, Rio Grande, and Rocky Mountain ASM TriBranch Meeting held April 6-7, 2018 in Durango, CO.

Either Caimi or Hathaway will be giving a presentation on this research at the Winter Technical Regional of the Southwest Region of the National Speleological Society, to be held 1 December 2018 at the University of New Mexico, Albuquerque, NM. We will invite ELMA staff to attend.

6. Evaluations:
Both WNPA and ELMA staff have been exceptionally supportive and helpful throughout this project. The reports requested are not overly onerous and the collaboration with the Park is

enjoyable and provides open communication between resource managers and project scientists.

Poster Drafted for El Malpais Interpretive Staff