

APPENDIX: Wyoming Wild Sheep Foundation - GIA

Project Title: What is the Respiratory Microbiome of Wild Sheep?

Detailed Budget

	Cost to be funded by WY-WSF Grant	Cost to be Funded by other cooperators
Equipment	\$0.00	
Services	\$16,000.00	\$4,000 (AK Dept of Fish & Game)
Publishing	\$2,000.00	
Monitoring	\$0.00	
Supplies	\$7,000.00	\$5,000 (WY Game & Fish)
Other(specify)	\$7,000.00	
Totals	\$32,000.00	

Services

Library Prep and Sequencing (University of Minnesota):

Free-ranging: 120 animals x 2 (1 nasal and 1 tonsil per animal) = 240 reactions = \$12,000

Captive: 38 samples x 2 (1 nasal and 1 tonsil per animal) = 76 reactions = \$4,000

Dall sheep: 70 expected samples (1 nasal each) = \$4,000

Publishing

Journal of Wildlife Disease or similar peer-reviewed journal: \$2000

Other

Travel to local and regional meetings: mileage/airfare, hotel, registration, per diem meals, \$3000

Macbook Laptop for microbiome analysis, \$4000

Supplies

DNA extraction: \$3,000

Gloves/Tubes/Buffer/96 well plates/expendables: \$4,000

OTHER ORGANIZATIONS PROVIDING FINANCIAL AID OR SUPPORT OF THE PROJECT (Include any pending amounts applied for.):

Wyoming Game & Fish: providing tissue collection swabs, transport media and manpower to collect samples. These supply costs are approximately \$5,000.

Alaska Dept of Fish and Game: providing tissue collection swabs, transport media, shipping costs, and manpower to collect samples. Will cover \$4,000 associated with microbiome sequencing costs.

Sources Cited

1. Butler, C. J. *et al.* Assessing respiratory pathogen communities in bighorn sheep populations: Sampling realities, challenges, and improvements. *PloS one* **12**, e0180689 (2017).
2. Cassirer, E. F. *et al.* Pneumonia in bighorn sheep: Risk and resilience. *The Journal of Wildlife Management* **82**, 32–45 (2018).
3. Butler, C. J. *et al.* Respiratory pathogens and their association with population performance in Montana and Wyoming bighorn sheep populations. *PLOS ONE* **13**, e0207780 (2018).
4. Cassirer, E. F., Manlove, K. R., Plowright, R. K. & Besser, T. E. Evidence for strain-specific immunity to pneumonia in bighorn sheep. *The Journal of Wildlife Management* **81**, 133–143 (2017).
5. Cassirer, E. F. & Sinclair, A. R. E. Dynamics of Pneumonia in a Bighorn Sheep Metapopulation. *Journal of Wildlife Management* **71**, 1080–1088 (2007).
6. Amat, S., Subramanian, S., Timsit, E. & Alexander, T. W. Probiotic bacteria inhibit the bovine respiratory pathogen *Mannheimia haemolytica* serotype 1 in vitro. *Lett. Appl. Microbiol.* **64**, 343–349 (2017).
7. Vonaesch, P., Anderson, M. & Sansonetti, P. J. Pathogens, microbiome and the host: emergence of the ecological Koch's postulates. *FEMS microbiology reviews* **42**, 273–292 (2018).
8. McDanel, T. G., Kuehn, L. A. & Keele, J. W. Evaluating the microbiome of two sampling locations in the nasal cavity of cattle with bovine respiratory disease complex (BRDC). *Journal of animal science* **96**, 1281–1287 (2018).

Tables and Figures

	NASAL	TONSIL
WYOMING		
Jackson	26	26
Absaroka	15	15
Whiskey Mtn	41	41
Sybille Captive	10	14
NEBRASKA		
Pine Ridge	27	27
Wildcat Hills	20	20
ALASKA		
13A, 14A, and 14C	22	
TOTAL	161	143

Table 1. Nasal and tonsillar swabs collected during pathogen surveillance during the winter 2018-2019. Swabs are stored in glycerol preservation media in -80° C freezer.

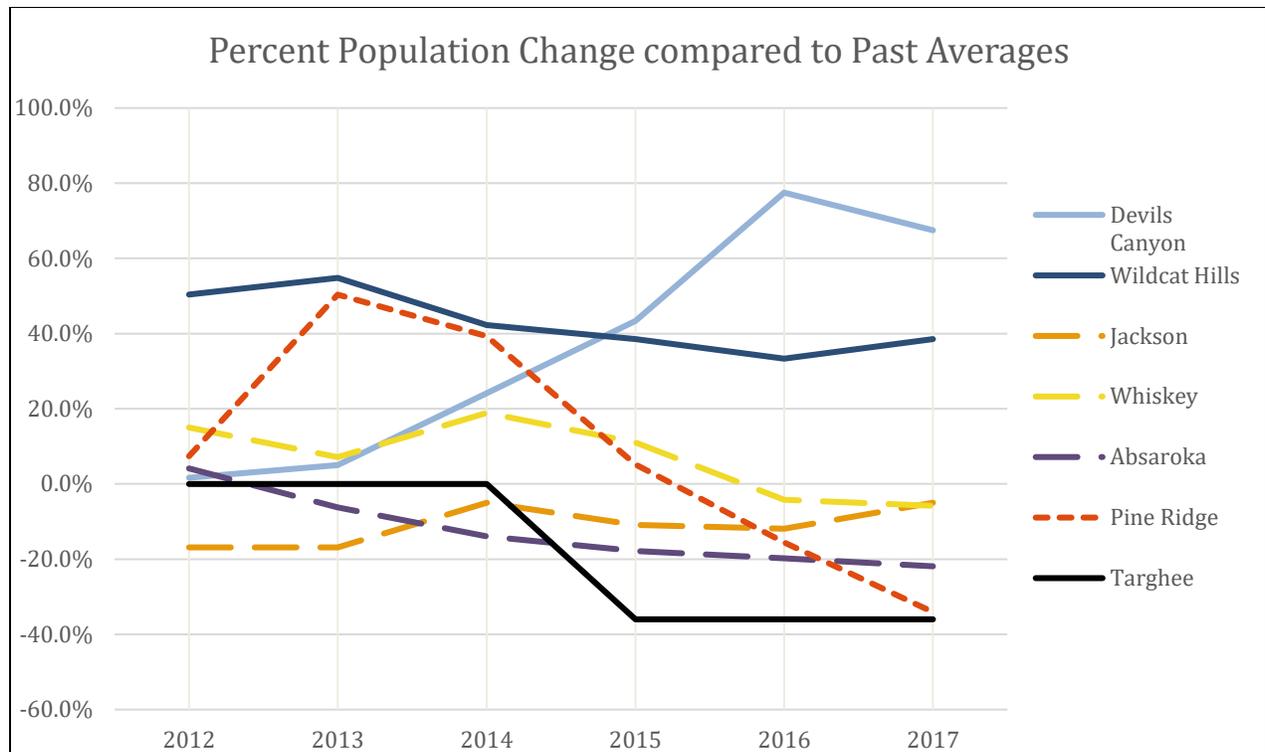


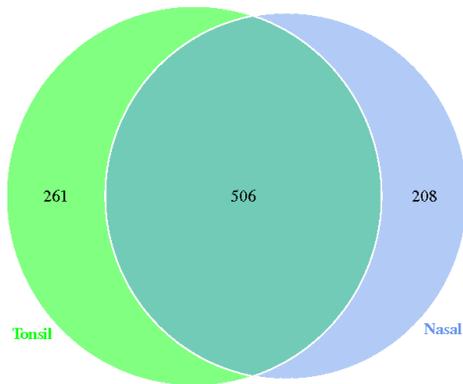
Figure 1. Yearly cumulative population change (λ) evaluated from published populations estimates. Initial population datum was calculated from a 5-year population average (2007-2011). Wyoming populations, Jackson, Whiskey Mtn, Absaroka, Targhee, and Devils Canyon are reported as an average of the 3-year population estimate. Solid lines are populations reported as generally healthy with positive population growth and above historical population abundance. Dashed lines are populations in varying states of recovery from pneumonia epizootics. The population health status of Targhee is not published, but the population has a negative trend. Samples from Devils Canyon and Targhee would be opportunistically collected and analyzed.

Nebraska and Wyoming Bighorn Sheep Herds



Figure 2. General distribution of study populations of Wyoming and Nebraska bighorn sheep. Population boundaries are adjoining in Wyoming, but limited connectivity is thought to exist.

A



B

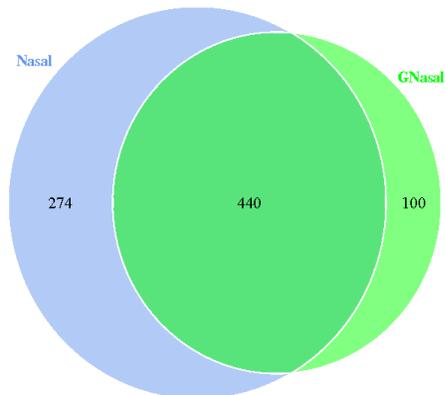


Figure 3. Venn Diagrams for preliminary data on alpha diversity from microbiome analysis of nasal and tonsil swabs (A), and of nasal swabs from animals of two different herd units (B). Alpha diversity measures the number of different types of bacteria and how evenly they are distributed. 3A represents the number of bacteria that are unique to the tonsil (261) vs the nasal (208), and how many are shared (506) from animals in the same herd. 3B represents the number of bacteria that are unique and shared between nasal samples from two different herds. In this figure bacteria are identified to the species level.

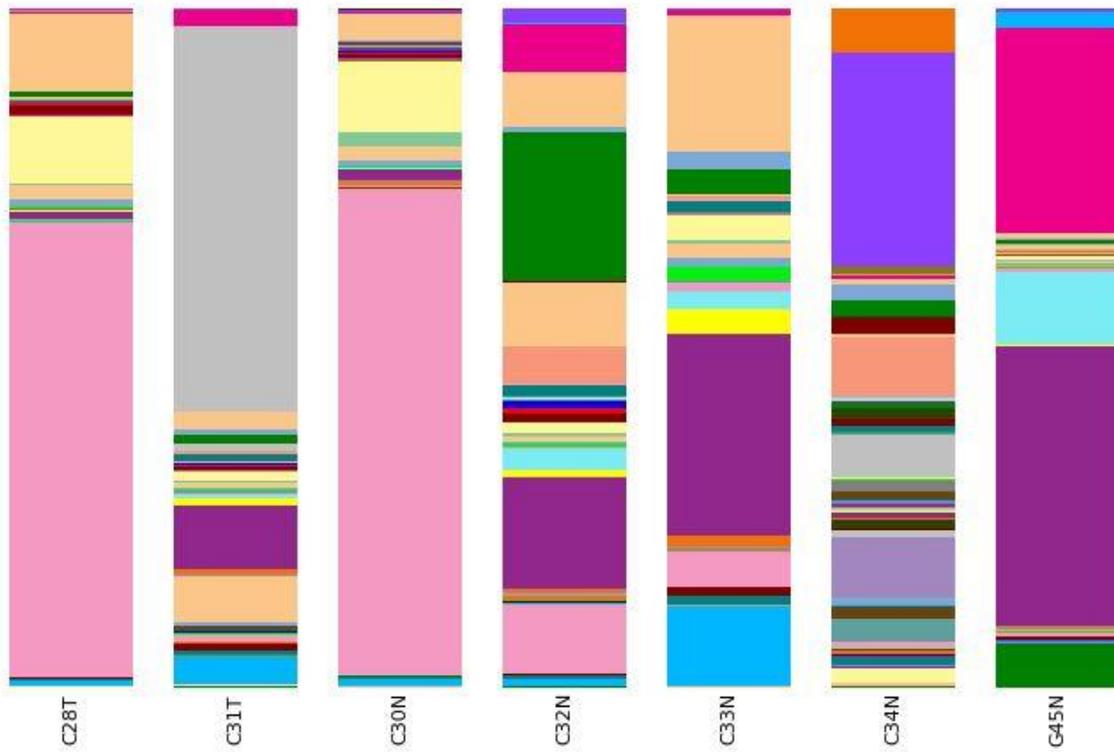


Figure 4. Microbiome analysis showing the proportion of bacterial OTUs per animal. Identification of bacteria is made to the Family level. Notable bacteria families are: Lactobacillus (straw), Pasteurelleacea (grey), Moraxellaceae (magenta), Streptomyces (hot pink), Mycoplasma (dark grey; detected but not visible).