

Longitudinal characterization of captive Wyoming toad

(*Anaxyrus baxteri*) microbiome



Veterinary Research
Scholars Program
University of Missouri

Nicole Scarberry¹, Zachary L. McAdams^{2,3}, Derek Benson⁴, Jason Herrick⁴, Brandon Moore⁵, and Aaron C. Ericsson^{2,3}
College of Veterinary Medicine¹, Molecular Pathogenesis and Therapeutics program², MU Metagenomics Center³, University of Missouri, Columbia, Missouri
Omaha's Henry Doorly Zoo and Aquarium, Amphibian Conservation Center⁴, Omaha, Nebraska
Department of Biology, Stephens College⁵, Columbia, Missouri



Background

- 1987- Thought to be extinct until small population found
- 1989- Last 10 individuals brought to Cheyenne Mountain Zoo
- 1991- Declared Extinct in the Wild
- 1993- Establishment of *ex-situ* breeding program
 - Presently 5 AZA zoos and 2 USFWS facilities
- 1995- First captive animals released back into the wild
 - Annual wild releases for over 20 years
- Mortenson Lake National Wildlife Refuge, Laramie Basin, Wyoming
- Habitat loss vs. *Batrachochytrium dendrobatidis* (*Bd*)
- Mucosal protection against *Bd* infections

Study design

Samples from breeding population at Omaha's Henry Doorly Zoo and Aquarium's (OHDZA) Amphibian Conservation Center (ACC).

Comparisons of Microbiome Samples:

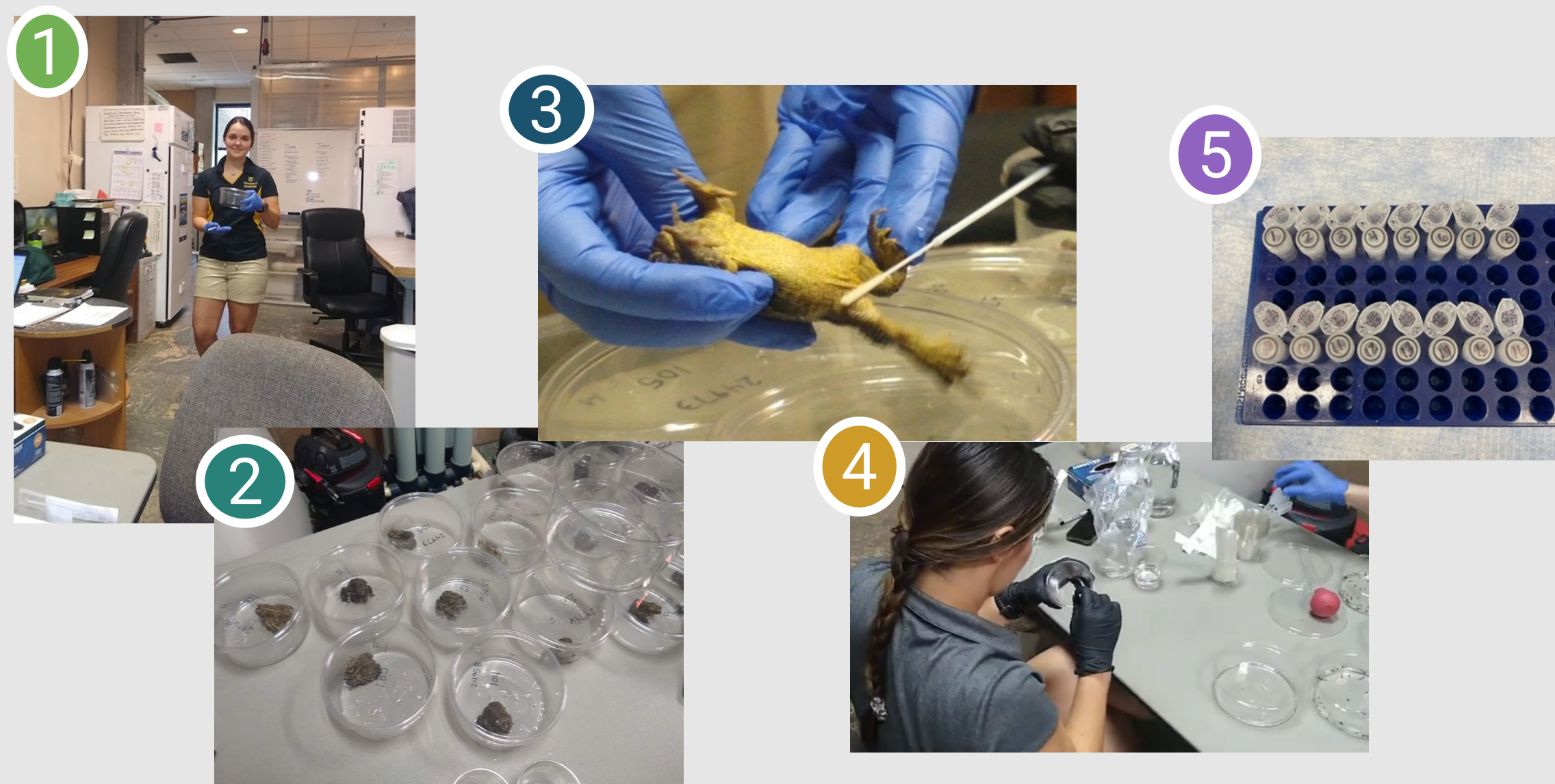
- Adult Breeder Toads vs. Tadpoles
- Male vs. Female (Adults)
- Across time
- Cohort and Housing differences (Tadpoles)
- Captive housed vs. Wild caught Adults (*pending*)

Goal: Husbandry modifications to improve survival among reintroduction population following release

Animals	Samples Collected	Sample Collection Timepoints			Totals
		Post-hatching	Mid-development	Pre-release	
Tadpoles	Mucosal Swab: Room 2 (Cohort A & F)	8	8	8	24
	Mucosal Swab: Room 8 (Cohort P & O)	8	8	8	24
Adults	Mucosal Swab	8	8	8	24
	Cloacal Swab	8	8	8	24
Totals		32	32	32	96

Table 1: Distribution of samples for microbiome analysis

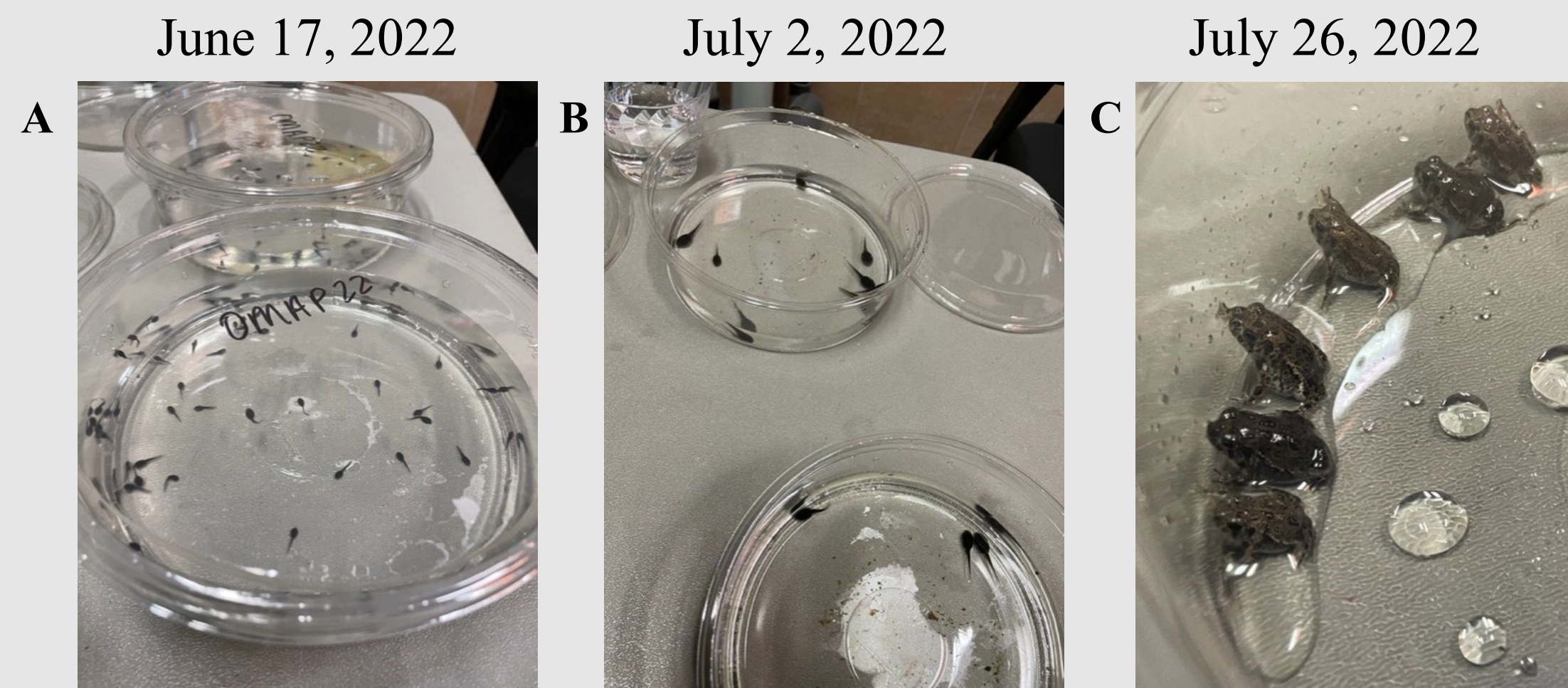
Methods



- Samples obtained from ACC at OHDZA
- Animals removed from isolation units to sampling area in individual containers (max time 1 hr)
- Toads washed with DI water, 60 "swipe" protocol along ventrum, dosum, mouth, and legs; Additional cloacal swab protocol
- Tadpoles washed with DI water, water briefly drained off to facilitate 10 "swipe" protocol of pooled samples
- Samples transported back to lab for DNA extraction and analysis

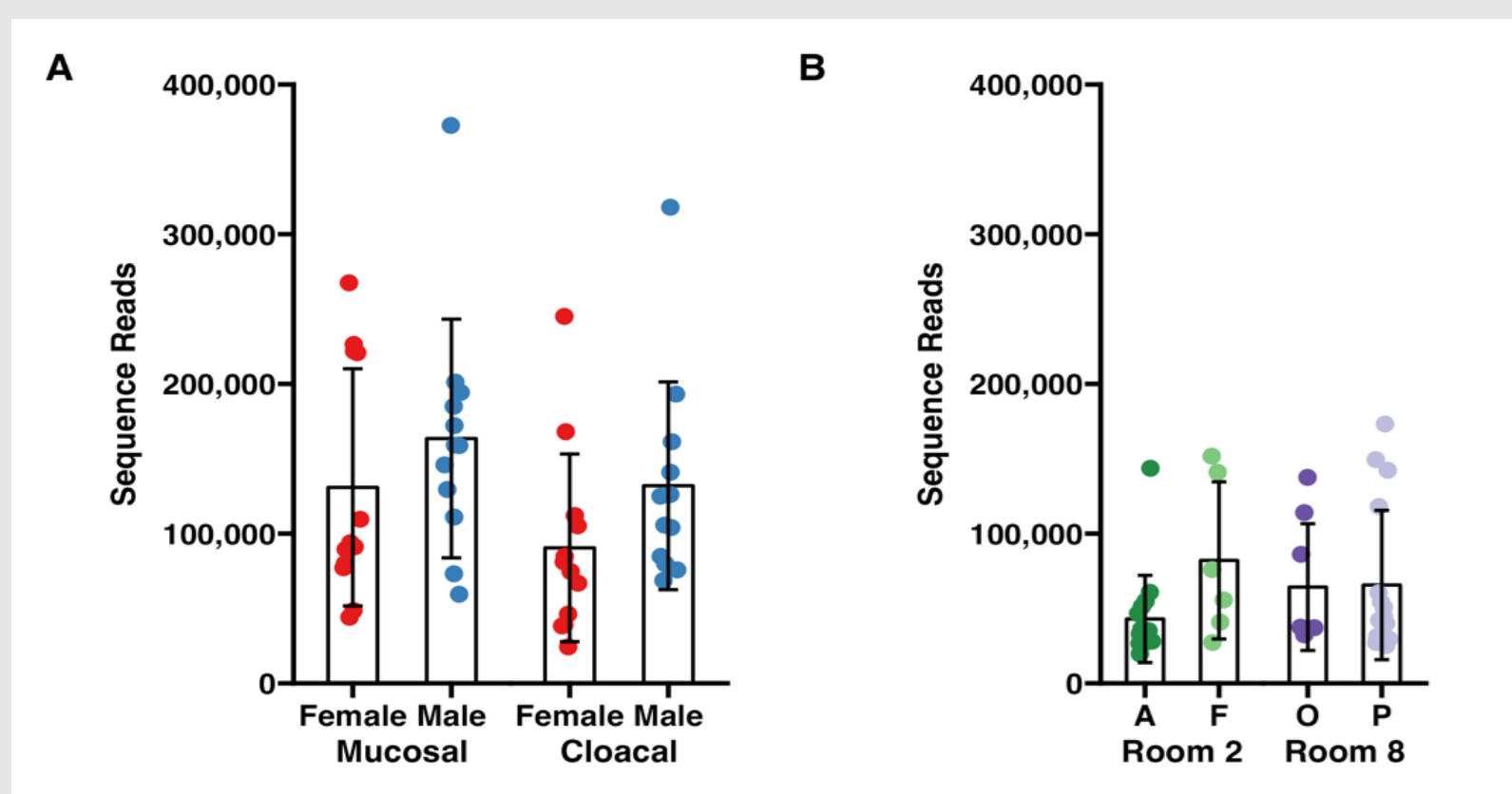
Longitudinal perspective of tadpoles throughout sampling

Figure 1. Photographs of tadpoles used for sample collection at 1-5 days post-hatch (A), two weeks later (B), and two days post-metamorph (C).



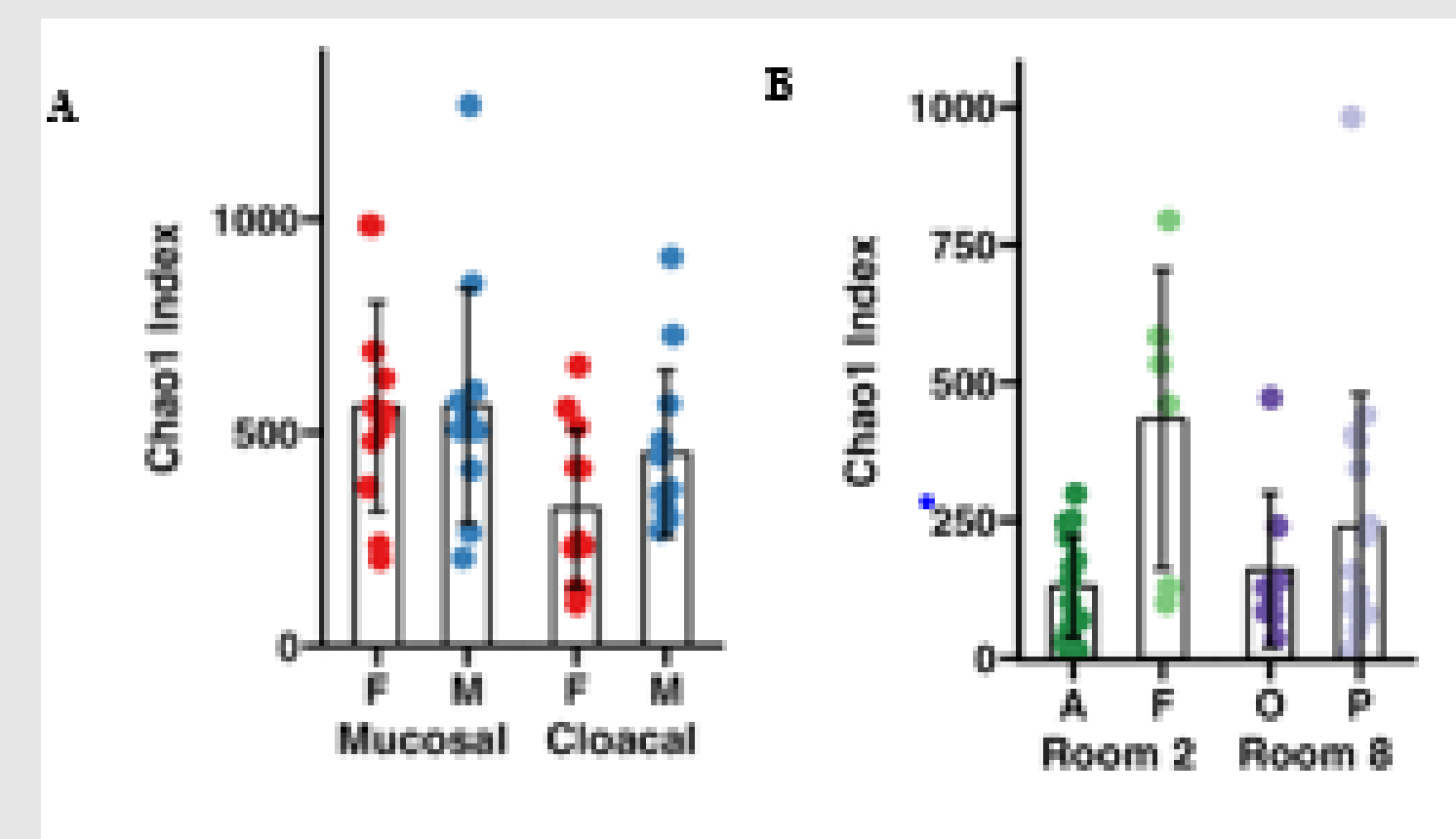
Successful amplification of all samples

Figure 2. Dot plots showing the total number of high-quality sequence read counts per sample from adults (A) or tadpoles (B).



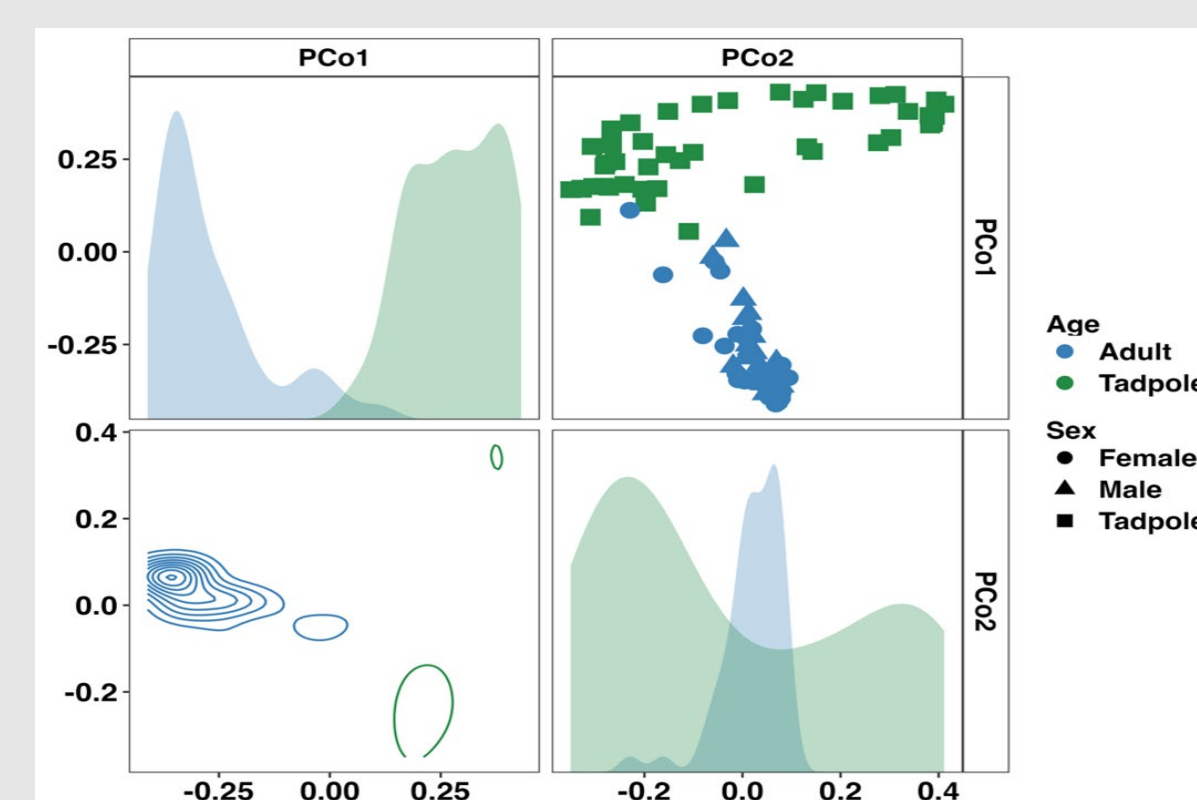
Adults and tadpoles-richness

Figure 3. Dot plots showing the microbial richness detected in samples from adults (A) or tadpoles (B).



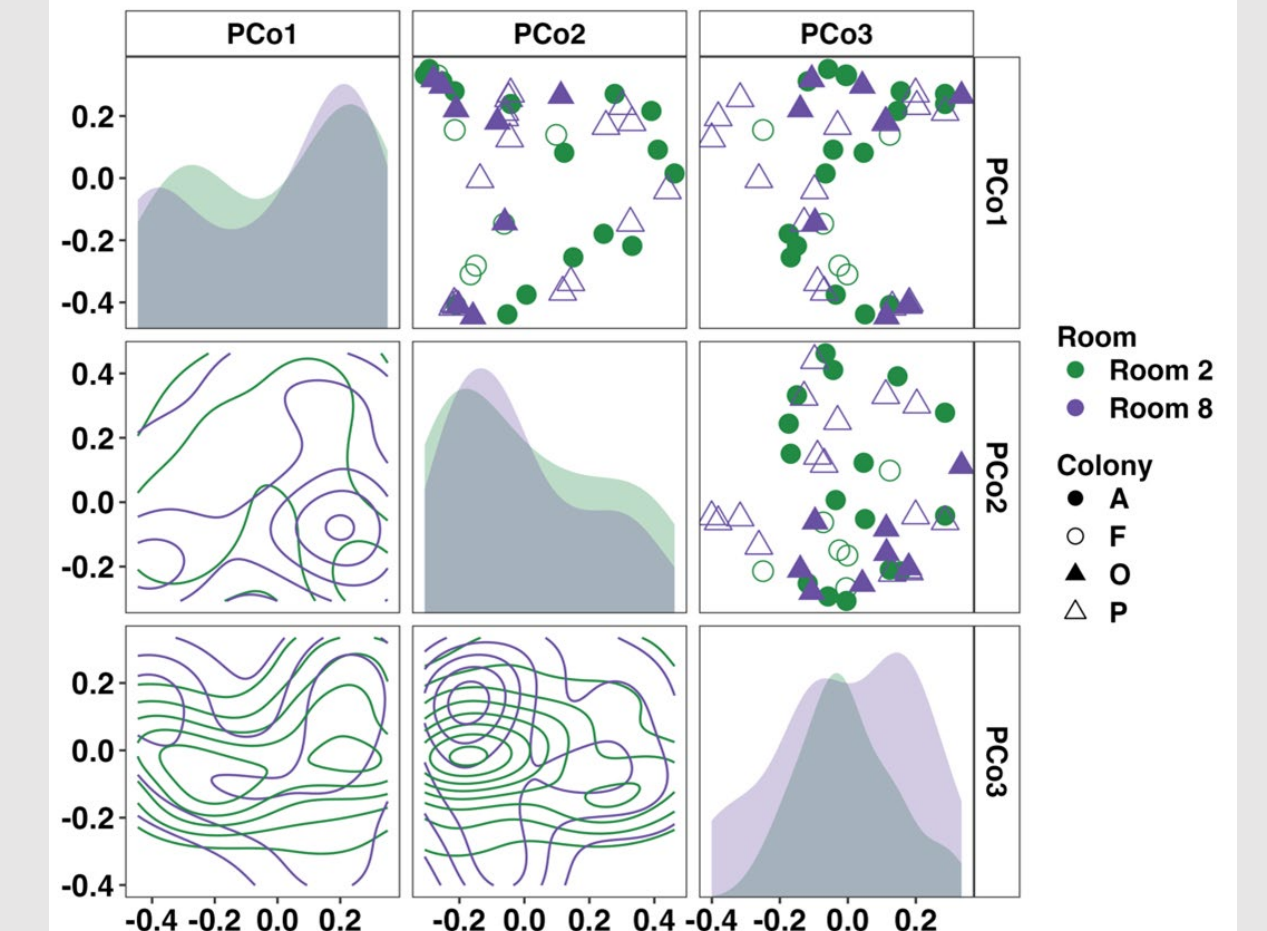
Adults and tadpoles- beta-diversity

Figure 4. Principal coordinate analysis matrix using weighted Bray-Curtis distances depicting significant differences in community composition between adult and tadpole Wyoming toads along the first (22.75%) and second (9.06%) principal coordinates. $F = 23.0$, $p < 0.001$. One-way PERMANOVA.



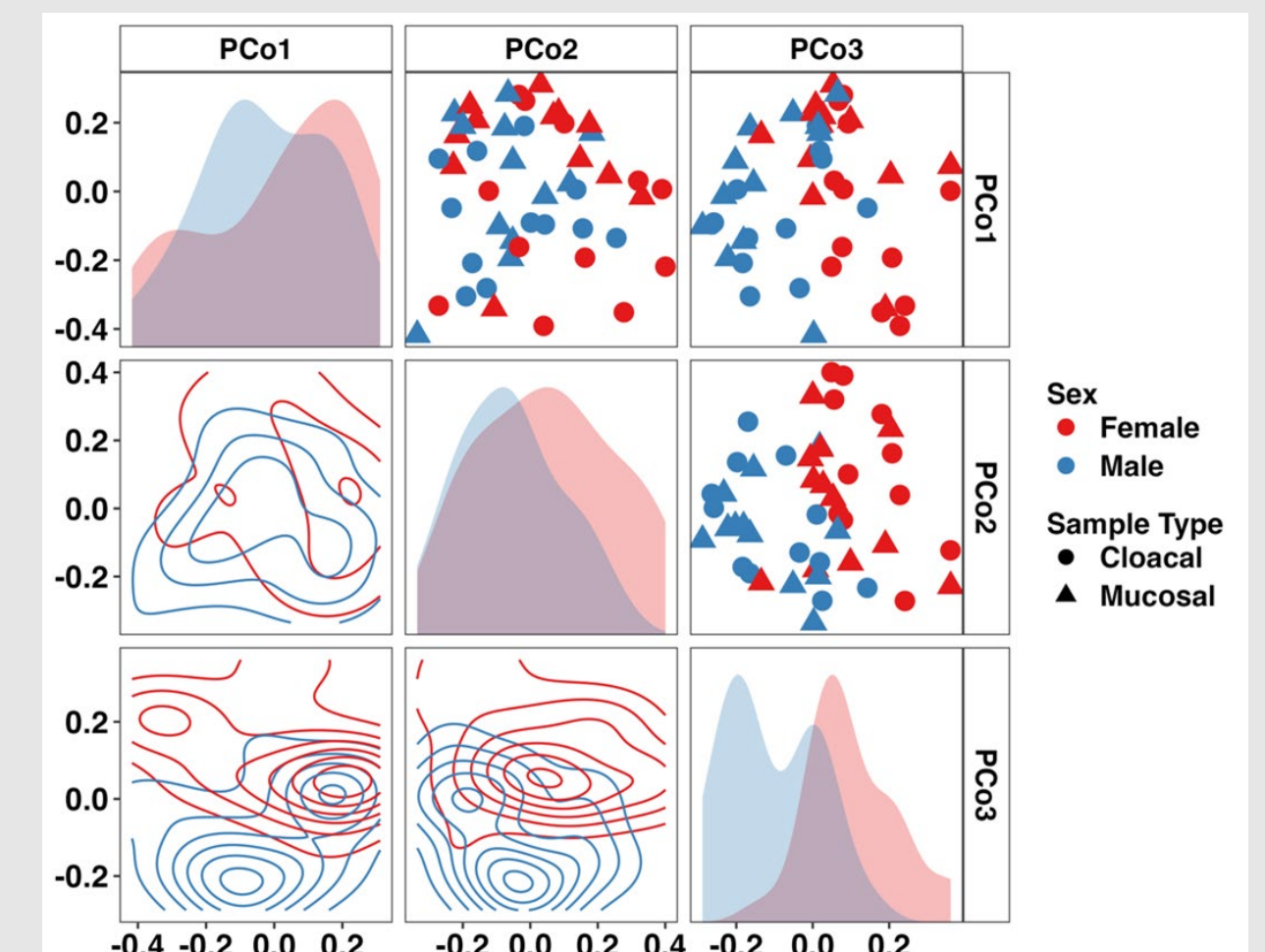
Tadpole rooms and cohort comparison

Figure 5. Principal coordinate analysis matrix using weighted Bray-Curtis distances depicting differences in community composition between Wyoming tadpole colonies along the first (19.41%), second (13.43%), and third (7.89%) principal coordinates. Significant colony-dependent differences were observed. Room: $p = 0.053$, $F = 1.7$; Room:Colony: $p = 0.021$, $F = 1.6$. Nested two-factor PERMANOVA.



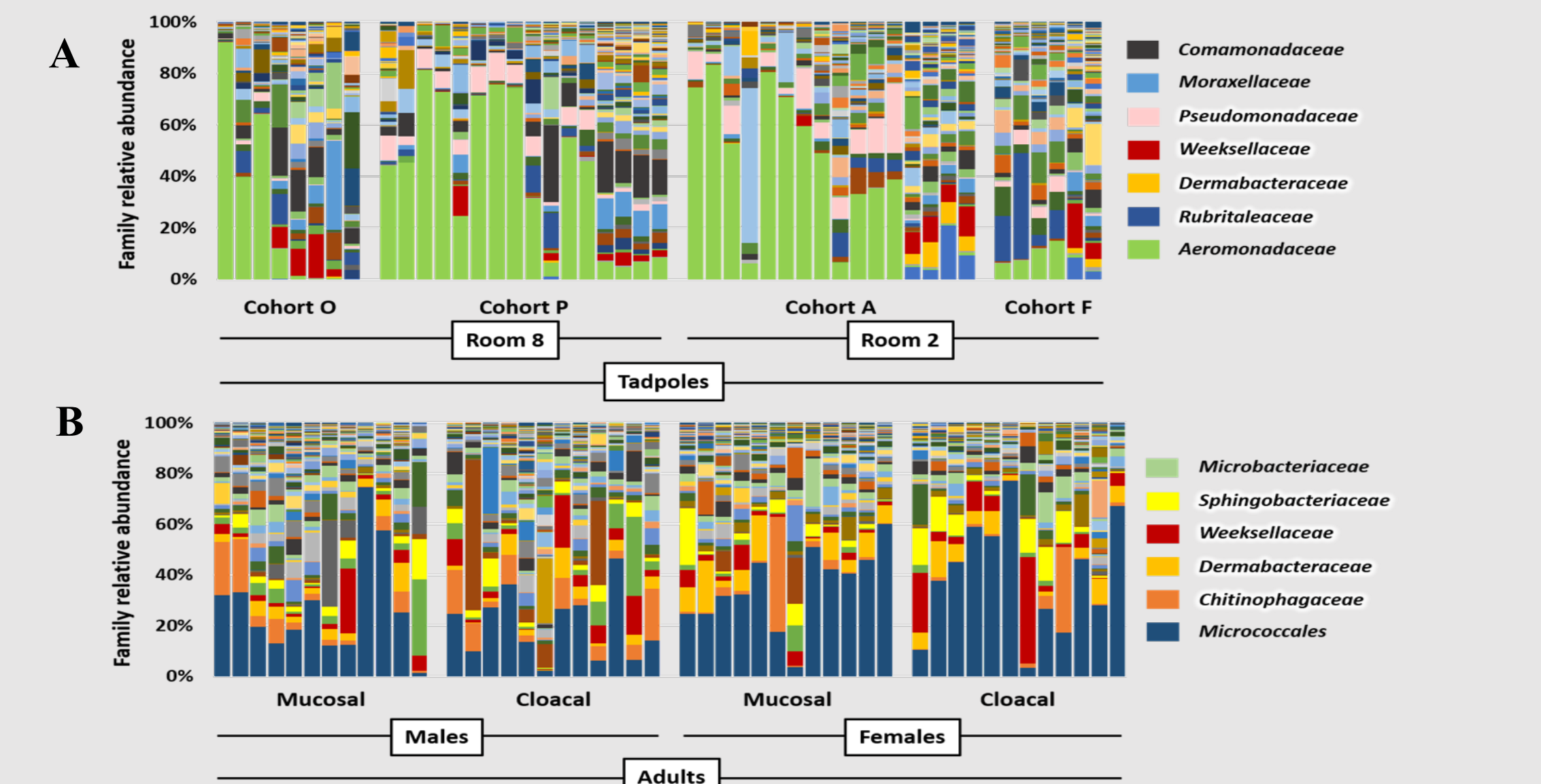
Adult sex-dependent and sample type comparison

Figure 5. Principal coordinate analysis matrix using weighted Bray-Curtis distances depicting differences in community composition between sex and sample type in adult Wyoming toads along the first (16.31%), second (13.45%), and third (9.85%) principal coordinates. Sex- ($p < 0.001$, $F = 3.6$) and sample type-dependent ($p = 0.014$, $F = 1.9$) effects on community composition were observed. Two-way PERMANOVA.



Age-dependent relative abundance

Figure 5. Relative abundance plot showing microbial make up on skin mucosa of tadpoles (A) and adults (B).



Conclusions

- Tadpoles and adults had differences in taxa, richness, and distribution
- Male and female adult populations had significant variance

Moving forward:

- Comparing with wild individuals in Laramie Basin (partnering with University of Wyoming)
- Husbandry modifications → toad probiotics?
- What does this mean for *Bd*?

Acknowledgements

- Huge thank you to OHDZA's Amphibian Conservation Center, The Wyoming Toad Recovery Team, U.S. Fish and Wildlife Service, the MU Metagenomics Center, and the MU College of Veterinary Medicine's Veterinary Research Scholars Program (VRSP)
- Stipend support for Nicole Scarberry was provided by the Morris Animal Foundation through the Veterinary Student Scholars grant.