

# Exploring the Relationship Between a Fungal Pathogen and the Skin Microbiome in Northern Two Lined Salamanders

Will Wasielewski<sup>1</sup>, Julia Fregonara<sup>1</sup>, Jesse Garrett-Larsen<sup>1</sup>, Erica Berent<sup>1</sup>, Nathaniel Newman<sup>1</sup>, Vincent Gilleylen<sup>1</sup>, Martin Edwards<sup>1</sup>, Caileigh Briggs<sup>1</sup>, Corinne Richards-Zawacki<sup>2</sup>, Miranda Kosowsky<sup>2</sup>, Richard Lehtinen<sup>1</sup>  
([rlehtinen@wooster.edu](mailto:rlehtinen@wooster.edu))<sup>1</sup>

<sup>1</sup> The College of Wooster, Biology Department

<sup>2</sup> University of Pittsburgh, Department of Biological Sciences

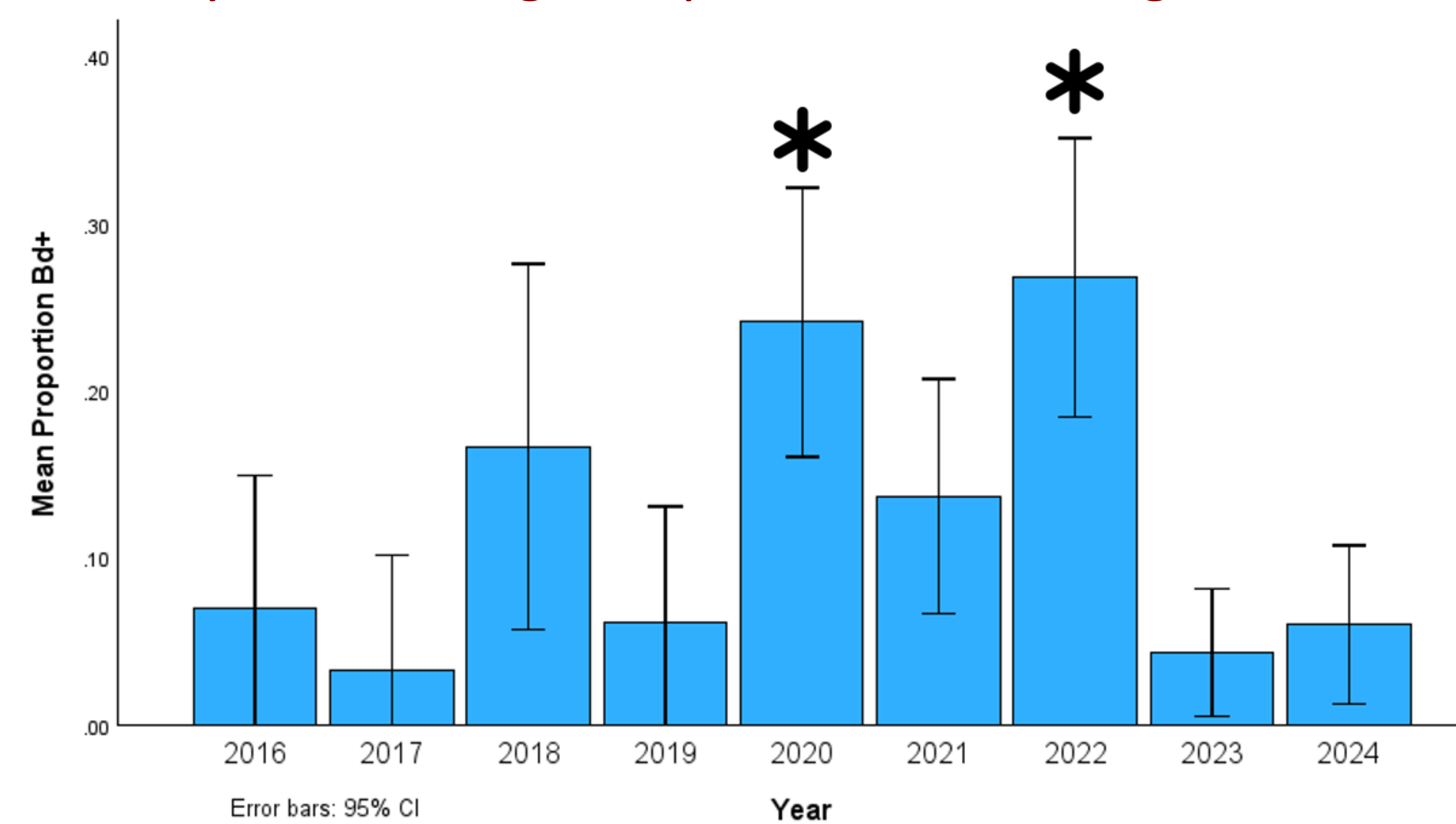
## Background

*Batrachochytrium dendrobatidis* (Bd) is an emerging fungal skin pathogen of amphibians. To understand its effect on salamander populations, we have been monitoring Bd infection frequency in a local northern two lined salamander (*Eurycea bislineata*) population for the past 9 years. We have also compared the microbiomes of Bd+ and Bd- individuals using metagenomic sequencing.

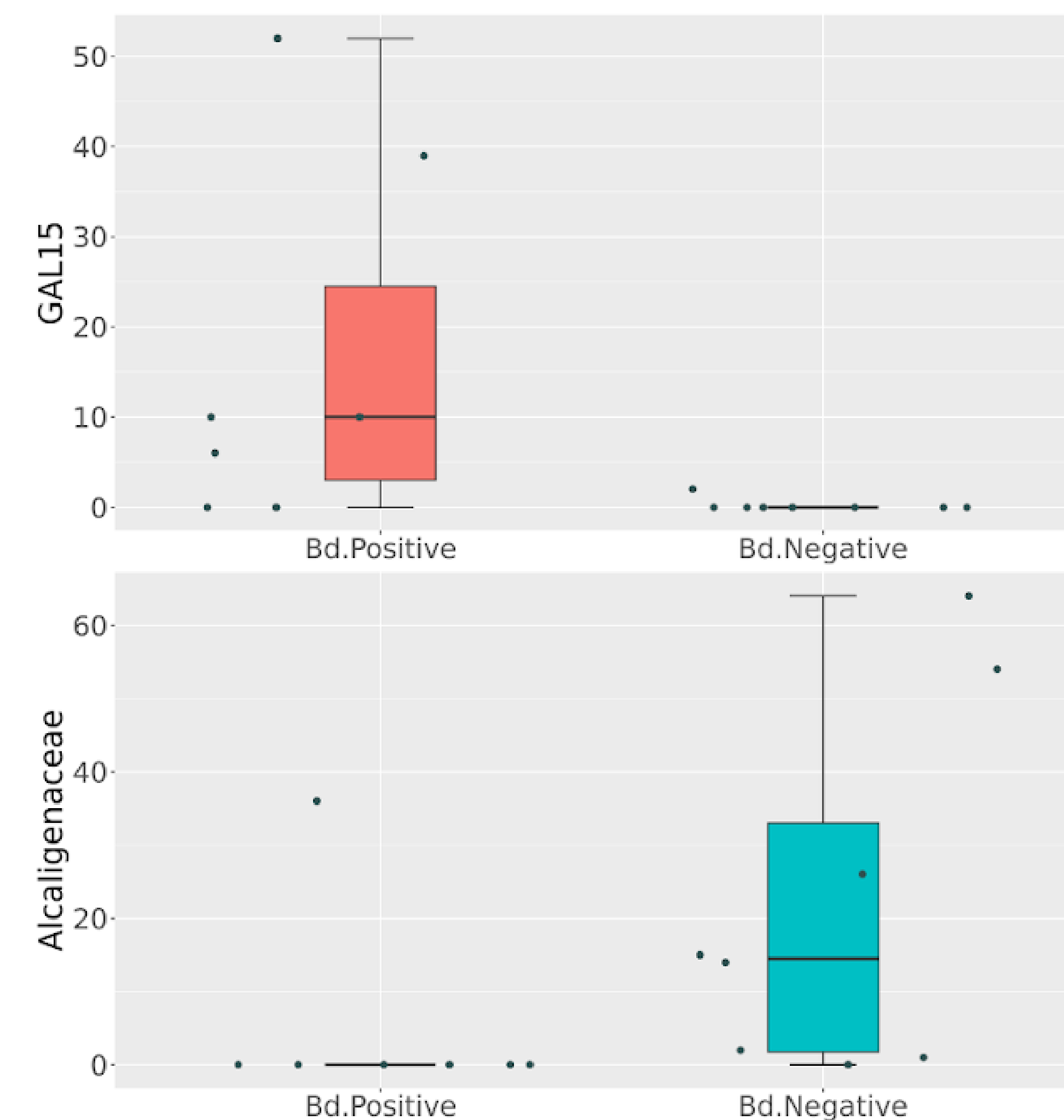
## Methods

Streams at Wooster Memorial Park (Wayne, Co., Ohio, 40.812, -82.030) were sampled during the spring, summer and fall of each year. Individuals were captured with new, sterile gloves and their skin was swabbed for 30 seconds each.

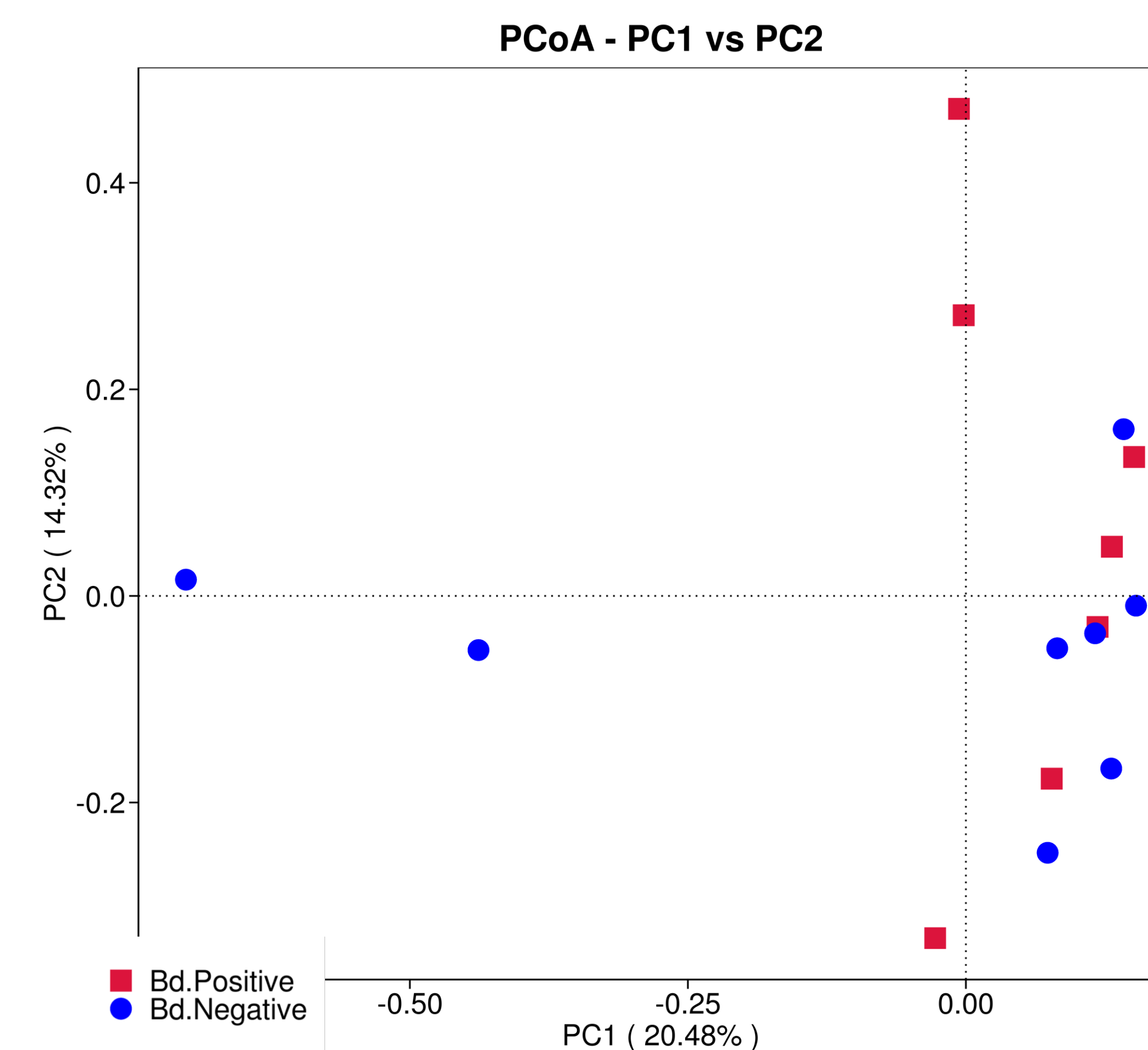
DNA was extracted from the swabs, and Bd presence was assayed using qPCR at the University of Pittsburgh. Then, some confirmed Bd+ and Bd- DNA extracts were sent for 16s v3-v4 metagenomic sequencing and bioinformatic analysis by the sequencing company Novogene.



**Figure 1. Bd Prevalence Over Time.** Wild northern two-lined salamanders in Wooster Memorial Park (n=704) were surveyed for Bd presence using qPCR assays on DNA extracted from skin swabs each year from 2016 to 2024. Asterisks denote significantly different years.



**Figure 2. Differentially Abundant Taxa in Bd positive versus Bd negative Salamander Microbiomes** DNA extracted from Bd positive (n=7) and Bd negative (n=8) salamanders was sequenced by Novogene via 16s v3-v4 metagenomic sequencing. The R program Genseq identified significantly higher levels of the uncultured GAL15 phylum (also known as Syuimicrobiota) in Bd positive salamanders ( $p = 0.006$ ), and significantly higher levels of the family Alcaligenaceae in Bd negative salamanders ( $p < 0.0001$ ).



**Figure 3. Principal coordinate analysis of Bd positive versus Bd negative microbiomes** DNA extracted from Bd positive (n=7) and Bd negative (n=8) salamanders was sequenced by Novogene via 16s v3-v4 metagenomic sequencing. Overall composition of the Bd positive and negative microbiomes were compared by applying multidimensional scaling based on Bray Curtis diversity index

## Results

Across all years, 96 (13.6%) of samples tested positive for Bd (n = 704). A multiple logistic regression model indicated that Bd frequency differed between years ( $p = 0.011$ ) and creeks ( $p = 0.009$ ) but not between seasons ( $p = 0.11$ ) (fig. 1). This model had a p value  $< 0.001$ , a chi-square value of 82.303 and R square values of 0.110 (Cox and Snell test) and 0.201 (Nagelkerke test)

Genera known to possess Bd-inhibiting species were found on every sample sequenced. Overall, Bd+ and Bd- community composition was not found to be statistically different according to Anosim, MRPP or Adonis tests (p values  $> 0.5$ ). However, bacteria of the phyla Syuimicrobiota were significantly more common on Bd+ salamanders, while bacteria of the family Alcaligenaceae were more common on Bd- salamanders (fig 2).

## Acknowledgements

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