Amphibian Fungal Pathogen *Batrachochytrium dendrobatidis* surveillance in Bedford County, Virginia

LIBERTY UNIVERSITY

Jaden Seman, Regan Robinson, and Dr. Matthew Becker PhD

Introduction

Background:

Batrachochytrium dendrobatidis is a fungal pathogen that primarily impacts amphibians. Beginning in the 1970's and continuing into the 1980's, it has been responsible for the extinction and endangerment of many species across the globe, from North and South America to Africa and Australia (Catenazzi et al., 2011). However, emerging data in the past 20 years indicates that many species, particularly those native to the eastern United States exhibit both Bd tolerance and even immunity (Fuchs et al., 2017). Evidence now suggests that a mechanism of protection for these amphibians begins with their microbiome, and that immunity can be harnessed for application in probiotic treatment (Becker et al., 2015).

Purpose:

1. There is currently a lack of information surrounding the prevalence of Bd in the Bedford county area. While its presence is generally known, specific species and locations remain unconfirmed.
2. Research is currently being performed on the microbiomes of various local species to search for inhibitory bacteria, but specific targeting of especially resistant amphibians may assist in this process.

Objectives:

1. To surveil the Bedford County are to determine precise levels of Bd prevalence.
2. To determine which local amphibian species are susceptible to and immune to Bd infection
3. To analyze the presence of Bd across different locations and habitats
4. To identify species of amphibian that tolerate Bd infection and can provide the basis for future treatment research

Hypothesis:

We expected to see Bd infection in at least several of the amphibian species. Additionally, we expected location and habitat distribution of Bd infection to be consistent across location, and more prevalent in aqueous habitats, such as the pond and stream.

Methods

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Sampling: Samples of various species (Table 1) were obtained from two sites in Bedford County, Watson Pond and White Oak Ridge (Figure 1). Specimens were caught using sterile gloves then rinsed with sterile water to ensure that only resident microbiota remained. Each individual was then swabbed and released. Environmental samples were also obtained.

Table 1. Species and location data from sampling.

Site	Species	Expected Sample Size	Habitat
Watson Pond	*Anaxyrus americanus*	2 (A) 4 (J)	Terrestrial/Aquatic
	Desmognathus fuscus	21 (A) 2 (J)	Aquatic (stream)
	Desmognathus monticola	14 (A) 3 (J)	Aquatic (stream)
	Eurycea cirrigera	8 (A) 3 (J)	Terrestrial/Aquatic
	Gyrinophilus porphyriticus	3 (A) 2 (J)	Terrestrial
	Lepomis macrochirus	1	Aquatic (pond)
	Lithobates palustris	4	Terrestrial
	Notophthalmus viridescens	18 (A) 2(J)	Aquatic (pond)
	Plethodon glutinosus	3 (A) 4 (J)	Terrestrial
	Plethodon hubrichti	18	Terrestral
White Oak Ridge	*Notophthalmus viridescens*	2 (J)	Terrestrial/Aquatic
	Plethodon cinereus	12	Terrestrial
	Plethodon glutinosus	6	Terrestrial
	Plethodon hubrichti	10	Terrestrial

DNA Extraction: DNA was extracted using the freeze-thaw method.

qPCR Real-time analysis: Quantitative Polymerase Chain Reaction was performed using an Integrated DNA Technologies kit and according to their protocols. This amplified only Bd DNA and allowed us to detect fluorescence relative to the amount of starting DNA (Figure 2).

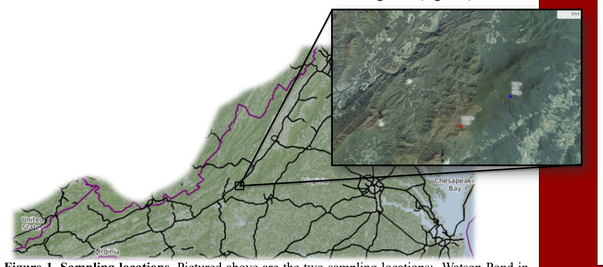

Figure 1. Sampling locations. Pictured above are the two sampling locations: Watson Pond in red and White Oak Ridge in blue. Images generated by Caltopo Mapping Software.

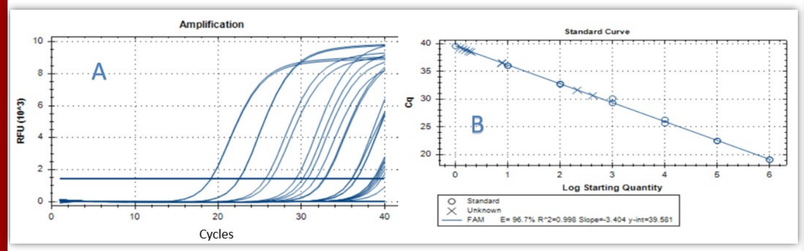

Figure 2. qPCR data and standard curve. (A) Pictured above are the curves read by the qPCR machine as DNA is replicated and fluoresces. Measurements of how many cycles it took to reach a certain fluorescence, which is represented by the horizontal line, are obtained. (B) The number of PCR cycles required to make a sample fluoresce can be applied to standards of known Bd DNA concentration to generate a standard curve allowing for the calculation of any unknown sample within the range of the curve.

Figure 3. *Desmognathus fuscus* species. Our findings showed the species with the most Bd prevalence is D. fuscus. Photo by Dr. Paul Sattler.

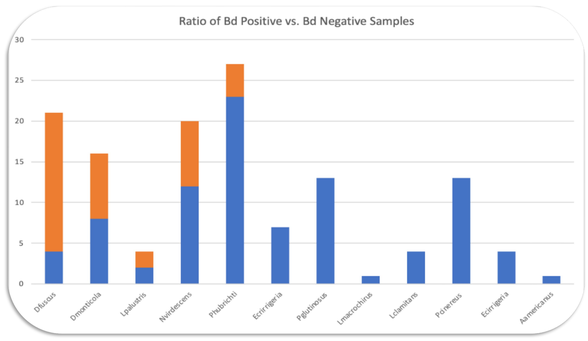

Figure 4. Bd positivity ratios of samples species. The figure above shows the list of species and the total number of samples we obtained from each. Shown in blue is the proportion of negative samples, while orange represents the number of positive samples.

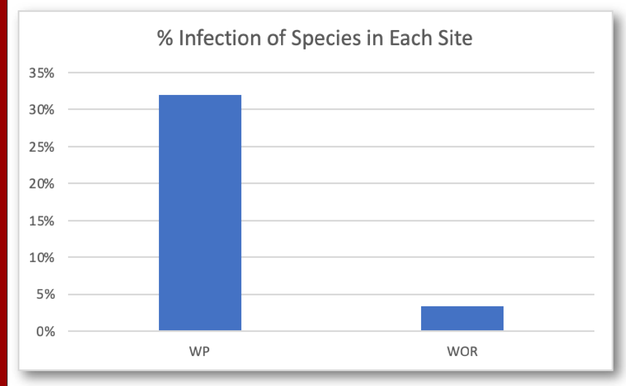

Figure 6. Variation in infection rates across geographic location. The bar graph above shows the percentage of amphibians testing positive for Bd at each of the sampling locations. White Oak Ridge (WOR) had an extremely low positive rate at under five percent, while Watson Pond (WP) was much higher at over 30 percent.

Figure 8. P. hubrichti. Plethodon hubrichti, or the Peaks of Otter Salamander, is a terrestrial salamander only found in Central Virginia. It was found to have a low Bd infection rate. From "Peaks of Otter Salamander," 2010, https://calphotos.berkeley.edu/cgi/img_query?enlarge=0000+0000+0410+0606 CC BY-NC-SA 3.0

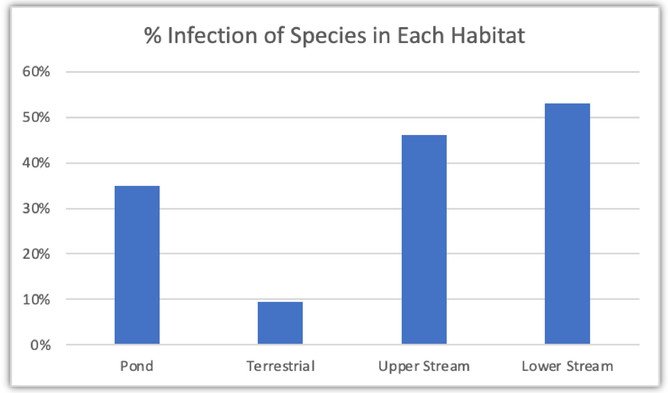

Figure 5. Infection Percentage across various habitats. This figure quantifies the percentage of amphibians in each type of habitat, including terrestrial, still water, and streams. Terrestrial had a significantly lower percentage of infections than did any of the aquatic habitats, and the stream habitats both outpaced the pond in infection rates.

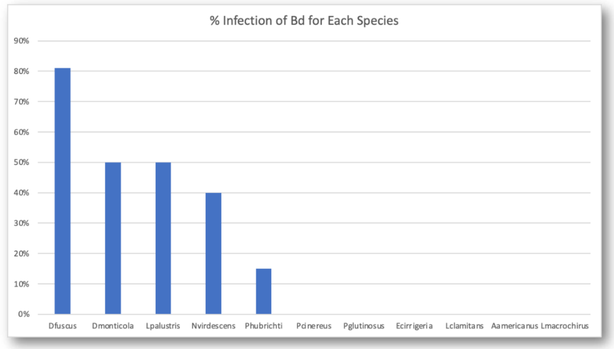

Figure 7. Bd infection rates in various species. Of the 11 species sampled, 5 of them at least had some individuals test positive. These include D. fuscus, D. monticola, L. palustris, N. virdescens, and P. hubrichti. D. fuscus had the highest rate of positive test at over 80 percent. Those that did not have any members test positive are likely to show some resistance to the disease.

Figure 9. D. monticola. Desmognathus monticola, or the seal salamander, was found to carry Bd. From "Seal Salamander" by Leif Van Laar, 2014 https://commons.wikimedia.org/wiki/File:Seal_salamander_(Desmognathus_monticola) CC BY-SA4.0

Results and Discussion

Results: Our results show that Bd is present in the Bedford county area on several different species. These include D. fuscus (Figure 3), D. monticola (Figure 9), L. palustris, N. virdescens, and P. hubrichti (Figure 8). Those species not exhibiting any Bd infection include E. cirrigeria, G. porphyriticus, P. glutinosis, L. macrochirus, L. clamitans, and P. cinereus. The highest rates of infection occurred in D. fuscus, at over 80 percent of individuals infected, with D. monticola and L. palustris closely following at a 50 percent infection rate (Figure 7).

Other results of note concern the location and habitat of affected amphibians. Those individuals living in the terrestrial environment showed a significantly lower infection rate of under 10 percent, while the pond, upper and lower streams had infection rates of 35, 45, and 52 percent, respectively (Figure 5). Additionally, Watson pond had species test positive at a rate of 32 percent, while White Oak Ridge had less than 5 percent of samples return positive (Figure 6).

Discussion: First, we have succeeded in identifying appropriate targets for microbiome analysis. The 6 species that had 0 members test positive are worth studying in further detail, as it can be reasonably assumed that they exhibit some level of immunity to Bd. Additionally, the prevalence of Bd in the various habitats (pond, terrestrial, stream) is consistent with what we expect based on the life cycle of Bd. Since it is primarily an aquatic organism, terrestrial salamanders saw a significantly lower infection rate than did their aquatic counterparts.

One result of our study that was unexpected was the stark contrast between the two locations. Since both Watson Pond and White Oak Ridge experience similar climates and are at similar elevations, it was expected that the location would have little impact on the incidence of Bd. However, this was clearly not the case, as seen in Figure 6. This begs us to ask why this is the case. A variety of explanations are plausible: the species distribution may be different and therefore there are fewer vectors of Bd, White Oak Ridge may have certain geographic barriers that largely prevent mixing of outside populations, or those species that reside in White Oak Ridge are largely resistant to Bd. The explanation of this observation is worth further consideration.

Figure 10. P. glutinosis. P. glutinosis, or the Northern Slimy Salamander, was not found to have any infection amongst our samples. From "Northern Slimy Salamander" by Patrick Coin, https://en.wikipedia.org/wiki/Northern_slimy_salamander#/media/File:Plethodon_cylindraceusPCA20060409-3183A.jpg CC BY-SA 2.5

Future Work

1. Compare Bd presence and intensity to various locations around the globe, including Ecuador.
2. What, if any, environmental differences contribute to the stark contrast in Bd infection at Watson pond versus White Oak Ridge?
3. Characterize the microbiome of the resistant species.
4. Determine any Bd inhibitory bacteria and compounds.
5. Explore probiotic treatment options for both local and global amphibian populations.

References

Becker, M. H., Walke, J. B., Murrill, L., Woodhams, D. C., Reinert, L. K., Rollins-Smith, L. A., Burzynski, E. A., Umile, T. P., Minbiole, K. P., & Belden, L. K. (2015). Phylogenetic distribution of symbiotic bacteria from Panamanian amphibians that inhibit growth of the lethal fungal Pathogenbatrachochytrium Dendrobatidis. *Molecular Ecology*, *24*(7), 1628–1641. https://doi.org/10.1111/mec.13135

Catenazzi, A., Lehr, E., Rodriguez, L. O., & Vredenburg, V. T. (2010). Batrachochytrium dendrobatidis and the collapse of Anuran species richness and abundance in the upper Manu National Park, southeastern Peru. *Conservation Biology*. https://doi.org/10.1111/j.1523-1739.2010.01604.x

Fuchs, L. F., Tupper, T. A., Sepapur, T., Aguilar, R., & Bozarth, C. A. (2017). A Survey of the Pathogenic Fungus, Batrachochytrium dendrobatidis, at the Smithsonian Environmental Research Center, Anne Arundel County, Maryland. *Catesbeiana*, *37*, 32-39.