Current Status of Fungal and Viral Infections of the Peaks of Otter Salamander and other Amphibians in Central Virginia

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Introduction

Background

- Chytridiomycosis is a skin disease caused by pathogens infecting amphibians globally (Robinson et al., 2020). Our research focuses on the aquatic chytrid fungi *Batrachochytrium dendrobatidis* (Bd) and *B. salamandrivorans* (Bsal). Both of these lethal pathogens have caused immense population declines and extinctions globally (Springborn et al., 2022).
- Bd affects all amphibians (frogs, salamanders, caecilians) everywhere they occur, while Bsal only affects salamanders in Europe. Bsal has not been detected in North America despite extensive surveys (Spitzen-van der Sluijs et al., 2016).
- Another problematic disease is the ranaviral disease caused by ranavirus, an emerging viral pathogen infecting mostly amphibians but also reptiles, and fish populations (Brunner et al., 2005).
- The main species focused on in this research is the Peaks of Otter salamander (*Plethodon hubrichti*), a salamander endemic to a small range in the Peaks of Otter, VA (Figure 1). Monitoring the occurrence of chytridiomycosis and ranaviral disease in this species is essential in maintaining biodiversity in Virginia.

Purpose

1. While the presence of Bd is known in Virginia, there is currently a lack of information surrounding the prevalence of Bd, Bsal, and ranavirus where the Peaks of Otter salamander occurs.
2. Research is currently being performed on the microbiomes of amphibian species in the Peaks of Otter area to search for bacteria that kill Bd and Bsal. Identifying amphibians that resist infection will assist in this process.

Objectives

- To survey amphibian diseases in the Peaks of Otter for precise levels of Bd, Bsal, and ranavirus prevalence.
- To assess the health of Peaks of Otter salamander populations.
- To determine if habitat type affects infection prevalence and intensity.

Hypothesis

We expect that most sampled species will be infected with Bd, especially those in aquatic environments. We do not expect to see Bsal in any of the species sampled. Additionally, we expect a higher prevalence of Bd infection in the Peaks of Otter salamander when found near water bodies.

Methods

Field collection

- Nine field sites were chosen throughout the range of the Peaks of Otter salamander (Figure 2A). We included sites that were both near and far from water bodies (ponds, streams, creeks) to determine if habitat type (wet vs. dry) affects infection prevalence.
- All sympatric amphibian species found inhabiting each site along with the Peaks of Otter salamander were sampled (Figure 2). We sampled both aquatic and terrestrial amphibians to determine if microhabitat use affects infection prevalence.
- To sample for *Bsal* and *Bd*, all amphibians were collected by hand with sterile gloves. We swabbed the ventral, dorsal, hind legs, lateral sides, and tails of each amphibian five times to collect cutaneous microbes (Figure 2c). For *ranavirus* testing, tail clips were obtained to analyze tissue samples. All samples were stored at -20C until further processing.

Pathogen Testing

- Microbial DNA was then extracted from the gathered samples and isolated using Zymo Quick-DNA Miniprep Plus Kit.
- We conducted multiple Quantitative Polymerase Chain (qPCR) reactions using a standard probe-based protocol to determine the infection intensity of *Bd* (Boyle et al., 2004), *Bsal* (Blooi et al., 2013), Ranavirus (Pico et al., 2007).

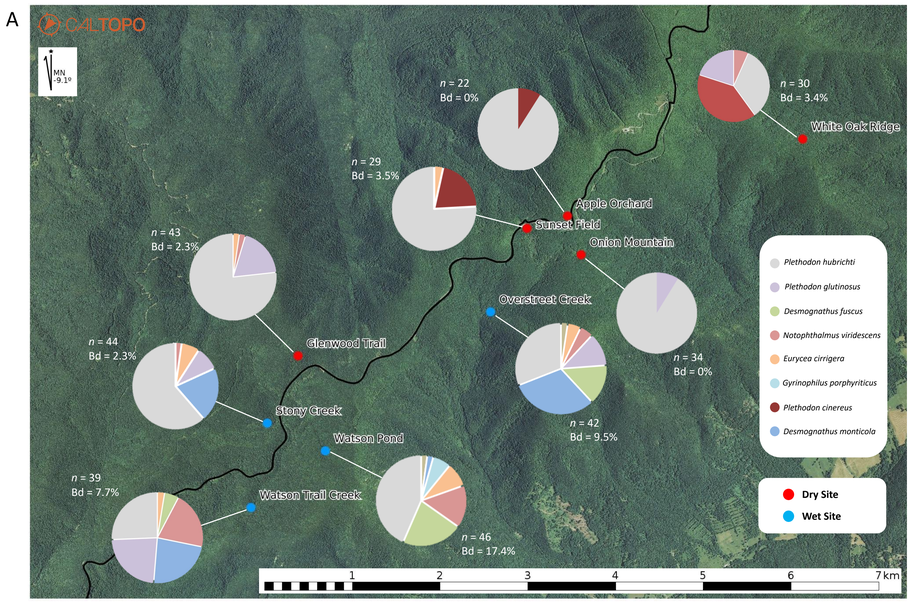
A

B

C

Figure 1. Study Species (A) *Plethodon hubrichti*, (B) *P. cinereus,* and (C) *Notophthalmus viridescens*. These salamanders are commonly found in the Blue Ridge Mountains of Central Virginia. Photos by Dr. Matthew H. Becker.

A



B

C

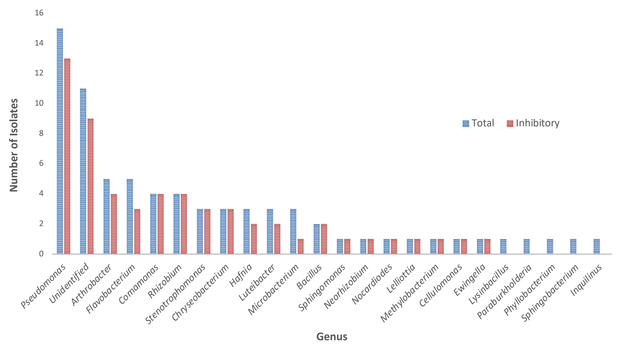
Figure 2. Salamanders were sampled at nine field sites. (A) Pathogen testing results for each field site. Each site is labeled with sample size (*n*), Bd prevalence, habitat type, and species. At each field site, (B) the research team would collect and (C) swab each amphibian and immediately return it to the exact site of collection. Credits: Map generated from CalTopo.com reproduced with permission (NAIP Imagery as the base layer - USDA Farm Service Agency) and both photos by the authors.

Table 1. Measured prevalence of Bd on amphibian species collected from the Peaks of Otter survey.

Species	Habitat	Sample Size	Prevalence
Anaxyrus americanus	Terrestrial	2	0.50
Gyrinophilus porphyriticus	Aquatic	3	0.00
Plethodon cinereus	Terrestrial	8	0.00
Desmognathus fuscus	Aquatic	18	0.33
Desmognathus monticola	Aquatic	32	0.09
Eurycea cirrigera	Semiaquatic	12	0.08
Notophthalmus viridescens (adult)	Aquatic	7	0.57
Notophthalmus viridescens (juv.)	Terrestrial	12	0.08
Plethodon glutinosus	Terrestrial	29	0.00
Plethodon hubrichti	Terrestrial	176	0.01
Total		299	0.06

Table 2. Measured prevalence of Bsal on amphibian species collected from the Peaks of Otter survey (55 out of 299 samples have been processed)

Species	Habitat	Sample Size	Prevalence
Anaxyrus americanus	Terrestrial	1	0
Gyrinophilus porphyriticus	Aquatic	3	0
Plethodon cinereus	Terrestrial	3	0
Desmognathus fuscus	Aquatic	4	0
Desmognathus monticola	Aquatic	9	0
Eurycea cirrigera	Semiaquatic	4	0
Notophthalmus viridescens (adult)	Aquatic	7	0
Notophthalmus viridescens (juv.)	Terrestrial	2	0
Plethodon glutinosus	Terrestrial	3	0
Plethodon hubrichti	Terrestrial	19	0
Total		55	0



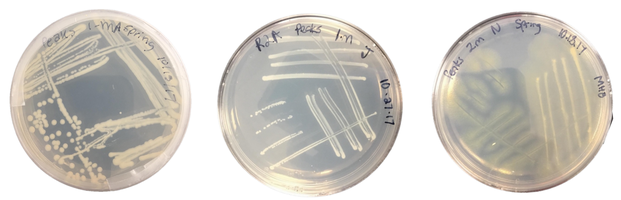


Figure 3. The anti-Bd activity of the Peaks of Otter salamander microbiome. Cultures (n=73) were isolated and tested with an *in vitro* co-culture assay to identify inhibitory isolates (Becker et al., unpublished data). (B) Pictures of *Plethodon hubrichti* bacterial isolates (M.H. Becker).

Results

Bd results

- In this study, *Batrachochytrium dendrobatidis* was observed in 18 out of 299 amphibians (6% prevalence). We only detected Bd in two out of 176 (1% prevalence) Peaks of Otter salamanders (Table 1).
- Bd was detected most often on adult *Notophthalmus viridescens* (57% prevalence) and *Desmognathus fuscus* (33% prevalence)
- Amphibians at field sites close to a water body had a higher Bd infection prevalence (9.4%) than "dry" sites (1.6%). This result is likely due the higher abundance of aquatic and semi-aquatic species at wet sites. These species had a higher infection prevalence than terrestrial species (Table 2).

Bsal results

- We are currently conducting *Batrachochytrium salamandrivorans* qPCR assays. We have not detected Bsal in the 55 samples tested (Table 3).

Conclusions and Future Work

Conclusions

- The prevalence of Bd throughout the distribution of the Peaks of Otter salamander (6%) is relatively low compared to other areas in central Virginia (range 0-86%, Becker et al., unpublished data; Hughey et al. 2014).
- Bd is an aquatic organism and therefore was found higher in aquatic and semi-aquatic species. However, there was no difference in Bd prevalence among terrestrial species found at "dry" vs. "wet" sites. These findings are consistent with the expected results since the terrestrial organisms did not show a high prevalence of Bd.
- Despite inhabiting sites with infected amphibians, we only detected Bd in two Peaks of Otter salamanders. This suggests that the Peaks of Otter salamander is unaffected by Bd throughout its distribution. *Plethdon hubrichti* are terrestrial and thus the aquatic zoospore Bd is less likely to affect this species. In addition, the bacteria on the skin or the immune system of the *P. hubrichti* potentially play a large role in the low prevalence of Bd in this species. Currently, work is being done on the microbiome that plays in this salamander's defense against fungal infections. Microbiome sampling has been performed and shown that *P. hubrichti* have antifungal bacteria with inhibitory effects (Figure 3).
- There was zero prevalence of Bsal in all species as expected since Bsal has not been detected in the United States despite extensive surveillance by the USGS (https://armi.usgs.gov/Bsal-studies/).

Future Work

1. Determine if Bsal and ranavirus is present in amphibians inhabiting the Peaks of Otter and surrounding areas.
2. Determine implications and conservation actions if Bsal is detected.
3. Investigate sources of potential antifungal probiotics that would effectively help treat infected amphibians.
4. Investigate why the pathogens affect certain amphibians while leaving some unharmed.

References and Acknowledgments

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