Prevalence of *Ranavirus* and *Batrachochytrium dendrobatidis* in Hellbenders of Tennessee and Arkansas

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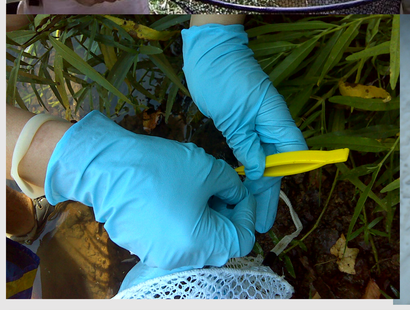
Introduction

The Hellbender, *Cryptobranchis allenganiensis*, is a large aquatic salamander containing two subspecies, the Ozark Hellbender, *C. a. bishopi,* and the Eastern Hellbender, *C. a. alleganiensis,* from the Ozark mountains and eastern U.S., respectively. Both subspecies have seen population declines over the past 25 years, especially in *C. a. bishopi* which is federally endangered. Habitat degradation and possibly low genetic diversity may lead to secondary infections with amphibian pathogens such as *Ranavirus* and *Batrachochytrium dendrobatidis* (Bd). This is of particular interest in Ozark populations where physical deformities of distal limbs is widespread among adults. The objective of this study was to determine prevalence of these pathogens in both subspecies as a first step in understanding the role of emerging amphibian pathogens in *C. alleganiensis* declines.

Swabbing for Bd



Tail notch for ranavirus testing

Results

We captured a total of 101 individuals; 39 Ozark (*C. a. bishopi*) and 62 Eastern (*C. a. alleganiensis*). Overall, for *C. a. bishopi*, we detected 18% prevalence of Bd and no cases of ranaviral infections; for *C. a. alleganiensis,* we detected 13% prevalence of Bd and 3% prevalence of ranavirus (Fig 1). There were more Bd infections for both populations in 2011 compared with 2012 with no significant difference (Table 2). Furthermore, there was no significant difference in prevalence of either pathogen between subspecies. We observed one *C. a. alleganiensis* with skin lesions that we later identified as positive for Bd. Additionally, we observed leeches parasitizing many individuals and identified one *C. a. allenganiensis* leech and host positive for ranavirus with similar CT values.

Table 1. Total captures and positive infections. Note: no co-infections were identified

Subspecies	Streams	No. Captures	Bd	RV
C. a .alleganiensis	9	62	8	2
C. a. bishopi	1	39	7	0

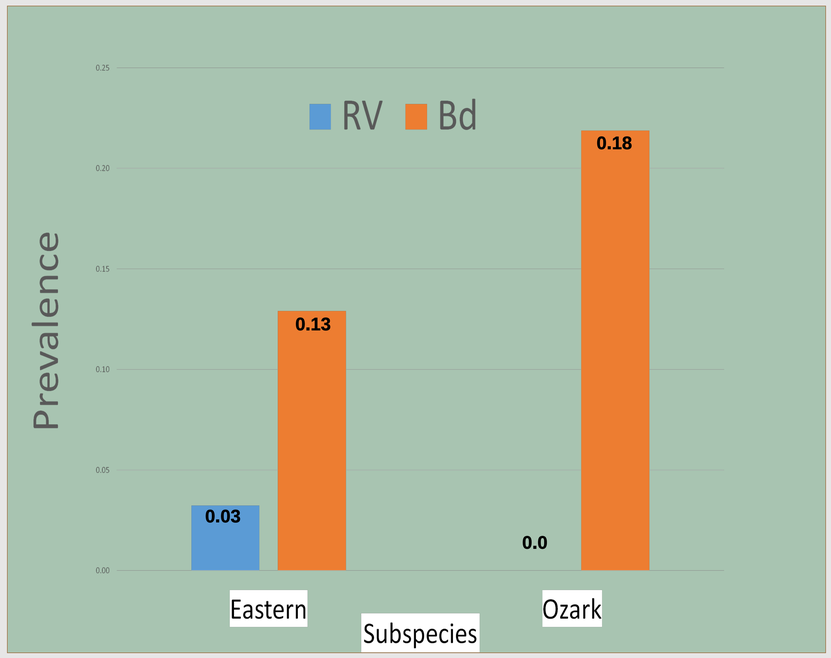


Figure 1. Percent Prevalence of pathogens in each subspecies. No significant difference between groups.

Table 2. Percent Infection by Year

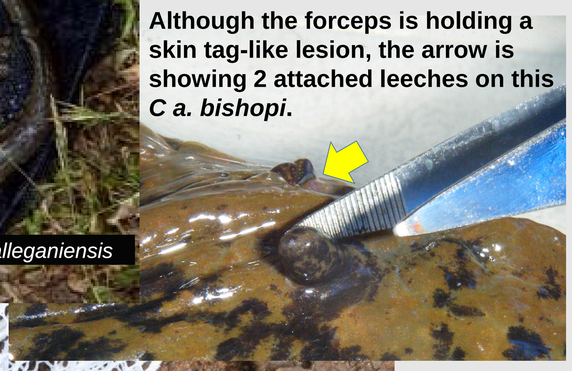
Subspecies	Bd		Ranavirus	
	2011	2012	2011	2012
C. a. alleganiensis	18.2	11.8	0.0	3.9
C. a. bishopi	19.4	12.5	0.0	0.0

Methods

We collected tail tissue and skin swabs from 40 *C. a. bishopi* individuals in the Eleven Point River watershed of Randolph County, AR over two sampling periods from 2011 to 2012. We performed skin swabs for collection of Bd since it is known to infect epidermis. We collected whole tissue from tail notches for detection of ranavirus. Samples were stored immediately in 70% ethanol. Genomic DNA was extracted from the tail notches and swab samples using commercially available kits (DNeasy Blood and Tissue Kit, Qiagen Inc., Valencia, California, USA). A TaqMan real-time quantitative PCR (qPCR) was performed following Picco et al. (2007) for ranavirus testing and Boyle et al. (2004) for *Bd* testing. Four controls were included for each PCR run and included two positive controls and two negative controls. Extraction controls were also included. We determined Ct value thresholds for presence/absence analysis.

Emaciated ranavirus-positive (by qPCR) *C.a. alleganiensis*

Although the forceps is holding a skin tag-like lesion, the arrow is showing 2 attached leeches on this *C a. bishopi*.



Bd-positive (by qPCR) *C.a. alleganiensis* with skin lesions (arrows)

Discussion

- First step in understanding the role of emerging amphibian pathogens in Hellbenders
- Need continued monitoring especially for ranavirus which is documented to cause sporadic outbreaks in amphibians
- Bd could play a role in secondary infections due to stress
- Investigation is needed in leech transmission
- Histology and other methods needed for disease analyses

Acknowledgements

We thank Bill Reeves of TWRA for SWG funding and Arkansas Game and Fish Commission Section 6 funding that helped support the project.

References

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Picco AM, Brunner JL, Collins JP. 2007. Susceptibility of the endangered California tiger salamander, *Ambystoma californiense*, to *Ranavirus* infection. Journal of Wildlife Diseases 43:286–290.