Crayfish as a biological reservoir for the amphibian skin pathogen *Batrachochytrium dendrobatidis*

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Background

The interactions between the fungal pathogen Bd and organisms other than amphibians are not well understood. Crayfish could be an effective model for Bd biomonitoring and potential treatment of this fungus, which negatively impacts wildlife populations.

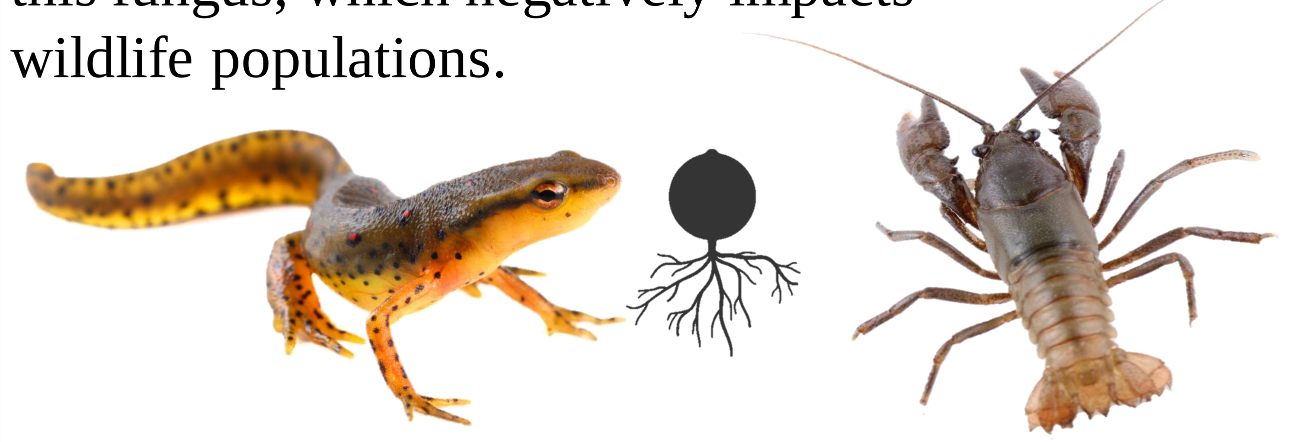

Figure 1. Newts (left) are a known biological reservoir of Bd (center) in local aquatic systems. **Crayfish (right), although documented as Bd reservoirs, haven't been recorded to be Bd reservoirs in Virginia.** Bd figure from Biorender, other images by the authors.

Purpose

This project aims to determine if local crayfish (*Cambarus sp.*) are susceptible to Bd infection and can act as biological reservoirs for the pathogen.

Hypothesis:
Given previous evidence of crayfish carrying Bd (Brannely, et.al., 2015), we hypothesize that local crayfish (*Cambarus sp.)* will contract Bd when exposed to the fungus in a laboratory setting.

Research Question: Are crayfish potential biological reservoirs for Bd in Central Virginia?

Figure 2. Top Left: Example of sampled crayfish (*Cambarus sp.*); Bottom Left: Field collection in kingfisher pond for newts (*Notophthalmus viridescens*); Right: Juvenile crayfish found at one of the sampling sites. Images by the authors.

Methods

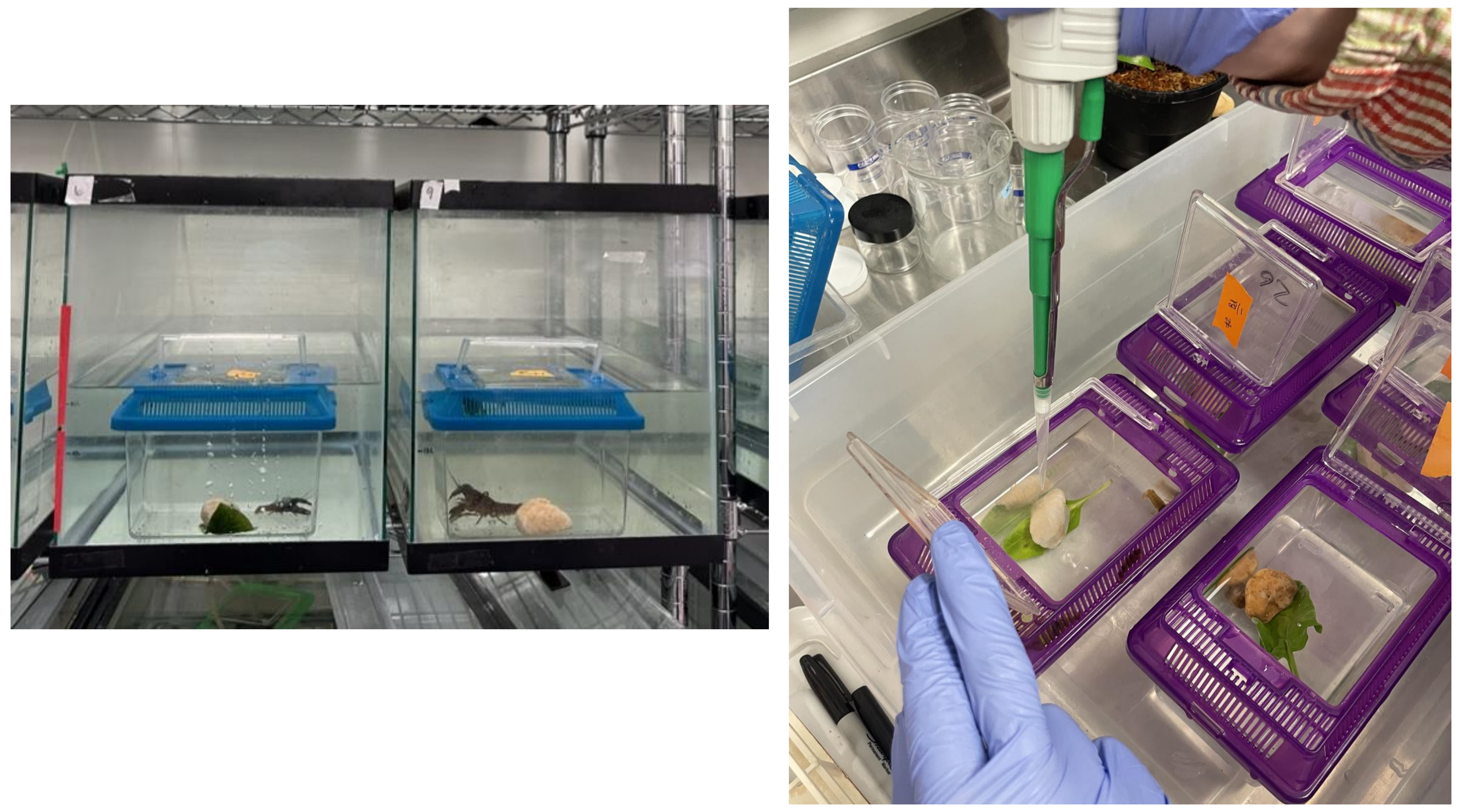

Figure 3. Control crayfish (left) in tanks and inoculation of experimental crayfish (right). Images by authors.

- Twelve crayfish (*Cambarus sp.)* will be divided randomly and equally into pathogen exposed and control groups.
- The exposed treatment of crayfish will contain a solution of Bd (~100,000 cells/ml) for two weeks. Control crayfish will experience similar conditions except without the presence of Bd.
- After two weeks, all crayfish will be swabbed again to evaluate the intensities of any Bd infection. Crayfish will then be placed into new individual containers with pathogen-free water.
- The experiment will continue with weekly swabbing and real-time PCR (qPCR) evaluation of swabs for signs of infection.

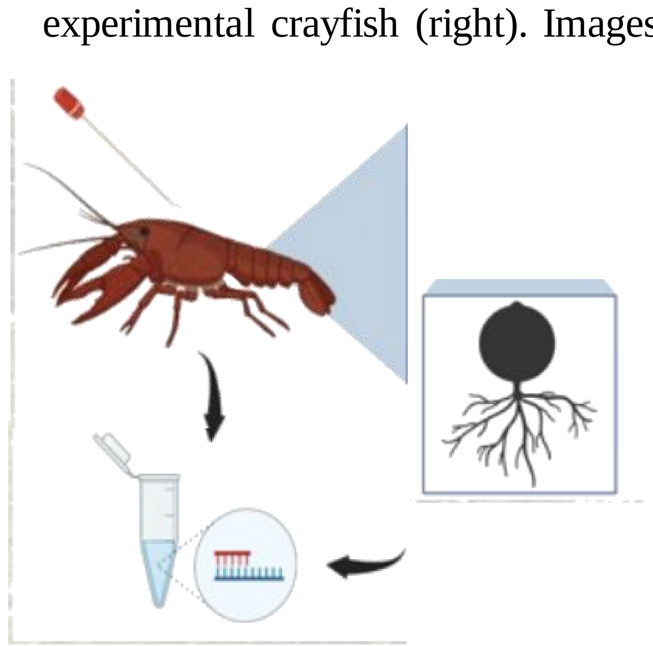

Figure 4. Methods to assess Bd prevalence & abundance on crayfish carapace before exposure to high Bd concentrations (n=12); Bd DNA will be extracted using the Zymo Quick DNA miniprep Plus Kit and quantified using qPCR. Figure developed in Biorender.

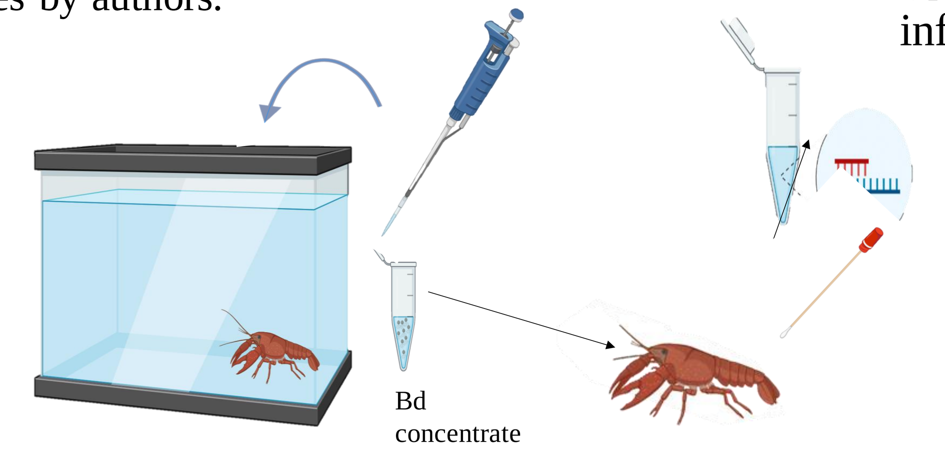

Figure 5. Six crayfish will be exposed to 100,000 Bd cells/ml. An additional six crayfish will act as controls. The control and experimental crayfish will be put into individual tanks of RO water for 4 weeks. Bd prevalence & abundance will be surveyed on each crayfish carapace on days 14 and 28 (n=12); DNA will be extracted using the Zymo Quick DNA miniprep Plus Kit and quantified using qPCR. Figure developed in Biorender.

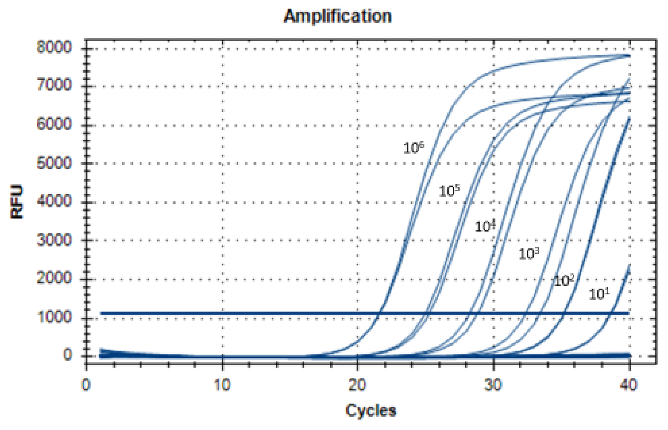

Figure 6. qPCR standard curve example for Bd prevalence and abundance identification. Standard curve generated from qPCR thermal cycler software.

Preliminary Findings

In a recent investigation of the Kingfisher Pond and Opossum Creek freshwater ecosystem, we did not detect signs of infection on the carapace or gastrointestinal tract of crayfish (n=60). In contrast, we detected Bd on 95% of the newts (n=20). The presence of a highly infected newt population in conjunction with a Bd-free crayfish population, suggests possible Bd resistance among these crayfish. This anomaly necessitates further research, given the documented Bd infection among other crayfish populations.

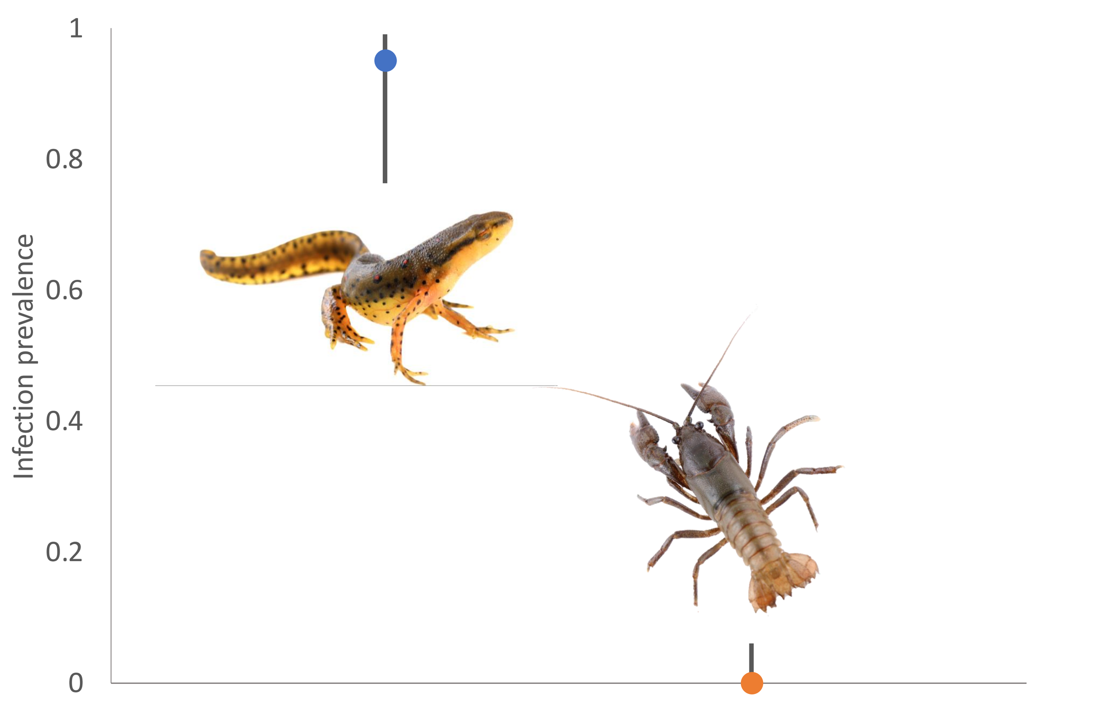

Figure 7. Bd infection prevalence and Wilson 95% confidence intervals for Newt (n=20) and crayfish (n=60) populations. Images belong to the authors.

Expected Results

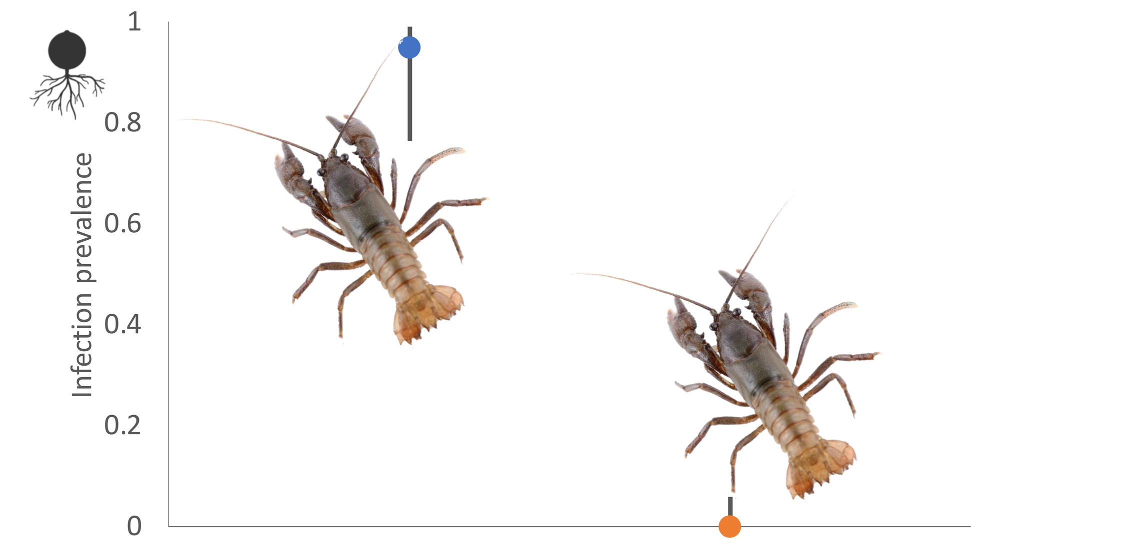

Figure 8. Bd infection prevalence and Wilson 95% confidence intervals is expected to show high levels of infection in exposed crayfish (n=6) and not detected in control crayfish (n=6) . Figure developed by authors.

We expect that the crayfish exposed to Bd will become infected based upon findings in a recent study (Brannely, et al., 2015).

Batrachochytrium dendrobatidis (Bd) infection in amphibians has been widely studied. However, the way in which the fungal pathogen affects other organisms is not well understood. Crayfish are a model organism to further study this pathogen in biological reservoirs other than amphibians and may provide valuable insight into treating susceptible amphibians.

Acknowledgements and References

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Voyles, J., Johnson, L. R., Briggs, C. J., Cashins, S. D., Alford, R. A., Berger, L., ... & Rosenblum, E. B. (2012). Temperature alters reproductive life history patterns in *Batrachochytrium dendrobatidis*, a lethal pathogen associated with the global loss of amphibians. Ecology and evolution, 2(9), 2241-2249.

Historical newt data and Bd methods cited from Dr. Becker's Lab from the following presentation:
Kang, J., Whitney, S., & Becker, M. (2019). Effect of seasonality on the prevalence and infection intensity of the fungal pathogen *Batrachochytrium dendrobatidis* in a population of eastern newts [Poster Session]. Liberty University Research Symposium.

Additional Protocol Sources:
Boyle, D. G., Boyle, D. B., Olsen, V., Morgan, J. A. T., & Hyatt, A. D. (2004). Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. Diseases of aquatic organisms, 60(2), 141-148.

Map of Hydaway Retrieved from: https://media.defense.gov/2016/Dec/14/2001679241/-1/-1/1/NAO-2007-02834_VICINITY_MAP%20.PDF

Biorender was used to generate figures supporting methodology; standard curve was generated from the qPCR thermal cycler, and other images were taken by the authors.