

# Bd Prevalence in the Local Crayfish Population

By Gibson Huff



Harris, K. (2023). *Crayfish* [Photograph]. Unpublished.

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# Background

- Crayfish are ubiquitous in local freshwater ecosystems



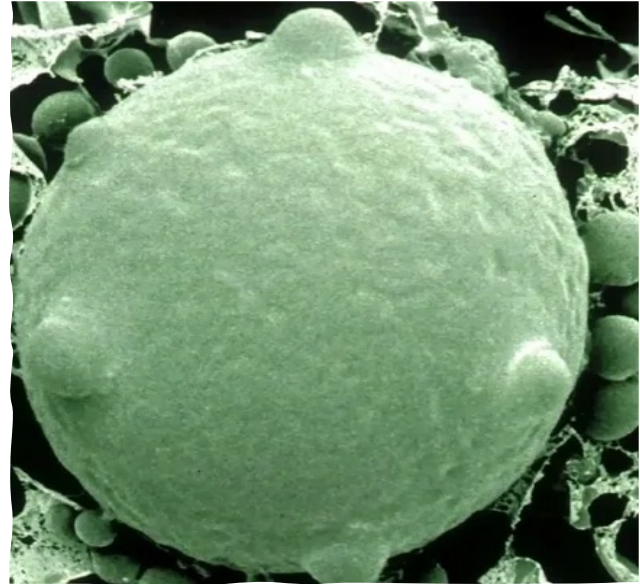
Harris, K. (2023). *Crayfish* [Photograph]. Unpublished.



Wikimedia Commons. (n.d.). *Crayfish underwater*. Retrieved from [https://commons.wikimedia.org/wiki/File:Crayfish\\_underwater\\_on\\_bottom\\_of\\_river.jpg](https://commons.wikimedia.org/wiki/File:Crayfish_underwater_on_bottom_of_river.jpg)

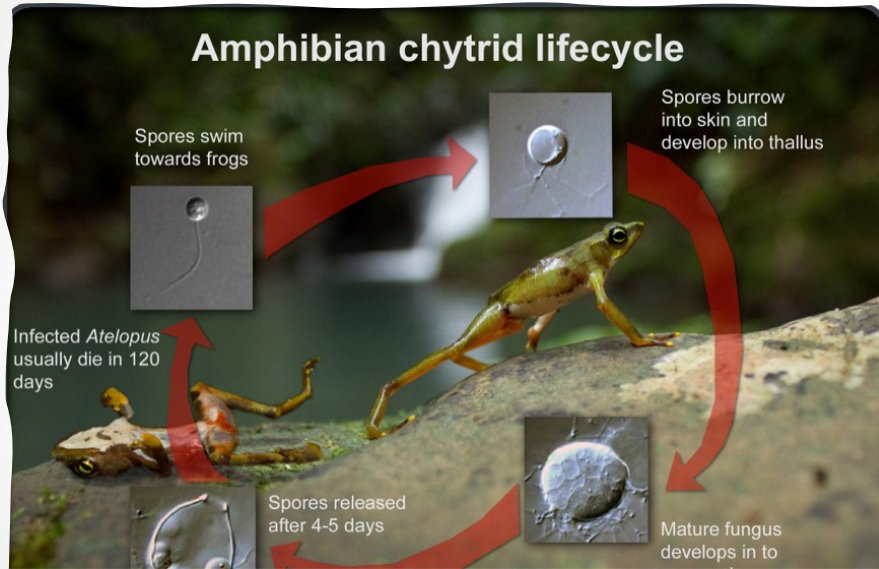
# Background

- Batrachochytrium dendrobatids (Bd) is a detrimental chytrid fungus that significantly contributes to the global decline of amphibian populations by causing deadly infections



Britannica. (n.d.). *Amphibian chytridiomycosis*. Retrieved from <https://www.britannica.com/science/amphibian-chytridiomycosis>

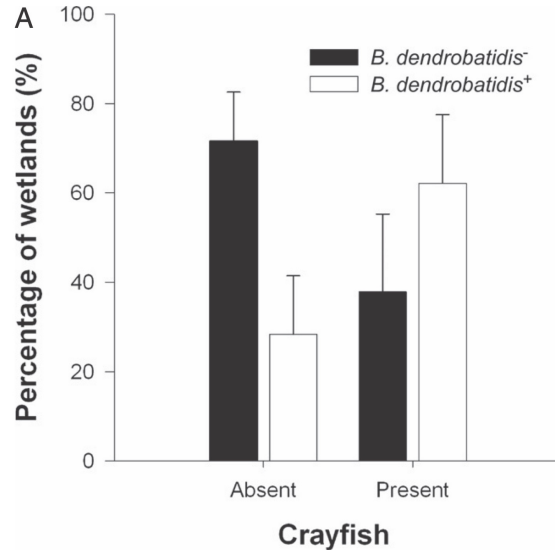
# Background



Gratwicke, B. (2014). *Amphibian chytrid life cycle*. Flickr.  
<https://www.flickr.com/photos/briangratwicke/14115178249>

- *Bd* had been shown to have 100% mortality rate in frogs and also have negative impacts in newts

# Background



McMahon, T. A., Brannnelly, L. A., Chatfield, M. W., Johnson, P. T., Joseph, M. B., McKenzie, V. J., Richards-Zawacki, C. L., Venesky, M. D., & Rohr, J. R. (2013). Chytrid fungus *Batrachochytrium dendrobatidis* has nonamphibian hosts and releases chemicals that cause pathology in the absence of infection. *Proceedings of the National Academy of Sciences of the United States of America*, 110(1), 210–215. <https://doi.org/10.1073/pnas.1200592110>

## Abstract

**“Batrachochytrium dendrobatidis, a pathogenic chytrid fungus implicated in worldwide amphibian declines..... crayfish** (*Procambarus* spp. and *Orconectes virilis*), which are syntopic with many amphibian species, are **possible hosts for *B. dendrobatidis***”.

McMahon, T. A., Brannnelly, L. A., Chatfield, M. W., Johnson, P. T., Joseph, M. B., McKenzie, V. J., Richards-Zawacki, C. L., Venesky, M. D., & Rohr, J. R. (2013). Chytrid fungus *Batrachochytrium dendrobatidis* has nonamphibian hosts and releases chemicals that cause pathology in the absence of infection. *Proceedings of the National Academy of Sciences of the United States of America*, 110(1), 210–215. <https://doi.org/10.1073/pnas.1200592110>



# Background

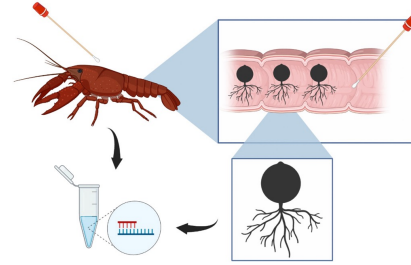
- In our first two semesters of research, we just collected 20 crayfish and checked to see if they had Bd through running qPCR



Harris, K. (2023). *Crayfish Collection Map*. Unpublished.

# Background

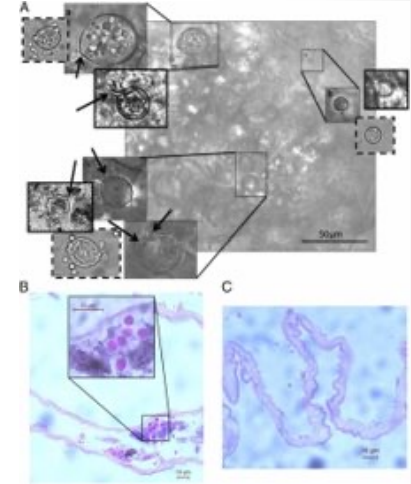
- In our two previous semesters, we swabbed the intestinal tract to find Bd, but this didn't work both times
- Was it due to human error?
- Was it because of the temperature?



Piddock, A. (2022). *Crayfish carapace and GI tract swabbing* [Image]. Created using BioRender.



Piddock, A. (2022). *Crayfish GI tract dissection* [Photograph]. Unpublished.



Harris, K. (2022). *Crayfish GI tract dissection* [Image]. Unpublished.

# Background

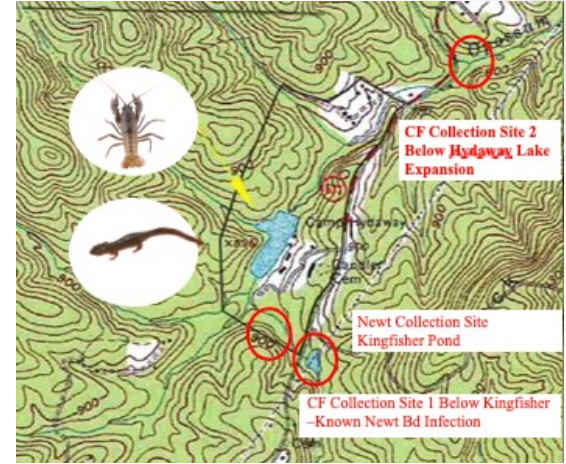
- this past summer, we only swabbed the carapace (outer shell) to prevent sacrificing crayfish and follow what the data is telling us
- Newts were previously found through the literature to be positive for Bd, so we used them as a positive control
- Third overall attempt at trying to find Bd in crayfish; if the newts had it and crayfish did not then this would reject our hypothesis



Huff, G. (2023). *Crayfish being swabbed* [Image]. Created using BioRender.



Harris, K. (2023). *Newt* [Photograph]. Unpublished.

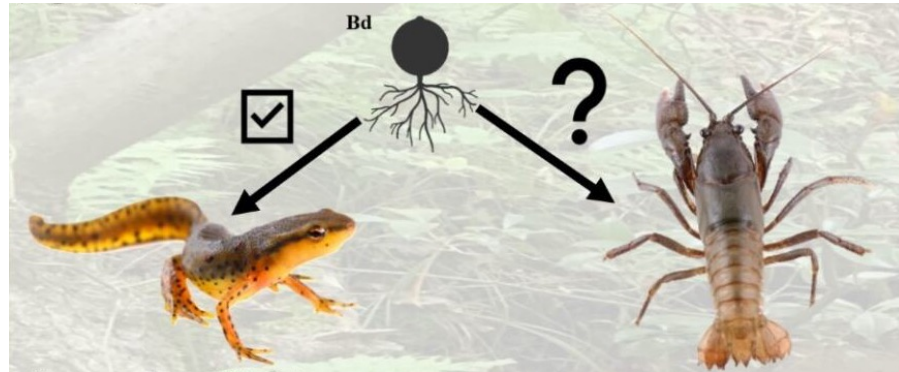


Harris, K. (2023). *Crayfish and Newt Collection Map*. Unpublished.



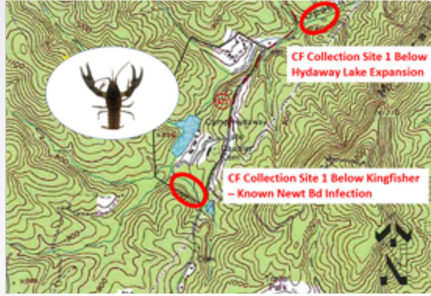
# Hypothesis

If both crayfish and newts in our local streams are positive for Bd, then the crayfish most likely serve as vectors for the pathogen.



Huff, G. (2023). *Bd correlation between crayfish and newts* [Image]. Unpublished.

# Crayfish Collection Location



Harris, K. (2023). *Crayfish Collection Map*. Unpublished.



Piddock, A. (2023). *Crayfish collection* [Photograph]. Unpublished.



Piddock, A. (2023). *Crayfish collection* [Photograph]. Unpublished.



Piddock, A. (2023). *Crayfish collection* [Photograph]. Unpublished.

- 20 crayfish were collected at each respective stream connected to kingfisher

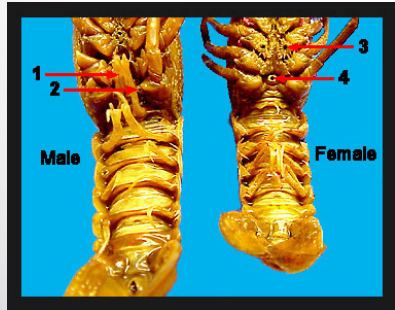
# Crayfish Collection Method



Piddock, A. (2023). *Crayfish collection* [Photograph]. Unpublished.



Harris, K. (2023). *Crayfish* [Photograph]. Unpublished.



Electric Crayfish. (n.d.). *Sexing*. Retrieved from <http://electriccrayfish.weebly.com/sexing.html>

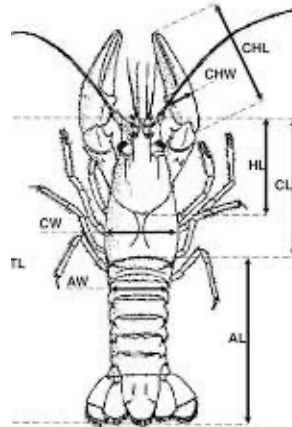


Piddock, A. (2023). *Crayfish collection* [Photograph]. Unpublished.

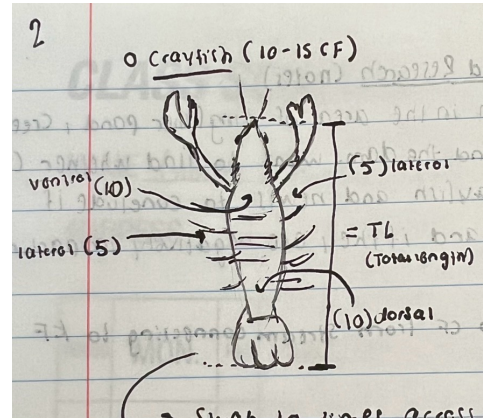
- Sex was recorded (10 males and 10 females)
- Total length (TL), sex, blotted wet mass (BWM) (g) recorded

# Crayfish Swabbing Method

Crayfish were obtained and swabbing techniques were conducted on the carapace; each crayfish was swabbed 10 times across the ventral surface (bottom shell), 5 times across each lateral surface (sides) and 10 times across the dorsal surface (top shell) for a total of 30 swabs per crayfish



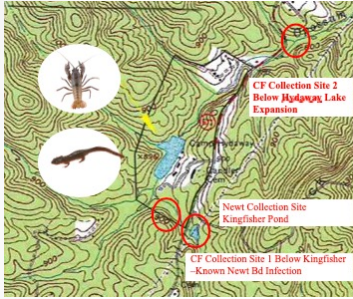
ResearchGate. (2005). *Scheme of measurements taken from investigated crayfish* [Image]. Retrieved from [https://www.researchgate.net/figure/Scheme-of-measurements-taken-from-investigated-crayfish-TL-total-body-length-AL-abdomen\\_fig2\\_239533326](https://www.researchgate.net/figure/Scheme-of-measurements-taken-from-investigated-crayfish-TL-total-body-length-AL-abdomen_fig2_239533326)



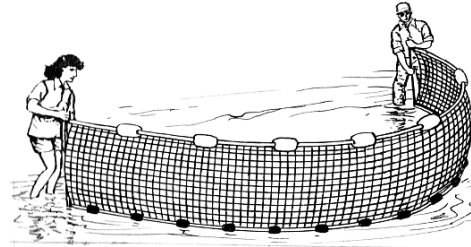
Huff, G. (2023). *Crayfish swabbing method* [Drawing]. Unpublished.



# Newt Collection Location



Harris, K. (2023). *Crayfish and Newt Collection Map*. Unpublished.



Wikipedia contributors. (n.d.). *Seine fishing* [Image]. Retrieved from [https://en.wikipedia.org/wiki/Seine\\_fishing](https://en.wikipedia.org/wiki/Seine_fishing).



Piddock, A. (2023). *Newt collection* [Photograph]. Unpublished.



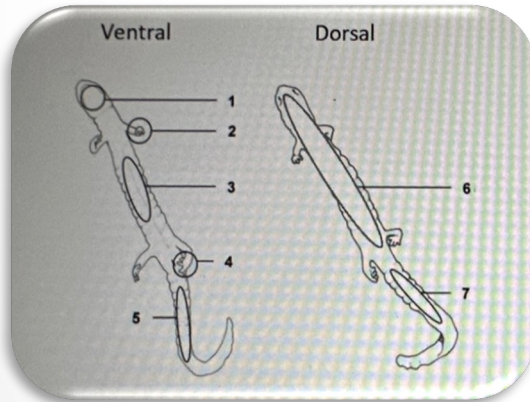
Harris, K. (2023). *Newt* [Photograph]. Unpublished.

- 20 newts were collected at kingfisher pond
- Newts were collected best using the seine method (giant net)

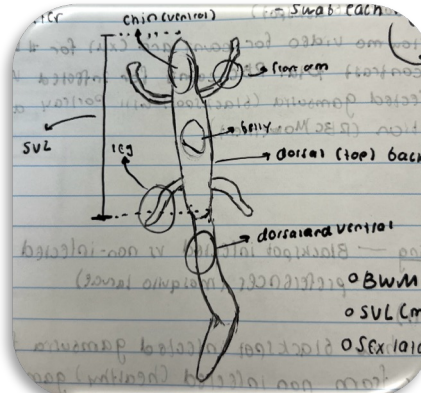


# Newt Swabbing Method

- BWM (g), SVL (mm) and Sex was recorded
- Swabs were taken of each newt respectively: Each newt was swabbed 5 times across the chin, one front arm, belly, one back leg and the vent (good place to swab; digestive and reproductive systems)



Harris, K. (2023). *Newt swabbing protocol* [Photograph]. Unpublished.



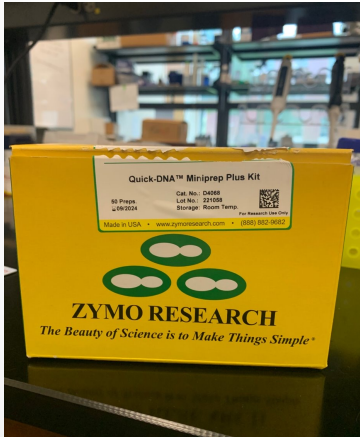
Huff, G. (2023). *Newt swabbing method* [Drawing]. Unpublished.



Harris, K. (2023). *Newt* [Photograph]. Unpublished.

# DNA Extraction and qPCR

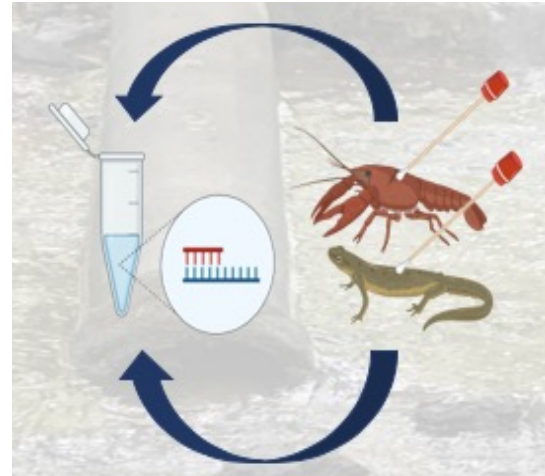
- Swabs were then placed in a microcentrifuge tube and the back was cut off
- DNA was extracted using the Zymo Quick DNA miniprep Plus Kit and amplified using qPCR



Piddock, A. (2023). *Zymo quick DNA miniprep plus kit* [Photograph]. Unpublished.



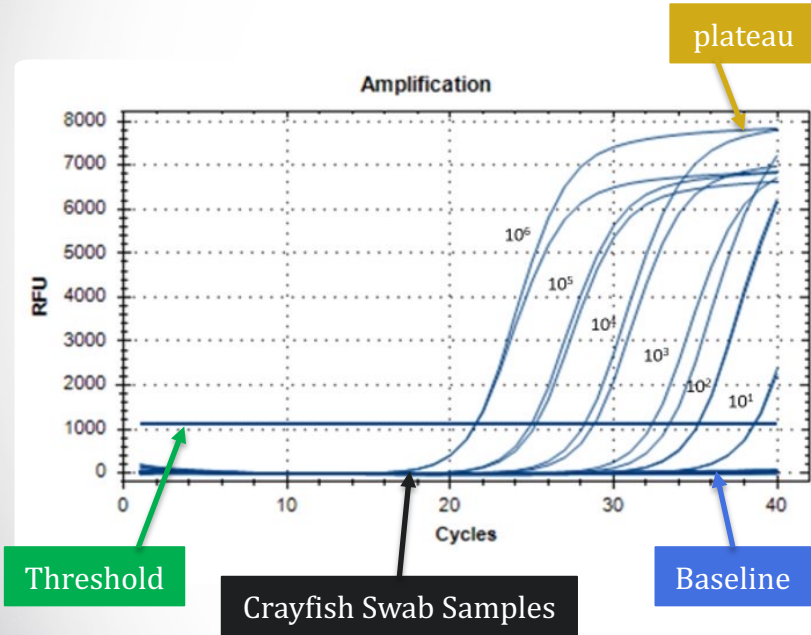
Huff, G. (2023). *Crayfish and newt samples qPCR* [Photograph]. Unpublished.



Huff, G. (2023). *Crayfish and newt swab transfer to centrifuge* [Image]. Created using BioRender.

# qPCR Newt Results

- Newts June: n=20
- \*Newts 95% infection



Harris, K. (2023). *Crayfish and Newt qPCR results [Graph]*. Presented at the Virginia Academy of Science (VAS) Fall Symposium at Virginia Union University.

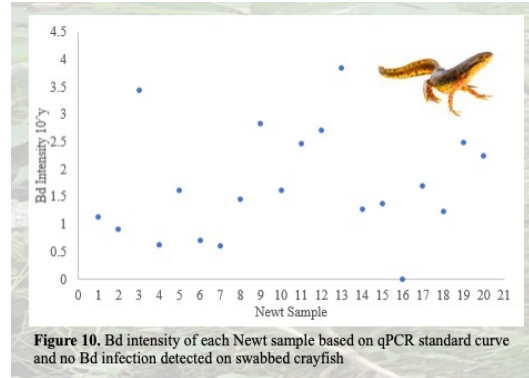
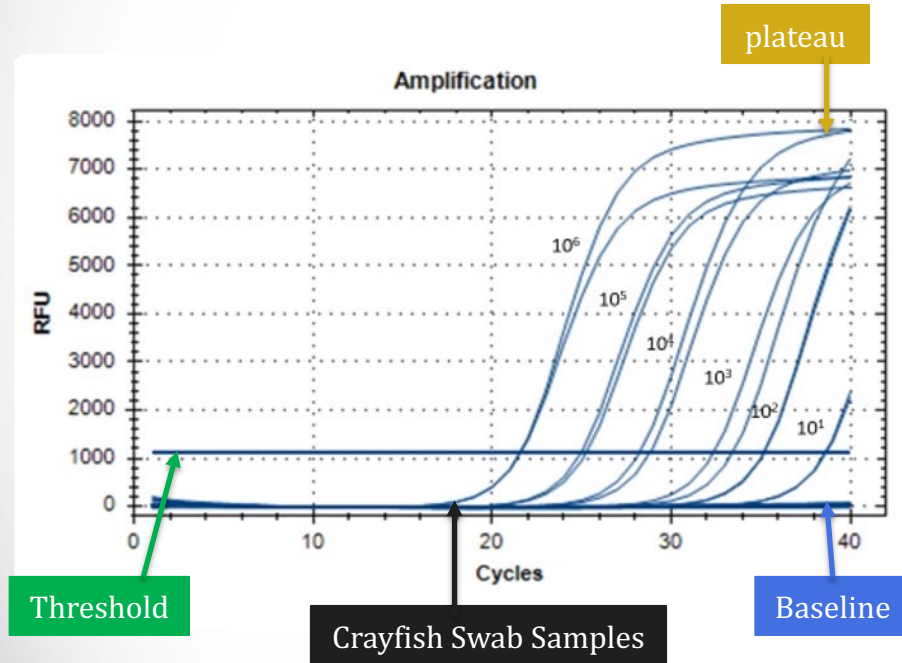


Figure 10. Bd intensity of each Newt sample based on qPCR standard curve and no Bd infection detected on swabbed crayfish

Huff, G. (2023). *Bd intensity vs newt samples [Graph]*. Presented at the Virginia Academy of Science (VAS) Fall Symposium at Virginia Union University.

# qPCR Crayfish Results



Harris, K. (2023). *Crayfish and Newt qPCR results [Graph]*. Presented at the Virginia Academy of Science (VAS) Fall Symposium at Virginia Union University.

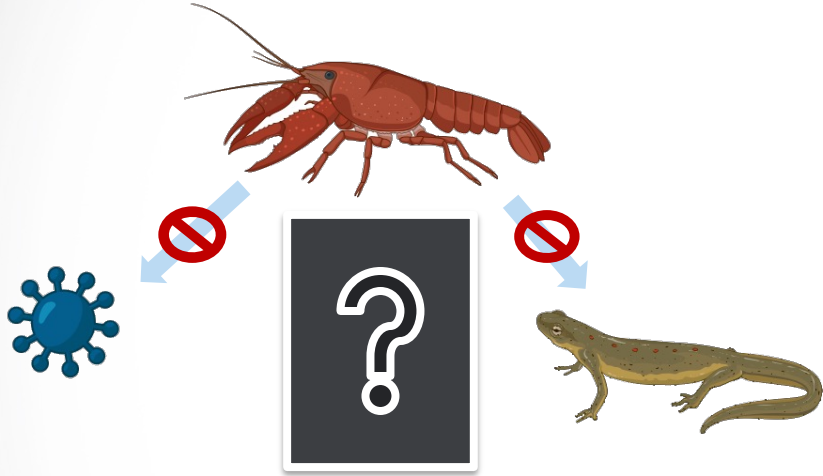
- Crayfish September: n=20
  - Crayfish April: n=20
  - Crayfish June: n=20
  - \*No infection identified



Harris, K. (2023). *Crayfish* [Photograph]. Unpublished.

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# Follow the Data

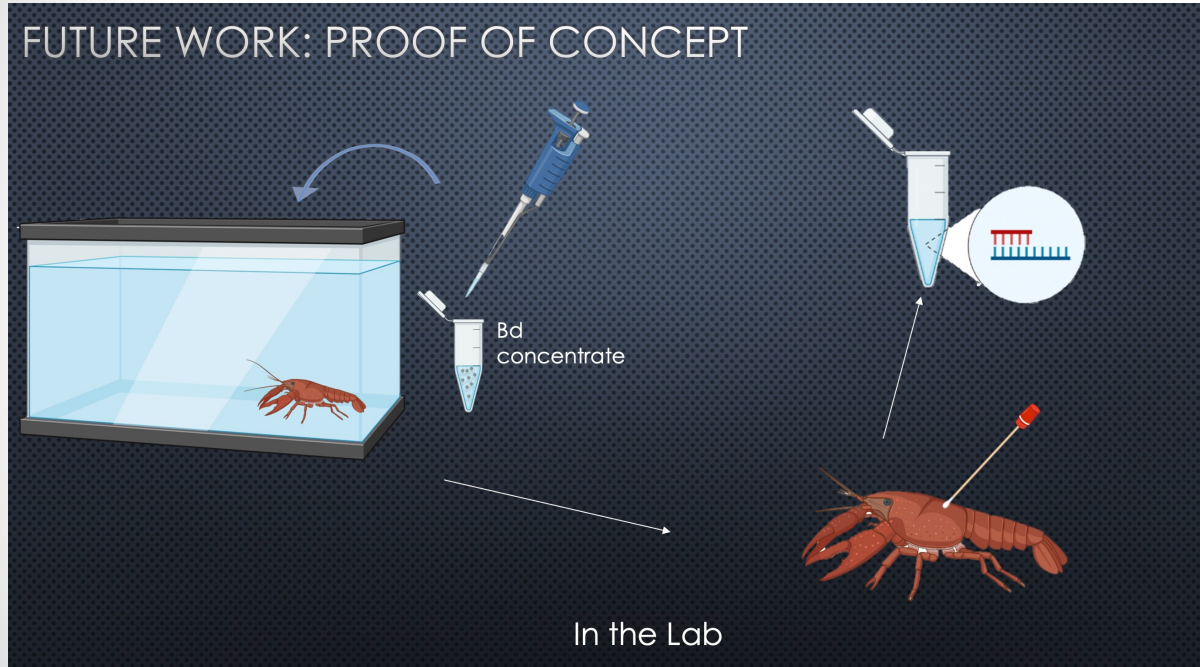


Huff, G. (2023). *Correlation between Bd, crayfish, and newts* [Image]. Created using BioRender.

- Why did 19 out of our 20 newts have positive hits for the fungal pathogen and none of our 20 crayfish get a single hit?
- Is it that the crayfish are not ingesting the fungal pathogen?
- Is it that the crayfish are not vectors in our local streams and prove the literature wrong in our location?
- we are still trying to prove the literature correct for our local streams



# Future Work



- Inoculating crayfish in a tank to test further vector possibilities (proof of concept)

Huff, G. (2023). *Proof of concept, implementing Bd into tank* [Image]. Created using BioRender.

# Future Work



Huff, G. (2023). *Crayfish inoculation in crates at kingfisher pond* [Image]. Created using BioRender.

- Inoculation in crates at kingfisher (in the field)
- Previous collection had the two streams that attached to kingfisher, not right in the pond



Harris, K. (2023). *Crayfish Collection Map*. Unpublished.