Research on the Chytrid Fungus in NH Amphibian Populations

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Introduction

- Batrachochytrium Dendrobatidis (Bd) is a fungus in the phylum, Chytridiomycota, and is characterized by its motile, flagellated spores.
- Bd causes chytridiomycosis in amphibians which is a disease that causes mortality, due to an osmotic balance in the skin and asystolic cardiac arrest.
- Bd has caused many frog species to become extinct and has slowly moved its way across the world.
- Our research shows how Bd has spread into NH and the water bodies it has infected.

Materials and Methods

Field Collections: Specimens were swabbed on sides, belly, legs, and feet. Samples were stored in alcohol at -20℃.

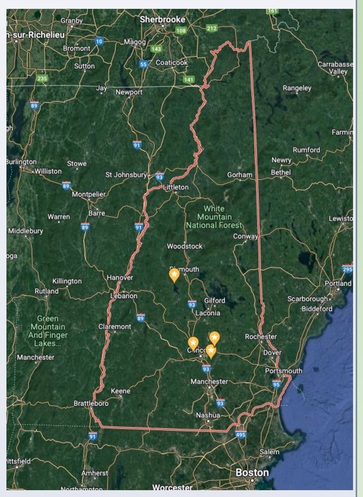
DNA Extraction: Extraction was performed using Qiagen DNeasy Blood & Tissue Kit. This process washes away cellular components and purifies DNA for PCR.

PCR: Polymerase Chain Reaction, used for amplifying the DNA into millions of copies to be seen in gel. Positive control is Bd sample isolated from CZEUM. Negative control is pure H2O.

Gel Electrophoresis: Electrophoresis is a technique used to separate DNA based on size and electrical charge. Pores within gel are where PCR products with dye are placed, then an electrical current runs through to separate products. DNA ladder of Bd is used to compare if samples had Bd present within them.

Sampling Locations and Procedures

Samples were found in Pembroke, Hebron, Concord, and Chichester, NH.



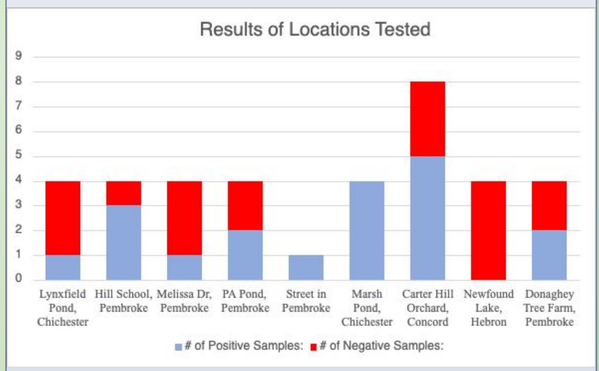
Positive Control of Batrachochytrium Dendrobatidis (Bd), obtained from CZEUM.

Frog Sample #11, Green Frog, isolated from PA Pond, Pembroke, NH.

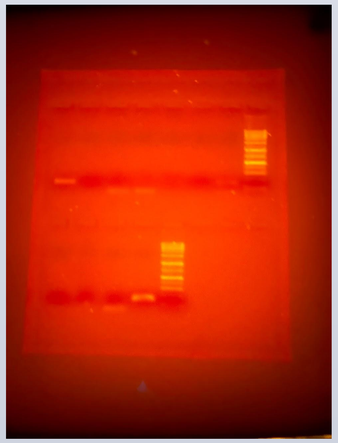
Lab Results

Location:	# of Samples Tested:	# of Positive Samples:
Lynxfield Pond, Chichester, NH	3	1
Hill School, Pembroke, NH	4	3
Melissa Drive, Pembroke, NH	4	1
PA Pond, Pembroke, NH	4	2
Street in Pembroke, NH (Rainy Night)	1	1
Marsh Pond, Chichester, NH	4	4
Carter Hill Orchard, Concord, NH	8	5
Newfound Lake, Audubon Gray Rocks, Hebron, NH	4	0
Donaghey Tree Farm, Pembroke, NH	4	2
9 Locations Tested	36	19

Results show that nineteen out of thirty-six samples were positive.



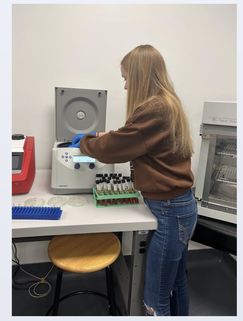
Results showing number of positive and negative samples found at each location.



Results from Gel Electrophoresis: Positives are represented by bright yellowish band. DNA ladders present on both ends to compare.

Lab Procedures

Pipetting PCR products with dye to be seen in agar gel.



Centrifuge machine used for DNA Extraction process. The centrifuge spins samples rapidly to allow DNA pellet to form on bottom.

Conclusions

- Our data shows that nineteen of thirty-six samples tested positive for Bd throughout NH.
- The data confirms that Bd is present in NH waterbodies and could potentially be causing the decline of NH amphibians.
- New strategies must be proposed to mitigate Bd to ensure the safety of our NH amphibian populations.

Literature Cited

- *Collection of Zoosporic Eufungi at the University of Michigan (CZEUM)*
- *Berger, Lee (1998). Chytridiomycosis Causes Amphibian Mortality Associated with Population Declines in the Rain Forests of Australia and Central America. Proceedings of the National Academy of Sciences of the United States of America, 95(15), 9031–9036.*
- *Niederle, M. (2019). Skin-associated lactic acid bacteria from North American bullfrogs as potential control agents of Batrachochytrium dendrobatidis. PloS one, 14(9), e0223020. https://doi.org/10.1371/journal.pone.0223020*