Chytrid Fungus in NH Frog Populations

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Background

Chytrid fungus, scientifically known as *batrachochytrium dendrobatidis*, is a fungus mostly found in aquatic environments. It is transferable between frogs and tadpoles, or through contact with infected waters. Chytrid fungus can be poisonous and deadly. It is a fungus that kills slowly, which makes it spread abundantly. The spread is caused by the migration of frogs from pond to pond before their death. Some frogs are resistant to chytrid such as the American bullfrog or the African clawed frog. However, they are still carriers that can spread this fungus to other non-resistant frogs, which may lead to a major frog pandemic and decrease in the population.

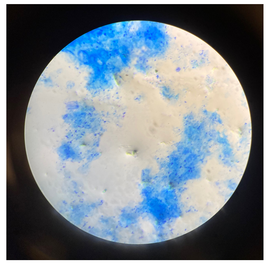

Figure I: Stain of chytrid fungus culture, seen under a microscope.

Introduction

Biology students at SNHU have decided to swab the frogs in ponds near campus and perform DNA extraction, polymerase chain reaction (PCR), and gel electrophoresis to uncover any cases of chytrid fungus in local frog populations.

Method

This project has had multiple stages, conducted over the past few years. Using frog DNA samples which have been collected over the past few summers, the following procedures have been run in the lab:

I. **DNA extraction**: The Qiagen DNeasy Blood and Tissue Kit is used for this step. The sample is vortexed and transferred to a centrifuge, to form a tissue pellet. The alcohol in the tube is then drawn out from the sample, which is followed by another centrifuge of the sample. With the addition of a buffer from the kit, we are left with the frog's fully extracted DNA sample.

II. **PCR**: Typically, one week later, a mix known as a master mix is created and added to the sample. The mix is also added to positive control (chytrid DNA) and to a negative control (nuclease-free water). The samples were run in the PCR thermocycler under the following conditions for a total of about 2.5 hours:

	Temp (C)	Time (m:ss)	
Initial denature	93	10:00	
Denaturing segment	93	:45	Repeat with steps 3+4, 40x
Annealing segment	60	:45	Repeat with steps 2+4, 40x
Elongation segment	72	1:00	Repeat with steps 2+3, 40x
Final Extension	72	10:00	
Hold step	4	Until tubes are removed	

Table 1: PCR steps which includes temperature, and time

III. Gel Electrophoresis:
Two weeks after the PCR is completed, we remove the samples from the freezer and allow them to reach room temperature. We then vortex them to ensure they are completely liquid. We then make an agarose gel and add it to the electrophoretic chamber, which is equip with a cathode and an anode at opposite ends. A loading dye and samples are added individually to the gel. Lastly, in a dark room, the gel is put under a blue UV light, to observe for any presence of chytrid.

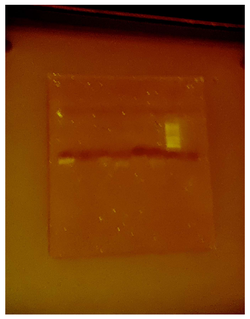

Figure II: Image of gel under blue light, with positive bands for presence of chytrid.

Graphs

Collection Site	Number of Samples Tested	Numbers of Positive Samples
House Pond	12	10
Dorrs Drainage	3	3
Dorrs Pond - Right	11	6
Marsh Pond	6	5
Lynx Field Pond	3	0
Hill School	4	3
PA Pond	4	2
Carter Hill Orchard	4	3
Melissa Drive	3	0
Misc. Locations	3	2

Table II: This table shows collection site, the number of sample we tested and the number of positive samples we recorded during the research.

Discussion

- Is the chytrid fungus found in Southern New Hampshire becoming more prevalent?
- Understanding of chytrid fungus and its effects on frogs, as well as on our environment, may help determine any future environmental catastrophic such as the extinction of frogs.
- Outside sources claim that chytrid has been associated with morbidity, mortality, and decline of frogs.

References:

Frog chytrid fungus. (2020, October 14). NSW Environment and Heritage. https://www.environment.nsw.gov.au/topics/animals-and-plants/native-animals/native-animal-facts/frogs/threats-to-frogs/frog-chytrid-fungus

Gervasi's, S. S., Urbina, J., Hua, J., Chestnut, T., Rely, R. A., & Blaustein, A. R. (2013, March 29). *Experimental Evidence for American Bullfrog (Lithobatescatesbeianus) Susceptibility to Chytrid Fungus(Batrachochytrium dendrobatidis)*. Ecohealth.

Longcore, J. R., Longcore, J. E., Pessier, A. P., & Halteman, W. A. (2017, April 1). *Chytridiomycosis Widespread in Anurans of Northeastern United States.* BioOne. https://bioone.org/journals/journal-of-wildlife-management/volume-71/issue-2/2006-345/Chytridiomycosis-Widespread-in-Anurans-of-Northeastern-United-States/10.2193/2006-345.short?tab=ArticleLink

Penn Vet | Fact Sheet Detail. (n.d.). https://www.vet.upenn.edu/research/centers-laboratories/research-initiatives/wildlife-futures-program/resources/fact-sheets/fact-sheet-detail/chytridiomycosis-(bd)