

Survey of New Hampshire Amphibians for Chytrid fungus



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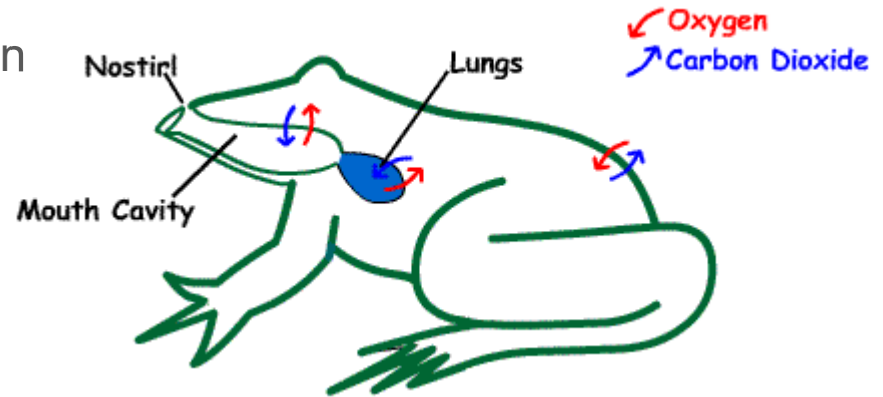
Background

- *Batrachochytrium dendrobatidis* a.k.a. Chytrid fungus causes Chytridiomycosis, a disease affecting frogs and other amphibians
- Chytridiomycosis is responsible for the decline and extinction of over 200 amphibian species



How Chytrid affects breathing

- Chytridiomycosis affects keratin layer of amphibian skin
- Reduces CO_2 and O_2 gas exchange, resulting in death
- Causes a thickening of skin layer
- Amphibians use cutaneous respiration



Research Question

- Are New Hampshire amphibians affected by chytrid fungus?



Field Collections

100+ samples collected from

- Marsh Pond - Chichester, NH
- Lynxfield Pond - Chichester, NH
- Carter Hill Orchard Pond - Concord, NH
- Several local ponds of Pembroke, NH
- Dorrs Pond - Livingston Park, Manchester NH



Collection Procedures

- Skin swab of frogs sides, belly, legs, and feet

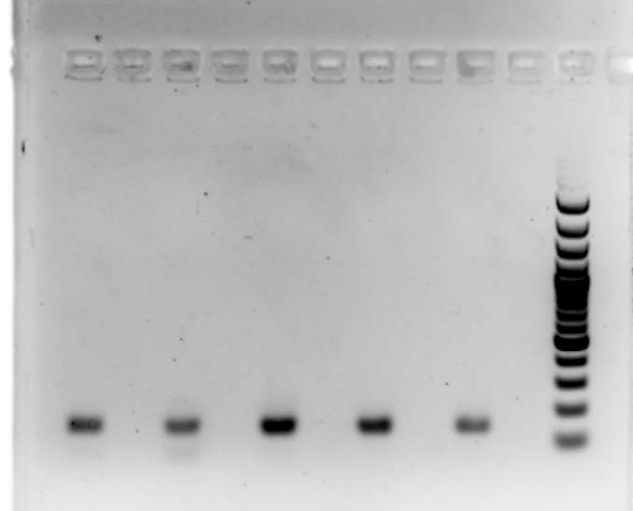
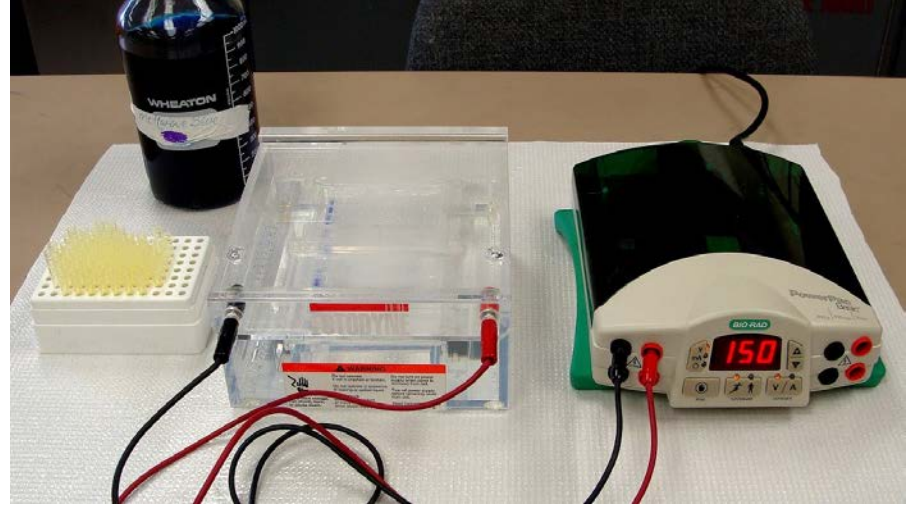


- Stored in Alcohol vial at -20°C



Laboratory Protocols

- DNA extractions
 - A modified Qiagen Kit extraction
 - Removes other tissues and purifies DNA for amplification by PCR
- PCR protocol
- Gel electrophoresis - a technique used to separate DNA fragments according to their size.



PCR (Polymerase Chain Reaction)

Add required reagents (primers or mastermix) and template(DNA) to PCR tubes.

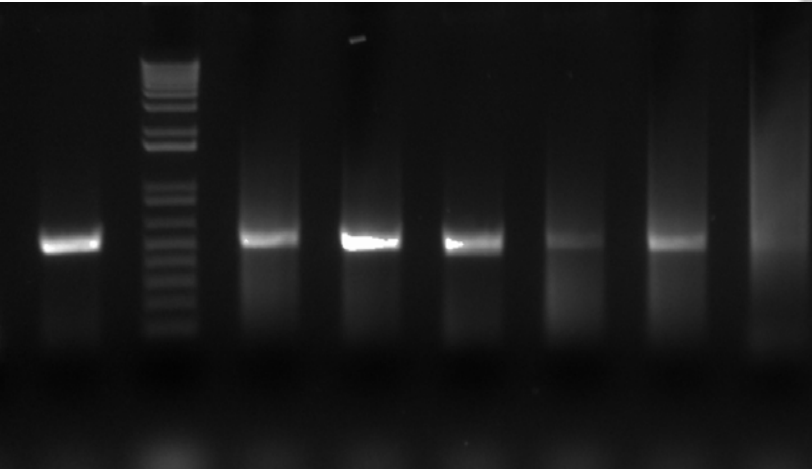
Thermal cycler (changing temperatures) - separates DNA to allow primers to bind. Multiple cycles increases DNA amount.

Evaluate amplified DNA by agarose gel electrophoresis followed by staining.

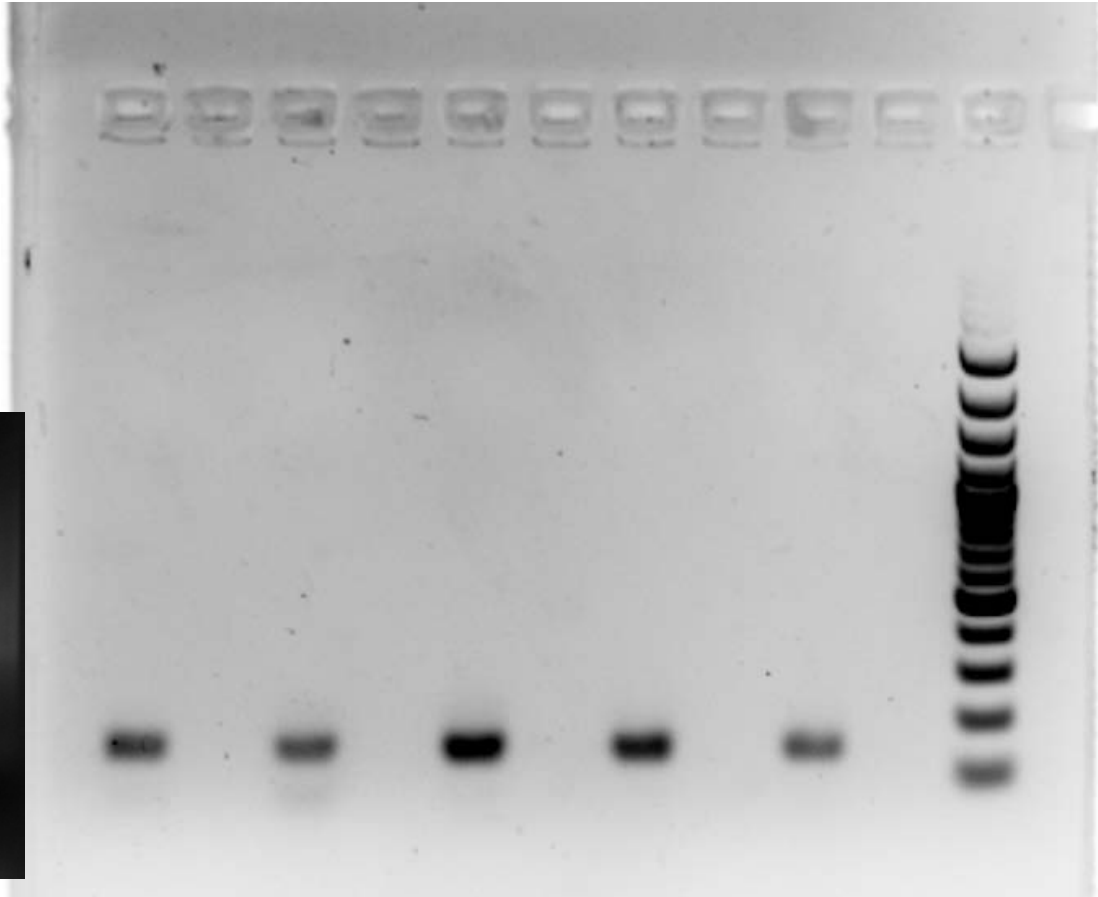


Gels

- Optimization of PCR:
 - Annealing temperature
 - Number of cycles



Smear bands from Internet



PCR gel photo from 3.17th

Preliminary Results

- 7 frogs have tested positive for chytrid so far
 - Lab work to be continued, when COVID-safe!

Location	Total Frogs Sampled	Positive Results (as of March 2021)	Number of Samples Still to be Tested
Chichester, NH	10	0	10
Concord, NH	6	0	6
Manchester, NH	22	3	19
Pembroke, NH	64	4	60
Totals	102	7	95

Future Directions

- Are New Hampshire amphibians affected by chytrid fungus?

Frogs are testing positive, but no signs of severe infection

- Frogs may be asymptomatic
- Seasonality may play a role

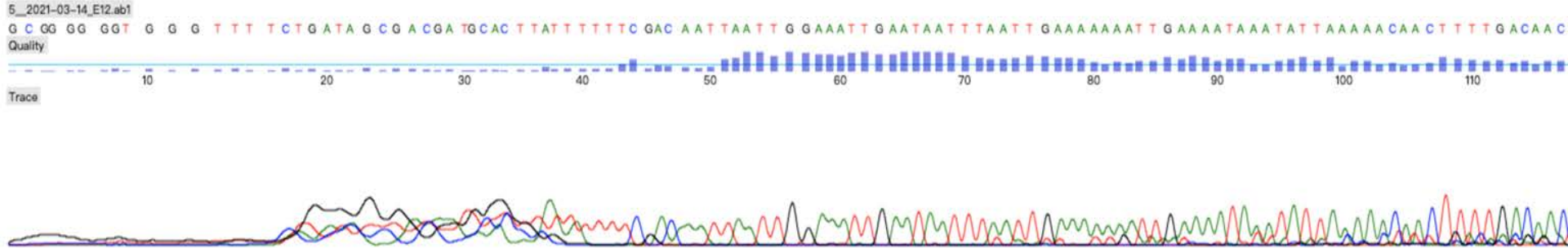


American Bullfrog

Future Directions

- Samples still to process - continue with DNA extraction and PCR tests
- Lab work and sequencing at Quintara Biolabs

PCR & Sequencing - shows the DNA sequence



Literature Cited

- https://www.environment.gov.au/system/files/resources/279bf387-09e0-433f-8973-3e18158febb6/files/c-disease_1.pdf
- <https://cwhl.vet.cornell.edu/disease/chytridiomycosis#:~:text=Chytridiomycosis%20is%20an%20infectious%20disease,of%20frogs%20and%20other%20amphibians.>
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