Examining Rates of Chytrid Fungal Infections in Male and Female Amphibians

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Introduction

As SNHU students working to complete the Biology Capstone, we performed DNA extraction, polymerase chain reaction (PCR), and gel electrophoresis on samples collected from frogs in environments near campus in order to uncover any cases of chytrid fungus in local frog communities.Studying chytrid fungus deepens our understanding of fungal biology, ecology, and its effects on ecosystems. Particularly, research in frog populations provides crucial insights into disease ecology and transmission patterns. These discoveries guide conservation efforts aimed at protecting endangered amphibians from fungal diseases.

Background

Chytrids, belonging to the fungal order Chytridiomycota, possess both sexually and asexually produced cells endowed with flagella, rendering them distinctively motile among fungi. These organisms primarily thrive as saprophytes, deriving nutrients from decomposing plant matter in aquatic habitats. Among them, species like Batrachochytrium dendrobatidis (Bd) and Batrachochytrium salamandrivorans (Bsal) are known pathogens, causing afflictions in frogs and salamanders, respectively. The emergence of chytrid infections has emerged as a primary driver of amphibian population decline worldwide since the late 1990s, impacting over 700 species globally, especially in mountainous terrains where stream networks aid in disease dissemination. Infected male frogs exhibit altered sexual behavior, notably changes in vocalization patterns, potentially appealing to females due to associated reproductive endeavors. Various factors, including temperature fluctuations, influence both frog behavior and chytrid proliferation, with infected males displaying heightened calling efforts, possibly influenced by selective pressures and the imperative to offset the diminished lifespan caused by fungal infection.

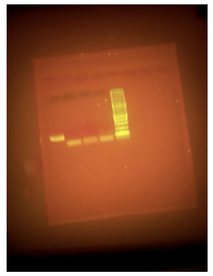
Methods

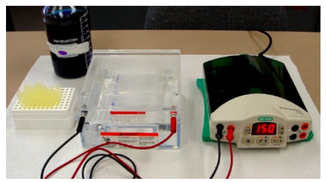
Frogs were swabbed at various locations to collect DNA samples.

DNA extraction:
Samples are centrifuged and vortexed to separate DNA from the swab. A pellet of DNA is formed and transferred to a microcentrifuge tube. Buffer ATL and buffer AL are utilized to purify the DNA, getting rid of unwanted material. Buffer AW1 and AW2 are used to wash the DNA. Buffer AE is used to elute DNA from the filter. All buffers come from the Qiagen DNeasy Blood and Tissue Kit.

PCR:
Each sample is added to a strip tube that contains a master mix (GoTaq, forward and reverse primers and nuclease-free water). A positive of the Chytrid DNA and negative control of water are also put in tubes with the master mix. The strip tube is then placed in the PCR thermocycler on chytrid run 2 to amplify positive chytrid DNA.

Gel electrophoresis:
An agarose gel is made and loaded with dye. The gel is poured and wells are created. Samples resulting from PCR are mixed with loading dye and placed into wells. The gel rig is connected to a cathode and anode for 20 minutes, pulling the samples. The smaller the DNA fragments, the further they will travel down the well. The gel is removed and analyzed under UV light. The samples are compared to a positive control, which is identifiable by the presence of a band.





Results

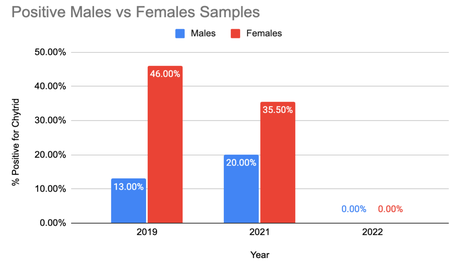


Figure 1. Percent of total positive samples of males and females in the years 2019, 2021, and 2022.

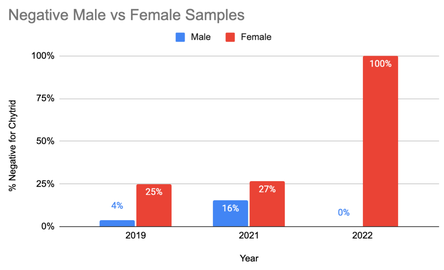


Figure 2. Percent of total negative samples of males and females in the years 2019, 2021, and 2022.

Discussion and Future Direction

These results are preliminary. Based on the first two years, we are finding more female samples than males, as seen in the graphs above. The results suggest variation in transmission of the fungus across genders. Future studies can include understanding mechanisms of susceptibility, immune response, behavior differences, hormones, and genetic diversity among locations.

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