**White Paper for *Bd* Research Using Data Science**

*Introduction*

Emerging infectious diseases are a primary driver of global amphibian declines (1,2). Diseases that are thought to have caused a massive loss of amphibian diversity include: ranaviruses, chytrid fungi, and bacterial pathogens (3–6). Many of these pathogens are sensitive to changes in temperature (7). Changes in thermal environment or variation in thermal regimes can alter the disease-dynamics that amphibians face by causing shifts in pathogen physiology, host immunity, and host behavior (8,9).

Chytridiomycosis, an infectious disease caused by the chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*) is a leading contributor to amphibian population declines (3,10). *Bd* has a two-stage life cycle that consists of a substrate-dependent immobile sporangium and a free-living uniflagellated, motile zoospore (10,11). Infection occurs during the motile zoospore stage of the pathogen’s life cycle (11). The motile zoospores encyst on a substrate, such as the keratinized tissue found in amphibian larval mouthparts or on adult epidermis, and then mature into a zoosporangium (10–12). Zoosporangia produce motile zoospores and then release the new motile zoospores into the environment to re-infect the same host or transmit to another individual host ((12); Fig. 2). Once infection is established within a host, the epidermis is disrupted, and an imbalance of electrolytes leads to cardiac arrest and death (13). Understanding the variation of physiological responses in these two life stages to temperature may explain patterns of disease among host populations (14).

Pathogen physiology, including zoospore production and population growth varies along thermal gradients (15,16). These physiological variations are likely to shift pathogenicity, host responses to infection, and disease outcomes (17). Such physiology studies on *Bd* isolates are extremely relevant considering zoospores are the infectious life stage of *Bd* and temperature-induced increases in zoospore densities may alter virulence and disease development. But what we currently know comes from multiple isolates within a single geographic region or across large-scale geographic locations that explore differences among temperate and tropical isolates (11,16,17). Examining responses of *Bd* to thermal environments from isolates that originate from fine-scale source populations, along a latitudinal gradient, will help to distinguish differences in functional traits that allow *Bd* to be so adaptive and lethal for amphibians across the globe (18).

To understand differential physiology across a finer scale latitudinal gradient, we used cryo-archived libraries of *Bd* isolates collected across the U.S. to measure variation in growth and reproductive traits. I hypothesized that isolates from various latitudes of the U.S. would differ from one another in growth rates and reproductive characteristics across a range of temperatures. To characterize growth and reproduction differences among isolates, I quantified growth rates, zoospore densities, and culture viability for five isolates. I quantified the thermal profile of these isolates by measuring each isolates' thermal minimum, optimum, and maximum (19,20). I predicted that each isolate would exhibit different quantities of zoospores produced at peak growth, times to peak growth in culture, and minimum, optimum, and maximum temperatures of performance for that respective isolate. Understanding if there are isolates from a particular region or latitude that have a constrained thermal range may shed light on amphibian susceptibility within these regions.

*Methods*

To organize and optimize data collection and analyses, it would be beneficial to use biological data science techniques to write a script that extracts and organizes raw data from optical density readers to an excel sheet. When collecting data, I use a plate reader that takes a measurement of the optical density (OD) of my isolates and gives an integer value in the layout of a 96 well plate. However, for data processing, I need to take half of the measurements for each row and transfer them to excel as a single column of positive readings, while the other half are transferred to the excel sheet in a separate column as negative readings. Additionally, each row often represents a unique isolate ID and therefore needs to be sorted accordingly for OD readings. Typically, I do this by hand using a copy and paste technique, with a secondary check of the values at the end of the data transfer. This technique allows for more error in the incorrect transfer of data values and can lead to slower data processing if I must pull up the original readings’ file and compare to my excel sheet with abnormalities are found.

For this project, I will write a python script that will extract the correct values from specific wells and write them to a new file that can be organized by my specification. This script will be versatile for the many plate experiments that I run back-to-back to ensure that data is being written correctly from the reader to an organized out file of my own design. This will streamline data collection and organization, by eliminating the need to perform a quality assurance check by hand multiple times per plate reading. If I am able, I will put the commands for value extraction within a loop, so that I may apply it to multiple files of the same setup. This project will require if/else statements as well to have checkpoints that will alert me to contamination wells for each plate reading so that any contamination can be eliminated from the data frame.

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