

University of Nevada, Reno

Climate Change Induced Temperature Effects on the Thermal Biology of *Batrachochytrium dendrobatidis* and Disease Dynamics of Chytridiomycosis

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in
Ecology, Evolution, and Conservation Biology

By Ciara Sheets

Dissertation Advisor: Dr. Jamie Voyles

August 2023

© Copyright by Ciara N. Sheets 2018 All Rights Reserved



THE GRADUATE SCHOOL

We recommend that the dissertation
prepared under our supervision by

entitled

be accepted in partial fulfillment of the
requirements for the degree of

Advisor

Committee Member

Committee Member

Committee Member

Graduate School Representative

Markus Kemmelmeier, Ph.D., Dean
Graduate School

Abstract

The study of disease ecology aims to understand the complex interactions among hosts, environments, and pathogens which result in a final disease outcome. An area of research that has been expanded within this field in recent years is the impact of climate change and global warming. Climate change impacts are of particular concern as the alterations of a host or pathogen's physiology to more variable or warm environments have been found to be highly influential of disease outcomes in many disease systems. To understand the influence of climate change on disease systems, researchers have assessed the thermal responses of a given pathogen or host in constant laboratory conditions, which may be difficult to relate to more complex, natural environments, or variable field conditions that may be difficult to disentangle direct cause and effect of individual environmental factors on physiological traits. A primary focus of this dissertation is to incorporate the complexities of variable temperatures predicted with climate change conditions in experimental evolution that can assess the implications of climate change on a pathogen known as *Batrachochytrium dendrobatidis* (*Bd*) and the resulting disease outcomes within the chytridiomycosis system. In the first chapter of this dissertation, I conduct a literature review of the impact climate change may have on disease systems and the role that temperature has on the thermal biology and adaptive potential of pathogens and hosts within a given disease system. In the second chapter, I assess and establish the characteristics of thermal biology for multiple isolates of *Bd* that will be used in later chapters. In the third chapter, I use the knowledge of the thermal biology of the isolate from New Mexico to understand patterns of seasonal infection intensity observed in the field. In the last chapter, I assess the physiological responses and adaptive potential of previously studied isolates within this dissertation when experimentally evolved to climate change simulations.

Acknowledgements

First, I'd like to acknowledge and thank my advisor Jamie Voyles; being a graduate student in this lab has allowed me to pursue this research. To the directors of the program, Lee Dyer and Marjorie Matocq, thank you for all that you do to cultivate a rewarding graduate program. The community that this program has provided through my peers exceeded all expectations of what my graduate degree would entail.

To my friends, family, and colleagues, I cannot thank you enough for being on this journey with me and never leaving my side. To my amazing partner, Russell Godkin, you have been a never-ending source of comfort and an unshakable pillar of support. A special shoutout to my friends Lauren Benedict and Augusta Phillips for providing endless support that was life-saving either for motivation, external processing, or problem shooting in R. A special thank you to previous lab mates of the Voyles lab (Alexa, Gabby, Keely, Abby, and Jordan), you all made the lab environment feel like a family unit at times of stress and struggle and have never been unwilling to help in times of need. To my other friends in this program that helped with moments of celebration, problem solving, or critiquing, thank you! Without the support of my friends and family, the last five years would not have been possible, and I will always be more grateful than I can convey.

Finally, I would like to thank the funding sources that made this dissertation possible: Jerry and Betty Wilson, the Dickerson scholarship sponsors, the UNR college of science, and the UNR graduate school. The second chapter of this dissertation was published in *Frontiers Veterinary Science* journal and had minor adjustments made for the purposes of this dissertation.

Table of Contents

Abstract	i
Acknowledgements	ii
List of Tables	v
List of Figures	vii

Chapter Summaries	1
--------------------------------	---

Chapter 1: A literature review of predicted climate change impacts on the thermal biology of pathogen evolution and disease dynamics for chytridiomycosis system

Climate change and infectious disease.....	4
Climate change and temperature effects.....	5
Temperature effects on thermal biology.....	7
Temperature effects on pathogen growth and reproduction.....	12
Temperature effects on host immune responses.....	15
Summary.....	18
References.....	20

Chapter 2: Thermal performance curves of multiple isolates of *Batrachochytrium dendrobatidis*, a lethal pathogen of amphibians

Abstract.....	33
Introduction.....	34
Methods.....	39
Results.....	44
Discussion.....	49

References.....	55
-----------------	----

**Chapter 3: Case study investigating effects of colder temperatures and seasonal differences
on *Bd* originating from New Mexico**

Introduction.....	65
Methods.....	68
Results.....	74
Discussion.....	79
References.....	84

**Chapter 4: The effects of climate change on the physiology and pathogenicity of a lethal
fungal pathogen (*Batrachochytrium dendrobatidis*) of amphibians**

Introduction.....	91
Methods.....	95
Results.....	103
Discussion.....	104
References.....	124
Conclusion.....	131
References.....	144

List of Tables

Table 2-1. Genotyping for isolates of <i>Batrachochytrium dendrobatidis</i> , amphibian host, and geographic origins.....	40
Table 2-2. Population growth viability (MTT) across temperatures (means, SE, r, and K).....	46
Table 2-3. Zoospore density descriptive difference of means using t-tests between genotypes....	47
Table 2-4. Zoospore densities across temperatures (means, SE, r, and K).....	48
Table 3-1. <i>Batrachochytrium dendrobatidis</i> prevalence data for NM.....	76
Table 3-2. <i>Batrachochytrium dendrobatidis</i> intensity of infection data for NM.....	76
Table 4-1. Experimental overview for series of experiments within this study.....	94
Table 4-2. Tukey <i>post Hoc</i> results for ANOVA to compare the difference in maximum whole culture viability of the Optimum lineage among temperature treatments.....	106
Table 4-3. Tukey <i>post Hoc</i> results for ANOVA to compare the difference in maximum zoospore densities of the Optimum lineage among temperature treatments.....	109
Table 4-4. Tukey <i>post Hoc</i> results of the LA lineages grown across the thermal range for maximum whole culture viability.....	114
Table 4-5. Tukey <i>post Hoc</i> results of the LA lineages grown across the thermal range for maximum zoospore densities.....	118
Table 4-6. Tukey <i>post Hoc</i> results for ANOVA to compare lineages inhibition of maximum whole culture viability when grown in the presence of host skin secretions.....	122
Table 4-7. Tukey <i>post Hoc</i> results for ANOVA to compare lineages inhibition of maximum zoospore densities when grown in the presence of host skin secretions.....	122

List of Figures

Figure 1-1. Thermal performance curve defined.....	9
Figure 1-2. Theoretical changes in thermal performance curves.	10
Figure 1-3. Current competing hypotheses for the evolution of maximum growth in relation to the temperature range that performance occurs.....	11
Figure 2-1. Traditional thermal performance curve parameters for a given trait.....	37
Figure 2-2. Mean annual air temperatures throughout the United States (highest temperatures in black, lowest temperatures in white).....	38
Figure 2-3. Thermal performance curves for isolates of <i>Batrachochytrium dendrobatidis</i> (<i>Bd</i>)..	44
Figure 2-4. Logistic growth curve parameter estimates for each isolate and temperature combination.....	49
Figure 3-1. Fecundity thermal performance curves of published <i>Batrachochytrium dendrobatidis</i> isolates.....	68
Figure 3-2. A) Actual water temperature from New Mexico field site and B) simulated temperatures closely matching the field water temperature data shown in panel A.....	71
Figure 3-3. A) Seasonal prevalence and B) seasonal intensity of infection of <i>Batrachochytrium dendrobatidis</i> in amphibians in New Mexico in 2017-2019.....	75
Figure 3-4. Boxplots of New Mexico isolate simulated seasonal trait responses.....	77
Figure 3-5. Constant versus fluctuating temperature maximum zoospore densities and whole culture viability for New Mexico.....	79
Figure 4-1. Experiment 3- predicted changes in <i>Batrachochytrium dendrobatidis</i> (<i>Bd</i> 's) thermal performance curve for the Ancestor versus evolved lineages of <i>Bd</i>	95
Figure 4-2. Modeled current and future amphibian body temperatures within a 24-hour period for May (late winter) in Ohio, near where the <i>Batrachochytrium dendrobatidis</i> isolate was collected.....	96

Figure 4-3. Three simulated temperature profiles for <i>Batrachochytrium dendrobatidis</i> experimentally evolved <i>in vitro</i>	98
Figure 4-4. Experimental design for the serial passaging experiments.....	102
Figure 4-5. Boxplots show the maximum whole culture viability of the Current (orange) and Future (blue) lineages for each <i>Batrachochytrium dendrobatidis</i> isolate in the three thermal treatments: A) stable optimal conditions (21°C), B) the current fluctuating conditions (5-25°C), and C) the future fluctuating conditions (8-28°C).....	107
Figure 4-6. Boxplots show the maximum zoospore densities of the Current (orange) and Future (blue) lineages for each <i>Batrachochytrium dendrobatidis</i> isolate in the three thermal treatments: A) stable Optimum conditions (21°C), B) the Current fluctuating conditions (5-25°C), and C) the Future fluctuating conditions (8-28°C).....	110
Figure 4-7. The <i>Batrachochytrium dendrobatidis</i> isolate from OH responses between Current (orange) and Future (blue) lineages after one month acclimation period.....	112
Figure 4-8. The <i>Batrachochytrium dendrobatidis</i> isolate from OH fecundity responses between Current (orange) and Future (blue) lineages before and after one month acclimation period.....	113
Figure 4-9. Thermal performance curve (TPC) of maximum whole culture viability for LA isolate across lineages.....	116
Figure 4-10. Maximum whole culture viability for LA isolate across lineages at temperatures surrounding the thermal maxima.....	117
Figure 4-11. Thermal performance curves of maximum zoospore densities for LA isolate across the Ancestor (green), Future (blue), and Hot (red) lineages.....	120
Figure 4-12. Maximum zoospore densities for LA isolate across the Ancestor (green), Future (blue), and Hot (red) lineages at temperatures surrounding the thermal maxima.....	121

Figure 4-13. The boxplots show the growth of the Ancestor, Future, Hot, and Optimum lineages
A) maximum whole culture viability or B) maximum zoospore production in the presence of host
skin secretions.....123

Chapter Summaries

Chapter 1: A literature review of predicted climate change impacts on the thermal biology of pathogen evolution and disease dynamics for chytridiomycosis system. In chapter 1, I review the existing literature for the impact and effects of climate change and temperature on disease systems. Specifically, I review (1) the study of climate change within infectious disease systems, (2) the effects of climate change focused through temperature changes on disease systems, (3) how changing temperatures impact thermal biology of pathogens and hosts, (4) the effects of temperature changes on the growth and reproductive physiology of pathogens specifically, and (5) the effects of temperature on host immune responses. The purpose of this literature review is to assess the status of research being done to disentangle the impact of changing temperatures from climate change on both host and pathogen aspects of the disease triad. This review sets the stage to introduce the theories and hypotheses being worked with throughout my remaining thesis chapters to predict future responses of pathogens and their hosts to changing temperatures and make conclusions about the impact climate change may have on the Chytridiomycosis disease system.

Chapter 2: Thermal performance curves of multiple isolates of *Batrachochytrium dendrobatidis*, a lethal pathogen of amphibians. In this chapter, I assess the thermal responses of physiological traits across multiple *Bd* isolates to establish characteristics of *Bd*'s thermal biology across a latitudinal gradient that allows for comparison across a temperature gradient. The second aim of this chapter is to assess the potential correlation between latitudinal position of origin (as a proxy for regional temperature differences) or genotype grouping and patterns of thermal performance across isolates of *Bd*. I predicted that the isolates of *Bd* would have differences in their responses to temperature due to location of origin along the latitudinal gradient. Specifically, I predicted that isolates originating from more northern latitudes would have a lower thermal optimum

temperature and a lower performance maximum at that thermal optimum temperature, while isolates from the southern latitudes would have higher thermal optimum temperatures and a higher performance maximum at that thermal optimum temperature. These predictions of response to temperature by latitude are again expected due to the differences in annual mean air temperatures across the latitudes assessed. Additionally, I predicted that genotype would not sufficiently explain differences in thermal responses of these isolates to temperatures due to a small sample size of available isolates to test.

Chapter 3: Case study investigating effects of variable colder temperatures and seasonal

differences on *Bd* originating from New Mexico. In the third chapter, I tested whether the isolate of *Bd* from New Mexico showed a preference for winter seasonal field conditions compared to summer seasonal field conditions. I also tested this isolate within different field conditions to better understand the pathogen's physiology in variable temperatures that match seasons where infection intensity or prevalence is high. I predicted that this isolate's maximum whole culture viability and zoospore densities would be similar to the previous chapter's study, in which the isolate only had high maximum zoospore densities and fecundity at the cooler temperatures of its thermal range. Therefore, I predicted that the isolate from New Mexico would show higher maximum zoospore densities and whole culture viability in the simulated winter field conditions compared to the summer field conditions. While my hypothesis anticipated the winter field conditions to be more favorable for pathogen growth and reproductive traits than the summer field conditions, I also hypothesized that warmer variable temperatures simulated for the summer field season would not prevent the pathogen from growing entirely.

Chapter 4: Using Experimental evolution to test the effects of climate change on the growth and reproduction of a lethal fungal pathogen (*Batrachochytrium dendrobatidis*). In the fourth and

final chapter, I tested the ability of the *Bd* isolates used in Chapter 2 to respond to climate change by designing an experimental evolution study that simulated climate change conditions.

Additionally, I assessed the impact climate change may have on pathogenicity within the chytrid system by testing the effect that experimental evolution had on the isolate's ability to grow in the presence of host skin secretions to simulate pathogenicity effects. I predicted that isolate responses to experimental evolution in climate change conditions would be different among isolates. However, I predicted that isolates which showed a preference for a warmer thermal optimum and thermal maximum would show a favorable response to conditions simulating warmer, future climate conditions than the cooler, current climate conditions. Additionally, I predicted that the isolates which showed a favorable response to the simulated future climate conditions would show differences between the evolved and ancestral lineages in pathogen growth inhibition when grown in the presence of host skin secretions.

Chapter 1: A literature review of predicted climate change impacts on the thermal biology of pathogen evolution and disease dynamics for chytridiomycosis system

Climate change and infectious disease

Emerging infectious diseases (EIDs) are defined in two different ways: (1) diseases that were previously unknown in a given host population or (2) diseases that have shifted in their geographic range or in their severity (Ogden et al., 2017; Patz, 1996). EIDs have been increasing in frequency in plants, animals, and humans around the world at an extraordinary rate over the past few decades (Daszak et al., 2001; Gebreyes et al., 2014; Jones et al., 2008; Patz, 1996). As such, EIDs pose serious threats to the health and stability of plant, animal, and human populations (Cutler et al., 2010; McMichael, 2015; Smolinski et al., 2003). Some of the most impactful consequences of EIDs include the loss of biodiversity and the decrease in corresponding ecological function that results from the loss of species interactions (Daszak, 2000). To better protect and restore vulnerable ecosystems, the mechanisms driving disease emergence must be understood. A cause of increasing disease emergence is the warming effects of anthropogenic induced climate change (Altizer et al., 2013; Wu et al., 2014).

Environmental conditions and climate warming may influence the rate and mechanisms of disease emergence globally (Daszak et al., 2000; Jones et al., 2008; Patz, 1996). The influence of climate warming on EIDs results from changes in the interactions among hosts, pathogens, and their shared environments (Epstein, 2001). Specifically, shifts in environmental conditions can lead to modifications in host and pathogen physiology, behavior, and their interactions with one-another (Bradshaw & Holzapfe, 2006; Dobson, 2009). For example, the diseases Hanta pulmonary syndrome (caused by Hanta virus) and meningococcal meningitis (caused by the fungus *Nisseria meningitidis*) have exhibited both seasonal and episodic outbreaks as a result of climate change (Greenwood et al., 1984; Wenzel, 1994). In these two disease systems, episodic outbreaks are likely due to phenological adjustments in host distribution and movement (e.g.,

migration; Greenwood et al., 1984; Wenzel, 1994). In other disease systems, such as malaria (caused by *Plasmodium* species), climate change has been linked to alterations of pathogen physiology (e.g., pathogen growth rates), which may increase disease severity (Paaijmans et al., 2012). However, the cause-and-effect relationships among host, pathogen, and their shared environment are frequently complex (Epstein, 2001). Thus, the effects of climate change on EIDs are challenging to determine, depending on the nature and severity of environmental changes, as well as the range of host and pathogen responses, that can occur in a given system (Epstein, 2001; Patz, 1996).

Although there are many climate factors that may be altered by climate change impacting disease dynamics (Dobson, 2009; Hedlund et al., 2014), increasing temperature is thought to be one of the most important for infectious disease (Duncan et al., 2011; Paaijmans et al., 2012; WHO, 2018). Temperatures may be altered by slow climactic shifts or rapid extreme weather events (e.g., heat waves; Easterling, 2000) and create increased opportunity for disease emergence (Epstein, 2001; Roth et al., 2010). Temperature changes can also work in tandem with other climate factors, such as precipitation or humidity, to indirectly exacerbate the severity of infectious diseases (Hedlund et al., 2014). For example, climate modifications that increase mean seasonal temperatures, while simultaneously reducing mean seasonal precipitation, could lead to severe droughts and more frequent wildfires (Reid et al., 2016). A resulting drought or wildfire may cause host immunosuppression, potentially making hosts more susceptible to emerging pathogens (Reid et al., 2016). As this example shows, to predict how climate change will affect the emergence and epidemiology of EIDs, it will be necessary to narrowly focus in on prospective mechanisms of how specific climate factors, such as changes in temperature, alter host and/or pathogen responses (Epstein, 2001; Wu et al., 2014).

Climate change and temperature effects

To focus on mechanistic shifts that occur in a disease system as a result of climate warming, it is first important to specify how temperature change is measured (Buckley & Huey, 2016; Tourneur & Meunier, 2020). There are different ways of measuring temperature and frequently the terms used to describe temperature measurements or profiles are specific to a given system (Bozinovic et al., 2011; Vasseur et al., 2014). For example, it may be essential for some disease systems to examine how temperature change may be occurring over specified increments of time (e.g., daily, seasonal, and annual periods; Vasseur et al., 2014). While there is general consensus over some of these terms (e.g., daily temperature changes being a 24-hour period and annual periods consisting of 12 months based off the Julian calendar), other terms are more ambiguous and difficult to define [e.g., the definition of “seasonal” periods will vary depending on the global region, but are often defined by specific weather conditions (e.g. rainy seasons or frosts) or animal events (e.g., migration or breeding)]. How temperature measurements are described and parameterized will determine how scientific results can be interpreted across systems (Bozinovic et al., 2011).

Two commonly used measures of temperature change in the EID literature are temperature variation and mean temperatures (Bozinovic et al., 2011; Sinclair et al., 2016; Tourneur & Meunier, 2020; Vasseur et al., 2014). Temperature variation is described by changes in the thermal range (i.e., the difference between the maximum and minimum temperatures of the measured time period) within a 24-hour day (Vasseur et al., 2014). Mean temperatures are described as any shift in averages across relevant time periods (Vasseur et al., 2014). Changes in temperature means or variability can affect the physiology and behavior of hosts and pathogens (Deutsch et al., 2008; Kingsolver et al., 2013). For example, a change in daily mean temperature that exceeds a pathogen’s thermal maximum may limit pathogen growth and, subsequently, disease development (Bennett et al., 1992). Increased seasonal mean temperatures may result in resource limitation for hosts and/or pathogens (Patz, 1996). Changes in temperature variation,

such as predicted higher daily maximum temperatures (Karl et al., 1993), may result in increases or decreases in essential activities (e.g., metabolism, growth, reproduction, etc.) for both hosts and pathogens (Patz, 1996). While both changes in temperature variation or means can influence organismal physiology (Parmesan, 2006), some evidence suggests that changes in temperature variation can have a greater influence on host or pathogen fitness than changes in mean temperatures (Altizer et al., 2013; Bozinovic et al., 2011; Easterling, 2000). Therefore, many predictive climate change models focus on understanding changes in temperature variation rather than on increases in daily means alone (Bozinovic et al., 2011; Duncan et al., 2011; Tourneur & Meunier, 2020; Vasseur et al., 2014).

Temperature effects on thermal biology

Temperature has regulating effects on the biology and evolution of most organisms due to thermodynamic constraints (Angilletta et al., 2010; Huey & Kingsolver, 1989). However, it is still unclear what mechanisms underlie how the laws of thermodynamics scale up to constrain organism-level performance across temperatures (Kingsolver & Woods, 2016). The leading hypothesis that describes the mechanistic adaptive potential for thermal physiology in the face of climate change is the thermodynamics constraint hypothesis, known as the “hotter is better” theory (Angilletta et al., 2010; Kingsolver et al., 2013; Knies et al., 2009). The hotter is better theory holds that low temperatures reduce the efficiency of biochemical reaction rate that allows organisms to perform tasks such as cellular growth or reproduction (Angilletta et al., 2010). Colder temperatures cause a reduction in the speed or abundance of chemical reactions that regulate cellular responses and create a lower possible maximum output (Kingsolver & Woods, 2016). These constraints prevent cold-adapted organisms from achieving the same performance maximum as warm-adapted species (Angilletta et al., 2010). The thermodynamics constraints of a given organism are best illustrated with thermal performance curves (TPC; Angilletta, 2006; Schulte et al., 2011; Fig 1-1).

TPCs reflect measurements of an organism's performance across a range of temperatures (Bennett et al., 1992; Kawecki et al., 2012; Fig. 1-1). TPCs use a variety of measurements to describe an organism's performance, including thermal optimum, thermal breadth, critical thermal limits, and a performance maximum (Clusella-Trullas et al., 2011; Knies et al., 2006; Schulte et al., 2011; Fig. 1-1). The thermal optimum (T_{opt}) is the temperature at which maximum performance of a physiological trait is achieved (Angilletta, 2006; Schulte et al., 2011). Thermal breadth (T_{br}) is defined as the range of temperatures at which an organism can perform (Schulte et al., 2011; Sinclair et al., 2016). Critical thermal limits consist of a maximum (CT_{max}) and minimum (CT_{min}) temperature thresholds (Angilletta, 2006; Knies et al., 2006; Sinclair et al., 2016). The performance maximum (P_{max}) is the level of performance at the optimal temperature (Bennett & Lenski, 1999; Schulte et al., 2011). These parameters are used to measure a wide range of traits (e.g., reproduction or growth) across thermal conditions (Angilletta, 2006; Kingsolver & Woods, 2016). TPCs are important because they allow for estimates of the thermal sensitivity of different organismal traits (Bennett & Lenski, 1999; Khelifa et al., 2019). Additionally, they facilitate a way to understand eco-evolutionary processes operating at the population and species levels (Knies et al., 2006).

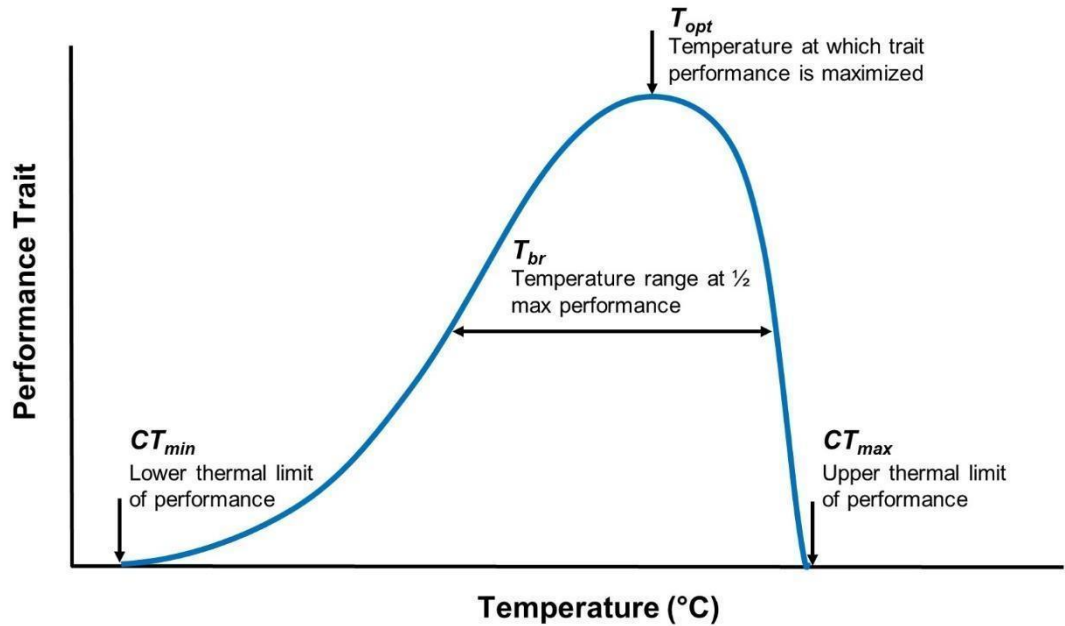


Figure 1-1. Thermal performance curve defined. A thermal performance curve showing the performance of a given trait (e.g., growth rate) across temperature range and featuring key thermal characteristics of the trait: Critical thermal minimum and maximum (CT_{min} & CT_{max}), respectively; thermal optimum (T_{opt}); Thermal breadth (T_{br}). From Sheets et al., 2021, based on Huey & Stevenson 1979.

Comparing TPCs among populations and species has facilitated the study of evolution of thermal sensitivity and spurred debate about thermodynamic processes (Angilletta et al., 2010; Frazier et al., 2006; Nowakowski et al., 2020). For example, many interspecific studies using TPCs have not found evidence to support the “hotter is better” theory and a contrasting theory has emerged (Angilletta et al., 2010; Knies et al., 2009). Specifically, the theory of biochemical adaptation asserts that organisms can compensate for thermodynamic constraints (Angilletta et al., 2010; Fig. 1-2). This contrasting theory predicts that species adapted to colder temperature perform just as well at their optimum temperature as their warm-adapted counterparts, possibly due to biochemical buffering that negates the effects of lower temperatures on the rates of chemical reactions (Angilletta et al., 2010). These two opposing theories are continually

reassessed and form the basis of our understanding of thermal constraints, which set the boundaries of an organism's thermal biology (Frazier et al., 2006).

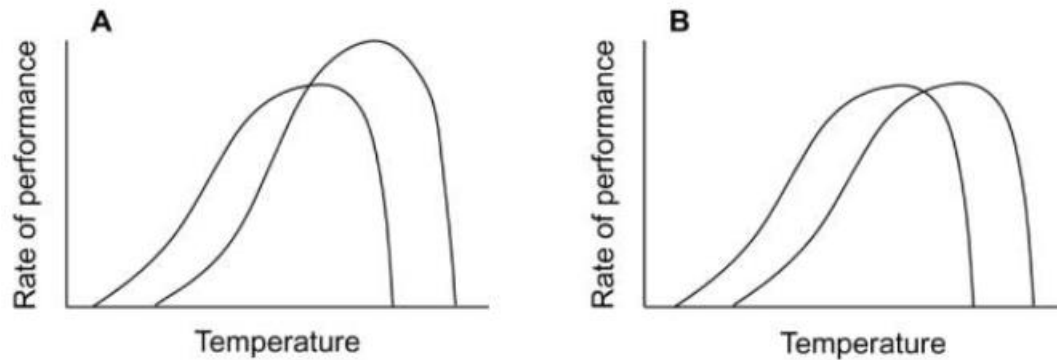


Figure 1-2. Theoretical changes in thermal performance curves. (A) Performance curves that show thermodynamic constraint hypothesis where performance is maximized at higher temperatures, or (B) the biochemical adaptation hypothesis, where adaptation to higher temperatures does not maximize performance more than cooler temperatures. Figure from Angilletta et al., 2010.

The debate over “hotter is better” has also generated additional discussion regarding tradeoffs that organisms have when performance is maximized (Gilchrist, 1995; Kingsolver et al., 2013). Specifically, the generalist-specialist tradeoff is thought to work in tandem with the hotter is better hypothesis (Gilchrist, 1995). For organisms that support the hotter is better hypothesis, the maximum fitness is optimized at the cost of a narrower T_{br} , making them specialists within a given thermal niche (Berger et al., 2014). Whereas generalists have lower maximum fitness but maintain a higher thermal breadth for performing essential activities (Gabriel & Lynch, 1992; Gilchrist, 1995), across a wide range of thermal environments. For more variable thermal environments or changes in temperature variation, it is believed that selection pressures favor

generalists (Berger et al., 2014). While more constant environments or changes in the means of temperature over time are more likely to favor specialists (Gilchrist, 1995).

The generalist-specialist tradeoff is believed to exist for all organisms but has been contradicted or lacking for some species (Kingsolver & Woods, 2016; Knies et al., 2009; Sinclair et al., 2016). Evidence that the generalist-specialist tradeoff is not universal has been supported by the idea that “hotter is better *and broader*” (Knies et al., 2009). Studies that show support of the hotter is better and broader theory suggest that warm-adapted organisms may also have a greater thermal breadth (Fig. 1-3) than cold-adapted individuals (Knies et al., 2009). If more species than previously known show no evidence of a generalist-specialist tradeoff, then former predictions of biological responses to climate change may be inaccurate (Buckley & Huey, 2016; Sinclair et al., 2016).

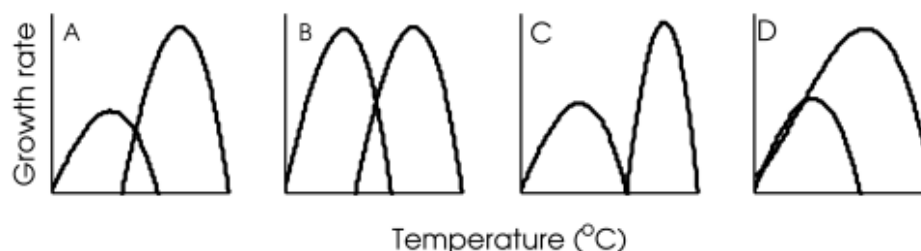


Figure 1-3. Current competing hypotheses for the evolution of maximum growth in relation to the temperature range that performance occurs. (A) Hotter is better shows that maximum growth rate results from adaptation to higher temperatures. (B) If (A) is not true, then adaptation to high temperatures will result in equal maximal growth rates. (C), Generalist-specialist trade-offs show that adapting to higher temperatures will result in a narrow temperature range. (D), If (C) is not true, then adapting to the higher temperatures will result in wider temperature ranges. Figure from Knies et al., 2009.

The extent of an organism’s evolutionary ability to withstand temperature changes resulting from climate change will depend on the adaptive potential of the organism’s thermal sensitivity (Huey et al., 2012; Huey & Kingsolver, 1989). Generally, organisms that have a

broader thermal breadth are predicted to be better equipped to adapt to climate change (Berger et al., 2014). However, when outcomes are less predictable for thermal adaptation, theories such as the climate variability hypothesis attempt to explain how variation in annual temperatures and fluctuating environmental conditions may shape the evolution of an organism's TPC (Kefford et al., 2022). These thermal constraint concepts are especially important for the study of pathogens, hosts, and their responses to climate change (i.e., increases in temperature means and variability) because adjustments in thermal breadth or thermal sensitivity will likely influence pathogenicity, transmission, host susceptibility and resistance, as well as other characteristics that are important for the disease. The thermal performance traits that can be measured for microbial pathogens, such as growth rate, are also more accurate to make predictions of fitness in the face of climate change (Khelifa et al., 2019; Nowakowski et al., 2020).

Temperature effects on pathogen growth and reproduction

In many disease systems, temperature influences pathogen traits, such as growth and reproduction (Bennett et al., 1992; Knies et al., 2006, 2009). These traits are temperature-dependent and have cascading effects on (1) pathogenicity/virulence and (2) transmission (Ewald, 1994; Hudson et al., 2002; Kilpatrick et al., 2008). Also, because growth and reproductive traits are often used as a measure of pathogen fitness, these measures can help understand how temperature may mediate evolution of a pathogen (Ewald, 1994; Laine, 2008).

For many disease studies, investigators are interested in how temperature may influence pathogenicity. Pathogenicity is defined as the ability of a pathogen to invade (or cause infection) and cause harm or negative effects for the host (Ebert & Herre, 1996; Thomas & Elkinton, 2004). Virulence is produced from an interaction among the pathogen, the host, and their shared environment and conveys the severity of the disease (Shapiro-Ilan et al., 2005). Although a variety of definitions for "virulence" exist in the literature (e.g., the pathogen-mediated morbidity and mortality of the host species; Levin, 1996), my working definition of "virulence" defines it as

a property of the disease, rather than of the pathogen alone. Hereafter, I will use the term “pathogenicity” to mean a pathogen-based trait and the term “virulence” to refer to the property of the host-pathogen interaction.

Pathogenicity may be indirectly influenced by temperature through changes in the pathogen’s physiology, such as growth rate, mobility, or adherence properties (Casadevall & Pirofski, 2001; Peterson, 1996). When temperatures are optimal or fluctuate more frequently in the optimal range of the pathogen, the growth rate and reproduction efforts of pathogens can be increased, resulting in a more severe disease or more harm to the host (Ebert, 1998; Kutz et al., 2005). The resulting severity of disease may be observed beyond a pathogen-load threshold, where a critical number of infectious cells cause host morbidity and/or mortality (Anderson & May, 1979; Ebert & Bull, 2007). For example, in the chytridiomycosis system, it has been hypothesized that seasonality effects of disease outbreaks are related to temperature where temperature-mediated pathogen reproduction is reduced and pathogenicity decreases (Berger et al., 2004; Kriger & Hero, 2007; Sonn et al., 2019). On the other hand, in seasons when temperatures are more optimal for cold-adapted pathogen reproduction, such as lower temperatures in mid-winter-early spring, infection intensity could increase due to higher pathogen loads and a resulting higher threshold level of pathogens in host organisms (Kriger & Hero, 2007). Pathogenicity can be best predicted by assessing pathogen-loads rather than just prevalence data (Vredenburg et al., 2010). Temperature changes from climate change may result in seasonal shifts of pathogen reproduction peaks, the timing for reaching infection threshold loads, and therefore the timing and severity of disease occurrence within host populations (Dobson, 2009; Raffel et al., 2013).

Transmission is defined as the mechanistic process by which a pathogen is transferred from a source or reservoir to a host and has been linked to trade-offs concerning virulence (Ebert & Bull, 2003). While virulence and transmission are commonly thought to be associated, there is

evidence of the temperature effects on each of these disease factors that may operate independently (Day, 2001, 2003; Elliot, 2003). Transmission is also altered by temperature through changes to life history traits such as growth and reproduction (Duncan et al., 2011; Gillooly, 2001). Higher mean temperatures may increase disease transmission via increase in pathogen growth rates (Duncan et al., 2011; Elderd & Reilly, 2014). For example, in a study that measured temperature effects on transmission of baculovirus among armyworms, the investigators found evidence that increased temperature altered the feeding and development rates of the worm, which increased transmission and outbreak intensity (Elder & Reilly, 2014). Other studies show that increased temperature variability reduces pathogen growth and results in decreased transmission success (Duncan et al., 2011). Temperature can improve other interactive traits of the pathogen, such as the mobility of free-living larval life stages, which ultimately enhances transmission efficiency and infectivity (Nguyen et al., 2020). Studies of malaria parasites predict that increased mean temperatures will increase parasite replication and development time (Mordecai et al., 2013; Paaijmans et al., 2012; Shapiro et al., 2017). To predict how temperature variation from climate change will affect transmission, the interactive effects of temperature should be measured for pathogen physiological traits such as growth, metabolism, mobility, and reproduction *in vivo* and *in vitro* (Fels & Kaltz, 2006; Mordecai et al., 2013; Paaijmans et al., 2012; Rohr et al., 2011).

To understand the evolutionary processes and the significance of temperature effects over time on pathogenicity and transmission, serial passage experiments can be used for pathogens (Zbinden et al., 2008). Serial passage experiments have been used traditionally to show the experimental evolution of disease factors such as pathogenicity or virulence through observing alterations to physiological traits of the pathogen (Chapuis et al., 2011; Voyles et al., 2014). Serial passage experiments involve passaging an organism in specific environmental conditions for multiple generations to measure the performance or response of the physiological traits before

and after the experimental evolution (Woo & Reifman, 2014). These studies can often answer questions about the evolutionary responses or adaptive potential of organisms to multiple factors, including competition, nutrition, reproduction, and temperature (Kawecki et al., 2012). For example, serial passaging of *E. coli* strains has been conducted in various thermal conditions to study the experimental evolution of pathogen growth and reproduction to temperature changes (Bennett & Lenski, 1999). Serial passage experiments are also useful to show evidence of existing trade-offs (e.g., pathogenicity vs. transmission or specialists vs. generalists) between various factors of disease dynamics (Ebert, 1998). For example, after serial passaging of an organism, changes in TPCs may demonstrate that the P_{max} of a trait increases at the T_{opt} at the cost of the overall T_{br} (Bennett et al., 1992). While experimental evolution is not exempt from limitations and caveats (e.g., laboratory experiments being artificial and lacking natural complexities), this approach has been pivotal in the study of pathogen adaptation under variable environmental conditions (Kawecki et al., 2012).

Temperature effects on host immune responses

An additional factor of pathogen-host relationships that is affected by climate-change induced temperature changes is the host immune responses to pathogens (Altizer et al., 2013; Dobson, 2009). Both innate and adaptive host immune defenses may be temperature dependent and thus impacted by changes in temperature (Ellner et al., 2007; Raffel et al., 2006; Seppälä & Jokela, 2011; Sonn et al., 2019). Innate host immune defenses consist of physiological, cellular, chemical, or physical non-specific, immune responses that occur within the host as a primary response to prevent disease (Butler et al., 2013; Raffel et al., 2006). Adaptive immune defenses are a secondary line of defense that involves specific immune responses to a pathogen after the immune system has been exposed to the pathogen (Butler et al., 2013). For both branches of the

immune system, the direct effects of temperature on immune responses and function are of concern for climate change research (Altizer et al., 2013; Butler et al., 2013).

Temperature plays a distinct role in immune responses that differs between ectotherms and endotherms (Butler et al., 2013). Ectotherms are unable to regulate their own body temperature, therefore, biological processes such as immune functions are highly temperature sensitive (Ferguson et al., 2018; Mondal & Rai, 2001; Raffel et al., 2006; Wright & Cooper, 1981). For example, immune responses can be directly affected if temperature changes fall outside of an ectotherm's thermal tolerance, rendering immune responses non-functional (Barber et al., 2016; Butler et al., 2013; Leicht et al., 2013). One study showed that when amphibians were exposed to extreme heat waves, host lymphocyte production was significantly reduced, which researchers suggest may have implications for susceptibility to amphibian pathogens (Wright & Cooper, 1981). Other studies have shown that increased temperatures that reach the critical maximum temperature of an ectotherm could reduce an ectotherm's immunity defenses against pathogens, such as coral fungus (*Aspergillus sydowii*) and protozoan (*Aplanochytrium sp.*; Altizer et al., 2013).

In contrast, endotherms can regulate their body temperatures and buffer minor changes in temperature within their thermal environment (Butler et al., 2013; Shephard & Shek, 1998). Temperature changes are still able to affect immune responses, through physiological stress related mechanisms (Adamo & Lovett, 2011; Butler et al., 2013). For example, if temperatures drop to extreme lows for extended periods of time, an endotherm experiences physiological stress which can lead to reductions of leukocyte production, natural killer (NK) cell counts, and cytolytic activity (Shephard & Shek, 1998; Shu et al., 1993; Won & Lin, 1995). There are fewer differences in the effects of temperature on innate immune responses between endotherms and ectotherms compared to the adaptive immune responses (Butler et al., 2013). Temperature changes thus impact ectotherms and endotherms to varying degrees depending on the severity and

longevity of change (Butler et al., 2013; Raffel et al., 2006). It is hypothesized that immune functions are influenced more severely at temperatures that lie further away from an organism's T_{opt} than at temperature changes near the optimum (Barber et al., 2016; Raffel et al., 2006). To identify where these T_{opt} , CT_{min} , and CT_{max} for immune functions lie, TPCs can be applied to traits relating to immune function (Padfield et al., 2020).

TPCs can be used to understand what effects increased temperatures or temperature fluctuation from climate warming may have on the immune responses of host species (Murdock et al., 2012). For example, observing the immune responses of a host across a TPC can demonstrate at which temperatures immune functions are reduced, optimized, or lost completely (Adamo & Lovett, 2011; Martin et al., 2010). When host individuals (e.g., stickleback fish) are exposed to their CT_{max} for short periods of time long-term immune deficiency can occur (Dittmar et al., 2014; Harvell et al., 2007; Roth et al., 2010; Leicht & Seppälä, 2014). Other studies have used TPCs to examine the temperatures at which immune enzyme activity can be improved, enabling the host to combat a faster immune response against infection (Adamo & Lovett, 2011). As climate change is predicted to increase temperature means and fluctuation, using TPCs to assess the risks of hosts immune responses may be important for disease predictions (Butler et al., 2013).

Increased temperature shifts due to climate change affect heat stress and acclimation of the host and their immune functions, which results in changes to resistance or susceptibility of host to their pathogens (Martin et al., 2010; Rohr et al., 2018). Therefore, the overall effect of temperature changes on host immunity is dependent upon environment, immune function, and pathogen dynamics (Raffel et al., 2006). The effect of temperature on host immune function can be highly variable and unpredictable depending on the system considered. Therefore, more systems should be studied for temperature effects of immunity (Altizer et al., 2013).

Summary: Climate change induced temperature effects on disease dynamics of infectious diseases

To show the effects of climate change on pathogen biology and physiology, studies implement a variety of methods and approaches using primary climate factors, such as temperature (Clusella-Trullas et al., 2011; Schulte et al., 2011). To analyze changes in physiological traits in variable thermal conditions, ecologists use TPCs and experimental evolution (Khelifa et al., 2019; Sinclair et al., 2016). From these studies, evidence suggests that changes in temperature, such as increased daily means or daily variation, can lead to changes in growth and reproduction of the pathogens of many natural environments (Kingsolver & Woods, 2016; Sinclair et al., 2016). Increased temperatures from climate change that alter pathogen physiology may lead to cases of increased pathogenicity and transmission (Elderl & Reilly, 2014). Increasing growth or reproduction ultimately affects the interaction of a pathogen with the host and its environment (Adamo & Lovett, 2011; Leggett et al., 2017). An advantage of studying the thermal biology of pathogens using this experimental evolution, is that multi-generational effects can be analyzed to investigate evolutionary processes across populations of pathogens (Kingsolver et al., 2013; Knies et al., 2009). The thermal performance traits that can be measured for pathogens, such as growth rate, also allow for accurate predictions of organismal fitness in the face of climate change (Altizer et al., 2013; Khelifa et al., 2019; Knies et al., 2006).

Climate-change induced temperature changes influence both pathogen and host traits that determine disease outcomes (Adamo & Lovett, 2011; Casadevall & Pirofski, 2001). If pathogen traits are influenced in a way that reduces pathogen fitness, while host traits are influenced to increase host fitness, then the likelihood of disease is reduced overall (Butler et al., 2013; Nowakowski et al., 2020). Disease dynamics are complex and coevolutionary factors between pathogen and host traits must be considered independently and in combination (Rohr et al., 2018; Woodhams et al., 2008). For example, when temperature changes alter aspects of the host's

immune response (e.g., resistance), temperature is indirectly changing the evolution of both the host and pathogen through selection (Ebert, 1998; Zbinden et al., 2008). The more robust immune response the host can maintain in changing temperatures, the more adaptations pathogens will need to establish within a host and cause infection (Dobson, 2009; Martin et al., 2010).

Understanding what immune functions are altered by climate change either *in vitro* or *in vivo*, while also assessing the impact climate change will have on the pathogen's biology, is essential to understanding the ultimate risk of disease within systems for the future (Altizer et al., 2013).

References

- Adamo, S. A., & Lovett, M. M. E. (2011). Some like it hot: The effects of climate change on reproduction, immune function and disease resistance in the cricket *Gryllus texensis*. *Journal of Experimental Biology*, 214(12), Article 12. <https://doi.org/10.1242/jeb.056531>
- Altizer, S., Ostfeld, R. S., Johnson, P. T. J., Kutz, S., & Harvell, C. D. (2013). Climate Change and Infectious Diseases: From Evidence to a Predictive Framework. *Science*, 341(6145), Article 6145. <https://doi.org/10.1126/science.1239401>
- Anderson, R. M., & May, R. M. (1979). Population biology of infectious diseases: Part I. *Nature*, 280(5721), Article 5721. <https://doi.org/10.1038/280361a0>
- Angilletta, M. J. (2006). Estimating and comparing thermal performance curves. *Journal of Thermal Biology*, 31(7), Article 7. <https://doi.org/10.1016/j.jtherbio.2006.06.002>
- Angilletta, M. J., Huey, R. B., & Frazier, M. R. (2010). Thermodynamic Effects on Organismal Performance: Is Hotter Better? *Physiological and Biochemical Zoology*, 83(2), Article 2. <https://doi.org/10.1086/648567>
- Barber, I., Berkhout, B. W., & Ismail, Z. (2016). Thermal Change and the Dynamics of Multi-Host Parasite Life Cycles in Aquatic Ecosystems. *Integrative and Comparative Biology*, 56(4), Article 4. <https://doi.org/10.1093/icb/icw025>
- Bennett, A. F., Lenski, R. E., & Mittler, J. E. (1992). Evolutionary Adaptation to Temperature. I. Fitness Responses of *Escherichia coli* to Changes in its Thermal Environment. *Evolution*, 46(1), Article 1. <https://doi.org/10.2307/2409801>
- Berger, D., Walters, R. J., & Blanckenhorn, W. U. (2014). Experimental evolution for generalists and specialists reveals multivariate genetic constraints on thermal reaction norms. *Journal of Evolutionary Biology*, 27(9), 1975–1989. <https://doi.org/10.1111/jeb.12452>
- Berger, L., Speare, R., Hines, H., Marantelli, G., Hyatt, A., McDonald, K., Skerratt, L., Olsen, V., Clarke, J., Gillespie, G., Mahony, M., Sheppard, N., Williams, C., & Tyler, M. (2004).

- Effect of season and temperature on mortality in amphibians due to chytridiomycosis. *Australian Veterinary Journal*, 82(7), Article 7. <https://doi.org/10.1111/j.1751-0813.2004.tb11137.x>
- Bozinovic, F., Bastías, D. A., Boher, F., Clavijo-Baquet, S., Estay, S. A., & Angilletta, M. J. (2011). The Mean and Variance of Environmental Temperature Interact to Determine Physiological Tolerance and Fitness. *Physiological and Biochemical Zoology*, 84(6), 543–552. <https://doi.org/10.1086/662551>
- Bradshaw, W. E., & Holzapfe, C. M. (2006). Climate Change: Evolutionary Response to Rapid Climate Change. *Science*, 312(5779), Article 5779. <https://doi.org/10.1126/science.1127000>
- Buckley, L. B., & Huey, R. B. (2016). How Extreme Temperatures Impact Organisms and the Evolution of their Thermal Tolerance. *Integrative and Comparative Biology*, 56(1), Article 1. <https://doi.org/10.1093/icb/icw004>
- Butler, M. W., Stahlschmidt, Z. R., Ardia, D. R., Davies, S., Davis, J., Guillette, L. J., Johnson, N., McCormick, S. D., McGraw, K. J., & DeNardo, D. F. (2013). Thermal Sensitivity of Immune Function: Evidence against a Generalist-Specialist Trade-Off among Endothermic and Ectothermic Vertebrates. *The American Naturalist*, 181(6), 761–774. <https://doi.org/10.1086/670191>
- Casadevall, A., & Pirofski, L. (2001). Host-Pathogen Interactions: The Attributes of Virulence. *The Journal of Infectious Diseases*, 184(3), Article 3. <https://doi.org/10.1086/322044>
- Chapuis, É., Pagès, S., Emelianoff, V., Givaudan, A., & Ferdy, J.-B. (2011). Virulence and Pathogen Multiplication: A Serial Passage Experiment in the Hypervirulent Bacterial Insect-Pathogen *Xenorhabdus nematophila*. *PLoS ONE*, 6(1), Article 1. <https://doi.org/10.1371/journal.pone.0015872>
- Clusella-Trullas, S., Blackburn, T. M., & Chown, S. L. (2011). Climatic Predictors of

- Temperature Performance Curve Parameters in Ectotherms Imply Complex Responses to Climate Change. *The American Naturalist*, 177(6), Article 6.
<https://doi.org/10.1086/660021>
- Cutler, S. J., Fooks, A. R., & van der Poel, W. H. M. (2010). Public Health Threat of New, Reemerging, and Neglected Zoonoses in the Industrialized World. *Emerging Infectious Diseases*, 16(1), Article 1. <https://doi.org/10.3201/eid1601.081467>
- Daszak, P. (2000). Emerging Infectious Diseases of Wildlife—Threats to Biodiversity and Human Health. *Science*, 287(5452), Article 5452.
<https://doi.org/10.1126/science.287.5452.443>
- Daszak, P., Cunningham, A. A., & Hyatt, A. D. (2001). Anthropogenic environmental change and the emergence of infectious diseases in wildlife. *Acta Tropica*, 78(2), Article 2.
[https://doi.org/10.1016/S0001-706X\(00\)00179-0](https://doi.org/10.1016/S0001-706X(00)00179-0)
- Day, T. (2001). Parasite Transmission Modes and the Evolution of Virulence. *Evolution*, 55(12), Article 12.
- Day, T. (2003). Virulence evolution and the timing of disease life-history events. *Trends in Ecology & Evolution*, 18(3), Article 3. [https://doi.org/10.1016/S0169-5347\(02\)00049-6](https://doi.org/10.1016/S0169-5347(02)00049-6)
- Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C., & Martin, P. R. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences*, 105(18), Article 18.
<https://doi.org/10.1073/pnas.0709472105>
- Dittmar, J., Janssen, H., Kuske, A., Kurtz, J., & Scharsack, J. P. (2014). Heat and immunity: An experimental heat wave alters immune functions in three-spined sticklebacks (*Gasterosteus aculeatus*). *Journal of Animal Ecology*, 83(4), Article 4.
<https://doi.org/10.1111/1365-2656.12175>
- Dobson, A. (2009). Climate variability, global change, immunity, and the dynamics of infectious

- diseases. *Ecology*, 90(4), Article 4. <https://doi.org/10.1890/08-0736.1>
- Duncan, A. B., Fellous, S., & Kaltz, O. (2011). Temporal variation in temperature determines disease spread and maintenance in *Paramecium* microcosm populations. *Proceedings of the Royal Society B: Biological Sciences*, 278(1723), Article 1723. <https://doi.org/10.1098/rspb.2011.0287>
- Easterling, D. R. (2000). Climate Extremes: Observations, Modeling, and Impacts. *Science*, 289(5487), Article 5487. <https://doi.org/10.1126/science.289.5487.2068>
- Ebert, D. (1998). Infectivity, Multiple Infections, and the Genetic Correlation Between Within-host Growth and Parasite Virulence: A reply to Hochberg. *Evolution*, 52(6), Article 6. <https://doi.org/10.1111/j.1558-5646.1998.tb02267.x>
- Ebert, D., & Bull, J. J. (2003). Challenging the trade-off model for the evolution of virulence: Is virulence management feasible? *Trends in Microbiology*, 11(1), Article 1. [https://doi.org/10.1016/S0966-842X\(02\)00003-3](https://doi.org/10.1016/S0966-842X(02)00003-3)
- Ebert, D., & Bull, J. J. (2007). The evolution and expression of virulence. In S. C. Stearns & J. C. Koella (Eds.), *Evolution in Health and Disease* (pp. 153–168). Oxford University Press. <https://doi.org/10.1093/acprof:oso/9780199207466.003.0012>
- Ebert, D., & Herre, E. A. (1996). The evolution of parasitic diseases. *Parasitology Today*, 12(3), Article 3. [https://doi.org/10.1016/0169-4758\(96\)80668-5](https://doi.org/10.1016/0169-4758(96)80668-5)
- Elder, B. D., & Reilly, J. R. (2014). Warmer temperatures increase disease transmission and outbreak intensity in a host-pathogen system. *Journal of Animal Ecology*, 83(4), Article 4. <https://doi.org/10.1111/1365-2656.12180>
- Elliot, S. L. (2003). The virulence–transmission trade-off and virulence management. *Trends in Microbiology*, 11(5), Article 5. [https://doi.org/10.1016/S0966-842X\(03\)00071-4](https://doi.org/10.1016/S0966-842X(03)00071-4)
- Ellner, S. P., Jones, L. E., Mydlarz, L. D., & Harvell, C. D. (2007). Within-Host Disease Ecology in the Sea Fan *Gorgonia ventalina*: Modeling the Spatial Immunodynamics of a Coral-

- Pathogen Interaction. *The American Naturalist*, 170(6), Article 6.
<https://doi.org/10.1086/522841>
- Epstein, P. R. (2001). Climate change and emerging infectious diseases. *Microbes and Infection*, 8.
- Ewald, P. W. (1994). *Evolution of Infectious Disease*. Oxford University Press.
- Fels, D., & Kaltz, O. (2006). Temperature-dependent transmission and latency of *Holospora undulata*, a micronucleus-specific parasite of the ciliate *Paramecium caudatum*. *Proceedings of the Royal Society B: Biological Sciences*, 273(1589), Article 1589.
<https://doi.org/10.1098/rspb.2005.3404>
- Ferguson, L. V., Kortet, R., & Sinclair, B. J. (2018). Eco-immunology in the cold: The role of immunity in shaping the overwintering survival of ectotherms. *Journal of Experimental Biology*, 221(13), jeb163873. <https://doi.org/10.1242/jeb.163873>
- Frazier, M. R., Huey, R. B., & Berrigan, D. (2006). Thermodynamics Constrains the Evolution of Insect Population Growth Rates: “Warmer Is Better.” *The American Naturalist*, 168(4), Article 4. <https://doi.org/10.1086/506977>
- Gabriel, W., & Lynch, M. (1992). The selective advantage of reaction norms for environmental tolerance. *Journal of Evolutionary Biology*, 5(1), Article 1.
<https://doi.org/10.1046/j.1420-9101.1992.5010041.x>
- Gebreyes, W. A., Dupouy-Camet, J., Newport, M. J., Oliveira, C. J. B., Schlesinger, L. S., Saif, Y. M., Kariuki, S., Saif, L. J., Saville, W., Wittum, T., Hoet, A., Quessy, S., Kazwala, R., Tekola, B., Shryock, T., Bisesi, M., Patchanee, P., Boonmar, S., & King, L. J. (2014). The global one health paradigm: Challenges and opportunities for tackling infectious diseases at the human, animal, and environment interface in low-resource settings. *PLoS Neglected Tropical Diseases*, 8(11), Article 11. PubMed.
<https://doi.org/10.1371/journal.pntd.0003257>

- Gilchrist, G. W. (1995). Specialists and Generalists in Changing Environments. I. Fitness Landscapes of Thermal Sensitivity. *The American Naturalist*, 146(2), Article 2. JSTOR.
- Gillooly, J. F. (2001). Effects of Size and Temperature on Metabolic Rate. *Science*, 293(5538), Article 5538. <https://doi.org/10.1126/science.1061967>
- Greenwood, B. M., Bradley, A. K., Blakebrough, I. S., Wali, S., & Whittle, H. C. (1984). Meningococcal Disease and Season in Sub-Saharan Africa. *The Lancet*, 323(8390), Article 8390. [https://doi.org/10.1016/S0140-6736\(84\)91830-0](https://doi.org/10.1016/S0140-6736(84)91830-0)
- Harvell, D., Altizer, S., Cattadori, I. M., Harrington, L., & Weil, E. (2009). Climate change and wildlife diseases: When does the host matter the most? *Ecology*, 90(4), Article 4. <https://doi.org/10.1890/08-0616.1>
- Hedlund, C., Blomstedt, Y., & Schumann, B. (2014). Association of climatic factors with infectious diseases in the Arctic and subarctic region – a systematic review. *Global Health Action*, 7(1), Article 1. <https://doi.org/10.3402/gha.v7.24161>
- Hudson, P. J., Rizzoli, A., Grenfell, B. T., Heesterbeek, H., & Dobson, A. P. (2002). *The Ecology of Wildlife Diseases*. Oxford University Press.
- Huey, R. B., Kearney, M. R., Krockenberger, A., Holtum, J. A. M., Jess, M., & Williams, S. E. (2012). Predicting organismal vulnerability to climate warming: Roles of behaviour, physiology and adaptation. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 367(1596), Article 1596. <https://doi.org/10.1098/rstb.2012.0005>
- Huey, R. B., & Kingsolver, J. G. (1989). Evolution of thermal sensitivity of ectotherm performance. *Trends in Ecology & Evolution*, 4(5), Article 5. [https://doi.org/10.1016/0169-5347\(89\)90211-5](https://doi.org/10.1016/0169-5347(89)90211-5)
- Huey, R. B., & Stevenson, R. D. (1979). Integrating Thermal Physiology and Ecology of Ectotherms: A Discussion of Approaches. *American Zoologist*, 19(1), 357–366. <https://doi.org/10.1093/icb/19.1.357>

- Jones, K. E., Patel, N. G., Levy, M. A., Storeygard, A., Balk, D., Gittleman, J. L., & Daszak, P. (2008). Global trends in emerging infectious diseases. *Nature*, *451*(7181), Article 7181. PubMed. <https://doi.org/10.1038/nature06536>
- Karl, T. R., Jones, P. D., Knight, R. W., Kukla, G., Plummer, N., Razuvayev, V., Gallo, K. P., Lindsey, J., Charlson, R. J., & Peterson, T. C. (1993). *Asymmetric Trends of Daily Maximum and Minimum Temperature*. *185*, 19.
- Kawecki, T. J., Lenski, R. E., Ebert, D., Hollis, B., Olivieri, I., & Whitlock, M. C. (2012). Experimental evolution. *Trends in Ecology & Evolution*, *27*(10), Article 10. <https://doi.org/10.1016/j.tree.2012.06.001>
- Khelifa, R., Blanckenhorn, W. U., Roy, J., Rohner, P. T., & Mahdjoub, H. (2019). Usefulness and limitations of thermal performance curves in predicting ectotherm development under climatic variability. *Journal of Animal Ecology*, *88*(12), Article 12. <https://doi.org/10.1111/1365-2656.13077>
- Kilpatrick, A. M., Meola, M. A., Moudy, R. M., & Kramer, L. D. (2008). Temperature, Viral Genetics, and the Transmission of West Nile Virus by *Culex pipiens* Mosquitoes. *PLoS Pathogens*, *4*(6), Article 6. <https://doi.org/10.1371/journal.ppat.1000092>
- Kingsolver, J. G., Diamond, S. E., & Buckley, L. B. (2013). Heat stress and the fitness consequences of climate change for terrestrial ectotherms. *Functional Ecology*, *27*(6), Article 6. <https://doi.org/10.1111/1365-2435.12145>
- Kingsolver, J. G., & Woods, H. A. (2016). Beyond Thermal Performance Curves: Modeling Time-Dependent Effects of Thermal Stress on Ectotherm Growth Rates. *The American Naturalist*, *187*(3), Article 3. <https://doi.org/10.1086/684786>
- Knies, J. L., Izem, R., Supler, K. L., Kingsolver, J. G., & Burch, C. L. (2006). The Genetic Basis of Thermal Reaction Norm Evolution in Lab and Natural Phage Populations. *PLoS Biology*, *4*(7), Article 7. <https://doi.org/10.1371/journal.pbio.0040201>

- Knies, J. L., Kingsolver, J. G., & Burch, C. L. (2009). Hotter Is Better and Broader: Thermal Sensitivity of Fitness in a Population of Bacteriophages. *The American Naturalist*, 173(4), 419–430. <https://doi.org/10.1086/597224>
- Kruger, K. M., & Hero, J. -M. (2007). Large-scale seasonal variation in the prevalence and severity of chytridiomycosis. *Journal of Zoology*, 271(3), 352–359. <https://doi.org/10.1111/j.1469-7998.2006.00220.x>
- Kutz, S. J., Hoberg, E. P., Polley, L., & Jenkins, E. J. (2005). Global warming is changing the dynamics of Arctic host–parasite systems. *Proceedings of the Royal Society B: Biological Sciences*, 272(1581), 2571–2576. <https://doi.org/10.1098/rspb.2005.3285>
- Laine, A.-L. (2008). Temperature-mediated patterns of local adaptation in a natural plant–pathogen metapopulation. *Ecology Letters*, 11(4), Article 4. <https://doi.org/10.1111/j.1461-0248.2007.01146.x>
- Leggett, H. C., Cornwallis, C. K., Buckling, A., & West, S. A. (2017). Growth rate, transmission mode and virulence in human pathogens. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 372(1719), Article 1719. <https://doi.org/10.1098/rstb.2016.0094>
- Leicht, K., Jokela, J., & Seppälä, O. (2013). An experimental heat wave changes immune defense and life history traits in a freshwater snail. *Ecology and Evolution*, 3(15), Article 15. <https://doi.org/10.1002/ece3.874>
- Leicht, K., & Seppälä, O. (2014). Infection success of *Echinoparyphium aconiatum* (Trematoda) in its snail host under high temperature: Role of host resistance. *Parasites & Vectors*, 7(1), Article 1. <https://doi.org/10.1186/1756-3305-7-192>
- Levin, B. (1996). The Evolution and Maintenance of Virulence in Microparasites. *Emerging Infectious Diseases*, 2(2), Article 2. <https://doi.org/10.3201/eid0202.960203>
- Martin, L. B., Hopkins, W. A., Mydlarz, L. D., & Rohr, J. R. (2010). The effects of anthropogenic global changes on immune functions and disease resistance:

- Ecoimmunology and global change. *Annals of the New York Academy of Sciences*, 1195(1), Article 1. <https://doi.org/10.1111/j.1749-6632.2010.05454.x>
- McMichael, A. J. (2015). Extreme weather events and infectious disease outbreaks. *Virulence*, 6(6), Article 6. <https://doi.org/10.4161/21505594.2014.975022>
- Mondal, S., & Rai, U. (2001). In vitro effect of temperature on phagocytic and cytotoxic activities of splenic phagocytes of the wall lizard, *Hemidactylus flaviviridis*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 129(2–3), 391–398. [https://doi.org/10.1016/S1095-6433\(00\)00356-1](https://doi.org/10.1016/S1095-6433(00)00356-1)
- Mordecai, E. A., Paaijmans, K. P., Johnson, L. R., Balzer, C., Ben-Horin, T., de Moor, E., McNally, A., Pawar, S., Ryan, S. J., Smith, T. C., & Lafferty, K. D. (2013). Optimal temperature for malaria transmission is dramatically lower than previously predicted. *Ecology Letters*, 16(1), Article 1. <https://doi.org/10.1111/ele.12015>
- Murdock, C. C., Paaijmans, K. P., Bell, A. S., King, J. G., Hillyer, J. F., Read, A. F., & Thomas, M. B. (2012). Complex effects of temperature on mosquito immune function. *Proceedings of the Royal Society B: Biological Sciences*, 279(1741), Article 1741. <https://doi.org/10.1098/rspb.2012.0638>
- Nguyen, K. H., Gemmell, B. J., & Rohr, J. R. (2020). Effects of temperature and viscosity on miracidial and cercarial movement of *Schistosoma mansoni*: Ramifications for disease transmission. *International Journal for Parasitology*, 50(2), Article 2. <https://doi.org/10.1016/j.ijpara.2019.12.003>
- Nowakowski, A. J., Peadar, J. M., Tuberville, T. D., Buhmann, K. A., & Todd, B. D. (2020). Thermal performance curves based on field movements reveal context-dependence of thermal traits in a desert ectotherm. *Landscape Ecology*, 35(4), Article 4. <https://doi.org/10.1007/s10980-020-00986-x>
- Ogden, N., AbdelMalik, P., & Pulliam, J. (2017). Emerging infectious diseases: Prediction and

- detection. *Canada Communicable Disease Report*, 43(10), Article 10.
<https://doi.org/10.14745/ccdr.v43i10a03>
- Paaijmans, K. P., Blanford, S., Chan, B. H. K., & Thomas, M. B. (2012). Warmer temperatures reduce the vectorial capacity of malaria mosquitoes. *Biology Letters*, 8(3), Article 3.
<https://doi.org/10.1098/rsbl.2011.1075>
- Padfield, D., Castledine, M., & Buckling, A. (2020). Temperature-dependent changes to host–parasite interactions alter the thermal performance of a bacterial host. *The ISME Journal*, 14(2), Article 2. <https://doi.org/10.1038/s41396-019-0526-5>
- Parmesan, C. (2006). Ecological and Evolutionary Responses to Recent Climate Change. *Annual Review of Ecology, Evolution, and Systematics*, 37(1), Article 1.
<https://doi.org/10.1146/annurev.ecolsys.37.091305.110100>
- Patz, J. A. (1996). Global Climate Change and Emerging Infectious Diseases. *JAMA: The Journal of the American Medical Association*, 275(3), Article 3.
<https://doi.org/10.1001/jama.1996.03530270057032>
- Peterson, J. W. (1996). Bacterial Pathogenesis. In S. Baron (Ed.), *Medical Microbiology* (4th ed.). University of Texas Medical Branch at Galveston.
<http://www.ncbi.nlm.nih.gov/books/NBK8526/>
- Raffel, T. R., Rohr, J. R., Kiesecker, J. M., & Hudson, P. J. (2006). Negative effects of changing temperature on amphibian immunity under field conditions. *Functional Ecology*, 20(5), Article 5. <https://doi.org/10.1111/j.1365-2435.2006.01159.x>
- Raffel, T. R., Romansic, J. M., Halstead, N. T., McMahon, T. A., Venesky, M. D., & Rohr, J. R. (2013). Disease and thermal acclimation in a more variable and unpredictable climate. *Nature Climate Change*, 3(2), 146–151. <https://doi.org/10.1038/nclimate1659>
- Reid, C. E., Brauer, M., Johnston, F. H., Jerrett, M., Balmes, J. R., & Elliott, C. T. (2016). Critical Review of Health Impacts of Wildfire Smoke Exposure. *Environmental Health*

- Perspectives*, 124(9), Article 9. <https://doi.org/10.1289/ehp.1409277>
- Rohr, J. R., Civitello, D. J., Cohen, J. M., Roznik, E. A., Sinervo, B., & Dell, A. I. (2018). The complex drivers of thermal acclimation and breadth in ectotherms. *Ecology Letters*, 21(9), Article 9. <https://doi.org/10.1111/ele.13107>
- Rohr, J. R., Dobson, A. P., Johnson, P. T. J., Kilpatrick, A. M., Paull, S. H., Raffel, T. R., Ruiz-Moreno, D., & Thomas, M. B. (2011). Frontiers in climate change–disease research. *Trends in Ecology & Evolution*, 26(6), Article 6. <https://doi.org/10.1016/j.tree.2011.03.002>
- Roth, O., Kurtz, J., & Reusch, T. B. H. (2010). A summer heat wave decreases the immunocompetence of the mesograzer, *Idotea baltica*. *Marine Biology*, 157(7), Article 7. <https://doi.org/10.1007/s00227-010-1433-5>
- Schulte, P. M., Healy, T. M., & Fangué, N. A. (2011). Thermal Performance Curves, Phenotypic Plasticity, and the Time Scales of Temperature Exposure. *Integrative and Comparative Biology*, 51(5), Article 5. <https://doi.org/10.1093/icb/icr097>
- Seppälä, O., & Jokela, J. (2011). Immune defence under extreme ambient temperature. *Biology Letters*, 7(1), Article 1. <https://doi.org/10.1098/rsbl.2010.0459>
- Shapiro, L. L. M., Whitehead, S. A., & Thomas, M. B. (2017). Quantifying the effects of temperature on mosquito and parasite traits that determine the transmission potential of human malaria. *PLOS Biology*, 15(10), Article 10. <https://doi.org/10.1371/journal.pbio.2003489>
- Shapiro-Ilan, D. I., Fuxa, J. R., Lacey, L. A., Onstad, D. W., & Kaya, H. K. (2005). Definitions of pathogenicity and virulence in invertebrate pathology. *Journal of Invertebrate Pathology*, 88(1), 1–7. <https://doi.org/10.1016/j.jip.2004.10.003>
- Shephard, R. J., & Shek, P. N. (1998). Cold exposure and immune function. *Canadian Journal of Physiology and Pharmacology*, 76(9), 828–836. <https://doi.org/10.1139/y98-097>

- Shu, J., Stevenson, J. R., & Zhou, X. (1993). Modulation of cellular immune responses by cold water swim stress in the rat. *Developmental & Comparative Immunology*, *17*(4), 357–371. [https://doi.org/10.1016/0145-305X\(93\)90007-D](https://doi.org/10.1016/0145-305X(93)90007-D)
- Sinclair, B. J., Marshall, K. E., Sewell, M. A., Levesque, D. L., Willett, C. S., Slotsbo, S., Dong, Y., Harley, C. D. G., Marshall, D. J., Helmuth, B. S., & Huey, R. B. (2016). Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? *Ecology Letters*, *19*(11), Article 11. <https://doi.org/10.1111/ele.12686>
- Smolinski, M. S., Hamburg, M. A., & Lederberg, J. (2003). *Microbial Threats to Health: Emergence, Detection, and Response* (p. 19-22). National Academies Press. <https://doi.org/10.17226/10636>
- Sonn, J. M., Utz, R. M., & Richards-Zawacki, C. L. (2019). Effects of latitudinal, seasonal, and daily temperature variations on chytrid fungal infections in a North American frog. *Ecosphere*, *10*(11). <https://doi.org/10.1002/ecs2.2892>
- Thomas, S. R., & Elkinton, J. S. (2004). Pathogenicity and virulence. *Journal of Invertebrate Pathology*, *85*(3), Article 3. <https://doi.org/10.1016/j.jip.2004.01.006>
- Tourneur, J., & Meunier, J. (2020). Variations in seasonal (not mean) temperatures drive rapid adaptations to novel environments at a continent scale. *Ecology*, *101*(4), Article 4. <https://doi.org/10.1002/ecy.2973>
- Vasseur, D. A., DeLong, J. P., Gilbert, B., Greig, H. S., Harley, C. D. G., McCann, K. S., Savage, V., Tunney, T. D., & O'Connor, M. I. (2014). Increased temperature variation poses a greater risk to species than climate warming. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1779), 20132612. <https://doi.org/10.1098/rspb.2013.2612>
- Voyles, J., Johnson, L. R., Briggs, C. J., Cashins, S. D., Alford, R. A., Berger, L., Skerratt, L. F., Speare, R., & Rosenblum, E. B. (2014). Experimental evolution alters the rate and temporal pattern of population growth in *Batrachochytrium dendrobatidis*, a lethal fungal

- pathogen of amphibians. *Ecology and Evolution*, 4(18), 3633–3641.
<https://doi.org/10.1002/ece3.1199>
- Wenzel, R. P. (1994). A New Hantavirus Infection in North America. *New England Journal of Medicine*, 330(14), Article 14. <https://doi.org/10.1056/NEJM199404073301410>
- WHO. (2018, June 1). *Heat and Health*. <https://www.who.int/news-room/fact-sheets/detail/climate-change-heat-and-health>
- Won, S. J., & Lin, M. T. (1995). Thermal stresses reduce natural killer cell cytotoxicity. *Journal of Applied Physiology*, 79(3), 732–737. <https://doi.org/10.1152/jappl.1995.79.3.732>
- Woo, H. J., & Reifman, J. (2014). Quantitative Modeling of Virus Evolutionary Dynamics and Adaptation in Serial Passages Using Empirically Inferred Fitness Landscapes. *Journal of Virology*, 88(2), Article 2. <https://doi.org/10.1128/JVI.02958-13>
- Woodhams, D. C., Alford, R. A., Briggs, C. J., Johnson, M., & Rollins-Smith, L. A. (2008). Life-history trade-offs influence disease in changing climates: Strategies of an amphibian pathogen. *Ecology*, 89(6), Article 6. <https://doi.org/10.1890/06-1842.1>
- Wright, R. K., & Cooper, E. L. (1981). Temperature effects on ectotherm immune responses. *Developmental & Comparative Immunology*, 5, 117–122. [https://doi.org/10.1016/0145-305X\(81\)90016-1](https://doi.org/10.1016/0145-305X(81)90016-1)
- Wu, X., Tian, H., Zhou, S., Chen, L., & Xu, B. (2014). Impact of global change on transmission of human infectious diseases. *Science China Earth Sciences*, 57(2), Article 2.
<https://doi.org/10.1007/s11430-013-4635-0>
- Zbinden, M., Haag, C. R., & Ebert, D. (2008). Experimental evolution of field populations of *Daphnia magna* in response to parasite treatment. *Journal of Evolutionary Biology*, 21(4), Article 4. <https://doi.org/10.1111/j.1420-9101.2008.01541.x>

Chapter 2: Thermal performance curves of multiple isolates of *Batrachochytrium dendrobatidis*, a lethal pathogen of amphibians

Abstract

Emerging infectious disease is a key factor in the loss of amphibian diversity. In particular, the disease chytridiomycosis has caused severe declines around the world. The lethal fungal pathogen that causes chytridiomycosis, *Batrachochytrium dendrobatidis* (*Bd*), has affected amphibians in many different environments. One primary question for researchers grappling with disease-induced losses of amphibian biodiversity is what abiotic factors drive *Bd* pathogenicity in different environments. To study environmental influences on *Bd* pathogenicity, I quantified responses of *Bd* phenotypic traits (e.g., viability, zoospore densities, growth rates, and carrying capacities) over a range of environmental temperatures to generate thermal performance curves. I selected multiple *Bd* isolates that belong to a single genetic lineage but that were collected across a latitudinal gradient, which served as a proxy for environmental temperature or climate differences. For the population viability, I found that the isolates had similar thermal optima at 21°C, but there was considerable variation among the isolates in maximum viability at that temperature. Additionally, I found the densities of infectious zoospores varied among isolates across all temperatures. Our results suggest that temperatures across a geographic thermal cline may explain some of the variation in *Bd* viability through vertical changes in maximal performance. However, the same pattern was not evident for other reproductive parameters (zoospore densities, growth rates, fecundity), underscoring the importance of measuring multiple traits to understand variation in pathogen responses to environmental conditions. I suggest that variation among *Bd* isolates that consist of two genetic variants may be due to environmental factors may be an important determinant of disease dynamics for amphibians across a range of diverse environments.

Introduction

Emerging infectious diseases are a primary driver of global amphibian declines (1). Disease outbreaks from ranaviruses, chytrid fungi, and bacterial pathogens have contributed to an unprecedented loss of global amphibian diversity (2–4). Therefore, understanding what factors influence the emergence, spread, pathogenicity, and ecology of these pathogens is important for amphibian conservation (5). Many of these pathogens are strongly influenced by their local environments, and corresponding changes in pathogen phenotypic traits (e.g., reproductive rates, pathogen persistence in the environment) can alter disease risks for susceptible amphibian host species (6–8). By investigating how a pathogen responds to its environment, as well as the genotypic and phenotypic variation that underpins those responses, I can begin to unravel the disease dynamics that threaten amphibians (9, 10).

Chytridiomycosis is one such infectious disease that is lethal to many amphibian species and has caused global declines in susceptible species (1, 11). The disease is caused by the fungal pathogens, *Batrachochytrium dendrobatidis* (*Bd*) (12) and *Batrachochytrium salamandrivorans* (*Bsal*) (13). However, *Bd* has spread globally and impacted far more amphibian host species than *Bsal*, making it a priority pathogen for study (1). Since its discovery in 1999, *Bd* has spread rapidly through multiple naïve amphibian communities, causing mass mortality events, and even the complete extinction of amphibian species (11). No other pathogen is known to have had such a ubiquitous effect on such a broad range of host species and in so many different environments (1, 14, 15). As a result, *Bd*-related declines have been called, “the most spectacular loss of biodiversity due to disease in recorded history” (11).

Bd has a two-stage life cycle that consists of a substrate-dependent immobile sporangium and a free-living unflagellated, motile zoospore (12, 16). Infection occurs during the motile zoospore stage of the pathogen's life cycle (12, 16). 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 (12, 17, 18). Zoosporangia produce motile zoospores and then release the new zoospores into the environment to re-infect the same host or transmit to another individual host (18). Once infection is established within a host, increases in infection intensity (or pathogen load) in amphibian skin is a key feature of pathogenesis (19, 20). As such, understanding the factors that regulate *Bd* growth and reproductive rates is integral to resolving questions concerning pathogenesis and the disease ecology of this lethal disease system (21, 22).

Recent phylogenetic analyses indicate that there are several major lineages of *Bd* that are genetically distinct (23–25). One lineage that has garnered considerable attention from the scientific community, due to its high lethality, is the Global Panzootic Lineage (*Bd*GPL) (23, 24, 26). Genomic sequencing of many *Bd* isolates within this lineage has shown that it contains substantial genetic diversity, including two genetic clades (*Bd*GPL1 and *Bd*GPL2) (26–28). These *Bd* isolates are defined as a pure culture of *Bd* that has been isolated from an infected host via microbiology techniques. With a global distribution, including many regions throughout the United States, *Bd*GPL occurs in a wide range of amphibian habitats and causes disease in diverse microclimates and thermal environments (2, 29). As such, researchers have focused on resolving the factors that determine variation among *Bd*GPL isolates to understand how temperature may mediate disease dynamics (21, 22, 30, 31). To date, while there have been many studies that document variation in the thermal biology among isolates from within the *Bd*GPL clade, no clear patterns have emerged that can explain the extent of variation among and within *Bd*GPL isolates across diverse thermal environments (30, 31). This outstanding question may be most appropriately investigated by generating thermal performance curves, which would allow for additional comparative investigations within the *Bd*GPL lineage.

Thermal performance curves (TPCs) are widely used to measure an organism's performance across a range of temperatures, estimate the thermal sensitivity of different traits,

and facilitate an understanding of ecological and evolutionary processes that may explain an organism's success within a given environment (32–34). TPCs include measures of thermal optimum [i.e., temperature optimum (T_{opt})], critical thermal minimum (CT_{min}), critical thermal maximum (CT_{max}), and thermal tolerance range (also known as thermal breadth; T_{br}) (Fig. 2-1). Temperature sensitive parameters that determine an organism's TPC frequently vary with geographic clines (e.g., latitude, Fig. 2-1), reflecting local adaptation (34, 35). Latitude can be used as a proxy for environmental temperatures as there are differences in the mean annual air temperatures across latitudes, from the Northern latitudes to the Southern latitudes in the U.S. (5; Fig. 2-2). TPC models (e.g., vertical or horizontal changes) offer a framework to consider the adaptive potential for temperature-sensitive organisms (36, 37). For example, horizontal changes toward a higher T_{opt} would provide evidence in support of the “hotter is better” hypothesis, which predicts that organisms will adapt to thermal conditions according to thermodynamic constraints (e.g., with higher T_{opt} in latitudes where mean temperatures are higher) (38–40) (Fig. 2-2).

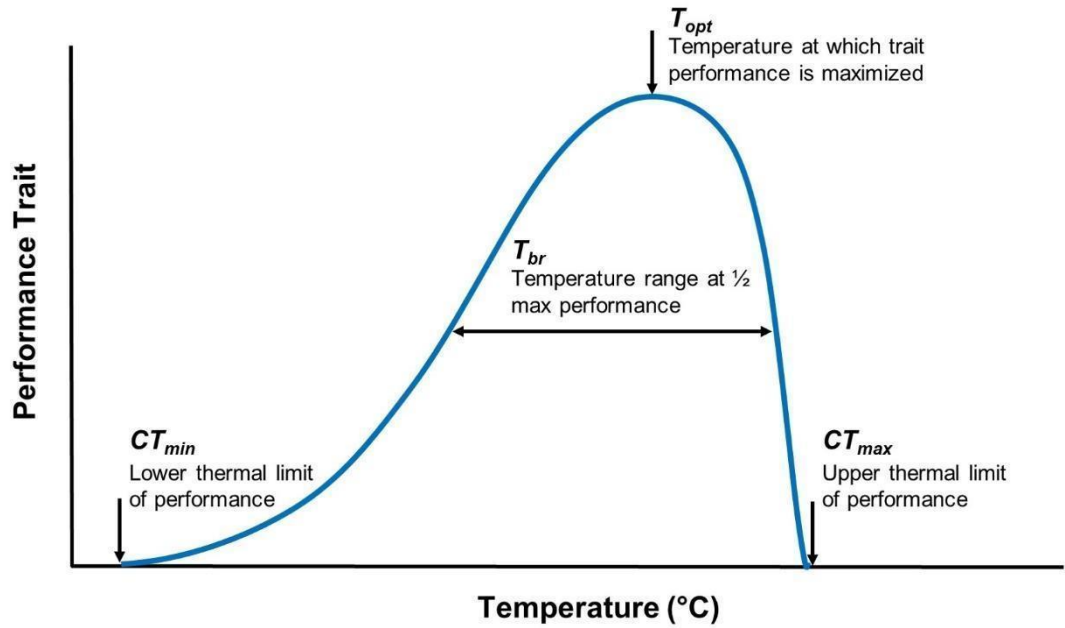


Figure 2-1. Traditional thermal performance curve parameters for a given trait. When a performance curve is generated, the performance of a trait is plotted against a temperature range. The thermal breadth (T_{br}), also referred to as the thermal tolerance range, is the temperature range at which a level of performance is achieved. A thermal optimum (T_{opt}) is the temperature at which trait performance is maximized. The critical thermal minimum (CT_{min}) and maximum (CT_{max}) are the lower and upper thermal limits of a trait's performance, respectively.

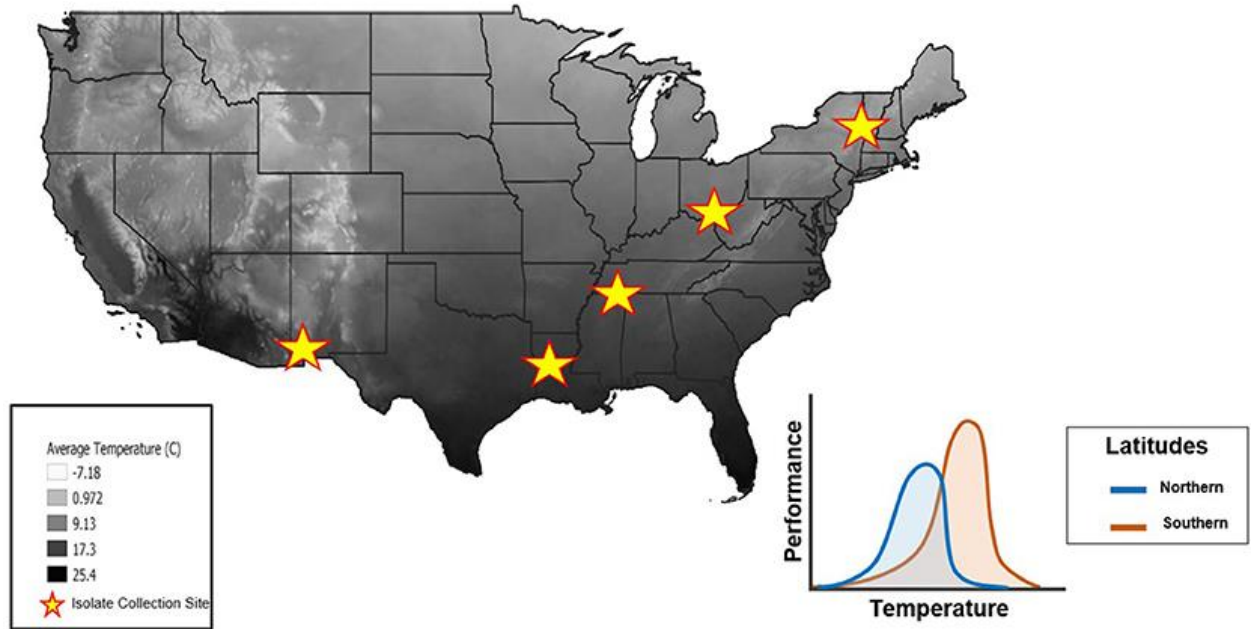


Figure 2-2. Mean annual air temperatures throughout the United States (highest temperatures in black, lowest temperatures in white). Stars represent the locations where isolates were collected from across a latitudinal gradient, which serves as a proxy for a geographic thermal cline. The thermal constraint hypothesis (“hotter is better”) predicts that isolates from Northern latitudes will have lower maximal performance at a lower T_{opt} (blue curve) whereas isolates from Southern latitudes will have a higher maximal performance at a higher T_{opt} due to adaptations from local temperature regimes (orange curve). Temperature data from the National Forest Climate Change Maps website to generate this figure in QGIS software (41).

It is generally thought that *Bd* has a thermal tolerance range of 2–28 °C (42), with a T_{opt} of 17–25 °C (8, 43), and CT_{min} , and CT_{max} of 2–5 and 25–28 °C, respectively (42, 44). Mounting evidence suggests that *Bd* isolates differ in their thermal optima (8, 42, 44), but experimental approaches have not yet explored this idea by comparing isolates collected across a thermal cline (45). I predicted that the TPCs of *Bd* isolates collected along a latitudinal gradient or thermal cline would differ due to thermal constraints in each region. More specifically, I expected that isolates from northern latitudes (meaning lower mean annual air temperatures) would have a

lower T_{opt} and exhibit a lower maximum performance at that temperature. In contrast, I expected isolates from southern latitudes (meaning higher mean annual air temperatures) to have a higher T_{opt} and higher performance at that temperature (Fig. 2-2). To test these predictions, I generated TPCs for five *Bd* isolates collected across a latitudinal gradient. The sites that these isolates originate from were chosen to represent a latitudinal gradient and to take advantage of habitats available on or near DOD installations as funding for the field work of related projects came from the Department of Defense.

Materials and Methods

Bd Isolate Collection and Maintenance

I used five different *Bd* isolates that fall within two genotypes and originated from amphibians in the United States (Table 2-1). The collection of *Bd* isolates from the United States provides an ideal repertoire for investigating phenotypic variation and differences in TPCs for multiple reasons. First, previous work using a microfluidic PCR genotyping method (one that targets ~200 loci) suggested that the *Bd*GPL I have represented in the data is the primary lineage found in North America. Second, the collection of *Bd* isolates that originate from amphibians in the United States is large, with numerous isolates from across the country, spanning a latitudinal gradient (25). Only one isolate was available from each origin site of this study due to the challenges of isolating *Bd in vitro* and cryopreservation (65).

Table 2-1. Genotyping for isolates of *Batrachochytrium dendrobatidis*, amphibian host, and geographic origins

Genotype	Isolate	Location	Host Species Name	Latitude
GPL1	Louisiana (LA)	New Orleans, LA	<i>Acris crepitans</i>	29.9511° N
GPL1	Tennessee (TN)	Memphis, TN	<i>Lithobates sphenoccephala</i>	35.1495° N
GPL1	Vermont (VT)	VT	<i>Lithobates clamitans</i>	44.5588° N
GPL2	New Mexico (NM)	NM	<i>Lithobates catesbeianus</i>	34.5199° N
GPL2	Ohio (OH)	Toledo, OH	<i>Lithobates pipiens</i>	41.6528° N

All isolates were cryoarchived and subsequently revived according to standard protocols (46) prior to the beginning of the experiment. Following isolate revival, I cultured the *Bd* isolates in tryptone/gelatin hydrolysate/lactose (TGhL) liquid growth media in 75 cm² tissue culture flasks (47). I incubated each isolate at 21°C and monitored them through the *Bd* life cycle until the point of peak zoospore densities (42). Once each culture flask reached peak zoospore density, 2 mL of culture was transferred to a new culture flask containing 13 mL of fresh TGhL media for standard passage. I used a biosafety cabinet for all laboratory work involving these isolates (e.g., passaging, experimental setup).

Generating Thermal Performance Curves

I filtered each of the five cultures using sterile filter paper (Whatman Qualitative Filter Papers, Grade 3) and used a vacuum filtration pump to remove zoosporangia (47). With the remaining filtrate, I quantified zoospores using a hemocytometer and diluted each culture with TGhL to a concentration of $\sim 50 \times 10^4$ zoospores/mL (48). I inoculated the cultures of each isolate containing only zoospores into 96-well-plates. I then added 50 μ L of additional TGhL media to each well. I included five negative control wells with 50 μ L of 50×10^4 zoospores/mL heat-killed zoospores and 50 μ L of TGhL media for each isolate (48). I filled the perimeter wells of the plate with 150 μ L TGhL media to provide a buffer against culture evaporation (45).

To establish a thermal profile for each respective isolate, I incubated all isolates at multiple stable temperatures (4, 12, 17, 21, 25, 26, and 27 °C). Because I used only zoospores to

start the growth experiments, I were able to track and quantify several parts of the *Bd* life cycle as they occurred at different time points in these different temperature conditions. Specifically, by tracking cultures for multiple successive days, I were able to measure the change in population growth, time to maximum zoospore densities, zoospore densities, and calculate fecundity (49). At multiple time points following experimental set up (Day 0), I randomly selected five wells ($N = 5$) for each of two destructive measures: zoospore counts and viability assays (49).

To quantify zoospore densities, I manually withdrew 20 μL of culture and counted live zoospores using a hemocytometer (48). Following these counts, I omitted those wells for the remainder of the experiment (48). To measure population growth, I conducted a standard viability assay, which measures the amount of live cells in a culture or sample (45). The MTT viability assay is a standard microbiological technique where a yellow tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) is reduced to purple MTT-formazan crystals in metabolically active and live cells (50). These crystals can be solubilized, and the color change can be quantified by reading culture absorbance at 570 nm (45). I added 20 μL of MTT to each experimental and negative control wells of the plate selected for that day and incubated the plate at 21°C for 2 h (45). After incubation, I added 140 μL of the stop-reagent to stop the reaction and solubilize the MTT-formazan crystals (45). To interpret viability, I then read culture absorbance at 570 nm using a Biotek EL x 800 Absorbance Reader.

Bd Isolate Genotyping

I genotyped the isolates using an amplicon sequencing approach according to published protocols (51). Briefly, I extracted DNA following the manufacturer's protocol for the Qiagen DNeasy Blood and Tissue kit. Next, to prepare raw DNA extracts for sequencing, I cleaned each using an isopropanol precipitation and preamplified each in two separate PCR reactions, each containing 96 primer pairs. Primers were designed to target 150–200 base pair regions of the *Bd* nuclear and mitochondrial genome (51). After preamplification, samples were cleaned using

EXOSap-it™ (ThermoFisher Scientific) and diluted 1:5 in water. Finally, I cleaned and diluted products from the two preamplification reactions, combined in equal proportions, and sent to the University of Idaho IBEST Genomics Resources Core, where they were loaded into a Fluidigm June LP 192.24 IFC (Fluidigm Inc.) for amplification and barcoding. Amplified products were pooled and sequenced on an Illumina MiSeq.

Raw sequences were processed as previously described (25, 51). Raw reads were joined via FLASH [(52); v.1.2.11] and consensus sequences for each sample/amplicon combination were called using the reduce amplicons R script (https://github.com/msettles/dbcAmplicons/blob/master/scripts/R/reduce_amplicons.R). Here, consensus sequences use IUPAC ambiguity codes to indicate multiple alleles at a locus. I compared the consensus sequences of each of our five isolates to 21 previously published *Bd* samples using a phylogenetic approach. I selected previously published reference sequences to represent every known major *Bd* lineage (25). To create a phylogeny, I used a gene tree to species tree approach: first aligning all sequences for each amplicon using MUSCLE [(53); v.3.32], then creating a tree for each amplicon using RAxML [(54); v.8.2.11] to search for the best scoring ML tree from 100 bootstrap replicates. Afterwards, I used newick utils [(55); v.1.6] to collapse all nodes in each amplicon tree with <10 bootstrap support. I then input a total of 190 amplicon trees with collapsed branches into Astral-III [(56); v.5.5.9], which estimates an unrooted species tree given a set of unrooted gene trees using the multispecies coalescent model.

Statistical Analysis

For all statistical analyses, I used R version 3.4.3 (57). I used QGIS software and the “ggplot2” package within R to generate figures. Summary statistics reported in the figures and the tables include means \pm standard error (SE) of the viability, zoospore densities, or fecundity measure among isolates or between genotypes. I analyzed the performance of each isolate when grouped by genetic variant and independently to compare differences among isolates at T_{opt} ,

CT_{min} , and CT_{max} temperature treatments. I used Analysis of Variance (ANOVA) and Games Howell *post-hoc* tests to make comparisons in mean maximum viability (OD following the MTT assay), mean maximum zoospore densities, mean fecundity, and time to maximum zoospore densities. I used a non-parametric *post-hoc* test when there was a violation of the homogenous variance assumption for each of the traits compared among isolates. To calculate mean maximum viability and maximum zoospore densities, I used measures from within the 2–6day period at which cultures exhibited maximum viability or zoospore densities in each temperature condition.

To make comparisons of fecundity, I calculated the ratio of zoospores densities to mean culture viability. Within the fecundity calculations, all viability measurements that were <0.005 were considered zeros to ensure that fecundity ratios were not artificially inflated. For statistical analyses, I log-transformed the fecundity metric and added a correction factor of 1 to accommodate for the wells that had zero zoospores. For comparing genetic variants in viability, zoospore densities, fecundity, and time to maximum zoospore densities, I used Welch's *t*-test because I had unequal variance after grouping by genotype. I used a Bonferroni correction after running the *t*-test at each temperature experiment for comparisons of MTT across the thermal range to reduce the likelihood of a type-1 error.

To further quantify the differences across temperatures, I fit a logistic growth curve to the normalized optical density measurements (i.e., *Bd* viability) data time series for each isolate-temperature combination. This approach allowed us to estimate the intrinsic growth rate (r) and carrying capacity (K). I used the resulting estimates for r and K to quantify differences among isolates over the range of temperatures considered. To calculate 95% confidence intervals for these estimates, I used likelihood profile-based methods (58, 59). I attempted to constrain r estimates to follow a Johnson–Lewin (J–L) curve as a function of temperature to characterize the thermal breadth of each isolate (59). See the [Supplementary Material](#) for details.

Results

Differential Responses to Temperature Between Genetic Variants

The genetic sequencing revealed that the isolates in this study belong to the *Bd*GPL clades 1 or 2 (Table 2-1). These two clades are referred to as *Bd*GPL1 and *Bd*GPL2 (25). When I grouped the isolates by genetic lineage, I found no differences in viability between *Bd*GPL1 and *Bd*GPL2 lineages at the T_{opt} , 21°C [$t_{(57.51)} = 0.91$, $p = 0.37$; Fig. 2-3A]. Furthermore, there were no significant differences in viability between *Bd*GPL1 and *Bd*GPL2, except at the low temperature of 4 °C and the high temperature at 27 °C (Fig. 2-3A, Table 2-2).

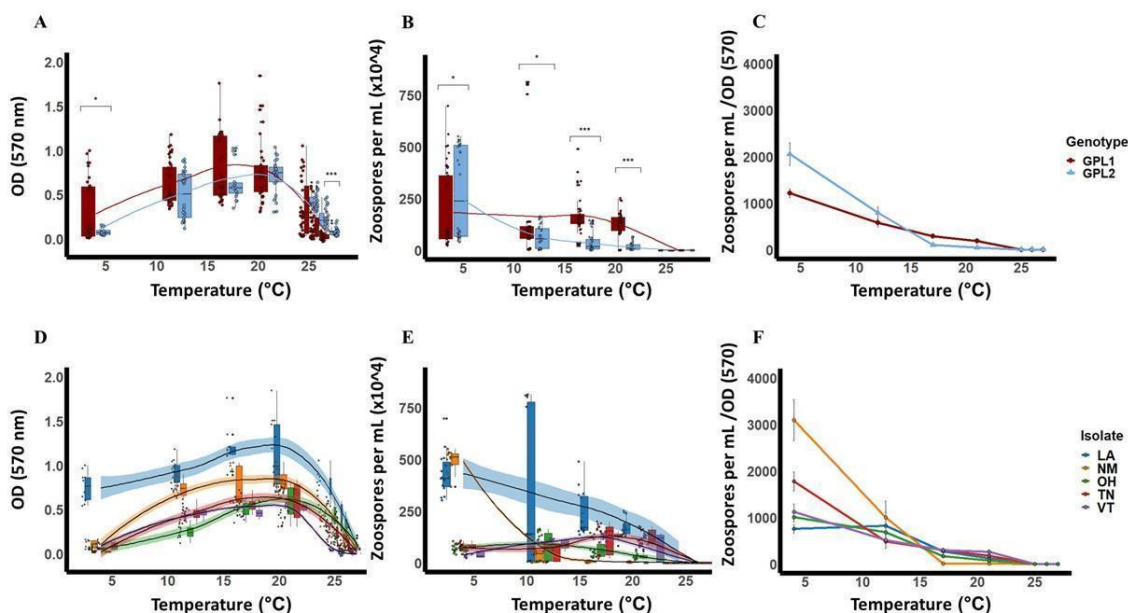


Figure 2-3. Thermal performance curves for isolates of *Batrachochytrium dendrobatidis* (*Bd*). Five isolates were collected from across a geographic thermal cline in the United States, genotyped, and tested for these responses across the known thermal range for *Bd* (4–27 °C). Data show means (\pm SE) for *Bd* viability (A), zoospore densities (B), and fecundity (calculated as optical density/zoospore densities) (C), for two *Bd* genotypes, Global Panzootic Lineage 1 and Global Panzootic Lineage 2 (*Bd*GPL1 and *Bd*GPL2; top row, red and blue). Additionally, data show means (\pm SE) for *Bd* viability (D), zoospore densities (E), and fecundity (F), for each of the five isolates that were collected in Louisiana (LA, blue), New Mexico (NM,

orange), Ohio (OH, green), Tennessee (TN, red), and Vermont (VT, purple). Significance levels are indicated by the asterisks as such: * < 0.05, ** < 0.01, *** < 0.001.

Table 2-2. Population growth viability (MTT) across temperatures (means, SE, r, and K).

Isolate ID	4°C			12°C			17°C			21°C			25°C			26°C			27°C		
	Mean (±SE)	r	K	Mean (±SE)	r	K	Mean (±SE)	r	K	Mean (±SE)	r	K	Mean (±SE)	r	K	Mean (±SE)	r	K	Mean (±SE)	r	K
GPL1 LA	0.75 ± 0.053	0.2591	0.6246	0.91 ± 0.036	0.5867	0.8337	1.19 ± 0.050	0.8546	1.275	1.12 ± 0.104	1.504	1.048	0.78 ± 0.046	0.9155	0.9878	0.40 ± 0.049	0.482	107300	0.02 ± 0.007	0.4817	0.0462
VT	0.09 ± 0.013	0.1644	2.022	0.46 ± 0.016	0.2312	0.4757	0.47 ± 0.021	0.4321	0.6248	0.53 ± 0.011	0.3991	0.602	0.04 ± 0.003	0.9506	0.0716	0.02 ± 0.004	5.431	0.0035	0.00 ± 0.001	1.433	5.725
TN	0.02 ± 0.002	0.07248	3.661	0.45 ± 0.019	0.8495	0.4357	0.57 ± 0.027	0.7361	0.6317	0.63 ± 0.052	0.8277	0.6702	0.23 ± 0.019	1.017	0.3259	0.14 ± 0.018	1.314	0.1533	0.06 ± 0.005	1.874	0.0533
GPL2 NM	0.10 ± 0.015	0.1939	0.1047	0.74 ± 0.023	0.6297	0.5608	0.76 ± 0.076	0.8781	0.8241	0.82 ± 0.029	0.806	0.882	0.40 ± 0.018	1.324	0.4455	0.21 ± 0.021	1.187	0.2709	0.07 ± 0.007	1.083	0.075
OH	0.06 ± 0.005	0.1305	0.0996	0.25 ± 0.019	0.3071	0.2313	0.52 ± 0.029	0.9106	0.5445	0.60 ± 0.046	0.8264	0.6487	0.44 ± 0.036	1.498	0.3906	0.21 ± 0.032	1.451	0.2994	0.10 ± 0.012	0.9965	0.1123

I also measured zoospore densities for the two genetic lineages in all temperatures because the capacity to generate high zoospore densities is thought to be a critical factor for disease development (21). I found patterns in our measures of zoospore densities that differed from those in our viability assays (Fig. 2-3B). There were significant differences between *BdGPL1* and *BdGPL2* in zoospore densities at every temperature where zoospores were produced (Fig. 2-3B, Table 2-3). Specifically, *BdGPL1* had higher zoospore densities than *BdGPL2* at all temperatures except 4 °C (Table 2-3). I found that fecundity was significantly different between *BdGPL1* and *BdGPL2* at three temperatures: 4 °C [$t(182.81) = -3.2, p = 0.002$], 17 °C [$t(162.63) = 6.039, p \leq 0.001$], and 21 °C [$t(131.85) = 6.9127, p \leq 0.001$] (Fig. 2-3C). There were no significant differences between *BdGPL1* and *BdGPL2* in the time to maximum zoospore densities at any temperature except 21 °C [$t(22.29) = -2.7584, p = 0.01$].

Table 2-3. Zoospore density descriptive difference of means using t-tests between genotypes.

	4 °C			12 °C			17 °C			21 °C			25 °C			26 °C			27 °C			
	t	df	p	t	df	p	t	df	p	t	df	p	t	df	p	t	df	p	t	df	p	
Genotype	-2.0148	56	0.049	2.4708	51	0.017	8.035	67.4	<0.001	11.56	54.3	<0.001	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Differential Responses to Temperature Among the *Bd* Isolates

All isolates exhibited maximum viability at 21°C. However, there were differences among isolates in their mean viability at the T_{opt} of 21°C [ANOVA, $F_{(4,63)} = 15.94, P < 0.001$, [Table 2-2](#), [Fig. 2-3D](#)]. The isolate from Louisiana exhibited the greatest mean viability in the T_{opt} , 21°C ([Table 2-2](#), [Fig. 2-3D](#)), as well as at every other temperature treatment except 27 °C ([Table 2-2](#), [Fig. 2-3D](#)). The isolate from Vermont exhibited the lowest viability except in the low temperature treatments of 4 and 12 °C ([Table 2-2](#), [Fig. 2-3D](#)).

I found that there were differences among the *Bd* isolates in their viability in both low and high temperature conditions ([Table 2-2](#), [Fig. 2-3D](#)). For the lowest temperature treatment, all

isolates exhibited minimal growth at 4 °C but there were differences among the isolates in viability at that temperature [Table 2-2; ANOVA, $F_{(4,45)} = 146.6$, $P < 0.001$]. The isolates also differed in their responses to high temperature treatments (Table 2-2, Fig. 2-3D). The isolate from Ohio exhibited significantly greater viability at the highest temperature treatment of 27 °C [ANOVA, $F_{(4,70)} = 25.82$, $P < 0.001$; Game's Howell, $P < 0.01$], whereas the isolate from Vermont had low viability at 26 °C and was not viable at 27 °C (Fig. 2-3D).

The patterns found in zoospore densities among isolates also differed from viability results (Fig. 2-3E). Specifically, two of the isolates produced their maximum zoospore densities at the low temperatures of 4 and 12 °C (Fig. 2-3E, Table 2-4). Notably, for the New Mexico isolate, zoospore densities were highest at 4 °C and were dramatically lower at all other temperatures (Table 2-4, Fig. 2-3E). Accordingly, the New Mexico isolate exhibited the highest fecundity (zoospores per viability measure) at 4 °C (Fig. 2-3F). All isolates exhibited a similar pattern, with higher fecundity in lower temperatures, but it was most pronounced in the New Mexico isolate at 4 °C. In addition, I found that the time to maximum zoospore densities differed among isolates at 4 °C [ANOVA, $F_{(4,70)} = 250.9$, $P < 0.001$] and 21°C [ANOVA, $F_{(4,70)} = 48.36$, $P < 0.001$]. I also found that, although the cultures were viable and growth measurements increased at the higher temperatures of 25, 26, and 27 °C, none of the isolates produced zoospores at these high temperatures (Fig. 2-3E).

Table 2-4. Zoospore densities across temperatures (means, SE, r, and K).

Isolate ID		4 °C	12 °C	17 °C	21 °C	25 °C	26 °C	27 °C
		Mean (±SE)	Mean (±SE)	Mean (±SE)	Mean (±SE)	Mean (±SE)	Mean (±SE)	Mean (±SE)
GPL1	LA	431.9 ± 27.141	314.7 ± 92.641	235.7 ± 27.795	172.8 ± 10.806	NA	0	0
	VT	38.9 ± 3.998	89.1 ± 5.996	127.3 ± 4.525	84.6 ± 16.238	NA	0	0
	TN	78.4 ± 4.296	56.1 ± 10.333	119.7 ± 17.980	120.6 ± 9.395	NA	0	0
GPL2	NM	492.1 ± 13.133	37.4 ± 6.995	4.5 ± 0.885	2.8 ± 0.433	NA	0	0
	OH	70.3 ± 4.853	89.2 ± 16.559	65.7 ± 11.233	30.7 ± 3.596	NA	0	0

I then assessed how these r and K estimates varied with temperature for each isolate. The overall trend for all isolates is r estimates that increase and then plateau for temperatures up to 21 °C (Fig. 2-4A). However, for higher temperatures (25–27 °C), the r estimates are larger and more variable both across isolates and in terms of having larger confidence intervals. The intrinsic growth rates at the higher temperatures, however, do not yield much long-term growth. The corresponding K estimates also increase and plateau at ~21°C, but then markedly decline at the higher temperatures (Fig. 2-4B). The combined effect is a short-lived exponential growth phase that quickly reaches a relatively low upper bound at these high temperatures (Supplementary Materials).

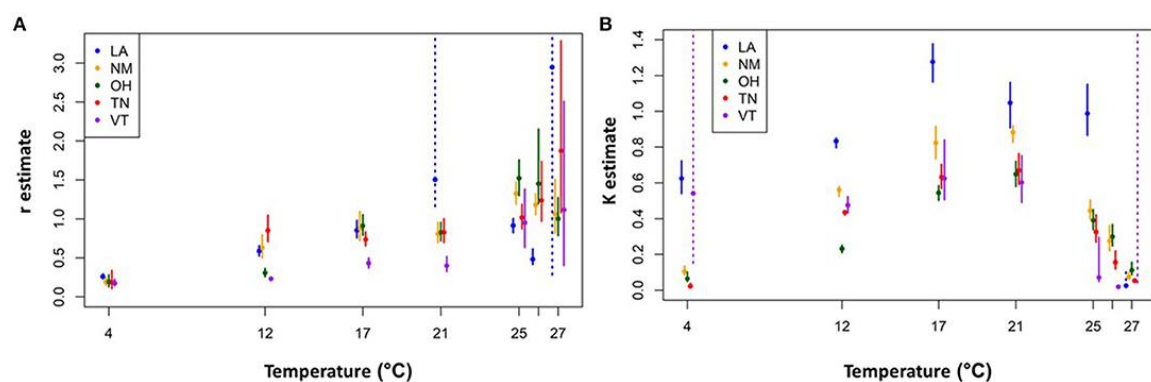


Figure 2-4. Logistic growth curve parameter estimates for each isolate and temperature combination.

Intrinsic exponential growth rate (r) estimates (A) carrying capacity (K) estimates (B). Solid circles indicate the best fit parameter values and vertical bars show the 95% confidence intervals. The dashed vertical bars indicate situations in which the confidence interval for the parameter of interest had a poorly resolved upper end point due to parameter identifiability issues (e.g., for time series data in the exponential growth phase, all values of K above a certain threshold will give equally good fits). Not shown, for clarity: VT-26 °C r estimate is 8.58, CI = (4.61, 14.12). See [Supplementary Material](#) for further details.

Discussion

Chytridiomycosis is a disease that has impacted amphibians in a wide range of environmental conditions (21, 60). Past studies have attempted to link *Bd* phenotypic patterns with environmental factors in order to understand how abiotic factors might mitigate or exacerbate disease (5, 30, 43, 61). For example, both Becker et al. (30) and Greener et al. (21) documented considerable phenotypic variation for isolates within the *Bd*GPL that was associated with differential pathogenicity in common susceptible host species (*Lithobates sylvaticus* and *Alytes obstetricans*, respectively). In addition, Lambertini et al. (22) and Muletz-Wolz et al. (31) demonstrated phenotypic variation in morphological characteristics (e.g., zoosporangia size) in multiple isolates from within the *Bd*GPL lineage. However, to date, studies that have tried to link pathogen traits to environmental predictors have not been able to account for the extent of phenotypic variation among *Bd* isolates across different thermal environments [e.g., *Bd* growth has not been linked to any environmental parameters, such as mean annual temperature, mean annual precipitation, elevation, etc., (22, 31)].

I predicted that quantifying *Bd* growth and reproductive traits from isolates of the same genotype but collected across a latitudinal gradient as a proxy for temperature (representing different mean annual air temperature regimes as shown in Fig. 2-2), might show distinct TPCs. I conducted temperature experiments to measure traits related to growth, reproduction, and fitness across the known thermal range of *Bd* and generated TPCs for five isolates from within the *Bd*GPL lineage. Our results reveal informative similarities and differences in several of the measured traits between two genetic lineages (*Bd*GPL1 and *Bd*GPL2) and among five *Bd* isolates.

I found that there was no obvious geographic pattern that could explain the distribution of genetic variants of *Bd*GPL collected across a latitudinal gradient within the United States. Three of our isolates nested within the *Bd*GPL1 clade and each originated from a different latitude (Table 2-1). Two of the isolates nested within the *Bd*GPL2 clade and similarly originated from different latitudes. Both genetic variants had the same T_{opt} of 21 °C, but the maximum viability

differed between *Bd*GPL1 and *Bd*GPL2. In addition, while both genetic variants had maximum zoospores densities and fecundity at low temperatures (4 °C), there were differences between *Bd*GPL1 and *Bd*GPL2 in these key reproductive traits. These findings corroborate previous studies that suggest variation exists even within a single *Bd* lineage (21, 30). My results suggest that there is less variation between *Bd* genotypes than if variation is compared across individual isolates. By grouping the isolates into genotypes, I increased the replication to assess thermal variation of *Bd*. The additional replicates within a genotype allows for increased confidence on the findings for thermal variation of *Bd* for the two measured traits. However, it is important to note that while I increased my replication within a genotype, my sample sizes of isolates within each genotype are likely inadequate for broad generalizability of *Bd*'s thermal variation. To more generally investigate the level of thermal performance variation that exists within and among genotypes, more research that increases the sample sizes (e.g., number of isolates) will be necessary. The addition of more *Bd* isolates to assess thermal variation among isolates and across genotypes would allow greater confidence in general patterns of thermal variation to be observed (65).

I suggest that there are likely numerous factors contributing to variation within *Bd*GPL in addition to thermal conditions. For example, each isolate for this study was collected from a unique host species (Table 2-1), with each host species occupying habitats that differ in a multitude of factors, including water pH, drying periods, microbiome composition, and other seasonality effects that likely have a large impact on *Bd* (5, 30, 43). Although it is impractical for *Bd* researchers to eliminate all confounding variables for *Bd* isolate origin, I should nevertheless make efforts to treat isolates identically following isolation (e.g., during laboratory maintenance) and acknowledge these limitations for resolving questions concerning differential pathogenicity.

Additionally, I acknowledge that there are limitations in only assessing a single isolate from each site origin. Understanding patterns or mechanisms of variation among genetic clades of

Bd may be achieved when more replicates can be studied at a single site of origin. For example, more isolates from each location may reveal whether the mean thermal variation among *Bd* isolates is drastically different or if there is in fact less variation among a larger sample of *Bd* isolates. There are limitations in the number of unique isolates that have been successfully found and maintained *in vitro*, and future studies should attempt to combine the information from previously measured isolates with new, unique isolates within this genetic clade as they become available to assess whether there are general patterns of thermal performance across the genetic variants or geographic orientation of *Bd*GPL.

I also found some patterns in the responses of *Bd* to temperature when assessing differences among all five isolates. To begin with, I found that the overall patterns of viability were similar and exhibited a T_{opt} at the intermediate temperature of 21°C. However, within each temperature, the isolates frequently differed from each other in their maximal viability, zoospore densities, fecundity, growth rates, and carrying capacities. These differences were pronounced at either end of the thermal spectrum, at low (4 °C) and high (26 and 27 °C) temperatures. For example, the temperature of the T_{opt} for zoospores densities is lower than 21°C, with far more zoospores produced in low temperatures (4 and 12°C), for a subset of the isolates. Furthermore, the fecundity of *Bd* was highest in low temperatures for every isolate. These findings are in line with those from previous studies that suggest understanding *Bd* responses (particularly zoospore production) in low temperatures is important to resolving the complexities of the fundamental niche and the disease ecology of *Bd* (42, 49, 62).

Additionally, I observed interesting patterns of *Bd* viability, growth rates, and carrying capacities at both extremes of the thermal range, making it difficult to determine the true CT_{max} and CT_{min} . Notably, I found the greatest complexity in thermal responses at the CT_{max} ; most of the *Bd* isolates (all except Vermont) exhibited at least some zoosporangia development, and early exponential growth (r), in the high temperature treatments (25, 26, and even 27 °C). Yet none of

the isolates produced any zoospores and growth could not be sustained for the duration of the experiment. In addition, I found that for the higher temperatures ($>21^{\circ}\text{C}$) considered in this experiment, the r estimates did not decline as one might expect. Rather, it was the K estimates that seemed to decline over the upper temperatures. As a result, the r estimates (constrained to follow a J–L curve) were overfit to the mid-range temperature data, causing unrealistically high CT_{max} and T_{opt} estimates, and poor r estimates, for high and low temperatures. Our findings for the higher temperature treatments differ from some previous studies that found no *Bd* growth at temperatures above 24°C (31, 43, 62). Thus, our findings that *Bd* can remain viable at high temperatures, but fail to produce zoospores, underscore the importance of using a viability assay to investigate additional questions concerning *Bd* responses to temperature (45).

Taken together, the variation in TPCs of the maximum viability of *Bd* isolates collected across a latitudinal gradient did not fit a pattern that could be explained by the “hotter is better” hypothesis. All isolates had the same T_{opt} for viability at 21°C . Instead, our viability results suggest that a vertical difference model may better explain the patterns for the TPCs of all five isolates. Namely, our viability measurements, and results from carrying capacities (K) among isolates, provide some evidence that mean temperatures across latitudes may influence the maximal performance of *Bd*. The isolates from northern latitudes (i.e., Vermont & Ohio), where mean temperatures are generally lower ($\sim 4\text{--}12^{\circ}\text{C}$; 61), exhibited lower viability and carrying capacities across temperatures, including at their T_{opt} . In contrast, the isolates from Louisiana, New Mexico, and Tennessee, in more southern latitudes where mean temperatures are generally higher ($\sim 14\text{--}25^{\circ}\text{C}$; 61), exhibited increased viability and carrying capacities across temperatures, including at their T_{opt} . As such, our evidence indicating a vertical difference in TPCs suggest that the mean temperatures experienced by amphibians across a latitudinal gradient may influence maximal viability—but not the T_{opt} or CT_{max} —of *Bd*. Other hypotheses of thermal constraints may be influencing *Bd*'s thermal responses such as the theory of biochemical adaptation or climate

variability hypothesis, reviewed in chapter 1. I note, however, that our results for our other reproductive parameters, including zoospore densities and fecundity, did not exhibit a similar pattern, underscoring the importance of measuring multiple traits to gain a full understanding of the complexities of *Bd* responses to temperature (37, 38).

Disease ecologists are concerned with how changes in environmental factors, such as temperature gradients, may influence disease dynamics through alterations in the biology of pathogens such as *Bd* (63, 64). Environmental influences on *Bd* traits such as growth and reproduction may ultimately influence the disease outcomes of chytridiomycosis (42, 44). For example, temperature conditions within local environments may increase viability, zoospores densities, fecundity, growth rates, or carrying capacities of *Bd*, leading to higher infectivity, and greater threat of disease for vulnerable amphibians (49). The threat of biodiversity loss for amphibian communities may be exacerbated from diseases like chytridiomycosis in the coming decades (63). To intervene in the continued population declines of amphibians, I must understand how pathogen biology is mediated across different environments, and within and among genetic lineages. I must also determine what environmental factors are driving the disease dynamics responsible for the disease-induced losses of amphibian biodiversity.

References

1. Scheele BC, Pasmans F, Skerratt LF, Berger L, Martel A, Beukema W, et al. Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. *Science*. (2019) 363:1459–63. doi: 10.1126/science.aav0379

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

2. Berger L, Speare R, Daszak P, Green DE, Cunningham AA, Goggin CL, et al. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proc Natl Acad Sci USA*. (1998) 95:9031–6. doi: 10.1073/pnas.95.15.9031

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

3. Bradford DF. Mass mortality and extinction in a high-elevation population of rana muscosa. *J Herpetol*. (1991) 25:174–77. doi: 10.2307/1564645

[CrossRef Full Text](#) | [Google Scholar](#)

4. Miller D, Gray M, Storfer A. Ecopathology of ranaviruses infecting amphibians. *Viruses*. (2011) 3:2351–73. doi: 10.3390/v3112351

[CrossRef Full Text](#) | [Google Scholar](#)

5. Sonn JM, Utz RM, Richards-Zawacki CL. Effects of latitudinal, seasonal, and daily temperature variations on chytrid fungal infections in a North American frog. *Ecosphere*. (2019) 10:2892. doi: 10.1002/ecs2.2892

[CrossRef Full Text](#) | [Google Scholar](#)

6. Hamilton PT, Richardson JML, Govindarajulu P, Anholt BR. Higher temperature variability increases the impact of *Batrachochytrium dendrobatidis* and shifts interspecific interactions in tadpole mesocosms. *Ecol Evol*. (2012) 2:2450–9. doi: 10.1002/ece3.369

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

7. Berger L, Speare R, Hines HB, Marantelli G, Hyatt AD, McDonald KR, et al. Effect of season and temperature on mortality in amphibians due to chytridiomycosis. *Aust Vet J.* (2004) 82:434–

9. doi: 10.1111/j.1751-0813.2004.tb11137.x

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

8. Bradley PW, Brawner MD, Raffel TR, Rohr JR, Olson DH, Blaustein AR. Shifts in temperature influence how *Batrachochytrium dendrobatidis* infects amphibian larvae. *PLoS ONE.* (2019) 14:e0222237. doi: 10.1371/journal.pone.0222237

doi: 10.1371/journal.pone.0222237

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

9. Altizer S, Ostfeld RS, Johnson PTJ, Kutz S, Harvell CD. Climate change and infectious diseases: from evidence to a predictive framework. *Science.* (2013) 341:514–9. doi:

10.1126/science.1239401

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

10. Daskin JH, Alford RA, Puschendorf R. Short-term exposure to warm microhabitats could explain amphibian persistence with *Batrachochytrium dendrobatidis*. *PLoS ONE.* (2011)

6:e26215. doi: 10.1371/journal.pone.0026215

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

11. Skerratt LF, Berger L, Speare R, Cashins S, McDonald KR, Phillott AD, et al. Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. *Ecohealth.* (2007)

4:125. doi: 10.1007/s10393-007-0093-5

[CrossRef Full Text](#) | [Google Scholar](#)

12. Longcore JE, Pessier AP, Nichols DK. *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. *Mycologia.* (1999) 91:219–27. doi: 10.2307/3761366

[CrossRef Full Text](#) | [Google Scholar](#)

13. Martel A, Spitzen-van der Sluijs A, Blooi M, Bert W, Ducatelle R, Fisher MC, et al.

Batrachochytrium salamandrivorans sp. nov. causes lethal chytridiomycosis in amphibians. *Proc Natl Acad Sci USA*. (2013) 110:15325–9. doi: 10.1073/pnas.1307356110

[CrossRef Full Text](#) | [Google Scholar](#)

14. Lambert MR, Womack MC, Byrne AQ, Hernández-Gómez O, Noss CF, Rothstein AP, et al.

Comment on “Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity.” *Science*. (2020) 367:eaay1838. doi: 10.1126/science.aay1838

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

15. Scheele BC, Pasmans F, Skerratt LF, Berger L, Martel A, Beukema W, et al. Response to

comment on “Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity.” *Science*. (2020) 367:eaay2905. doi: 10.1126/science.aay2905

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

16. Berger L, Hyatt AD, Speare R, Longcore JE. Life cycle stages of the amphibian chytrid

Batrachochytrium dendrobatidis. *Dis Aquat Organ*. (2005) 68:51–63. doi: 10.3354/dao068051

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

17. Berger L, Speare R, Skerratt L. Distribution of *Batrachochytrium dendrobatidis* and

pathology in the skin of green tree frogs *Litoria caerulea* with severe chytridiomycosis. *Dis Aquat Organ*. (2005) 68:65–70. doi: 10.3354/dao068065

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

18. Van Rooij P, Martel A, D'Herde K, Brutyn M, Croubels S, Ducatelle R, et al. Germ tube

mediated invasion of *Batrachochytrium dendrobatidis* in amphibian skin is host dependent. *PLoS ONE*. (2012) 7:e41481. doi: 10.1371/journal.pone.0041481

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

19. Voyles J, Young S, Berger L, Campbell C, Voyles WF, Dinudom A, et al. Pathogenesis of chytridiomycosis, a cause of catastrophic amphibian declines. *Science*. (2009) 326:582–5. doi: 10.1126/science.1176765

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

20. Vredenburg VT, Knapp RA, Tunstall TS, Briggs CJ. Dynamics of an emerging disease drive large-scale amphibian population extinctions. *Proc Natl Acad Sci USA*. (2010) 107:9689–94. doi: 10.1073/pnas.0914111107

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

21. Greener MS, Verbrugghe E, Kelly M, Blooi M, Beukema W, Canessa S, et al. Presence of low virulence chytrid fungi could protect European amphibians from more deadly strains. *Nat Commun*. (2020) 11:5393. doi: 10.1038/s41467-020-19241-7

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

22. Lambertini C, Becker CG, Jenkinson TS, Rodriguez D, da Silva Leite D, James TY, et al. Local phenotypic variation in amphibian-killing fungus predicts infection dynamics. *Fungal Ecol*. (2016) 20:15–21. doi: 10.1016/j.funeco.2015.09.014

[CrossRef Full Text](#) | [Google Scholar](#)

23. Farrer RA, Weinert LA, Bielby J, Garner TWJ, Balloux F, Clare F, et al. Multiple emergences of genetically diverse amphibian-infecting chytrids include a globalized hypervirulent recombinant lineage. *Proc Natl Acad Sci USA*. (2011) 108:18732–6. doi: 10.1073/pnas.1111915108

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

24. Rosenblum EB, James TY, Zamudio KR, Poorten TJ, Ilut D, Rodriguez D, et al. Complex history of the amphibian-killing chytrid fungus revealed with genome resequencing data. *Proc Natl Acad Sci USA*. (2013) 110:9385–90. doi: 10.1073/pnas.1300130110

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

25. Byrne AQ, Vredenburg VT, Martel A, Pasmans F, Bell RC, Blackburn DC, et al. Cryptic diversity of a widespread global pathogen reveals expanded threats to amphibian conservation.

Proc Natl Acad Sci USA. (2019) 116:20382–7. doi: 10.1073/pnas.1908289116

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

26. Schloegel LM, Toledo LF, Longcore JE, Greenspan SE, Vieira CA, Lee M, et al. Novel, panzootic and hybrid genotypes of amphibian chytridiomycosis associated with the bullfrog trade: Chytrid Genotypes and the Bullfrog Trade.

Mol Ecol. (2012) 21:5162–77. doi:

10.1111/j.1365-294X.2012.05710.x

[CrossRef Full Text](#) | [Google Scholar](#)

27. James TY, Toledo LF, Rödder D, Silva Leite D, Belasen AM, Betancourt-Román CM, et al.

Disentangling host, pathogen, and environmental determinants of a recently emerged wildlife disease: lessons from the first 15 years of amphibian chytridiomycosis research. *Ecol Evol*.

(2015) 5:4079–97. doi: 10.1002/ece3.1672

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

28. Basanta MD, Byrne AQ, Rosenblum EB, Piovia-Scott J, Parra-Olea G. Early presence of

Batrachochytrium dendrobatidis in Mexico with a contemporary dominance of the global panzootic lineage. *Mol Ecol*. (2021) 30:424–37. doi: 10.1111/mec.15733.

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

29. Briggs CJ, Knapp RA, Vredenburg VT. Enzootic and epizootic dynamics of the chytrid

fungal pathogen of amphibians. *Proc Natl Acad Sci USA*. (2010) 107:9695–700. doi:

10.1073/pnas.0912886107

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

30. Becker CG, Greenspan SE, Tracy KE, Dash JA, Lambertini C, Jenkinson TS, et al. Variation in phenotype and virulence among enzootic and panzootic amphibian chytrid lineages. *Fungal Ecol*.

(2017) 26:45–50. doi: 10.1016/j.funeco.2016.11.007

[CrossRef Full Text](#) | [Google Scholar](#)

31. Muletz-Wolz CR, Barnett SE, DiRenzo GV, Zamudio KR, Toledo LF, James TY, et al. Diverse genotypes of the amphibian-killing fungus produce distinct phenotypes through plastic responses to temperature. *J Evol Biol.* (2019) 32:287–98. doi: 10.1111/jeb.13413

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

32. Angilletta MJ. Estimating and comparing thermal performance curves. *J Therm Biol.* (2006) 31:541–5. doi: 10.1016/j.jtherbio.2006.06.002

[CrossRef Full Text](#) | [Google Scholar](#)

33. Huey RB, Kingsolver JG. Evolution of thermal sensitivity of ectotherm performance. *Trends Ecol Evol.* (1989) 4:131–5. doi: 10.1016/0169-5347(89)90211-5

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

34. Schulte PM, Healy TM, Fangué NA. Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. *Integr Comp Biol.* (2011) 51:691–702. doi: 10.1093/icb/ucr097

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

35. Khelifa R, Blanckenhorn WU, Roy J, Rohner PT, Mahdjoub H. Usefulness and limitations of thermal performance curves in predicting ectotherm development under climatic variability. *J Anim Ecol.* (2019) 88:1901–12. doi: 10.1111/1365-2656.13077

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

36. Knies JL, Izem R, Supler KL, Kingsolver JG, Burch CL. The genetic basis of thermal reaction norm evolution in lab and natural phage populations. *PLoS Biol.* (2006) 4:40201. doi: 10.1371/journal.pbio.0040201

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

37. Kingsolver JG. The well-temperated biologist. *Am Nat.* (2009) 174:755–68. doi: 10.1086/648310

[CrossRef Full Text](#) | [Google Scholar](#)

38. Angilletta MJ, Huey RB, Frazier MR. Thermodynamic effects on organismal performance: is hotter better? *Physiol Biochem Zool.* (2010) 83:197–206. doi: 10.1086/648567

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

39. Frazier MR, Huey RB, Berrigan D. Thermodynamics constrains the evolution of insect population growth rates: “warmer is better.” *Am Nat.* (2006) 168:512–20. doi: 10.1086/506977

[CrossRef Full Text](#) | [Google Scholar](#)

40. Gaitán-Espitia JD, Belén Arias M, Lardies MA, Nespolo RF. Variation in thermal sensitivity and thermal tolerances in an invasive species across a climatic gradient: lessons from the land snail *cornu aspersum*. *PLoS ONE.* (2013) 8:70662. doi: 10.1371/journal.pone.0070662

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

41. U.S. Forest Service. *National Forest Climate Change Maps* (2019).

[Google Scholar](#)

42. Voyles J, Johnson LR, Rohr J, Kelly R, Barron C, Miller D, et al. Diversity in growth patterns among strains of the lethal fungal pathogen *Batrachochytrium dendrobatidis* across extended thermal optima. *Oecologia.* (2017) 18:363–73. doi: 10.1007/s00442-017-3866-8

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

43. Piotrowski JS, Annis SL, Longcore JE. Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. *Mycologia.* (2004) 96:9–15. doi: 10.2307/3761981

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

44. Stevenson LA, Alford RA, Bell SC, Roznik EA, Berger L, Pike DA. Variation in thermal performance of a widespread pathogen, the amphibian chytrid fungus *Batrachochytrium dendrobatidis*. *PLoS ONE.* (2013) 8:e73830. doi: 10.1371/journal.pone.0073830

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

45. Lindauer A, May T, Rios-Sotelo G, Sheets C, Voyles J. Quantifying *Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans* viability. *Ecohealth*. (2019) 16:346–50.

doi: 10.1007/s10393-019-01414-6

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

46. Boyle DG, Boyle DB, Olsen V, Morgan JAT, Hyatt AD. Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time

Taqman PCR assay. *Dis Aquat Organ*. (2004) 60:141–8. doi: 10.3354/dao060141

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

47. Voyles J. Phenotypic profiling of *Batrachochytrium dendrobatidis*, a lethal fungal pathogen of amphibians. *Fungal Ecol*. (2011) 4:196–200. doi: 10.1016/j.funeco.2010.12.003

[CrossRef Full Text](#) | [Google Scholar](#)

48. Voyles J, Johnson LR, Briggs CJ, Cashins SD, Alford RA, Berger L, et al. Temperature alters reproductive life history patterns in *Batrachochytrium dendrobatidis*, a lethal pathogen associated with the global loss of amphibians. *Ecol Evol*. (2012) 2:2241–9. doi: 10.1002/ece3.334

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

49. Lindauer AL, Maier PA, Voyles J. Daily fluctuating temperatures decrease growth and reproduction rate of a lethal amphibian fungal pathogen in culture. *BMC Ecol*. (2020) 20:18. doi:

10.1186/s12898-020-00286-7

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

50. Levitz SM, Diamond RD. A rapid colorimetric assay of fungal viability with the tetrazolium salt MTT. *J Infect Dis*. (1985) 152:938–45. doi: 10.1093/infdis/152.5.938

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

51. Byrne AQ, Rothstein AP, Poorten TJ, Erens J, Settles ML, Rosenblum EB. Unlocking the story in the swab: a new genotyping assay for the amphibian chytrid fungus *Batrachochytrium dendrobatidis*. *Mol Ecol Resour*. (2017) 17:1283–92. doi: 10.1111/1755-0998.12675

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

52. Magoc T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*. (2011) 27:2957–63. doi: 10.1093/bioinformatics/btr507

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

53. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* (2004) 32:1792–7. doi: 10.1093/nar/gkh340

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

54. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*. (2014) 30:1312–3. doi: 10.1093/bioinformatics/btu033

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

55. Junier T, Zdobnov EM. The Newick utilities: high-throughput phylogenetic tree processing in the UNIX shell. *Bioinformatics*. (2010) 26:1669–70. doi: 10.1093/bioinformatics/btq243

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

56. Zhang C, Rabiee M, Sayyari E, Mirarab S. ASTRAL-III: polynomial time species tree reconstruction from partially resolved gene trees. *BMC Bioinform.* (2018) 19:153. doi: 10.1186/s12859-018-2129-y

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

57. R-Core-Team. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing (2019).

[Google Scholar](#)

58. Eisenberg MC, Hayashi MAL. Determining identifiable parameter combinations using subset profiling. *Math Biosci.* (2014) 256:116–26. doi: 10.1016/j.mbs.2014.08.008

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

59. Venzon DJ, Moolgavkar SH. A method for computing profile-likelihood-based confidence intervals. *Appl Stat.* (1988) 37:87. doi: 10.2307/2347496

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

60. Olson DH, Aanensen DM, Ronnenberg KL, Powell CI, Walker SF, Bielby J, et al. Mapping the Global Emergence of *Batrachochytrium dendrobatidis*, the Amphibian Chytrid Fungus. *PLoS ONE*. (2013) 8:e56802. doi: 10.1371/journal.pone.0056802

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

61. Kärvemo S, Meurling S, Berger D, Höglund J, Laurila A. Effects of host species and environmental factors on the prevalence of *Batrachochytrium dendrobatidis* in northern Europe. *PLoS ONE*. (2018) 13:199852. doi: 10.1371/journal.pone.0199852

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

62. Woodhams DC, Alford RA, Briggs CJ, Johnson M, Rollins-Smith LA. Life-history trade-offs influence disease in changing climates: strategies of an amphibian pathogen. *Ecology*. (2008) 89:1627–39. doi: 10.1890/06-1842.1

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

63. Rohr JR, Dobson AP, Johnson PTJ, Kilpatrick AM, Paull SH, Raffel TR, et al. Frontiers in climate change–disease research. *Trends Ecol Evol*. (2011) 26:270–7. doi: 10.1016/j.tree.2011.03.002

[CrossRef Full Text](#) | [Google Scholar](#)

64. Rohr JR, Raffel TR, Romansic JM, McCallum H, Hudson PJ. Evaluating the links between climate, disease spread, and amphibian declines. *Proc Natl Acad Sci USA*. (2008) 105:17436–41. doi: 10.1073/pnas.0806368105

[PubMed Abstract](#) | [CrossRef Full Text](#) | [Google Scholar](#)

65. Fisher, M. C., & Garner, T. W. J. (2020). Chytrid fungi and global amphibian declines. *Nature Reviews Microbiology*, 18(6), 332–343. <https://doi.org/10.1038/s41579-020-0335-x>

Chapter 3: Case study investigating effects of variable colder temperatures and seasonal differences on *Bd* originating from New Mexico

Introduction

Emerging infectious diseases (EIDs) are a leading cause of amphibian population declines and die-off events globally (Blaustein et al., 2010; Daszak et al., 1999; Daszak et al., 2003). While several diseases can potentially lead to amphibian population declines, chytridiomycosis is one EID that has been definitively linked to die-off events in recent decades (Berger et al., 1998; Bosch et al., 2001; Kamoroff & Goldberg, 2017). Research has been conducted for several decades to understand the driving factors of the chytridiomycosis disease system, focusing on the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) (Alford et al., 2007; Berger et al., 1998; Sapsford et al., 2013). *Bd* infects amphibians in diverse environments, and therefore, has been extensively researched for its sensitivity to environmental conditions (Piotrowski et al., 2004; Pounds et al., 2006; Stevenson et al., 2013; Voyles et al., 2017).

Bd and its amphibian hosts live in complex environments that are impacted by thermal variation, including daily and seasonal fluctuations (Raffell et al., 2006; Sonn et al., 2019). Within the chytridiomycosis system, I see evidence of seasonal patterns in infection intensity, prevalence, and mortality (Berger et al., 2004; Raffell et al., 2006; Sapsford et al., 2013). For example, in tropical environments, higher rates of infection are generally observed in the seasons with lower mean temperatures (Woodhams & Alford, 2005). In contrast, some research has suggested that seasons with higher mean temperatures may lead to lower intensity of infection due to temperatures that are less favorable to *Bd* growth and reproduction compared to cooler seasons (Sonn et al., 2019). However, warmer seasons are not always associated with low levels of *Bd* infection across temperate landscapes (Forrest et al., 2015; Knapp et al., 2011). Seasonality can impact both pathogen and host physiology, but temperature effects on pathogen growth and

reproduction are thought to be highly influential on disease outcomes (Piotrowski et al., 2004; Stevenson et al., 2013). Therefore, continuing to investigate the impacts that seasonal environmental changes can have on *Bd* may be beneficial for understanding this lethal EID and for mitigating the disease impacts in areas where conservation actions need to be planned accordingly.

Environmental temperatures may alter disease outcomes within the chytridiomycosis system by influencing *Bd* reproductive physiology (Fernandez-Loras et al., 2019; Rohr & Raffel, 2010; Stevenson et al., 2013; Sheets et al., 2021). For example, studies have shown that *Bd* growth and reproduction are influenced when temperature is experimentally manipulated for *Bd* *in vitro* (Kásler et al., 2022; Piotrowski et al., 2004; Sheets et al., 2021; Voyles et al., 2017). Specifically, temperature alters the quantity or viability of motile zoospores, which are responsible for navigating in an aqueous environment to infect a host (Lindauer et al., 2020; Stevenson et al., 2013; Sheets et al., 2021; Voyles et al., 2017). Temperature also impacts other *Bd* growth traits, such as sporangium size and whole culture growth (Becker et al., 2017; Lambertini et al., 2016; Muletz-Wolz et al., 2019; Stevenson et al., 2013, Sheets et al. 2021). The impact that temperature has on *Bd* growth and reproduction occurs synergistically with the effects of temperature on host physiology, ultimately influencing disease outcomes via changes in virulence or pathogenicity (Sapsford et al., 2013; Woodhams & Alford, 2005).

In the past, studies that explore the effect of temperature on *Bd* growth and reproduction test hypotheses using constant temperatures (Piotrowski et al., 2004; Stevenson et al., 2014). However, more recent studies have suggested that understanding *Bd* responses to fluctuating daily temperature conditions can provide ecologically relevant results for understanding the impact that seasonal thermal environments can have on pathogen physiology (Greenspan et al., 2023; Raffel et al., 2015; Vasseur et al., 2014; Lindauer et al. 2020). For example, in two studies that simulated different fluctuating temperatures, *Bd* growth and reproduction was reduced in

treatments with a higher daily mean temperature compared to treatments with a lower daily mean temperature (Lindauer et al., 2020; Sonn et al., 2019). While studying constant temperatures *in vitro* can show a basic relationship between temperature and *Bd*'s physiology, researchers suggest that simulating daily or seasonal temperature variability should be a topic of future research (Gajewski et al., 2021).

When considering temperature variability, at least two factors can be assessed. First, the range of temperature variation can be defined as the difference between the minimum and maximum temperatures for a given time period (e.g., daily temperatures; Beyer, 1885). Second, the rate of change can be defined as the time required for temperature to decrease or increase to the minimum or maximum temperatures (e.g., if it takes 4 hours to reach the maximum daily temperature one day but 8 hours to reach the maximum daily temperature on another day; Beyer, 1885). The extent of how the range of variability and rate of change in variable temperatures may alter pathogen physiology is not fully understood and likely depends on other climatic factors for a given study area (Gajewski et al., 2021; Greenspan et al., 2023).

To further investigate the effect of fluctuating temperatures, and how they may underpin seasonality in chytridiomycosis, I collated temperature and infection data that was collected from amphibian populations in southern New Mexico, a region where several amphibian host species are threatened by chytridiomycosis (Christman & Jennings, 2018; Hinderer et al., 2021). I then simulated field temperature conditions in incubators to study the responses of *Bd* isolate from New Mexico (NM) in *in vitro* experiments. I selected this isolate because I previously found that it had an unusual thermal performance curve (TPC) relative to *Bd* isolates from other parts of the United States (Sheets et al., 2021). Namely, the NM *Bd* isolate exhibited optimal fecundity and greater zoospore densities in low temperatures, approaching the critical thermal minimum for *Bd* (Fig. 3-1). This evidence suggests that the NM *Bd* isolate has a thermal optimum closer to 4°C, which may influence seasonal infection patterns in this region (Sheets et al., 2021).

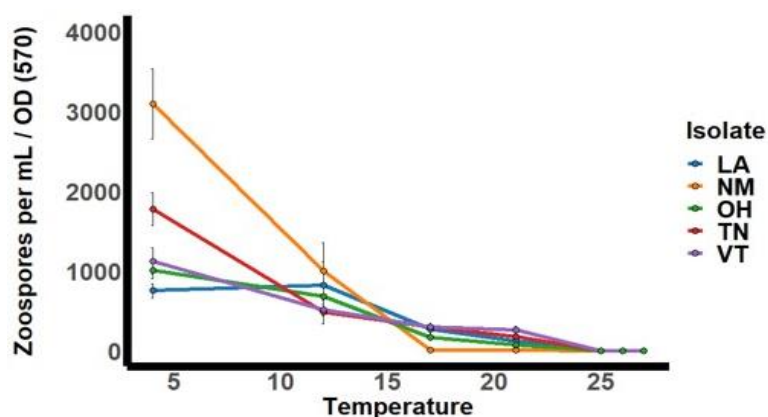


Figure 3-1. Fecundity thermal performance curves of published *Batrachochytrium dendrobatidis* (*Bd*) isolates. The *Bd* isolate from New Mexico (NM, Orange line) produces more infectious zoospores per viable whole culture population measured at 4°C and 12°C, with a markedly decreased fecundity approaching 17°C. (figure modified from Sheets et al. 2021).

In this study, I focused on understanding changes in whole culture viability and zoospore densities under fluctuating thermal conditions that mimicked field conditions. I hypothesized that under fluctuating simulated winter conditions, *Bd* isolate from New Mexico would show a higher maximum zoospore densities and whole culture viability compared to fluctuating simulated summer conditions. Additionally, I hypothesized that maximum whole culture viability and maximum whole culture viability of the *Bd* isolate from New Mexico would be lower at each fluctuating seasonal condition compared to constant temperatures due to the challenges *Bd* may experience in adjusting to daily temperature variability.

Methods

Field collection for infection related data

I used field data collected from individual amphibians of *Lithobates blairi*, *L. chiricahuensis*, and *Ambystoma mavortium* captured in sites in southern New Mexico.

Specifically, I collated data on species, site, time and date of collection, and qPCR data generated from a diagnostic swab to test for the presence of *Bd*. To collect a swab for *Bd* detection, field researchers captured an individual and swabbed (Medical Wire #M113) five times across each of the dorsal surfaces, ventral surfaces, and each digit of the hands and feet (Wilber et al., 2022). I stored the swabs in a centrifuge tube and froze them in -80°C , until they could be used for DNA extraction and qPCR (Wilber et al., 2022). Collaborators conducted standard protocols for DNA extraction and qPCR (Hyatt et al., 2007), in conjunction with minor modifications, these methods were published for the other sites across the U.S. that are located near Department of Defense installations (i.e., PA, LA, TN, and VT), and were also conducted the same way and at the same time for the New Mexico samples (Wilber et al., 2022). I used a $5\ \mu\text{L}$ sample of extracted DNA for qPCR. Because the sample was only $5\ \mu\text{L}$ out of $200\ \mu\text{L}$ extracted DNA, I multiplied the DNA copy number by a dilution factor of 40 to get the quantity of DNA (genomic equivalents) on an individual swab. I then added 1 and log transformed the value of the *Bd* DNA copy number to estimate the intensity of infection for each individual amphibian. I calculated infection prevalence by determining how many individuals were positive for *Bd* infection per number of sampled individuals across seasons (i.e., winter, spring, summer, fall). Winter data were collected from December through March, spring data were collected April through May, summer data were collected June through August, and fall data were collected from October-November.

Temperature profile selection

Field researchers collected temperature data from ephemeral or permanent pools used by amphibians. They placed hobo loggers (Onset Computer Corporation, Bourne, MA) into the pond water at a depth where amphibians were observed. The hobos collected temperature data in the water every 30 minutes or every hour each day for three years. I used a subset of the water temperature data to design thermal treatments that simulated real conditions experienced by frogs

in the ponds. Specifically, I used the water temperatures for three months of winter and three months of summer over 2 consecutive years (2018-2019) because this time coincided with positive *Bd* infection results from frogs at the same site location (Fig. 3-2A, 3-2B).

From the data subset, I simulated the exact field water temperatures measured by the hobos every hour in a 2-week period of summer and a 3-week period of winter in environmental chambers in 2018 (Fig. 3-2C). I set incubators (Conviro) to produce air temperatures that simulated the hobo water temperature data exactly every hour for the entire 2-3 week period. The thermal treatments that simulated “winter” conditions had temperatures fluctuating daily between 6°C and 18°C, with a daily mean temperature of 10.6°C. The thermal treatment that simulated summer field conditions had daily fluctuating temperatures between 14°C and 32°C, with a daily mean temperature of 21.7°C. While temperature data from August 2018 was as high as 40°C, I capped the simulated summer temperatures at 32°C to prevent the cultures from being heat killed. Once I set the incubation chambers to simulate the exact environmental temperatures measured by the hobos, I grew the *Bd* isolate from New Mexico in 96-well plates in each of two seasonal conditions (winter and summer) for 2-3 weeks.

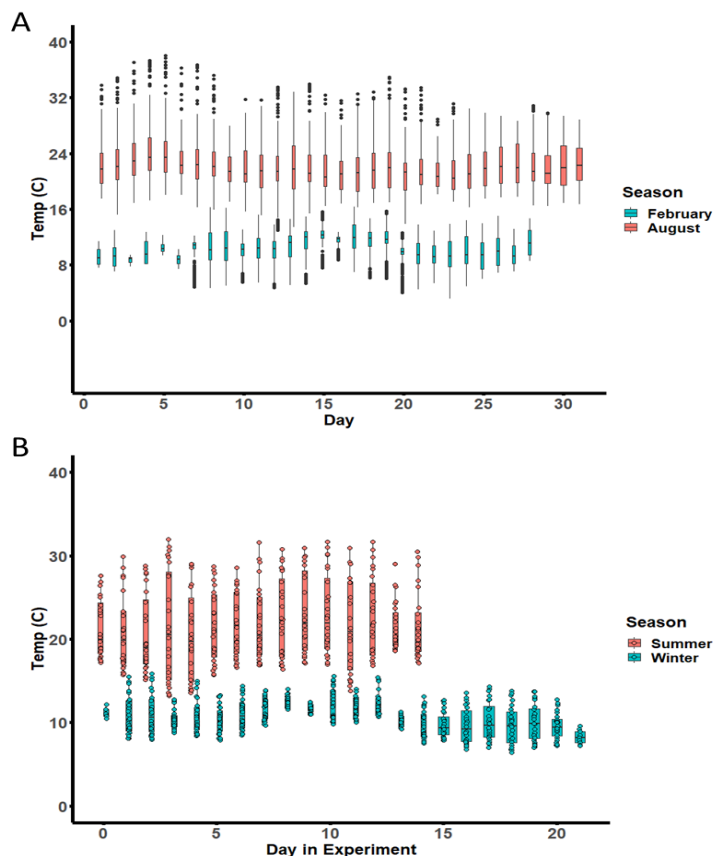


Figure 3-2. A) Water temperatures from New Mexico field site and B) simulated temperatures closely matching the field water temperature data shown in panel A. (A) Field water temperatures measured by hobos every hour for the month of February (blue, winter) and August (red, summer) for two consecutive years (2018, 2019). (B) Temperature profiles simulated in lab. Winter conditions (blue), consisting of three weeks of hourly temperatures, were simulated in an environmental chamber (Conviron) to match the fluctuating temperatures observed on February 7-28th in the field for 2018. Summer conditions (red), consisting of two weeks of hourly temperatures, were simulated in an environmental chamber (Conviron) to match the fluctuating temperatures seen in the field for August 9-23rd, 2018. The temperature was adjusted within the chambers in real time to match the actual time and temperature measured by the hobos in the field each day.

Isolate culturing and plate design

I selected the *Bd* isolate NMBF_003 from New Mexico. This isolate was collected from a bullfrog (*Lithobates catesbeianus*) in 2017 and maintained in stock culture collections (Longcore, 1999). For this study, I revived the isolate from cryo-archives and passaged it using standard protocols, where I grew the isolate in a 75 cm² flask with tryptone/gelatin hydrolysate/lactose (TGhL) media (Boyle et al., 2004; Piotrowski et al., 2004). I passaged the isolate five times before I set up the experiment.

To set up the *in vitro* growth experiment, I filtered out zoosporangia using a standard protocol (Voyles, 2011). I diluted the filtrate containing zoospores with TGhL media to a concentration of 50 x 10⁴ zoospores mL⁻¹. I inoculated the filtered zoospore filtrate into 24-48 wells of 18 clear, flat-bottomed 96-well plates. I set up each plate with experimental wells containing live zoospores and negative control wells containing heat-killed zoospores. Additionally, I pipetted 50 µl of TGhL media and 50 µl of culture inoculum into each sample well. I randomly assigned and then placed eight plates into the environmental chamber (Conviron) simulating “winter” conditions and nine plates into the environmental chamber (Conviron) simulating “summer” conditions. I randomly selected plates for each environmental chamber.

Quantification of growth and reproductive traits

I quantified growth and reproductive traits by measuring whole culture viability and zoospore densities. On the days of maximum culture growth (determined using light microscopy), I used a MTT colorimetric cellular viability assay to assess growth via maximum whole culture viability (Lindauer et al., 2019). I collected optical density (OD) data of the viability assay with a microplate reader (Biotek ELx800 Absorbance) that uses a 570 nm wavelength filter (Lindauer et

al., 2019). I performed manual zoospore counts to quantify motile zoospore densities using a hemocytometer. I measured traits of maximum whole culture viability and maximum zoospore densities every other day after initial zoospore release for each thermal treatment group. The maximum period of each trait consists of 2 days of data collection where quantities of each measured response was highest over a single life cycle.

Statistical analysis

I completed all statistical analyses using R version 4.0.2 (R Core Team, 2020). To compare differences in the *Bd* load among the four field seasons (i.e., winter, summer, spring, fall), I conducted a non-parametric Kruskal Wallace test (Fig. 3-3). These statistics were appropriate because the field data for the intensity of infection were not normally distributed (Shapiro test: $p = 0.025$) but were equal in variance (Fligner test: $p = 0.49$).

To estimate whether prevalence in the field differed among four seasons, I assessed the likelihood that an individual would be infected out of a sampled population. I indicated infected individuals as a 1 and uninfected individuals with a 0 for each season in the data. I conducted a logistic regression using the generalized linear model (GLM) function from the package "lme4" (Bates et al., 2015) using the "logit" link. The logit link is used to model the probability of success by converting a linear combination of covariate values to a probability scale. This is appropriate for my logistic regression because I have set up a binomial response variable (i.e., infected or not infected). I included season as a categorical fixed effect. I plotted the residuals using the "DHARMA" package, which is applicable to use for assessing the goodness of fit for GLMs, such as my model with the prevalence data. To report whether the effect of season on prevalence was statistically significant, I conducted an ANOVA test using the "car" package (Fox & Weisberg, 2019) and reported the χ^2 value for season. I used the "car" package in addition to the "lme4" package to assess the statistical significance of the seasonal prevalence model, which

are appropriate because the “lme4” package fits random effects associated with non-nested grouping factors and the “car” package calculates a p-value for each covariate. Finally, to evaluate whether the differences in prevalence among the four seasons were statistically significant, I calculated the estimated marginal means using the “emmeans” package (Lenth, 2020) and conducted pairwise comparisons of the four seasons using a Tukey-adjusted *post hoc* test on my GLM model. This was fitting because estimated marginal means estimate the mean response value given the covariates used in the model. I used the “ggemmeans” function from the “ggeffects” package, which is correct because it wraps the output from the emmeans package into ggplot2 to visualize prevalence across the four seasons. These statistical tests were reported as odds ratios, standard errors, z-ratio, and p-values.

To analyze our reproductive growth (maximum whole culture viability) and (maximum zoospore densities) traits *in vitro* with simulated seasonal conditions, I conducted the Welch t-test. I conducted Welch’s t-test to compare whole culture viability and maximum zoospore densities between simulated winter and summer conditions. I also compared the maximum whole culture viability and zoospore density data from this experiment with the corresponding data from *Bd* growth in constant temperatures using a Welch t-test (Sheets et al., 2021).

Results

Field data: prevalence and intensity of infection

For seasonal prevalence, I found a significant difference among seasons (GLM, $\chi^2_3 = 63.6$; $P < 0.001$; Fig. 3-3A). A *post hoc* Tukey-adjusted pairwise comparisons showed that there were significant differences in seasonal prevalence among all seasons except between spring and winter, as well as winter and summer (Table 3-1). Specifically, *Bd* prevalence was higher in the spring compared to the summer and higher in the summer compared to the fall (Table 3-1). I found that *Bd* intensity of infection was highest in winter months, when temperatures were at

seasonal lows ($\bar{X} = 7.5 \pm 0.37$ (SE); Fig. 3-3B). For intensity of infection, there were differences among seasons (ANOVA, $F_{(3,59)} = 28.9$, $P < 0.001$). A Tukey *post hoc* test showed that all seasons were statistically different from each other, with the exception of spring compared to summer, which was not statistically different (Tukey *post hoc*, $F_{(3,59)} = 28.9$, $P = 0.53$; Fig. 3-3B; Table 3-2).

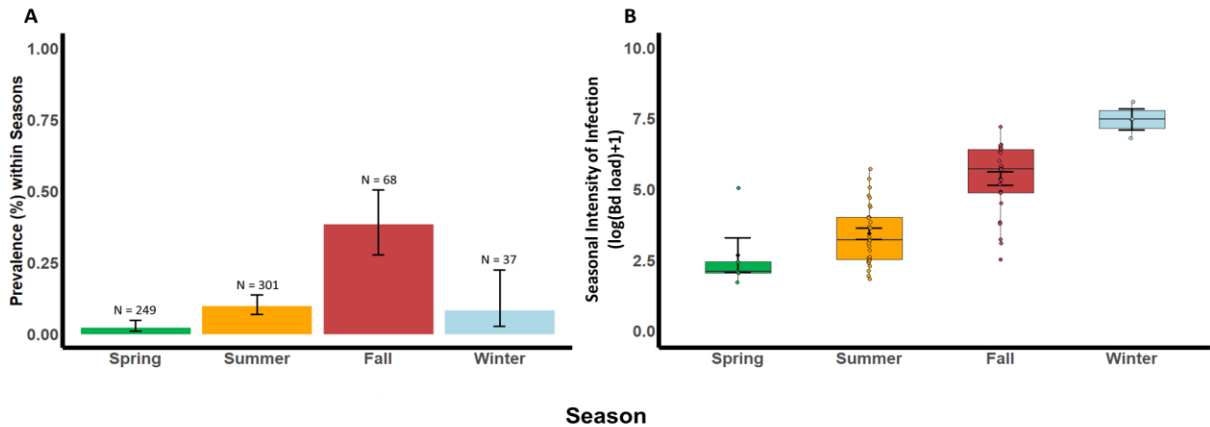


Figure 3-3. A) Seasonal prevalence and B) seasonal intensity of infection of *Batrachochytrium dendrobatidis* in amphibians in New Mexico in 2017-2019. Field researchers collected diagnostic samples from three host species (*Lithobates blairi*, *L. chiricahuensis*, *Ambystoma mavortium*) during spring, summer, fall, and winter sampling effort seasons. The intensity of infection was determined using qPCR to estimate the *Bd* load values. Prevalence is shown as the proportion of host samples that tested positive for *Bd* divided by the total number of samples with 95% confidence intervals. Intensity of infection results are shown with standard error bars for each seasonal group. The sample size (N) for each season is reported above each bar in panel A.

Table 3-1. *Bd* prevalence data for New Mexico. The table shows the pairwise comparisons of the Tukey-adjusted *post hoc* test conducted on the GLM data. The odds ratios were predicted using “ggeffects”. The table shows the differences among seasons indicated by the Odds ratio and p values.

Contrast Pairs	Odds Ratio	SE	z Ratio	P value
Fall/Spring	30.2	15.6	6.6	<0.001
Fall/Summer	5.8	1.8	5.5	<0.001
Fall/Winter	7.0	4.6	2.99	0.015
Spring/Summer	0.2	0.1	-3.4	0.005
Spring/Winter	0.2	0.2	-1.9	0.2
Summer/Winter	1.2	0.77	0.3	0.99

Table 3-2. *Bd* intensity of infection data for NM. The table shows the pairwise comparisons of the Tukey-adjusted *post hoc* test conducted on the ANOVA data. This table indicates that there are differences among seasons in the intensity of infection, except in the case of spring compared to summer and fall compared to winter.

Contrast Pairs	P value
Fall/Spring	< 0.001
Fall/Summer	< 0.001
Fall/Winter	0.02
Spring/Summer	0.53
Spring/Winter	< 0.001
Summer/Winter	< 0.001

Growth and reproduction of *Batrachochytrium dendrobatidis* in vitro

I found that when *Bd* was grown in simulated winter and summer field conditions, it exhibited differences in maximum whole culture viability and maximum zoospore densities

between thermal treatments. Specifically, the NM *Bd* isolate showed differences in the maximum whole culture viability between the winter and summer thermal treatments. When grown at simulated winter conditions, the *Bd* isolate from New Mexico showed a higher maximum whole culture viability than when grown in summer conditions (Welch t-test: $t(43.4) = 2.5$, $p = 0.02$; Winter: $\bar{X} = 0.69 \pm 0.02$ (SE); Summer: $\bar{X} = 0.63 \pm 0.02$ (SE); Fig. 3-4B). In addition, I found that maximum zoospore densities were higher in the winter conditions compared to the summer conditions (Welch t-test: $t(13.7) = 21$, $p < 0.001$; Winter: $\bar{X} = 81.5 \pm 3.3$ (SE); Summer: $\bar{X} = 7.2 \pm 1.2$ (SE); Fig. 3-4A).

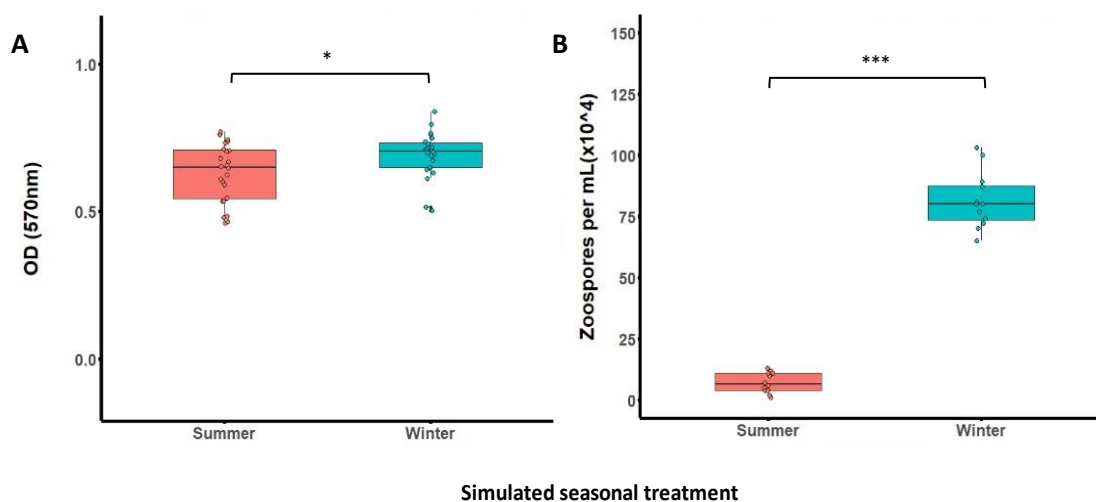


Figure 3-4. Boxplots of the *Batrachochytrium dendrobatidis* isolate from New Mexico simulated seasonal trait responses. A) boxplots show the maximum whole culture viability via optical density (OD) readings at 570 nm and B) boxplots show the maximum zoospore densities at the two simulated seasonal treatments. Both boxplots are showing results for the New Mexico isolate. The pink boxes indicate the simulated summer field conditions, and the blue boxes indicate the simulated winter field conditions. Each boxplot consists of data from a 2-day maximum period during the thermal experiment. Significance labels are shown with * marks to indicate degrees of significance between lineages as follows: * < 0.05 , ** < 0.01 , *** < 0.001 .

In vitro: constant versus fluctuating temperatures

There was no significant difference between the maximum whole culture viability compared to the results of the culture incubated at constant 12°C from chapter 2 (Welch t-test: $t(26.1) = -1.81$, $p = 0.082$; Winter: $\bar{X} = 0.7 \pm 0.02$ (SE); 12°C: $\bar{X} = 0.7 \pm 0.02$ (SE); Fig. 3-5A). However, the *Bd* isolate from New Mexico had lower maximum whole culture viability when grown in summer conditions that fluctuated from 13-32°C over three weeks with a daily average temperature of 21.7°C compared to growth in constant 21°C (Welch t-test: $t(27.3) = -5.41$, $p < 0.001$; Summer: $\bar{X} = 0.6 \pm 0.2$ (SE); 21°C: $\bar{X} = 0.8 \pm 0.03$ (SE); Fig. 3-5A).

I found that the fluctuating winter and summer maximum data were significantly different from the constant temperature which most closely matches the daily average temperature of each, respective simulated season. The maximum zoospore densities of the fluctuating winter season were higher than at constant 12°C (Welch t-test: $t(19.8) = 5.7$, $p \leq 0.001$; Winter: $\bar{X} = 81.5 \pm 3.3$ (SE); 12°C: $\bar{X} = 37.4 \pm 7.0$ (SE); Fig. 3-5B). The maximum zoospore densities in fluctuating summer conditions were also higher than maximum zoospore densities at constant 21°C (Welch t-test: $t(13.9) = 3.5$, $p < 0.01$; Summer: $\bar{X} = 7.2 \pm 1.2$ (SE); 21°C: $\bar{X} = 2.8 \pm 0.4$ (SE); Fig. 3-5B).

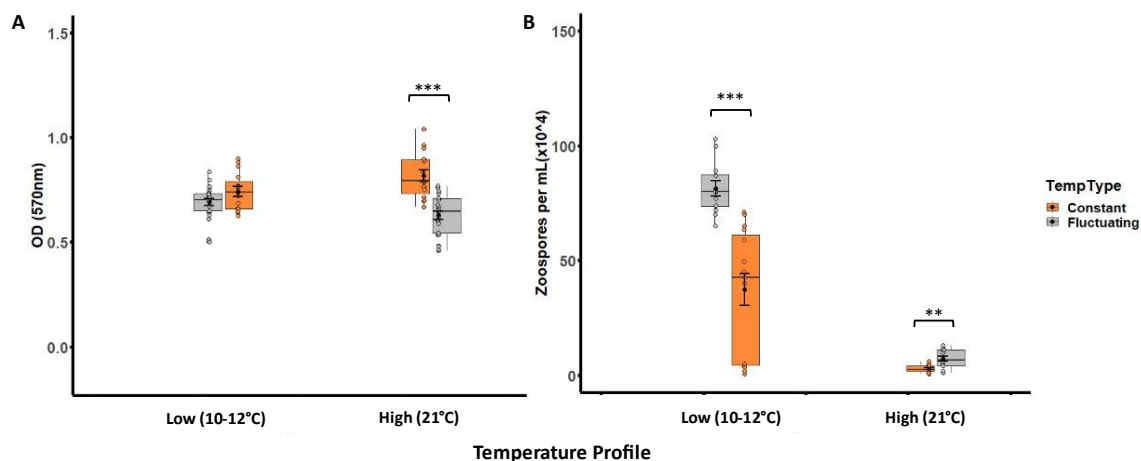


Figure 3-5. Constant versus fluctuating temperature maximum zoospore densities and whole culture viability for the New Mexico site. A) Boxplots show the maximum whole culture viability via optical density (OD) readings at 570nm and B) boxplots show the maximum zoospore densities of the two simulated fluctuating seasonal treatments compared to the constant temperatures this isolate was previously grown in. The summer fluctuating conditions varied each day and had a daily average temperature of 21.7°C, while the winter fluctuating conditions varied each day and had a daily average temperature of 10.6°C. Both simulated, fluctuating conditions are shown in gray. The constant temperatures most closely matching the daily average of each simulated season are shown in orange (constant temperature data used from Sheets et al., 2021). Each boxplot consists of data from a 2-3 day maximum period during the thermal experiment. Significance labels are shown with * marks to indicate degrees of significance between lineages as follows: * <0.05, ** <0.01, *** <0.001.

Discussion

Understanding how variable environmental temperatures influence pathogen physiology and subsequent infection rates is important to understanding disease dynamics (Gajweski et al., 2021; Stevenson et al., 2013). The aim of this study was to test a *Bd* isolate from the New Mexico region and relate its established thermal physiology to the seasonal temperatures measured in the wild where *Bd* has caused disease in amphibian hosts (Christman & Jennings, 2018). To do this, I

collected water temperature data and infection data from multiple sites in southern New Mexico and simulated the daily fluctuating temperatures measured in winter and summer where *Bd* was detected. I then grew the *Bd* isolate from New Mexico in those simulated winter and summer conditions to understand its thermal responses to fluctuating seasonal conditions. I compared the seasonal simulation data to data from previously tested constant temperatures, which was similar to the daily average temperature of each respective seasonal condition.

From field data collected over a three-year period at the study area, I found that the prevalence of infection was higher in the summer and fall seasons than in winter and spring, with fall having the highest *Bd* prevalence overall. My results suggest that infection patterns within amphibian populations in this specific region exhibit a seasonal pattern in *Bd* infection prevalence and intensity, which is similar to other studied temperate regions that found an effect of season on prevalence or intensity of infection (Kinney et al., 2011; Petersen et al., 2016; Sonn et al., 2019). For example, Sonn et al., (2019) showed that prevalence was influenced by seasonal climate variation. I observed differences in seasonal prevalence that may be influenced by the climate variation observed and simulated from daily water temperatures. However, I found that the seasonal patterns for *Bd* prevalence and intensity of infection differed from other studies. For example, I found that the intensity of infection was highest in the fall and winter seasons compared to the spring and summer seasons as reported in other studies (Petersen et al., 2016; Wilber et al., 2022). In addition, there was no decrease in the prevalence and intensity of infection from the spring to summer seasons as exhibited in other study areas across the U.S. (Petersen et al., 2016; Wilber et al., 2022). The unique characteristics of this study area have led to variations in the observed seasonal patterns compared to other research. This underscores the importance of considering local climate variation and specific ecological factors when interpreting infection dynamics in amphibian populations.

I found that fluctuating thermal conditions altered the growth and reproduction of the *Bd* isolate from New Mexico. In previous laboratory experiments, the NM *Bd* isolate did not produce zoospores at the temperatures of 17°C or greater, and generally had higher zoospore densities and fecundity at lower temperatures (Sheets et al., 2021). Additionally, this NM isolate had a thermal maximum temperature (CT_{max}) of 25°C for growth and reproductive traits (Sheets et al., 2021). However, my results suggest that this isolate was able to withstand and complete a full life cycle of growth and reproduction in fluctuating summer conditions, where the isolate was repeatedly exposed to temperatures above its thermal maximum for several hours. When grown in fluctuating seasonal conditions, the *Bd* isolate from New Mexico showed reduced maximum zoospore densities and whole culture viability in the summer conditions compared to the winter conditions. These results of comparing *Bd* growth and reproductive traits in winter versus summer conditions align with my predictions.

I found that *Bd* survived in higher temperatures than expected based on its known thermal range (Piotrowski et al., 2004). For example, simulated summer field temperatures, which exceeded 35°C for several hours each day, did not prevent *Bd* growth as might be predicted based on the critical thermal maximum (CT_{max}) of this isolate (Sheets et al., 2021). The maximum whole culture viability was not reduced following exposure to extreme temperatures above the CT_{max} temperature of 25°C (Sheets et al., 2021). Researchers have questioned if recovery (i.e., ability to resume growth and reproduction by exposure to lower temperatures after being at high temperatures) may enable *Bd*'s heat tolerance (i.e., ability to survive temporarily at higher temperatures; Kásler et al., 2022). One factor that may have influenced the *Bd*'s response to simulated summer temperatures are the cooler temperatures experienced during the early morning or middle of the night, which could have allowed the isolate to recover from potential "heat shock" effects (Kásler et al., 2022). My study serves as an initial step to determine which factors

of temperature variation within seasonal dynamics are responsible for *Bd*'s heat tolerance and recovery.

Previous studies have examined whether mean, constant temperatures are the best way to assess the thermal performance of *Bd* compared to variable temperatures (Greenspan et al., 2023). The results of my study supported my predictions that the isolate from NM had reduced maximum whole culture viability in fluctuating summer conditions (with an average daily temperature of 21.7°C) compared to maximum whole culture viability at a constant 21°C. These results of the summer fluctuating condition treatment compared to the constant 21°C results corroborate previous studies (Lindauer et al., 2020). However, fluctuating summer conditions did not show a reduction in maximum zoospore densities when compared to the constant 21°C in my study, contradicting previous studies with other *Bd* isolates (Lindauer et al., 2020). When comparing trait responses in winter conditions that had an average daily temperature of 10.6°C with a constant temperature of 12°C, I found no differences in maximum culture viability between the two temperature treatments but an increase in maximum zoospore densities. The statistical difference in maximum zoospore densities between these two treatment groups may be due to the difference in mean temperature. However, my results suggest that the relationship between fluctuating daily temperatures and *Bd* physiological traits will likely be difficult to predict and support literature suggesting an incorporation of variable temperatures when studying *Bd* physiology in regard to disease dynamics (Raffel et al., 2006; Stevenson et al., 2013).

Overall, in this study, I found support for the hypothesis that seasonal temperature fluctuations impact *Bd* growth and reproduction (Raffel et al., 2013; Sonn et al., 2019). This study shows that temperature variability across seasons can alter the outcomes of *Bd* reproductive physiology, and the subsequent predictions that can be made about disease dynamics in wild amphibian populations (Gajweski et al., 2021; Raffel et al., 2013; Stevenson et al., 2013). Not only do variable versus constant temperatures impact our understanding of *Bd* growth and

reproduction but seasonal sampling at a local or regional scale can aid in anticipating *Bd* die-off events (Berger et al., 2004).

Understanding the complexities that variable temperatures and seasonal conditions have on pathogen growth and reproduction can allow disease researchers to better adjust for effective local conservation actions (Rohr et al., 2008). For example, management practices for mitigating the effects of chytridiomycosis should use the available information on regional seasonal effects to facilitate coordinating actions of animal movement (i.e., animal translocation or release) with appropriate timing of low prevalence or intensity of infection in the future. This study has a focus on *in vitro* physiological responses of *Bd*, however future studies are needed to accommodate gaps of knowledge in select seasons with mixed results as well as incorporating host defense aspects into our understanding of seasonal disease dynamics (Petersen et al., 2016; Sonn et al., 2019). Generally understanding the impact of variable temperatures on pathogen physiology is equally important to mitigate the effects of emerging infectious diseases causing amphibian population declines (Daszak et al., 1999).

References

- Alford, R. A., Bradfield, K. S., & Richards, S. J. (2007). Global warming and amphibian losses. *Nature*, 447(7144), Article 7144. <https://doi.org/10.1038/nature05940>
- Bates, D., Machler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J Stat Softw* 67, 1–48. 10.18637/jss.v067.i01.
- Becker, C. G., Greenspan, S. E., Tracy, K. E., Dash, J. A., Lambertini, C., Jenkinson, T. S., Leite, D. S., Toledo, L. F., Longcore, J. E., James, T. Y., & Zamudio, K. R. (2017). Variation in phenotype and virulence among enzootic and panzootic amphibian chytrid lineages. *Fungal Ecology*, 26, 45–50. <https://doi.org/10.1016/j.funeco.2016.11.007>
- Berger, L., Speare, R., Daszak, P., Green, D. E., Cunningham, A. A., Goggin, C. L., Slocombe, R., Ragan, M. A., Hyatt, A. D., McDonald, K. R., Hines, H. B., Lips, K. R., Marantelli, G., & Parkes, H. (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*, 95(15), 9031–9036. <https://doi.org/10.1073/pnas.95.15.9031>
- Berger, L., Speare, R., Hines, H., Marantelli, G., Hyatt, A., McDONALD, K., Skerratt, L., Olsen, V., Clarke, J., Gillespie, G., Mahony, M., Sheppard, N., Williams, C., & Tyler, M. (2004). Effect of season and temperature on mortality in amphibians due to chytridiomycosis. *Australian Veterinary Journal*, 82(7), 434–439. <https://doi.org/10.1111/j.1751-0813.2004.tb11137.x>
- Blaustein, A. R., Walls, S. C., Bancroft, B. A., Lawler, J. J., Searle, C. L., & Gervasi, S. S. (2010). Direct and Indirect Effects of Climate Change on Amphibian Populations. *Diversity*, 2(2), 281–313. <https://doi.org/10.3390/d2020281>
- Bosch, J., Martínez-Solano, I., & García-París, M. (2001). Evidence of a chytrid fungus infection involved in the decline of the common midwife toad (*Alytes obstetricans*) in protected

areas of central Spain. *Biological Conservation*, 97(3), 331–337.

[https://doi.org/10.1016/S0006-3207\(00\)00132-4](https://doi.org/10.1016/S0006-3207(00)00132-4)

Boyle, D., Boyle, D., Olsen, V., Morgan, J., & Hyatt, A. (2004). Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. *Diseases of Aquatic Organisms*, 60, 141–148.

<https://doi.org/10.3354/dao060141>

Christman, B. L., & Jennings, R. D. (2018). Distribution of the Amphibian Chytrid Fungus *Batrachochytrium dendrobatidis* (Bd) in New Mexico. New Mexico Department of Game and Fish, Santa Fe, New Mexico, USA.

Daszak, P., Berger, L., Cunningham, A. A., Hyatt, A. D., Green, D. E., & Speare, R. (1999). Emerging Infectious Diseases and Amphibian Population Declines. *Emerging Infectious Diseases*, 5(6), 735–748. <https://doi.org/10.3201/eid0506.990601>

Daszak, P., Cunningham, A. A., & Hyatt, A. D. (2003). Infectious disease and amphibian population declines. *Diversity & Distributions*, 9(2), 141–150. <https://doi.org/10.1046/j.1472-4642.2003.00016.x>

Ferguson, L. V., & Sinclair, B. J. (2020). Thermal Variability and Plasticity Drive the Outcome of a Host-Pathogen Interaction. *The American Naturalist*, 195(4), 603–615. <https://doi.org/10.1086/707545>

Fernández-Loras, A., Boyero, L., Correa-Araneda, F., Tejedo, M., Hettyey, A., & Bosch, J. (2019). Infection with *Batrachochytrium dendrobatidis* lowers heat tolerance of tadpole hosts and cannot be cleared by brief exposure to CTmax. *PLOS ONE*, 14(4), e0216090. <https://doi.org/10.1371/journal.pone.0216090>

Forrest, M. J., Edwards, M. S., Rivera, R., Sjöberg, J. C., & Jaeger, J. R. (2015). HIGH PREVALENCE AND SEASONAL PERSISTENCE OF AMPHIBIAN CHYTRID FUNGUS INFECTIONS IN THE DESERT-DWELLING AMARGOSA TOAD,

- ANAXYRUS NELSONI. *Herpetological Conservation and Biology*, 10(3), 917–925.
- Fox, J., and Weisberg, S. (2019). *An R Companion to Applied Regression* 3rd ed. (Sage).
- Gajewski, Z., Stevenson, L. A., Pike, D. A., Roznik, E. A., Alford, R. A., & Johnson, L. R. (2021). Predicting the growth of the amphibian chytrid fungus in varying temperature environments. *Ecology and Evolution*, 11(24), 17920–17931.
<https://doi.org/10.1002/ece3.8379>
- Greenspan, S. E., Roznik, E. A., Edwards, L., Duffy, R., Berger, L., Bower, D. S., Pike, D. A., Schwarzkopf, L., & Alford, R. A. (2023). Constant-temperature predictions underestimate growth of a fungal amphibian pathogen under individual host thermal profiles. *Journal of Thermal Biology*, 111, 103394.
<https://doi.org/10.1016/j.jtherbio.2022.103394>
- Hinderer, R. K., Litt, A. R., & McCaffery, M. (2021). Habitat selection by a threatened desert amphibian. *Ecology and Evolution*, 11(1), 536–546. <https://doi.org/10.1002/ece3.7074>
- Hyatt, A., Boyle, D., Olsen, V., Boyle, D., Berger, L., Obendorf, D., Dalton, A., Kriger, K., Hero, M., Hines, H., Phillott, R., Campbell, R., Marantelli, G., Gleason, F., & Colling, A. (2007). Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. *Diseases of Aquatic Organisms*, 73, 175–192.
<https://doi.org/10.3354/dao073175>
- Kamoroff, C., & Goldberg, C. (2017). Using environmental DNA for early detection of amphibian chytrid fungus *Batrachochytrium dendrobatidis* prior to a rapid die-off. *Diseases of Aquatic Organisms*, 127(1), 75–79. <https://doi.org/10.3354/dao03183>
- Kásler, A., Ujszegi, J., Holly, D., Jaloveczki, B., Gál, Z., & Hettyey, A. (2022). In vitro thermal tolerance of a hypervirulent lineage of *Batrachochytrium dendrobatidis*: Growth arrestment by elevated temperature and recovery following thermal treatment. *Mycologia*, 114(4), 661–669. <https://doi.org/10.1080/00275514.2022.2065443>

- Kinney, V. C., Heemeyer, J. L., Pessier, A. P., & Lannoo, M. J. (2011). Seasonal Pattern of *Batrachochytrium dendrobatidis* Infection and Mortality in *Lithobates areolatus*: Affirmation of Vredenburg's "10,000 Zoospore Rule." *PLoS ONE*, 6(3), e16708. <https://doi.org/10.1371/journal.pone.0016708>
- Knapp, R. A., Briggs, C. J., Smith, T. C., & Maurer, J. R. (2011). Nowhere to hide: Impact of a temperature-sensitive amphibian pathogen along an elevation gradient in the temperate zone. *Ecosphere*, 2(8), art93. <https://doi.org/10.1890/ES11-00028.1>
- Kruger, K. M., & Hero, J. -M. (2007). Large-scale seasonal variation in the prevalence and severity of chytridiomycosis. *Journal of Zoology*, 271(3), 352–359. <https://doi.org/10.1111/j.1469-7998.2006.00220.x>
- Lambertini, C., Becker, C. G., Jenkinson, T. S., Rodriguez, D., da Silva Leite, D., James, T. Y., Zamudio, K. R., & Toledo, L. F. (2016). Local phenotypic variation in amphibian-killing fungus predicts infection dynamics. *Fungal Ecology*, 20, 15–21. <https://doi.org/10.1016/j.funeco.2015.09.014>
- Lenth, R. (2020). emmeans: estimated marginal means, aka least-squares means.
- Lindauer, A. L., Maier, P. A., & Voyles, J. (2020). Daily fluctuating temperatures decrease growth and reproduction rate of a lethal amphibian fungal pathogen in culture. *BMC Ecology*, 20(1), 18. <https://doi.org/10.1186/s12898-020-00286-7>
- Muletz-Wolz, C. R., Barnett, S. E., DiRenzo, G. V., Zamudio, K. R., Toledo, L. F., James, T. Y., & Lips, K. R. (2019). Diverse genotypes of the amphibian-killing fungus produce distinct phenotypes through plastic responses to temperature. *Journal of Evolutionary Biology*, 32(3), 287–298. <https://doi.org/10.1111/jeb.13413>
- O'Brien, C., Van Riper, C., & Myers, D. E. (2009). Making Reliable Decisions in the Study of Wildlife Diseases: Using Hypothesis Tests, Statistical Power, and Observed Effects. *Journal of Wildlife Diseases*, 45(3), 700–712. <https://doi.org/10.7589/0090-3558->

45.3.700

- Petersen, C. E., Lovich, R. E., Phillips, C. A., Dreslik, M. J., & Lannoo, M. J. (2016). Prevalence and Seasonality of the Amphibian Chytrid Fungus *Batrachochytrium dendrobatidis* Along Widely Separated Longitudes Across the United States. *EcoHealth*, *13*(2), 368–382. <https://doi.org/10.1007/s10393-016-1101-4>
- Piotrowski, J. S., Annis, S. L., & Longcore, J. E. (2004). Physiology of *Batrachochytrium dendrobatidis*, a Chytrid Pathogen of Amphibians. *Mycologia*, *96*(1), Article 1. <https://doi.org/10.2307/3761981>
- Pounds, A. J., Bustamante, M. R., Coloma, L. A., Consuegra, J. A., Fogden, M. P. L., Foster, P. N., La Marca, E., Masters, K. L., Merino-Viteri, A., Puschendorf, R., Ron, S. R., Sánchez-Azofeifa, G. A., Still, C. J., & Young, B. E. (2006). Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature*, *439*(7073), Article 7073. <https://doi.org/10.1038/nature04246>
- Raffel, T. R., Halstead, N. T., McMahon, T. A., Davis, A. K., & Rohr, J. R. (2015). Temperature variability and moisture synergistically interact to exacerbate an epizootic disease. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1801), 20142039. <https://doi.org/10.1098/rspb.2014.2039>
- Raffel, T. R., Rohr, J. R., Kiesecker, J. M., & Hudson, P. J. (2006). Negative effects of changing temperature on amphibian immunity under field conditions. *Functional Ecology*, *20*(5), Article 5. <https://doi.org/10.1111/j.1365-2435.2006.01159.x>
- Raffel, T. R., Romansic, J. M., Halstead, N. T., McMahon, T. A., Venesky, M. D., & Rohr, J. R. (2013). Disease and thermal acclimation in a more variable and unpredictable climate. *Nature Climate Change*, *3*(2), 146–151. <https://doi.org/10.1038/nclimate1659>
- R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.

- Rohr, J. R., Raffel, T. R., Romansic, J. M., McCallum, H., & Hudson, P. J. (2008). Evaluating the links between climate, disease spread, and amphibian declines. *Proceedings of the National Academy of Sciences*, *105*(45), Article 45. <https://doi.org/10.1073/pnas.0806368105>
- Rohr, J. R., & Raffel, T. R. (2010). Linking global climate and temperature variability to widespread amphibian declines putatively caused by disease. *Proceedings of the National Academy of Sciences*, *107*(18), 8269–8274. <https://doi.org/10.1073/pnas.0912883107>
- Sapsford, S. J., Alford, R. A., & Schwarzkopf, L. (2013). Elevation, Temperature, and Aquatic Connectivity All Influence the Infection Dynamics of the Amphibian Chytrid Fungus in Adult Frogs. *PLoS ONE*, *8*(12), e82425. <https://doi.org/10.1371/journal.pone.0082425>
- Sheets, C. N., Schmidt, D. R., Hurtado, P. J., Byrne, A. Q., Rosenblum, E. B., Richards-Zawacki, C. L., & Voyles, J. (2021). Thermal Performance Curves of Multiple Isolates of *Batrachochytrium dendrobatidis*, a Lethal Pathogen of Amphibians. *Frontiers in Veterinary Science*, *8*. <https://www.frontiersin.org/article/10.3389/fvets.2021.687084>
- Shoo, L. P., Olson, D. H., McMenamin, S. K., Murray, K. A., Van Sluys, M., Donnelly, M. A., Stratford, D., Terhivuo, J., Merino-Viteri, A., Herbert, S. M., Bishop, P. J., Corn, P. S., Dovey, L., Griffiths, R. A., Lowe, K., Mahony, M., McCallum, H., Shuker, J. D., Simpkins, C., ... Hero, J.-M. (2011). Engineering a future for amphibians under climate change: Engineering a future for amphibians. *Journal of Applied Ecology*, *48*(2), 487–492. <https://doi.org/10.1111/j.1365-2664.2010.01942.x>
- Sonn, J. M., Utz, R. M., & Richards-Zawacki, C. L. (2019). Effects of latitudinal, seasonal, and daily temperature variations on chytrid fungal infections in a North American frog. *Ecosphere*, *10*(11). <https://doi.org/10.1002/ecs2.2892>
- Stevenson, L. A., Alford, R. A., Bell, S. C., Roznik, E. A., Berger, L., & Pike, D. A. (2013). Variation in Thermal Performance of a Widespread Pathogen, the Amphibian Chytrid

Fungus *Batrachochytrium dendrobatidis*. *PLoS ONE*, 8(9), e73830.

<https://doi.org/10.1371/journal.pone.0073830>

Vasseur, D. A., DeLong, J. P., Gilbert, B., Greig, H. S., Harley, C. D. G., McCann, K. S., Savage,

V., Tunney, T. D., & O'Connor, M. I. (2014). Increased temperature variation poses a greater risk to species than climate warming. *Proceedings of the Royal Society B: Biological Sciences*, 281(1779), 20132612. <https://doi.org/10.1098/rspb.2013.2612>

Biological Sciences, 281(1779), 20132612. <https://doi.org/10.1098/rspb.2013.2612>

Voyles, J. (2011). Phenotypic profiling of *Batrachochytrium dendrobatidis*, a lethal fungal pathogen of amphibians. *Fungal Ecology*, 4(3), 196–200.

<https://doi.org/10.1016/j.funeco.2010.12.003>

Voyles, J., Johnson, L. R., Rohr, J., Kelly, R., Barron, C., Miller, D., Minster, J., & Rosenblum,

E. B. (2017). Diversity in growth patterns among strains of the lethal fungal pathogen

Batrachochytrium dendrobatidis across extended thermal optima. *Oecologia*, 184(2),

Article 2. <https://doi.org/10.1007/s00442-017-3866-8>

Wilber, M. Q., Ohmer, M. E. B., Altman, K. A., Brannelly, L. A., LaBumbard, B. C., Le Sage, E.

H., McDonnell, N. B., Muñoz Torres, A. Y., Nordheim, C. L., Pfab, F., Richards-

Zawacki, C. L., Rollins-Smith, L. A., Saenz, V., Voyles, J., Wetzel, D. P., Woodhams, D.

C., & Briggs, C. J. (2022). Once a reservoir, always a reservoir? Seasonality affects the pathogen maintenance potential of amphibian hosts. *Ecology*, 103(9).

<https://doi.org/10.1002/ecy.3759>

Woodhams, D. C., & Alford, R. A. (2005). Ecology of Chytridiomycosis in Rainforest Stream

Frog Assemblages of Tropical Queensland. *Conservation Biology*, 19(5), 1449–1459.

<https://doi.org/10.1111/j.1523-1739.2005.004403.x>

Chapter 4: Using Experimental evolution to test the effects of climate change on the growth and reproduction of a lethal fungal pathogen (*Batrachochytrium dendrobatidis*)

Introduction

Experimental evolution is a technique that scientists use to investigate evolutionary dynamics through the manipulation of organismal traits and processes (Bennett & Lenski, 1993; Bennett & Houghs, 2009). For example, disease ecologists use experimental evolution to answer questions concerning the evolutionary processes of organisms that contribute to disease dynamics (Ebert, 1998). Generally, disease ecologists are interested in understanding how the environment, host, and pathogen interact influence disease outcomes (Altizer et al., 2013). By subjecting pathogens and their hosts to controlled experimental conditions, researchers can observe and measure the effects of specific environmental and genetic factors on the evolution of infectious diseases (Fay et al., 2021; Kawecki et al., 2012). Experimental evolution has shown thermal adaptation is possible in various microorganisms, including bacteria, fungi, and viruses (Bennett & Lenski, 2007; Ebert, 1998; Fay et al., 2021; Lambrechts et al., 2011; Voyles et al., 2014). Thus, this approach has led to many important discoveries and has helped shed light on key questions related to the emergence, spread, or virulence of infectious diseases (Lambrechts et al., 2011).

Global warming and climate change are predicted to influence pathogen evolution in many infectious disease systems (Altizer et al., 2013; Fay et al., 2021; Lafferty, 2009; Rohr et al., 2008, 2011). Studies have examined short-term responses of pathogens to temperature changes, but few have investigated the effects of sustained high temperatures on pathogen evolution over longer periods (Lenski, 2017). Further research is needed to understand the potential long-term impacts of environmental changes on pathogen evolution (Lenski, 2017). One effective method to investigate either short-term or long-term experimental evolution for pathogens to climate changes is using serial

passage experiments (SPEs; Lenski, 2017). SPEs are used when conducting experimental evolution studies that aim to track changes in pathogen genotypes, phenotypes, or general physiological trait responses (Bazin 2003; Woo & Reifman, 2014). The timing and conditions of propagation can influence the direction of shifts in pathogens' abilities to exploit available resources (Ford et al., 2002; Somerville et al., 2002; Voyles et al., 2014).

The rising temperatures associated with global warming can affect pathogen growth, reproduction, and infectivity, as shown in different controlled experiments (Ford & Chintala, 2006; Robin et al., 2017; Voyles et al., 2012; Wu et al., 2022). Higher environmental temperatures may act as a selective pressure for pathogens, particularly those that prefer cooler temperatures (Bennett et al., 2021). However, the specific impacts that climate change may have on pathogen evolution in terms of pathogen-host interactions remain poorly understood (Cohen et al., 2019; Lafferty & Mordecai, 2016; Rohr et al., 2011; Schampera et al., 2022). Predicting the adaptive potential of any organism to changing environments is muddled by trade-offs that exist among physiological traits and multivariable stressors that synergistically act on the study organism (Deardorff et al., 2011; Gunderson et al., 2016; Kelly, 2022). Ultimately, understanding how environmental conditions affect pathogen evolution is relevant for predicting future pathogen dynamics under climate change conditions (Altizer et al., 2013).

The chytridiomycosis system provides a unique opportunity to study rapid adaptation to climate change in a highly lethal pathogen that poses a serious threat to amphibians (Rohr et al., 2010, 2011). Chytridiomycosis is again caused by the pathogen *Batrachochytrium dendrobatidis* (*Bd*) and has two life stages (Berger et al., 2005; Longcore et al., 1999). Each life stage develops in a distinct location relative to the infected host and has been shown to be affected by temperature (Berger et al., 2005; Woodhams et al., 2008; Voyles et al., 2012). Several hypotheses have been proposed and debated to understand *Bd*'s response to climate change (Cohen et al., 2019; Rohr et al., 2008; Rohr et

al., 2018; Raffel et al., 2013). As daily temperatures are predicted to exceed the critical thermal maximum of *Bd* isolates, it is possible that *Bd* will experience heat stress that leads to decreased survival rates, potentially benefiting susceptible amphibian host populations (Cohen et al., 2019; Daskin et al., 2011; Neely et al., 2020). Alternatively, *Bd* may rapidly evolve to have a higher thermal tolerance and become more pathogenic to amphibian host species (Hamilton et al., 2012). Serial passaging of *Bd* isolates in multiple thermal treatments is a promising approach to study the adaptive potential of fluctuating temperatures on pathogen traits (Fay et al., 2021). By understanding the potential outcomes of *Bd* adaptation to changing temperatures, I may be better equipped to predict and manage the spread of this deadly pathogen under climate change conditions (Rohr et al., 2008).

I conducted a series of studies where I assessed *Bd* growth (maximum whole culture viability) and reproductive (maximum zoospore densities) responses to climate change by serially passaging multiple *Bd* isolates over many life cycles in simulated climate change conditions (Fig. 4-4). For experiment 1, I hypothesized that the *Bd* lineage experimentally evolved in warmer, “Future” climate change conditions would have increased maximum whole culture viability and increased maximum zoospore densities compared to the *Bd* lineage experimentally evolved in cooler, “Current” climate change conditions following serial passaging, indicating potential adaptation to future climate change conditions (Table 4-1). For experiment 2, I hypothesized that an isolate showing evidence for potential adaptation (i.e., increased response in maximum whole culture or maximum zoospore densities after SPE) to Future climate change conditions would maintain increased maximum whole culture viability or maximum zoospore densities after an additional acclimation period (Table 4-1). For experiment 3, I compared the evolved *Bd* lineage that showed evidence for potential adaptation to the Ancestor *Bd* isolate lineage to determine if the evolved lineage differed in its thermal performance curves (TPC) for maximum whole culture viability and maximum zoospore densities. For experiment 3, I hypothesized that the “Future” lineage would have

a wider thermal breadth, higher maximum whole culture viability, and higher maximum zoospore densities compared to the Ancestor lineage of the assessed isolate (Table 4-1; Fig. 4-1). Additionally, for experiment 3, I hypothesized that the “Hot” lineage would have a higher thermal optimum temperature indicating a horizontal change in the TPC. For experiment 4, I hypothesized that an isolate showing increased maximum zoospore densities in Future climate change conditions would have greater whole culture viability and maximum zoospore densities in the presence of inhibitory host skin secretions compared to its Ancestor lineage (Table 4-1).

Table 4-1: Experimental overview for series of experiments within this study. Within this study there are a series of experiments that can be categorized and explained in four parts. In this table, each part of the study has been defined by the question the experiment was addressing and the subsequent hypotheses for that question.

Part I: Fully factorial experimental evolution to climate change	Part II: Acclimation effect experiment after experimental evolution	Part III: Ancestral versus evolved TPC comparisons	Part IV: Experimental evolution affect to pathogenicity
<u>Question:</u> Will <i>Bd</i> rapidly adapt to increased mean temperatures in simulated climate change conditions?	<u>Question:</u> Are the results of experimental evolution evidence of potential evolution or acclimation effects?	<u>Question:</u> Did the experimental evolution to climate change conditions cause a shift in <i>Bd</i> 's TPC?	<u>Question:</u> Did the experimental evolution to climate change conditions cause a change in pathogen growth inhibition in the presence of host defenses?
<u>Hypotheses:</u> Increased temperatures from future fluctuating climate conditions will result in the rapid adaption of <i>Bd</i> through increased growth and reproduction.	<u>Hypotheses:</u> The increased growth and reproduction of <i>Bd</i> observed after experimental evolution will remain after an additional acclimation period, indicating potential adaptation rather than acclimation.	<u>Hypotheses:</u> Experimental evolution to future climate change conditions will result in a horizontal shift in <i>Bd</i> 's TPC where potential adaptation is observed.	<u>Hypotheses:</u> Experimental evolution to future climate change conditions will result in decreased pathogen growth inhibition when grown in the presence of host skin secretions.

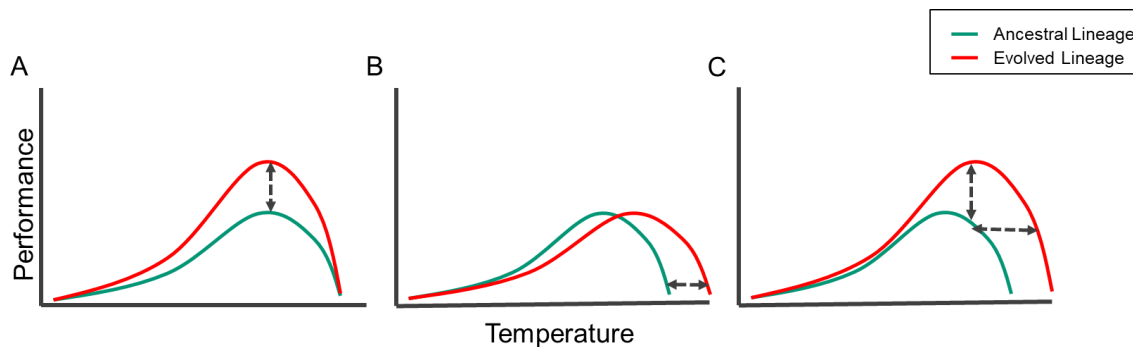


Figure 4-1. Predicted changes in *Batrachochytrium dendrobatidis*' thermal performance curve for the Ancestor versus evolved lineages of *Bd*. A) Displays a vertical change in the TPC after experimental evolution that would represent the P_{max} being changed at the T_{opt} but the CT_{min} and CT_{max} are unchanged. B) Displays a horizontal change in the TPC that would represent the P_{max} being unchanged at the T_{opt} but the CT_{min} and CT_{max} are altered. C) Displays both a horizontal and vertical change in the TPC that would represent the P_{max} being changed at the T_{opt} , the CT_{min} , and CT_{max} being altered. The predictions of the changes for my experiment are shown in each scenario with the Ancestor lineage in green and an evolved lineage of choice being shown in red. I predicted that the Hot lineage would show a change in the TPC similar to that shown in B and the Future lineage would show a change in the TPC as shown in either A or C.

Methods

For all experiments: Predicting amphibian body temperatures under climate change

Using models from a collaborator, I obtained estimates of mean spring frog body temperatures under current and future projected climates. Amphibian body temperatures for a mean day for each month of the year were calculated in Niche Mapper™, using downscaled microclimate data and biophysical parameters for a typical Ranid frog, following previous studies (Bartelt et al., 2010; Fig. 4-2). For current conditions, collaborators used downscaled WorldClim2 global temperature data (50-year average, WorldClim2, 30 second resolution; Fick & Hijmans, 2017). For future conditions, collaborators used temperatures predicted in 2070 (downscaled IPCC5 data at 30 second resolution, calibrated using WorldClim 1.4 database, global climate model (GCM)

HadGEM2-ES (HE), representative concentration pathway (RCP) 60; Fick & Hijmans, 2017). Niche Mapper™ is a Fortran program that employs two mechanistic models, one to model microclimate in a region of interest, and one to model body temperatures of ectothermic organisms in that same region.

To determine relevant experimental temperature conditions to simulate climate change, collaborators modeled amphibian body temperature in the month of May at a field site near Ohio (Linesville, Pennsylvania). At this time of year, both *Bd* prevalence and host densities are high in wild populations near the location where the *Bd* isolate originated. To simulate the predicted fluctuating current and future thermal conditions, I used programmable environmental chambers (Conviron) that fluctuate in temperature over a 24-hour period (Fig. 4-3).

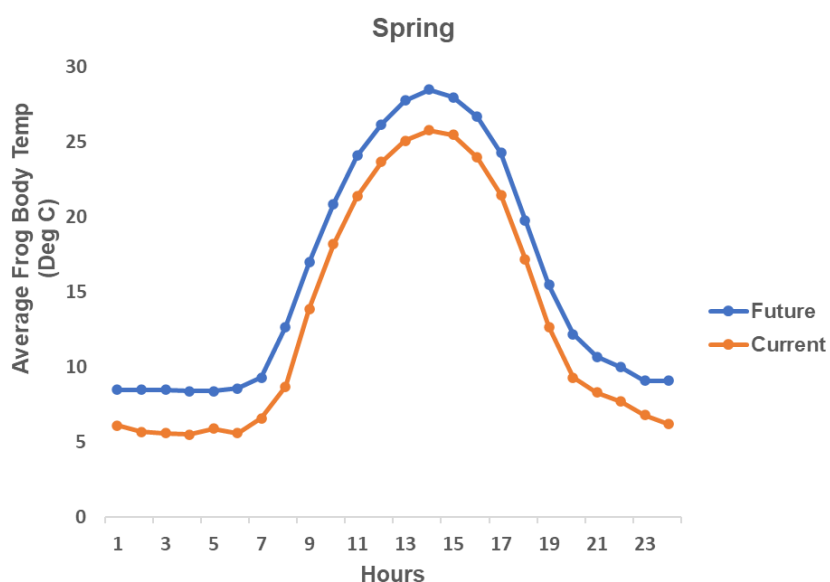


Figure 4-2. Modeled current and future amphibian body temperatures within a 24-hour period for May (early spring) in Ohio, near where the *Bd* isolate was collected. The current temperature profile (orange) of average frog body temperatures fluctuates between 5-25°C in a 24-hour period, while the future temperature profile (blue) fluctuates between 8-28°C.

For all experiments: Experimental evolution with *Bd*

I serially propagated each of five *Bd* isolates from a previous study (Sheets et al., 2021) in three lineages per isolate. Specifically, I assigned all the propagated lineages to a respective environmental chamber and experimentally evolved each lineage through serial passaging in one of three programmed climate conditions: 1) simulated “Current” thermal treatment (fluctuating from 5-25°C daily), 2) a simulated “Future” thermal treatment (fluctuating from 8-28°C daily), or 3) a stable, “Optimum” thermal treatment (control; 21°C; Fig. 4-3). Additionally, I serially passaged a *Bd* isolate collected from Louisiana (LA) in a stable, maxima “Hot” thermal treatment (control; 27°C). I selected the critical thermal maximum (27°C) for serial passage of LA *Bd* isolate “Hot” lineage for testing in experiments 3 and 4.

I serially passaged *Bd* in 75² cm flasks with TGhL media 45-70 times to complete the experimental evolution of each isolate (Voyles et al., 2014). I considered a “passage” to be the point in time when I observed the maximum sporangia growth and zoospore densities in a flask using light microscopy, which indicated the time when actively growing culture could be transferred to a new flask with fresh media (Longcore et al., 1999). I cryo-archived 4-6 aliquots of each isolate (Boyle et al., 2004) and its respective lineages every ten passages until I reached the 45th passage, concluding the initial serial passage experiment (Fig. 4-4). I cryo-archived each isolate and its lineage between 70-100 passages as an additional extension to the end point of our initial study.

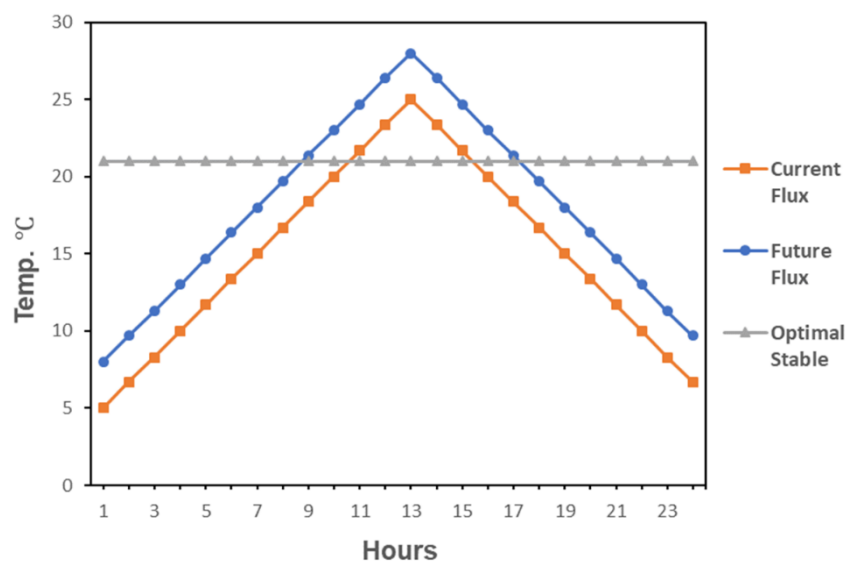


Figure 4-3. Three simulated temperature profiles for *Batrachocytrium dendrobatidis* experimentally evolved *in vitro*. The Optimal temperature profile (gray) stays at a constant, stable 21°C. The Current fluctuating temperature profile (orange) fluctuates between 8-28°C. The Future fluctuating temperature profile (blue) fluctuates between 5-25°C each day. Each temperature profile was simulated within its own environmental chamber. These conditions are consistent throughout the serial passage experiment and fluctuate over 24-hour periods in even intervals when fluctuation is applicable.

For all experiments: Quantifying *Bd* growth (maximum whole culture viability) and reproductive (maximum zoospore densities) responses

For all experiments, I monitored the growth of cultures until I observed the day of maximum zoospore densities using light microscopy. On that day, I filtered out zoosporangia using a standard protocol for each lineage of each isolate (Voyles, 2011). I diluted the filtrate containing zoospores with TGH media to a concentration of 50×10^4 zoospores mL^{-1} for each respective lineage of each isolate. I inoculated the zoospore filtrate of each lineage into ten wells in a clear, flat-bottomed 96-well plate. I repeated the same design for 20-40 plates (depending on the experiment) and each plate was randomly labeled for the day of data collection. I set up each row of plate containing a lineage

with five experimental wells containing live zoospores and five negative control wells containing heat-killed zoospores. I pipetted 50 μ l of TGhL media and 50 μ l of culture inoculum into each sample well. I randomly assigned plates into the environmental chamber (Convion) that corresponded with a specific temperature treatment.

For data collection, I used one plate each day to quantify maximum whole culture viability and/or maximum zoospore densities throughout the experiment. To measure maximum whole culture viability, I used a MTT viability assay (Lindauer et al., 2019). I measured maximum zoospore densities by counting zoospores using a hemocytometer from five randomly selected wells of the 96-well plate (Voyles et al., 2011).

Experiment 1: Comparison of evolved lineages

After 45 passages in Optimum, Future, or Current temperature conditions, I measured maximum whole culture viability and maximum zoospore densities in a fully factorial design across temperature treatments (Fig. 4-4). As stated above, each row of the 96-well plate contained an isolate and one of its three lineages to be quantified at the assigned temperature treatment over the course of a full life cycle.

Experiment 2: Acclimation study post experimental evolution

I used the OH isolate and its lineages to determine whether *Bd*'s response to Future climate conditions was due to potential adaptation or acclimation. I focused on the OH isolate because it showed higher maximum zoospore densities (which is thought to be important for pathogenicity; Langhammer et al., 2013) in the Future lineage across all temperature treatments compared to the Current lineage. Additionally, this isolate had high maximum zoospore densities in general at some of the tested thermal conditions. I tested for acclimation effects by allowing the Optimum, Future, and

Current lineages to grow in the Optimum temperature treatment (21°C) for ~10 passages before retesting each lineage's maximum whole culture viability and maximum zoospore densities at 21°C (Fig. 4-4). I also tested the fecundity of the OH isolate for this experiment by examining the ratio of maximum zoospore densities to maximum whole culture viability (maximum zoospore densities divided by whole culture viability). I added 1 to each maximum zoospore density entry to ensure there were no zeros when assessing the ratio. To calculate fecundity after acclimation, I used the maximum zoospore densities and maximum whole culture viability after 10 passages in Optimum conditions (21°C).

Experiments 3 and 4

I used the LA isolate for the remaining experiments because I had limited success in reviving the cryopreserved aliquots of the other isolates. The LA isolate was an ideal isolate to continue experimentation because it showed higher maximum zoospore densities in the Future lineage across multiple temperature treatments compared to the Current lineage and it had the highest maximum zoospore densities in general at all of the tested thermal conditions. I revived all evolved lineages for the isolate from LA from a cryo-archived state (Boyle et al., 2003). However, the Future lineage was only able to revive at passage number 70. Therefore, I further serially passaged the Optimum lineage in the Optimum conditions to match the passage number of the Hot and Future lineages at p. 70 (Fig. 4-4).

Experiment 3: Ancestor versus evolved lineage Thermal Performance Curve comparisons

After the initial experimental evolution of 45 passages (Experiment 1), I used the LA *Bd* isolate to test for differences between the Evolved and Ancestor lineages. Specifically, I tested for changes in thermal profile characteristics, such as the thermal optimum (T_{opt}), critical thermal

maximum (CT_{max}), and performance maximum (P_{max}) *in vitro*. After 70 passages, I generated a TPC of the maximum whole culture viability and/or maximum zoospore densities for comparison among the Optimum, Hot, Future, and Ancestor lineages. I used the Ancestor lineage to compare changes in maximum whole culture viability and maximum zoospore densities after experimental evolution. I revived the Future, Optimum, Hot and Ancestor lineage simultaneously for comparisons between Ancestor and Evolved lineages.

To assess the thermal sensitivity of the Ancestor versus Evolved lineages, I followed the same protocols and design for thermal profile experiments and culturing as a previous study (Sheets et al., 2021). I tested *Bd* responses at seven temperatures from the *Bd* thermal range: 4°C, 12°C, 17°C, 21°C, 25°C, 27°C, and 28°C. Using these temperatures, I generated TPCs for each lineage of the LA isolate to assess changes in the T_{opt} , CT_{max} , and P_{max} for maximum whole culture viability and maximum zoospore densities due to serial passaging at simulated climate change conditions.

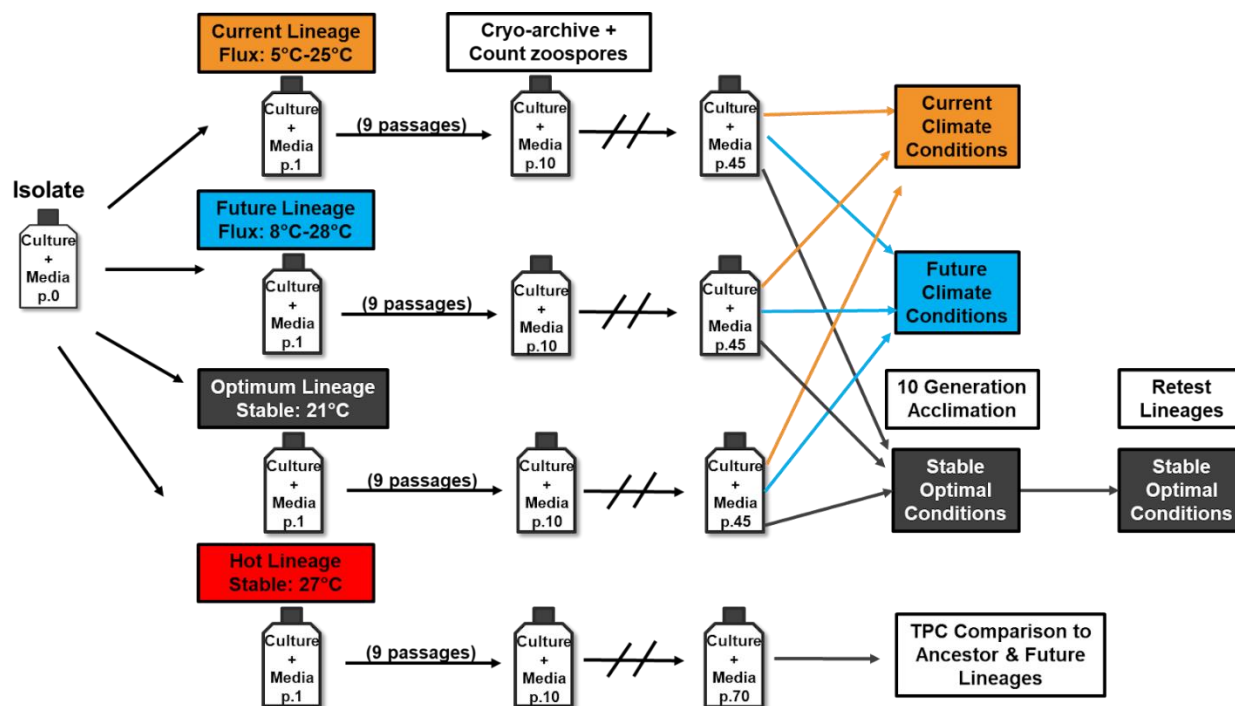


Figure 4-4. Experimental design for the serial passaging experiments. At the end of the experimental evolution, which completed at passage 45 (p. 45), I grew each evolved lineage of each isolate in culture flasks in the conditions they were evolved in as well as the two conditions never experienced before (indicated by colored arrows). Every 10 passages, aliquots of each culture were cryo-archived and diluted to a standard concentration of $50-70 \times 10^4$ zoospores per mL. For the OH isolate, I passaged the Current, Future, and Optimum lineages in the stable optimal conditions to allow for an acclimation period. After 30 days, I measured maximum whole culture viability and maximum zoospore densities of the OH isolate. I propagated the LA isolate into a Hot lineage and serially passaged for 70 passages to test for the effects of selection on the thermal maxima. After the Hot lineage passaging, the Future, Hot, and Optimum lineages of the LA isolate were compared to the Ancestor lineage across the thermal range.

Experiment 4: Growth inhibition assay in amphibian skin secretions

To determine if there were differences in traits that may be related to pathogenicity (i.e., the ability to grow in the presence of host skin secretions) between the Ancestor and evolved lineages, I

compared the Future, Hot, and Optimum lineages of the LA *Bd* isolate with the corresponding Ancestor lineage in the presence of skin secretions collected from live amphibians. Collaborators collected skin secretions from *Lithobates sphenoccephalus* that were captured in the wild (LeSage et al., 2022). Collaborators collected the skin secretions by stimulating the amphibian's sympathetic nervous system using an injection of the hormone norepinephrine (NE; 40 nmol per gram body mass; LeSage et al., 2022). The injection of NE causes the amphibian host to release stored skin secretions from their cutaneous granular glands (Rollins-Smith 2005). The samples were frozen at xx temp and transported to the University of Nevada, Reno, where I lyophilized and reconstituted the samples in 1 mL of sterile HPLC-grade water (add a ref for this method).

For the *Bd* growth inhibition assay, I used ten skin secretion samples that I mixed together to ensure the same skin secretion mixture was used across lineages. Specifically, I mixed 0.5 mL of reconstituted skin secretion sample together via vortexing. Then, I filter-sterilized the solution using (Cobetter, 0.5µm Hydrophilic Syringe Filters) to remove all bacteria and avoid contamination. I used 96 well plates to grow the *Bd* lineages in the presence of the sterile skin secretions. I inoculated each lineage into a row of the 96 well plate that consisted of 4 wells containing live *Bd* (30 µL), media (50 µL), and the skin secretion sample mixture (20 µL). I also included 4 wells containing heat killed *Bd* (30 µL), media (50 µL), and the skin secretion sample mixture (20 µL) as a negative control. Lastly, I included 4 wells containing only media (50 µL) and live *Bd* (30 µL) as a positive control. At the time of initial plate setup, I placed all plates in an environmental chamber set at a constant 21°C.

Statistical Analysis

For all statistical analyses, I used R version 4.0.2 (R Core Team, 2020). I used the “ggplot2” package within R to generate figures. Summary statistics reported in the figures include means of the maximum whole culture viability and maximum zoospore densities measured among isolates or

lineages. In all experiments, I used the measures from within the 2day period at which cultures exhibited maximum viability or zoospore densities in each thermal treatment condition to calculate maximum whole culture viability and maximum zoospore densities. In Experiment 1, I conducted standard t-tests used in R studio. I used a Welch t-test to compare the maximum whole culture viability and maximum zoospore densities of the Future and Current lineages at each thermal condition. I used an ANOVA with a Tukey *post hoc* test to compare the Optimum lineage's maximum whole culture viability or maximum zoospore densities among thermal treatment groups. In Experiment 2, I used an ANOVA with a Tukey *post hoc* test to compare the maximum whole culture viability, maximum zoospore densities, and fecundity among the Future, Current, and Optimum lineages to one another before or after acclimation. In Experiment 3, I analyzed the maximum whole culture viability and maximum zoospore densities of each lineage to compare for differences among lineages at several temperature treatments. I used an ANOVA and Tukey *post hoc* tests to make comparisons in maximum whole culture viability (optical density following the MTT assay) and maximum zoospore densities (zoospores per mL). In Experiment 4, I analyzed the differences in maximum whole culture viability and maximum zoospore densities among lineages using ANOVA and Tukey *post hoc* tests. I conducted an ANOVA to compare among lineages after normalizing each lineage's raw data by subtracting each individual data point of the positive control maximum whole culture viability and maximum zoospore densities from the individual data point of the experimental maximum whole culture viability and maximum zoospore densities for each lineage.

Results

Experiment 1: Comparison of evolved lineages

Following 45 passages of *Bd* in experimental thermal conditions, I compared the maximum whole culture viability and maximum zoospore densities for the Future and Current lineages grown at

the three temperature treatments used in the experimental evolution: Optimum, stable 21°C (Fig. 4-5 A), Current, fluctuating 5-25°C (Fig. 4-5 B), and Future, fluctuating 8-28°C (Fig. 4-5 C). I found that the maximum whole culture viability of the Future lineages was higher than the Current lineage viability for four of the five isolates across the tested temperature treatments. Specifically, the Future lineage for the Vermont (VT) isolate had higher maximum whole culture viability in all temperature treatments compared to the Current lineage (21°C: $t(13.3) = -5.45$, $p < 0.001$; 5-25°C: $t(16.7) = -11.09$, $p < 0.001$; and 8-28°C: $t(16.9) = -7.66$, $p < 0.001$; Fig. 4-5 A,B,C). I observed a similar pattern with the Future lineage of the Tennessee (TN) isolate, except that results were not significantly different between the two lineages at Optimum 21°C and the Future 8-28°C fluctuating temperature treatment (21°C: $t(17.99) = -1.04$, $p = 0.31$; 5-25°C: $t(15.15) = -6.66$, $p < 0.001$; and 8-28°C: $t(17.52) = -1.54$, $p = 0.14$; Fig. 4-5 A,C). The OH isolate showed differences between the evolved lineages in which, again, the Future lineage had higher maximum whole culture viability than the Current lineage at the Future 8-28°C fluctuating temperature treatment (8-28°C: $t(17.9) = -3.97$, $p < 0.001$; Fig. 4-5 C). Notably, the New Mexico (NM) isolate was the only isolate which showed the Current lineage having higher maximum viability than the Future lineage in both Current and Future fluctuating temperature treatments (5-25°C: $t(16.04) = 4.23$, $p < 0.001$; and 8-28°C: $t(17.8) = 3.17$, $p < 0.01$; Fig. 4-5 B,C). Additionally, I found that only the VT isolate showed that there was no difference in whole culture viability for the control Optimum lineage across thermal treatments Table 4-2; Fig. 4-5 A,B,C). The other 4 isolates showed differences in maximum the whole culture viability of the Optimum lineage grown at 21°C compared to the Optimum lineage grown at the other two fluctuating conditions in no straightforward pattern (Table 4-2).

Table 4-2. Tukey *post Hoc* results for ANOVA to compare the difference in maximum whole culture viability of the Optimum lineage among temperature treatments.

Isolate	Temperature Treatment Comparison	Difference	p-value
LA	Optimum (21°C) - Current (5-25°C)	-0.104	0.19
LA	Optimum (21°C) - Future (8-28°C)	0.102	0.19
LA	Current (5-25°C) - Future (8-28°C)	-0.206	0.004
NM	Optimum (21°C) - Current (5-25°C)	0.150	0.001
NM	Optimum (21°C) - Future (8-28°C)	0.078	0.13
NM	Current (5-25°C) - Future (8-28°C)	0.072	0.18
OH	Optimum (21°C) - Current (5-25°C)	0.409	< 0.001
OH	Optimum (21°C) - Future (8-28°C)	0.375	< 0.001
OH	Current (5-25°C) - Future (8-28°C)	0.034	0.73
TN	Optimum (21°C) - Current (5-25°C)	0.155	0.09
TN	Optimum (21°C) - Future (8-28°C)	0.261	0.002
TN	Current (5-25°C) - Future (8-28°C)	-0.106	0.29
VT	Optimum (21°C) - Current (5-25°C)	-0.024	0.82
VT	Optimum (21°C) - Future (8-28°C)	-0.026	0.80
VT	Current (5-25°C) - Future (8-28°C)	0.002	0.99

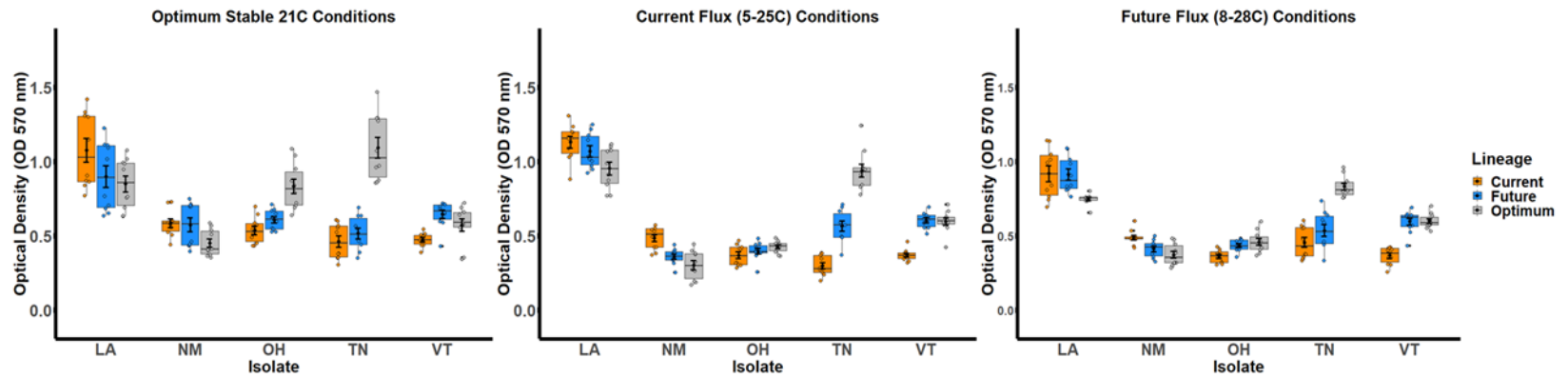


Figure 4-5. Boxplots show the maximum whole culture viability of the Current (orange), Future (blue), and Optimum (grey) lineages for each *Batrachochytrium dendrobatidis* isolate in the three thermal treatments: A) stable optimal conditions (21°C), B) the current fluctuating conditions (5-25°C), and C) the future fluctuating conditions (8-28°C). Maximum whole culture viability is measured using optical density (OD) readings at 570 nm wavelengths for quantification of MTT viability assay. Each boxplot consists of data from a 2-day maximum period during the thermal experiment.

I also compared the maximum zoospore densities between the Future and Current lineages at the three thermal treatments for all isolates. For multiple isolates, the maximum zoospore densities of the Future lineages were higher than the Current lineages across the tested temperature treatments. Specifically, the Future lineage for the OH isolate had higher maximum zoospore densities in all temperature treatments than the Current lineage (21°C: $t(8.6) = -2.51$, $p = 0.04$; 5-25°C: $t(8.4) = -7.72$, $p < 0.001$; and 8-28°C: $t(8.1) = -7.72$, $p < 0.001$; Fig. 4-6 A,B,C). I observed a similar pattern with the Future lineage of the LA *Bd* isolate, except that results were not significantly different between the Future and Current lineages in the Future 8-28°C fluctuating temperature treatment (21°C: $t(9.98) = -4.03$, $p < 0.01$; 5-25°C: $t(9.94) = -2.27$, $p = 0.047$; and 8-28°C: $t(13.53) = -1.43$, $p = 0.18$; Fig. 4-6 A,B,C). For the VT isolate, I only found significant differences between the Current and Future lineages at the Future 8-28°C temperature treatment, where the Future lineage had higher maximum zoospore densities than the Current lineage (8-28°C: $t(7.7) = -7.71$, $p < 0.001$; Fig. 4-6 C). The NM *Bd* isolate was the only isolate that showed the Current lineage having higher maximum zoospore densities than the Future lineage in the Future 8-28°C fluctuating temperature treatment (8-28°C: $t(13.48) = 2.4$, $p = 0.03$; Fig. 4-6 C). However, in the Optimum 21°C thermal treatment, the Future lineage had higher maximum zoospore densities than the Current lineage for the NM isolate (21°C: $t(11.27) = 1.92$, $p = 0.08$; Fig. 4-6 A). Additionally, I found that for the control Optimum lineage, no isolate showed the same response in whole culture viability across thermal treatments Table 4-3; Fig. 4-5 A,B,C). The five isolates showed differences in maximum the whole culture viability of the Optimum lineage grown at 21°C compared to the Optimum lineage grown at the other two fluctuating conditions in no straightforward pattern (Table 4-3).

Table 4-3. Tukey *post Hoc* results for ANOVA to compare the difference in maximum zoospore densities of the Optimum lineage among temperature treatments.

Isolate	Temperature Treatment Comparison	Difference	p-value
LA	Optimum (21°C) - Current (5-25°C)	149.438	< 0.001
LA	Optimum (21°C) - Future (8-28°C)	226.688	0.02
LA	Current (5-25°C) - Future (8-28°C)	-77.250	0.27
NM	Optimum (21°C) - Current (5-25°C)	17.5	0.04
NM	Optimum (21°C) - Future (8-28°C)	20.5	0.02
NM	Current (5-25°C) - Future (8-28°C)	-3.0	0.89
OH	Optimum (21°C) - Current (5-25°C)	-150.625	< 0.001
OH	Optimum (21°C) - Future (8-28°C)	-28.125	< 0.001
OH	Current (5-25°C) - Future (8-28°C)	-122.500	0.12
TN	Optimum (21°C) - Current (5-25°C)	-88.0	0.99
TN	Optimum (21°C) - Future (8-28°C)	-86.875	0.13
TN	Current (5-25°C) - Future (8-28°C)	-1.125	0.14
VT	Optimum (21°C) - Current (5-25°C)	-23.625	0.12
VT	Optimum (21°C) - Future (8-28°C)	74.250	0.03
VT	Current (5-25°C) - Future (8-28°C)	97.875	0.79

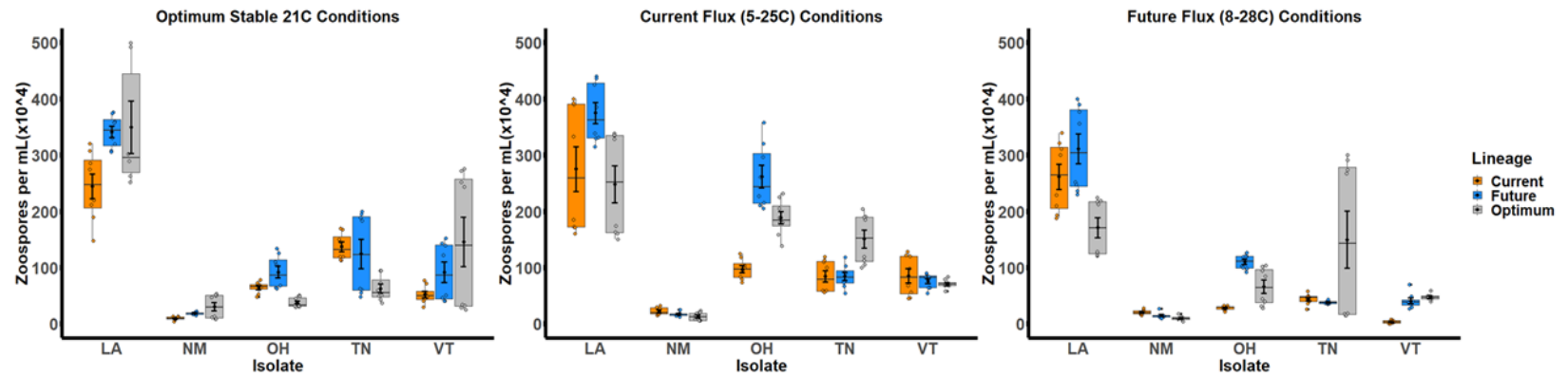


Figure 4-6. Boxplots show the maximum zoospore densities of the Current (orange) and Future (blue) lineages for each *Batrachochytrium dendrobatidis* isolate in the three thermal treatments: A) stable Optimum conditions (21°C), B) the Current fluctuating conditions (5-25°C), and C) the Future fluctuating conditions (8-28°C). Zoospore densities are shown as zoospores per mL with a concentration of 1×10^4 . Each boxplot consists of data from a 2-day maximum period during the thermal experiment.

Experiment 2: Acclimation study post experimental evolution

After one month of incubation at the Optimum temperature treatment (21°C), the Current, Future, and Optimum lineages showed higher maximum whole culture viability for the Current and Optimum lineages than the Future lineages when grown at Optimum 21°C temperature treatment after a month of additional acclimation (ANOVA, $F(2,27) = 7.4$, $p = 0.003$; Current – Future: Tukey, $p = 0.002$; Optimum – Current: Tukey, $p = 0.06$; Optimum – Future: Tukey, $p = 0.34$; Fig. 4-7 A). However, after I incubated each lineage at the Optimum 21°C temperature treatment for 10 passages, the Future lineage of the OH *Bd* isolate had higher maximum zoospore densities than the Current and Optimum lineages when grown in the Optimum 21°C temperature treatment (ANOVA, $F(2,21) = 9.7$, $p = 0.001$; Current – Future: Tukey, $p = 0.002$; Optimum – Current: Tukey, $p = 0.88$; Optimum – Future: Tukey, $p = 0.005$; Fig. 4-7 B). There were no differences in fecundity between the Current and Future lineages before acclimation (ANOVA, $F(2,21) = 13.9$, $p < 0.001$; Current – Future: Tukey, $p = 0.32$; Optimum – Current: Tukey, $p = 0.004$; Optimum – Future: Tukey, $p < 0.001$; Fig. 4-8 A). However, after the additional acclimation period of 10 passages in Optimum thermal conditions, I found that the Future lineage had significantly higher fecundity than the Current lineage when grown in the Optimum 21°C temperature treatment (ANOVA, $F(2,21) = 22.5$, $p < 0.001$; Current – Future: Tukey, $p < 0.001$; Optimum – Current: Tukey, $p = 0.44$; Optimum – Future: Tukey, $p < 0.001$; Fig. 4-8 B).

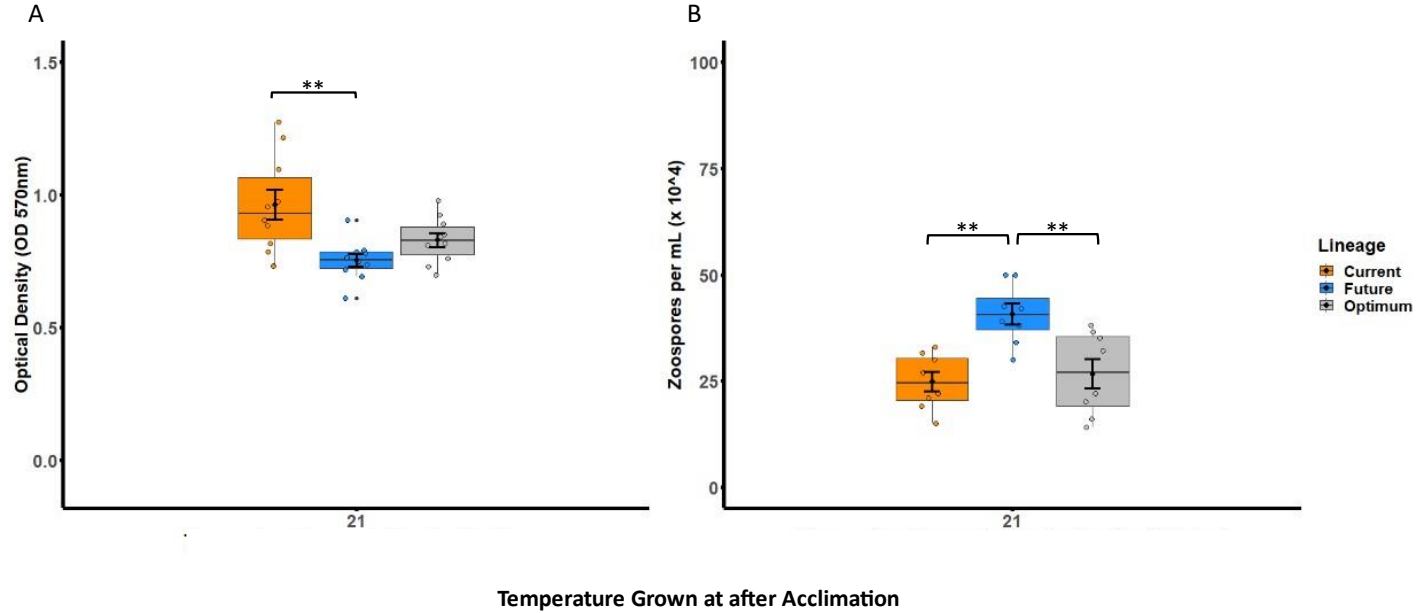


Figure 4-7. The *Batrachochytrium dendrobatidis* isolate from OH responses between Current (orange), Future (blue), and Optimum (grey) lineages after one month acclimation period. Boxplots show A) the maximum whole culture viability via optical density (OD) readings at 570 nm for quantification of MTT viability and B) the maximum zoospore densities as zoospore per mL with a concentration of 1×10^4 in the Optimum thermal treatment: stable Optimum conditions (21°C) after a month-long acclimation period in the Optimum temperature profile. Each boxplot consists of data from a 2-day maximum period during the thermal experiment. Significance labels are shown with * marks to indicate degrees of significance between lineages as follows: * < 0.05, ** < 0.01, *** < 0.001.

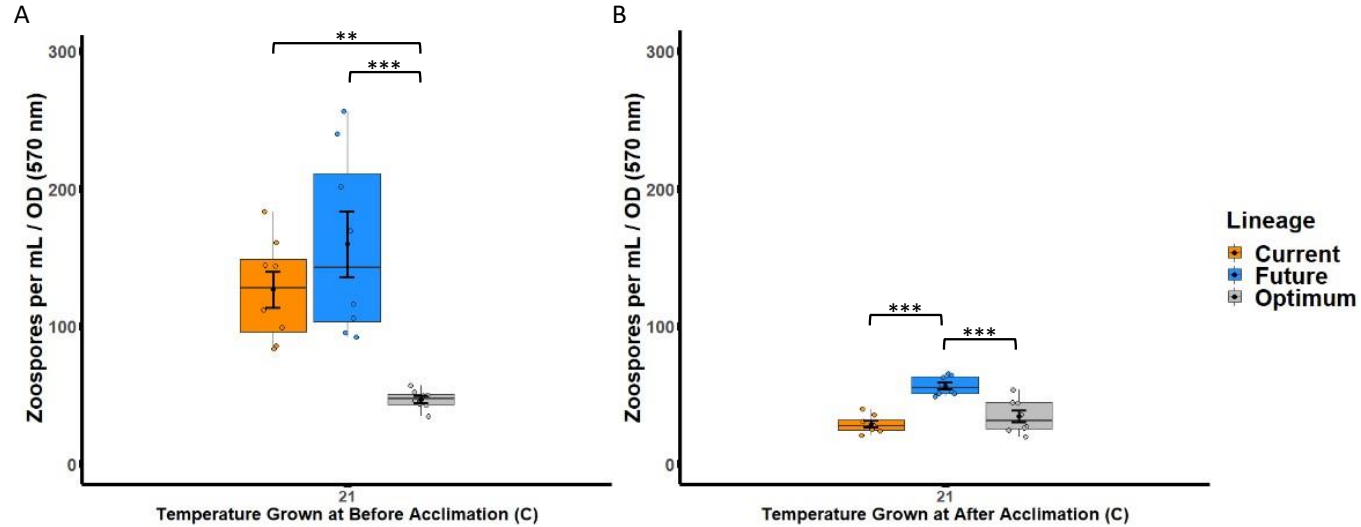


Figure 4-8. The *Batrachochytrium dendrobatidis* isolate from OH fecundity responses between Current (orange) and Future (blue) lineages before and after one month acclimation period. Boxplots show A) fecundity as a ratio of zoospores per mL to optical density of whole culture viability before the acclimation period and B) fecundity as a ratio of zoospores per mL to optical density of whole culture viability after the acclimation period grown in the Optimum thermal treatment: stable Optimum conditions (21°C). Each boxplot consists of data from a 2-day maximum period during the thermal experiment. Significance labels are shown with * marks to indicate degrees of significance between lineages as follows: * <0.05, ** <0.01, *** <0.001.

Experiment 3: Ancestral versus evolved lineage Thermal Performance Curve comparisons

I found that there were differences between the Ancestor and Evolved lineages when I tested for maximum whole culture viability and maximum zoospore densities after incubation at five temperatures across their thermal range. For the whole culture viability measured of the LA *Bd* isolate, I found that the Hot lineage had lower maximum whole culture viability than the Future, Optimum, and Ancestor lineages at 4°C, 12°C, and 21°C (4°C: ANOVA, $F(3,35) = 79.9$, $p < 0.001$; 12°C: ANOVA, $F(3,35) = 19.3$, $p < 0.001$; 21°C: ANOVA, $F(3,35) = 7.5$, $p < 0.001$; Fig. 4-9 & 4-10). The T_{opt} was difficult to determine for maximum whole culture viability of the Future, Optimum, and Ancestor lineages because the P_{max} was similar for multiple temperatures for each lineage. The T_{opt} for the Hot lineage's maximum whole culture viability was 17°C. At 27°C, the Future and Optimum lineages had higher maximum whole culture viability than both the Ancestor and Hot lineages (27°C: ANOVA, $F(3, 36) = 35.8$, $p < 0.001$; Fig. 4-10). All four lineages approached their CT_{max} at 28°C. The Optimum and Ancestor lineages were statistically similar in their maximum whole culture viability at 4°C, 12°C, and 21°C (Table 4-4).

Table 4-4. Tukey *post Hoc* results of the LA lineages grown across the thermal range for maximum whole culture viability.

Lineage Comparison	Temperature Treatment	Difference	p-value
Future-Ancestor	4	0.095	0.22
Hot-Ancestor	4	-0.585	< 0.001
Stable-Ancestor	4	-0.073	0.46
Hot-Future	4	-0.680	< 0.001
Stable-Future	4	-0.168	0.009
Stable-Hot	4	0.512	< 0.001
Future-Ancestor	12	-0.203	0.002
Hot-Ancestor	12	-0.514	< 0.001
Stable-Ancestor	12	0.069	0.56
Hot-Future	12	-0.311	< 0.001

Stable-Future	12	0.272	< 0.001
Stable-Hot	12	0.584	< 0.001
Future-Ancestor	17	0.017	0.98
Hot-Ancestor	17	0.032	0.89
Stable-Ancestor	17	0.147	0.01
Hot-Future	17	0.015	0.99
Stable-Future	17	0.129	0.03
Stable-Hot	17	0.115	0.07
Future-Ancestor	21	-0.019	0.97
Hot-Ancestor	21	-0.186	< 0.001
Stable-Ancestor	21	-0.029	0.91
Hot-Future	21	-0.166	0.003
Stable-Future	21	-0.009	0.99
Stable-Hot	21	0.156	0.006
Future-Ancestor	25	-0.029	0.96
Hot-Ancestor	25	-0.316	< 0.001
Stable-Ancestor	25	-0.297	< 0.001
Hot-Future	25	-0.287	< 0.001
Stable-Future	25	-0.268	< 0.001
Stable-Hot	25	0.018	0.99
Future-Ancestor	27	0.355	< 0.001
Hot-Ancestor	27	-0.029	0.94
Stable-Ancestor	27	0.355	< 0.001
Hot-Future	27	-0.384	< 0.001
Stable-Future	27	0.001	0.99
Stable-Hot	27	0.385	< 0.001
Future-Ancestor	28	0.064	0.12
Hot-Ancestor	28	-0.008	0.99
Stable-Ancestor	28	0.088	0.02
Hot-Future	28	-0.071	0.06
Stable-Future	28	0.025	0.81
Stable-Hot	28	0.096	0.007

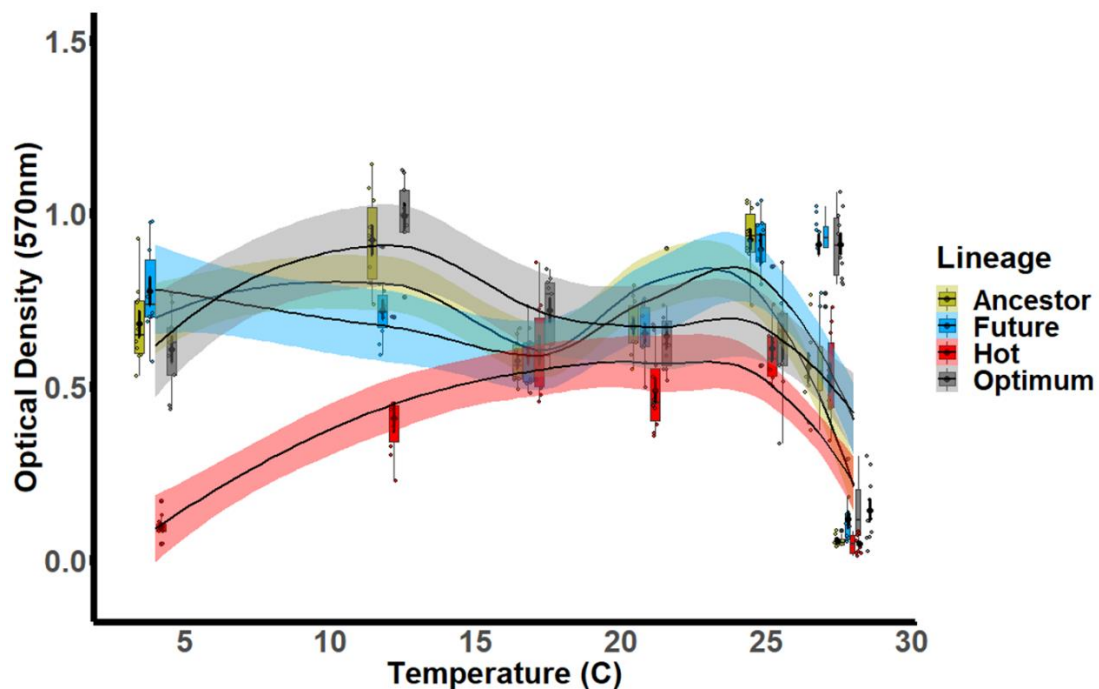


Figure 4-9. Thermal performance curve (TPC) of maximum whole culture viability for LA isolate across lineages. Boxplots show the maximum whole culture viability of the Ancestor (green), Future (blue), and the Hot (red) lineages for the OH isolate across the TPC. Maximum whole culture viability is measured using optical density (OD) readings at 570 nm wavelengths for quantification of MTT viability assay. Each boxplot consists of data from a 2-day maximum period during the thermal experiment.

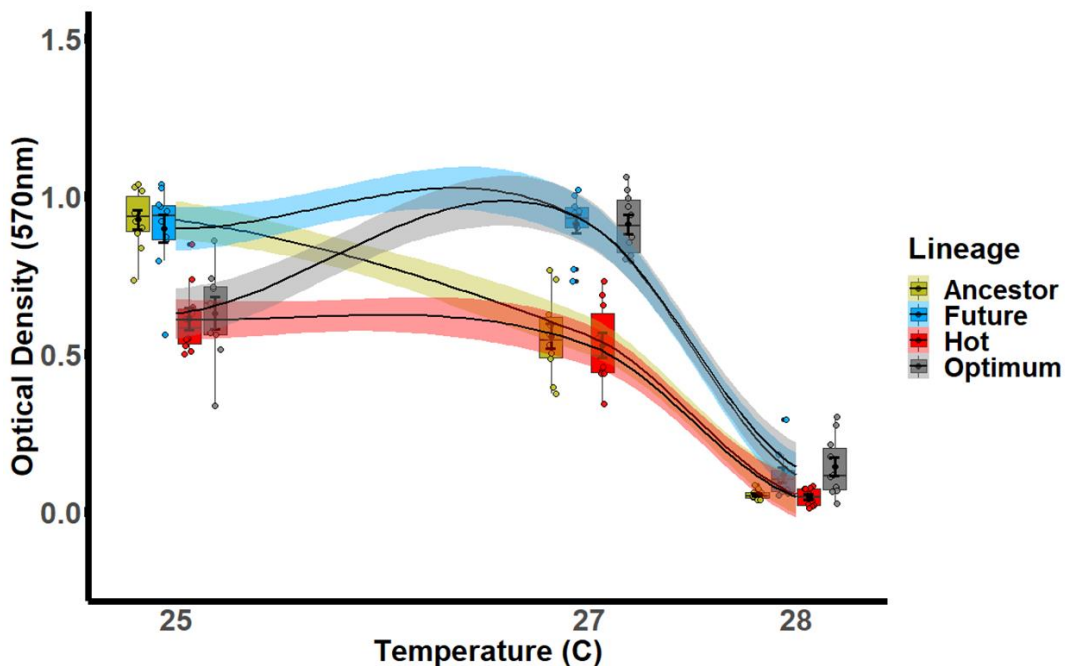


Figure 4-10. Maximum whole culture viability for LA isolate across lineages at temperatures surrounding the thermal maxima. Boxplots show the maximum whole culture viability for the Ancestor (green), Future (blue), and Hot (red) lineages of the LA isolate at temperatures surrounding the thermal maxima. Maximum whole culture viability is measured using optical density (OD) readings at 570 nm wavelengths for quantification of MTT viability assay. Each boxplot consists of data from a 2-day maximum period during the thermal experiment.

For maximum zoospore densities, I observed that the T_{opt} was 12°C for the Future, Optimum, and Ancestor lineage, but not the Hot lineage (Fig. 4-11). The Hot lineage had the highest maximum zoospore density at 17°C, making its T_{opt} 17°C. The Ancestor and Future lineages were significantly higher in maximum zoospore density at both 4°C and 12°C than the Hot and Optimum lineages (4°C: ANOVA, $F(3,41) = 30.4$, $p < 0.001$; 12°C: ANOVA, $F(3,36) = 19.3$, $p < 0.001$; Table 4-5; Fig. 4-11). The Optimum lineage had significantly higher maximum zoospore densities than the other three lineages at 17°C (17°C: ANOVA, $F(3, 36) = 13.4$, $p < 0.001$; Table 4-5). At 21°C, the Ancestor and Hot lineages had higher maximum zoospore densities than the Future and Optimum lineages (21°C: ANOVA, $F(3,36) = 20.99$, $p < 0.001$; Fig.

4-11). The Hot and Optimum lineages had a lower P_{max} than both the Future and Ancestor lineages at their respective T_{opt} . The Future lineage had a significantly lower P_{max} at its T_{opt} compared to the Ancestor lineage (12°C: ANOVA, $F(3,36) = 19.3$, $p < 0.01$; Fig. 4-11). At the higher temperature of 25°C, approaching the isolate's CT_{max} , I found that the Hot lineage had highest maximum zoospore densities compared to the Future, Optimum, and Ancestor lineages (25°C: ANOVA, $F(3,36) = 19.6$, $p < 0.001$; Table 4-5; Fig. 4-12). At 27°C, the Hot lineage reached its CT_{max} , (Fig. 4-12). The Future, Optimum, and Ancestor lineages had low maximum zoospore densities at 27°C, with the Future lineage having higher maximum zoospore densities than the Ancestor and Optimum lineages (27°C: ANOVA, $F(3,36) = 70.6$, $p < 0.001$; Table 4-5; Fig. 4-12). All lineages had reached their CT_{max} at 28°C and there were no differences among the isolates at this temperature (28°C: ANOVA, $F(3,36) = 1.2$, $p = 0.32$; Table 4-5; Fig. 4-12). The Optimum lineage was statistically similar in maximum zoospore densities compared to the Ancestor at the temperature approaching the CT_{max} of 25°C, 27°C, and 28°C (Table 4-5; Fig. 4-12).

Table 4-5. Tukey *post Hoc* results of the LA lineages grown across the thermal range for maximum zoospore densities.

Lineage Comparison	Temperature Treatment	Difference	p-value
Future-Ancestor	4	-43.70	0.38
Hot-Ancestor	4	-204.80	< 0.001
Stable-Ancestor	4	-184.47	< 0.001
Hot-Future	4	-161.10	< 0.001
Stable-Future	4	-140.77	< 0.001
Stable-Hot	4	20.33	0.84
Future-Ancestor	12	-213.20	0.01
Hot-Ancestor	12	-441.25	< 0.001
Stable-Ancestor	12	-408.90	< 0.001
Hot-Future	12	-228.05	0.007
Stable-Future	12	-195.70	0.03

Stable-Hot	12	32.35	0.96
Future-Ancestor	17	-56.50	0.23
Hot-Ancestor	17	17.20	0.93
Stable-Ancestor	17	-149.85	< 0.001
Hot-Future	17	73.70	0.07
Stable-Future	17	-93.35	0.01
Stable-Hot	17	-167.05	< 0.001
Future-Ancestor	21	-114.70	0.002
Hot-Ancestor	21	-7.35	0.99
Stable-Ancestor	21	-199.70	< 0.001
Hot-Future	21	107.35	0.004
Stable-Future	21	-85.00	0.03
Stable-Hot	21	-192.35	< 0.001
Future-Ancestor	25	7.15	0.33
Hot-Ancestor	25	25.00	< 0.001
Stable-Ancestor	25	-4.65	0.68
Hot-Future	25	17.85	< 0.001
Stable-Future	25	-11.80	0.04
Stable-Hot	25	-29.65	< 0.001
Future-Ancestor	27	10.80	< 0.001
Hot-Ancestor	27	-3.45	0.01
Stable-Ancestor	27	-1.20	0.68
Hot-Future	27	-14.25	< 0.001
Stable-Future	27	-12.00	< 0.001
Stable-Hot	27	2.25	0.17
Future-Ancestor	28	-0.30	0.45
Hot-Ancestor	28	0.05	0.99
Stable-Ancestor	28	-0.05	0.99
Hot-Future	28	0.35	0.31
Stable-Future	28	0.25	0.60
Stable-Hot	28	-0.10	0.96

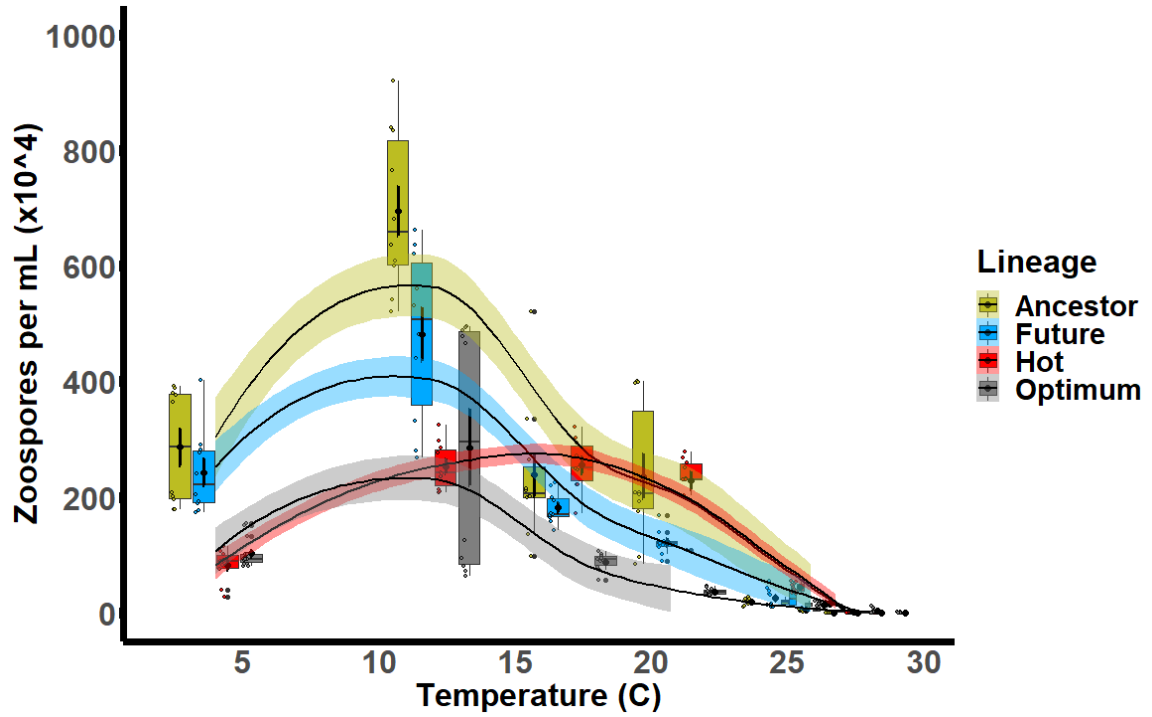


Figure 4-11. Thermal performance curves of maximum zoospore densities for LA isolate across the Ancestor (green), Future (blue), and Hot (red) lineages. Zoospore densities are shown as zoospores per mL with a concentration of 1×10^4 . Each boxplot consists of data from a 2-day maximum period during the thermal experiment.

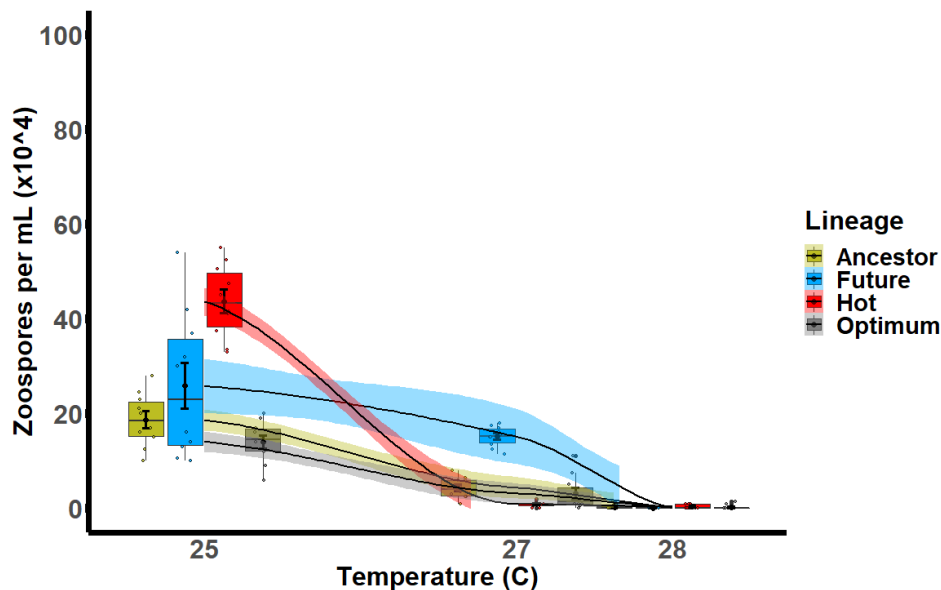


Figure 4-12. Maximum zoospore densities for LA isolate across the Ancestor (green), Future (blue), and Hot (red) lineages at temperatures surrounding the thermal maxima. Zoospore densities are shown as zoospores per mL with a concentration of 1×10^4 . Each boxplot consists of data from a 2-day maximum period during the thermal experiment.

Experiment 4: Growth inhibition assay in amphibian skin secretions

After growing the Ancestor, Future, Hot, and Optimum lineages in the presence of frog skin secretions, I found that there were differences among the lineages in responses of maximum whole culture viability (ANOVA, $F(3, 28) = 11.6$, $p < 0.001$) and maximum zoospore densities (ANOVA, $F(3, 28) = 5.1$, $p = 0.006$). I found that the Future lineage had significantly higher maximum whole culture viability compared to the Ancestor and Optimum lineages when grown in the presence of skin secretions (Table 4-6; Fig. 4-13 A). Additionally, the Hot lineage had significantly higher whole culture viability compared to the Optimum lineage when grown in the presence of host skin secretions (Table 4-6). I observed that the maximum zoospore densities of the Ancestor lineage did not significantly differ from those of the other lineages (Table 4-7; Fig. 4-13 B). However, the Hot lineage had significantly higher maximum zoospore densities when

grown in the presence of skin secretions compared to the Future and Optimum lineages (Table 4-7; Fig. 4-13 B).

Table 4-6. Tukey *post Hoc* results for ANOVA to compare lineages inhibition of maximum whole culture viability when grown in the presence of host skin secretions.

Lineage Comparison	Difference	p-value
Future-Ancestor	0.20	0.004
Hot-Ancestor	0.07	0.58
Optimum-Ancestor	-0.10	0.23
Hot-Future	-0.13	0.08
Optimum-Future	-0.31	<0.001
Optimum-Hot	-0.17	0.016

Table 4-7. Tukey *post Hoc* results for ANOVA to compare lineages inhibition of maximum zoospore densities when grown in the presence of host skin secretions.

Lineage Comparison	Difference	p-value
Future-Ancestor	-28.9	0.77
Hot-Ancestor	63.9	0.16
Optimum-Ancestor	-43.9	0.47
Hot-Future	92.8	0.02
Optimum-Future	-15.0	0.96
Optimum-Hot	-107.8	0.006

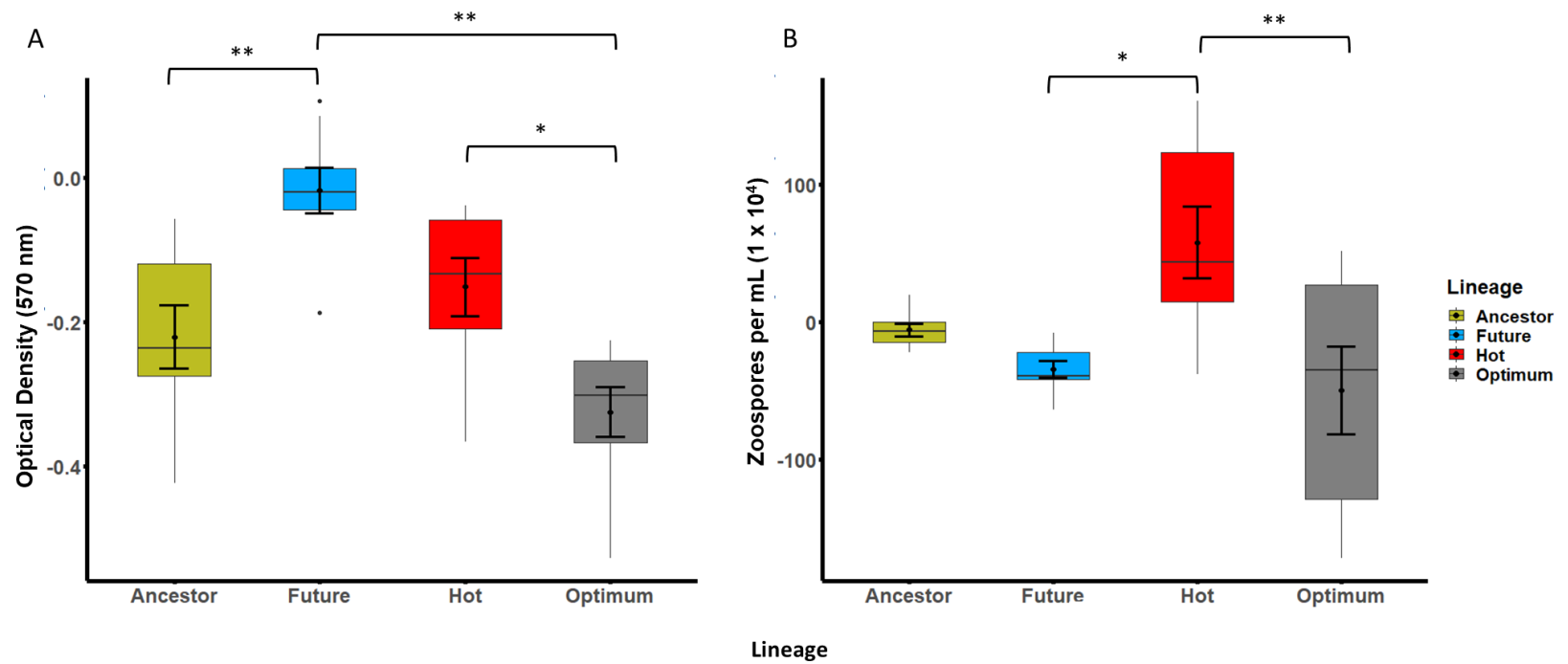


Figure 4-13. The boxplots show the growth of the Ancestor, Future, Hot, and Optimum lineages A) maximum whole culture viability or B) maximum zoospore production in the presence of host skin secretions. The data are normalized by subtracting the positive control response quantities (no skin secretions) from the experimental response quantities (grown with skin secretions). The mean of each boxplot is indicated by the bolded black error bars. Maximum whole culture viability is measured using optical density (OD) readings at 570 nm wavelengths for quantification of MTT viability assay. Zoospore densities are shown as zoospores per mL with a concentration of 1×10^4 . Each boxplot consists of data from a 2-day maximum period during the thermal experiment. Significance labels are shown with * marks to indicate degrees of significance between lineages as follows: * < 0.05 , ** < 0.01 , *** < 0.001 .

Discussion

Climate change is expected to impact a wide range of biological systems, including disease dynamics in a wide range of different infectious disease systems (Altizier et al., 2013; Raffel et al., 2013; Rohr, 2011). The purpose of this study was to assess the responses of a lethal fungal pathogen of amphibians (*Bd*) for maximum whole culture viability and maximum zoospore densities to climate change conditions. The responses that *Bd* may have to climate change conditions in physiological traits related to growth and reproduction is still not understood and has been hypothesized in the literature (Cohen et al., 2013; Rohr et al., 2011). I used experimental evolution and serially passaged five isolates of *Bd* in current and predicted temperatures to understand the responses of *Bd* in simulated thermal conditions. My results suggest that the effect of elevated temperatures that amphibians may encounter with climate change did not uniformly alter *Bd* growth and reproduction. Rather, the patterns of *Bd* responses differed among isolates, among the experimentally derived lineages, among thermal conditions, as well as between the traits that I measured (maximum whole culture viability, maximum zoospore densities, and fecundity). Additionally, my results suggest that serial passaging may impact the zoospore densities over time for some isolates.

I found two important outcomes from Experiment 1. First, temperature conditions for the serial passage experiments can impact the physiological responses of *Bd*. Second, a majority of the *Bd* isolates tested in this study had higher maximum whole culture viability or maximum zoospore densities when passaged in higher “future” conditions. To first understand the general effects of serial passaging in different temperature treatments on the maximum whole culture viability and maximum zoospore densities of *Bd*, I included a control lineage that was serially passaged at 21°C. The results from the control lineage after experimental evolution suggest that *Bd* has different responses (in maximum whole culture viability and maximum zoospore

densities) depending on whether an isolate is grown in stable Optimum, lower mean Current fluctuating, or higher mean Future fluctuating thermal conditions. Additionally, I found that four of the five *Bd* isolates showed higher maximum whole culture viability and/or maximum zoospore densities for the higher Future lineage compared to the lower Current lineage. The results of this experiment broadly indicated that SPEs can be an effective method for testing the effects of temperature conditions on *Bd* physiology over time with additional research being required to understand the general impact of SPEs on *Bd* physiological responses without temperature treatment as a factor.

However, the responses to the temperature treatments were inconsistent among the different isolates. While I found that some isolates of *Bd* respond to Future thermal conditions with increased maximum whole culture viability or maximum zoospore densities, other isolates responded with decreased maximum whole culture viability and maximum zoospore densities. For example, the *Bd* isolate from New Mexico had higher maximum whole culture viability and maximum zoospore densities in the Current thermal treatment compared to the Future temperature conditions. This isolate may have experienced heat stress in the higher, Future temperature conditions that led to decreased survival rates (i.e., decreased maximum whole culture viability or zoospore densities), which has been observed in other studies (Kásler et al., 2022). Given the different outcomes that I observed across multiple *Bd* isolates, it was important to consider the possibility that there is an effect of long-term acclimation and further research is required.

In Experiment 2, I aimed to determine whether the changes observed in *Bd* isolates after experimental evolution could be due to adaptation or acclimation. The results of the acclimation experiment were mixed depending on the trait (maximum whole culture viability, max zoo density or fecundity) I assessed. Specifically, I found that the Future lineage maintained higher maximum zoospore densities compared to the Current and Optimum lineages. However, the

Future lineage did not exhibit higher maximum whole culture viability compared to the Current and Optimum lineages. Yet, these two traits co-vary and therefore I calculated fecundity and found that the Future lineage had higher fecundity compared to the Current and Optimum lineages. Due to the mixed results of the acclimation test across traits, this experiment suggests there are inconclusive results to determine whether *Bd* shows an adaptive response to Future climate change conditions after experimental evolution.

The findings in Experiment 3 suggest that experimentally evolving *Bd* isolates at constant or fluctuating higher temperatures can impact the thermal performance curves. When I assessed the TPCs of the lineages propagated in different thermal conditions, I found three interesting results. First, I found that there was an effect of serial passaging on the LA isolate based on the results of the Ancestor and Optimum lineage comparisons. These results suggest that there may be an effect of SPEs or long-term passaging on *Bd* physiology *in vitro* even when the effect of temperature treatments is removed, which has been discussed in the *Bd* literature (Langhammer et al., 2013). Second, there was little evidence that temperatures simulating climate change conditions will cause a decrease in survival of *Bd* as some previous studies have suggested (Cohen et al., 2019; Daskin et al., 2011). My results comparing the TPCs of the Future lineage to the Ancestor lineages suggest that at least the *Bd* from LA can maintain its thermal breadth or P_{max} at its T_{opt} following increasing temperatures induced by climate change conditions. These results for the Future lineage led me to conclude that evolving at Future climate change conditions did not show consistent evidence of changes in the TPC. Third, I found that serially passaging a lineage at *Bd*'s CT_{max} , with no temperature variability or opportunity of recovery, did show a change in the T_{opt} for maximum zoospore densities. Therefore, further investigation is needed to determine if the observed differences among lineages after experimental evolution are due to an artifact of long-term passaging *in vitro* or if they represent genuine effects of climate change.

For Experiment 4, experimental evolution at Hot or Future conditions allowed for reduced *Bd* growth inhibition in the presence of skin secretions compared to the Ancestor. Specifically, in the response of maximum whole culture viability, I found support for my hypothesis that suggests that evolving in Future climate conditions allowed for increased growth in the presence of inhibitory skin secretions. My results also suggest that evolving *Bd* in high temperatures (Hot conditions) may increase maximum zoospore densities in the presence of inhibitory host skin secretions, which may be indicative of their pathogenicity (Fisher et al., 2021). These results are different from the conclusions of Experiment 3, which may be due to the additional factors introduced with the exposure to skin secretion that have a complex composition of antimicrobial peptides that impact pathogen growth independently of temperature (Rollins-Smith et al., 2005). If higher fluctuating climate conditions can influence *Bd* to produce higher maximum zoospore densities, even in the presence of host defenses, then disease outcomes involving isolates that can rapidly adapt to different thermal conditions may be exacerbated.

Discovering predictable patterns in the responses of *Bd* to climate change may help in general assessments of amphibian population projections in the future. For example, predicted decreased survival rates after exposure to climate change conditions could potentially benefit amphibian host populations (Cohen et al., 2019; Daskin et al., 2011; Neely et al., 2020). The findings of this study provide insights into the potential impacts of climate change on *Bd* and how it might respond to changing thermal conditions. For example, my results suggest that some *Bd* isolates may be capable of adaptation to increasing mean daily temperatures while maintaining their T_{opt} and P_{max} for whole culture viability and zoospore densities across the thermal range. Additionally, my study suggests that serially passaging *Bd* in high temperatures can impact *Bd* maximum whole culture viability and maximum zoospore densities in the presence of host skin secretions and may alter host-pathogen interactions. However, the effects of evolving at either fluctuating or stable Hot conditions near the thermal maxima had mixed results across

experiments and measured traits, suggesting a lack of consistent, predictable responses to climate change among multiple *Bd* isolates. This study will allow researchers to build upon our results and approach to better understand *Bd* responses to climate change and variable temperatures.

Multiple factors may be contributing to the inconsistencies among traits that I documented in this study. Although zoospore densities are likely linked to whole culture viability, I found mixed results for maximum zoospore densities and maximum whole culture viability in response to temperature in nearly all my experiments. This finding has also been reported in previous studies where temperatures differentially affected *Bd* traits (Muletz-Wolz et al., 2019; Sheets et al., 2021; Sinclair et al., 2016; Schulte et al., 2011; Schulte, 2015). Muletz-Wolz et al. (2019) suggested that phenotypic plasticity in physiological trait responses to temperature (for a single genotype, isolate, or lineage) could provide an explanation for observed inconsistencies. Additionally, it is currently not understood whether these differences could be due to life history characteristics. For example, temperature may be affecting each of these life-stages differently based on where they develop throughout the infection process. Zoospores have a free-living stage where they can navigate away from the host tissue, whereas the sporangia do not ever completely leave the host tissue (Berger et al., 2005). This difference in location and timing of development may result in different thermal selection pressures that could affect the two life stages differently. The role of these factors discussed, such as phenotypic plasticity of *Bd*, the effect of long-term culturing maintenance, individual variation among *Bd* isolates, and innate selection pressures occurring at the level of life-history traits is not resolved by this study. Additionally, other related traits such as encystment rates or quantities of zoospores released per mature sporangia may help future researchers to understand if there are distinct or varying selection pressures occurring for these two traits when exposed to variable temperatures.

Theories of thermal biological constraints, such as the climate variability hypothesis, suggest that the numerous and diverse elements of climate conditions (e.g., variable annual

conditions) make it unlikely that scientists can make straightforward predictions about the responses of different species to climate change (Kefford et al., 2022). My study supports this assertion because I observed that climate change thermal treatments led to mixed results in *Bd*'s responses across multiple experiments. My long-term study also shows that a shift in temperature (i.e., increasing mean fluctuating temperatures) alters maximum whole culture viability or maximum zoospore densities of *Bd*. Under the “hotter is better hypothesis”, researchers may predict that a warmer climate would select for organisms to change their T_{opt} to a warmer temperature while increasing the P_{max} (Angiletta, 2006, 2009). My study does not provide results that are consistent with this hypothesis and therefore, climate variability hypothesis may be more relevant to the *Bd* system.

Additional long-term experimental evolution studies are needed to fully capture the effects of climate change on *Bd* in additional *in vitro* as well as *in vivo* experiments. My study suggests that the responses of *Bd* to serial passaging *in vitro* are inconsistent. Further investigation of additional isolates would allow for the assessment of variation in *Bd* responses to variable temperatures, climate change, and experimental evolution using serial passaging techniques. Future studies could investigate the genetic changes that occur if *Bd* adapts to climate change to understand the mechanisms that lead to changes in thermal sensitivity for growth and reproductive traits. For example, researchers can assess cryo-archived aliquots of different lineages over time to examine the effect of passaging, time, and cryo-preservation techniques on *Bd* responses. Comparisons between evolved and ancestral lineages have provided insights into specific trait changes, such as zoospore densities, that can increase overall reproductive output. However, more research is needed to understand the impact of climate change on other physiological traits, such as motility, spore size, and maturation rate. Furthermore, comparisons between evolved and ancestral lineages in the context of host biology could provide insights into

differences in pathogenicity in live hosts. Studies that expose live hosts to ancestral and evolved lineages at different thermal treatments can help understand changes in disease dynamics.

Finally, the insights gained from my study on adaptation of *Bd* to changing temperatures are not limited to the chytridiomycosis disease system alone. Instead, these insights offer important implications for understanding the impacts of climate change on other infectious diseases that may also rapidly adapt and affect host populations, community dynamics, and entire ecosystems. My study has shown the results of using experimental evolution, specifically serial passage experiments, to predict the effects of variable temperatures and other climate change factors on pathogen physiology. As discussed above, the effects of complex factors of a changing climate on disease dynamics are difficult to assess with short-term *in vitro* studies. However, by incorporating ecologically relevant environmental factors in long-term experimental evolution studies, researchers can better understand the relationship between pathogen physiology and a changing environment. Thus, our model system provides a valuable framework for advancing our understanding of the complex and dynamic relationships among infectious pathogens, their hosts, and the changing environment.

References

- Altizer, S., Ostfeld, R. S., Johnson, P. T. J., Kutz, S., & Harvell, C. D. (2013). Climate Change and Infectious Diseases: From Evidence to a Predictive Framework. *Science*, *341*(6145), 514–519. <https://doi.org/10.1126/science.1239401>
- Angilletta, M. J. (2006). Estimating and comparing thermal performance curves. *Journal of Thermal Biology*, *31*(7), 541–545. <https://doi.org/10.1016/j.jtherbio.2006.06.002>
- Angilletta, M. J. (2009). *Thermal Adaptation: A Theoretical and Empirical Synthesis*. OUP Oxford.
- Bartelt, P. E., Klaver, R. W., & Porter, W. P. (2010). Modeling amphibian energetics, habitat suitability, and movements of western toads, *Anaxyrus (=Bufo) boreas*, across present and future landscapes. *Ecological Modelling*, *221*(22), 2675–2686. <https://doi.org/10.1016/j.ecolmodel.2010.07.009>
- Bazin, H. (2003). A brief history of the prevention of infectious diseases by immunisations. *Comparative Immunology, Microbiology and Infectious Diseases*, *26*(5–6), 293–308. [https://doi.org/10.1016/S0147-9571\(03\)00016-X](https://doi.org/10.1016/S0147-9571(03)00016-X)
- Bennett, A. F., & Hughes, B. S. (2009). Microbial experimental evolution. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *297*(1), R17–R25. <https://doi.org/10.1152/ajpregu.90562.2008>
- Bennett, A. F., & Lenski, R. E. (1993). EVOLUTIONARY ADAPTATION TO TEMPERATURE II. THERMAL NICHEs OF EXPERIMENTAL LINES OF *ESCHERICHIA COLI*. *Evolution*, *47*(1), 1–12. <https://doi.org/10.1111/j.1558-5646.1993.tb01194.x>
- Bennett, A. F., & Lenski, R. E. (2007). An experimental test of evolutionary trade-offs during temperature adaptation. *Proceedings of the National Academy of Sciences*, *104*(suppl_1), 8649–8654. <https://doi.org/10.1073/pnas.0702117104>

- Bennett, J. M., Sunday, J., Calosi, P., Villalobos, F., Martínez, B., Molina-Venegas, R., Araújo, M. B., Algar, A. C., Clusella-Trullas, S., Hawkins, B. A., Keith, S. A., Kühn, I., Rahbek, C., Rodríguez, L., Singer, A., Morales-Castilla, I., & Olalla-Tárraga, M. Á. (2021). The evolution of critical thermal limits of life on Earth. *Nature Communications*, 12(1), 1198. <https://doi.org/10.1038/s41467-021-21263-8>
- Boyle, D., Boyle, D., Olsen, V., Morgan, J., & Hyatt, A. (2004). Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. *Diseases of Aquatic Organisms*, 60, 141–148. <https://doi.org/10.3354/dao060141>
- Bradley, P. W., Brawner, M. D., Raffel, T. R., Rohr, J. R., Olson, D. H., & Blaustein, A. R. (2019). Shifts in temperature influence how *Batrachochytrium dendrobatidis* infects amphibian larvae. *PLOS ONE*, 14(9), e0222237. <https://doi.org/10.1371/journal.pone.0222237>
- Cohen, J. M., Civitello, D. J., Venesky, M. D., McMahon, T. A., & Rohr, J. R. (2019). An interaction between climate change and infectious disease drove widespread amphibian declines. *Global Change Biology*, 25(3), 927–937. <https://doi.org/10.1111/gcb.14489>
- Cohen, J. M., McMahon, T. A., Ramsay, C., Roznik, E. A., Sauer, E. L., Bessler, S., Civitello, D. J., Delius, B. K., Halstead, N., Knutie, S. A., Nguyen, K. H., Ortega, N., Sears, B., Venesky, M. D., Young, S., & Rohr, J. R. (2019). Impacts of thermal mismatches on chytrid fungus *Batrachochytrium dendrobatidis* prevalence are moderated by life stage, body size, elevation and latitude. *Ecology Letters*, 22(5), 817–825. <https://doi.org/10.1111/ele.13239>
- Daskin, J. H., Alford, R. A., & Puschendorf, R. (2011). Short-Term Exposure to Warm Microhabitats Could Explain Amphibian Persistence with *Batrachochytrium dendrobatidis*. *PLoS ONE*, 6(10), Article 10.

<https://doi.org/10.1371/journal.pone.0026215>

- Deardorff, E. R., Fitzpatrick, K. A., Jerzak, G. V. S., Shi, P.-Y., Kramer, L. D., & Ebel, G. D. (2011). West Nile Virus Experimental Evolution in vivo and the Trade-off Hypothesis. *PLoS Pathogens*, 7(11), e1002335. <https://doi.org/10.1371/journal.ppat.1002335>
- Ebert, D. (1998). Experimental Evolution of Parasites. *Science*, 282(5393), 1432–1436. <https://doi.org/10.1126/science.282.5393.1432>
- Fay, R. L., Ngo, K. A., Kuo, L., Willsey, G. G., Kramer, L. D., & Ciota, A. T. (2021). Experimental Evolution of West Nile Virus at Higher Temperatures Facilitates Broad Adaptation and Increased Genetic Diversity. *Viruses*, 13(10), 1889. <https://doi.org/10.3390/v13101889>
- Fernández-Loras, A., Boyero, L., Correa-Araneda, F., Tejedo, M., Hettyey, A., & Bosch, J. (2019). Infection with *Batrachochytrium dendrobatidis* lowers heat tolerance of tadpole hosts and cannot be cleared by brief exposure to CTmax. *PLOS ONE*, 14(4), e0216090. <https://doi.org/10.1371/journal.pone.0216090>
- Fick, S.E. and R.J. Hijmans, 2017. Worldclim 2: New 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*
- Fisher, M. C., Pasmans, F., & Martel, A. (2021). Virulence and Pathogenicity of Chytrid Fungi Causing Amphibian Extinctions. *Annual Review of Microbiology*, 75(1), 673–693. <https://doi.org/10.1146/annurev-micro-052621-124212>
- Ford, S., Chintala, M., & Bushek, D. (2002). Comparison of in vitro-cultured and wild-type *Perkinsus marinus*. I. Pathogen virulence. *Diseases of Aquatic Organisms*, 51, 187–201. <https://doi.org/10.3354/dao051187>
- Ford, S. E., & Chintala, M. M. (2006). Northward expansion of a marine parasite: Testing the role of temperature adaptation. *Journal of Experimental Marine Biology and Ecology*, 339(2), 226–235. <https://doi.org/10.1016/j.jembe.2006.08.004>

- Gunderson, A. R., & Leal, M. (2016). A conceptual framework for understanding thermal constraints on ectotherm activity with implications for predicting responses to global change. *Ecology Letters*, *19*(2), 111–120. <https://doi.org/10.1111/ele.12552>
- Hamilton, P. T., Richardson, J. M. L., Govindarajulu, P., & Anholt, B. R. (2012). Higher temperature variability increases the impact of *Batrachochytrium dendrobatidis* and shifts interspecific interactions in tadpole mesocosms. *Ecology and Evolution*, *2*(10), Article 10. <https://doi.org/10.1002/ece3.369>
- Kawecki, T. J., Lenski, R. E., Ebert, D., Hollis, B., Olivieri, I., & Whitlock, M. C. (2012). Experimental evolution. *Trends in Ecology & Evolution*, *27*(10), 547–560. <https://doi.org/10.1016/j.tree.2012.06.001>
- Kefford, B. J., Ghalambor, C. K., Dewenter, B., Poff, N. L., Hughes, J., Reich, J., & Thompson, R. (2022). Acute, diel, and annual temperature variability and the thermal biology of ectotherms. *Global Change Biology*, *28*(23), 6872–6888. <https://doi.org/10.1111/gcb.16453>
- Kelly, M. W. (2022). Experimental evolution reveals complex responses to environmental change. *Proceedings of the National Academy of Sciences*, *119*(43), e2214263119. <https://doi.org/10.1073/pnas.2214263119>
- Langhammer, P. F., Lips, K. R., Burrowes, P. A., Tunstall, T., Palmer, C. M., & Collins, J. P. (2013). A Fungal Pathogen of Amphibians, *Batrachochytrium dendrobatidis*, Attenuates in Pathogenicity with In Vitro Passages. *PLoS ONE*, *8*(10), e77630. <https://doi.org/10.1371/journal.pone.0077630>
- Lafferty, K. D. (2009). Calling for an ecological approach to studying climate change and infectious diseases. *Ecology*, *90*(4), 932–933. <https://doi.org/10.1890/08-1767.1>
- Lafferty, K. D., & Mordecai, E. A. (2016). The rise and fall of infectious disease in a warmer world. *F1000Research*, *5*, 2040. <https://doi.org/10.12688/f1000research.8766.1>

- Lambrechts, L., Paaijmans, K. P., Fansiri, T., Carrington, L. B., Kramer, L. D., Thomas, M. B., & Scott, T. W. (2011). Impact of daily temperature fluctuations on dengue virus transmission by *Aedes aegypti*. *Proceedings of the National Academy of Sciences*, *108*(18), 7460–7465. <https://doi.org/10.1073/pnas.1101377108>
- Lenski, R. E. (2017). Experimental evolution and the dynamics of adaptation and genome evolution in microbial populations. *The ISME Journal*, *11*(10), 2181–2194. <https://doi.org/10.1038/ismej.2017.69>
- Le Sage, E. H., Ohmer, M. E. B., LaBumbard, B. C., Altman, K. A., Reinert, L. K., Bednark, J. G., Bletz, M. C., Inman, B., Lindauer, A., McDonnell, N. B., Parker, S. K., Skerlec, S. M., Wantman, T., Rollins-Smith, L. A., Woodhams, D. C., Voyles, J., & Richards-Zawacki, C. L. (2022). Localized carry-over effects of pond drying on survival, growth, and pathogen defenses in amphibians. *Ecosphere*, *13*(9). <https://doi.org/10.1002/ecs2.4224>
- Lindauer, A. L., Maier, P. A., & Voyles, J. (2020). Daily fluctuating temperatures decrease growth and reproduction rate of a lethal amphibian fungal pathogen in culture. *BMC Ecology*, *20*(1), 18. <https://doi.org/10.1186/s12898-020-00286-7>
- Lindauer, A., May, T., Rios-Sotelo, G., Sheets, C., & Voyles, J. (2019). Quantifying *Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans* Viability. *EcoHealth*, *16*(2), 346–350. <https://doi.org/10.1007/s10393-019-01414-6>
- Longcore, J. E., Pessier, A. P., & Nichols, D. K. (1999). *Batrachochytrium dendrobatidis* gen. Et sp. Nov., a chytrid pathogenic to amphibians. *Mycologia*, *91*(2), 219–227. <https://doi.org/10.1080/00275514.1999.12061011>
- Muletz-Wolz, C. R., Barnett, S. E., DiRenzo, G. V., Zamudio, K. R., Toledo, L. F., James, T. Y., & Lips, K. R. (2019). Diverse genotypes of the amphibian-killing fungus produce distinct phenotypes through plastic responses to temperature. *Journal of Evolutionary Biology*,

32(3), 287–298. <https://doi.org/10.1111/jeb.13413>

- Neely, W. J., Greenspan, S. E., Ribeiro, L. P., Carvalho, T., Martins, R. A., Rodriguez, D., Rohr, J. R., Haddad, C. F. B., Toledo, L. F., & Becker, C. G. (2020). Synergistic effects of warming and disease linked to high mortality in cool-adapted terrestrial frogs. *Biological Conservation*, 245, 108521. <https://doi.org/10.1016/j.biocon.2020.108521>
- R-Core-Team. R: A Language and Environment for Statistical Computing. Vienna: R Foundation for Statistical Computing (2019).
- Raffel, T. R., Romansic, J. M., Halstead, N. T., McMahon, T. A., Venesky, M. D., & Rohr, J. R. (2013). Disease and thermal acclimation in a more variable and unpredictable climate. *Nature Climate Change*, 3(2), 146–151. <https://doi.org/10.1038/nclimate1659>
- Robin, C., Andanson, A., Saint-Jean, G., Fabreguettes, O., & Dutech, C. (2017). What was old is new again: Thermal adaptation within clonal lineages during range expansion in a fungal pathogen. *Molecular Ecology*, 26(7), 1952–1963. <https://doi.org/10.1111/mec.14039>
- Rohr, J. R., Civitello, D. J., Cohen, J. M., Roznik, E. A., Sinervo, B., & Dell, A. I. (2018). The complex drivers of thermal acclimation and breadth in ectotherms. *Ecology Letters*, 21(9), Article 9. <https://doi.org/10.1111/ele.13107>
- Rohr, J. R., Dobson, A. P., Johnson, P. T. J., Kilpatrick, A. M., Paull, S. H., Raffel, T. R., Ruiz-Moreno, D., & Thomas, M. B. (2011). Frontiers in climate change–disease research. *Trends in Ecology & Evolution*, 26(6), 270–277. <https://doi.org/10.1016/j.tree.2011.03.002>
- Rohr, J. R., & Raffel, T. R. (2010). Linking global climate and temperature variability to widespread amphibian declines putatively caused by disease. *Proceedings of the National Academy of Sciences*, 107(18), 8269–8274. <https://doi.org/10.1073/pnas.0912883107>
- Rohr, J. R., Raffel, T. R., Romansic, J. M., McCallum, H., & Hudson, P. J. (2008). Evaluating the links between climate, disease spread, and amphibian declines. *Proceedings of the*

National Academy of Sciences, 105(45), 17436–17441.

<https://doi.org/10.1073/pnas.0806368105>

Rollins-Smith, L. A., Reinert, L. K., O’Leary, C. J., Houston, L. E., & Woodhams, D. C. (2005).

Antimicrobial Peptide Defenses in Amphibian Skin. *Integrative and Comparative Biology*, 45(1), Article 1. <https://doi.org/10.1093/icb/45.1.137>

Schampera, C., Agha, R., Manzi, F., & Wolinska, J. (2022). Parasites do not adapt to elevated

temperature, as evidenced from experimental evolution of a phytoplankton–fungus system. *Biology Letters*, 18(2), 20210560. <https://doi.org/10.1098/rsbl.2021.0560>

Schulte, P. M. (2015). The effects of temperature on aerobic metabolism: Towards a mechanistic

understanding of the responses of ectotherms to a changing environment. *Journal of Experimental Biology*, 218(12), 1856–1866. <https://doi.org/10.1242/jeb.118851>

Schulte, P. M., Healy, T. M., & Fangué, N. A. (2011). Thermal Performance Curves, Phenotypic

Plasticity, and the Time Scales of Temperature Exposure. *Integrative and Comparative Biology*, 51(5), Article 5. <https://doi.org/10.1093/icb/icr097>

Sheets, C. N., Schmidt, D. R., Hurtado, P. J., Byrne, A. Q., Rosenblum, E. B., Richards-Zawacki,

C. L., & Voyles, J. (2021). Thermal Performance Curves of Multiple Isolates of *Batrachochytrium dendrobatidis*, a Lethal Pathogen of Amphibians. *Frontiers in*

Veterinary Science, 8. <https://www.frontiersin.org/article/10.3389/fvets.2021.687084>

Sinclair, B. J., Marshall, K. E., Sewell, M. A., Levesque, D. L., Willett, C. S., Slotsbo, S., Dong,

Y., Harley, C. D. G., Marshall, D. J., Helmuth, B. S., & Huey, R. B. (2016). Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? *Ecology Letters*, 19(11), 1372–1385.

<https://doi.org/10.1111/ele.12686>

Somerville, G. A., Beres, S. B., Fitzgerald, J. R., DeLeo, F. R., Cole, R. L., Hoff, J. S., & Musser,

J. M. (2002). In Vitro Serial Passage of *Staphylococcus aureus*: Changes in Physiology,

- Virulence Factor Production, and *agr* Nucleotide Sequence. *Journal of Bacteriology*, 184(5), 1430–1437. <https://doi.org/10.1128/JB.184.5.1430-1437.2002>
- Stevenson, L. A., Alford, R. A., Bell, S. C., Roznik, E. A., Berger, L., & Pike, D. A. (2013). Variation in Thermal Performance of a Widespread Pathogen, the Amphibian Chytrid Fungus *Batrachochytrium dendrobatidis*. *PLoS ONE*, 8(9), e73830. <https://doi.org/10.1371/journal.pone.0073830>
- Voyles, J. (2011). Phenotypic profiling of *Batrachochytrium dendrobatidis*, a lethal fungal pathogen of amphibians. *Fungal Ecology*, 4(3), 196–200. <https://doi.org/10.1016/j.funeco.2010.12.003>
- Voyles, J., Johnson, L. R., Briggs, C. J., Cashins, S. D., Alford, R. A., Berger, L., Skerratt, L. F., Speare, R., & Rosenblum, E. B. (2012). Temperature alters reproductive life history patterns in *Batrachochytrium dendrobatidis*, a lethal pathogen associated with the global loss of amphibians. *Ecology and Evolution*, 2(9), 2241–2249. <https://doi.org/10.1002/ece3.334>
- Voyles, J., Johnson, L. R., Briggs, C. J., Cashins, S. D., Alford, R. A., Berger, L., Skerratt, L. F., Speare, R., & Rosenblum, E. B. (2014). Experimental evolution alters the rate and temporal pattern of population growth in *Batrachochytrium dendrobatidis*, a lethal fungal pathogen of amphibians. *Ecology and Evolution*, 4(18), 3633–3641. <https://doi.org/10.1002/ece3.1199>
- Voyles, J., Johnson, L. R., Rohr, J., Kelly, R., Barron, C., Miller, D., Minster, J., & Rosenblum, E. B. (2017). Diversity in growth patterns among strains of the lethal fungal pathogen *Batrachochytrium dendrobatidis* across extended thermal optima. *Oecologia*, 184(2), 363–373. <https://doi.org/10.1007/s00442-017-3866-8>
- Woo, H. J., & Reifman, J. (2014). Quantitative Modeling of Virus Evolutionary Dynamics and Adaptation in Serial Passages Using Empirically Inferred Fitness Landscapes. *Journal of*

Virology, 88(2), Article 2. <https://doi.org/10.1128/JVI.02958-13>

Woodhams, D. C., Alford, R. A., Briggs, C. J., Johnson, M., & Rollins-Smith, L. A. (2008). Life-history trade-offs influence disease in changing climates: Strategies of an amphibian pathogen. *Ecology*, 89(6), Article 6. <https://doi.org/10.1890/06-1842.1>

Wu, E.-J., Wang, Y.-P., Yang, L.-N., Zhao, M.-Z., & Zhan, J. (2022). Elevating Air Temperature May Enhance Future Epidemic Risk of the Plant Pathogen *Phytophthora infestans*. *Journal of Fungi*, 8(8), 808. <https://doi.org/10.3390/jof8080808>

Conclusion

Temperature is an environmental factor that has been studied for its role in altering organismal biology and disease dynamics for centuries (Beyer, 1885; Murray, 1883). Through decades of study, researchers have shown that temperature affects organismal traits, such as growth, reproduction, respiration, etc., in ways that can impact evolutionary potential over time (Allen, 1923; Bennett & Lenski, 1993; Lenski et al., 2017). Studies have shown that changes in temperature on a daily, seasonal, or annual basis can result in different evolutionary trajectories for many organisms (Bennett & Lenski, 1993; Tourneur & Meunier, 2020).

Temperatures are expected to increase and/or become more variable due to the effects of climate change (Epstein, 2001). Researchers are interested in whether these temperature changes associated with global climate change will affect disease outcomes through the alteration of host-pathogen interactions, particularly in systems where the pathogen is temperature-sensitive (Barber et al., 2016; Raffel et al., 2013).

Chytridiomycosis provides a model system to test hypotheses about the ways in which climate change and increased daily temperatures may impact pathogen-host biology (Rohr et al., 2011). This is because the pathogen that causes chytridiomycosis, *Batrachochytrium dendrobatidis* (*Bd*) is a temperature-sensitive fungal pathogen (Piotrowski et al., 2004) that infects an ectothermic amphibian host (Berger et al., 1998; Longcore et al., 1999).

In this dissertation, I assessed the effects of temperature on *Bd* to establish thermal biology characteristics, thermal sensitivity, and thermal tolerance. Additionally, I used the thermal biology information of *Bd* to further investigate how changes in temperature and regional temperature profiles shape its adaptive potential. My findings have broad implications for the predictions of *Bd* adaptation in the context of climate change. In addition, my findings are applicable for understanding and informing conservation efforts in the fight to mitigate the disease-induced declines faced by many amphibian species (Berger et al., 1998). Finally, my

findings investigate the impacts of variable temperatures and selection of thermal maxima temperatures, which can be used in other disease systems.

First, my research aimed to synthesize the current literature related to temperature effects on ectotherm biology within disease systems. There is a relatively large literature that focuses on how temperature (constant and variable) impacts an ectothermic host or its pathogen (Duncan et al., 2011; Huey & Kingsolver, 1989; Rohr et al., 2018). However, I conclude that more research could be useful when trying to understand general patterns of how changing temperatures can impact disease outcomes. Similarly, there are additional research directions for scientists to investigate the effects of changing climates and temperature variations on host-pathogen interactions (Rohr et al., 2011). For example, researchers can strive to understand how temperature may impact disease outcomes when one or more pathogens are causing infection simultaneously (co-infections; Barber et al., 2016; Herczeg et al., 2021). Chytridiomycosis is a unique disease system that may serve to be a model system to further the investigation of climate change impacts on ectotherms facing declines from disease (Alford et al., 2007).

Second, in Chapter 2, I showed how isolates of *Bd* spanning a latitudinal gradient each have unique thermal performance curves (TPC) and characteristics across the thermal range of *Bd*. My study suggests that *Bd*, even within the same genotype or genetic clade, has different thermal maxima, thermal minima, thermal optima, and maximum performance for a given trait (Sheets et al., 20213). The findings of this chapter provide support for existing literature, which indicates that neither geographic location nor genotype can fully explain the patterns of thermal performance (Becker et al., 2017; Lambertini et al., 2016; Muletz-Wolz et al., 2019; Sonn et al., 2019). These results highlight the variability of thermal sensitivity in *Bd* across different genetic lineages and locally evolved isolates (Muletz-Wolz et al., 2019; Lambertini et al., 2016). Considering the literature and the findings of my dissertation, it would be valuable for future research to investigate the up or down regulation of genes related to thermal tolerance of specific

genes associated with growth and reproduction in *Bd* when exposed to various temperature profiles. This approach would contribute to a better understanding of the mechanisms underpinning *Bd* physiological responses to temperature. Additionally, expanding the range of isolates available for laboratory studies could provide insights into general patterns of thermal biology for *Bd* under variable temperature conditions.

Third, my dissertation shows that when examining a case study for *Bd* within a region, researchers can understand important physiological responses, whether assessing constant or fluctuating temperature conditions. Recent literature has been concerned with how experiments with constant or fluctuating temperature studies *in vitro* can be best implemented to understand the effects of global warming on *Bd* (Gajewski et al., 2021; Greenspan et al., 2023). The third chapter showed that when the New Mexico isolate of *Bd* was grown in variable temperature conditions (similar to the temperature profile the isolate would experience in the wild) simulated summer temperatures reduced growth and reproductive traits maximum output compared to the winter season. However, importantly, the simulated summer temperatures did not kill *Bd*. Yet, my findings support the notion that increased variable temperatures may reduce *Bd* growth and reproduction (Lindauer et al., 2020), but understanding the effects of regional environmental conditions is also important to predict changes in disease dynamics.

Lastly, I conducted experiments to investigate the effects of climate change, specifically increased mean daily temperatures and temperature fluctuations, on *Bd* physiology. The findings revealed that fluctuating temperatures can indeed influence *Bd* physiology, but there is still much to understand about how *Bd* will respond to climate change conditions. Some isolates of *Bd* showed potential adaptation to increased fluctuating temperatures following long-term serial passaging, but there was no clear pattern in the growth and reproductive responses under these simulated climate change conditions. The relationship between climate change and pathogenicity-related factors was complex, with mixed results on whether adaptation occurred through

increased growth and reproductive responses. Further research is needed to explore the effects of simulated climate change conditions on host dynamics and the adaptation of *Bd*, including studying alterations in gene expression. Based on the findings of this final chapter, I suggest that this research has broad implications for understanding the impact of thermal biology in a changing environment on disease dynamics in chytridiomycosis as well as other disease systems.

References

- Alford, R. A., Bradfield, K. S., & Richards, S. J. (2007). Global warming and amphibian losses. *Nature*, 447(7144), Article 7144. <https://doi.org/10.1038/nature05940>
- Allen, P. W. (1923). The Attenuation of Bacteria Due to Temperature Shock. *Journal of Bacteriology*, 8(6), 555–566. <https://doi.org/10.1128/jb.8.6.555-566.1923>
- Altizer, S., Ostfeld, R. S., Johnson, P. T. J., Kutz, S., & Harvell, C. D. (2013). Climate Change and Infectious Diseases: From Evidence to a Predictive Framework. *Science*, 341(6145), 514–519. <https://doi.org/10.1126/science.1239401>
- Barber, I., Berkhout, B. W., & Ismail, Z. (2016). Thermal Change and the Dynamics of Multi-Host Parasite Life Cycles in Aquatic Ecosystems. *Integrative and Comparative Biology*, 56(4), Article 4. <https://doi.org/10.1093/icb/icw025>
- Becker, C. G., Greenspan, S. E., Tracy, K. E., Dash, J. A., Lambertini, C., Jenkinson, T. S., Leite, D. S., Toledo, L. F., Longcore, J. E., James, T. Y., & Zamudio, K. R. (2017). Variation in phenotype and virulence among enzootic and panzootic amphibian chytrid lineages. *Fungal Ecology*, 26, 45–50. <https://doi.org/10.1016/j.funeco.2016.11.007>
- Bennett, A. F., & Lenski, R. E. (1993). EVOLUTIONARY ADAPTATION TO TEMPERATURE II. THERMAL NICHEs OF EXPERIMENTAL LINES OF *ESCHERICHIA COLI*. *Evolution*, 47(1), 1–12. <https://doi.org/10.1111/j.1558-5646.1993.tb01194.x>
- Beyer, H. G. (1885). The influence of variations of temperature upon the rate and the work of the heart of the slider terrapin (*Pseudemys rugosa*). *Proceedings of the United States National Museum*.
- Duncan, A. B., Fellous, S., & Kaltz, O. (2011). Temporal variation in temperature determines disease spread and maintenance in *Paramecium* microcosm populations. *Proceedings of*

the Royal Society B: Biological Sciences, 278(1723), Article 1723.

<https://doi.org/10.1098/rspb.2011.0287>

Epstein, P. R. (2001). Climate change and emerging infectious diseases. *Microbes and Infection*, 8.

Fisher, M. C., Pasmans, F., & Martel, A. (2021). Virulence and Pathogenicity of Chytrid Fungi Causing Amphibian Extinctions. *Annual Review of Microbiology*, 75(1), 673–693.

<https://doi.org/10.1146/annurev-micro-052621-124212>

Gajewski, Z., Stevenson, L. A., Pike, D. A., Roznik, E. A., Alford, R. A., & Johnson, L. R. (2021). Predicting the growth of the amphibian chytrid fungus in varying temperature environments. *Ecology and Evolution*, 11(24), 17920–17931.

<https://doi.org/10.1002/ece3.8379>

Greenspan, S. E., Roznik, E. A., Edwards, L., Duffy, R., Berger, L., Bower, D. S., Pike, D. A., Schwarzkopf, L., & Alford, R. A. (2023). Constant-temperature predictions underestimate growth of a fungal amphibian pathogen under individual host thermal profiles. *Journal of Thermal Biology*, 111, 103394.

<https://doi.org/10.1016/j.jtherbio.2022.103394>

Herczeg, D., Ujszegi, J., Kásler, A., Holly, D., & Hettyey, A. (2021). Host–multiparasite interactions in amphibians: A review. *Parasites & Vectors*, 14(1), 296.

<https://doi.org/10.1186/s13071-021-04796-1>

Huey, R. B., & Kingsolver, J. G. (1989). Evolution of thermal sensitivity of ectotherm performance. *Trends in Ecology & Evolution*, 4(5), Article 5.

[https://doi.org/10.1016/0169-5347\(89\)90211-5](https://doi.org/10.1016/0169-5347(89)90211-5)

Lambertini, C., Becker, C. G., Jenkinson, T. S., Rodriguez, D., da Silva Leite, D., James, T. Y., Zamudio, K. R., & Toledo, L. F. (2016). Local phenotypic variation in amphibian-killing

fungus predicts infection dynamics. *Fungal Ecology*, 20, 15–21.

<https://doi.org/10.1016/j.funeco.2015.09.014>

Lenski, R. E. (2017). Experimental evolution and the dynamics of adaptation and genome evolution in microbial populations. *The ISME Journal*, 11(10), 2181–2194.

<https://doi.org/10.1038/ismej.2017.69>

Lindauer, A. L., Maier, P. A., & Voyles, J. (2020). Daily fluctuating temperatures decrease growth and reproduction rate of a lethal amphibian fungal pathogen in culture. *BMC Ecology*, 20(1), 18. <https://doi.org/10.1186/s12898-020-00286-7>

Muletz-Wolz, C. R., Barnett, S. E., DiRenzo, G. V., Zamudio, K. R., Toledo, L. F., James, T. Y., & Lips, K. R. (2019). Diverse genotypes of the amphibian-killing fungus produce distinct phenotypes through plastic responses to temperature. *Journal of Evolutionary Biology*, 32(3), 287–298. <https://doi.org/10.1111/jeb.13413>

Murray, N. (1883). *Studies from the Biological Laboratory: V.1 -5; 1877/78-1891/93*. N. Murray, Johns Hopkins University.

Piotrowski JS, Annis SL, Longcore JE. Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. *Mycologia*. 2004;96:9–15.

Raffel, T. R., Romansic, J. M., Halstead, N. T., McMahon, T. A., Venesky, M. D., & Rohr, J. R. (2013). Disease and thermal acclimation in a more variable and unpredictable climate. *Nature Climate Change*, 3(2), 146–151. <https://doi.org/10.1038/nclimate1659>

Rohr, J. R., Civitello, D. J., Cohen, J. M., Roznik, E. A., Sinervo, B., & Dell, A. I. (2018). The complex drivers of thermal acclimation and breadth in ectotherms. *Ecology Letters*, 21(9), Article 9. <https://doi.org/10.1111/ele.13107>

Rohr, J. R., Dobson, A. P., Johnson, P. T. J., Kilpatrick, A. M., Paull, S. H., Raffel, T. R., Ruiz-Moreno, D., & Thomas, M. B. (2011). Frontiers in climate change–disease research.

Trends in Ecology & Evolution, 26(6), Article 6.

<https://doi.org/10.1016/j.tree.2011.03.002>

Sheets, C. N., Schmidt, D. R., Hurtado, P. J., Byrne, A. Q., Rosenblum, E. B., Richards-Zawacki, C. L., & Voyles, J. (2021). Thermal Performance Curves of Multiple Isolates of *Batrachochytrium dendrobatidis*, a Lethal Pathogen of Amphibians. *Frontiers in Veterinary Science*, 8. <https://www.frontiersin.org/article/10.3389/fvets.2021.687084>

Sonn, J. M., Utz, R. M., & Richards-Zawacki, C. L. (2019). Effects of latitudinal, seasonal, and daily temperature variations on chytrid fungal infections in a North American frog. *Ecosphere*, 10(11). <https://doi.org/10.1002/ecs2.2892>

Stevenson, L. A., Alford, R. A., Bell, S. C., Roznik, E. A., Berger, L., & Pike, D. A. (2013). Variation in Thermal Performance of a Widespread Pathogen, the Amphibian Chytrid Fungus *Batrachochytrium dendrobatidis*. *PLoS ONE*, 8(9), e73830.

<https://doi.org/10.1371/journal.pone.0073830>

Tourneur, J., & Meunier, J. (2020). Variations in seasonal (not mean) temperatures drive rapid adaptations to novel environments at a continent scale. *Ecology*, 101(4), Article 4.

<https://doi.org/10.1002/ecy.2973>