THERMAL BIOLOGY OF PARASITES AND THEIR HOSTS IN THE LABORATORY AND CLASSROOM

by

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A dissertation submitted in partial fulfillment of the
requirements for the degree of

DOCTOR OF PHILOSOPHY IN BIOLOGICAL AND BIOMEDICAL SCIENCES

2024

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In memory of my mom, and for everyone (even those with four legs) who gave me a reason to keep going.

ACKNOWLEDGMENTS

To my adviser, Dr. Thomas Raffel, thank you for supporting me through multiple degrees and hardships. You showed me how to be a good scientist, educator, mentor, and friend. I will always have the skills you instilled in me, and I hope to pass on your teachings.

To my committee members, Dr. Keith Berven and Dr. Shaun Moore, thank you for your mentorship, valuable input, and guidance through my educational journey.

To all past and present lab members including Karie Altman, Sean Brady, Ryan McWhinnie, Andrea Nadjarian, Jay Noelker, Vitoria Ruozzi, and Jason Sckrabulis, thank you for your advice, support and friendship. To the undergrads for their help: Jenna Agosta, Carson Evans, Grace Fielhauer, Gina Ghose, Autumn Gratti, Jacob Heabeart, Alejandro Hernandez, Sajjad Kamil, Declan McCrary, Amanda McKeon, Alex Orille, Melissa Ostrowski, Galilee Rogers, Holly Russell, Keshav Seetharaman, Leah Shamoun, Vanessa Six, Kyle Spengler, Rima Stepanian, Julia Tituskin, Levi Trumbore, and Jazalyn Williams.

To Mike Benard, Kristen Cecala, David Dimitrie, Tim Krynak, Ana Locci, Joyce Longcore, Pat Lorch, and Paul Pira for help obtaining animals and cultures. To Holly Greiner-Hallman, Ava McDowell, and Gary Miller for their assistance, cooperation, and guidance. To the OU Department of Biological Sciences faculty, staff, instructors, and students for their assistance and cooperation.

Funding for this work was provided by NSF (IOS-1651888). Use of animals and Bd was approved by OU IACUC (18011; Appendix A) and OU IBC (2759-13, Appendix B). Human subjects work was deemed Exempt by OU IRB (FY2021-369; Appendix C).

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ABSTRACT

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The threat of climate change makes it increasingly important for biologists and the public to understand how organisms respond to temperature. The Metabolic Theory of Ecology (MTE) predicts that temperature should affect organism performance, with implications for species interactions and ecosystems that span disciplines including mathematics, chemistry, and biology. Temperature strongly impacts parasitic diseases of ectothermic hosts, with important implications for diseases of humans and wildlife. It is thus vital to examine metabolic responses of hosts and parasites to temperature in research laboratories and classroom education. I used MTE-based models to describe thermal responses of the pathogen *Batrachochytrium dendrobatidis* (Bd) and its amphibian hosts (*Notophthalmus viridescens*, eastern red-spotted newts). I measured newt breath rate and oxygen consumption (Chapter 2), as well as Bd zoospore swimming speed and critical thermal maximum (Chapter 3), to parameterize MTE-based models of host and parasite thermal performance. I conducted a controlled-temperature Bd infection experiment with newts collected from different latitudes (Chapter 4). In all three experiments, I also used temperature shifts to test for thermal acclimation effects. I

hypothesized that newts and Bd would exhibit beneficial acclimation, but that Bd would acclimate to a new temperature faster because of its smaller mass and faster metabolism. I found little evidence of thermal acclimation effects on host performance, but my results suggested that the Bd either shows no acclimation or very rapid (< 3 minutes) acclimation responses. I also combined scholarship of research with scholarship of learning by developing an online teaching lab activity that introduces students to MTE-based thermal models by contrasting thermal responses in humans versus frogs (Chapter 5). I conducted a controlled experiment to compare learning outcomes for the online versus the face-to-face version of the activity (Chapter 6). Results varied, but students generally achieved similar learning outcomes in both versions of this activity.

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LIST OF ABBREVIATIONS

AIC	Akaike Information Criterion
ASW	Artificial Spring Water
BA	Boltzmann-Arrhenius
BB	Ball bearing
Bd	*Batrachochytrium dendrobatidis*
BIO 1201	Introductory Biology Laboratory class at Oakland University
BS	Bachelor of Science
Bsal	*Batrachochytrium salamandrivorans*
CI	Confidence interval
CLASS–bio	Colorado Learning Attitudes about Science Survey – Biology
COVID–19	Coronavirus disease
CT_{max}	Critical thermal maximum
CT_{max}^{50}	Critical thermal maximum, 50% zoospore immobility
CT_{max}^{100}	Critical thermal maximum, 100% zoospore immobility
df	Degrees of freedom
E_a	Activation energy
ERUPT	Explanatory, Relevant, Understandable, Plausible, and Testable
H_2O_2	Hydrogen peroxide
IACUC	Institutional Animal Care and Use Committee
IBC	Institutional Biosafety Committee
IRB	Institutional Review Board

MTE	Metabolic Theory of Ecology
NSF	National Science Foundation
O_2	Oxygen
OU	Oakland University
PUB	Project Upward Bound
qPCR	Quantitative polymerase chain reaction
SD	Standard deviation
SE	Standard error
SS	Sharpe-Schoolfield
STEM	Science, Technology, Engineering, and Mathematics
SVL	Snout-vent length
TA	Teaching assistant
TM	Thermal metabolism
TPC	Thermal performance curve

CHAPTER ONE

BACKGROUND AND SIGNIFICANCE

1.1 Introduction

Anthropogenic climate change and its effects on infectious diseases are increasingly important problems for humans and wildlife (Altizer et al., 2013; Carlson et al., 2022). Climate-disease interactions can be complex and difficult to predict, especially for diseases of ectothermic hosts whose body temperatures change as external temperatures fluctuate (Molnár et al., 2017; Raffel et al., 2013). Making sense of this complexity requires multidisciplinary approaches with input from diverse disciplines including climatology, epidemiology, community ecology, and thermal biology (Rohr et al., 2011). The Metabolic Theory of Ecology (MTE) combines mathematics, chemistry, and biology to provide a powerful framework for understanding temperature effects on biological processes including climate-disease interactions (Brown et al., 2004; Molnár et al., 2017). Recent work has shown that MTE-based models can successfully describe the temperature dependence of helminth and fungal diseases of frogs (Sckrabulis et al., In prep.; Sckrabulis et al., 2022), but these approaches have not yet been tested for other groups of ectothermic hosts. There is also a need to develop new and better ways to communicate key MTE insights about organism responses to climate change to the general public. The first core goal of my dissertation research was to explore MTE-based approaches to describing the temperature dependent of host-parasite interactions in salamanders, using chytridiomycosis (*Batrachochytrium dendobatidis* = "Bd") in red-spotted newts (*Notophthalmus viridescens*) as a model system. The second core goal of

my research was to design and evaluate new activities for introducing undergraduate students to MTE-based models for describing ectotherm responses to temperature.

MTE postulates that an organism's metabolic rate, which is heavily influenced by body temperature, determines the rate of most biologically relevant activities and thus organism fitness (Brown et al., 2004). MTE predicts that ectotherms, whose body temperatures vary with environmental temperature, should have increased metabolic rates at warmer temperatures according to the Arrhenius equation for temperature-dependence of chemical reactions (Gillooly et al., 2001). Because MTE predicts that metabolic rate drives ecological relationships and disease dynamics, it is important to conduct scientific inquiries of MTE to understand metabolic responses to the temperature shifts that climate change causes. It is also vital to educate the scientific community and the general public about these far-reaching ecological responses and their consequences. Thus, I used MTE-based models in my research to describe the thermal biology of amphibian disease, and in developing educational activities introducing students to the interdisciplinary nature of thermal biology.

Amphibians are hosts for important emerging parasites, and their parasite-host interactions are also sensitive to alterations due to climate change (Raffel et al., 2013). A significant challenge for understanding temperature dependence of parasitism is that both parasites and their hosts have individual thermal responses, such that the measurable outcome of the interaction (i.e., the parasite's population growth rate in or on a host) should depend on the thermal mismatch between a parasite's ability to infect a host (infectivity) and the host's ability to resist infection (J. M. Cohen et al., 2017; Sckrabulis et al., 2022).However, it is difficult to quantify these separate effects of temperature on

parasite infectivity and host resistance (Molnár et al., 2017). One proposed strategy for disentangling parasite and host thermal performance curves, which are measures of metabolic function across a range of temperatures, is to use MTE-based models of thermal performance, which can be partially parameterized based on the performance curve for a metabolic proxy of each organism measured independently of the other organism (e.g., swimming velocity of the parasite infective stage; Sckrabulis et al., 2022). To estimate parameters for a MTE-based model of Bd infection in *N. viridescens*, I collected separate measurements of metabolic proxies for parasite and host thermal responses, in addition to measuring the overall temperature dependence of infection, and used these data to parameterize MTE-based thermal performance curves for both pathogen and host.

My focal parasite, the fungal pathogen Bd is responsible for chytridiomycosis and is a massive threat to amphibian populations (Bower et al., 2017; Fisher et al., 2009). Bd has already caused the extinction of many amphibian populations (Bower et al., 2017), and with ongoing and impending climate change, it is important to examine chytridiomycosis responses to temperature change (J. M. Cohen et al., 2017). This will help to inform wildlife management practices to prevent the destruction of aquatic and terrestrial communities that rely on amphibians for energy and nutrient exchanges (Capps et al., 2015; Stoler & Relyea, 2013). My focal host species was the eastern red-spotted newt (*Notophthalmus viridescens*), which is thought to act as an important reservoir host species for Bd (Rothermel et al., 2016). I used Bd and *N. viridescens* to develop thermal performance curves and investigate parasite and host metabolic responses to temperature.

To fully understand host and parasite thermal responses, it is also important to examine whether the thermal performance curve of either species is affected by adaptive responses to past temperatures (i.e., thermal acclimation). Thermal acclimation responses are a type of phenotypic plasticity involving changes to an organism's thermal performance curve following extended exposure to a given temperature (Raffel et al., 2013). Thermal acclimation responses may be particularly important for understanding climate-disease interactions in fluctuating temperature environments (Raffel et al. 2013). The temperature variability hypothesis of Rohr et al. (2011) postulates that pathogens may have an advantage over ectotherm hosts following a recent shift in temperature, due to their smaller body masses and thus MTE-predicted faster thermal acclimation to the new temperature. Raffel et al. (2013) hypothesized that parasites, with their smaller masses and faster metabolisms, might acclimate faster to temperature shifts than their larger hosts, resulting in higher pathogen growth rates immediately following a temperature shift. This hypothesis assumes that both the host and the parasite will exhibit beneficial acclimation responses, meaning each will perform better at a given temperature if they have already been acclimated to this temperature, relative to an unacclimated host or parasite (Huey et al., 1999; Wilson & Franklin, 2002). This assumption is consistent with the findings of Rohr et al. (2018), who demonstrated that acclimation rate has a negative correlation with body size. Thermal acclimation has been occasionally documented in microorganisms, but the experimental acclimation time has commonly been for longer periods of time, over a span of days to weeks (Bennett & Lenski, 1997; Tian et al., 2022; Tveit et al., 2023). Small vertebrates take 3–13 days to acclimate critical thermal maximum (Brett, 1944; Hutchison, 1961), so scaling down by mass, Bd

zoospores should take only 8–34 minutes to acclimate. This short time frame is supported by research that shows microscopic parasites can demonstrate rapid thermal shift responses in heat shock protein expression as quickly as 20–30 minutes (Alcina et al., 1988; Kalinina et al., 1988; Krobitsch et al., 1998), and alterations in transcription and luciferase activity levels on the scale of just several hours (Engstler & Boshart, 2004; Fang & McCutchan, 2002).

Thermal studies on Bd suggest that optimal growth in culture occurs at 17–25 °C with a peak in zoospore production at 100 h post-inoculation, but that cooler temperatures (7–10 °C) result in higher, longer-lived zoospore production peaking at approximately 250 h after inoculation (Woodhams et al., 2008). Raffel et al. (2015) found evidence of acclimation effects on Bd infection in juvenile *N. viridescens*, consistent with the temperature variability hypothesis (Raffel et al., 2013; Rohr et al., 2011). I therefore incorporated treatments into my experimental design to test for thermal acclimation effects on Bd infection with adult *N. viridescens*. I predicted that adult newts would have similar thermal acclimation effects to those found for juvenile newts in Raffel et al. (2015). I also hypothesized that newts from higher latitudes (i.e., Ohio) would have greater resistance to Bd infection than newts from lower latitudes (i.e., Tennessee), due to local adaptation to presumably higher levels of Bd infection in a cooler location.

In addition to exploring the potential for MTE-based models to describe temperature dependence of chytridiomycosis in salamanders, my dissertation project explored approaches to teaching core concepts about thermal biology and Metabolic Theory. As the general public becomes increasingly interested in climate change impacts (McCright et al., 2016), it has become increasingly important to educate the public about

these concerns and about the interdisciplinary nature of science (Falk & Dierking, 2019; Irwin, 2020; Labov et al., 2010). MTE presents excellent opportunities to introduce undergraduate students to the interdisciplinary nature of thermal biology and of science in general, and to the increasing importance of mathematics to understanding biological systems (Labov et al., 2010). Understanding metabolic responses to temperature is also generally important for undergraduate students studying biological sciences, whether they plan to become ecologists or medical practitioners. Overall, a better understanding and use of MTE can lead to more effective management practices and better outcomes for ecosystems (Munch et al., 2023; Tomlinson et al., 2014). Active and hands-on learning techniques are also important for development of scientific skills (Freeman et al., 2014; Goldstein & Flynn, 2011). Toward this goal, it is important to incorporate interdisciplinary and inquiry-based activities into undergraduate education (Tripp & Shortlidge, 2019). Unfortunately, relatively few graduate students have opportunities to incorporate scholarship of teaching and learning into their doctoral studies, even though many graduate students seek careers in education.

While serving as a teaching and research assistant, I had an opportunity to help develop an NSF-sponsored laboratory activity for BIO 1201 (Introductory Biology Lab). This activity was designed to introduce students to core MTE concepts and skills by having them conduct experiments measuring chemical and metabolic responses to temperature in frogs and humans. With the outbreak of COVID–19 and subsequent shift to online learning in 2020, I took the lead in developing an online version of this lab activity, using virtual experiments (e.g., observations of videos) and a series of "quiz" modules to help maintain the inquiry-based nature of the original lab activity. Published

studies have shown that it is possible for online simulation activities to achieve similar learning outcomes to in-person lab activities (Darrah et al., 2014; Krüger et al., 2022; Lei et al., 2021; Perera et al., 2017); however, these studies sometimes lack rigorous control treatments. To help address this knowledge gap, I collaborated with the BIO 1201 instructor to design and implement a controlled experiment to compare the effectiveness of face-to-face versus online versions of this lab activity.

CHAPTER TWO

USING RESPIROMETRY AND BREATH RATE TO QUANTIFY NEWT METABOLIC RESPONSES TO TEMPERATURE

2.1 Introduction

Global climate change is associated with large changes in organism phenologies and projected range limits, resulting in potential threats to the persistence of many species (Burrows et al., 2011; Hughes, 2000; Walther et al., 2002). This is especially concerning for ectotherms like amphibians that might experience local population extirpation or species extinction due to direct and indirect responses to temperature shifts (Deutsch et al., 2008; Parmesan, 2006; Walther et al., 2002). To properly gauge how amphibian populations will respond to climate change, it is necessary to study the impacts of changing temperature on amphibian physiology. One method of doing this is to apply the Metabolic Theory of Ecology (MTE), which posits that temperature impacts organism metabolisms, and has far-reaching consequences on organism fitness and performance from the microscopic to the ecosystem scale (Brown et al., 2004; Gillooly et al., 2001). Using this framework, it might be possible to predict amphibian responses to climate change by examining the effects of temperature on whole-body metabolic rate.

Local adaptation can play a significant role in organism fitness, where populations have evolved through natural selection to perform better at temperatures that are closer to their local climate. Various studies have found differences in metabolisms and thermal performance of amphibian from difference latitudes, consistent with genetic adaptation to local climate conditions. Amphibians collected from different latitudes have been found

to exhibit significant differences in temperature dependence of metabolic rates, susceptibility to infection, thermal tolerances, and thermal performance curves (Cohen et al., 2019; DeLong et al., 2018; Deutsch et al., 2008; Sunday et al., 2011; Wilson, 2001), although latitudinal differences are not always present in some metrics of thermal performance (John-Alder et al., 1989). Consistent with genetic adaptation to local climate conditions, red-spotted newts (*Notophthalmus viridescens*) from higher latitudes (i.e. a cooler climate) show increased locomotor performance at lower temperatures whereas newts collected from lower latitudes (i.e. a warmer climate) showed increased locomotor performance at higher temperatures (Mineo & Schaeffer, 2014). However, it is not always clear that these among-population differences are due to local genetic adaptations, because it is also possible for organisms to exhibit plastic responses that allow for adaptation within an organism's lifetime (i.e., without the need for natural selection across generations; Angilletta, 2009). For example, thermal acclimation responses can result in improved amphibian performance at a given temperature following extended exposure to this temperature (i.e., the beneficial acclimation hypothesis; Angilletta, 2009). One approach to teasing apart local adaptation versus plastic responses is by conducting a common garden experiment, by bringing organisms from multiple locations into a common environment and testing for phenotypic plasticity in a controlled environment. Applying this approach, Mineo & Schaeffer (2014) showed that newts also exhibit beneficial acclimation of their locomotor performance curves. In the current study, we used a similar common garden experiment, sourcing red-spotted newts from populations at two widely different latitudes to examine local adaptation and thermal acclimation responses of the thermal performance curve for whole-body metabolic rate.

Researchers use a variety of proxies for metabolism, including physiological and biochemical measurements (Dell et al., 2014; Holmborn et al., 2009; Molnár et al., 2017). Unfortunately, there is evidence that various proxies for whole-body metabolism have different responses to thermal change (Dell et al., 2011). Thus, when studying metabolic responses to varying temperatures, it is important to select a proxy of metabolic performance that scales similarly with temperature to true metabolism. Prior studies of red-spotted newt thermal performance focused on measuring thermal limits (i.e., CT_{max}; Hutchison, 1961), resistance to Bd infection (Raffel et al 2015), or thermal performance of locomotion (Mineo & Schaeffer, 2014). The most direct proxy of metabolic rate is oxygen consumption, which should be directly proportional to energy consumption in an aerobic organism (Brown et al., 2004). There are several established methods of measuring oxygen consumption, including closed and open respirometry. A closed system measures the difference in oxygen or carbon dioxide before and after placing the organism in an airtight container or mask apparatus, whereas an open or "flow-through" system pumps air through the airtight container at a consistent low rate, replenishing the respired air and continuously measuring flow rate and oxygen depletion or carbon dioxide production (Macfarlane, 2017). In the current study, we used a custom-built flow-through respirometry system (McWhinnie et al., 2021) to allow our animals to exhibit normal resting behavior and avoid unnecessary stress due to oxygen depletion that might negatively impact our results (Lighton & Halsey, 2011; Nowack et al., 2020). However, measuring whole-body metabolic rate (i.e., oxygen consumption) can be difficult (Brown et al. 2004), and it can be easier to measure a metabolic proxy such as breath rate. We therefore also measured temperature dependence of breath rate as an

indirect proxy for metabolic rate in this study, for comparison with the direct measurements of oxygen consumption. Using breath rate measurements to estimate metabolism could help simplify research methodology for future thermal and climate change research. However, because amphibians respire through cutaneous, buccal, and pulmonary methods, it is possible that breath rate might scale differently with temperature compared to oxygen consumption, as was observed in *Xenopus laevis* (McWhinnie et al., 2021).

We conducted a common garden experiment to measure effects of temperature, thermal acclimation, and local adaptation on two measures of metabolic rate (breath rate and oxygen consumption) in adult *N. viridescens*. We compared these two respirometry methods and hypothesized that newts would exhibit both (1) genetic adaptation to local climate and (2) beneficial acclimation responses. We also hypothesized that newts would demonstrate similar metabolic scaling patterns across a natural range of temperatures using oxygen consumption rates and breath rates as metabolic proxies.

2.2 Methods

2.2.1 Newt collection and maintenance

Newts were collected from three source populations in Ohio (41.41720° N, -81.42898° W; 41.49945° N, -81.41763° W; 41.45709° N, -81.33080° W) and three source populations in Tennessee (35.21936° N, -85.97371° W; 35.20899° N, -85.95834° W; 35.22474° N, -85.94534° W). Newts were transported by car in individual containers and arrived at our lab at Oakland University (Rochester, Michigan) within 48 hours of collection. Five newts from each population were swabbed for *Batrachochytrium dendrobatidis* (Bd) infections upon arrival and given heat treatment (30 °C for three

days) to clear them of pre-existing infections, as described in Chapter 4. Newts were allowed to reacclimate to room temperature following heat treatment for at least two weeks prior to acclimation for this experiment. Newts were placed in communal plastic bins separated by source population with no more than 10 newts per container, maintained with weekly water changes and fed blackworms *ad libitum*. Each bin contained 4 L of dechlorinated (using Kordon AmQuel Plus Ammonia Detoxifier) tap water, a 33 x 13 cm plexiglass ramp to provide a dry ("land") space, and plastic aquarium plant leaves to provide enrichment. Lights were kept on a 12:12 light cycle.

During the experiment, each newt was housed individually in a 1.9 L plastic container with 400 mL dechlorinated water and a 13 x 8 cm plexiglass ramp (Fig. 2.1).

2.2.2 Temperature acclimation and performance

Each newt's individual housing container was placed within a custom-built Styrofoam incubator (as described in Altman et al., 2016) set to one of three "acclimation temperatures", to allow the amphibian's metabolism to become acclimated to cold, middle, or warm temperatures (8, 17, and 25 °C, respectively). After two weeks of acclimation, each newt was moved to a "performance" incubator (Exo Terra reptile egg incubator, Model PT2445, Hagen, Mansfield, MA, USA) for measurement of oxygen consumption and breath rate at one of seven "performance temperatures" (8, 12, 16, 20, 24, 28, 32 °C). Temperatures were selected from the natural temperature range experienced by *N. viridescens* (Raffel et al., 2006). For any acclimation to performance temperature shift larger than 12°C , newts were allowed to adjust to an intermediate "step temperature" of 21°C for two hours to avoid temperature shock.

Within two hours of being shifted to its new performance temperature, we measured each newt's oxygen consumption rate and breath rate for a duration of 40 minutes using a flow-through respirometry system developed by McWhinnie et al., (2021). During respirometry measurements, each newt was housed in a flow-through air system with air input from an aquarium air pump. Air passed through two empty 1 L muffling jars prior to reaching the newt's chamber, to even out air flow before flowing into the newt's container (McWhinnie et al., 2021; Fig. 2.2). Air flowing out of each newt's container was directed through a tube filled with Drierite to remove moisture, and then to a custom-made oxygen and air flow sensor (McWhinnie et al., 2021; Fig. 2.2). At the beginning of each trial, oxygen sensors were run for 20 minutes to warm up while newts were allowed to equilibrate their body temperatures to the new incubator temperature. During the experiment, air flow was shifted eight times over the course of 40 minutes to either the newt's container (5-minute duration) or bypassing the container (3-minute duration). This allowed us to obtain oxygen measurements for both air that has been exposed to the newt breathing in its container and the baseline "control" air flowing from the aquarium bubbler for easy comparison (Fig. 2.3). The newt's container acted as a closed system during the 3 minute bypass periods, but the risk of hypoxia was negligible because oxygen levels never decreased by more than 0.1 %. Oxygen consumption was measured in mL O_2 min^{-1} based on the air flow rate of the system and the difference in O_2 concentration between the air exposed to the newt and the air bypassing the newt.

After measuring oxygen consumption, each newt was filmed for at least 10 seconds to measure breath rate, as determined by the number of buccal pumps per

second. The breath rate videos did not occur during the oxygen measurement period, to avoid air flow fluctuations when the incubator was jostled and opened, as we had seen in preliminary trials. Each newt was then transferred back to its 1.9 L container and placed in a second custom-built Styrofoam incubator set to its assigned performance temperature. At 3 and 7 days into the performance period, each newt was temporarily removed from its incubator and placed into a clear container with wet paper towel that had been equilibrated to the performance incubator temperature. The newts were allowed to sit for at least 5 minutes to adjust to the new visual environment, and then each newt was filmed again through the clear container wall to determine breath rate.

2.2.3 Statistical Methods

We conducted statistical analyses using program R Version 4.0.3 (R Core Team, 2020). We used the lmer function from the lme4 package (Bates et al., 2015) to generate linear-mixed effects models to test for main and interactive effects of lognormal transformed mass, performance temperature, acclimation temperature, and latitude-of-origin on lognormal transformations of oxygen consumption or breath rate, treating incubator and source population as random effects in each model. Performance temperature was transformed to inverse temperature in Kelvin. We tested for interactions between performance temperature × acclimation temperature because positive interactions are predicted by the beneficial acclimation hypothesis. We used the Anova function from the car package to generate type II F statistics for each model (Fox & Weisberg, 2019). Nonsignificant interactions and predictors were removed from the models before generating E_a estimates for oxygen consumption and breath rates, as described in (McWhinnie et al., 2021).

2.3 Results

Oxygen consumption and breath rate both increased with performance temperature and mass, but we found no significant thermal acclimation or latitude-of-origin effects on either metabolic proxy (Table 2.1). Oxygen consumption rates and breath rates showed similar temperature scaling patterns (Fig. 2.4), with E_a estimates of 0.471 ± 0.047 eV and 0.252 ± 0.019 eV, respectively (± SE throughout). When analyzed separately, the oxygen consumption rates of newts from Ohio and Tennessee yielded E_a estimates of 0.478 ± 0.059 eV and 0.436 ± 0.072 eV, respectively. The breath rates newts from Ohio and Tennessee yielded E_a estimates of 0.251 ± 0.019 eV and 0.212 ± 0.053 eV, respectively.

2.4 Discussion

We found no evidence of thermal acclimation effects on newt oxygen consumption and breath rate. These results are contrary to our prediction that we would see evidence of beneficial acclimation (i.e. Ohio newts would be adapted to cooler climates and show increased performance at lower temperatures, while Tennessee newts would be adapted to warmer climates and show increased performance at higher temperatures). This suggests that the geographic and plastic variation in locomotory performance observed by Mineo & Schaeffer (2014) was not driven by differences in underlying metabolic responses. Therefore it seems more likely that the differences shown in Mineo & Schaeffer (2014) were caused by genetic differences and plasticity in physiological responses that are specific to locomotion. This contradicts the common assumption of MTE based models that physiological rate processes are tightly bound to underlying metabolic rates (Brown et al., 2004). While this does not mean that MTE

should be discarded, this highlights the importance of remembering that this is a critical simplifying assumption of MTE based models and this assumption may not always be met.

While our experimental design did not allow us to distinguish between genetic adaptation and developmental plasticity, we nevertheless found no differences in reversible plasticity (i.e. metabolic thermal performance curves) between newts from different latitudes.

We hesitate conclude that there are no metabolic latitudinal differences based on climate, because there are likely many differences between the two locations, and our study sites were spatially confounded where "climate" was not spatially interspersed on the landscape. While the Ohio and Tennessee sites were ~800 km apart, it is possible that there were confounding factors that made the local climates more similar than anticipated. For instance, higher elevation is associated with increases in ectotherm metabolic rates (Plasman et al., 2020), and the elevations of our sites were fairly similar (304.8 m and 548.6 m above sea level for Ohio and Tennessee, respectively). The Ohio sites were also close to an urban center (Cleveland), and the Tennessee sites were more rural. More urban environments can lead to differences in resource availability, anthropogenic light during nighttime hours, and increases in microclimate temperature that all might lead to changes in local physiological adaptation (Vardi et al., 2023). It would therefore be necessary to include sites from a wider range of locations and collect more detailed climate data to disentangle the effects (and determine whether they exist) of latitude alone on newt metabolic rates.

Contrary to what MTE would suggest, we found a positive correlation between mass and metabolic rates (Brown et al., 2004). This might have been driven by confoundment with latitude, because Ohio newts were slightly larger than Tennessee newts (2.88 and 2.58 g, respectively). There might have also been confoundment with performance temperature treatment. Newts were separated in approximately equal ratios into treatments according to source population, but newts were not separated by mass.

Our $E_a$estimates fall within the established range of E_a across all organisms (Dell et al. 2011. Oxygen consumption gave a higher E_a estimate that is more in line with the expected value of 0.65 eV (Dell et al., 2011), but both estimates will be useful as alternative estimates of the activation energy for host resistance to infection, to determine which provides a better fit to Bd infection data (Chapter 3) when modeling the host-parasite thermal mismatch.

2.5 Acknowledgements

Thank you to Galilee Rogers for her assistance at every step of the way in this project. This work would not have been possible without Kristen Cecala for assistance obtaining newts from the Tennessee populations, and Mike Bernard, Pat Lorch, and Paul Pira for assistance obtaining newts from Ohio populations. Thank you also to David Dimitrie, Tim Krynak, and Ana Locci for their permissions for collecting newts. Thank you to Levi Trumbore for help obtaining, transporting, and caring for newts, as well as for help with experiments. Thank you to Carson Evans, Amanda McKeon, Alex Orille, Melissa Ostrowski, Holly Russell, Keshav Seetharaman, Julia Tituskin, and Jazalyn Williams for their help caring for animals and conducting experiments. Thank you to Jay Noelker for his assistance with analysis. Funding for this project was provided by the National

Science Foundation (IOS 1651888). Use of animals was approved by the Oakland University IACUC (18011; Appendix A). Collection of animals was approved by Ohio Division of Wildlife (scientific collection permit 21-067) and Tennessee Wildlife Resources Agency (scientific collection permit 1296).

2.6 Chapter 2 tables

Table 2.1: Linear mixed-effects model outputs for oxygen and consumption. Mass was log transformed, performance temperature was inverse temperature (K) multiplied by Boltzmann's constant, and acclimation temperature was in °C. We used Kenward-Rogers degrees of freedom (df), which can be non-integers in a mixed effects model with an unbalanced design.

Response	Predictor	F	df	p
Oxygen consumption	Mass	37.1	1, 47.8	< 0.001
	Performance temperature	88.9	1, 47.3	< 0.001
	Acclimation temperature	2.3	1, 16.7	0.145
	Latitude (state)	0.3	1, 4.9	0.623
Breath rate	Mass	9.0	1, 148.6	0.003
	Performance temperature	153.2	1, 168.6	< 0.001
	Acclimation temperature	0.2	1, 18.6	0.694
	Latitude (state)	2.1	1, 10.3	0.177

2.6 Chapter 2 figures

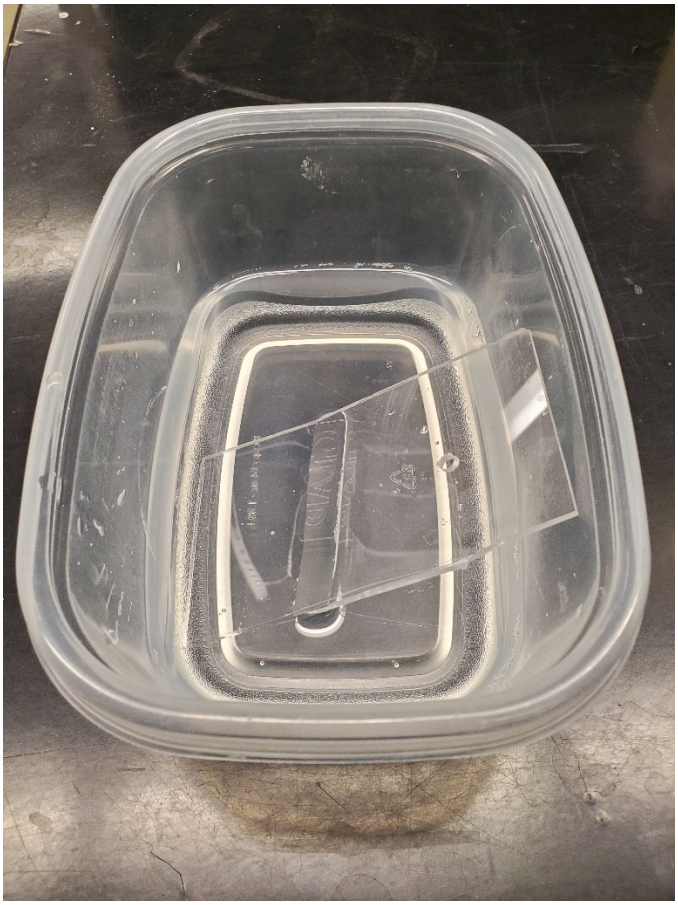

Figure 2.1: Newt individual housing container with 400 mL dechlorinated water and a plexiglass ramp to provide dry “land”. When housing a newt, the container would be securely covered with a perforated lid, allow free exchange of air.

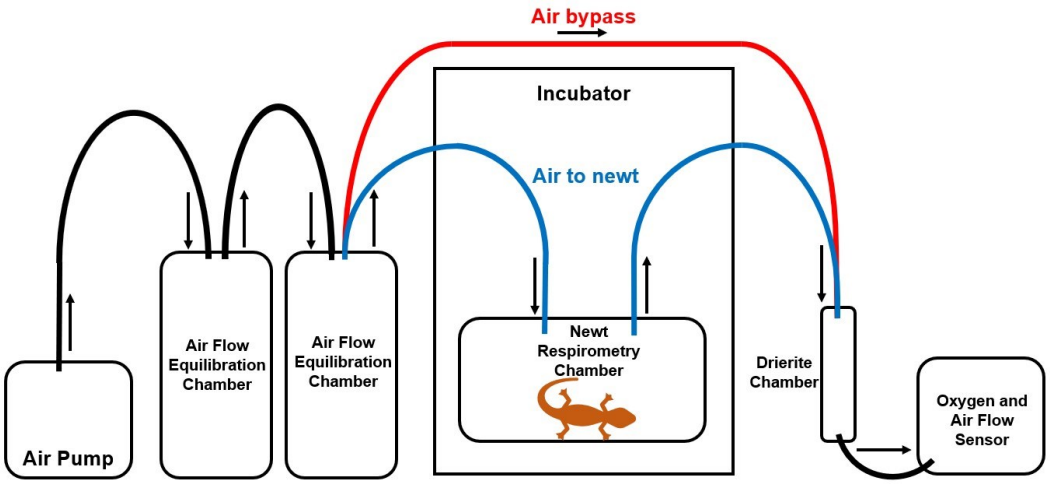

Figure 2.2: Respirometry setup. Arrows designate the flow of air from the air pump, through the system, to the oxygen and air flow sensor. The blue lines represent when air flow was shifted to the newt respirometry chamber, and the red lines represent when air flow was shifted to bypass the newt to establish the baseline oxygen concentration of air from the pump.

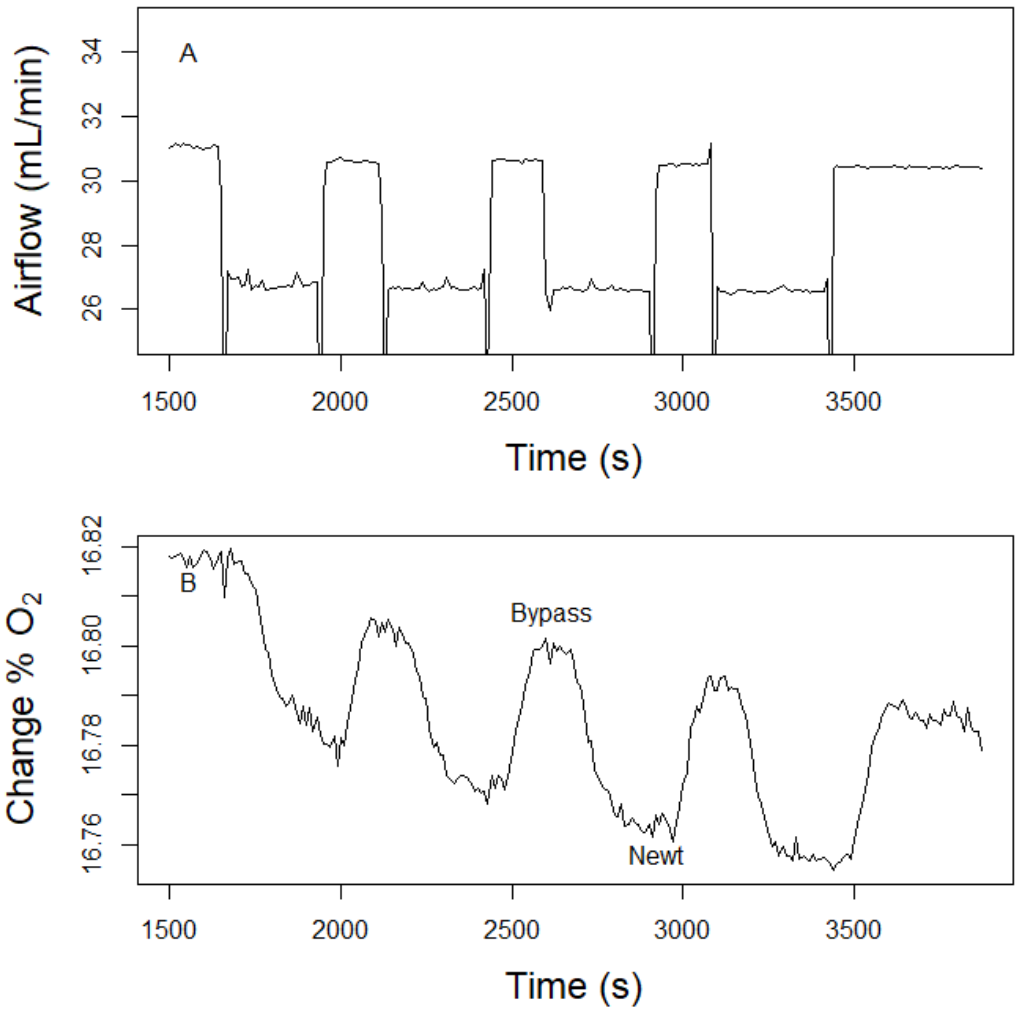

Figure 2.3: Example of a newt respirometry measurement, depicting (A) air flow and (B) percent change in oxygen. The peaks in the two graphs represent when air was flowing through the bypass ("Bypass"), and the troughs represent when air was flowing through the newt container ("Newt"). We incorporated a time delay in our measurements to account for the time it took for the air to flow through each incubator's tubes and Drierite container. We then used spline-fitting to estimate the oxygen in the air for both the bypass and the newt container to account for changes in room oxygen percentage over the course of the measurement, depicted above as decreasing through time.

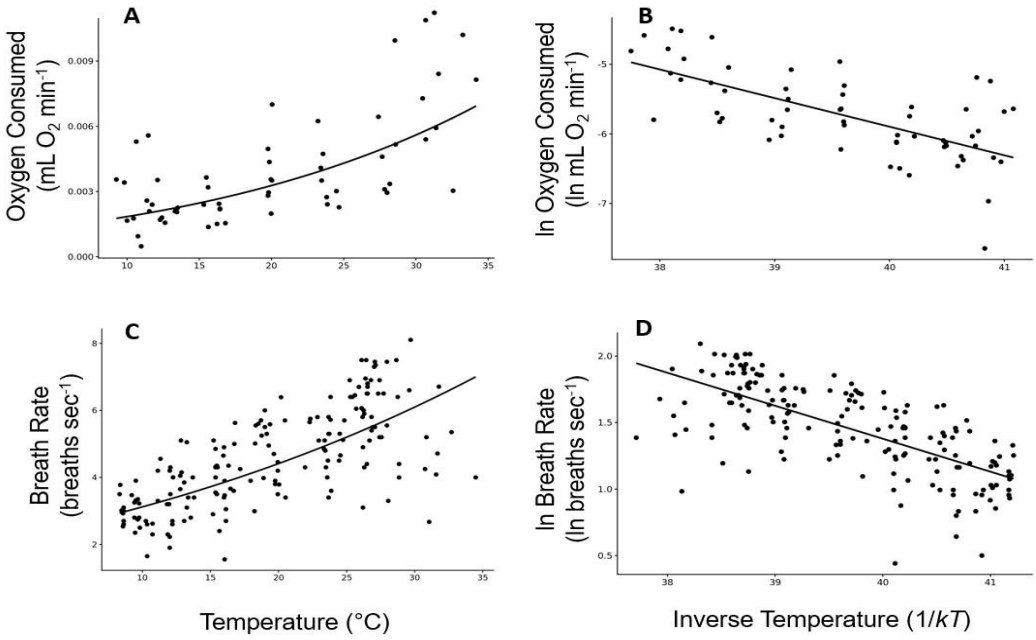

Figure 2.4: Temperature scaling of oxygen consumption rates and breath rates of eastern red-spotted newts (*N. viridescens*). Panel A shows newt oxygen consumption rate across a range of temperatures (°C). Panel B shows the natural log of oxygen consumption rate across a range of inverse temperatures where k is Boltzmann's constant 8.62×10^{-5} eV K^{-1} and T is temperature in Kelvin. Panel C shows newt breath rate across a range of temperatures (°C). Panel D shows the natural log of newt breath rate across a range of inverse temperatures where k is Boltzmann's constant 8.62×10^{-5} eV K^{-1} and T is temperature in Kelvin.

CHAPTER THREE

RAPID THERMAL ACCLIMATION (OR LACK THEREOF) IN ZOOSPORES OF AN AMPHIBIAN PATHOGEN

3.1 Abstract

Thermal acclimation effects on locomotory performance have been widely documented for macroscopic organisms, but such responses remain largely unexplored in microorganisms. Metabolic theory of ecology (MTE) predicts faster responses in smaller organisms, with potential consequences for host-parasite interactions in variable temperature environments. We investigated thermal acclimation effects on zoospores from the amphibian fungal pathogen *Batrachochytrium dendrobatidis* (Bd), quantifying: (1) thermal performance for maximum zoospore velocity and (2) high temperatures needed to immobilize 50% (CT_{max}^{50}) or 100% (CT_{max}^{100}) of zoospores. We obtained measurements within 18 minutes following a shift from one of three experimental acclimation temperatures. We found a positive effect of performance temperature on zoospore velocity but no clear effects of experimental acclimation, except for a curvilinear effect on CT_{max}^{50} that might have been driven by a confoundment with zoospore density. However, we observed a significant positive effect of the trial start temperature on CT_{max}^{50}, consistent with a rapid acclimation response to the start temperature on a time scale of ~1–6 minutes (i.e., too rapid for our experimental acclimation treatments to detect). To explore the plausibility of such a rapid response, we analyzed published acclimation times for potential amphibian hosts and calculated MTE-predicted acclimation times of 1.38–16.72 minutes when scaled to the size of a zoospore.

We concluded that Bd zoospores either have constitutive thermal responses (i.e., no acclimation) or fully acclimate to new temperatures within ~10 minutes of a shift, either of which might provide an advantage over slow-acclimating hosts in variable temperature environments.

3.2 Introduction

The threat of climate change makes it increasingly important for biologists to understand organism adaptations to temperature, including plastic responses to temperature known as thermal acclimation responses. Thermal acclimation has been extensively researched in macroorganisms such as amphibian and insects (Carilo Filho et al., 2022; Rohr et al., 2018), but less is known about how acclimation affects performance characteristics of single-celled microorganisms. The Metabolic theory of ecology (MTE) predicts that smaller organisms should have more rapid physiological responses, including thermal acclimation, due to the negative scaling relationship between body mass and metabolic rate ($B \propto M^{-0.25}$; Gillooly et al. 2001, Brown et al. 2004, Raffel et al. 2013, Rohr et al. 2018). This has potentially important implications for pathogenic microorganisms, such as the amphibian fungal pathogen *Batrachochytrium dendrobatidis* (Bd). The temperature variability hypothesis of Rohr & Raffel (2010) postulates that pathogens have an advantage over their hosts in variable temperature conditions, due to delays in host acclimation following unpredictable temperature shifts (Raffel et al., 2013, 2015). This hypothesis assumes that parasites have either (1) more rapid acclimation responses than their hosts or (2) constitutively expressed thermal adaptations (Raffel et al., 2013, p. 201, 2015; i.e., no acclimation responses; Rohr & Raffel, 2010). Field evidence and infection experiments have found some support for the

temperature variability hypothesis for Bd infection of amphibian hosts (Raffel et al., 2013, 2015; Rohr & Raffel, 2010). However, relatively little is known about whether and how thermal acclimation responses influence metabolic or locomotory performance of microorganisms like Bd, or how these responses affect the ability of pathogens to infect hosts (Raffel et al., 2013).

Thermal acclimation is a type of temperature-induced phenotypic plasticity, typically defined as within-generation changes in an organism's thermal tolerances or thermal performance curve following experimental exposure to warm or cool temperatures (Angilletta, 2009). A thermal performance curve (TPC) measures quantitative changes in some performance metric across a range of temperatures within an organism's ecologically relevant thermal range (Angilletta, 2009). In macroscopic organisms, thermal tolerances and performance curves are often quantified based on changes in locomotory function. For example, thermal performance curves of amphibians are often quantified based on jumping distance or maximum velocity (Dell et al., 2014), and critical thermal maximum (CT_{max}) is often quantified as the high temperature above which frogs or salamanders fail to right themselves (Lutterschmidt & Hutchison, 1997; Pottier et al., 2022). Thermal acclimation can have different effects on an organism's TPC depending on the species and how performance is measured. The beneficial acclimation hypothesis postulates that acclimation to a given temperature will increase performance at that temperature, relative to an unacclimated organism (Wilson & Franklin, 2002). Consistent with beneficial acclimation, many species have improved high temperature tolerance (i.e., increased CT_{max}) following extended exposure to warm temperature (Rohr et al., 2018). However, beneficial acclimation is not universal,

especially for TPC changes within an organism's normal temperature range (Huey et al., 1999; Wilson & Franklin, 2002). Other common acclimation patterns include "cooler is better" or "warmer is better" effects, in which the TPC is overall higher following acclimation to cooler or warmer temperatures (respectively), or "optimal temperature" effects, in which the TPC is higher following acclimation to an intermediate temperature (Altman et al., 2016; McWhinnie et al., 2021; Wilson & Franklin, 2002). In principle, an organism could also have constitutive rather than induced adaptations to temperature variation, in which case there would be no measurable effects of experimental acclimation treatments on an organism's thermal tolerances or TPC.

Relatively few studies have investigated within-generation thermal acclimation responses in microorganisms (Addy et al., 1998; Crowther & Bradford, 2013; Heinemeyer et al., 2006; Malcolm et al., 2008; C. H. Robinson, 2001; but see: P. M. Robinson & Morris, 1984). This might be in part because such acclimation effects should only be detectable during a short window of time following an experimental shift in temperature, making them difficult to study (Rohr et al., 2018). According to MTE, biological process times including time to acclimation should increase with organism body mass according to a quarter-power scaling relationship ($Biological\ time \propto Mass^{0.25}$; Gillooly et al., 2001). Rohr et al. (2018) found indirect evidence for this scaling relationship, showing that acclimation effects on CT_{max} were more likely to be detected in small animals like stoneflies (2.0×10^{-5} kg) when ramping trials were conducted with faster heating rates (i.e., shorter measurement times). Thus, we are more likely to detect thermal acclimation if the measurement is collected soon after shifting an organism to a new temperature. Most studies of "thermal acclimation" in single celled

microorganisms have been conducted on time frames of days to weeks, measuring changes in thermal tolerances or TPCs over multiple generations (Bennett & Lenski 1997, Hall et al. 2010, Tian et al. 2022, Tveit et al. 2023). Although these responses are likely to represent a type of multi-generational phenotypic plasticity in some cases (Bennett & Lenski, 1997), we cannot entirely rule out the possibility that these effects were caused by genetic evolution rather than a classic thermal acclimation response. Indeed, some of these authors explicitly define "thermal acclimation" as encompassing both phenotypic plasticity and genetic evolution (Tian et al. 2022, Tveit et al. 2023). This focus on longer time scales and evolutionary responses might be in part because microbial performance is often quantified based on population growth rates in culture or competitive ability, precluding within-generation measurements (although respiration is also commonly measured; Bennett & Lenski, 1997; Crowther & Bradford, 2013; Hall et al., 2010; Malcolm et al., 2008; Tian et al., 2022; Tveit et al., 2023). A number of studies have documented thermal acclimation responses in fungi based on various performance metrics (e.g., radial growth of hyphal cultures; Addy et al., 1998; Crowther & Bradford, 2013; Dell et al., 2013; Fargues et al., 1997; Heinemeyer et al., 2006; Malcolm et al., 2008; Ouedraogo et al., 1997; Robinson, 2001; Robinson & Morris, 1984; Vidal et al., 1997). Hyphal fungi are often considered unicellular due to cytoplasmic streaming between hyphal segments; however they have relatively large "body" sizes and the ability to close off septa between compartments (Bleichrodt et al., 2015). Thus, hyphal fungi might be expected to have comparable acclimation responses to multicellular organisms with similar biomass per individual mycelium. To our knowledge, no empirical studies have directly tested for within-generation thermal acclimation effects on thermal

tolerances or TPCs of single celled microbes with independently motile cells. Our lack of data for acclimation effects on microbe locomotory performance is a particularly important knowledge gap, due to the likely association between infective stage velocities and pathogen infectivity (e.g., Bd zoospores; Canter & Jaworski 1981, Appiah et al. 2005).

Much of what we know about microorganism thermal responses comes from studies of heat shock protein expression (Alcina et al., 1988, Kalinina et al., 1988, Krobitsch et al., 1998, Schlesinger et al., 1982). Although this is not a direct measure of organism performance, heat shock proteins are generally assumed to protect cells from high temperature stress (Bakthisaran et al., 2015; Ikwegbue et al., 2017). Altered heat shock protein expression has been observed as quickly as 1–15 minutes (i.e., <1 generation) following a temperature shift in bacteria (Travers & Mace, 1982, Neidhardt et al., 1982, Pellon et al., 1982, Yamamori et al., 1982) and 10–30 minutes for microscopic eukaryotes including yeasts, amoebae, and kinetoplastid flagellates (Alcina et al., 1988, Finkelstein & Strausberg, 1982, Kalinina et al., 1988, Krobitsch et al., 1998, Loomis & Wheeler, 1982). Vector-borne parasitic protozoans of humans have been found to exhibit significant changes in gene transcription within 1–4 hours following a sudden shift to a new host body temperature (Engstler & Boshart, 2004; Fang & McCutchan, 2002). Taken together, these findings suggest that single celled organisms likely do exhibit classic acclimation responses (i.e., within-generation plasticity) and that these might occur on the rapid time scales predicted by MTE.

In the current study, we tested for thermal acclimation effects on high-temperature thermal tolerance and thermal performance of Bd zoospores in culture, focusing on

performance metrics that could be quantified soon (within 20 minutes) after removing zoospores from one of three acclimation temperatures (i.e., hopefully before zoospores could become fully acclimated to the new "performance" temperature). To quantify changes in heat tolerance, we used the ramping method to quantify temperatures at which 50% or 100% of visible zoospores stopped swimming (CT_{max}^{50} and CT_{max}^{100}, respectively). To quantify changes in thermal performance, we measured the maximum velocity of zoospores at one of eight performance temperatures using video microscopy. We also fit MTE-based models of thermal performance to the zoospore velocity data to obtain estimates of Bd's metabolic activation energy (E_a), a key metabolic parameter (Molnár et al., 2017). To explore MTE predictions for thermal acclimation times in microorganisms, we analyzed published values for acclimation times in potential amphibian hosts of Bd and generated predicted times for an organism the size of a Bd zoospore. Based on the beneficial acclimation hypothesis, we predicted that CT_{max}^{50} and CT_{max}^{100} measurements would be higher following warm temperature acclimation, and that zoospore velocities would be faster at a given temperature if they had recently been acclimated to that temperature.

3.3 Methods

3.3.1 Culturing Bd

Bd strain JEL423, isolated from *Peltophryne lemur* in 2004 (Farrer et al., 2011), was obtained from Joyce Longcore in 2016 and grown in 1% tryptone broth for approximately 10 generations prior to long term −80 °C storage as frozen stocks (Boyle et al., 2003). We used all Bd within one month after thawing from stock, to minimize evolutionary changes in response to culture conditions(Kumar et al., 2020; Voyles et al.,

2014). Prior to each experimental trial, we thawed and grew Bd in sterile 1% tryptone broth solution for one week at 21 °C. Then 500 µL of this culture was spread onto each of several sterile 1% tryptone agar plates and grown for one week at 21 °C. We placed each plate into an Exo Terra reptile egg incubator (Model PT2445, Hagen, Mansfield, MA, USA) or a commercial countertop beverage center (Model SCR114L, Summit Commercial, Felix Storch, Inc., Bronx NY, USA) to achieve lower temperatures, set to low, middle, and high acclimation temperatures (9, 16.5, 24 °C), and allowed the plate to grow for three days. For both experiments, six acclimation incubators were arranged in a randomized block design with two incubators per temperature treatment. We used digital aquarium thermometers to confirm actual temperatures were near our target temperatures and used Onset HOBO loggers to record acclimation temperature readings in the incubators every 30 minutes. We calculated the mean HOBO temperature throughout each acclimation period for use in analyses. At the end of a 3-day acclimation period, each plate was flooded with 3 mL artificial spring water (ASW; Cohen et al., 1980) that had been pre-equilibrated to the acclimation temperature. The flooded plate remained in the acclimation incubator for another 20 minutes to allow zoosporangia to release zoospores into the water.

3.3.2 Measuring zoospore critical thermal maximum

To measure zoospore CT_{max}^{50} and CT_{max}^{100}, we conducted temperature ramping trials by placing samples on a microscope slide fitted into a top stage incubation chamber (system by Okolab SRL, Naples, Italy). Prior to beginning the ramping trial, we fitted a microscope slide into the top stage incubation chamber. We cleaned the slide with chilled 70% ethanol, cooling the slide to a target temperature of 20 °C. After the ethanol was

fully dry, we transferred 20 µL sample from the flooded agar plate (described above) to the slide and inserted the Oko machine's miniature temperature probe directly into the sample droplet to measure temperature in real time. It was difficult to achieve a precise trial start temperature for every trial, so the start temperature varied between 15–25 °C. We recorded the start temperature for each trial, allowing us to investigate start temperature as a potentially important covariate (Terblanche et al., 2007).

We then sealed the top stage incubation chamber and positioned it over the objective lens of an inverted Nikon Diaphot compound microscope at 200x magnification. We recorded the approximate zoospore density (zoospores per field of view) and proportion of zoospores moving based on an observer estimate. To begin the ramping process, a second investigator turned on the stage heater, resulting in gradual heating of the sample at a rate of approximately 1.8 °C per minute (range of 0.8 to 2.8 °C per minute). The observer watched the zoospores continuously throughout the ramping trial and noted times when 50% (CT_{max}^{50}) and 100% (CT_{max}^{100}) of the zoospores had stopped moving. A second investigator recorded the stage temperature at these times. It took approximately 30 seconds to set up for each ramping trial, after which it took 2.5 ± 1.2 (mean ±SD) minutes to reach CT_{max}^{50} and 8.5 ± 4.7 minutes to reach CT_{max}^{100}, corresponding to total times since removal from acclimation temperature of 3 and 9 minutes, respectively.

An advantage of using real-time observations in lieu of video analysis for CT_{max} measurements was that it allowed us to observe zoospore movement based on the full field of view and variable focal depth. However, it increased the risk that subjective judgements of observers might influence the results. To control for potential differences among observers, both observers went through a training period to confirm that their

estimates correlated with estimates by the first author. To control for effects of observer bias on zoospore density, proportion moving, and CT_{max} estimates, observers were blinded to acclimation treatment and current temperature readings. To verify the consistency of observer zoospore density estimates, we conducted a follow-up image analysis from a subset of trials (temporal blocks E–J). We obtained photos using a GoPro fitted into the ocular lens of the microscope. The GoPro did not record the entire field of vision nor depth of focus we observed manually, resulting in lower numbers of zoospores being visible in photographs; nevertheless, this allowed us to confirm that our observer estimates were positively correlated with an objective alternative measure of zoospore density ($r = 0.786$, df = 23, $p < 0.001$).

3.3.3 Measuring zoospore velocity

To measure velocity at various performance temperatures, we collected water containing zoospores from flooded agar plates (described above), added the sample to a microtube, and vortex mixed it. We used a hemacytometer to quickly determine the density of zoospores in each inoculum and then diluted each inoculum with water (already equilibrated to the acclimation temperature) to a target concentration of 15 zoospores nL^{-1} and a final volume of 1 mL. When Bd cultures did not produce enough zoospores for 15 zoospores nL^{-1}, we used a minimum concentration of 7 zoospores nL^{-1}, resulting in zoospore concentrations between 7–15 zoospores nL^{-1}. To verify that zoospore concentration did not affect the velocity results, we tested for an effect of zoospore concentration in our final statistical model. We were unable to use a stage heater to maintain temperatures during microscopy for this experiment, because the available stage heater only allows for temperature set points of >25 °C. Instead, we

placed each 1 mL tube of diluted zoospores into a performance incubator set to one of eight target performance temperatures (7, 10, 13, 16, 19, 22, 25, 28 °C). Nine performance incubators were arranged in a randomized block design, with temperature treatments re-randomized for each of six temporal blocks of the experiment. After allowing ten minutes to ensure each tube was fully equilibrated to the new performance temperature, 40 µL was transferred to a hemacytometer and cover-slipped for video microscopy. To help maintain the target performance temperature throughout video microscopy, the thermal inertia of each hemacytometer was increased by placing it within a foam cutout on top of a 150-mm diameter Petri dish filled with gel wax (Fig. 3.1). The gel wax Petri dish was equilibrated to the performance temperature prior to each trial. Each replicate was recorded three times for ten seconds at 400x magnification using a Leica DM 1000 microscope, a Leica DFC450 C digital microscope camera and VirtualDub screen recording software (VirtualDub 2021). We obtained nearly all videos within 15 minutes after moving zoospores from their original acclimation temperature to their new performance temperature, for a mean time of 14.7 ± 1.0 (SD) minutes at the new performance temperature prior to obtaining measurements. Hemacytometers gradually equilibrated to room temperature during video microscopy, but we were unable to measure continuous changes in sample temperature during trials. To obtain better estimates of real time temperature at the time of each video, we conducted a separate experiment to determine the rate at which hemacytometers equilibrated to room temperature from a given start temperature. Samples of 40 µL water were taken from incubators set to 9, 16.5, or 24 °C, and placed onto a gel-wax-stabilized hemacytometer at the same temperature as described above. The miniature temperature probe used in the

CT_{max} ramping measurements was then placed into the water sample and a cover slip was laid on top. The gel wax block and hemacytometer were placed on a microscope stage to simulate a video microscopy session, and hemacytometer temperature was recorded every minute for 10 minutes. We used nonlinear least squares to fit the data to the following equation:

$$T\{t\} = T_R + (T_0 - T_{R)}^{-rt},$$

where T is sample temperature (in °C) as a function of time, T_R is room temperature, T_0 is the initial performance temperature (mean of the three most recent HOBO logger measurements in the performance incubator), r is the rate of increase or decrease in temperature, and t is the time in minutes since the sample was removed from the performance incubator (Newton's law of cooling; Lienhard IV & Lienhard V 2020; Fig. 3.2). Room temperature was a consistent average of 21.6 °C and was assumed constant in the model, making r the only free parameter with an estimated value of 0.048 ± 0.001 SE. We used this model to estimate the real-time hemacytometer temperature for each video from the primary zoospore velocity experiment, based on the start (incubator) temperature and the time since each sample was taken from its performance incubator.

We analyzed zoospore velocity videos using the *wrMTrck* plug-in (Table 3.1; Pederson, 2011) for ImageJ (Schneider et al., 2012), recording the mean velocity of the fastest zoospore from each video. Each video was manually checked to confirm that the *wrMTrck* software accurately tracked at least the fastest 10 zoospores for at least one second and did not re-label the zoospores during zoospore collisions or when a zoospore moved out of the frame of view. We did not use videos if they were out of focus, or if the software did not accurately track zoospores. Prior to statistical analyses, we calculated the

average value from the three videos for each replicate sample. We also recorded the estimated proportion of zoospores moving in each video.

3.3.4 Analysis of experimental data

All statistical analyses and models were implemented in Program R Version 4.0.3 (R Core Team, 2020). CT_{max} and zoospore velocity data were analyzed using linear regression models (function "lm"), with variable significance evaluated with Type II F tests using the "Anova" function from the "car" package (Fox & Weisberg, 2019). Model residuals were approximately normal and regression diagnostics generally supported model assumptions.

For the CT_{max} experiment, the primary explanatory variables we tested were acclimation temperature and start temperature. Zoospore density varied among replicates, so we also tested models with and without zoospore density as a covariate. CT_{max}^{50} and zoospore density appeared curvilinear with respect to acclimation temperature, so we tested for quadratic effects of acclimation temperature on these variables, centering acclimation temperatures around zero to avoid marginality errors. We noted one datapoint with an excessively high zoospore density, as well as five datapoints that had only 50–60% zoospores initially moving. Out of concern that these data might not be fully comparable to other datapoints, we ran our final models with and without these data included.

For zoospore velocity measurements, we used polynomial regression to test for linear and quadratic effects of acclimation temperature and performance temperature, as well as the interaction between acclimation temperature and performance temperature. The purpose of the interaction term was to test the beneficial acclimation hypothesis, which

predicts a positive interaction between these variables (Altman et al., 2016). These regression models included a quadratic effect of performance temperature to account for curvilinearity of the TPC. For the purposes of this analysis, both acclimation and performance temperatures were centered around zero to avoid marginality problems when interpreting linear terms.

To estimate key MTE parameters for maximum zoospore velocity, we used nonlinear least squares (nls function in the stats package; R Core Team, 2020) to fit a Boltzmann-Arrhenius (BA) and a reduced Sharpe-Schoolfield (SS) model as described by Sckrabulis et al. (2022). We compared AIC values for models assuming normal or lognormal error structures for both the BA and SS models as described by Xiao et al. (2011). BA and SS models were formulated as the following functions of performance temperature (T):

$$BA\{T\} = P_{T0} \cdot e^{-\frac{E_a}{k}\left(\frac{1}{T}-\frac{1}{T_0}\right)},$$

$$SS\{T\} = BA\{T\} \cdot \left(1 + e^{-\frac{E_d^H}{k}\left(\frac{1}{T_d^H}-\frac{1}{T}\right)}\right)^{-1},$$

where T is the performance temperature in degrees kelvin, P_{T0} is the BA-predicted performance at standardization temperature T_0 = 283.15 K (10 °C), E_a is the activation energy in eV, k is Boltzmann's constant, T_d^H is the high temperature for deactivation of performance, and E_d^H is the deactivation energy in eV.

3.3.5 Analysis of literature data – acclimation times for amphibian hosts of Bd

To explore MTE predictions for acclimation times at the scale of a zoospore, we analyzed thermal acclimation times from the published literature for potential amphibian hosts of Bd. We focused on three articles featuring adult amphibians that measured the

timing of acclimation effects on CT_{max} (Brattstrom, 1970; Brattstrom & Lawrence, 1962; lack of amphibian righting response or onset of spasms; Hutchison, 1961). Time to full acclimation was defined in this analysis as the time it took for CT_{max} to equilibrate to a new value following a temperature shift (see Fig. 3.3). A single "measurement" was defined as time to full acclimation for a single combination of temperature treatments within an acclimation experiment (Fig. 3.3). If CT_{max} had not equilibrated by the end of an experiment, we assumed time to acclimation was greater than the experiment's duration and recorded this as the minimum time to full acclimation. Whether or not these "minimum acclimation times" were included in our analysis did not affect the range of predicted zoospore acclimation times. If amphibian body mass was not available from within the same published study, we used species mass estimates from the AmphiBIO database (Oliveira et al., 2017). The AmphiBIO database lacked mass values for three species, which were therefore left out of the mass-scaling analysis. To scale down each acclimation times to an organism the size of a zoospore (diameter = 4 μm; Longcore et al., 1999), we used the following equation (Gillooly et al., 2001):

$$\ln(acclimation\ time) = 0.25\ \times \ln(body\ mass) + C.$$

First, we solved for the constant C for each acclimation time measurement based on the body mass of each amphibian species. Next, we calculated the scaled down acclimation time for a zoospore's body mass, assuming zoospores are spheres with a density approximately equal to that of water (i.e., zoospore mass $\approx 4/3\pi r^3 = 3.35\times10^{-11}$ g).

3.4 Results

3.4.1 Critical thermal maximum results

Half of the Bd zoospores became immobile (CT_{max}^{50}) at 26.59 ± 2.27 °C (± SD; range 22–32 °C) and 100% were immobile at 31.71 ± 2.89 °C (range 25–37 °C; Fig. 3.4). There was an apparent curvilinear effect of acclimation temperature on both measures of thermal tolerance, especially CT_{max}^{50} (Fig. 3.4). Zoospores acclimated to middle temperatures (16.5 °C) appeared to exhibit higher CT_{max}^{50} values than those acclimated to low (9 °C) or high temperatures (24 °C; Fig. 3.4). When tested alone, acclimation temperature had a significant quadratic effect on CT_{max}^{50} ($F_{1, 45} = 5.8$, $p = 0.021$); however, this effect became nonsignificant when zoospore density and start temperature were added to the model ($F_{1, 43} = 0.6$, $p = 0.430$). There was no significant quadratic effect of acclimation temperature for CT_{max}^{100} ($F_{1, 47} = 1.1$, $p = 0.293$). We therefore left quadratic effects of acclimation temperature out of final models presented in Table 3.2. There was no significant linear effect of acclimation temperature on CT_{max}^{50} or CT_{max}^{100} in any of these models (all p values greater than 0.05; Table 3.2).

Start temperature had a significant positive effect on CT_{max}^{50} and a nonsignificant trend toward a positive effect on CT_{max}^{100} (Table 3.2; Fig. 3.5). Zoospore density had significant positive effects on both CT_{max}^{50} and CT_{max}^{100} (Table 3.2; Fig. 3.5). Whether zoospore density was included in these models did not qualitatively change the effects of start temperature on CT_{max}^{50} or CT_{max}^{100}. There was a curvilinear effect of acclimation temperature on zoospore density ($F_{1, 47} = 27$, $p < 0.001$), similar to the apparent curvilinear effect of acclimation temperature on CT_{max}^{50} (Fig. 3.4). We tried running

models with and without datapoints with unusually high zoospore densities, low proportion moving, or high heating rates (1 sample with zoospore density >550 per field of view, 5 samples with <70% of zoospores moving, and 1 sample with a heating rate >4 °C per minute). Removing these datapoints did not qualitatively change the effects of acclimation temperature or zoospore density, but start temperature effects became more pronounced (CT_{max}^{50} $F_{1, 38} = 11.8$, $p = 0.001$; CT_{max}^{100} $F_{1, 39} = 4.9$, $p = 0.033$).

3.4.2 Zoospore velocity results

Zoospore velocities increased with performance temperature (linear effect: $F_{1,42} = 112.1$, $p < 0.001$; Fig. 3.6) and appeared to level off at higher temperatures, resulting in a curvilinear pattern with peak zoospore velocities between 22-29 °C (Fig. 3.6). The curvilinearity of the performance curve was supported by a significant quadratic effect of performance temperature on maximum zoospore velocity ($F_{1, 40} = 7.7$, $p = 0.008$). There were no significant linear ($F_{1,42} = 0.9$, $p = 0.355$) or quadratic ($F_{1,41} = 1.0$, $p = 0.312$) effects of acclimation temperature on maximum zoospore velocity, and there was no significant interaction between acclimation temperature and performance temperature ($F_{1,40} = 1.1$, $p = 0.309$). The initial proportion of zoospores moving had no significant relationship with either maximum zoospore velocity ($F_{1, 42} = 1.8$, $p = 0.188$) or performance temperature ($F_{1, 42} = 0.4$, $p = 0.526$), and there was no significant relationship between zoospore concentration and maximum zoospore velocity ($F_{1,42} = 0.01$, $p = 0.917$) .

Out of the MTE-based models for the zoospore velocity TPC, the SS model with lognormal error provided good description of the TPC (Fig. 3.6) and generated the lowest AIC (lognormal SS: 282.2, normal SS: 283.1; lognormal BA: 288.8; normal BA: 290.1).

The lognormal BA model generated an E_a = 0.2059 ± 0.0199 eV (± SE) and P_{T0} = 53.16 ± 0.93 μm·s^{-1}, at a standardization temperature (T_0) of 10 °C. The lognormal SS model generated E_a = 0.6883 ± 0.7372 eV, P_{T0} = 63.98 ± 62.24 μm·s^{-1}, E_d^H = 0.847 ± 0.229 eV, T^H = 289.2 ± 27.0 °C.

3.4.3 MTE predictions for zoospore acclimation times (literature data analysis)

We reviewed measurements of time to full acclimation of in potential amphibian hosts of Bd from three published papers (Brattstrom, 1970; Brattstrom & Lawrence, 1962; Hutchison, 1961), generating 94 measurements from 23 amphibian species as described above and in Fig. 3.3. The number of individual animals included in each measurement was not always available. Here "measurement" refers to a single combination of temperature treatments in a CT_{max} acclimation experiment. Acclimation times ranged from 1–3 days for increases in temperature and 1–6 days for decreases in temperature in species with body masses ranging from 0.8–467.8 g (masses obtained from the published papers or from the AmphiBIO database; Oliveira et al. 2017). Nearly all measurements following an increase in temperature came to a new equilibrium CT_{max} by the end of each experiment. Acclimation times were generally longer in the 32 measurements that followed a decrease in temperature, with 21 (66%) of these measurements failing to come to an equilibrium CT_{max} prior to the end of the experiment (usually 48 or 72 hours). This allowed us to calculate an expected acclimation time range of 1.38–9.16 minutes for increases in temperature and 2.22–16.72 minutes for decreases in temperature.

3.5 Discussion

We did not find clear evidence that experimental acclimation treatments affected CT_{max}^{50} , CT_{max}^{100} , or zoospore velocity. These results suggest that Bd zoospores either (1) have constitutive responses to temperature or (2) fully acclimate to new temperatures before we were able to complete our measurements (i.e., ~3 min for CT_{max}^{50}, ~9 min for CT_{max}^{100}, and ~15 min for zoospore velocity). However, ramping trials with higher start temperatures had significantly higher CT_{max}^{50}. Start temperature effects are commonly observed in studies that use ramping trials to measure critical thermal limits (Kingsolver & Umbanhowar, 2018; Terblanche et al., 2007). While start temperature effects remain poorly understood, they might represent full or partial acclimation responses occurring on short time scales. The positive effect of start temperature on CT_{max}^{50} observed in the current study is consistent with the beneficial acclimation hypothesis, but only if zoospore acclimation occurs very rapidly. Zoospores were only at or near the start temperature for the first ~30 seconds of the ramping trial, and most CT_{max}^{50} measurements were completed in under 3 minutes, so any acclimation effects caused by exposure to the start temperature would have occurred in less than a minute.

Such a rapid thermal acclimation response might be plausible for an organism as small as a Bd zoospore. Our literature analysis of acclimation times for various frog species found that full acclimation took 24–144 hours (masses 0.8–106.3 g) following a decrease and 24–72 hours (masses 0.8–467.8 g) following an increase in temperature, which when scaled down to the size of a zoospore (assuming on a quarter-power scaling relationship) translates into 2.22–16.72 and 1.38–9.16 minutes, respectively. These predicted ranges might account for failure to detect effects of our experimental thermal

acclimation treatments, which took 1–18 minutes to complete. However, even these numbers might underestimate the rate at which zoospores could acclimate to new temperatures. Increased thermal tolerances referred to as "heat hardening" effects have been documented in some amphibian species (e.g. *Xenopus laevis* juveniles) after brief exposure to elevated temperatures (10 min followed by 2 hr room temperature interval for *X. laevis* study; Sherman & Levitis, 2003, Spotila et al., 1989). Assuming the juvenile *X. laevis* masses were approximately ~3.5 g, a 2.2 hour heat hardening time scale translates to 13.8 seconds when scaled down to the size of a Bd zoospore. Short thermal response time frames have been observed in other microscopic eukaryotes that showed changes in heat shock protein expression in as few as 20 – 30 minutes following a temperature shift (Alcina et al., 1988; Kalinina et al., 1988; Krobitsch et al., 1998). It is possible that we only observed start temperature effects on CT_{max}^{50} because these data were collected within 3 minutes of exposure to a particular start temperature, whereas CT_{max}^{100} measurements took ~9 minutes to complete by which time start-temperature effects had mostly dissipated (though, start temperature became a significant predictor of CT_{max}^{100} after removing some data points with extreme values for high zoospore density, low proportion moving, and high heating rate). Follow-up experiments would be necessary to confirm rapid Bd zoospore acclimation in response to an experimental acclimation treatment, perhaps with a much higher rate of temperature increase to complete ramping trials in under 1 minute.

There appeared to be a curvilinear effect of acclimation temperature on CT_{max}^{50} that would have been consistent with an "optimal temperature" acclimation response (Fig. 3.4). However, this pattern appears to have been caused by a confoundment between our

CT_{max}^{50} and zoospore density, and the quadratic effect of acclimation temperature became nonsignificant when zoospore density was added to the model (Fig. 3.4; Table 3.2). Zoospore density also had a curvilinear relationship with acclimation temperatures, with higher zoospore densities collected from cultures that were grown at an intermediate acclimation temperature of 16.5 °C. This is close to the optimal temperature range for Bd growth in culture (Piotrowski et al., 2004), which might account for higher zoospore production. Observers might have overestimated CT_{max}^{50} when zoospores were denser because it was more difficult to accurately estimate the proportion of zoospores moving. Zoospore density was also a positive predictor of CT_{max}^{100}, though there was no significant quadratic effect of acclimation temperature on this response variable. CT_{max}^{100} measurements should have been less subject to observer bias, but a positive effect of zoospore density makes biological sense because observing a larger number of zoospores increases the probability of at least one zoospore continuing to move as the temperature is ramped up.

Our overall measurements of Bd zoospore thermal tolerances, and the thermal performance curve for maximum zoospore velocity, were consistent with aspects of Bd thermal biology measured in prior studies (Stevenson et al., 2013; Voyles et al., 2017). Half of the zoospores became immobile at 26.59 °C (±2.27 SD) and 100% became immobile at 31.71 °C (±2.89 SD). These CT_{max} correspond well with Bd's known thermal limits, with 27 °C often cited for zero growth in culture (Longcore et al., 1999; Piotrowski et al., 2004) and 30 °C sometimes used as target temperature for heat treatments to clear frogs of Bd infection (Chatfield & Richards-Zawacki, 2011, McMahon et al., 2014). Consistent with MTE predictions, zoospore velocity was well-

described by an SS model, which assumes increased performance up to some T_{opt} above which performance decreases due to reversible enzyme inactivation (Molnár et al., 2017). Interestingly, high zoospore velocities persisted at temperatures well above the optimal ranges established for Bd growth rates in culture (Sheets et al., 2021; Voyles et al., 2017). Faster zoospores are likely to be better at infecting potential hosts (Appiah et al., 2005; Canter & Jaworski, 1981), so our results suggest Bd might be intrinsically better at infecting hosts at higher temperatures (not accounting for changes in host resistance; Raffel et al. 2013), at least until zoospores reach their CT_{max} and become immobile.

3.6 Conclusions

Although we found no clear effects of our experimental acclimation treatments, the presence of start temperature effects suggest that Bd zoospores might have undergone physiological acclimation within the 1–18 minutes it took to collect data following removal of zoospores from their acclimation treatments. Assuming this reflects a real pattern of thermal plasticity, it is worth considering whether an acclimation response on this time scale might have any relevant effects on infection dynamics in amphibian hosts. The temperature variability hypothesis of Rohr & Raffel (2010) assumes that pathogens have either: (1) constitutive thermal responses or (2) more rapid thermal acclimation responses than their hosts, following an unpredictable shift in temperature. Regardless of how one interprets our results, they are consistent with this assumption. These findings could have implications for climate change effects on Bd disease dynamics. If Bd zoospores are better equipped than their hosts to perform under variable temperature regimes, then we could see increases in chytridiomycosis infections and associated

amphibian declines as future climate conditions become more variable and unpredictable (Raffel et al., 2013).

3.7 Data availability

Code and data can be accessed here:

https://github.com/huntercraig248/ZoosporeAcclimation

3.8 Acknowledgements

Funding for this study was provided by the National Science Foundation (IOS1651888). Use of Bd was approved by the Oakland University IBC (#2759-13, Appendix B). Frozen Bd stocks were provided by Joyce Longcore (University of Maine). Experimental assistance was provided by Jenna Agosta, Alejandro Hernandez, and Sajjad Kamil, Declan McCrary, James Noelker, and Vanessa Six. Thank you to the Oakland University Department of Biological Sciences for TA support to HMC, JPS, and KAA. KAA was also supported by a King-Parks-Chavez Future Faculty Fellowship while completing this work.

3.9 Chapter 3 tables

Table 3.1: Settings used in the *wrMTrck* plug-in (Pederson, 2011) for imageJ (Schneider et al., 2012) for zoospore velocity analysis.

Setting description	Value
minSize	3
maxSize	600
maxVelocity	20
maxAreaChange	25
minTrackLength	7
bendThreshold	2
binSize	0
saveResultsFile	unchecked
showPathLengths	checked
showLabels	checked
showPositions	checked
showPaths	unchecked
showSummary	checked
roundCoord	unchecked
smoothing	unchecked
plotBendTrack	unchecked
rawData	0
bendDetect	0
FPS	0
backSub	0
threshMode	Otsu
fontSize	16

Table 3.2: Linear model outputs for the effects of acclimation temperature, start temperature, and zoospore density (zoospores per field of view at 200x magnification) on critical thermal maximum with 50% immobility and 100% immobility (CT_{max}^{50} and CT_{max}^{100}; df = degrees of freedom).

Response	Predictor	F	df	p
CT_{max}^{50}	Acclimation temperature	1.1	1, 44	0.292
	Start temperature	4.6	1, 44	0.038
	Zoospore density	4.5	1, 44	0.039
CT_{max}^{100}	Acclimation temperature	0.4	1, 46	0.553
	Start temperature	3.0	1, 46	0.092
	Zoospore density	16.2	1, 46	< 0.001

3.10 Chapter 3 figures

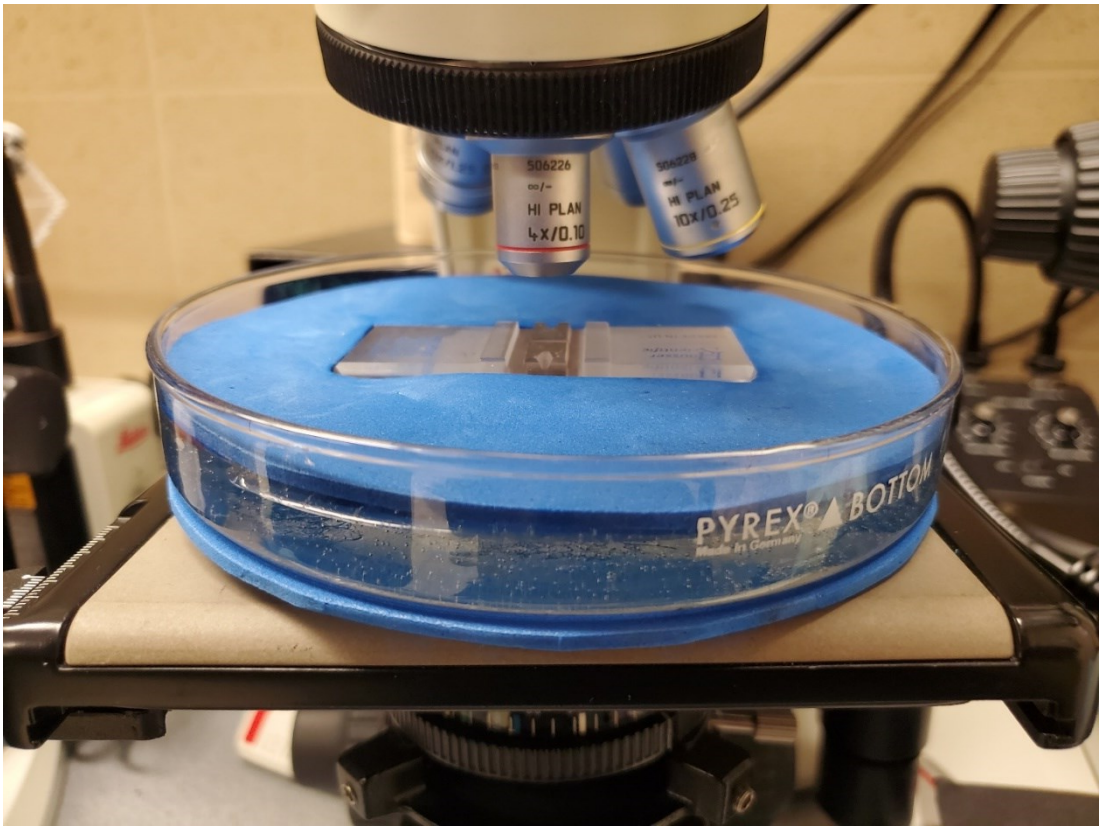

Figure 3.1: Setup to maintain sample temperature during video microscopy for zoospore velocity measurements. A hemacytometer is fitted into a foam cutout, placed on top of a 150-mm diameter glass Petri dish filled with gel wax. The purpose of the gel wax was to increase the thermal inertia of the hemacytometer, allowing for more consistent application of performance temperature treatments.

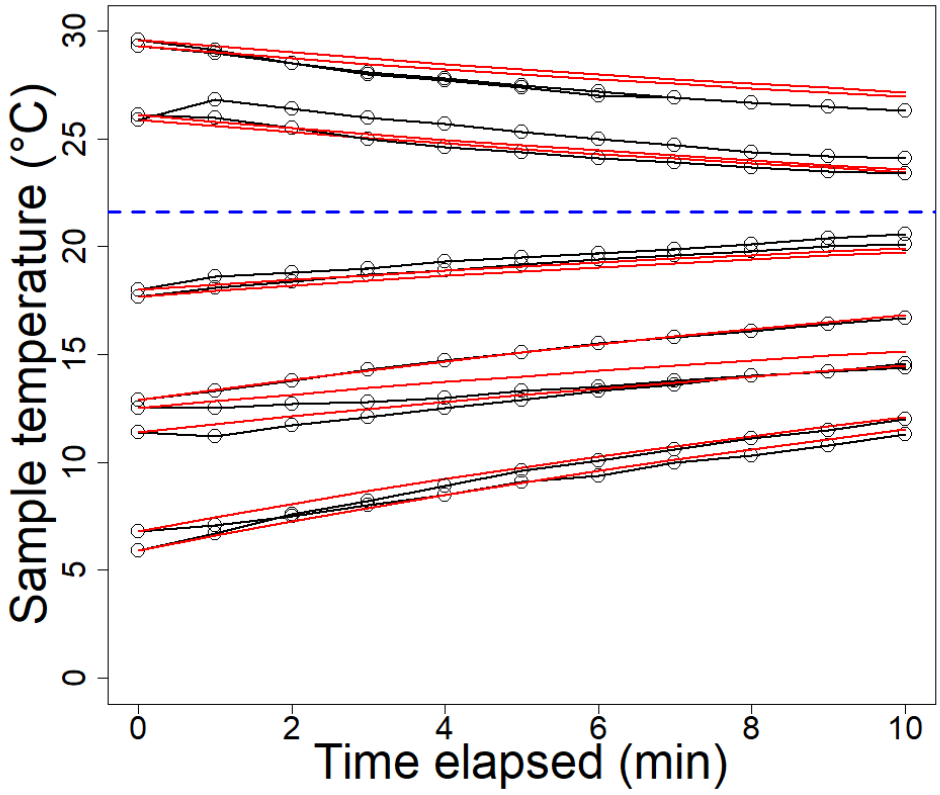

Figure 3.2: Time series temperature data for dummy samples (20 µL water) placed on hemacytometers fitted into gel wax dishes as shown in Fig. 3.1. The black circles and lines represent temperatures observed for individual hemacytometers (n = 10), and red curves represent the predictions of the fitted model describing Newton's law of cooling. The blue line shows room temperature (21.6 °C). We used the fitted model to adjust performance temperature estimates for the zoospore velocity trials to account for temperature equilibration to room temperature by the time of video microscopy. During zoospore velocity trials all videos were obtained within 7 minutes of removing hemacytometers (and gel wax) from their performance incubators.

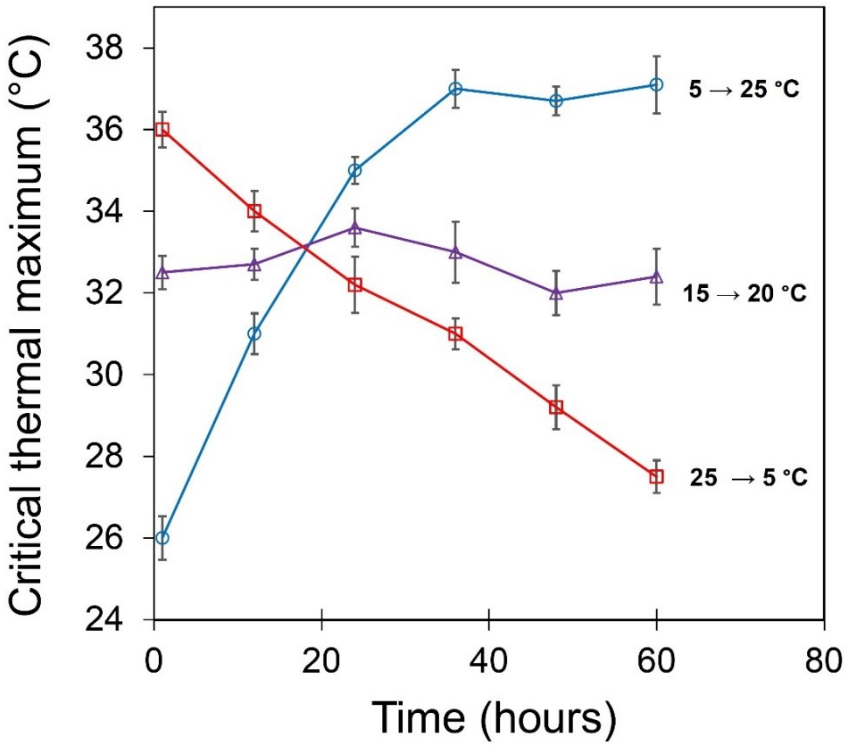

Figure 3.3: Hypothetical critical thermal maximum (CT_{max}) data representing typical patterns observed in published acclimation time experiments (Brattstrom, 1970; Brattstrom & Lawrence, 1962; Hutchison, 1961). As defined in our analysis, this graph would represent three "measurements" of thermal acclimation time for one or more amphibian species. Acclimation time was defined as the minimum number of hours it took from the time of the temperature shift (time 0) until CT_{max} stabilized at some new equilibrium. Equilibrium was considered stabilized at a given time point if the error bars for that datapoint overlapped with that of the next datapoint. Error bars representing ranges of observed CT_{max} values were included in approximately half of the analyzed figures. If error bars were not provided, we made our best judgement of acclimation time based on the available data. The blue line represents an increase in CT_{max} following an increase in acclimation temperature (from 5 to 25 °C). This curve would have generated an acclimation time estimate of 36 hours. The purple line represents no observed acclimation because there was no change in approximate CT_{max} post-temperature shift. The red line represents a decrease in CT_{max} following a decrease in acclimation temperature (from 25 to 5 °C), where the change in CT_{max} had not stabilized by the end of the experiment. In this case, the acclimation time would be considered "greater than 60 hours". In all published sources, each point on the graph represented the average of more than one individual animal, but sample sizes were not always provided.

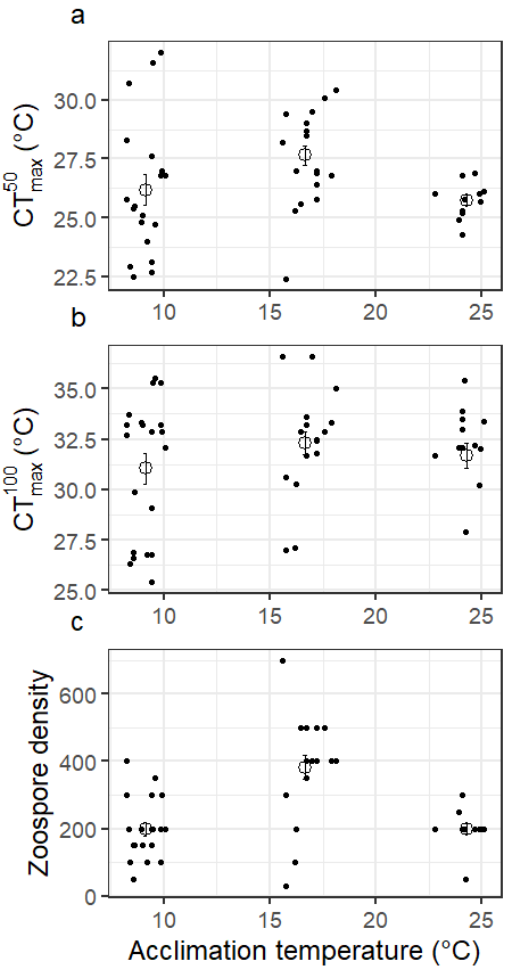

Figure 3.4: Zoospore critical thermal maximum and density estimates (zoospores per field of view at 200x magnification) plotted as a function of acclimation temperatures. Open circles represent the mean critical thermal maximum at 50% immobility (CT^{50}_{max}) (a), 100% immobility (CT^{100}_{max}) (b), and zoospore density (c) of each acclimation treatment with standard error bars. Closed circles represent the individual measurements, plotted at their actual acclimation temperatures as measured by HOBO loggers.

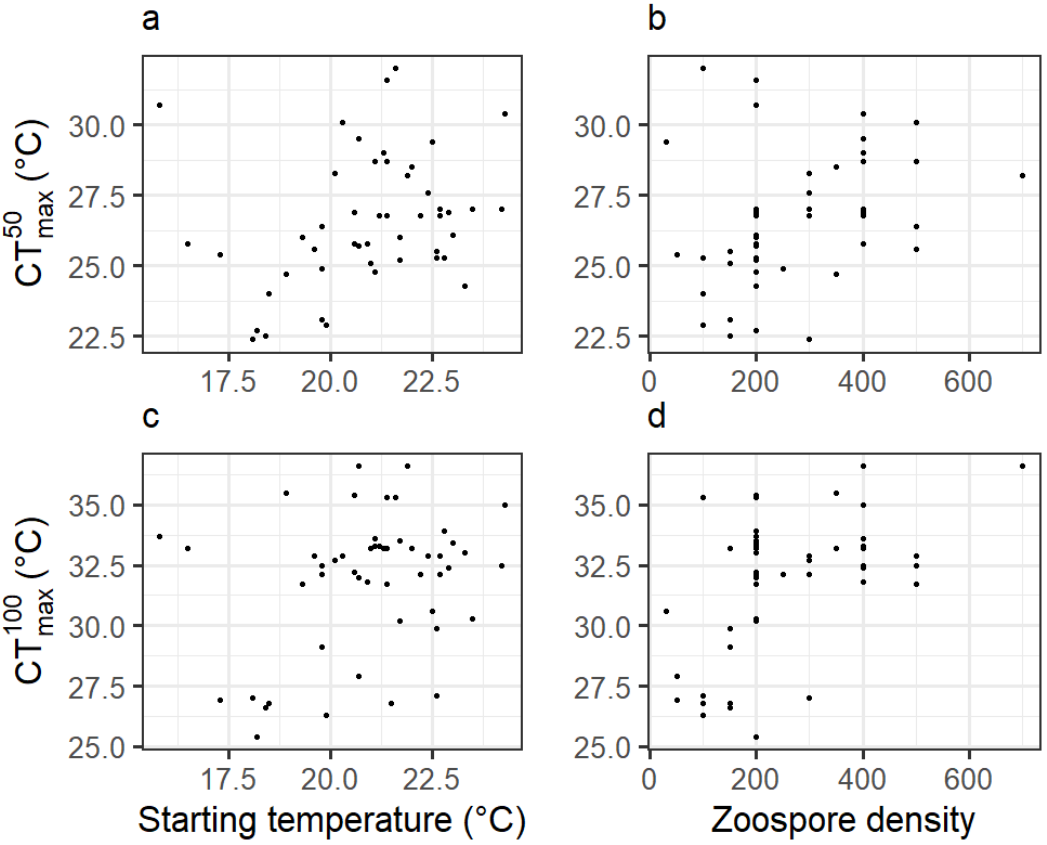

Figure 3.5: The effects of trial start temperature (a, c) and zoospore density (zoospores per field of view at 200x magnification; b, d) on zoospore critical thermal maximum with 50% immobility (CT^{50}_{max}; a, b) and 100% immobility (CT^{100}_{max}; c, d).

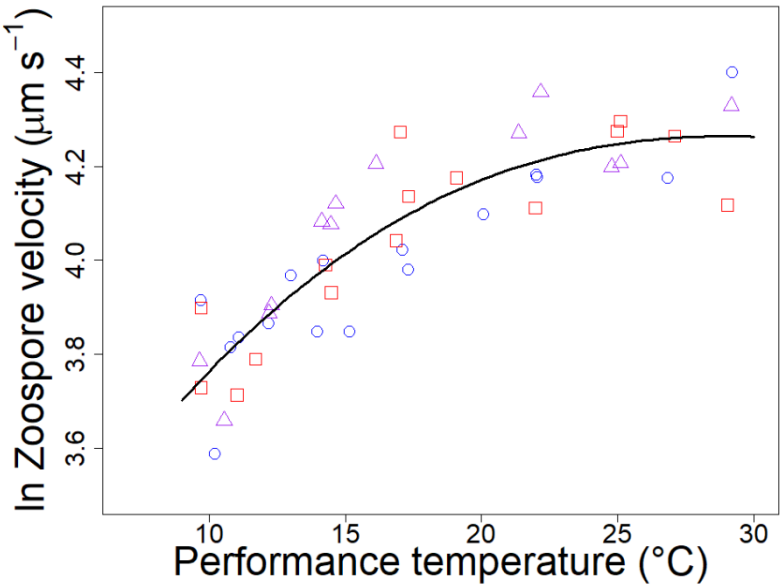

Figure 3.6: Thermal performance curve showing maximum zoospore velocity (µm s^{-1}) as a function of performance temperature (°C). The black curve shows the fitted Sharpe-Schoolfield model assuming a lognormal error distribution. Different acclimation treatments are indicated by point color and shape (blue circles = 9 °C; purple triangles = 16.5 °C; red squares = 24 °C).

CHAPTER FOUR

LACK OF CLEAR THERMAL ACCLIMATION OR LOCAL ADAPTATION EFFECTS ON CHYTRID INFECTION IN A NEWT COMMON GARDEN EXPERIMENT

4.1 Introduction

Temperature variability is expected to increase with global climate change, so it is important to understand how variable temperatures affect disease dynamics (Raffel et al., 2013). Chytridiomycosis is a temperature-dependent emerging disease caused by the fungal pathogen *Batrachochytrium dendrobatidis* (Bd) that threatens amphibian populations worldwide (Bower et al., 2017; Fisher et al., 2009). Juvenile red-spotted newts (*Notophthalmus viridescens*) showed lower resistance to Bd infection following a temperature shift, relative to newts already acclimated to the exposure temperature (Raffel et al., 2015). This result is consistent with the temperature variability hypothesis of Rohr and Raffel (2010), which postulated that pathogens might take advantage of adaptive delays in host thermal acclimation immediately following abrupt shifts in temperature. This hypothesis assumes that hosts exhibit beneficial acclimation of their immune responses, and that host immune system acclimation takes longer than pathogen acclimation to a new temperature (Raffel et al. 2013). Here we hypothesized that adult newts would also exhibit lower resistance to Bd infection following a temperature shift, consistent with seasonal changes in blood leukocytes of adult newts in the field (Raffel et al., 2006) and with the beneficial acclimation hypothesis of thermal biology.

Another outstanding question is whether hosts from different latitudes have local adaptations for temperature-dependent responses to infection (DeLong et al., 2018).

Because latitudinal differences in amphibian source populations are associated with differences in thermal metabolic responses (Cohen et al., 2019; Deutsch et al., 2008; Mineo & Schaeffer, 2014; Rohr & Raffel, 2010; Sunday et al., 2011; Wilson, 2001), amphibians from different latitudes might be expected to have different temperature-dependent responses to infection. Assuming adaptation to local climate conditions, we hypothesized that newts from higher latitudes would have relatively higher resistance to Bd infection at cooler temperatures, whereas newts from low latitudes would have relatively higher resistance at warmer temperatures. Furthermore, newts from higher latitudes might have higher levels of baseline exposure to Bd, given that this pathogen is typically associated with cooler climates (Li et al., 2013), potentially resulting in higher levels of acquired immunity in wild caught individuals (McMahon et al., 2014). We therefore hypothesized that Ohio newts would be locally adapted for greater resistance to Bd infection than Tennessee newts, due to local adaptation to presumably higher levels of Bd infection in this cooler latitude (Raffel et al., 2015).

We conducted a common garden infection experiment on red-spotted newts (*N. viridescens*) collected from different latitudes (Ohio and Tennessee), to investigate how source population, thermal acclimation responses, and temperature affect Bd infection in adults of this species.

4.2 Methods

4.2.1 Newt collection and maintenance

Newts were collected from three source populations in each state (Ohio and Tennessee). See Chapter 2 for information about transporting and maintenance, as these were the same newts used in the respirometry experiments. Within 48 hours of arriving at

the lab, five newts from each container were swabbed via sterile cotton swab three times each along the central abdomen, around the cloaca, and along the ventral surface of each hind foot pad. Swabs were stored in screw cap tubes at −20 °C until they could be further processed. Before handling each new newt, our gloved hands were rinsed in 10% bleach, then water with excess dechlorination agent (Kordon AmQuel Plus Ammonia Detoxifier) then regular water and dried as described by Raffel et al. (2013). Within two weeks of arrival at the lab, all newts were heat treated at 30 °C for three days in an attempt to clear any initial Bd infection (Chatfield & Richards-Zawacki, 2011) . We then returned newts to room temperature conditions for at least two weeks prior to acclimation for respirometry (Chapter 2). Newts were then kept at room temperature for two months after the respirometry performance period had ended, prior to acclimation for the present experiment.

During the Bd infection experiment, each newt was singly-housed in a 64 oz (~1.9 L) container with 400 mL dechlorinated water and a plexiglass ramp to allow it to climb out of the water. Newts were fed blackworms *ad libitum* throughout acclimation and performance.

4.2.2 Newt acclimation

Newts were re-randomized and acclimated to either 8 °C or 25 °C in twenty-four custom Styrofoam incubators arranged in a randomized block design, with two newt containers per incubator. Incubators were constructed as described by Altman et al. (2016). Newt containers were rotated and swapped within their incubators daily to mitigate microclimate effects within the incubator. Newts were tested in two temporal blocks that began 15 days apart. Each newt was acclimated to its acclimation test

temperature for 30 days prior to the performance period. Newts from different latitudes (Ohio vs Tennessee) were distributed evenly across temperature treatments. One container per incubator housed a HOBO temperature logger (Onset Computer Corporation, MA, USA) to track actual temperatures throughout the experiment. Newt water was changed weekly, using fresh water pre-heated or cooled to 8, 11, or 25 °C to be consistent with assigned temperature treatments. Throughout the experiment, newts were checked daily for mortality or signs of illness. Excessive skin sloughing, malaise, or lack of righting ability were considered alternative endpoints, but no newts exhibited this during the experiment. If a newt was found dead, it was immediately swabbed according to the procedure described above and preserved in 70% ethanol. Newt containers were rotated in position within the incubator daily, to even out any potential for microenvironmental differences within the incubators.

Seven days after the acclimation period began (four months after heat treatment), all newts were swabbed using the procedure described above to evaluate the potential for pre-existing Bd infections (i.e., infections that got past our heat treatment procedure). This corresponded to 25 and 40 days pre-experimental infection for newts from temporal blocks A and B, respectively.

4.2.3 Bd infection

After a 30 day acclimation period, each newt of temporal block A was weighed and then inoculated with 3×10^6 Bd zoospores in 3 mL ASW (Cohen et al., 1980). The Bd isolate was cultured, and zoospores collected as described for the Bd zoospore experiment (Chapter 3). Inoculum was prepared by quantifying zoospore densities with a hemacytometer and diluting to a density of 1 million zoospores per mL. Each newt was

inoculated by holding it above its housing container and applying 3 mL over its head and body. Inoculum was allowed to run off the newt's body into 400 mL of water in its housing container, and the newt was then returned to its water. The newt's container was then placed into its assigned performance incubator at one of three temperatures (8, 11, 25 °C). Twenty-four performance-temperature incubators were arranged on shelves in a randomized block design. Again, newts from different latitudes (Ohio vs Tennessee) were distributed evenly across temperature treatments. HOBO temperature loggers were again placed into newt containers, with one logger per incubator. Temporal block B newts were acclimated for 45 days and infected 15 days after temporal block A. All newts were swabbed every 14 days post-infection to keep track of Bd zoospore load on each newt. Six weeks post-infection, newts were swabbed, weighed, measured for SVL, euthanized via Orajel (benzocaine) application to the head/abdomen followed by decapitation, and preserved individually in 70% ethanol. Newt Bd load was calculated via qPCR of skin swabs and converted from gene copies to approximate number of Bd zoospores as described by Altman et al. (2019).

4.2.4 Statistical methods

I used the lmer function from the lme4 package (Bates et al., 2015) and the Anova function from the car package (Fox & Weisberg, 2019) to generate linear-mixed effects models in Program R Version 4.0.3 (R Core Team, 2020) to investigate effects of performance temperature, acclimation temperature, latitude (Ohio versus Tennessee), and time on Bd load, treating performance incubator, acclimation incubator, and source population as random effects.

4.3 Results

During the performance period (across all weeks post-experimental infection there were no significant effects of acclimation temperature or latitude on Bd load (Table 4.1) However, Bd load declined through time post-infection (Table 4.1, Fig. 4.1). In the full model that included all three post-experimental infection time points, there were no significant two-way interactions between any of the predictor variables (all p-values > 0.2), so interactions were removed (Table 4.1). There were no interactions between acclimation temperature and performance temperature ($F_{1,100.38}$, $p = 0.455$), or between acclimation temperature and time ($F_{1,82.88}$, $p = 0.930$).

Two weeks post-experimental infection, there was a significant negative effect of performance temperature on Bd load, and a near-significant positive effect of acclimation temperature on Bd load (Table 4.1, Fig. 4.2). There were no significant two-way interactions between any of the predictors (all p-values > 0.3).

Immediately upon arrival in the lab (i.e., within 48 hours of capture), newts from Ohio had significantly lower levels of infection than newts from Tennessee (geometric mean of ~140 zoospore equivalents and ~5350 zoospore equivalents, respectively; $F_{1,33.5}$, $p < 0.001$; Fig. 4.3). When re-swabbed during the acclimation period of the experiment (i.e., 4 months following heat treatment), newts had greatly reduced Bd load relative to their levels immediately following capture (Fig. 4.3). However, 30 % of newts from Ohio and 50 % from Tennessee still had detectable levels of Bd infection (~2 zoospore equivalents for Ohio and ~14 zoospore equivalents for Tennessee; Fig. 4.3). This was greater than a 99% reduction (on a log scale) in infection levels four months post-heat

treatment. Only three newts died throughout the performance period, with infection levels rarely going above 10,000 zoospore equivalents during the performance period.

4.4 Discussion

There was little evidence of acclimation or local adaptation (latitude-of-origin) effects on adult newt Bd resistance in this study, although there was a nonsignificant trend toward warm acclimated newts having higher levels of infection immediately post-experimental infection (Fig. 4.2). Assuming this is not simply a statistical artefact, this trend is more consistent with a "cooler is better" acclimation response than the hypothesized beneficial acclimation response. The overall negative effect of performance temperature on Bd load is consistent with prior studies of temperature dependent Bd or *Batrachochytrium salamandrivorans* (Bsal) infection in this species (Carter et al., 2021; Raffel et al., 2015). However, this temperature dependence became less pronounced over time as overall Bd levels decreased (Fig. 4.1).

There were no significant differences in post-exposure Bd infection levels in newts collected from states at different latitudes (Fig. 4.2). Field-caught newts from Tennessee had higher levels of infection shortly following capture than newts from Ohio (Fig. 4.3), contradicting a primary assumption of our second hypothesis. These differences in prior Bd infection may have affected the experimental results, especially since our heat treatment was not entirely successful at clearing animals of infection prior to the experiment (Fig. 4.1, 4.3). This result adds to growing evidence that heat treatment can effectively reduce Bd infection levels in amphibians, but that it is not a reliable way to fully clear animals of Bd infection (Brannelly, 2014; Chatfield & Richards-Zawacki, 2011). As a result of this study, we changed our practices and have applied a combination

of heat treatment and itraconazole treatment with wild-caught amphibians prior to conducting subsequent Bd infection experiments, to ensure animals are free of infection.

Mortality was low during the experiment, which adds to growing evidence that red-spotted newts may be relatively tolerant of Bd infection (e.g., Raffel et al., 2010).

4.5 Next steps

A future goal in the analysis of these data is to determine if these patterns can be reasonably described using a MTE-based thermal mismatch model. In a prior collaborative study in the Raffel lab (Sckrabulis et al., in prep), we estimated the infectivity curve for Bd infection in spring peepers (*Pseudacris crucifer*). However, a remaining key question is if the same method can be successfully applied to additional amphibian species. In the future we will use nonlinear least squares to fit my data from Chapter 4 to parameterize a MTE-based model (described above), to test the thermal mismatch hypothesis in this host-parasite system.

4.6 Acknowledgements

Thank you to Galilee Rogers for helping with every aspect of this experiment. Many thanks to those who helped collect and care for the newts (see Chapter 2). Funding for this project was provided by the National Science Foundation (IOS 1651888). Use of animals was approved by the Oakland University IACUC (18011; Appendix A). Collection of animals was approved by Ohio Division of Wildlife (scientific collection permit #21-067) and Tennessee Wildlife Resources Agency (scientific collection permit #1296). Use of Bd was approved by the Oakland University IBC (2759-13, Appendix B).

4.7 Chapter 4 tables

Table 4.1: Linear mixed-effects model outputs for natural log transformed Bd load (ln zoospore equivalents). Performance temperature and acclimation temperature were in °C, and time was treated as categorical based on weeks post-experimental infection. Model 1 used data from all time points post-experimental infection, and Model 2 used data from two weeks post-experimental infection. We used Kenward-Rogers degrees of freedom (df), which can be non-integers in a mixed effects model with an unbalanced design.

Model	Response	Predictor	F	df	p
1	ln Bd load	Performance temperature	2.5	1, 16.95	0.135
		Acclimation temperature	2.8	1, 14.03	0.115
		Latitude (state)	0.9	1, 11.59	0.366
		Time	13.9	1, 83.73	< 0.001
2	ln Bd load	Performance temperature	10.2	1, 18.72	0.005
		Acclimation temperature	3.8	1, 12.50	0.074
		Latitude (state)	0.05	1, 9.75	0.836

4.8 Chapter 4 figures

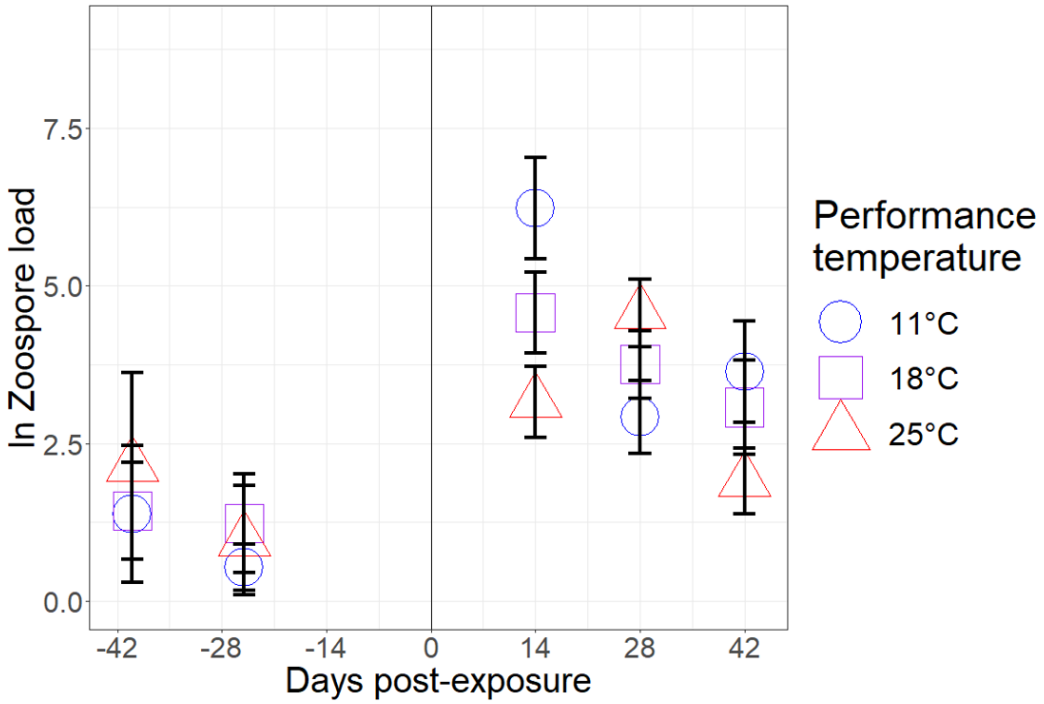

Figure 4.1: Bd infection levels in ln zoospore equivalents through time for different performance temperature treatments. The vertical line represents experimental infection and the shift to performance temperatures on Day 0. Negative days represent swabs taken during the acclimation period.

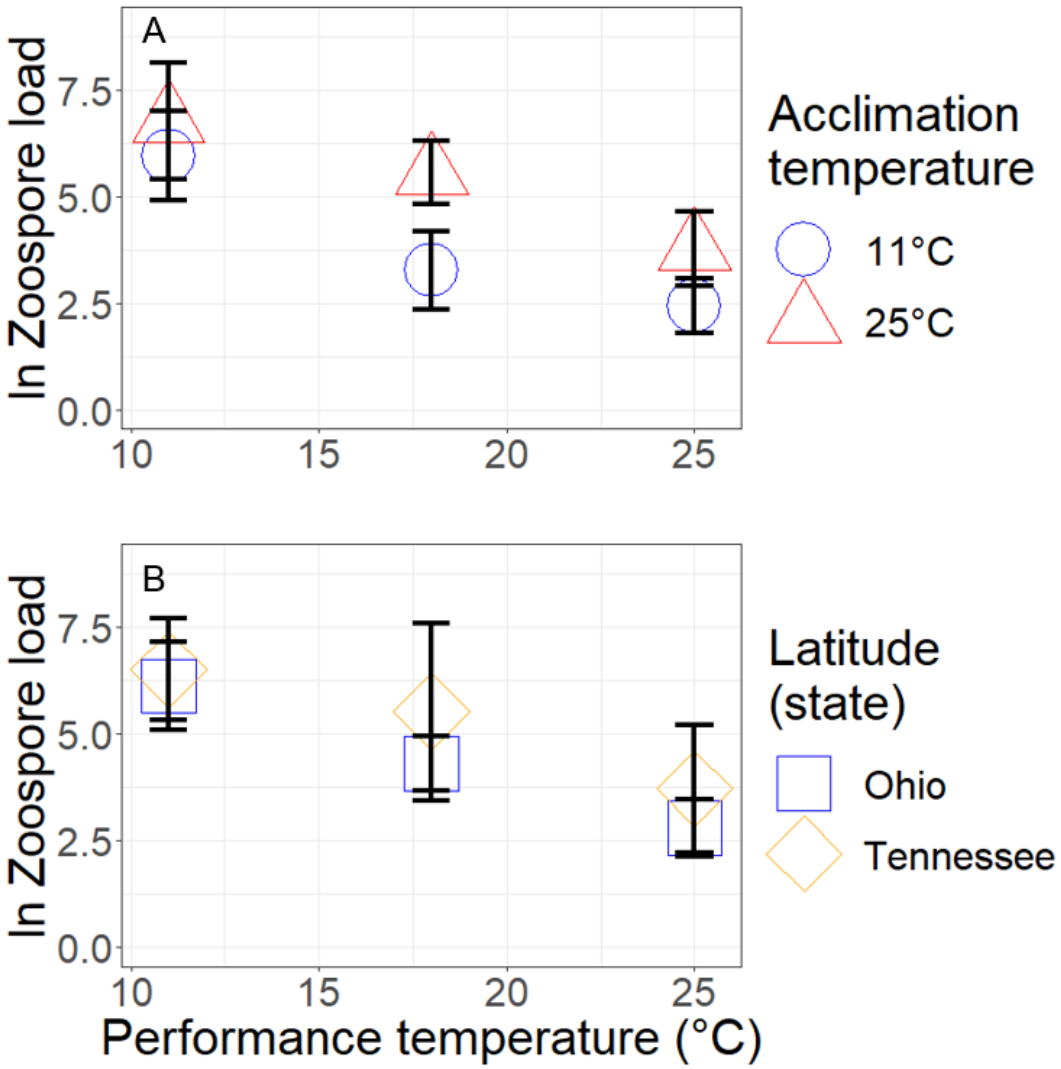

Figure 4.2: Thermal acclimation (A) and latitude (B) effects on Bd infection levels (in ln zoospore equivalents) at 2 weeks post-infection, as a function of performance temperature.

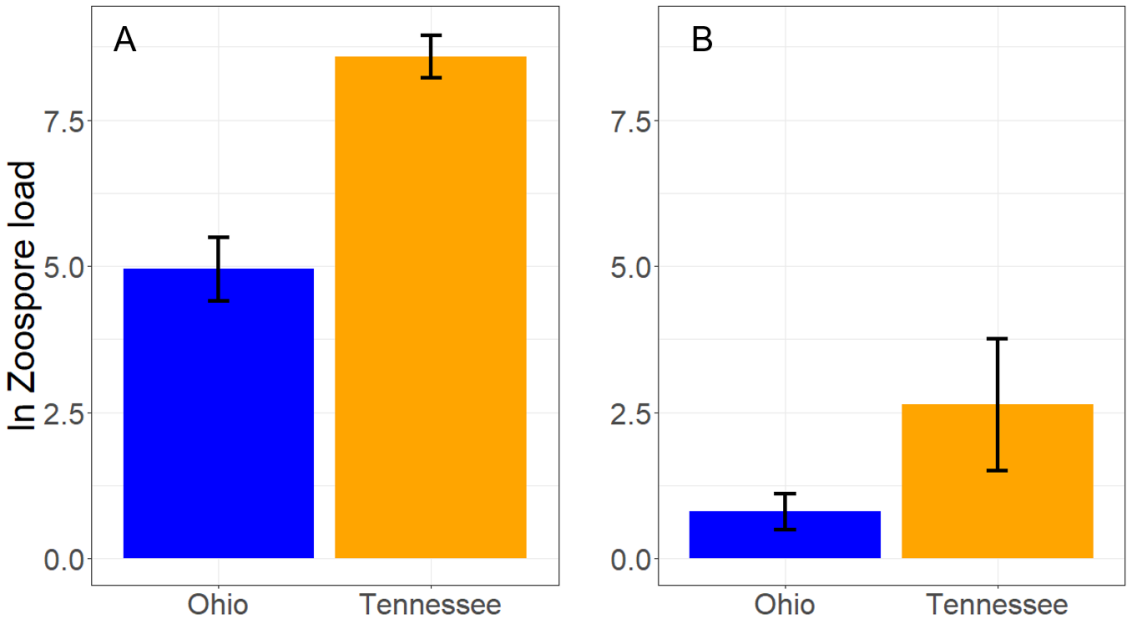

Figure 4.3: Bd infection levels in ln zoospore equivalents prior to experimental Bd exposure. (A) Before heat treatment (immediately following arrival in the laboratory) and (B) four months after heat treatment (during the acclimation period).

CHAPTER FIVE

DEVELOPMENT OF AN INTRODUCTORY BIOLOGY EDUCATIONAL ACTIVITY TO EXPLORE ORGANISMS' METABOLIC RESPONSES TO TEMPERATURE

5.1 Abstract

Active-learning and inquiry-based educational activities show great potential in keeping students engaged and improving learning outcomes in biology. One vital set of concepts for biology students to learn is how chemical and physical processes scale up to explain whole-organism processes, such as metabolic responses to temperature in different animal species. We developed a set of hands-on, inquiry-based laboratory activities to explore how thermodynamic principles, mathematics, and the Metabolic Theory of Ecology (MTE) help explain why ectotherms and endotherms have different metabolic responses to temperature. We developed six experimental activities and organized two versions of the full lesson: a version with simplified mathematics and extra instructional guidance designed for high school students in Project Upward Bound (PUB), and a more mathematically challenging and independent version with less instructional guidance aimed at undergraduate students. In the high school activity, PUB students were (1) introduced to the full activity, (2) separated into separate groups to conduct physics, chemistry, or biology experiments, and (3) brought back together to discuss connections between of what each group had learned. In the undergraduate activity, all students completed a series of chemistry and biology experiments and analyzed their results, using higher-level mathematical skills to compare and contrast quantitative outcomes of the various experiments. There was no change post-activity in

student responses to a published survey instrument designed to evaluate student attitudes about science; however, this was still promising because past studies usually found decreased student agreement with expert opinions between the start and end of a course (Semsar et al., 2011). Comparing undergraduate student dispositions about the activity itself between 2020/2021 and 2023/2024 revealed that later improvements to the activity seem to have improved responses overall.

5.2 Introduction

Active learning is an important pedagogy involving engaging students by using methods like group discussions, problem-solving, and investigations like inquiry-based learning to promote higher-order learning (Demaria et al., 2019; Lazonder & Harmsen, 2016). With inquiry-based learning, students apply their learning in real-world scenarios and experiments, enabling them to practice academic skills and create links between classroom learning and real-world applications (Spronken-Smith & Walker, 2010). Use of active-learning and inquiry-based instructional methods has been shown to promote student engagement and enhance learning, including improved knowledge retention and skill development (Demaria et al., 2019; Freeman et al., 2014; Lazonder & Harmsen, 2016). Active learning methods encourage deeper engagement, experimentation, and increased communication of ideas by students (Pedaste et al., 2015). Students engaged in active learning activities tend to perform better on examinations and are less likely to fail compared to those taught in standard lecture style (Freeman et al., 2014). Inquiry-based and active learning approaches are beneficial to students studying biology, helping them to develop mastery of concepts, problem-solving skills, critical thinking, and metacognitive abilities that will help them to become more successful scientists (Mutlu,

2020; Nunaki et al., 2019; Santana-Vega et al., 2020). However, inquiry-based learning can be difficult to implement successfully, due to the challenge of providing students with both freedom to explore their own solutions and sufficient guidance to encourage productive learning (Hmelo-Silver et al., 2007; Justice et al., 2009; Lazonder & Harmsen, 2016). Indeed, many laboratory classroom activities implemented in introductory Biology courses are procedural ("cookbook") in nature, providing few opportunities for students to practice higher-order cognitive skills (Wood, 2009). Fortunately, with time and effort it is possible to successfully incorporate inquiry-based learning into undergraduate Biology classes (Justice et al., 2009). Inquiry-based learning can be implemented in lecture courses, for example by using case studies, or as hands-on problem-solving activities in laboratory and field courses (Bergin et al., 2018; Kricsfalusy et al., 2018; Yoo & Park, 2015).

An important set of concepts for Biology undergraduates to master is the thermodynamic principles underlying how and why different types of organisms respond to temperature in different ways. Temperature affects all aspects of how biological systems work, with far-reaching implications for organism physiology, behavior, and fitness (Brown et al., 2004; Stanga et al., 2023). Mastering these concepts is thus critical for students studying biological sciences, whether they intend to pursue a career in ecology, molecular biology, microbiology, biomedical science, veterinary science, or medicine. Biology is multidisciplinary and relies on concepts and skills derived from mathematics, chemistry, and physics, so it is important to help students develop a deep appreciation for the mechanistic underpinnings of biological processes and the connections between biology and other STEM disciplines. However, students commonly

have fundamental misconceptions about core concepts such as how temperature affects chemical reactions or about energetic costs associated with maintaining thermal homeostasis (Bretz & Linenberger, 2012; Halim et al., 2018; Stanga et al., 2023), and they may underappreciate the importance of concepts and skills derived from other disciplines (e.g., mathematics). Inquiry-based laboratory activities can be designed to help students to confront misconceptions and develop a multidisciplinary skill set.

One way to achieve multidisciplinary learning outcomes and guide students to developing important scientific skills is to teach students about the Metabolic Theory of Ecology (MTE), which uses principles from mathematics, physics, chemistry, and biology to explain how temperature-dependent metabolic processes scale from the molecular and cellular levels up to population, community, and ecosystem levels (Brown et al., 2004). To explore MTE with high school science students and undergraduate biology students, and to achieve and assess specific learning outcomes covered in the BS in Biological Sciences program offered at Oakland University (Table 5.1), we created a laboratory activity examining chemical and physical drivers of temperature-induced metabolic responses in ectotherms (frogs) and endotherms (humans). The intended learning outcomes of these activities included: (1) knowledge related to thermal biology, thermodynamics, and how processes at one level of organization scale up to higher levels; (2) skills such as collecting accurate measurements and developing hypothesis statements; and (3) dispositions such as appreciating the inherent value of discovery and the importance of a broad knowledge of biology.

We designed three learning activities to address interrelated questions (Fig. 5.1) about organism responses to temperature (Biology), temperature dependence of chemical

reactions (Chemistry), or the production and conservation of heat energy (Physics). Each activity includes two experimental investigations and a series of discussion questions. We developed two alternative lesson plans based on these experiments, aimed at students at different levels. One version was designed for high school students in the Project Upward Bound (PUB) Summer Academy, in which students are divided into groups studying biology, chemistry, or physics. These students were (1) introduced to the overall lesson as a whole class (including a brief thermodynamics demonstration) and asked to develop and evaluate hypotheses related to their respective discipline, (2) divided into discipline-specific groups to conduct two guided experiments per group, and (3) brought back together to discuss connections between the observations made by each group and how they inform our understanding of big-picture questions about thermodynamics and thermal biology. Students were guided through this activity and associated lower-level (arithmetic and algebra) mathematics problems with the assistance of researchers and high school program mentors. The second version was designed for undergraduates enrolled in Introductory Biology Lab (BIO 1201) at Oakland University. These students worked more independently than the PUB students, conducting chemistry and biology experiments within a single lab period under the supervision of a single graduate teaching assistant. BIO 1201 students worked in groups to develop and evaluate hypotheses, complete experiments, and analyze data including the application of higher-level mathematics skills (exponents, logarithms, and linear regression).

Students were provided with opportunities for freedom of inquiry with their open-ended hypothesis development. Undergraduates work in small groups and are given assistance as needed by their teaching assistant throughout the activity and the data

analysis but are encouraged to problem-solve on their own with their fellow classmates. High school students are given extra guidance with a higher teacher:student ratio for hands-on procedures. High school students also complete data analysis as a class so that complex concepts are easier to address. Following the experiments, students are given follow-up lectures to help provide guidance about the concepts addressed by the activity. While we do not have data on the content that these activities replaced in either program, we nevertheless sought to create successful inquiry-based learning activities that engaged students and encouraged higher-order thinking.

5.3 Activity descriptions

Students were first given an introductory lecture defining key MTE terms and concepts, as well as a tutorial on how to create an effective hypothesis. Students were told that a good hypothesis should be described by the acronym ERUPT, meaning the statement should be explanatory, relevant, understandable, plausible, and testable (Table 5.2). The students were then given written instructions and directed to complete several experiments exploring temperature effects on ectotherm (frog) and endotherm (human) metabolisms and use statistical analysis to interpret the results. Undergraduate students completed three experiments, focusing on chemistry, ectotherm biology, and endotherm biology. High school students complete one of three sets of experiments, focusing on either physics, chemistry, or biology (ectotherm and endotherm biology). Students were given a series of questions to answer using calculations to interpret the results from their given experiment. For a detailed activity description and list of questions students are given, see the activity worksheet (5.7 Material availability). The group as a whole was then brought back together to discuss their results and explore the interconnectivity of the

different activities (Fig. 5.1), with high schoolers collaborating with the other groups to answer a series of questions relating the different activities.

5.3.1 Pre- and post-activity class discussions

The general goal of both versions of this activity (BIO 1201 and PUB) was for students to explore how different metabolic responses to temperature in animals with "cold-blooded" (ectothermic) or "warm-blooded" (endothermic) thermal strategies can be predicted based on (1) the temperature dependence of chemical reactions (ectotherm strategy) and (2) the energetic costs of maintaining a constant body temperature (endotherm strategy). Prior to conducting experiments, students are introduced to (1) key terminology (e.g., "metabolism", "homeothermic", "ectothermic", and "endothermic"), (2) key concepts (e.g., heat as a type of kinetic energy), and (3) the experimental setups they will use (e.g., the reaction vessel for measuring H2O2 decomposition, breath rate as a proxy for frog metabolism, and the cold pressor test for measuring human responses to cold exposure). As part of this activity, students develop hypotheses individually and in small groups to predict key experimental outcomes. Students then evaluate each other's hypothesis statements using the ERUPT rubric (described below).

After conducting experiments, students evaluate their predictions in light of the experimental results and answer a series of questions connecting the experiments in each discipline (e.g., Biology) to experiments related to the other two disciplines (e.g., Physics & Chemistry). Towards the end of the class period, the instructor brings everyone back together for a general discussion of the experimental results, including a brief follow up lecture covering key thermal biology and chemistry concepts related to interpreting the results.

5.3.2 Evaluating hypothesis statements with the ERUPT rubric

One particularly common flaw in hypothesis statements from students, textbooks, and teachers is that the writer confuses a hypothesis, which is an explanation of a pattern or phenomenon, with a prediction (Strode, 2015). Hypotheses can be generalizing, explaining a general pattern observed, or explanatory, providing a direct explanation of the pattern (McComas, 2002; Sonleitner, 1989; Strode, 2015). Predictions can then be generated from these explanations. We developed the ERUPT rubric to address this and other common issues that arise with creating a well-developed hypothesis statement. ERUPT is an acronym for five important components of a hypothesis: Explanatory, Relevant, Understandable, Plausible, and Testable. Students were told that a proper hypothesis statement should satisfy all five categories. It should be explanatory, answering a "how" or "why" question; it should be relevant to the original scientific question; it should be understandable to a non-specialist; it should be plausible based on known facts and logical reasoning; and it should be testable, generating operational and directional predictions. Hypothesis statements were gathered and scored using the rubric described in Table 5.2.

5.3.3 Physics Experiment #1 (PUB & BIO 1201): Energetic cost of heating water

In this experiment, students conduct a simple thermodynamics experiment to explore how expensive it is for homeotherms to raise or maintain their body temperatures, by testing how much kinetic energy it takes to raise the temperature of water (which has a similar specific heat capacity to an animal's body) compared to the same mass of copper BB's. Prior to conducting this experiment, students develop hypotheses to predict the relative effort needed to heat water versus copper. Students then

measure 300 g copper BB's into a plastic container and use a temperature probe to record the internal starting temperature of the copper. We used a Traceable thermometer probe, but any digital thermometer with a metal probe and precision of 0.1 °C should work just fine. A volunteer is then instructed to vigorously shake the container for a minute, after which the internal temperature of the BB's is recorded again. Next, students place 300 g (300 mL) water into a metal thermos and record the water temperature. The volunteer is then instructed to shake the thermos vigorously for one minute, after which the temperature is recorded again. Students then compare results from the two demonstrations. Because water has a much higher specific heat capacity than copper (Chekhovskoĭ et al., 2002; Manyà et al., 2011), its temperature change should be much lower. It therefore takes ~10.7 times more mechanical work to increase the temperature of water by the same amount as the copper BB's.

5.3.4 Physics Experiment #2 (PUB): Mechanical equivalence of heat energy

In the second physics experiment, students use a homemade Joule apparatus (Fig. 5.2) to measure how energetically expensive it is for a 3 kg mammal (e.g., an arctic hare) to increase its body temperature by 1 °C, relative to the energy required to maintain physical activity (e.g., hopping 1 m high). A Joule apparatus is a historically significant experimental device developed by James Joule, that converts gravitational potential energy into measurable heat energy (Joule, 1850). A Joule apparatus uses a system of pulleys and gears to connect one or more weights to a paddle that spins inside an insulated container of water. The 9 kg weights are dropped from a 1 m height, beginning with gravitational potential energy equal to the kinetic energy required for a 3 kg hare to complete three 1 m jumps.

Before conducting this experiment, students use standard thermodynamics equations to calculate how many weight drops it should take to raise the temperature of the water 0.1 °C (see 5.7 Material availability). For this experiment, we constructed a Joule apparatus using an ice cream maker, a series of pulleys, braided fishing line, and nylon twine that can be attached to a 9 kg weight (4 L jug containing sand & water) on either end of the apparatus. It was designed to be clamped across a 1.5 m wide lab bench or table and is operated by alternately attaching and dropping a weight from each end of the apparatus. This system was inexpensive to construct, though it required basic carpentry tools and skills including a jig saw and router. To avoid impeding paddle motion within the internal canister of the ice cream maker, temperature is monitored by inserting the probe into an insulated hole immediately external to the metal canister. The high-precision (0.01 C) temperature probe was necessary to detect the magnitude of observed temperature changes.

To conduct the experiment, students measure 3 L of water into the insulated canister. They then measure changes in the temperature of the water as they alternate dropping weights 1 m to the ground on each end of the apparatus, causing the paddle to spin. Students continued the experiment until they completed at least 32 drops, recording the water temperature every 4 drops. Students then compared their measurement to their predicted number of drops to raise the water temperature by 0.1 °C, which should be about 14.2 drops, not accounting for energy loss due to friction. In practice, our homemade Joule apparatus proved to be approximately 80% efficient. This means it took about 20% more effort to achieve a 0.1 °C increase than predicted by theory.

5.3.5 Chemistry Experiment #1 (PUB & BIO 1201): Temperature dependence of H_2O_2 decomposition

In this experiment, students simulate how ectotherm metabolisms respond to temperature by measuring the effect of temperature on an enzyme-assisted chemical reaction. The focal reaction is catalase-assisted hydrogen peroxide decomposition into water and oxygen, which is widely used in educational chemistry demonstrations (Cybulskis et al., 2016). Before conducting this experiment, students develop hypothesis statements to predict how the rate of a chemical reaction will respond to changes in temperature. Students measure rates of hydrogen peroxide decomposition at various temperatures using a reaction apparatus comprising an air-tight glass bottle, air line tubing, a thin metal tube for injecting 3% H_2O_2 solution, and an inverted column made from plastic test tubes (Fig. 5.3). To set up this experiment, students first measure 1 g freeze-dried beef liver powder and 200 mL phosphate-buffered saline and add both to the glass bottle. The bottle cap is modified to contain two tube adapters, one connected to a metal tube for injecting 3% H_2O_2 with a 5 mL syringe and the other connected to airline tubing connected to the inverted column. The cap also contains the probe for an aquarium thermometer for continuous monitoring of the internal reaction temperature. The column is suspended upside down in a deli cup filled with water, with a 60 mL gas syringe attached to a valve at the top for sucking out air and raising water to the top of the column.

To achieve each target temperature, students place the reaction bottle into a hot-water bath or ice-water and monitor the change in internal temperature as they swirl the liver/saline slurry. Once they have achieved the desired test temperature, students fill the

5 mL syringe with hydrogen peroxide and attach it to the reaction bottle cap, and then use the 60 mL gas syringe to suck water to the top of the gas-measurement tube. After recording the start temperature, students administer 5 mL of 3% H_2O_2 into the slurry and record the time it takes for the oxygen produced from the reaction to lower the waterline in the gas-measurement tube by 40 mL. Each group repeats this procedure for six temperatures between 10 and 35 °C (approximately 10, 15, 20, 25, 30, & 35 °C). One important learning outcome from this experiment is that the enzyme responsible for catalyzing this reaction, catalase, is not used up in the reaction. Thus, the source of the enzyme, beef liver, can be reused for each trial.

Based on collision theory, the expected experimental results are that chemical reactions at higher temperatures should occur faster, resulting in shorter times for the gas-measurement tube to fill with oxygen. For the PUB activity, students discussed the general patterns they observed and how they related to this prediction. For the BIO 1201 activity, students uploaded their class data to a custom-designed R Shiny application and used linear regression fit their natural log transformed data to the Arrhenius equation, allowing them to estimate the activation energy for hydrogen peroxide decomposition.

5.3.6 Chemistry Experiment #2 (PUB): Heat production by an exothermic reaction

In the second chemistry experiment, students explore how endotherms like humans respond to cold temperatures by converting chemical potential energy into body heat. Before conducting this experiment, students predict how much heat will be produced by an exothermic chemical reaction by using equations describing the difference in potential energy of the reactants and products of H_2O_2 decomposition. Students observe the heat produced by the catabolic H_2O_2 decomposition reaction as a

proxy for human body heat production. Prior to the experiment, Students use a similar setup to the first chemistry experiment, except that the oxygen produced from the reaction is allowed to escape the bottle (i.e., not connected to a column), and the bottle is placed in a neoprene sleeve to reduce heat loss to the outside environment. The students are instructed to swirl the bottle to homogenize the liver solution, and then add 5 mL of 3% H_2O_2. Students measure the temperature of the liver solution using the temperature probe every ten seconds for two minutes. Because hydrogen peroxide decomposition is an exothermic reaction, heat is released and the slurry raises in temperature over time. Assuming no heat loss occurs to the external environment, the temperature should raise by 0.5 °C, however some negligible heat loss does commonly occur despite the use of the neoprene sleeve.

Students then discuss the observed temperature change and compare their result to the theoretical prediction.

5.3.7 Biology Experiment #1 (PUB & BIO 1201): Temperature dependence of ectotherm breath rates

In the first biology experiment, students explore how ectotherms respond to differences in environmental temperature. This experiment involves measuring metabolic rates of ectotherms (Cuban tree frog, *Osteopilus septentrionalis*) across different temperatures using breath rate as a metabolic proxy. Any large frog species could be substituted for Cuban tree frogs, as long as their breath rate is slow enough to be observable with the naked eye. We have also successfully used American toads (*Anaxyrus americanus*) for this activity. Students observe frogs held in incubators set to different target temperatures and record their breath rate (number of buccal movements

per minute). Because ectotherm body temperatures change with environmental temperature, and chemical reaction rates increase with temperature (see above), MTE predicts that frogs held at higher temperatures will have faster metabolic rates and thus faster breath rates. When this pattern is not observed, it may be due to differences in frog masses (lower breath rate for larger frogs) or activity levels (higher breath rate for more-active frogs). An important prediction of MTE is that mass-specific metabolic rates scale with organism mass$^{-1/4}$, so smaller frogs will have faster breath rates at a given temperature than larger frogs.

To analyze the data, undergraduate students again upload their data to the R Shiny application to estimate the frogs' activation energy of metabolism. To account for frog mass effects, the application allows students to incorporate frog mass into the model. Alternatively, high school students discuss general patterns observed and how they compare to the qualitative predictions of their hypotheses and MTE.

5.3.8 Biology Experiment #2 (PUB & BIO 1201): Human response to cold-temperature exposure

In the second biology experiment, students examine the metabolic response of an endotherm (humans) to cold temperature exposure. In this experiment students measure heart rate as a metabolic proxy while conducting a version of the cold pressor test with a student volunteer. The cold pressor test is a widely-used tool to assess patient cardiovascular health to evaluate blood pressure or as a measure of pain tolerance (Lamotte et al., 2021; Mitchell et al., 2004; Velasco et al., 1997). To conduct the experiment, a human subject first sits in a relaxed posture for at least two minutes. Next, a second student measures their resting heart rate every 15 seconds using a pulse

oximeter (e.g. Zacurate Model 500DL) for one minute, after which the subject's body temperature is measured using an infrared thermometer (e.g Meijer instant forehead thermometer Model NT13) The subject then places their hand and/or forearm into an insulated container of cold (0 °C) water for 45 seconds (or until the subject decides they are finished). The second student continues recording heart rate every 15 seconds during cold exposure, and body temperature is recorded at the end of this period. After the subject removes their hand from the ice water, body temperature is measured again and heart rate recordings continue every 15 seconds for two minutes, followed by a final body temperature measurement is recorded. We used ice-water for the original version of this activity (2018 – 2021), consistent with a classic cold pressor test. In 2022, we designed a modified version of this activity using a set volume (1 L) of ice water with the ice filtered out, allowing students to calculate the amount of heat energy lost to the cold water during the test by measuring water temperature at the beginning and end of the test (see 5.7 Material availability).

Because humans are endothermic and homeothermic, subjects should maintain a constant body temperature throughout the cold pressor test. This is achieved by elevating their metabolic rates (and thus heart rate) and thus the rate of exothermic chemical reactions in their bodies, thereby generating heat to replace the heat energy lost to the cold water. Heart rate should then gradually go back to normal "resting" heart rate after the subject no longer has their hand in cold water. Students have consistently reported constant (or nearly constant) body temperatures and a multiple-degree increase in water temperature during the test (for the version with ice-free water), consistent with theoretical expectations. However, heart rate results are typically variable from one

student to the next, providing an opportunity for students to discuss potential reasons for the unexpected results. For instance, it is a common to observe an increase in heart rate immediately prior to the start of the test, followed by a slight decrease during cold exposure and (often) a further decrease during the post-exposure period. These students often report that they felt nervous prior to the start of cold exposure but relaxed once it began, having discovered that the cold pressor test was not as bad as expected.

For analysis, students calculate average, minimum, and maximum heart rate at each time point of the experiment. They then use Microsoft Excel to make a time-series graph to visualize how heart rates changed through time (mentors help with Excel during the PUB activity). Students then join a class discussion of how the human metabolic response to cold temperature compares and contrasts to the frog response.

5.4 Learning outcomes assessment

To assess knowledge outcomes, BIO 1201 students were given a post-activity quiz featuring higher and lower order quiz questions to assess their understanding of key concepts from the activity. To assess skills outcomes, students are quizzed on their ability to conduct graphical analysis. Students are also instructed to construct hypotheses about this lab activity, as well as another week later for another lab activity. These statements are collected and assessed using the ERUPT rubric (Table 5.2). To evaluate progress in developing an important hands-on skill, BIO 1201 students were also graded on their ability to accurately measure 10 mL water using a graduated cylinder.

5.5 Student perceptions of the activity

Throughout the history of this activity's implementation, we have used post-activity surveys to assess student dispositions about the activity itself, evaluating student

opinions about pros and cons of the activity (Table 5.3). Overall, PUB (high school) students responded to the post-activity survey with mixed results. 81.6% of students felt prepared to conduct the activity, and 67.3% of students indicated that they learned something. Furthermore, 69.4% of students were able to identify real-world connections to the concepts they learned. However, 75.5% of students indicated that something was unclear, and 71.4% of students provided suggestions for improvement.

We have since made several improvements to the activity in response to student and teacher feedback. We simplified the mathematical calculations expected of the PUB students, because it was not always possible for the teachers to introduce exponents and logarithms prior to the activity. In response to less enthusiasm from physics-focused PUB students, we added Physics Experiment #2 to the activity and had chemistry students take on Chemistry Experiment #2, which was originally the physics activity focused on heat production from a chemical reaction. We also simplified Biology Experiment #1 by shifting to just two target temperatures to compare frogs' responses to "cold" and "warm" temperatures. Due to time constraint issues, we divided the BIO 1201 activity into 2 weeks, with one week focusing on ectotherms (Chemistry Experiments #1 and #2, Biology Experiment #1), and a second week focusing on endotherms (Physics Experiment #1, Biology Experiment #2).

For BIO 1201 from 2020 to 2021 (N = 199), survey responses were mixed. Overall, 60.7% of respondents indicated they felt at least somewhat prepared to complete the activity prior to starting it, and 77.2% felt the materials and instructions were at least somewhat clearly communicated. For whether learning outcomes had been achieved, responses scored an average of 3.57 out of 5 (i.e. "somewhat"). After the later

improvements to the activity (after splitting into a 2 week activity), respondents from 2023 to 2024 (N = 89) indicated that 90.9% felt prepared to complete the activity, and 94.4% indicated the materials and instructions were clearly communicated. For whether learning outcomes had been achieved, responses scored an average of 4.12 out of 5 (i.e. "mostly"). Regardless of the year, respondents typically indicated that they enjoyed Biology Experiments #1 and #2 the most.

5.6 Student perceptions of science

To assess whether and how this activity altered undergraduate student attitudes about science in general, we implemented the Colorado Learning Attitudes about Science Survey–Biology (CLASS–bio; Semsar et al., 2011) before and after completion of the Thermal Metabolism activity. This survey is used to evaluate student views about science and how well they correlate with expert responses (Semsar et al., 2011). The survey is then re-administered following the activity to gauge if student attitudes about science changed after performing the laboratory activity. Questions are grouped into the following categories: Real-world Connection, Problem-solving difficulty, Effort, Conceptual Connections/Memorization, Problem-solving Strategies, and Reasoning. I analyzed the survey data, comparing before and after using Wilcox signed rank tests and t-tests in Program R (R Core Team, 2020). Undergraduate student responses to the CLASS–bio did not change significantly for any of the question categories. Normally, the CLASS–bio is administered at the beginning and ending of a semester (Semsar et al., 2011), so it is perhaps unlikely to expect significant changes after the implementation of just one learning activity. However, typical results of the survey show decreases in scores, indicating increased novice-like attitudes compared to initial survey

implementation (Semsar et al., 2011). Thus the lack of changes seen in the present study might suggest that the Thermal Metabolism activity did not negatively affect student dispositions. Of note however, is that values for all categories remained slightly negative, meaning while students showed no improvement in dispositions, they had an overall negative correlation with typical views held by professional scientists. The only exception to this rule was for Question 13, which did not fit into any of the categories, in which students tended to agree with scientific consensus. The question was, “It is important for the government to approve new scientific ideas before they can be widely accepted”.

5.7 Conclusions

These educational activities successfully used concepts from physics, chemistry, and biology to teach students about thermal metabolic processes. Students gain a basic understanding of thermodynamics principles, how chemical reactions respond to and produce changes in temperature, and how ectotherms and endotherms have opposite responses to temperature. This is vital in biological education because energy and chemical reactions are the foundations of biological processes (Stanga et al., 2023). Students also learn about MTE, an important ecological theory that explains metabolic responses to temperature across organismal scales(Brown et al., 2004). Furthermore, MTE is an opportune framework to teach students about the multidisciplinary nature of science because it combines thermodynamics concepts from physics, chemistry, and biology (Brown et al., 2004).

Learning these concepts can be challenging (Stanga et al., 2023), but inquiry-based active learning can be a useful way to teach these biological concepts by keeping

students engaged in the material while also increasing student performance (Demaria et al., 2019; Freeman et al., 2014; Lazonder & Harmsen, 2016). These activities successfully use inquiry-based techniques and active, hands-on learning to educate both high schoolers and undergraduates about metabolic processes, encompassing the underlying physics, chemistry, and biology processes involved.

Although high school students indicated that they had challenges conducting these activities, there were still many students who indicated that they had successfully learned something about these complex processes. While undergraduate students did not improve in terms of dispositions after completing the activity, they did not regress, as is commonly seen in introductory biology courses (Semsar et al., 2011). Therefore, these activities could potentially be seen as more successful than the typical introductory biology activity in encouraging expert-like opinions in undergraduate students.

5.8 Material availability

Presentation materials, worksheets, and quizzes can be found at https://github.com/huntercraig248/Thermal-Metabolism-and-Phylogenetics

5.9 Chapter 5 tables

Table 5.1: Constructive alignment table for the BIO 1201 Thermal Metabolism lab activity, including learning outcomes from the B.S. in Biological Sciences program that are fulfilled by this lab activity and our methods of assessment.

Category	Program Learning Outcome (Biology BS)	Activity	Summative assessment
Knowledge	Apply thermodynamics principles to biological processes	• Lectures • Chemistry & biology experiments • Follow-up discussion	• Knowledge-based quiz questions • Individual hypothesis statements (graded)
Skills	Use mathematical models and statistics to solve biological problems	• Data analysis (R Shiny app)	• Graphical analysis quiz question
	Construct hypotheses based on observations	• Hypothesis development activity	• Individual hypothesis statements (graded)
	Collect accurate weights and volumes	• Chemistry experiment	• Volumetric measurements
Dispositions	Consider new approaches to solving problems	• Chemistry & biology experiments • Problem sets & discussion questions (worksheets) • Pre- and post-activity class discussions	• CLASS–bio survey
	Appreciate the importance of biological knowledge and the inherent value of discovery		
	Change mind when presented with new evidence		

Table 5.2: The ERUPT hypothesis grading rubric used to evaluate students' hypothesis statements. Hypothesis statements were graded by H. Craig and assigned either 0, 1, or 2 points for each of the five categories based on the rubric explanations.

Category	Low (0 pt)	Mid (1 pt)	High (2 pt)
Explanatory	Does not offer an explanation; Does not provide necessary logical links	Vaguely answers a question; Some logical leaps	Answers a how or why question; Provides logical links; Is logically consistent
Relevant	Does not answer the specified research question	Answers a question similar or related to the specified question	Answers the specified research question
Understandable	Does not make sense; uses excessive jargon	Vaguely communicates idea; Uses jargon sparingly	Clearly communicates idea; Avoids jargon
Plausible	Unreasonable; Illogical based on available information	Somewhat reasonable without considering some available information	Reasonable based on available information
Testable	Hypothesis generates no testable prediction (e.g., supernatural explanation), or stated prediction does not follow from hypothesis.	Vaguely testable prediction (e.g. difficult to falsify)	Generates at least one prediction that is specific enough to be readily testable (e.g., directional or quantitative)

Table 5.3: Google form survey administered to students after the Thermal Metabolism activity. Questions 1 and 2 were scored on a scale of 1–5 with 1 meaning “not at all prepared” and 5 meaning “very prepared”. Questions 3–6 were also scored on a scale of 1–5, with 1 meaning “not at all”, 2 meaning “poorly”, 3 meaning “somewhat, 4 meaning “mostly”, and 5 meaning “entirely”. Questions 7–10 were open-ended short answer responses.

Q1	To what extent did you feel prepared to complete (or teach) the Thermal Metabolism Lab Activity, prior to starting it?
Q2	To what extent were the lab materials and instructions clearly communicated to you (or your students)?
Q3	To what extent did this lab activity help you (or your students) to achieve each of the following learning outcomes? [LO1: "Collect accurate measurements of body temperature, heart rate, respiration rate, and chemical reaction rate."]
Q4	To what extent did this lab activity help you (or your students) to achieve each of the following learning outcomes? [LO2: "Analyze data to estimate parameters for a mathematical model describing temperature dependence of a chemical reaction or of whole-body metabolism."]
Q5	To what extent did this lab activity help you (or your students) to achieve each of the following learning outcomes? [LO3: "Explain why metabolic chemical reactions are temperature-dependent, and how enzymes increase chemical reaction rates."]
Q6	To what extent did this lab activity help you (or your students) to achieve each of the following learning outcomes? [LO4: "Use thermodynamics and evolutionary biology to explain how and why frogs and humans have different metabolic responses to external temperature."]
Q7	Name one new thing you (or your students) learned about Biology and/or the practice of Science, through participation in this lab activity.
Q8	Name one part of this lab activity that was LEAST clear to you (or your students).
Q9	Name one part of this lab activity that you liked the most.
Q10	Name one thing you would suggest changing to make this activity better.

5.10 Chapter 5 figures

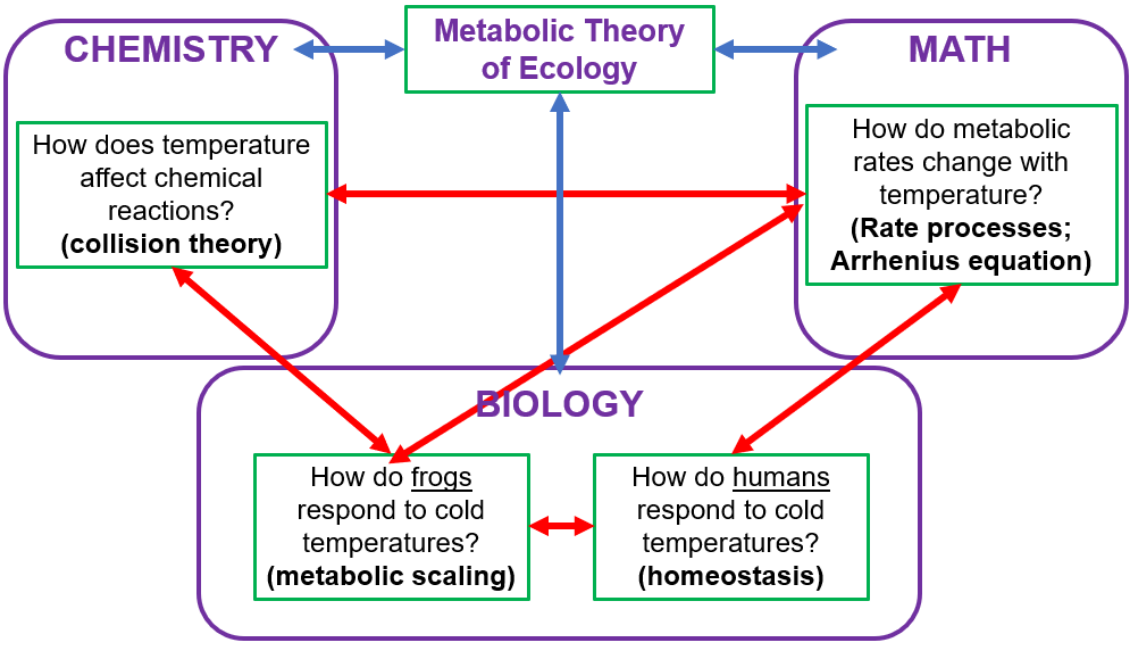

Figure 5.1: Connections explored in the Thermal Metabolism activity, using a series of questions that use a combination of mathematics, chemistry, and biology and relating them back to the Metabolic Theory of Ecology (Brown et al., 2004).

Figure 5.2: Dr. Raffel demonstrates to PUB students how to use the custom-built Joule apparatus to convert mechanical energy into heat energy. Designed to drop two 3 kg weights in succession to spin a paddle in the center bucket filled with water to raise the temperature of the water my adding mechanical energy to the system. Photo credit: Ava McDowell.

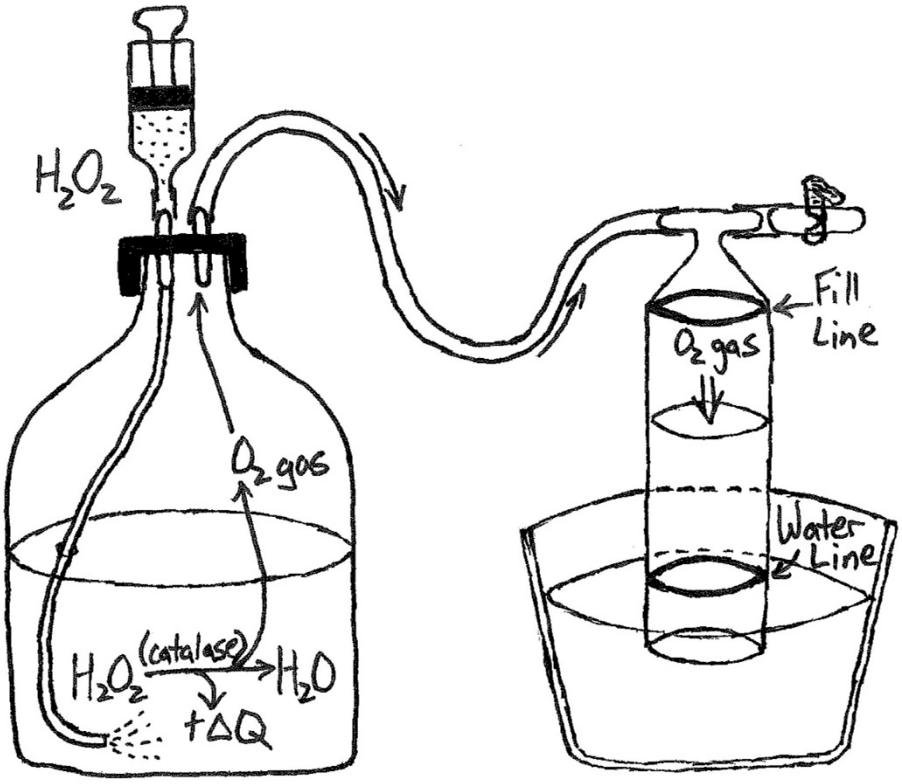

Figure 5.3: Illustration of the hydrogen peroxide decomposition chemical reaction closed-air system. A bottle is filled with saline and beef liver (to supply catalase), and water is pulled by negative pressure into the column on the right. Hydrogen peroxide (H_2O_2) is then added to the system to induce the reaction and oxygen is produced, which causes the water in the right column to go down by positive pressure. Illustration by T. R. Raffel.

CHAPTER SIX

COMPARING ONLINE AND FACE-TO-FACE LEARNING ENVIRONMENTS IN AN INTRODUCTORY BIOLOGY LABORATORY

6.1 Abstract

While there is debate over the effectiveness of online learning in the sciences, some evidence suggests that students can achieve similar learning outcomes compared with face-to-face learning, especially with regard to student grades on assignments and classes (Bettinger et al., 2017; Pei & Wu, 2019; Regmi & Jones, 2020; Richmond et al., 2017). However, some studies show the opposite results with regard to specific learning outcomes, especially for skill development, although there is a lack of research on the subject (Bettinger et al., 2017; Dutton et al., 2001; Richmond et al., 2017). We created two interactive online laboratory activities, based on pre-existing face-to-face activities, and conducted a direct comparison of the two versions to assess student knowledge, skill, and disposition learning outcomes. We hypothesized that students who conducted the activities face-to-face would demonstrate better scores, more developed skills, and improved dispositions compared to the online students. Our results indicated that students performed similarly well with regard to lower order questions and dispositions, however higher order, skill-based outcomes were generally better for face-to-face students. We conclude that the online activity was generally successful in helping students to achieve similar learning outcomes. However, for achieving skill-based learning outcomes, there is still value in participating in face-to-face classes with ample opportunities for student interactions with instructors.

6.2 Introduction

There has been much debate about the effectiveness of virtual science education, especially with regard to the teaching styles used and impacts on student dispositions (Bettinger et al., 2017; Regmi & Jones, 2020; Richmond et al., 2017). Some research suggests that student grades and retainment are lower for online courses(Bettinger et al., 2017; Dutton et al., 2001), while other research suggests online students achieve similar grades and are better off with online learning because of the enhanced flexibility and accessibility (Regmi & Jones, 2020; Richmond et al., 2017). Educators may be particularly skeptical that online delivery is effective for helping students to achieve the kinds of skill-based learning outcomes associated with laboratory courses in the sciences, especially due to the lack of research on the subject (Richmond et al., 2017) . Additionally, there is concern that students are not given appropriate peer-to-peer social learning opportunities in an online setting (Xu & Jaggars, 2013), although it is possible to achieve this by implementing online forums or group-discussions(Ghosh & Kleinberg, 2013; Kipling et al., 2023). However, there is growing evidence that online classes can achieve similar learning outcomes to face-to-face classes. In a meta-analysis of medical undergraduate education studies, Pei & Wu (2019) found that online students performed equally or better in assessments of both knowledge and skills when compared to face-to-face students. Darrah et al. (2014) found that virtual inquiry-based physics labs resulted in similar increases in test performance when compared to face-to-face labs. Lei et al. (2021) demonstrated that online nursing students performed better on mid-semester tests than face-to-face students, although differences in final exam grades were negligible.

Krüger et al. (2022) found that online science students learned more than their face-to-face counterparts, but that those who performed in-person experiments developed more interest in the subjects. Similarly, Visuddho et al. (2023) found that medical students had more knowledge and practical skill gains than face-to-face students, however student attitudes and satisfaction suffered. Despite this, there is contradicting evidence that student dispositions about science can also be improved with online courses (Perera et al., 2017).It has also been suggested that the best teaching practice is to use blended learning, combining benefits of both e-learning and face-to-face learning (Bernard et al., 2014; Cook et al., 2011; De Jong et al., 2013; Dziuban et al., 2018). For example, supplementing education with online simulations appears to increase knowledge, skills, and behaviors in health profession education (Cook et al., 2011; Visuddho et al., 2023). However, questions remain about whether students can develop important practical skills in an online setting and more research is needed on the subject (Richmond et al., 2017). Furthermore, there is contradicting evidence about student disposition learning outcomes, where some studies suggest student enthusiasm suffers in an online setting (Krüger et al., 2022; Visuddho et al., 2023), and other suggest student attitudes can be improved using online learning (Perera et al., 2017). Ultimately, more research is needed to evaluate student satisfaction in online learning environments (Lanzieri et al., 2023).

I conducted a replicated experimental comparison of face-to-face versus online versions of two laboratory activities in the undergraduate course, Introduction to Biology Laboratory (BIO 1201), to evaluate how effectively students achieved intended learning outcomes. I created asynchronous online versions of two laboratory activities based on existing in-person lab activities: (1) the Thermal Metabolism activity (hereafter referred

to as TM) described in Chapter 5 and (2) a Phylogenetics activity examining core evolutionary concepts and skills by using morphological and genetic characteristics of invertebrates to evaluate hypotheses about their evolutionary history. For our interactive online activity, we created "quizzes" in Moodle, a widely-used Learning Management System that can improve student performance and engagement, especially in STEM disciplines (Gamage et al., 2022; Moodle Project, 2023). To ensure all students received an approximately equal educational experience in a blended course format, I used a within-subject experimental design in which each lab section (and thus each student) was randomly assigned to complete one of two lab activities online and the other in person. I evaluated differences in student performance on assessments designed to evaluate learning for intended outcomes related to knowledge (e.g., thermodynamics concepts), skills (e.g., hypothesis development), and dispositions (e.g., attitudes about science) covered during the activities.

The intended learning outcomes of these activities included the ability to apply basic thermodynamics concepts to biological processes, the ability to collect accurate measurements, the ability to develop good hypothesis statements, and increased positive attitudes and enthusiasm about science (Chapter 5, Table 5.1). We hypothesized that students would demonstrate improved scores on conceptual quiz questions and more positive attitudes about science after conducting face-to-face activities, because they would be more invested in their activities and would gain practical hands-on experience. We predicted this because face-to-face students demonstrate more situational interest in subjects and overall satisfaction than online students (Krüger et al., 2022; Parsons-Pollard et al., 2008), and because students tend to prefer traditional lectures over online subject

matter (Lai, 2021).. We also predicted positive effects of face-to-face instruction for outcomes involving hands on skills (e.g., measuring volumes), which they had less opportunity to practice during online activities. This is because students were not guaranteed to have the resources needed to conduct the experiments at home (e.g. thermometers, measuring cups, or a microwave for heating water). Alternatively, we hypothesized that students might perform better on assessments of knowledge-based learning outcomes after conducting online activities, because these activities provide more consistent presentation of relevant information than is possible for in person delivery of material by teaching assistants with varied levels of experience. It was also possible that student learning outcomes would be roughly the same for in person and online instruction, because students in both groups were given access to the same information, and research suggests online students can achieve similarly or even better than face-to-face students in terms of grades, attitudes, and higher order skills, depending on the context of the study (Johnson, 2002; Jones & Long, Vena M, 2013; Krüger et al., 2022; Pei & Wu, 2019; Regmi & Jones, 2020; Richmond et al., 2017; Schulman & Sims, 1999; Sitzmann et al., 2006).

6.3 Methods

6.3.1 Overall experimental design

The eleven class sections of BIO 1201 were randomly divided into two treatments: Online TM and Face-to-Face Phylogenetics, or Face-to-Face TM and Online Phylogenetics. I used performance on assignments and quizzes as summative assessments from the laboratory activities to determine the effectiveness of the two teaching strategies. For formative assessments on the TM lab, I used a Google form survey (see

Chapter 5) and the Colorado Learning Attitudes About Science Survey–Bio (CLASS–Biology; Semsar et al., 2011) to assess students' understanding and views about the activities, as well as their views about science in general.

Online modules featured videos, multiple choice questions, and fill-in-the-blank questions incorporated into a Moodle quiz format. There were 15 different instructors teaching 22 half-sections of the class (due to COVID–19 Pandemic protocols, each of the 11 listed sections of the course was split in half across two rooms with two different instructors. When the same instructor taught multiple sections their sections were assigned different treatments to control for any impacts of the individual instructor on student performance and attitudes. We received IRB approval to conduct this project (Appendix C), and students were invited to sign a consent form (Appendix D) agreeing to allow their assignments, grades, survey responses, and demographic information to be collected and used for this research project. In total, 143 students gave consent for their data to be collected for this study. Procedures were developed and implemented to protect the privacy and integrity of participant data, and data were de-identified prior to conducting analyses.

6.3.2 Thermal metabolism activity

For both the online and the face-to-face versions of the TM activity, students were given access to the same informational materials and questions to check understanding. Additionally, teaching assistants were given a script to follow to ensure they covered the same information during lecture that was provided in the online lectures. Whether they followed the script perfectly varied, but they reported that on average, they followed the script and lesson plan with a score of 2.5 out of a scale of 1 ("exactly") to 5 ("not at all").

Students were first given an introductory lecture covering basic MTE concepts and introduced to the ERUPT rubric for developing and evaluating scientific hypotheses (see Chapter 5). Face-to-face students were given a supplemental worksheet (see 6.6 Material availability) to fill out as they completed the lecture and experiments. Online students instead answered these questions interspersed between lecture videos and interactive online activities. Students practiced developing and evaluating scientific hypotheses, either as part of a small-group activity using a think-pair-share design (face-to-face activity) or as an interactive individual activity (online activity). The interactive online activity covered the same basic materials, but without the group component of the face-to-face activity. Next, students completed the primary lab activity by conducting a series of experiments to explore temperature dependence of chemical reactions, the similar temperature dependence of ectotherm (frog) metabolic rates, and the metabolic responses of humans to cold-temperature exposure. Face-to-face students conducted the hydrogen peroxide decomposition experiment, the frog breath rate experiment, and the cold-pressor test experiment all described in Chapter 5. Online students instead conducted a similar chemistry experiment using the bicarbonate reaction (described below), the same frog breath rate experiment using pre-recorded videos of frogs held at different temperatures, and the same cold-pressor test experiment using a pre-recorded video of a human subject undergoing the test.

Because it would be impractical for online students to use the complicated hydrogen peroxide decomposition experiment setup (described in Chapter 5), they were instead provided with a pre-recorded video of the reaction taking place under a cold and a warm temperature. They were then encouraged (but not required) to use Alka-Seltzer

tablets (provided to the students prior to the lab activity) to conduct a similar experiment (the bicarbonate reaction) at home. In this experiment, students were directed to measure out specific volumes of either boiling water (100 °C) or ice water (0 °C) and mix them to create water of a specific temperature. This was repeated with different volumes to produce a range of temperatures (8.5 – 40 °C). Students placed an Alka-Seltzer tablet into the water mixture and recorded the amount of time it took for the tablet to fully dissolve in the water. While this alternative experiment was not the same as the original hydrogen peroxide experiment, it still allowed students to practice measuring out liquids, recording the time of a reaction, and observing the temperature dependence of a simple chemical reaction. As is seen with hydrogen peroxide decomposition, the bicarbonate reaction speeds up with higher temperatures.

One week following the TM activity, students were given a quiz covering the conceptual material covered during this activity. Two weeks after the TM activity, students developed hypothesis statements for another lab activity focused on Baker's yeast fermentation (referred to as the Cellular Metabolism activity) that included overlapping concepts to the TM activity. The Cellular Metabolism activity involved students forming hypotheses and designing and running an experiment where they recorded the amount of oxygen produced by Baker's yeast fermentation in different experimental treatments. Overlapping concepts included hypothesis development, measuring precise quantities of liquids, and possibly temperature dependence of metabolism (depending on the students' experimental design choices). Hypothesis statements developed for both activities (TM and Cellular Metabolism) were recorded and graded using the standardized ERUPT rubric described in Chapter 5, evaluating each

hypothesis on a 10-point scale based on the extent to which it was explanatory, relevant, understandable, plausible, and testable (0, 1, or 2 points per component).I personally evaluated each hypothesis statement using this rubric to remain consistent and relatively free of bias across all sections.

We implemented the CLASS–bio survey (Semsar et al., 2011) to assess students' dispositions and determine if they were at all influenced by participating in the TM activity. The survey was administered prior to the TM activity, and was then re-administered following the activity to gauge if student attitudes about science changed after performing either a virtual or a hands-on laboratory activity. Questions in the CLASS–bio survey are grouped into the following categories: Real-world Connection, Problem-solving difficulty, Effort, Conceptual Connections & Memorization, Problem-solving Strategies, and Reasoning (Semsar et al., 2011). I also used the post-TM lab Google form survey (see Chapter 5), to assess students' opinions and experiences with the TM activity.

6.3.3 Phylogenetics activity

In the Phylogenetics activity, students were given an introductory lecture defining key evolutionary terms and explanations of how to interpret cladograms. This lecture was interspersed with interactive questions to check student understanding of basic concepts. Face-to-face students were given a supplemental worksheet to complete during lecture (See 6.6 Material availability), while online students had breaks between recorded lecture videos to answer questions in the online Moodle module.

Students were then asked to identify morphological characteristics in eleven invertebrate species, using preserved specimens in the face-to-face activity or photos in

the online module. A demonstration was then given on how to use these morphological characteristics to create a simple cladogram using five of the eleven invertebrates. Finally, the students were instructed to create a cladogram with all eleven invertebrates, identifying all transitions and examples of analogous traits, to be turned in the following week. I personally scored every cladogram to remain consistent and relatively free of bias across all sections. Students were given full points if they developed a parsimonious cladogram based on the total number of transitions (20 points for 19 or fewer transitions with no mistakes). A single point was taken off for: each transition over 19, a missing or incorrectly placed transition, and each set of analogous traits not properly identified. The most parsimonious cladogram possible based on the species and traits listed in the assignment is shown in Fig. 6.1. Finally, students were given a quiz the following week to assess their understanding of basic concepts of evolution and phylogenetics.

6.3.4 Analysis

We used linear-mixed effects models or generalized linear binomial mixed-effects models, depending on the nature of the response variable, with random effects of half-class section. To emphasize any trends seen in the results, and because the CLASS–Bio data were neither ordinal nor normal, we used bootstrapping for the linear mixed-effects models using the lmeresampler package (Loy, n.d.; Bates et al., 2015; J. Fox & S. Weisberg, 2019; R Core Team, 2020) to generate confidence intervals to determine whether online or face-to-face students performed better on assessments of learning outcomes. To test for basic differences in the CLASS–Bio before and after conducting the TM activity, we used t-tests. We used linear models (for numeric responses) and

generalized linear binomial models (for positive or negative responses) to evaluate data from the post-TM lab Google form survey

6.4 Results

For the TM activity, there were no statistically significant differences between face-to-face and online delivery for the quality of hypotheses developed by students one week following the TM activity (Table 6.1, Fig. 6.2). However, in the second week following the TM activity, students with face-to-face delivery received higher scores for developing explanatory hypotheses, whereas students who received online delivery scored higher for developing plausible and testable hypotheses (Table 6.2, Fig. 6.3). Students performed similarly on quizzes across treatments (Table 6.3, Fig. 6.4-6.5). Face-to-face students were also less accurate than online students in volumetric measurements following the TM activity, contrary to my prediction (Fig. 6.6).

For the CLASS–bio (Semsar et al., 2011) survey instrument, students responded equally favorably (i.e. agreed with expert consensus) before and after conducting the TM activity (Table 6.4). One question category of interest is the students' problem-solving difficulty, or how difficult they perceive scientific problems to be, relative to established scientific professionals' perceptions of problems. Students who conducted the TM activity in an online setting showed an increase in their perception of problem-solving difficulty after completing the survey, in contrast to no change in this perception for students who conducted the activities in a face-to-face setting (Fig. 6.7). Otherwise, both groups responded similarly to all other CLASS–bio question categories before and after the TM activity, and indicating that there were no differences in the effects of face-to-face versus online delivery on student attitudes about science and biology (Fig. 6.7).

While face-to-face students indicated on the post-TM Google form survey (see Chapter 5, Table 5.3) that they felt more prepared going into the activities (question 1; $F_{1,70} = 5.82$, $p = 0.02$), a higher percentage of respondents indicated that some aspects of the activities were unclear relative to online students (100% versus 80%; question 8; $F_{1,71} = 5.56$, $p = 0.02$). Students with face-to-face and online delivery gave similar responses for whether they thought they had achieved the intended learning outcomes (Average response for Questions 3–6 in Chapter 5, Table 5.3; $F_{1,70} = 0.02$, $p = 0.90$). Face-to-face and online students also responded similarly with regard to whether instructions were clearly communicated (question 2; $F_{1,71} = 0.22$, $p = 0.64$), whether they indicated that they learned something of value (question 7; $F_{1,71} = 0.10$, $p = 0.76$), whether they indicated that they enjoyed something about the activity (question 9; $F_{1,71} = 1.88$, $p = 0.17$), and whether they suggested some part of the activity to change for future implementation (question 10; $F_{1,71} = 0.24$, $p = 0.62$). It is important to note that only 73 of the 143 students responded to this survey (59 online and 14 face-to-face students) and because of the small sample size of face-to-face students, we are hesitant to draw strong conclusions comparing the two activities based on these data.

For the Phylogenetics activity, students in the face-to-face version of the activity scored higher on their cladogram construction (a higher-order cognitive task; Figure 6.8). For other learning outcomes, students performed similarly in both versions of the activity (Figure 6.8).

6.5 Discussion

Student performance was generally similar for face-to-face and online students across most of the intended learning outcomes. Therefore we concluded that we

successfully used Moodle to develop interactive online laboratory activities that can help students achieve similarly when compared to face-to-face, hands-on interactive laboratory activities. Students in either version of the TM activity performed similarly on quizzes, with two exceptions. This suggests that the online activity format was equally successful compared to the face-to-face activity in educating students on basic thermodynamics concepts, as well as higher order tasks like data interpretation. This is consistent with prior studies that found virtual learning activities were as effective as face-to-face learning activities in terms of scores on assignments (Johnson, 2002; Jones & Long, Vena M, 2013; Krüger et al., 2022; Pei & Wu, 2019; Regmi & Jones, 2020; Richmond et al., 2017; Schulman & Sims, 1999; Sitzmann et al., 2006). However, there was one significant exception. Online students scored significantly higher on the question, "If you hold two frogs with different masses at the exact same body temperature, which do you predict will have a faster breathing rate?". According to MTE, the smaller frog should have a faster breathing rate. It is unclear why online students performed better for this question, but it is possible that the face-to-face activity did not clearly demonstrate this concept due to experimental error, whereas the specially selected online videos for breath rate analysis clearly demonstrated the intended patterns well. Furthermore, it is possible that because students did not directly investigate mass scaling in the lab and this was a concept covered in the lecture, there was more inconsistent delivery of this idea in face-to-face classes due to instructors' varying backgrounds and levels of training. Because we used different quiz questions to focus on different aspects of the activity's content, we are better able to suggest explanations for differences in student performance based on the context of the questions of concern. There was also a

potentially interesting trend that face-to-face students tended to score higher on a skill-based question interpreting a graph of organisms' metabolic rates, although this was only a trend that appeared in the 85% CI. If this trend represents a real difference, this supports our hypothesis that face-to-face students would be more likely to achieve skill-based learning outcomes because students are more likely to benefit from group work, interactions with instructors, and opportunities to practice skills in person.

Student retention of hypothesis-development concepts varied across treatments. Online students created more testable and plausible hypotheses, suggesting that they had a more clear and consistent experience in covering necessary background information that might be relevant to the Cellular Metabolism lab, including concepts of temperature dependence of metabolism and the functions of enzymes. Face-to-face students produced more explanatory hypotheses, possibly due to their increased opportunity to communicate an explanation with peers and instructors Thus, our recommendations for future classes performing these activities involved providing ample opportunities for student-student and student-instructor interactions when constructing hypothesis statements. We also suggested fully emphasizing all aspects of the ERUPT hypothesis rubric. With better focus on all components of a "good" hypothesis, future students will hopefully be able to perform equally well on hypothesis development, regardless of the version of the activity in which they participate.

Interestingly, online students performed better at conducting volumetric measurements. This was contrary to our hypothesis that face-to-face students would better achieve skill-based learning outcomes. This could be because the online students who conducted the bicarbonate Alka-Seltzer reaction at home ended up getting more

practice at measuring volumes of liquid compared to the face-to-face students who only conducted a couple of volumetric measurements in their hydrogen peroxide decomposition reaction experiment. However, this was a trend only seen in the 90% CI, so it is possible that this was a random-chance result and students would require further practice throughout their education to demonstrate significant improvements in this skill.

In the CLASS–Bio (Semsar et al., 2011), problem-solving difficulty appeared to increase for students after participating in the online activity, compared to students participating in the face-to-face activity, meaning online students had an increase in their perception that scientific problems are difficult to solve The reason for this is unclear, but it is possible that students had more opportunities for problem-solving in the face-to-face lab and therefore felt more confident. Alternatively, it is possible that students conducting the online lab experienced more confusion about problem-solving because they did not have as easy access to input from their instructor. Otherwise, students responded similarly to the CLASS–Bio question categories regardless of the version of the TM activity in which they participated. This suggests that both the face-to-face and the online versions of the activity resulted in similar disposition outcomes and thus the interactive online version was relatively successful in replicating the face-to-face version's impact on student dispositions. Additionally, comparisons of the pre- and post- activity CLASS–Bio were not significantly different, whereas the published results of introductory biology courses suggest that student dispositions tend to stray from expert consensus more after completing the semester (Semsar et al., 2011). This evidence suggests that the TM activity was generally more successful than the average introductory biology class insofar as it did not negatively affect student dispositions. However, it is important to note that

the CLASS–Bio is generally administered before and after an entire semester (Semsar et al., 2011), so the impacts of a single lab activity might be considered relatively insignificant.

For the Phylogenetics activity, face-to-face students performed better on the higher order tasks of creating a cladogram (Fig. 6.8), possibly because these students had more opportunities to ask questions and get input from the instructor. Thus, it is important for students to have appropriate guidance in online activities to achieve similar learning outcomes. However, students performed equally well on lower order tasks, suggesting that basic evolutionary concepts were still effectively taught in an asynchronous learning environment.

Across the intended knowledge, skill, and disposition learning outcomes of the TM and the Phylogenetics activities, face-to-face and online students performed similarly with a couple of important exceptions. This suggests that the online learning environment was generally successful in replicating the benefits of a face-to-face laboratory activity. One key difference between the two versions of the activities included online students having equal or better performance on lower order knowledge outcomes compared to face-to-face students. This is supported by the literature (Johnson, 2002; Jones & Long, 2013; Krüger et al., 2022; Pei & Wu, 2019; Regmi & Jones, 2020; Richmond et al., 2017; Schulman & Sims, 1999; Sitzmann et al., 2006), and suggests that the structured activities that encompass the intended knowledge outcomes were successfully implemented in the online versions of the activities. Face-to-face students performed better on certain higher order tasks (e.g. creating more explanatory hypotheses, successfully conducting graphical analysis, and creating more parsimonious cladograms).

This supports our hypothesis that face-to-face students would gain more practical skills than online students because of the increased opportunities for interaction with peers and instructors, as well as increased opportunities for practicing these skills in a classroom setting. Thus, while students achieved similarly for most of the intended learning outcomes of these activities, there is still evidence that students can gain more in terms of skill-based outcomes if they conduct a lab face-to-face with multiple opportunities to interact with others and practice hands-on skills. Although we did not have the time or resources to include peer-to-peer or student-teacher discussions in our online activity, this would be an important step moving forward to improve the lack of social aspects our activity offers. In the future, implementing a discussion forum for students to engage with each other and teachers, or a synchronous video group-discussion activity, might lead to more student engagement and reinforce higher order learning outcomes.

6.6 Material availability

Presentation materials, worksheets, and quizzes can be found at https://github.com/huntercraig248/Thermal-Metabolism-and-Phylogenetics

6.7 Chapter 6 tables

Table 6.1: Mixed-effects linear model outputs comparing hypothesis statements developed for the Thermal Metabolism quiz (see 6.6 Material availability, Thermal Metabolism quiz question 6) between face-to-face and online versions of the activity. See Fig. 6.2 for confidence intervals generated through bootstrapping.

Response Variable	F	df	p
Explanatory	1.56	1, 13.70	0.23
Relevant	0.09	1, 14.13	0.77
Understandable	0.07	1, 14.82	0.80
Plausible	0.36	1, 13.70	0.56
Testable	0.92	1, 13.70	0.35
Overall Score	0.63	1, 13.70	0.44

Table 6.2: Mixed-effects linear model outputs comparing hypothesis statements developed for the Cellular Metabolism activity (Fig. 6.3) between face-to-face and online versions of the TM activity. See Fig. 6.3 for confidence intervals generated through bootstrapping.

Response Variable	F	df	p
Explanatory	3.03	1, 17.23	0.10
Relevant	1.24	1, 17.23	0.28
Understandable	0.84	1, 18.24	0.37
Plausible	4.16	1, 17.47	0.06
Testable	1.87	1, 18.69	0.19
Overall Score	0.01	1, 19.77	0.94

Table 6.3: Mixed-effects linear model outputs comparing Thermal Metabolism-related student scores between face-to-face and online versions of the activity. Random effect of half-section was included to control for class differences. For individual questions, binomial models and chi-square tests were used because the responses (correct vs. incorrect) were binomial. See Fig. 6.4-6.6 for confidence intervals generated through bootstrapping. See 6.6 Material availability for quiz questions.

Response Variable	Test Statistic	df	p
Quiz Question 1	0.38 (χ^2)	1, 96	0.54
Quiz Question 2	0.87 (χ^2)	1, 96	0.35
Quiz Question 3	0.08 (χ^2)	1, 96	0.77
Quiz Question 4	0.11 (χ^2)	1, 96	0.74
Quiz Question 5	4.42 (χ^2)	1, 96	0.04
Quiz Question 7	0.17 (χ^2)	1, 87	0.68
Quiz Question 8	2.35 (χ^2)	1, 87	0.13
Quiz 3 Total Score	1.90 (F)	1,13.7	0.19
Volumetric Measurement Accuracy	2.30 (F)	1, 134	0.13

Table 6.4: T-test comparisons of the CLASS–Bio results between students taking the survey before and after completing the Thermal Metabolism activity. Response variables represent established categories of questions on the survey (Semsar et al., 2011).

Response Variable	T	df	p
Real world connection	-0.47	39.69	0.64
Problem-solving difficulty	0.22	39.86	0.83
Enjoyment	-0.25	38.15	0.80
Problem-solving effort	-0.44	39.77	0.66
Conceptual connections/Memorization	-0.93	39.10	0.36
Problem-solving strategies	-0.31	39.81	0.76
Reasoning	-1.31	34.82	0.20
Overall	-0.57	39.42	0.57

6.8 Chapter 6 figures

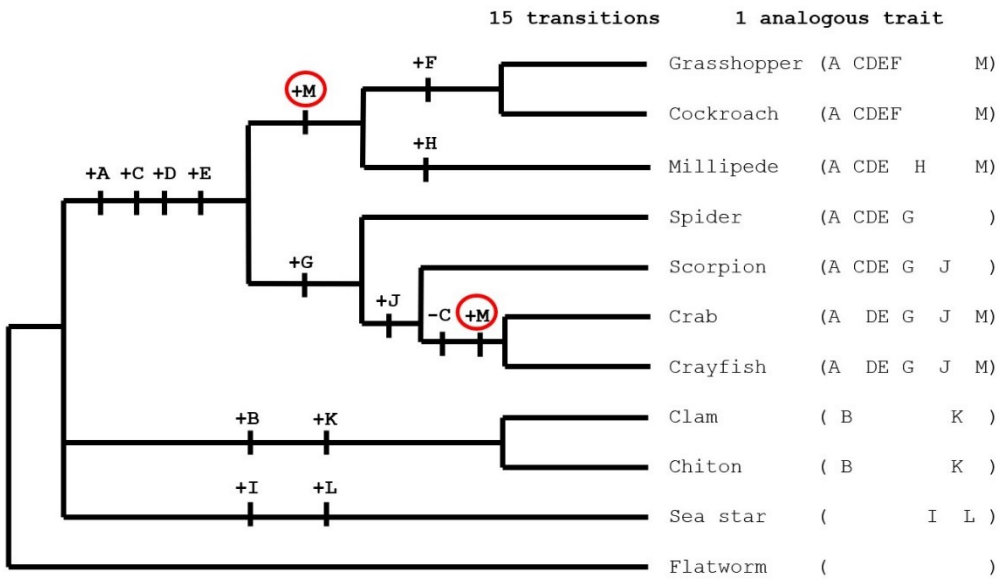

Figure 6.1: The most parsimonious cladogram possible given the species and traits listed in the Phylogenetics Activity assignment. Species are listed with their traits (listed by letter) on the right. Each addition or subtraction of a trait on the cladogram on the left counts as one transition, for a total of 15 transitions. There is 1 analogous trait (trait M), circled.

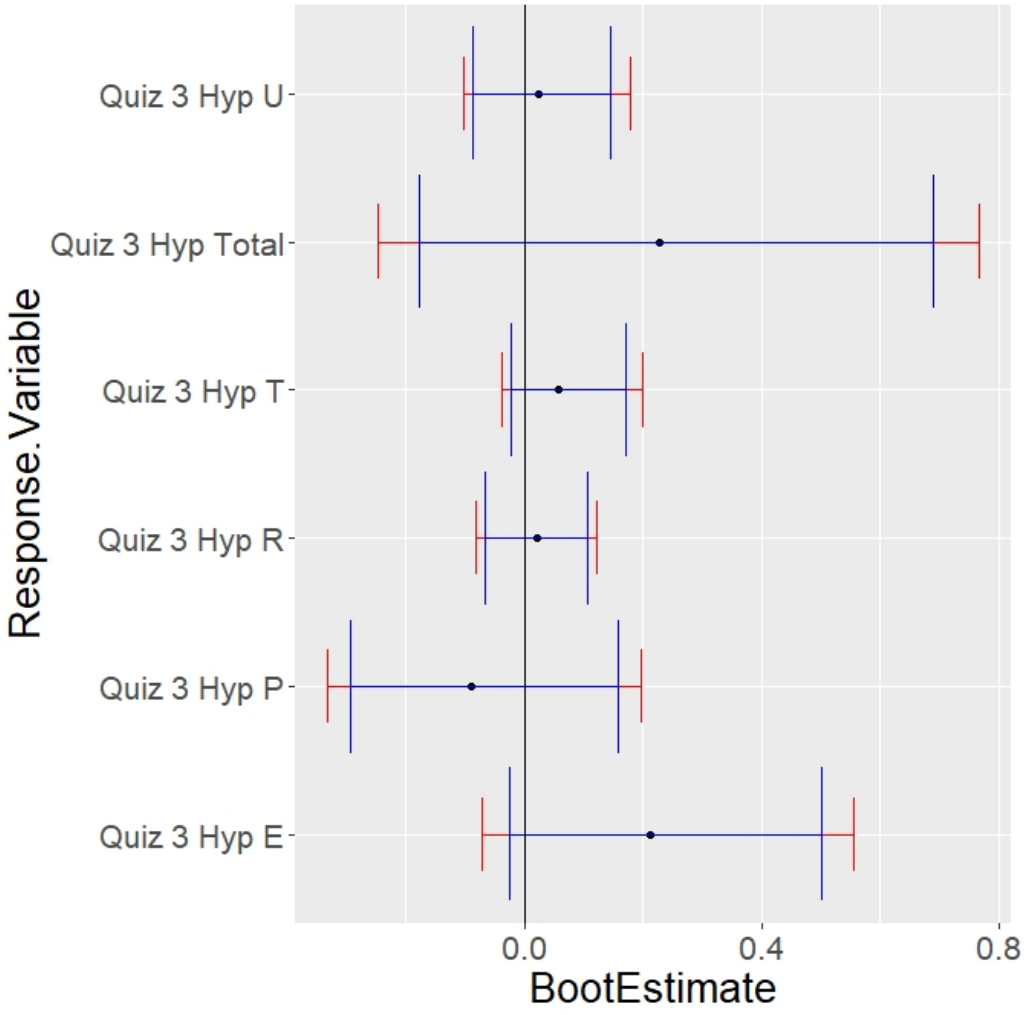

Figure 6.2: Confidence intervals generated through bootstrapping linear mixed-effects models evaluating overall scores of hypotheses developed about the Thermal Metabolism lab. Blue lines represent 90% CI and red lines represent 95% CI. Confidence intervals to the left of the 0 line indicate face-to-face students performed better, and confidence intervals to the right of the 0 line indicate online students performed better. This hypothesis was developed to answer question 7 on the post-Thermal Metabolism lab quiz administered 1 week after the activity (see 6.6 Material availability).

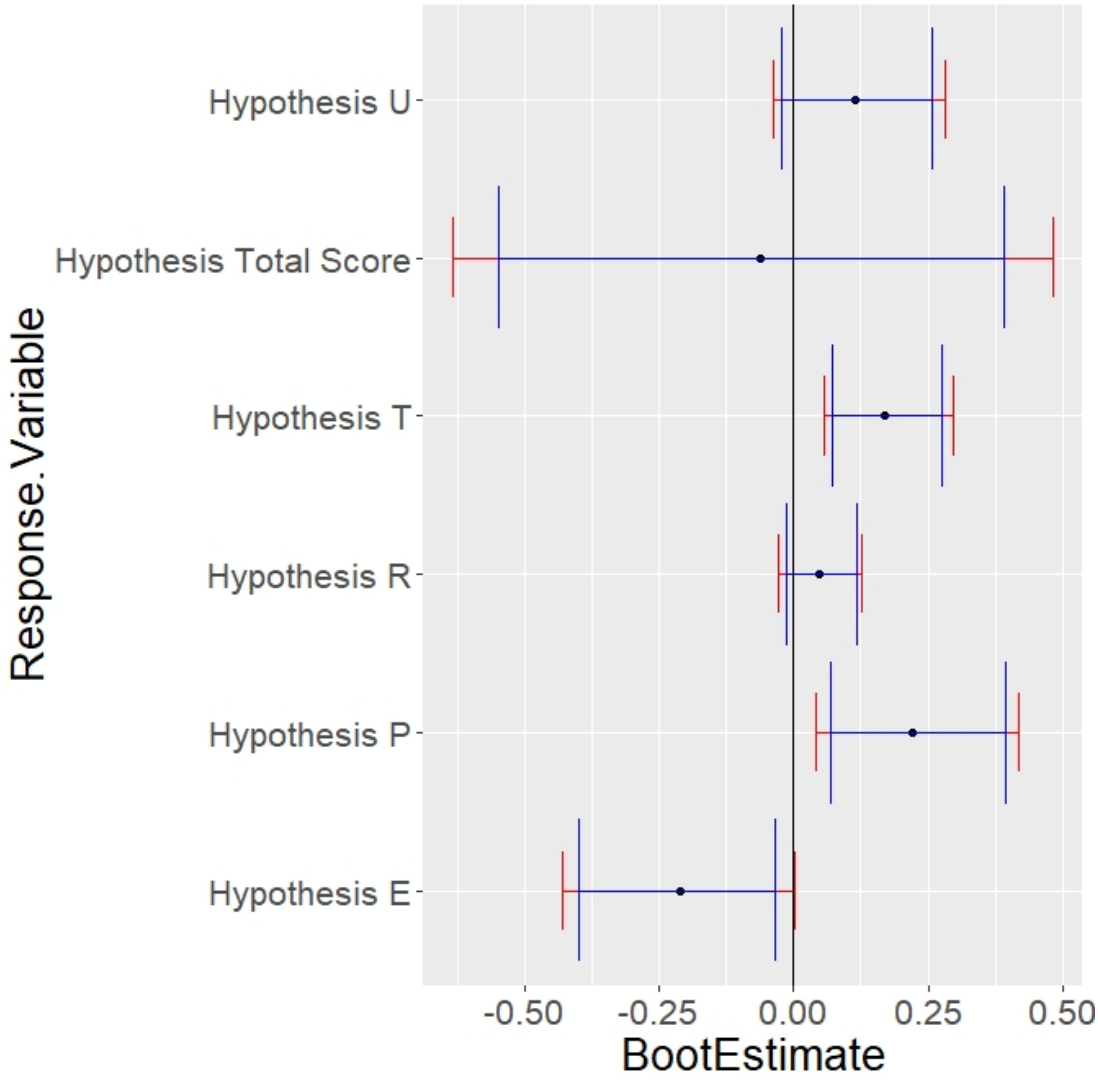

Figure 6.3: Confidence intervals generated through bootstrapping linear mixed-effects models evaluating overall scores of hypotheses developed for the Cellular Metabolism lab activity, conducted two weeks after the Thermal Metabolism lab activity. Blue lines represent 90% CI and red lines represent 95% CI.

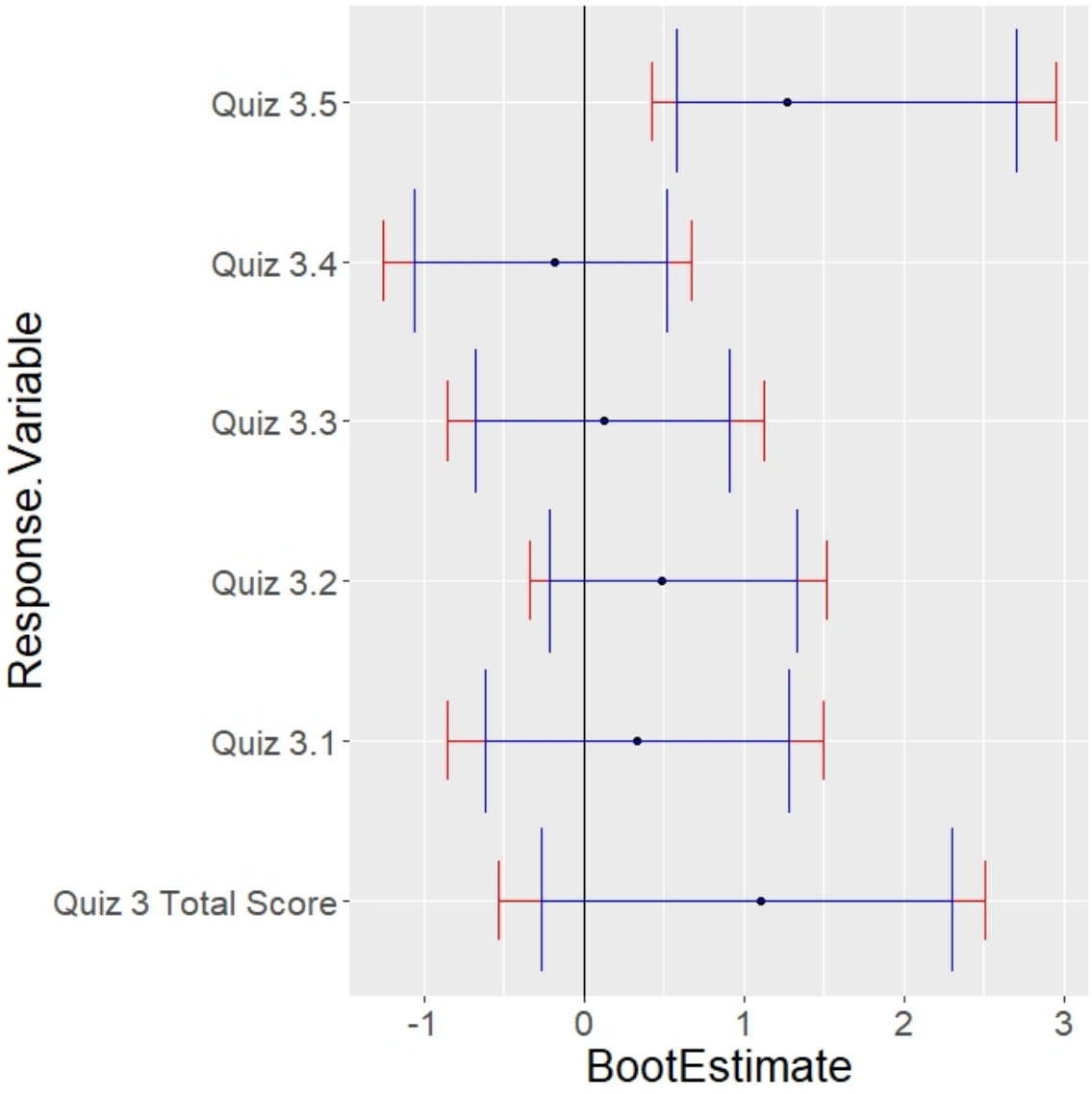

Figure 6.4: Confidence intervals generated through bootstrapping linear mixed-effects models evaluating overall scores of each question on the quiz administered one week following the Thermal Metabolism lab activity. Blue lines represent 90% CI and red lines represent 95% CI. See 6.6 Material availability for quiz questions.

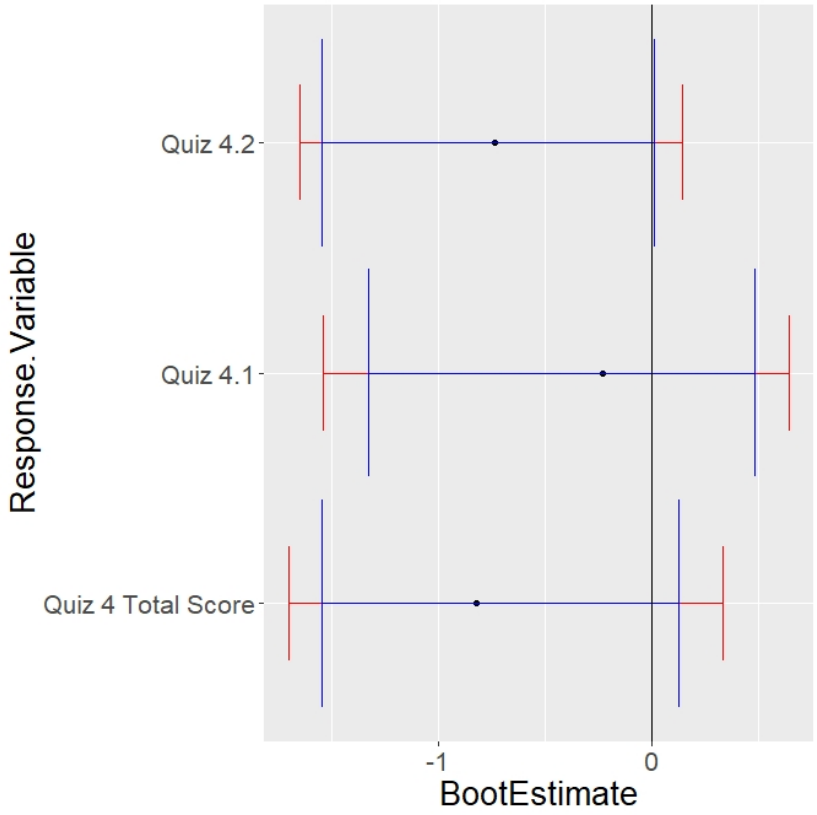

Figure 6.5: Confidence intervals generated through bootstrapping linear mixed-effects models evaluating overall scores of each question on the quiz administered two weeks following the Thermal Metabolism lab activity. Blue lines represent 90% CI and red lines represent 95% CI. See 6.6 Material availability, Thermal Metabolism quiz questions 7 and 8.

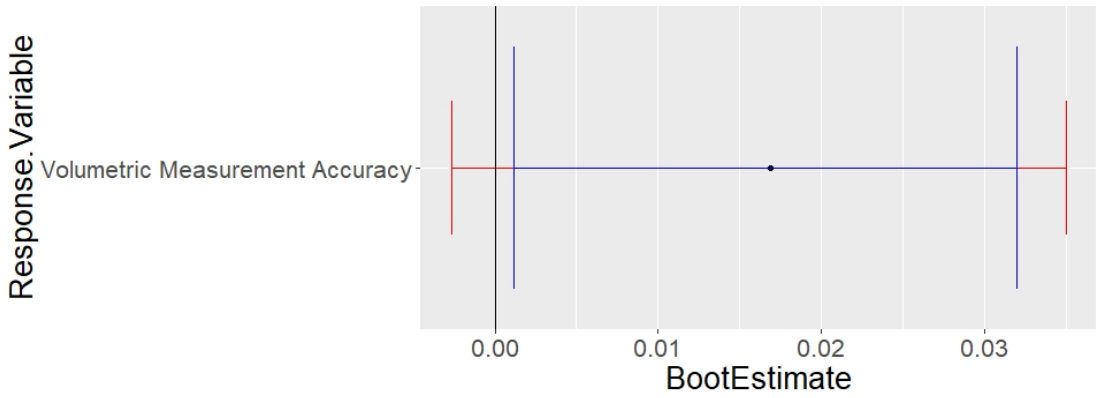

Figure 6.6: Confidence interval generated through bootstrapping linear mixed-effects model evaluating the students' accuracy in measuring out 10 mL water using a graduated cylinder 2 weeks following the Thermal Metabolism lab activity. Blue lines represent 90% CI and red lines represent 95% CI.

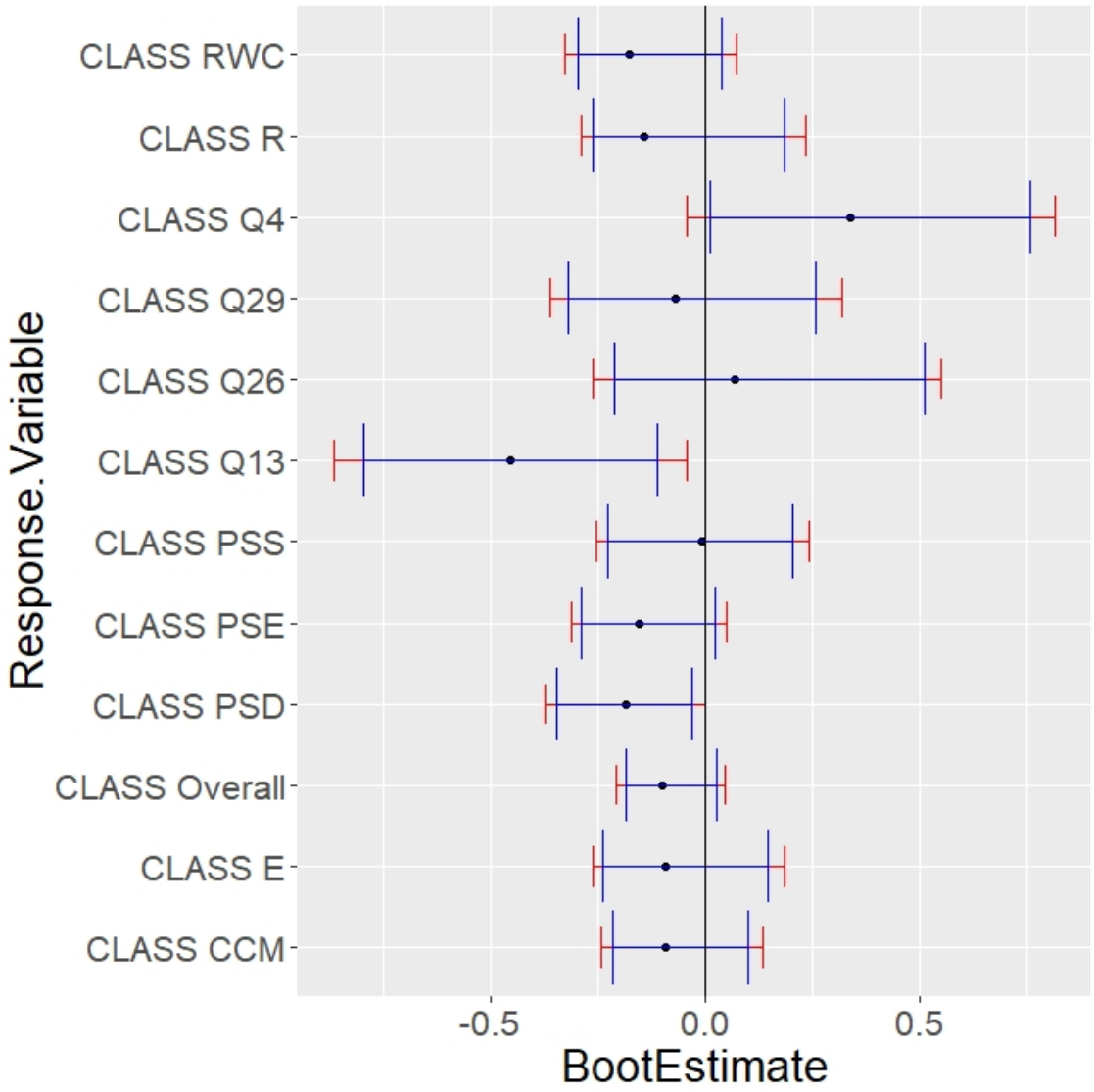

Figure 6.7: Confidence interval generated through bootstrapping linear mixed-effects model evaluating the students' change in responses to the CLASS–Bio survey pre– and post–Thermal Metabolism lab activity. Blue lines represent 90% CI and red lines represent 95% CI.

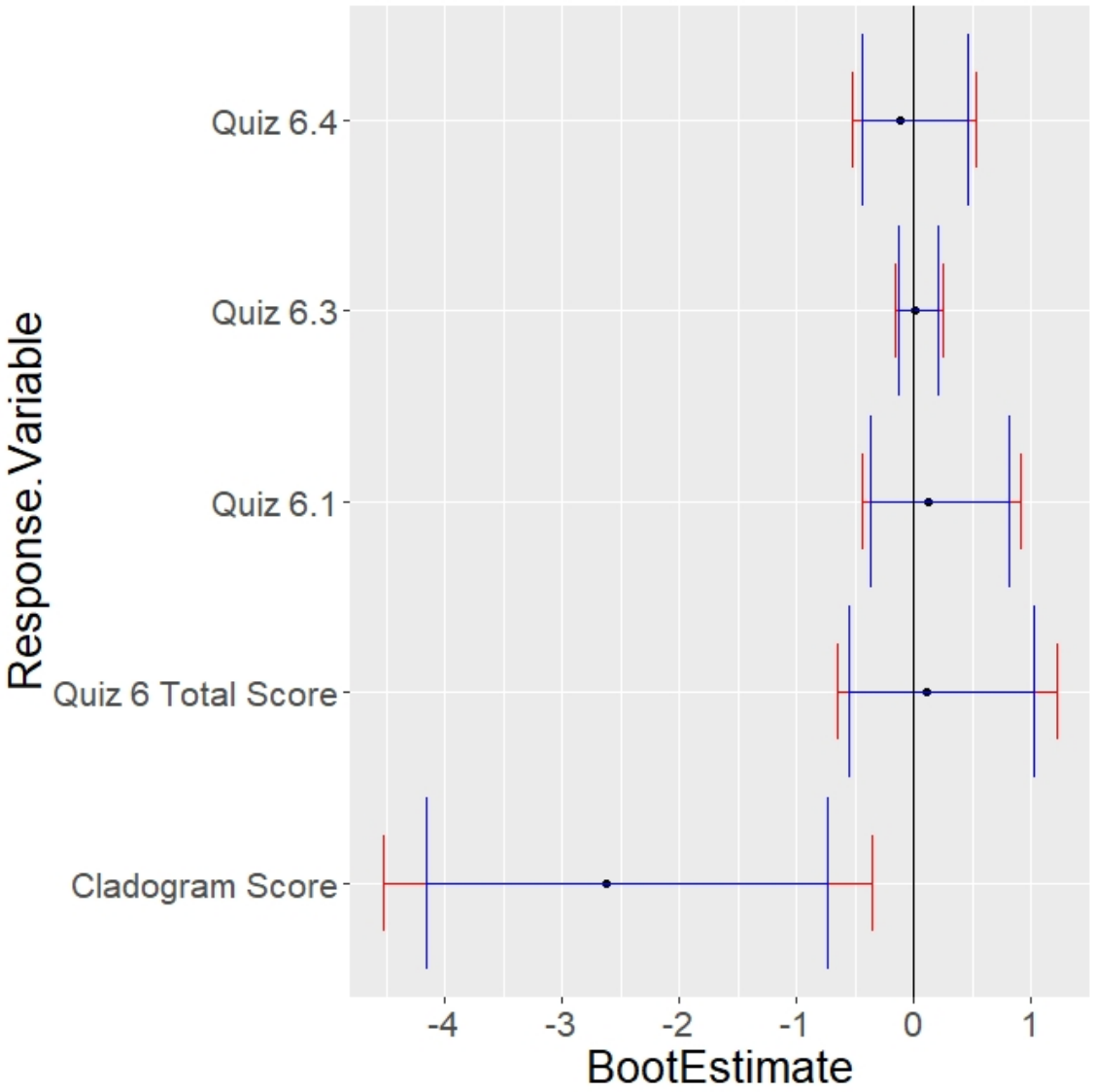

Figure 6.8: Confidence interval generated through bootstrapping linear mixed-effects model evaluating the students' performance on the post-Phylogenetics Activity quiz and the overall score on the 11-species cladogram developed for the activity. Blue lines represent 90% CI and red lines represent 95% CI. See 6.6 Material availability for Phylogenetics quiz questions.

APPENDIX A

IACUC APPROVAL DOCUMENT

Oakland University
Institutional Animal Care and Use Committee
Committee Action Record

January 16, 2018	4405
Committee Review Date	**RAM Number**

PROJECT TITLE

A Metabolic Theory Approach to the Thermal Biology of Parasitism

Approved [X] Disapproved [ ]

NEW PROJECT

[X] 18011 — **IACUC Project Approval Number**

Thomas Raffel, Ph.D. — **Principal Investigator**

REVISED PROJECT

Your Request for Revision of your Application for Use of Vertebrate Animals has been reviewed by IACUC and given full approval.

[ ] _____ — **New IACUC Project Approval Number***

_____ — **Principal Investigator**

*** Please use this new approval number, with the revision number indicated, in all future activities.**

Authorization for Project Commencement

Keith Williams — **IACUC Chairperson: Keith Williams, PhD**

1/20/2018 — **Date**

Approval Period: **February 1, 2018 through January 31, 2021**

Annual Review Dates: **February 1, 2019 and February 1, 2020**

Project Completion Summary Due Date: **January 31, 2021**

APPENDIX B

IBC APPROVAL DOCUMENT

OAKLAND UNIVERSITY

Institutional Biosafety Committee

May 19, 2021

Professor Thomas Raffel
Assistant Professor
Department of Biological Sciences

Reference: Addendum to IBC Application # 2759-13, "A Metabolic Theory Approach to the Thermal Biology of Parasitism"

Dear Professor Raffel:

The Institutional Biosafety Committee (IBC), responsible for the review of research involving materials of human origin, recombinant DNA, and biohazardous, infectious or toxic materials, has reviewed the original proposal, noted the revisions provided by you upon committee's request, and approved the revised project referenced above.

The proposed addendum to include a new procedure for treating chytridiomycosis-infected frogs with the drug itraconazole has been approved. The BSL-2 biosafety level and approval period of March 13, 2018 through March 12, 2023 has not changed.

Any unanticipated problems, changes in, or halting of the activities proposed in the project, as described, must be promptly reported to the IBC or the biosafety officer.

In addition, all faculty, staff and students involved in the project must be trained and qualified to conduct the project in a responsible manner. Please note, in this regard, that you, the principal investigator, are fully responsible at all times for limiting or restricting access to laboratories and for assessing, in each circumstance, who may enter or work in a laboratory when biohazardous materials are in use.

Please let me know if you have any questions. Thank you.

Sincerely,

Domenic Luongo, CHMM
Laboratory Compliance Manager

Office of Research Administration

371 Wilson Blvd, Rochester, MI 48309-4486
(248) 370-4898 | Fax: (248) 370-2973 | oakland.edu

APPENDIX C

DETERMINATION OF EXEMPT HUMAN SUBJECTS RESEARCH BY THE IRB

OAKLAND UNIVERSITY

Institutional Review Board

July 21, 2021

Protocol #: IRB-FY2021-369

Research Team:
Hunter Craig
Thomas Raffel
Holly Greiner-Hallman

Based on applicable federal regulations, the following study, "Comparing online and in-person biology laboratory class activities" has been determined to be Exempt, with the following categories Category 1. Research, conducted in established or commonly accepted educational settings, that specifically involves normal educational practices that are not likely to adversely impact students' opportunity to learn required educational content or the assessment of educators who provide instruction. This includes most research on regular and special education instructional strategies, and research on the effectiveness of or the comparison among instructional techniques, curricula, or classroom management methods.

Notes for Researcher(s):
The research is approved with the following understanding:

1. The in-person interaction between the researcher(s) and participants is occurring for educational purposes regardless of the research;
2. Researcher(s) will follow the Oakland University guidelines and procedures to mitigate the risk of COVID-19 exposure or transmission; and
3. Permission from the Chair of the Department of Biological Sciences to conducted the in-person research has been provided.

This submission includes the following approved documents:

- Consent Form (FERPA) V 7/21/21

Permission from Research Site(s):
Please note the following:

- This IRB exemption determination letter means that this research has met one or more of the federal criteria for exemption per 45 CFR 46.104- Exempt Research.
- Before the research is initiated, permission to conduct research at a given site must be obtained from all research locations listed in the IRB submission. You must keep copies of all such permission letters for your files.
- It is the responsibility of each researcher to follow all applicable policies and procedures of any outside institution where the research will be conducted.

Letter and Consent Document:
This letter along with the IRB approved (date-stamped) consent document can be found in Cayuse in the Submission Details page under Letters and Attachments, respectively.

Modifications:
Any changes to this exempt project must be reviewed by the IRB prior to initiation by submitting a MODIFICATION request. Do not collect data while the changes are being reviewed. Data collected during this time cannot be used in research.

Record Retention:
Exempt projects will be retained by the IRB office for three years after the last action on the project.

You are approved to start the research. Please retain a copy of this notification for your records.

If you have any questions, please contact the IRB office.

Thank you.

The Oakland University IRB

APPENDIX D

CONSENT FORM TO COLLECT STUDENT AND TEACHER INFORMATION FOR CHAPTER SIX

Information Sheet for Exempt Research
Study: Comparing online versus in-person laboratory activities in BIO 1201

Introduction

You are being asked to participate in an educational research study comparing in-person and online versions of laboratory activities implemented in BIO 1201. This study is being conducted by OU graduate student Hunter Craig as part of his doctoral dissertation research, under the direction of Dr. Tom Raffel (Associate Professor, Biological Sciences).

The purpose of this study is to assess the effectiveness of two online laboratory activities that were developed and implemented during the COVID-19 pandemic, in comparison with the original in-person versions of these same activities. To reduce the risk of continued disease transmission during the F2021 semester, each lab section will be randomly assigned the online version of one of these two lab activities.

Your participation in this study is completely voluntary and will not affect your present or future relationship with Oakland University, the Department of Biological Sciences, the researchers, or the instructors for this course. To ensure there will be no effect of this study on BIO 1201 grades, the list of study participants will be kept confidential from BIO 1201 instructors and teaching assistants until after final grades have been assigned.

What do I have to do?

Your decision to participate in this study will have no effect on the activities, assignments, or exams you will be asked to complete as a student or teaching assistant participating in BIO 1201. There is no extra time commitment for participating in this study beyond the time it takes for you to sign and submit this form. By signing this form, you give permission for the researchers to access your demographic and education records, including your class standing, major, overall GPA, science course GPA, BIO 1201 course grades, assignments, quizzes, exams, and survey responses. The researchers listed at the end of this document along with the research team will view these records after final grades have been submitted for this course and will remove your name from the records prior to analysis.

Are there any risks to me?

For this study, the only potential risk is breach of confidentiality; i.e., that someone who is not part of this research project may accidentally see your personal information. We will try to make sure that this does not happen by keeping your research records as confidential as possible, and by de-identifying your data prior to analysis. When the results of this research are published or presented at conferences, no information will be included that personally identifies you.

What are the benefits of this study?

This study will help guide teaching practices for in-person and online lab activities, to improve future BIO 1201 classes.

Will I receive anything for participating?

Students and teaching assistants who participate in this study will receive a $5 Amazon.com gift **card** via their oakland.edu email address, after the semester is over and final grades have been submitted. Students or TAs who choose not to participate, or decide to opt out later in the semester, will not receive a gift card.

What if I change my mind about participating?

You can choose to start or stop participating at any time until the last day of the Fall 2021 semester, by sending an email to one of the researchers listed below. Students who wish to join the study later in the semester can give consent by signing a copy of this form. After the class is over and data are compiled, it will not be possible to alter our list of participants because names and other identifying information will already have been removed from the data.

Who should I contact if I have questions about this study?

You may contact
For questions regarding your rights as a participant in human subject research, you may contact the Oakland University Institutional Review Board,

Your signature below means that you authorize Oakland University to disclose information from your education records as described in this Consent Form, for use in connection with the research study, to the researchers listed in this form.

Name of participant (print)	**OU email address**	**Signature of participant**	**Date**
_____	_____	_____	

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